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Antimycobacterial activity of new N^1-[1-[1-aryl-3-[4-(1*H*-imidazol-1-yl)phenyl]-3-oxo]propyl]-pyridine-2-carboxamidrazone derivatives

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ABSTRACT

N^1-[1-[1-aryl-3-[4-(1*H*-imidazol-1-yl)phenyl]-3-oxo]propyl]-pyridine-2-carboxamidrazone derivatives were design, synthesized and tested for their in vitro antimycobacterial activity. The new compounds showed a moderate antimycobacterial activity against the tested strain of *Mycobacterium tuberculosis* H37Ra and a significant antimycobacterial activity against several mycobacteria other than tuberculosis strains.

Tuberculosis (TB) is a chronic infection and one of the most widespread disease in the world and it's the second cause of death from a single infectious agent. The *M. tuberculosis* is the causative pathogen and the re-emergence of the extensively multidrug-resistant strains of *M. tuberculosis* (MDR) together with the high incidence of severe disseminated infections produced by mycobacteria other than tuberculosis (MOTT) necessitates the development of new antimycobacterial therapeutic agents. In this context, imidazole derivatives have shown antimycobacterial activity associated with good antifungal activity.[1,2] In general, the common goal of the azole antifungal agents is the inhibition of cytochrome P450-dependent lanosterol 14-α-demethylase (P450, CYP51). Interestingly, it has been shown that a CYP51-like gene of *M. tuberculosis* (MT) H_{37}Rv strain may also be exploited as a target for novel drugs.[3] This gene encodes a mycobacterial sterol 14-α-demethylase (MT $P450_{14DM}$), whose substrates are 14-α-methyl sterols, and that forms complexes with antifungal azole inhibitors of $P450_{DM}$.

To obtain compounds with both antifungal and antimycobacterial properties, we design and synthesized the series of derivatives **1a**–**n** in which, the imidazol-1-yl and the pyridine-2-carboxamidrazone nuclei are present simultaneously. We tested the combination of the two moieties because the first is essential for the antifungal activity of a number of azole drugs, and the latter is important for the antimycobacterial activity of a number of derivatives (Fig. 1).[4–10]

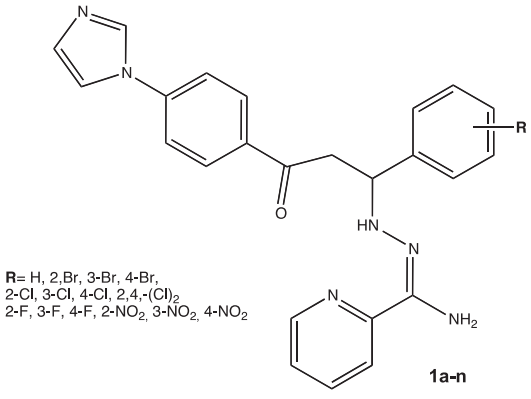

Figure 1. Structure of new derivatives **1a**–**n**.

The N^1-[1-[1-aryl-3-[4-(1*H*-imidazol-1-yl)phenyl]-3-oxo]propyl]-pyridine-2-carboxamidrazone derivatives **1a**–**n** (Table 1, supplementary data) were prepared by reacting pyridine-2-carboxamidrazone **3**, prepared from 2-cyanopyridine and hydrazine

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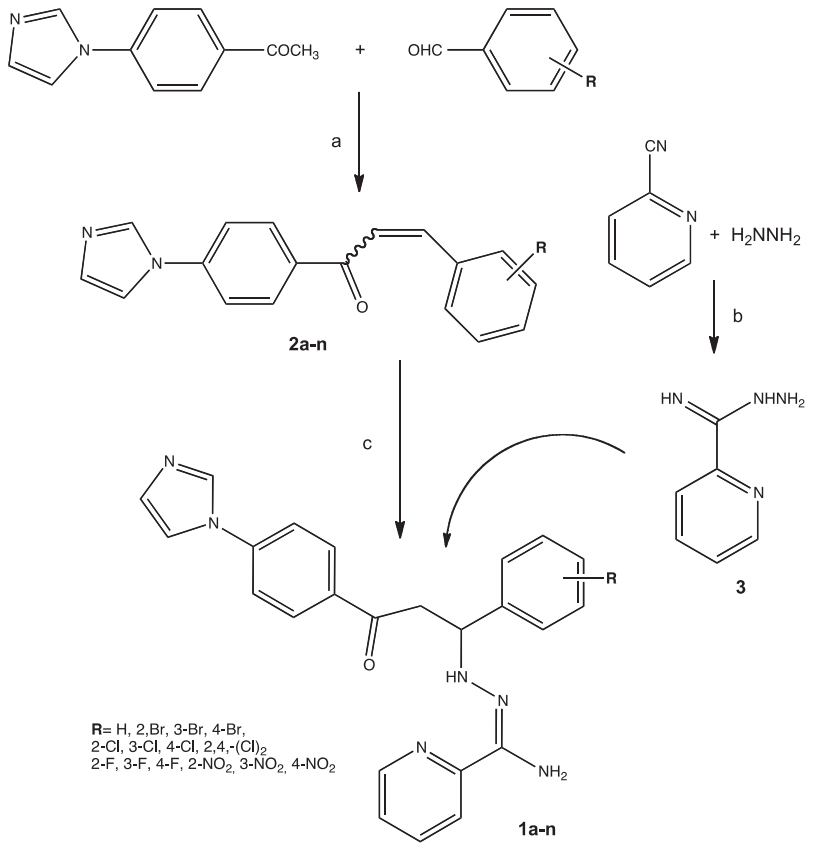

Scheme 1. Synthetic route for the synthesis of compounds **1a**–**n**. Reagents and conditions: (a) BF_3-AcOH 72 h rt; (b) EtOH 36 h 0 °C; (c) EtOH 48 h rt.

hydrate in accordance with a slightly modified method previously described,[11] with the corresponding 3-aryl-1-[4-(1*H*-imidazol-1-yl)phenyl]-propenones **2a**–**n**, which in turn were obtained, accordingly by a conventional procedure,[12] by treating 1-(4-imidazol-1-yl-phenyl)-ethanone **4** with aromatic aldehydes in the presence of the boron trifluoride-acetic acid complex (Scheme 1).

Compounds **1a**–**n** were preliminary tested against a Gram positive bacterial strain (*S. aureus* ATCC 25923), a Gram negative strain (*E. coli* ATCC 25922) and a fungal strain of *Candida albicans* 685. We performed the zone of inhibition assays for all the synthesized compounds in comparison with Cefalotin and Amphotericin B, as standard references.

As shown in Table 2, all the tested compounds (at dose of 20 μg) revealed an antibacterial activity, in particular towards the Gram positive strain of *S. aureus*, where all the chlorinated derivatives showed a inhibition zone ranging from 17 to 22 mm in comparison to Cefalotin (dose 30 μg; zone 31 mm), used as a standard reference. Regarding the antifungal activity (Table 2), all the compounds showed a weak inhibition towards *C. albicans* 865, clinical isolate strain, with diameter values ranging from 4 to 13 mm at 20 μg dose (Amphotericin B; 5 μg and 16 mm).

The derivatives **1a**–**n** were tested against a strain of *M. tuberculosis* H37Ra and in all cases were observed to result in *MIC values* ranging from 4.4 to 36 μM. Furthermore all the synthesized compounds were tested against seven strains of MOTT and several derivatives showed interesting antimycobacterial activity (Table 3). In particular, the 2,4-dichloro derivative **1h** showed the best MIC values towards *M. intracellulare* and *M. avium* (33.3 μM) and against *M. kansasii* (MIC = 4.2 μg/ml; INH=>467, 116 and 29.2 μM respectively). The entire series **1a**–**n** revealed a potent antimycobacterial activity towards *M. gordonae*, with the best values for the bromo derivatives **1c, d** (MIC = 2.0 μM), and *M. scrofulaceum* (MIC = 8.8 μM for all the nitro derivatives **1l-n**) compared to reference standard drug isoniazid (MIC = 116 μM).

Towards *M. marinum* and *M. Bovis*, once again, the bromo derivative **1d**, it proved to be the more active compound of the series (MIC = 8.2 μM).

Table 2
Antibacterial and antifungal activities of the tested compounds **1a**–**n**; zone inhibition test

Cpd.	R	*S. aureus ATCC 25923*	*E. coli ATCC 25922*	*C. albicans* 685
		Inhibition diameter (millimeter) after 24 h[a]		
1a	H	14	7	5
1b	2-Br	19	5	8
1c	3-Br	19	8	13
1d	4-Br	20	6	8
1e	2-Cl	20	6	7
1f	3-Cl	22	7	11
1g	4-Cl	20	8	9
1h	2,4-Cl_2	17	6	8
1i	2-F	14	7	5
1j	3-F	16	7	11
1k	4-F	20	6	6
1l	2-NO_2	13	6	4
1m	3-NO_2	13	5	10
1n	4-NO_2	13	5	5
INH		—	—	—
Cefalotin[b]		31	11	—
Amph. B[c]		—	—	16

[a] 20 μg dose cpd **1a**–**n**.
[b] 30 μg disk.
[c] 5 μg disk.

Table 3
Antimycobacterial activities (Minimum Inhibition Concentration: MIC) and cytotoxicity of the tested compounds **1a–n**

Cpd.	MIC[a] (μM)								CC_{50} Vero cells (μM)
	M. tuberculosis ATC 25197	*M. intracellulare* NCTC 10425	*M. gordonae* NCTC 10267	*M. scrofulaceum* NCTC 10803	*M. bovis* NCTC 10772	*M. kansasii* NCTC 10268	*M. marinum* CIP 6423	*M. avium* NCTC 08559	
1a	19.5	78.0	9.7	19.5	39.0	19.5	19.5	78.0	63.5
1b	32.7	131	8.2	16.3	32.7	16.3	16.3	65.4	29.0
1c	16.3	131	2.0	32.7	16.3	16.3	16.3	131	51.3
1d	16.3	131	2.0	16.3	8.2	4.1	8.2	131	298 (18)[b]
1e	36.0	144	18.0	18.0	18.0	18.0	18.0	72.0	53.2
1f	18.0	72.0	4.5	18.0	18.0	9.0	18.0	72.0	68.6
1g	9.0	72.0	4.5	18.0	18.0	9.0	18.0	72.0	78.8
1h	9.0	33.3	9.0	16.7	9.0	4.2	33.3	33.3	27.8
1i	17.6	74.7	4.7	18.7	18.7	18.7	18.7	74.7	62.1
1j	4.7	74.7	2.3	9.3	18.7	9.3	18.7	74.7	26.5
1k	9.3	74.7	18.7	18.7	18.7	9.3	18.7	74.7	78.8
1l	8.8	140	8.8	8.8	17.6	17.6	35.1	70.3	48.0
1m	4.4	>140	8.8	8.8	17.6	8.8	35.1	140	28.7
1n	4.4	>140	8.8	8.8	35.1	35.1	35.1	140	23.1
INH[c]	0.9	>467	116	116	7.3	29.2	116	116	>7000

[a] Minimum Inhibition Concentration, duplicate.
[b] Selectivity index (CC_{50}/MIC).
[c] Isoniazid.

All the synthesized compounds were also tested for their cytotoxicity against Vero cells (μM) with the derivative **1d** showing maximum selectivity index (CC_{50}/MIC) of 18.

Regarding the antitubercular activity towards *M. tuberculosis* H37Ra strain, the derivatives that present a substituent in the *meta*-position showed the lower MIC's values within the entire panel of compounds presented in this study (MIC = 4.7 and 4.4 μM, **1j, m**). On the other hand, the poorest results with higher MIC values, were obtained by the *ortho*-substituted derivatives (**1b**, **1e**, **1i**, **1l**) of our new pyridine-2-carboxamidrazone derivatives, suggesting that the position of the substituents on phenyl ring can modulate the antimycobacterial activity. In conclusion, the activity against MOTT strains is remarkable, when compared to that of reference standard drug isoniazid, in particular towards *M. intracellulare*, *M. kansasii* and *M. avium* (**1h**), *M. gordonae* (**1c, d** and **1j**) and *M. scrofulaceum* (**1j** and **1l-n**), while the antitubercular activity towards *M. tuberculosis* H37Ra is moderate except for nitro derivatives **1m, n** that showed the best MIC value (4.4 μM) of the entire series.

Subsequently, in order to investigate a possible interaction of the compounds with the common azole-drug-like target, a spectral analysis with the CYP51 (sterol 14α-demethylase) from *Mycobacterium tuberculosis* was performed. None of the novel compounds (**1a–n**) were able to bind the enzyme as efficiently as the reference standard, econazole (supplementary data). This observation can explain the low antifungal activity of our derivatives and suggests a different target for their antimycobacterial activity. Our molecules, probably, act as isoniazid-like drugs, being sufficient lipophilic ($LogP_{calc.}$ range = 3.09–4.21) they can be able to penetrate into the waxy layer of the mycobacterial cell wall inhibiting the mycolic acid synthesis and induce the cell death. This could also explain the good antibacterial activity against the tested strains of G+ and G− bacteria.

By our experience, from previously results obtained by our group,[5–10] we focused only on lipophilic and electron withdrawing substituents, which increase the lipophilicity of our molecules and can ensure a possible interaction with the mycobacteria cell wall, known to be highly hydrophobic, waxy, due to the presence of the mycolate-arabinogalactan-peptidoglycan layer. For this reason, some substituents like hydroxyl or amino were deliberately omitted due to their polar/hydrophilic profile. Furthermore, we have decided to retain only the 2-pyridine nucleus, linked to the amidrazone group; in fact we already proved that, at the contrary of picolinic acid hydrazide and its isoster isoniazid, the position of nitrogen atom in the piridinecarboxamidrazone derivatives, it does not seem to be crucial for the antimycobacterial activity.[5,6]

In particular, the final results suggest that: (i) notwithstanding the presence of the imidazole ring, none of the synthesized compounds possess a relevant antifungal property as supported also by results obtained from the CYP51 spectral analysis; (ii) a highly lipophilic scaffold, as starting calchone, is necessary for the activity against bacteria and mycobacteria in order to efficiently interact and/or penetrate into the cell wall and, possibly, increase the antimycobacterial activity; (iii) the *ortho* substitution, in general, lead to compounds gifted with worst antimycobacterial activity; on the contrary, the *ortho* and *para* positions seems to be more favourable for the mycobacteria inhibition; (iv) the 2,4-dichloro and the 4-bromo substituted derivatives (**1h** and **1d**, respectively) exhibited the most interesting activity against MOTT strains and the latter showed, also, the best cytotoxic profile; the nitro derivatives **1m,n** were observed to result the lower MIC value towards *M. tuberculosis* H37Ra strain; (v) it could be speculated that the hydrophilic pyridine-2-carboxyamidrazone scaffold, being structurally related to isoniazid, is the effective drug which acts inhibiting the mycolic acid synthesis and promotes the mycobacterial killing.

In conclusion, the newly synthesized compounds **1a–n** exhibited a weak antifungal activity, a good antibacterial activity against *E. coli* and *S. aureus* and, in particular, a remarkable high activity towards several MOTT strains with MIC values, in almost all cases, better than reference drug isoniazid. The antimycobacterial activity against the tested strain of *M. tuberculosis* H37Ra is appreciable.

Future studies will extend the strains panel for a more comprehensive and detailed knowledge of these promising compounds. Hence, these derivatives could serve as promising lead molecules for further generation of more potent antitubercular agents.

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Supplementary data

Supplementary data associated with this article can be found, in the online version, at http://dx.doi.org/10.1016/j.bmcl.2016.05.053.

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