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Antimycobacterial activity of 5-arylidene aromatic derivatives of hydantoin

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Abstract

Various 5-(chlorobenzylidene)-2-isoniazido and 5-(chlorobenzylidene)-2-amino substituted derivatives of imidazoline-4-one were synthesized and evaluated in the primary assay for their antimycobacterial activity against *Mycobacterium tuberculosis* H37Rv. Eight of them exhibited $>90\%$ inhibition in the primary screening at 12.5 μg/ml. For these primarily selected compounds the actual MIC and IC_{50} values were determined. Two of the isoniazid derivatives, for which MIC ≤ 3.13 μg/ml and SI > 10, were selected for further screening and investigated for efficacy in vitro in a TB-infected macrophage model. The most promising compound, 5-(3-chlorobenzylidene)-2-(isonicotinoylhydrazino)-imidazoline-4-one, with activity in vitro comparable with rifampin (MIC = 0.8 μg/ml, SI > 78) was tested in vivo in the animal tuberculosis model but exhibited insignificant activity. For several compounds the primary screening of antimycobacterial activity against *Mycobacterium avium* (ATCC 25291) was conducted as well, but none of them demonstrated satisfactory activity.

Keywords: 5-*Z*-Arylideno hydantoins; 5-Chlorobenzylidene-2-aminoimidazoline-4-ones; Antimicrobial activity; Antimycobacterial activity

1. Introduction

Research programs into the discovery of new antimycobacterial drugs and improving their evaluation criteria are under way in many laboratories [1–6]. Increasing resistance of *Mycobacterium tuberculosis* to currently available therapy and the large number of epidemic infections due to *Mycobacterium avium* complex have become an important health problem in several countries [7]. The knowledge of specific constituents of a mycobacterial cell and their biochemical roles as well as the knowledge of the mechanism of the action of available drugs have advanced considerably in recent years and may permit a more rational design of new drugs acting on specific targets.

One of the most active drugs, isoniazid (INH), continues to be well established for the treatment of tuberculosis. The mechanism of its action, as well as the mechanism conferring INH resistance, are not completely explained yet [6,8–10]. Isoniazid has bacteriostatic and bactericidal activity against *M. tuberculosis* and also against strains resistant to other antimycobacterial drugs. For these reasons the antimycobacterial pharmacophore moiety of INH is willingly introduced in different molecules to improve their activity against drug-sensitive strains and to fight against multi-drug resistant Mycobacteria.

The group of various 5-chlorobenzylidene derivatives of imidazoline-4-one with structure **I** was previously synthesized and evaluated in the primary assay for their antimycobacterial activity [11–14]. Four of those compounds exhibited $>90\%$ inhibition of *M. tuberculosis* growth and were selected for further tests. Now we present the synthesis and in vitro screening of new imidazoline-4-one derivatives, possessing amine or isoniazid residue (structures **I** and **II**). For active compounds from this and previous group [13] results of further assays against *M. tuberculosis* are described.

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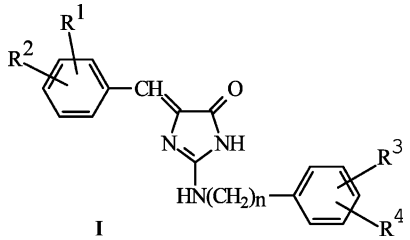

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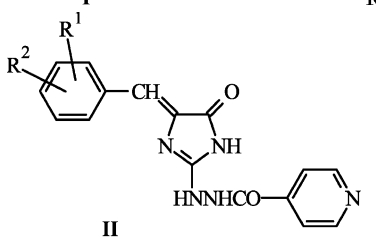
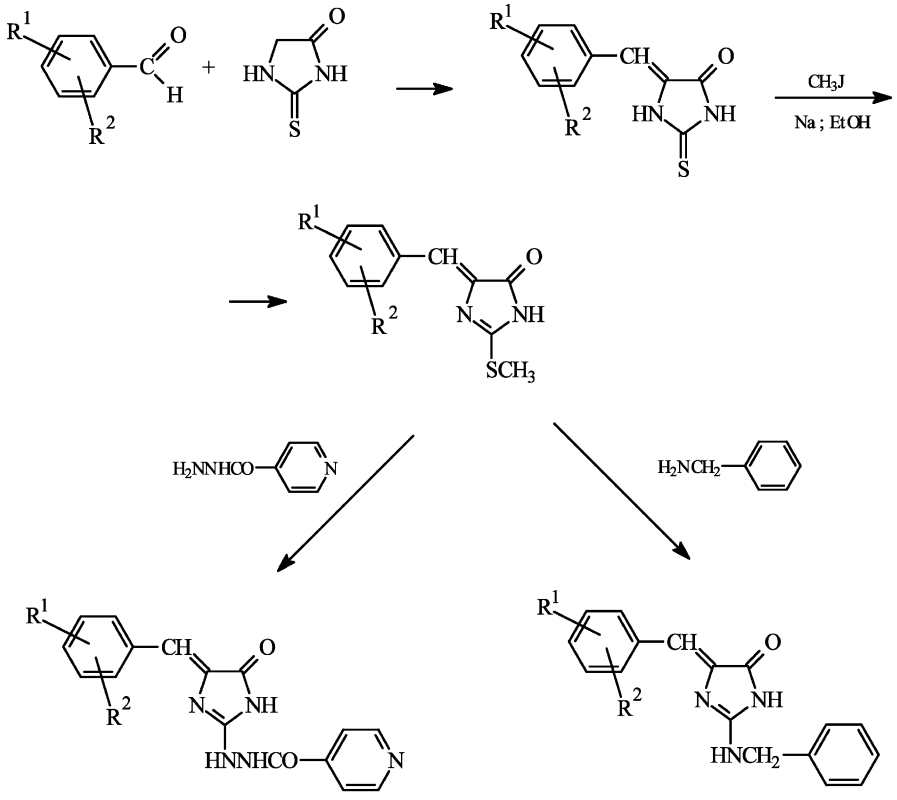


II

2. Chemistry

The synthesis of compounds **1**–**6** is presented in Scheme 1. The starting 5-(2-/, 3-/, 4-chlorobenzylidene)-, 5-(2,4-/, 2,6-dichlorobenzylidene)-2-thiohydantoins were prepared in the Knovenagel condensation of the appropriate benzaldehydes with 2-thiohydantoin according to the described procedure [15].

In the reaction of thiohydantoin derivatives with methyl iodide the intermediate methylthio-products were prepared and reacted with benzylamine or isoniazid to give the designed compounds. The reactions were carried out in toluene. Other cited compounds (**7**–**10**) were already described elsewhere [11–13].



1 – 5 **6**

1 R^1, R^2 = H, 2-Cl

2 R^1, R^2 = H, 3-Cl

3 R^1, R^2 = H, 4-Cl

4 R^1, R^2 = 2,4-diCl

5 R^1, R^2 = 2,6-diCl

6 R^1, R^2 = H, 4-Cl

Scheme 1.

3. Experimental

3.1. Chemistry

3.1.1. Material and methods

The chemical structures of the obtained compounds were confirmed by elemental and spectral analyses (IR, ^{1}H NMR). IR spectra were recorded with FT/IR-410 Spectrophotometer (Jasco Corp., Japan) using KBr discs. ^{1}H NMR spectra were determined with Varian Mercury 300 MHz spectrometer, in DMSO-d_6 solution with TMS as an internal standard. All chemical shifts are quoted in δ values. Elemental analyses (C, H, N) were within ± 0.3 from the theoretical values.

The purity of the compounds was checked by thin-layer chromatography performed with Merck silica gel GF_{254} aluminum sheets, using the developing system: 2-propanol:chloroform:aqueous ammonia, 11:9:2. Spots were detected by their absorption under UV light. The melting points (uncorrected) were determined on Mel-Temp melting point apparatus (Laboratory Devices Inc., USA).

Starting materials: 5-(2-chlorobenzylidene)-, 5-(3-chlorobenzylidene)-, 5-(4-chlorobenzylidene)-, 5-(2,6-dichlorobenzylidene)- and 5-(2,4-dichlorobenzylidene)-2-thiohydantoin were prepared according to the literature procedure [15]. The methods of synthesis of the compounds: 5-(2,4-dichlorobenzylidene)-2-(2,4-dichloroanilino)-imidazoline-4-one (**7**), 5-(2,4-dichlorobenzylidene)-2-(4-chloroanilino)-imidazoline-4-one (**8**), 5-(2-chlorobenzylidene)-2-(3-chloroanilino)-imidazoline-4-one (**9**) and 5-(2-chlorobenzylidene)-2-(4-chloroanilino)-imidazoline-4-one (**10**) are described in separate publications [11,13].

3.1.2. General procedure for synthesis of: 5-(2-chlorobenzylidene)-2-(isonicotinoylhydrazino)-imidazoline-4-one (*1*), 5-(3-chlorobenzylidene)-2-(isonicotinoylhydrazino)-imidazoline-4-one (*2*), 5-(4-chlorobenzylidene)-2-(isonicotinoylhydrazino)-imidazoline-4-one (*3*), 5-(2,4-dichlorobenzylidene)-2-(isonicotinoylhydrazino)-imidazoline-4-one (*4*), 5-(2,6-dichlorobenzylidene)-2-(isonicotinoylhydrazino)-imidazoline-4-one (*5*), 5-(4-chlorobenzylidene)-2-(benzylamino)-imidazoline-4-one (*6*)

To the stirred solution of sodium (0.04 mol) in 200 ml of ethanol arylidene-2-tiohydantoin (0.04 mol) and methyl iodide (0.04 mol) were added. After stirring at room temperature for 30 min the product was filtered off, washed with water and dried. Methylthio derivative was shown to be analytically pure.

A mixture containing 5 mmol of methylthio derivative of arylidene-2-thiohydantoin and 5.5 mmol of isoniazid or benzylamine in 30 ml of toluene was refluxed for 9 h, and then allowed to cool. The solid was isolated by suction and recrystallized from acetic acid (**1**–**5**) or methanol (**6**).

*3.1.2.1. 5-(2-Chlorobenzylidene)-2-(isonicotinoylhydrazino)-imidazoline-4-one (**1**).* Yield 35%, m.p. 270–273 °C, $R_f = 0.29$, ^{1}H NMR δ (ppm) 6.56 (s, 1H, Ar–CH=), 7.28–7.48 (m, 2H, H_{ar}-4, H_{ar}-5), 7.49 (def.d, 1H, $J =$ 7.1 Hz, H_{ar}-3), 7.78 (br.s, 2H, H_{ar}-3′, H_{ar}-5′), 8.30 (br.s, 1H, H_{ar}-6), 8.73 (def.d, 2H, $J = 5.8$ Hz, H_{ar}-2′, H_{ar}-6′), 10.81 (br.s, 1H, NH), 11.47 (br.s, 2H, N_3H, NHCO); IR ν (cm^{-1}): 3348 (–NH), 1724, 1691 (C=O), 1645 (C=N), 1628 (Ar–CH=). *Anal.* ($C_{16}H_{12}O_2N_5Cl \times 1.5$ CH_3COOH): C, H, N.

*3.1.2.2. 5-(3-Chlorobenzylidene)-2-(isonicotinoylhydrazino)-imidazoline-4-one (**2**).* Yield 23.5%, m.p. 303–305 °C, $R_f = 0.31$, ^{1}H NMR δ (ppm) 6.32 (s, 1H, Ar–CH=), 7.28–7.52 (m, 2H, H_{ar}-4, H_{ar}-5), 7.57–7.78 (m, 1H, H_{ar}-6), 7.79 (def.d, 2H, $J = 5.8$ Hz, H_{ar}-3′, H_{ar}-5′), 8.06 (br.s, 1H, H_{ar}-2), 8.74 (def.d, 2H, $J = 5.5$ Hz, H_{ar}-2′, H_{ar}-6′), 10.83 (br.s, 1H, NH), 11.19 (br.s, 1H, N_3H), 11.69 (br.s, 1H, NHCO); IR ν (cm^{-1}): 3420 (–NH), 1756, 1692 (C=O), 1636 (C=N), 1628 (Ar–CH=). *Anal.* ($C_{16}H_{12}O_2N_5Cl \times CH_3COOH$): C, H, N.

*3.1.2.3. 5-(4-Chlorobenzylidene)-2-(isonicotinoylhydrazino)-imidazoline-4-one (**3**).* Yield 80%, m.p. 301–303 °C, $R_f = 0{,}41$, ^{1}H NMR δ (ppm) 6.33 (s, 1H, Ar–CH=), 7.41 (def.d, 2H, $J = 7.7$ Hz, H_{ar}-3, H_{ar}-5), 7.78 (def.d, 2H, $J = 4.9$ Hz, H_{ar}-3′, H_{ar}-5′), 7.80 (br.s, 2H, H_{ar}-2, H_{ar}-6), 8.74 (def.d, 2H, $J = 4.8$ Hz, H_{ar}-2′, H_{ar}-6′), 10.90 (br.s, 1H, NH), 11.38 (br.s, 2H, N_3H, NHCO); IR ν (cm^{-1}): 3428 (–NH), 1730, 1690 (C=O), 1664 (C=N), 1628 (Ar–CH=). *Anal.* ($C_{16}H_{12}O_2N_5Cl \times CH_3COOH$): C, H, N.

*3.1.2.4. 5-(2,4-Dichlorobenzylidene)-2-(isonicotinoylhydrazino)-imidazoline-4-one (**4**).* Yield 66%, m.p. 268–270 °C, $R_f = 0.36$, ^{1}H NMR δ (ppm) 6.88 (s, 1H, Ar–CH=), 7.49 (d.d, 2H, $J_1 = 8.5$ Hz, $J_2 = 2.0$ Hz, H_{ar}-3′, H_{ar}-5′), 7.69–7.70 (m, 3H, H_{ar}-3, H_{ar}-5, H_{ar}-6), 8.85 (def.d, 2H, $J = 8.5$ Hz, H_{ar}-2′, H_{ar}-6′), 12.02 (br.s, 1H, NHCO); IR ν (cm^{-1}): 3461 (–NH), 1762, 1703 (C=O), 1630 (Ar–CH=). *Anal.* ($C_{16}H_{11}O_2N_5Cl_2$): C, H, N.

*3.1.2.5. 5-(2,6-Dichlorobenzylidene)-2-(isonicotinoylhydrazino)-imidazoline-4-one (**5**).* Yield 86%, m.p. 275–277 °C, $R_f = 0.27$, ^{1}H NMR δ (ppm) 6.07 (s, 1H, Ar–CH=), 7.24–7.43 (m, 3H, H_{ar}-3, H_{ar}-4, H_{ar}-5), 7.72 (def.d, 1H, $J = 5.8$ Hz, H_{ar}-3′), 7.78 (def.d, 1H, $J = 5.8$ Hz, H_{ar}-5′), 8.71 (def.d, 2H, $J = 4.7$ Hz, H_{ar}-2′, H_{ar}-6′), 10.08 (br.s, 1H, N_3H), 10.58 (d, 1H, NH), 11.77 (br.s, 1H, NHCO); IR ν (cm^{-1}): 3430 (–NH), 1753, 1687 (C=O), 1639 (Ar–CH=). *Anal.* ($C_{16}H_{11}O_2N_5Cl_2$): C, H, N.

*3.1.2.6. 5-(4-Chlorobenzylidene)-2-(benzylamino)-imidazoline-4-one (**6**).* Yield 48%, m.p. 237–238 °C, R_f = 0.86, ^{1}H NMR δ (ppm) 4.54 (br.s, 2H, –CH_2) 6.26 (s, 1H, Ar–CH=), 7.23–7.48 (m, 7H, H_{ar}-3, H_{ar}-5, H_{ar}-2′, H_{ar}-3′, H_{ar}-4′, H_{ar}-5′, H_{ar}-6′), 8.06 (def.d, 2H, J = 8.5 Hz, H_{ar}-2, H_{ar}-6), 10.92 (br.s, 1H, NH); IR ν (cm^{-1}): 3341 (–NH), 1702, 1672 (C=O), 1608 (Ar–CH=). *Anal.* ($C_{17}H_{14}ON_3Cl$): C, H, N.

3.2. Microbiology

3.2.1. In vitro evaluation of antimycobacterial activity [16–19]

Primary screening was conducted at 12.5 μg/ml against *M. tuberculosis* H37Rv (ATCC 27294; American Type Culture Collection, Rockville, MD) in BACTEC 12B medium using the BACTEC 460-radiometric system. Compounds demonstrating at least 90% inhibition were re-tested against *M. tuberculosis* H37Rv at lower concentration to determine the actual minimum inhibitory concentration (MIC) in the Microplate Alamar Blue Assay (MABA). The MIC was defined as the lowest concentration inhibiting 99% of the inoculum. Rifampin (Sigma Chemical Compound, St. Louis, MO) or isoniazid were included as a positive drug control.

Compounds were tested for cytotoxicity (IC_{50}) in VERO cells at concentrations less than or equal to 10 times the MIC. After 72 h exposure, viability was assessed on the basis of cellular conversion of 3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide (MTT) into a formazan product using the Promega CellTiter 96 Non-radioactive Cell Proliferation Assay. Compounds for which the selectivity index SI ($= IC_{50}$/MIC) > 10 were tested for killing of *M. tuberculosis* Erdman (ATCC 35801) in monolayers of mouse bone marrow macrophages at 4-fold increasing concentrations equivalent to 0.25, 1, 4 and 16 times the MIC.

The activity against singly-drug-resistant *M. tuberculosis* (strain resistant to isoniazid, rifampin, ethambutol, capreomycin and ciprofloksacin) as well as drug-sensitive *M. tuberculosis* (H37Rv and Erdman) for investigated compounds was determined.

The minimum bactericidal concentration (MBC) was determined for *M. tuberculosis* H37Rv, Erdman and for the rifampin- and isoniazid-resistant strains by subculturing onto drug-free solid media and enumerating colony forming units following exposure in supplemented Middlebrook 7H9 media to drug concentrations equivalent to and higher than the previously determined MICs of the respective strains.

3.2.2. Tuberculosis animal model

Prior to animal screening, the maximally tolerated dose (MTD) of an experimental compound was determined using C57BL/6 female mice by administration of a one-time dose/animal (oral gavage) of the compound at concentrations of 100, 300 or 1000 mg/kg. The compounds were dissolved in an appropriate vehicle (DMSO), administered in a solution if necessary. Three animals per dose were observed post-administration for a total of 1 week. The surviving mice were sacrificed day 7 post-administration and the critical organs were observed for evidence of drug toxicity.

Mice were exposed to an aerosol of *M. tuberculosis* Erdman (ATCC 35801), which deposited approximately 50 bacilli into the lungs of the animal. Test compounds were administered to groups of mice starting on day 20 post-inoculation. The dose of drug (25 mg/kg/day) was given intraperitoneally once a day; an additional group was given isoniazid as a positive control. The course of the infection and the effect of the compound were observed in the lungs and spleen on days 35 and 50 by plating homogenates of harvested organs on nutrient agar and determining bacterial numbers. The data is expressed as the log 10 protection provided by a given dose of the compound against the growth of the organism in the untreated control group.

An INH control group, administered via oral gavage at 25 mg/kg/day was included in each study.

3.2.3. Evaluation of anti-Mycobacterium avium activity

Primary screening was conducted against *Mycobacterium avium* (ATCC 25291) in Middlebrook 7H9 broth using the Microplate Alamar Blue Assay and in the BACTEC 460-radiometric system. Clarithromycin was included as a positive drug control.

4. Results

In order to find a potential antimycobacterial activity among 30 previously described [13] and 6 new amino- and isoniazid derivatives of 5-arylidene hydantoin, the compounds were tested according to the Tuberculosis Antimicrobial Acquisition and Coordinating Facility (TAACF) screening program using the BACTEC 460 radiometric system. Eight of them (**1**–**3**, **6**–**10**) showed at least a 90% inhibition of growth of *Mycobacterium tuberculosis* H37Rv at concentrations lower than 12.5 μg/ml, and were selected for further tests. The minimum inhibitory concentrations for these compounds were determined. The results are shown in Table 1.

Generally the monochloro substituted isoniazid derivatives were active against *M. tuberculosis* at concentrations equal to or lower than 3.13 μg/ml. The compound **2**, 5-(3-chlorobenzylidene)-2-(isonicotinoylhydrazino)-imidazoline-4-one (MIC = 0.8 μg/ml), was found to be almost as active as rifampin ($MIC_{RIF} = 0.5$ μg/ml). The compounds **6**–**10** showed in vitro MIC values versus *M. tuberculosis* higher than 12.5 μg/ml.

Table 1
Antibacterial activity against *M. tuberculosis*

Comp.	% Inhibition at 12.5 μg/ml of the compound	MIC vs. H37Rv (μg/ml)	IC_{50} (μg/ml)	SI (IC_{50}/MIC)
1	99	3.13 [a]	48.56 [a]	16 [a]
2	99	0.8 [b]	>62.5 [c]	>78 [c]
3	99	3.13 [a]	34.51 [a]	11 [a]
4	0	>12.5 [a]		
5	17	>12.5 [a]		
6	97	>12.5 [d]	31 [e]	<2.5 [e]
7	96	>12.5 [d]	23 [f]	<2 [f]
8	100	>12.5 [d]	*	
9	91	>12.5 [d]	*	
10	99	>12.5 [d]	*	

[a] $MIC_{RIF} = 0.25$ μg/ml. IC_{50} for INH > 1000; RIF = 30; DMSO = 0.0115.
[b] $MIC_{RIF} = 0.5$ μg/ml.
[c] $MIC_{RIF} = 0.25$ μg/ml. IC_{50} for INH > 1000; RIF = 69; DMSO = 0.0105.
[d] $MIC_{RIF} = 0.125$ μg/ml.
[e] $MIC_{RIF} = 0.125$ μg/ml. IC_{50} for INH > 1000; RIF = 116; DMSO = 0.0069.
[f] MIC Rifampin (RIF) = 0.125 μg/ml. IC_{50} for isoniazid (INH) > 1000; RIF = 120; DMSO = 0.0095.
* Insoluble in tissue culture medium, unable to test IC_{50}.

All the compounds except **4** and **5** were screened to assess cytotoxicity (IC_{50}) to a VERO cell line. Unfortunately, derivatives **8**, **9** and **10** demonstrated very low solubility in tissue culture medium, and the test could not be carried out. For the remaining compounds the selectivity index (SI) was calculated as it is presented in Table 1.

The isoniazid derivatives **1** and **2**, for which SI > 10, were tested for efficacy in vitro in a TB-infected macrophage model. The results are summarized in Table 2. The columns labeled EC_{90} and EC_{99} list the concentrations effecting 90 and 99% reduction in intramacrophage bacterial growth after 7 days relative to a drug-free control. The ratio EC_{99}/MIC compares the in vitro activity against the bacillus to the activity against the bacillus while it residues and flourishes within the target host cell. This value should be less than 4 for active agents. Under the experimental conditions the most active compound **2** exhibited the ratio EC_{99}/MIC comparable with positive controls: rifampin and isoniazid (Table 2).

For compounds **1**–**3** their in vitro activity (MIC) against five singly-drug-resistant strains was determined in the MABA assay. All the compounds showed high levels of activity for RMP-R, EMB-R, KM-R, and CIP-R strains, but were much less active against the isoniazid-resistant one (Table 3).

As shown in Table 4, the derivatives **1** and **3** have bacteriostatic rather than bactericidal profile of activity, as their MBC values are greater than MICs (MBC/MIC $\gg$ 1). Only **1** demonstrated bactericidal effect against rifampin resistant strain (MBC/MIC $\approx$ 1).

Compound **2** was tested in vivo in the animal tuberculosis model. It showed no toxicity under the experimental conditions. The maximally tolerated dose (MTD) is the highest one of all tested doses of **2** and it amounts to 1000 mg/kg. In the aerosol standard mouse model the compound **2** was considered inactive, as it yielded only 0.08 $\log_{10}$ reduction in bacterial counts in a lung tissue, as shown in Table 5. During the test the yellow plagues on/within peritoneum-enlarged spleens were observed.

Only compounds **6**–**10** were tested in the Microplate Alamar Blue Assay (MABA) against *Mycobacterium avium* as well, but none of them demonstrated satisfactory activity as shown in Table 6. All the compounds showed low inhibition at 12.5 μg/ml.

5. Discussion

Continuing our studies on the biological activity of imidazoline-4-one derivatives [14], and taking into consideration strong antitubercular properties of INH, we decided to synthesize a group of 5-arylidenehydantoins containing isoniazid or an aromatic amine substituent at 2 positions of the hydantoin ring, with a view to

Table 2
Macrophage assay

Comp.	EC_{90} (μg/ml)	EC_{99} (μg/ml)	EC_{99}/MIC
2	0.38	0.85	1.06 [a]
1	16.71	>50	>15.97 [b]

EC_{90} and EC_{99}, the lowest concentration effecting a 90 and 99% reduction in colony forming units at 7 days compared to drug-free controls.
[a] EC_{90} RIF = 0.077 μg/ml; EC_{99} RIF = 0.46 μg/ml.
[b] EC_{90} RIF = 0.11 μg/ml; EC_{99} RIF = 0.37 μg/ml; EC_{90} INH = 0.21 μg/ml; EC_{99} INH = 0.4 μg/ml.

Table 3
Singly-drug-resistant *M. tuberculosis* assay

Comp.	H37Rv MIC (μg/ml)	Erdman MIC (μg/ml)	INH-R MIC (μg/ml)	RMP-R MIC (μg/ml)	EMB-R MIC (μg/ml)	KM-R MIC (μg/ml)	CIP-R MIC (μg/ml)
1	≤1.56	3.13	50	≤1.56	≤1.56	≤1.56	≤1.56
2	≤0.39	0.78	25	≤0.39	≤0.39	≤0.39	≤0.39
3	3.13	12.5	100	6.25	3.13	3.13	3.13

H37Rv, drug-sensitive *M. tuberculosis* (ATCC 27294). Erdman, drug-sensitive *M. tuberculosis* (ATCC 35801). INH-R, isoniazid resistant *M. tuberculosis* (ATCC 35822). RMP-R, rifampin resistant *M. tuberculosis* (ATCC 35838). EMB-R, ethambutol resistant *M. tuberculosis*. KM-R, capreomycin resistant *M. tuberculosis*. CIP-R, ciprofloksacin resistant *M. tuberculosis*.

Table 4
Minimum bactericidal concentration (MBC) assay

Comp.	H37Rv MIC (μg/ml)	H37Rv MBC (μg/ml)	H37Rv MBC/MIC	RMP-R MBC (μg/ml)	INH-R MBC (μg/ml)
1	≤1.56	50	>32	1.56	>100
3	3.13	>100	>32	25	>100

Table 5
In vivo activity against *M. tuberculosis*

Compound	MTD assay					
	Tested doses (mg/kg)	Highest tolerated dose (mg/kg)	Toxicity issues (pathology)	Solubility issues	Comments	
2	100				IP injection with 26 gauge needle. No ill effects apparent	
	300	1000	none	suspension at all doses		
	1000					
	Murine TB model assay					
	Route	Dose (mg/kg)	Log protection		Control protection [a]	
			Lung	Spleen	Lung	Spleen
	IP injection	25	0.08	0.75	1.13	2.06

[a] Isoniazid; the results are referred to day 50.

Table 6
Antibacterial activity against *M. avium* [a]

Comp.	MIC (μg/ml)	Inhibition%
6	>12.5	42
7	>12.5	11
8	>12.5	66
9	>12.5	53
10	>12.5	71

[a] MIC Clarithromycin = 2 μg/ml (98% inhibition).

obtaining antimycobacterial agents. During the primary screening the activity of these compounds against *M. tuberculosis* was confirmed. In further studies, compounds **1**–**3** possessing in their structure isoniazid residue showed high levels of antimycobacterial activity in vitro. However, the most effective compound **2** (MIC = 0.8 μg/ml), the activity of which was comparable with rifampin in macrophage assay as well, was shown to be inactive in vivo. It is supposed that because of the very low solubility of this group of hydantoin derivatives, the compound **2** administered by IP injection (in the form of suspension) was not absorbed and was observed on/within peritoneum-enlarged spleens in the form of yellow plagues.

From the above data it can be concluded that **2** should be a leading compound for further development. Concurrently with structural modifications, attempts at improving the solubility and bioavailability of active in vitro compounds are going to be conducted.

The effectiveness of compounds **1**–**5** against *M. avium* complex is now being studied.

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