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Synthesis and antimycobacterial evaluation of new *trans*-cinnamic acid hydrazide derivatives

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Abstract—In this work, we report the synthesis and the antimycobacterial evaluation of new *trans*-cinnamic acid derivatives of isonicotinic acid series (**5**) and benzoic acid series (**6**), designed by exploring the molecular hybridization approach between isoniazid (**1**) and *trans*-cinnamic acid derivative (**3**). The minimum inhibitory concentration (MIC) of the compounds **5a**–**d** and **6c** exhibited activity between 3.12 and 12.5 μg/mL and could be a good start point to find new lead compounds against multi-drug resistant tuberculosis.

Tuberculosis (TB) remains among the world's great public health challenges. Worldwide resurgence of TB is due to two major problems: the AIDS epidemic, which started in the mid-1980s, and the outbreak of multi-drug resistant (MDR) TB. For example, the deadly combination of TB and HIV has led to a quadrupling of TB cases in several African and Asian countries.[1] MDR-TB, defined as resistance to at least isoniazid (**1**) and rifamycin (RIF) (**2**), two current first-line drugs, has increased morbidity and mortality with an overall increase in healthcare costs. It is estimate that 4% of all worldwide TB patients are resistant to at least one of the current first-line drugs (Fig. 1).

TB treatment is long, possesses important side-effects, and patients often interrupt it. The first-line treatment has some disadvantages such as important side effects and weak sterility problems and must be administered for 6–9 months. When standard treatments fail, second-line TB drugs are used, but these drugs have a far lower efficacy and require even longer administration periods (18–24 months) with higher cost (US $2500–3000), higher rates of adverse effects, and low cure rates (around 60%).[1]

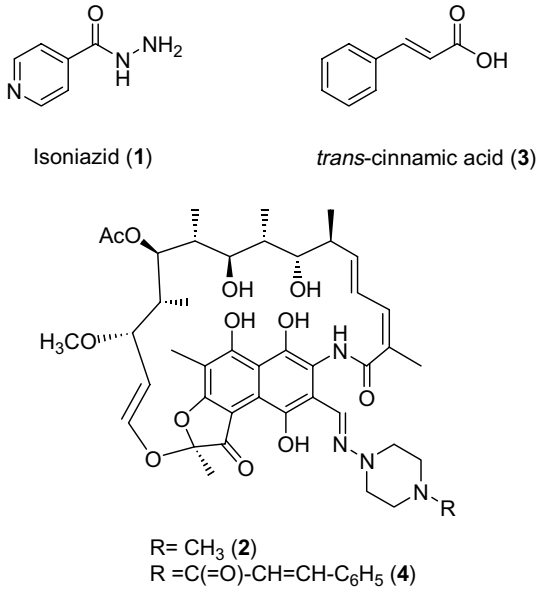

Figure 1. Chemical structures of compounds **1**–**4**.

TB is responsible for 20% of all deaths in adults, and each year there are about nine millions of new cases,

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of which 15% are children, and two millions of deaths, of which 450.000 are children. Globally, the number of TB cases is currently rising at 2% per year with the estimative of 32% of the world population, which have about two billion people, infected by latent TB. In the case of patients with AIDS, TB is the most common opportunistic infection and cause of death killing one of every three patients. Due to the increase of MDR-TB and AIDS cases worldwide and the lack of new drugs nowadays, there is an urgent need for new drugs to fight against this disease.

The goals of tuberculosis control are to cure active disease, prevent relapse, reduce transmission, and avert the emergence of drug resistance.

The literature indicates that many isoniazid derivatives[2,3] have shown important antimycobacterial activity. In addition *trans*-cinnamic acid (**3**) derivatives have a wide range of therapeutical importance, such as, antitumor activity,[4] antioxidant activity,[5] and antibacterial activity.[6] Promising results have been shown by Rastogi and coworkers[7] which reported the synergistic activity of *trans*-cinnamic acid (**3**) in drug combinations with isoniazid (**1**), RIF (**2**), and other known antimycobacterial agents against *Mycobacterium tuberculosis*. The increase of activity was even observed with drug resistant isolates.

Additionally, a cinnamyl rifamycin derivative (**4**) exhibits 2- to 8-fold lower MICs than those of (RIF) (**1**) for most of the 20 susceptible and multi-drug resistant *M. tuberculosis* strains tested, superior intracellular and in vivo activities compared with those of RIF (**1**).[8]

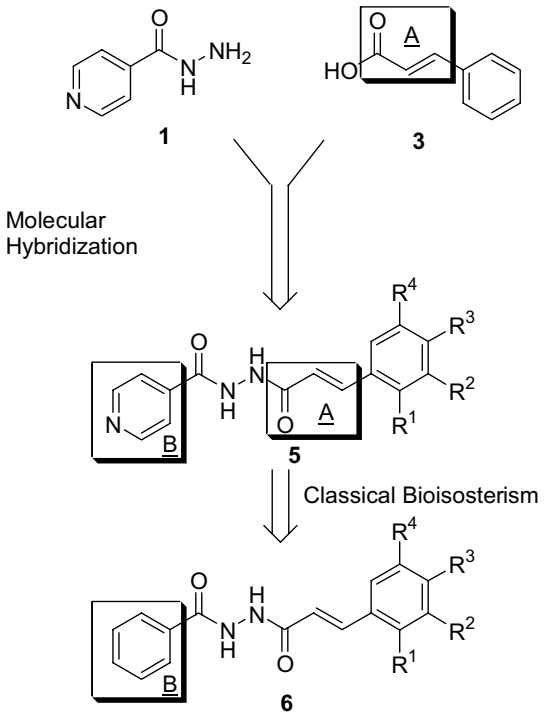


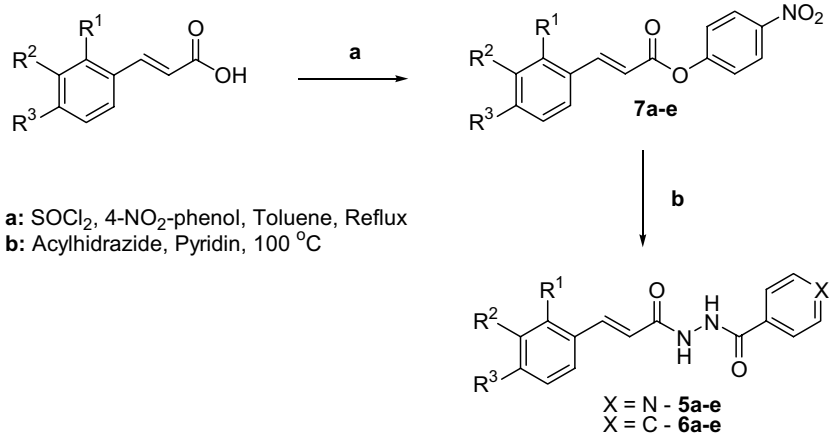
Figure 2. Design concept of new isonicotinic (**5**) and benzoic (**6**) acid *N*′-(3-phenyl-acryloyl)-hydrazides.

In our continuous program in the search of new potent and safe isoniazid derivatives, we decided to construct a new class of isonicotinic (**5**) and benzoic (**6**) acid *N*′-(3-phenyl-acryloyl)-hydrazide derivatives (Fig. 2) as attractive antitubercular agents, designed by molecular hybridization between isoniazid (**1**) and *trans*-cinnamic acid (**3**). The design concept of these compounds explored the introduction of the *trans*-cinnamic moiety (**A**) into isoniazid core structure aiming to potencialize its activity, possible by the incorporation of a different mode of action through a different target. Our second goal was to investigate the effects of the isosteric substitution of pyridine ring (**B**, Fig. 2), present in the more active derivatives of series (**5**), to a simple phenyl group, producing the corresponding benzoic derivatives of series (**6**).

The synthetic route used for the preparation of the title compounds is outlined in Scheme 1. Cinnamic acid derivatives were employed as starting material. In order to obtain more stable intermediates, we utilized 4-nitrophenol esters as general substrates. The 4-nitro-phenyl esters were prepared by treating the appropriate cinnamic acid with thionyl chloride and 4-nitro-phenol[9] resulting in a stoichiometric amount of the 4-nitro-phenyl esters. The target hydrazides were obtained, in good yields, by the nucleophilic substitution of the 4-nitrophenol moiety for the acyl hydrazide, as described in the general procedure.[10] The analysis of the ^{1}H NMR showed the two hydrazine protons as two singlets at 10.21–10.62 ppm and 10.70–10.90 ppm, and the ^{13}C NMR spectra were consistent with the presence of the two C=O signals at 163.91–166.02 ppm and 162.12–163.89 ppm.

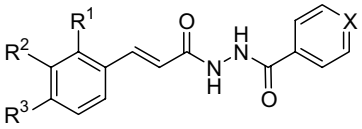
The antimycobacterial activities of compounds (**5a**–**e** and **6a**–**e**) were assessed against *M. tuberculosis* ATTC 2729411, using the microplate Alamar Blue assay (MABA)[11,12] (Table 1). This methodology is nontoxic, uses thermally stable reagent, and shows good correlation with proportional and BACTEC radiometric methods.[13,14] Briefly, 200 μl of sterile deionized water was added to all outer-perimeter wells of sterile 96-well plates (falcon, 3072: Becton Dickinson, Lincoln Park, NJ) to minimize evaporation of the medium in the test wells during incubation. The 96-well plates received 100 μL of the Middlebrook 7H9 broth (Difco laboratories, Detroit, MI, USA) and a serial dilution of the compounds **9**–**16** was made directly on the plate. The final drug concentrations tested were 0.01–10.0 μL/mL. Plates were covered and sealed with paraffin and incubated at 37 °C for 5 days. After this time, 25 μl of a freshly prepared 1:1 mixture of Alamar Blue (Accumed International, Westlake Ohio) reagent and 10% Tween 80 was added to the plate and incubated for 24 h. A blue color in the well was interpreted as no bacterial growth, and a pink color was scored as growth. The MIC (Minimal Inhibition Concentration) was defined as the lowest drug concentration, which prevented a color change from blue to pink.

Four of the five isonicotinic derivatives (Table 1) were sensitive in the minimum concentration tested



Scheme 1. Synthetic route for the preparation of the new isonicotinic (**5**) and benzoic (**6**) acid *N*′-(3-phenyl-acryloyl)-hydrazides.

Table 1. Antimycobacterial activities, melting points, log *P* measurements, and yields of isonicotinic (**5**) and benzoic (**6**) acid *N*′-(3-phenyl-acryloyl)-hydrazides



Entry	Compound	R^1	R^2	R^3	X	Yield (%)	Mp (°C)	log *P*[a]	MIC[b]
1	**5a**	H	H	H	N	82	221.6	2.12	3.12
2	**6a**	H	H	H	C	80	209.7	2.84	50
3	**5b**	H	H	NO_2	N	87	257.1	1.86	3.12
4	**6b**	H	H	NO_2	C	83	278.2	2.42	>100
5	**5c**	H	H	OCH_3	N	85	243.2	1.96	3.12
6	**6c**	H	H	OCH_3	C	80	234.5	2.96	12.5
7	**5d**	H	O–CH_2-O		N	83	252.2	2.26	3.12
8	**6d**	H	O–CH_2-O		C	83	225.6	2.76	25
9	**5e**	Cl	H	Cl	N	85	238.3	2.57	>100
10	**6e**	Cl	H	Cl	C	84	260.8	3.04	>100
11	Isoniazid	—	—	—	—	—	—	—	0.2

[a] Calculated by www.logp.com.
[b] Minimal Inhibition Concentration (MIC) is expressed in μg/mL.

(MIC = 3.12 μg/mL). All of the benzoic acid series (**6**) were less active than the corresponding ones of the series (**5**), reinforcing the pharmacophoric contribution of isonicotinic moiety to the mechanism of action against the *M. tuberculosis*. On the other hand, we are able to identify the interesting profile of 4-methoxy derivative (entry 6), which in spite of the absence of the pyridine framework was able to inhibit bacterial growth with a MIC = 12.5 μg/mL, indicating clearly the compensatory effect promoted by introduction of the *trans*-cinnamic subunit.

In conclusion, the antimicrobial activity of the two new series described here suggests that they may be selectively targeted to *M. tuberculosis* growths. They were effective in inhibiting *M. tuberculosis* infection at 3.12, 12.5, 25.0, and 50.0 μg/mL concentrations, and could be a good start point to further studies, as well as find new lead compounds with different framework which are also not cytotoxic to host cells at the same concentration.

Acknowledgments

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