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Synthesis and antimycobacterial activity of some N^1-[1-[3-aryl-1-(pyridin-2-, 3-, or 4-yl)-3-oxo]propyl]-2-pyridinecarboxamidrazones

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Abstract

N^1-[1-[3-aryl-1-(pyridin-2-,3-, and 4-yl)-3-oxo]propyl]-2-pyridinecarboxamidrazone derivatives were synthesized and tested for their in vitro antimycobacterial activity. Some compounds showed interesting activity against a strain of *Mycobacterium tuberculosis* and a strain of *Mycobacterium avium*. © 1999 Elsevier Science S.A. All rights reserved.

Keywords: Pyridine-2-carboxamidrazone; Antimycobacterial activity

1. Introduction

The increase of reported cases of tuberculosis even in developed countries, along with the emergence of multidrug-resistant (MDR) *Mycobacterium tuberculosis* strains, has refocused attention on this illness. An important factor in the re-emergence of tuberculosis was the spread of acquired immunodeficiency syndrome (AIDS). Another consequence of the spread of AIDS has been the emergence of infections associated with mycobacteria other than tuberculosis (MOTT), among which members belonging to the *M. avium* intracellular complex (MAC) frequently produce severe disseminated infections in HIV-infected patients. In previous papers [1–5] we described some pyridine-2-carboxamidrazone and pyridine-4-carboxamidrazone derivatives. Among those compounds some showed interesting in vitro activity against *M. tuberculosis* $H_{37}Rv$ and against strains of *M. tuberculosis* isolated from human bronchial aspirates, some of which were resistant to isoniazid, rifampicin and ofloxacin. It is noteworthy that some of those compounds exhibited inhibitory activity towards a human strain of *M. avium* resistant to the primary drugs isoniazid, rifampicin and ofloxacin. In consideration of the antimycobacterial properties of compounds characterized by the presence of the pyridine-2-carboxamidrazone moiety, we synthesized a series of new N^1-substituted pyridine-2-carboxamidrazone derivatives **22**–**42** (Table 1). All synthesized compounds were tested for their antimycobacterial activity towards a strain of *M. tuberculosis* and a strain of *M. avium*. The activity of these compounds against a strain of *Candida albicans* and one of *Escherichia coli* was also determined.

2. Chemistry

The synthesis of N^1-[1-[3-aryl-1-(pyridin-2-, 3-, or 4-yl)-3-oxo]propyl]-2-pyridinecarboxamidrazones (**22**–**42**) (Table 1) was carried out (Scheme 1) by adding 2-pyridinecarboxamidrazone (**1a**) to 1-aryl-3-(pyridin-2-yl)-propenones (**1**–**7**), 1-aryl-3-(pyridin-3-yl)-propenones (**8**–**14**) and 1-aryl-3-(pyridin-4-yl)-propenones (**15**–**21**), variously substituted on the phenyl residue. Pyridine-2-carboxamidrazone (**1a**) was prepared by direct action of hydrazine on 2-cyanopyridine, according to the previously proposed method [6]. The α,β-unsaturated ketones (**1**–**21**) (Table 2) were prepared by the conventional method described in Section 3.

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3. Experimental

3.1. Chemistry

Melting points (m.p.) 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 spectrophotometer; chemical shifts are reported as δ (ppm) relative to tetramethylsilane as the internal standard, deuterochloroform as the solvent. Reaction courses and product mixture were routinely monitored by thin-layer chromatography (TLC) 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 from the theoretical value.

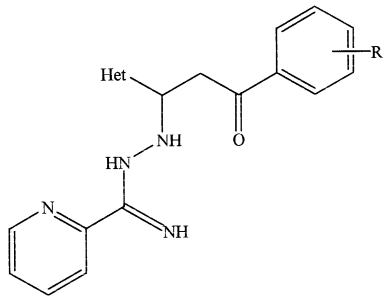
3.1.1. General procedure for the preparation of 1-aryl-3-(pyridin-2-,3-,or 4-yl)-propenones (**1**–**21**)

To a solution of 5 g (46 mmol) of 2-, 3- or 4-pyridinecarboxaldehyde in 5 ml of methanol and 9 ml of 10% sodium hydroxide, the appropriate acetophenone (23 mmol) was added dropwise under cooling (0–5°C) and stirring. After complete addition, the reaction mixture was stirred for 1–2 h keeping the temperature at about 10°C. The resulting solid was collected by filtration and washed thoroughly with water. The solid was then dried and recrystallized from ethanol (Table 2).

3.1.2. N^1-[1-[3-Phenyl-1-(pyridin-2-yl)-3-oxo]propyl]-2-pyridinecarboxamidrazone (**22**)

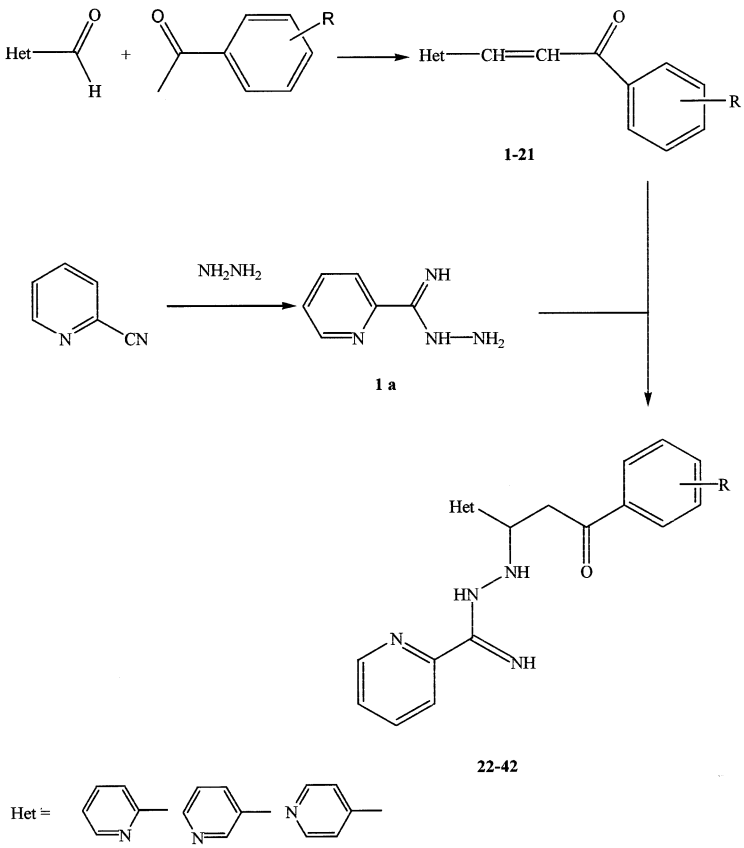
To the solution of the α,β-unsaturated ketone **1** in 20 ml of absolute ethanol, 0.60 g (4.4 mmol) of 2-pyridincarboxyamidrazone dissolved in 20 ml of the same solvent was added: the reaction mixture was stirred at

Table 1
Yields and melting points for compounds **22**–**42**



22-42

Comp.	Het	R	Yield (%)	M.p. (°C)	Formula
22	2-pyridyl	H	55	120	$C_{20}H_{19}N_5O$
23	2-pyridyl	3-Cl	35	122	$C_{20}H_{18}N_5OCl$
24	2-pyridyl	4-Cl	22	135	$C_{20}H_{18}N_5OCl$
25	2-pyridyl	3,4-Cl_2	50	126	$C_{20}H_{17}N_5OCl_2$
26	2-pyridyl	3-CH_3	31	98	$C_{21}H_{21}N_5O$
27	2-pyridyl	4-CH_3	39	125	$C_{21}H_{21}N_5O$
28	2-pyridyl	4-Ph	76	147	$C_{26}H_{23}N_5O$
29	3-pyridyl	H	21	124	$C_{20}H_{19}N_5O$
30	3-pyridyl	3-Cl	25	150	$C_{20}H_{18}N_5OCl$
31	3-pyridyl	4-Cl	21	140	$C_{20}H_{18}N_5OCl$
32	3-pyridyl	3,4-Cl_2	58	135	$C_{20}H_{17}N_5OCl_2$
33	3-pyridyl	3-CH_3	25	130	$C_{21}H_{21}N_5O$
34	3-pyridyl	4-CH_3	37	124	$C_{21}H_{21}N_5O$
35	3-pyridyl	4-Ph	36	160	$C_{26}H_{23}N_5O$
36	4-pyridyl	H	44	124	$C_{20}H_{19}N_5O$
37	4-pyridyl	3-Cl	40	137	$C_{20}H_{18}N_5OCl$
38	4-pyridyl	4-Cl	37	130	$C_{20}H_{18}N_5OCl$
39	4-pyridyl	3,4-Cl_2	40	150	$C_{20}H_{17}N_5OCl_2$
40	4-pyridyl	3-CH_3	47	125	$C_{21}H_{21}N_5O$
41	4-pyridyl	4-CH_3	38	160	$C_{21}H_{21}N_5O$
42	4-pyridyl	4-Ph	32	150	$C_{26}H_{23}N_5O$



Scheme 1.

room temperature (r.t.). After 24 h, the precipitate was collected by filtration and recrystallized from absolute ethanol to obtain 2.75 g (55%) of **22**; m.p. 120°C.

IR (Nujol, cm^{-1}): 3440, 3340, 3200; 1680; 1610. ^1H NMR ($CDCl_3$/TMS): δ 3.40 (dd, 1H, C*H*H, $J = 16.87$ and 5.82 Hz), 3.90 (dd, 1H, C*H*H, $J = 16.87$ and 7.69 Hz), 3.80–4.45 (br, s, 1H, NH, disappearing on deuteration), 5.0 (m, 1H, CH), 5.30 (s, 2H, NH, disappearing on deuteration), 7.20–8.70 (m, 13H, arom. and pyr.). MS: m/z 345 [M^+]. *Anal.* ($C_{20}H_{19}N_5O$): C, H, N.

In an analogous way the following compounds (**22**–**42**) have been prepared. Yields and melting point are reported in Table 1.

*3.1.3. N[1]-[1-[3-(3-Chlorophenyl)-1-(pyridin-2-yl)-3-oxo]propyl]-2-pyridinecarboxamidrazone (**23**)*

IR (Nujol, cm^{-1}): 3400, 3310, 3220; 1670; 1610. ^1H NMR ($CDCl_3$/TMS): δ3.65 (dd, 1H, C*H*H, $J = 16.75$ and 5.94 Hz), 3.90 (dd, 1H, CH*H*, $J = 16.75$ and 7.03 Hz), 3.70–4.30 (br, s, 1H, NH, disappearing on deuteration), 5.05 (m, 1H, CH), 5.30 (s, 2H, NH, disappearing on deuteration), 7.20–8.60 (m, 12H, arom. and pyr.). MS: m/z 378,380 [M^+]. *Anal.* ($C_{20}H_{18}N_5OCl$): C, H, N.

*3.1.4. N[1]-[1-[3-(4-Chlorophenyl)-1-(pyridin-2-yl)-3-oxo]propyl]-2-pyridinecarboxamidrazone (**24**)*

IR (Nujol, cm^{-1}): 3430, 3320, 3180; 1680; 1620. ^1H NMR ($CDCl_3$/TMS): δ 3.65 (dd, 1H, C*H*H, $J = 16.70$ and 5.92 Hz), 3.90 (dd, 1H, CH*H*, $J = 16.70$ and 7.02 Hz), 3.85–4.55 (br, s, 1H, NH, disappearing on deuteration), 5.10 (m, 1H, CH), 5.30 (s, 2H, NH, disappearing on deuteration), 7.20–8.60 (m, 12H, arom. and pyr.). MS: m/z 378,380 [M^+]. *Anal.* ($C_{20}H_{18}N_5OCl$): C, H, N.

*3.1.5. N[1]-[1-[3-(3,4-Dichlorophenyl)-1-(pyridin-2-yl)-3-oxo]propyl]-2-pyridinecarboxamidrazone (**25**)*

IR (Nujol, cm^{-1}): 3440, 3320, 3210; 1680; 1610. ^1H NMR ($CDCl_3$/TMS): (δ) 3.60 (dd, 1H, C*H*H, $J =$ 16.74 and 6.36 Hz), 3.85 (dd, 1H, CH*H*, $J = 16.74$ and 6.66 Hz), 3.80–4.75 (br, s, 1H, NH, disappearing on deuteration), 5.10 (m, 1H, CH), 5.35 (s, 2H, NH, disappearing on deuteration), 7.20–8.60 (m, 11H, arom. and pyr.). MS: m/z 414 [M^+]. *Anal.* ($C_{20}H_{17}N_5OCl_2$): C, H, N.

*3.1.6. N^{1}-[1-[3-(3-Methylphenyl)-1-(pyridin-2-yl)-3-oxo]propyl]-2-pyridinecarboxamidrazone (**26**)*

IR (Nujol, cm^{-1}): 3400, 3320, 3180; 1680; 1620. ^1H NMR ($CDCl_3$/TMS): δ 2.40 (s, 3H, CH_3), 3.70 (dd, 1H, C*H*H, $J = 16.77$ and 6.9 Hz), 3.85 (dd, 1H, CH*H*, $J = 16.77$ and 7.02 Hz), 3.75–4.35 (br, s, 1H, NH, disappearing on deuteration), 5.10 (m, 1H, CH), 5.40 (s, 2H, NH, disappearing on deuteration), 7.30–8.70 (m, 12H, arom. and pyr.). MS: m/z 359 [M^+]. *Anal.* ($C_{21}H_{21}N_5O$): C, H, N.

*3.1.7. N^{1}-[1-[3-(4-Methylphenyl)-1-(pyridin-2-yl)-3-oxo]propyl]-2-pyridinecarboxamidrazone (**27**)*

IR (Nujol, cm^{-1}): 3400, 3320, 3200; 1680; 1610. ^1H NMR ($CDCl_3$/TMS): (δ) 2.40 (s, 3H, CH_3), 3.65 (dd, 1H, C*H*H, $J = 16.92$ and 6.31 Hz), 3.90 (dd, 1H, CH*H*, $J = 16.92$ and 6.45 Hz), 3.70–4.30 (br, s, 1H, NH, disappearing on deuteration), 5.10 (m, 1H, CH), 5.30 (s, 2H, NH, disappearing on deuteration), 7.20–8.60 (m, 12H, arom. and pyr.). MS: m/z 359 [M^+]. *Anal.* ($C_{21}H_{21}N_5O$): C, H, N.

*3.1.8. N^{1}-[1-[3-(4-Biphenylyl)-1-(pyridin-2-yl)-3-oxo]-propyl]-2-pyridinecarboxamidrazone (**28**)*

IR (Nujol, cm^{-1}): 3420, 3320, 3220; 1680; 1620. ^1H NMR ($CDCl_3$/TMS): δ 3.10 (dd, 1H, C*H*H, $J = 16.18$ and 8.9 Hz), 3.70 (dd, 1H, CH*H*, $J = 16.18$ and 9.9 Hz), 3.80–4.70 (br, s, 1H, NH, disappearing on deuteration), 4.90 (m, 1H, CH), 5.30 (s, 2H, NH, disappearing on deuteration), 7.30–8.70 (m, 17H, arom. and pyr.). MS: m/z 421 [M^+]. *Anal.* ($C_{26}H_{23}N_5O$): C, H, N.

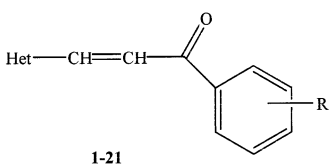
*3.1.9. N^{1}-[1-[3-Phenyl-1-(pyridin-3-yl)-1-oxo]-propyl]-2-pyridinecarboxamidrazone (**29**)*

IR (Nujol, cm^{-1}): 3440, 3300, 3200; 1670; 1620. ^1H NMR ($CDCl_3$/TMS): δ 3.30 (dd, 1H, C*H*H, $J = 17.10$ and 5.62 Hz), 4.0 (dd, 1H, CH*H*, $J = 17.10$ and 7.63 Hz), 3.80–4.60 (br, s, 1H, NH, disappearing on deuteration), 5.0 (m, 1H, CH), 5.40 (s, 2H, NH, disappearing on deuteration), 7.20–8.90 (m, 13H, arom. and pyr.). MS: m/z 345 [M^+]. *Anal.* ($C_{20}H_{19}N_5O$): C, H, N.

*3.1.10. N^{1}-[1-[3-(3-Chlorophenyl)-1-(pyridin-3-yl)-3-oxo]propyl]-2-pyridinecarboxamidrazone (**30**)*

IR (Nujol, cm^{-1}): 3400, 3300, 3200; 1680; 1620. ^1H NMR ($CDCl_3$/TMS): δ 3.35 (dd, 1H, C*H*H, $J = 16.70$ and 6.2 Hz), 4.00 (dd, 1H, CH*H*, $J = 16.70$ and 6.85 Hz), 4.10–4.80 (br, s, 1H, NH, disappearing on deuteration), 5.0 (m, 1H, CH), 5.35 (s, 2H, NH, disappearing on deuteration), 7.20–8.85 (m, 12H, arom. and pyr.). MS: m/z 378,380 [M^+]. *Anal.* ($C_{20}H_{18}N_5OCl$): C, H, N.

Table 2
Yields, melting points and IR data for compounds **1**–**21**



1-21

Comp.	Het	R	Yield (%)	M.p. (°C)	IR cm^{-1} (C=O)	Formula	Ref.
1	2-pyridyl	H	35	60–61	1670	$C_{14}H_{11}NO$	[7]
2	2-pyridyl	3-Cl	67	65	1665	$C_{14}H_{10}NOCl$	
3	2-pyridyl	4-Cl	85	85–86	1670	$C_{14}H_{10}NOCl$	[7]
4	2-pyridyl	3,4-Cl_2	92	154	1660	$C_{14}H_9NOCl_2$	[8]
5	2-pyridyl	3-CH_3	70	41	1670	$C_{15}H_{13}NO$	
6	2-pyridyl	4-CH_3	86	55	1675	$C_{15}H_{13}NO$	
7	2-pyridyl	4-Ph	68	130	1660	$C_{20}H_{15}NO$	
8	3-pyridyl	H	40	101–102	1670	$C_{14}H_{11}NO$	[7]
9	3-pyridyl	3-Cl	47	120	1680	$C_{14}H_{10}NOCl$	
10	3-pyridyl	4-Cl	63	129	1665	$C_{14}H_{10}NOCl$	[8]
11	3-pyridyl	3,4-Cl_2	33	145	1680	$C_{14}H_9NOCl_2$	[8]
12	3-pyridyl	3-CH_3	75	60	1680	$C_{15}H_{13}NO$	
13	3-pyridyl	4-CH_3	63	79	1670	$C_{15}H_{13}NO$	
14	3-pyridyl	4-Ph	36	140	1680	$C_{20}H_{15}NO$	
15	4-pyridyl	H	35	70–71	1660	$C_{14}H_{11}NO$	[7]
16	4-pyridyl	3-Cl	69	106–109	1675	$C_{14}H_{10}NOCl$	[9]
17	4-pyridyl	4-Cl	67	150	1670	$C_{14}H_{10}NOCl$	[8]
18	4-pyridyl	3,4-Cl_2	68	164	1670	$C_{14}H_9NOCl_2$	[8]
19	4-pyridyl	3-CH_3	21	113–114	1665	$C_{15}H_{13}NO$	[9]
20	4-pyridyl	4-CH_3	60	83–85	1660	$C_{15}H_{13}NO$	[10]
21	4-pyridyl	4-Ph	85	158	1670	$C_{20}H_{15}NO$	

*3.1.11. N¹-[1-[3-(4-Chlorophenyl)-1-(pyridin-3-yl)-3-oxo]propyl]-2-pyridinecarboxamidrazone (**31**)*

IR (Nujol, cm^{-1}): 3380, 3280, 3200; 1680; 1620. ^1H NMR ($CDCl_3$/TMS): δ 3.35 (dd, 1H, C*H*H, $J = 16.81$ and 6.43 Hz), 3.80 (dd, 1H, CH*H*, $J = 16.81$ and 6.51 Hz), 4.20–4.90 (br, s, 1H, NH, disappearing on deuteration), 5.30 (m, 1H, CH), 5.35 (s, 2H, NH, disappearing on deuteration), 7.10–8.80 (m, 12H, arom. and pyr.). MS: m/z 378, 380 [M^+]. *Anal.* ($C_{20}H_{18}N_5OCl$): C, H, N.

*3.1.12. N¹-[1-[3-(3,4-Dichlorophenyl)-1-(pyridin-3-yl)-3-oxo]propyl]-2-pyridinecarboxamidrazone (**32**)*

IR (Nujol, cm^{-1}): 3460, 3360, 3200; 1690; 1630. ^1H NMR ($CDCl_3$/TMS): δ 3.25 (dd, 1H, C*H*H, $J = 16.75$ and 5.98 Hz), 3.95 (dd, 1H, CH*H*, $J = 16.75$ and 7.01 Hz), 3.90–4.70 (br, s, 1H, NH, disappearing on deuteration), 5.0 (m, 1H, CH), 5.30 (s, 2H, NH, disappearing on deuteration), 7.15–8.80 (m, 11H, arom. and pyr.). MS: m/z 414 [M^+]. *Anal.* ($C_{20}H_{17}N_5OCl_2$): C, H, N.

*3.1.13. N¹-[1-[3-(3-Methylphenyl)-1-(pyridin-3-yl)-3-oxo]propyl]-2-pyridinecarboxamidrazone (**33**)*

IR (Nujol, cm^{-1}): 3440, 3280, 3180; 1680; 1610. ^1H NMR ($CDCl_3$/TMS): δ 2.40 (s, 3H, CH_3), 3.35 (dd, 1H, C*H*H, $J = 16.93$ and 5.75 Hz), 3.70 (dd, 1H, CH*H*, $J = 16.93$ and 7.27 Hz), 3.75–4.50 (br, s, 1H, NH, disappearing on deuteration), 5.0 (m, 1H, CH), 5.20 (s, 2H, NH, disappearing on deuteration), 7.30–8.85 (m, 12H, arom. and pyr.). MS: m/z 359 [M^+]. *Anal.* ($C_{21}H_{21}N_5O$): C, H, N.

*3.1.14. N¹-[1-[3-(4-Methylphenyl)-1-(pyridin-3-yl)-3-oxo]propyl]-2-pyridinecarboxamidrazone (**34**)*

IR (Nujol, cm^{-1}): 3440, 3310, 3200; 1680; 1620. ^1H NMR ($CDCl_3$/TMS): δ 2.40 (s, 3H, CH_3), 3.40 (dd, 1H, C*H*H, $J = 16.98$ and 5.5 Hz), 3.90 (dd, 1H, CH*H*, $J = 16.98$ and 7.54 Hz),), 3.80–4.70 (br, s, 1H, NH, disappearing on deuteration), 5.05 (m, 1H, CH), 5.30 (s, 2H, NH, disappearing on deuteration), 7.30–8.80 (m, 12H, arom. and pyr.). MS: m/z 359 [M^+]. *Anal.* ($C_{21}H_{21}N_5O$): C, H, N.

*3.1.15. N¹-[1-[3-(4-Biphenylyl)-1-(pyridin-3-yl)-3-oxo]propyl]-2-pyridinecarboxamidrazone (**35**)*

IR (Nujol, cm^{-1}): 3420, 3310, 3210; 1660; 1630. ^1H NMR ($CDCl_3$/TMS): δ 3.10 (dd, 1H, C*H*H, $J = 16.41$ and 8.6 Hz), 3.65 (dd, 1H, CH*H*, $J = 16.41$ and 10.21 Hz), 4.10–4.90 (br, s, 1H, NH, disappearing on deuteration), 4.90 (m, 1H, CH), 5.30 (s, 2H, NH, disappearing on deuteration), 7.20–8.90 (m, 17H, arom. and pyr.). MS: m/z 421 [M^+]. *Anal.* ($C_{26}H_{23}N_5O$): C, H, N.

*3.1.16. N¹-[1-[3-Phenyl-1-(pyridin-4-yl)-3-oxo]propyl]-2-pyridinecarboxamidrazone (**36**)*

IR (Nujol, cm^{-1}): 3440, 3340, 3200; 1680; 1620. ^1H NMR ($CDCl_3$/TMS): δ 3.35 (dd, 1H, C*H*H, $J = 16.99$ and 6.34 Hz), 4.10 (dd, 1H, CH*H*, $J = 16.99$ and 6.48 Hz), 3.95–4.85 (br, s, 1H, NH, disappearing on deuteration), 5.05 (m, 1H, CH), 5.50 (s, 2H, NH, disappearing on deuteration), 7.10–8.80 (m, 13H, arom. and pyr.). MS: m/z 345 [M^+]. *Anal.* ($C_{20}H_{19}N_5O$): C, H, N.

*3.1.17. N¹-[1-[3-(3-Chlorophenyl)-1-(pyridin-4-yl)-3-oxo]propyl]-2-pyridinecarboxamidrazone (**37**)*

IR (Nujol, cm^{-1}): 3400, 3330, 3220; 1685; 1600. ^1H NMR ($CDCl_3$/TMS): δ 3.30 (dd, 1H, C*H*H, $J = 16.75$ and 5.94 Hz), 3.90 (dd, 1H, CH*H*, $J = 16.75$ and 7.03 Hz), 3.75–4.40 (br, s, 1H, NH, disappearing on deuteration), 5.00 (m, 1H, CH), 5.35 (s, 2H, NH, disappearing on deuteration), 7.20–8.60 (m, 12H, arom. and pyr.). MS: m/z 378,380 [M^+]. *Anal.* ($C_{20}H_{18}N_5OCl$): C, H, N.

*3.1.18. N¹-[1-[3-(4-Chlorophenyl)-1-(pyridin-4-yl)-3-oxo]propyl]-2-pyridinecarboxamidrazone (**38**)*

IR (Nujol, cm^{-1}): 3440, 3320, 3200; 1680; 1630. ^1H NMR ($CDCl_3$/TMS): δ 3.30 (dd, 1H, C*H*H, $J = 16.85$ and 5.90 Hz), 3.85 (dd, 1H, CH*H*, $J = 16.85$ and 7.10 Hz), 3.75–4.40 (br, s, 1H, NH, disappearing on deuteration), 4.95 (m, 1H, CH), 5.35 (s, 2H, NH, disappearing on deuteration), 7.20–8.70 (m, 12H, arom. and pyr.). MS: m/z 378,380 [M^+]. *Anal.* ($C_{20}H_{18}N_5OCl$): C, H, N.

*3.1.19. N¹-[1-[3-(3,4-Dichlorophenyl)-1-(pyridin-4-yl)-3-oxo]propyl]-2-pyridinecarboxamidrazone (**39**)*

IR (Nujol, cm^{-1}): 3440, 3360, 3280; 1680; 1610. ^1H NMR ($CDCl_3$/TMS): δ 3.25 (dd, 1H, C*H*H, $J = 16.57$ and 9.1 Hz), 3.90 (dd, 1H, CH*H*, $J = 16.57$ and 9.87 Hz), 3.75–4.80 (br, s, 1H, NH, disappearing on deuteration), 4.95 (m, 1H, CH), 5.30 (s, 2H, NH, disappearing on deuteration), 7.10–8.85 (m, 11H, arom. and pyr.). MS: m/z 414 [M^+]. *Anal.* ($C_{20}H_{17}N_5OCl_2$): C, H, N.

*3.1.20. N¹-[1-[3-(3-Methylphenyl)-1-(pyridin-4-yl)-3-oxo]propyl]-2-pyridinecarboxamidrazone (**40**)*

IR (Nujol, cm^{-1}): 3440, 3300, 3200; 1670; 1620. ^1H NMR ($CDCl_3$/TMS): δ 2.50 (s, 3H, CH_3), 3.40 (dd, 1H, C*H*H, $J = 17.13$ and 6.31 Hz), 3.75 (dd, 1H, CH*H*, $J = 17.13$ and 6.52 Hz), 3.65–4.50 (br, s, 1H, NH, disappearing on deuteration), 5.05 (m, 1H, CH), 5.25 (s, 2H, NH, disappearing on deuteration), 7.10–8.70 (m, 12H, arom. and pyr.). MS: m/z 359 [M^+]. *Anal.* ($C_{21}H_{21}N_5O$): C, H, N.

*3.1.21. N¹-[1-[3-(4-Methylphenyl)-1-(pyridin-4-yl)-3-oxo]propyl]-2-pyridinecarboxamidrazone (**41**)*

IR (Nujol, cm^{-1}): 3400, 3280, 3200; 1680; 1620. ^1H NMR ($CDCl_3$/TMS): δ 2.45 (s, 3H, CH_3), 3.40 (dd, 1H, C*H*H, $J = 16.76$ and 5.95 Hz), 3.80 (dd, 1H, CH*H*, $J = 16.76$ and 7.83 Hz),), 3.70–4.40 (br, s, 1H, NH, disappearing on deuteration), 5.10 (m, 1H, CH), 5.30

Table 3
Activity of the carboxamidrazone derivatives **22–42** against *M. tuberculosis* $H_{37}Rv$ (MIC, μg/ml) [a]

Comp.	MIC (μg/ml)
22	4
23	16
24	4
25	2
26	2
27	1
28	8
29	16
30	8
31	1
32	16
33	2
34	1
35	32
36	8
37	4
38	2
39	2
40	8
41	8
42	16

[a] *M. tuberculosis* strain resulted sensitive to isoniazid (5 μg disk), rifampicin (25 μg disk) and ofloxacin (30 μg disk).

(s, 2H, NH, disappearing on deuteration), 7.20–8.90 (m, 12H, arom. and pyr.). MS: m/z 359 [M^+]. *Anal.* ($C_{21}H_{21}N_5O$): C, H, N.

3.1.22. N^1-[1-[3-(4-Biphenylyl)-1-(pyridin-4-yl)-3-oxo]-propyl]-2-pyridinecarboxamidrazone (**42**)

IR (Nujol, cm^{-1}): 3440, 3280, 3200; 1670; 1600. ^1H NMR ($CDCl_3$/TMS): δ 3.15 (dd, 1H, C*H*H, $J = 16.15$ and 9.2 Hz), 3.60 (dd, 1H, CH*H*, $J = 16.15$ and 10.76 Hz), 3.80–4.40 (br, s, 1H, NH, disappearing on deuteration), 4.95 (m, 1H, CH), 5.35 (s, 2H, NH, disappearing on deuteration), 7.20–8.80 (m, 17H, arom. and pyr.). MS: m/z 421 [M^+]. *Anal.* ($C_{26}H_{23}N_5O$): C, H, N.

3.2. Microbiology

The determination of antimycobacterial activity was performed by viable count employing the agar dilution method [11]. Midlebrook and Cohn 7H10 agar, supplemented with Midlebrook OADC enrichment, was used to prepare quadrant plates with serial dimethylsulfoxide twofold dilutions of the different chemicals tested.

We employed two strains of *Mycobacterium spp.*: *M. tuberculosis* reference strain $H_{37}Rv$ and *M. avium*, strain 485, from our bacterial collection, emulsified in diluting fluid containing 0.2% fatty acid free albumin and 0.02% polysorbate 80, pH 6.9.

Control plates were included with known antimycobacterial drugs; all plates were incubated at 35°C in 5% CO_2 for 3–4 weeks.

The minimal inhibitory concentration (MIC) was defined as the lowest chemical dilution associated with at least a 99% reduction in the number of the visible colonies. The results are shown in Tables 3 and 4.

The other microbial strains tested were *E. coli* strain ATCC 25922 and *C. albicans* strain C873, a recent clinical isolate. The *E. coli* strain was 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/ml. 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 microbial suspension was inoculated onto 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 strains or econazole (10 μg/disk) for *C. albicans*. All the plates were then incubated at 37°C overnight. The results obtained are shown in Table 5.

Table 4
Activity of the carboxamidrazone derivatives **22–42** against *M. avium* 485 (MIC, μg/ml) [a]

Comp.	MIC (μg/ml)
22	80
23	80
24	8
25	8
26	16
27	80
28	32
29	8
30	32
31	80
32	80
33	16
34	16
35	16
36	16
37	8
38	8
39	8
40	16
41	16
42	8

[a] *M. avium* strain resulted resistant to isoniazid (5 μg disk) and rifampicin (30 μg disk).

Table 5
Activity of the carboxamidrazone derivatives **22–42** against *E. coli* and *C. albicans* C873 (MIC, μg/ml) [a]

Comp.	MIC *E. coli* (μg/ml)	MIC *C. albicans* (μg/ml)
22	>160	20
23	>160	80
24	>160	40
25	>160	20
26	>160	80
27	>160	80
28	>160	>160
29	>160	>160
30	>160	80
31	>160	80
32	>160	80
33	>160	>160
34	>160	>160
35	>160	>160
36	80	80
37	>160	40
38	>160	80
39	>160	20
40	>160	80
41	>160	80
42	>160	>160

[a] E. *coli* strain resulted sensitive to ampicillin (10 μg disk). *C. albicans* strain resulted resistant to econazole (10 μg disk).

4. Results and discussion

A series of pyridine-2-carboxamidrazone derivatives (**22**–**42**) have been synthesized with the aim of evaluating their antimycobacterial activity (Table 3) towards a strain of *M. tuberculosis* ($H_{37}Rv$) sensitive to isoniazid and rifampicin and a strain of *M. avium* resistant to isoniazid and rifampicin. In these compounds the pyridine-2-carboxamidrazone moiety is linked through the N^1 atom to the position 3 of 1-aryl-3-(pyridin-2-, 3-, or 4-yl)-propan-1-ones. All the synthesized compounds exhibit interesting in vitro antimycobacterial activity against the tested strain *of M. tuberculosis* $H_{37}Rv$, their MIC values ranging from 1 to 32 μg/ml. From the obtained data, the most potent derivatives in the series are compounds **27**, **31** and **34**, whose MIC values (1 μg/ml) are very interesting. These compounds are characterized by the presence of 2- and 3-pyridyl residues and by the phenyl ring substituted with methyl or halogens on the *para* position. However, compounds with an appreciable degree of activity are present in all the series of 2-, 3-, and 4-pyridyl derivatives with substituents on different positions at phenyl residue. Of note is that some compounds exhibit a significant activity (MIC = 8 μg/ml) towards the tested strain of *M. avium* (Table 4). On the other hand, the activity of the synthesized compounds towards a strain of *C. albicans* and *E. coli* is very feeble or completely absent. This evidence confirms the importance of the 2-pyridinecarboxamidrazone moiety with respect to the antimycobacterial activity and suggests that other structural modifications of the groups linked to the N^1-amidrazone atom may produce more active compounds.

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