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Antimycobacterial activity of 5-arylidene derivatives of hydantoin

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Abstract

The synthesis of various 5-arylidene-2-thiohydantoins and results of the primary assay in vitro for their antimycobacterial activity is reported. Eight of those compounds exhibited >90% inhibition of *Mycobacterium tuberculosis* growth and for them the minimum inhibitory concentrations, cytotoxicity (IC_{50}) and the selectivity index values were determined. The most active structure, (5*Z*)-5-(1,1′-biphenyl-4-ylmethylene)-2-thioxoimidazolidin-4-one, showed MIC = 0.78 μg/ml. For all compounds log *P* and log *D* (pH 6.5) values were calculated.

Keywords: Arylidene 2-thiohydantoins; Antimycobacterial activity

1. Introduction

According to data of the World Health Organization [1] tuberculosis is considered to be the most chronic communicable disease in the world. The increasing resistance of *Mycobacterium tuberculosis* to currently available therapy and the large number of epidemic infections due to *Mycobacterium avium* complex are stimulating factors in the research of new active compounds. The studies on current antimycobacterial agents show the connection of the antitubercular activity with the presence of some suitable fragments of the agent molecule [2,3]. The list of these pharmacophores includes isonicotinic acid hydrazides, pyrazinoic acids, β-lactams, fluoroquinolones, compounds containing a thiocarbonyl group (thioamides, thioureas), compounds containing an alkylthio group bound to an electron-deficient atom of carbon, oxazolidinones, nitroimidazoles and others.

The interesting results of our previous studies on antibacterial and -mycobacterial activity of imidazoline-4-one derivatives [4–6] prompted us to continue our investigations in this field. In the present work we focused on the antimycobacterial effect of both previously synthesized [7–10] and new 5-arylidene-hydantoin derivatives bearing the thiocarbonyl group as a pharmacophore. The synthesis of the new compounds and the results of their in vitro screening against *M. tuberculosis* $H_{37}Rv$ are reported.

As a part of structure–activity relationship considerations we decided to determine lipophilicity, expressed as log *P* (octanol–water coefficient) and log $D_{6.5}$ (distribution coefficient at pH 6.5—pH of the small intestine) values.

2. Chemistry

The synthetic pathways leading to the new imidazolidine-4-one derivatives are illustrated in Scheme 1. The target compounds were prepared as a result of condensation of 2-thiohydantoin with suitable aldehydes (or ketones) according to two methods. The mixture of starting materials was refluxed in acetic acid with anhydrous sodium acetate (method 1 [11] compounds **1**–**45**) or in toluene with ammonium acetate (method B [12] compounds **46**–**48**) to give with good yields solid products **III**. Some benzaldehyde derivatives, needed as the starting material for the synthesis of **40**–**45**, were obtained by the alkylation of *m*- or *p*-hydroksybenzaldehyde (Scheme 2).

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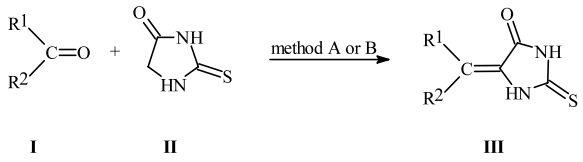


I II III

Scheme 1.

The purity of the synthesized compounds was checked by thin-layer chromatography. Their structures were confirmed by elemental analyses and spectral data (IR, ^{1}H NMR).

As the steric position of benzylidene substituent was restricted by C=C bond, the target compounds could occur in either *E*- or *Z*-configuration. However, the X-ray structure analysis as well as theoretical calculations of this type of compounds indicate *Z*-form [13].

For all structures the log *P* and log $D_{6.5}$ (pH of the small intestine) values were calculated by means of Pallas program [14].

3. Results and discussion

In order to find a potential antimycobacterial activity all the compounds were tested according to the Tuberculosis Antimicrobial Acquisition and Coordinating Facility (TAACF) screening program using a broth microdilution assay, the Microplate Alamar Blue Assay (MABA) [15]. Primary screening was conducted at 6.25 μg/ml. Results as well as log *P* combined and log *D* values prediction of the obtained compounds taken with Pallas program are presented in Tables 1 and 2. All compounds exhibited weak acidic properties.

Among all the compounds tested, eight structures demonstrated the high level of inhibition of *M. tuberculosis* (> 90%). These active compounds displayed lipophilicity ranged from 0.79 to 2.54 (mean 1.46), while, for comparison, log *P* of inactive derivatives (inhibition ≤ 10%) varied from −0.03 to 1.57 (mean 0.76). However, the calculated log *P* and log *D* values did not show any apparent correlation between structure and observed antimycobacterial activity indicating that the other than acidic properties and lipophilicity factors dominate in their mode of action.

On basis of the obtained screening results we can state, that among benzylidene substituted compounds (Table 1) 3- and 4-chloro/bromo/fluoro substituted structures (**2**, **3**, **10**–**13**) showed better effect against *M. tuberculosis* than 2-substituted group (**1**, **4**, **5**, **7**–**9**). The occurrence of additional halogen atom in benzylidene residue reduced activity (**4**–**8**, **14**). Alkyloxy substituents in the *meta* and particularly *para* position were advantageous as it was observed for **18**, **21**, **22**, **44** and **45**. Introducing of the additional aromatic fragment, especially joined by the oxygen atom, to the phenyl ring (Table 2, **36**, **40**–**43**) caused the strong increase of activity. The substitution of hydrogen atom in double bond of benzylidene residue (**46**–**48**) totally decreased the antimycobacterial effect.

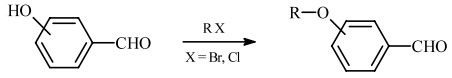
Compounds, which inhibited *M. tuberculosis* growth to extent higher than 90% were classified to further tests. The minimum inhibitory concentrations (MICs), cytotoxicity (IC_{50}) and the selectivity index ($SI = IC_{50}/MIC$) values for them were determined (Table 3). Four structures showed MIC in vitro equal or lower than 3.13 μg/ml and for the most active biphenyl derivative, **36**, MIC was 0.78 μg/ml, while for the reference rifampicin, in this test, MIC was equal to 0.0075 μg/ml (for isoniazid MIC = 0.025–0.05 μg/ml). As it is shown, investigated compounds displayed in tests too high cytotoxicity—for all of them (except **35**) calculated SI values were lower than 10 and according to TAACF program, they were not advanced to the further tests.

4. Experimental

4.1. Chemistry

4.1.1. Materials and methods

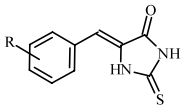
M.p.s were measured on a Mel.-Temp. II (LD Inc., USA) apparatus. The tlc was performed on Merck silica gel GF_{254} precoated tlc Al sheets; the used solvent systems were: (A) CH_2Cl_2–EtOAc (1:1); (B) $C_6H_5CH_3$–C_3H_6O (20:1.5); (C) CH_2Cl_2. Spots were visualized by UV absorption at 254 nm. Infrared spectra were measured with FT IR 410 spectrometer (JASCO) in KBr pellets. The ^{1}H NMR spectra were performed on a Bruker AC-200F spectrometer or on VARIAN MERCURY 300 MHz spectrometer or on a Bruker DPX 400 Avance (400 MHz) spectrometer in DMSO-d_6 using TMS as an internal standard (chemical shifts are reported in δ units). The elemental analyses (C, H, N) performed at the Department of Pharmaceutical Chemistry of the Jagiellonian University, Kraków (Poland) were within ±0.4% from the theoretical values.



R = CH_2=$CHCH_2$-, $C_6H_5CH_2$-, 4-Cl-$C_6H_4CH_2$-, 2,4-diCl-$C_6H_3CH_2$-, $C_6H_5CH_2CH_2$-

Scheme 2.

Table 1
Antimyconbacterial activity of benzylidene hydantoins



Comp.	R	% Inhibition*,**	log P_{comb}	log $D_{6.5}$	Comp.	R^1	% Inhibition*,**	log P_{comb}	log $D_{6.5}$
1	2-Cl	20	0.86	0.83	**17**	2,3-diOCH_3	0	0.07	0.04
2	3-Cl	71	0.92	0.90	**18**	2,4-diOCH_3	88	0.04	0.01
3	4-Cl	86	0.87	0.84	**19**	2,5-diOCH_3	57	0.04	0.01
4	2,6-diCl	0	1.57	1.54	**20**	3,4-diOCH_3	0	0.04	0.01
5	2,3-diCl	10	1.41	1.38	**21**	4-OC_2H_5	87	0.56	0.53
6	3,4-diCl	66	1.57	1.54	**22**	4-OC_3H_7	95	1.07	1.04
7	2,4-diCl	6	1.65	1.62	**23**	3-CH_3	88	0.68	0.66
8	2-Cl-6-F	0	1.01	0.99	**24**	4-CH_3	26	0.58	0.55
9	2-Br	26	1.02	0.99	**25**	3-CF_3	42	1.36	1.33
10	3-Br	85	1.08	1.06	**26**	4-CF_3	92	1.30	1.28
11	4-Br	83	1.03	1.00	**27**	4-$(CH_3)_2CH$	89	1.44	1.41
12	3-F	73	0.40	0.37	**28**	2,5-diCH_3	52	1.00	0.97
13	4-F	75	0.29	0.27	**29**	2,5-diCH_3-4-OCH_3	43	1.07	1.04
14	3,5-diF	55	0.63	0.61	**30**	4-$COOCH_3$	8	0.30	0.27
15	3-NO_2	29	0.12	0.09	**31**	4-OH	46	-0.41	-0.43
16	4-NO_2	58	0.06	0.04	**32**	3-OH	11	-0.30	-0.38

* % Inhibition at 6.25 μg/ml of the compound; ** MIC for rifampicin (RIF): 0.015-0.125 μg/ml; MIC for isoniazid (INH): 0.025-0.05 μg/ml

4.1.2. Preparation of 2-thiohydantoins

The 2-thiohydantoin derivatives were obtained as recently described [4,5] or in analogy to the described procedures [6,7]. The following new derivatives were prepared:

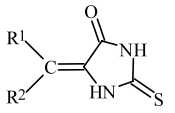
*4.1.2.1. Z-5-(3,4-Dichlorobenzylidene)-2-thiohydantoin (**6**).* Obtained as described in Ref. [9], the reaction mixture was refluxed for 2.0 h, m.p. 260–262 °C (yellow crystals from AcOH), yield 78%; R_f (A) 0.52; R_f (B) 0.84; ^{1}H NMR (400 MHz): δ 6.46 (s, 1H, ArCH=), 7.63–7.71 (m, 2H, H-5′, H-6′), 8.03 (s, 1H, H-2′), 12.35 (s, 1H, N_1–H), 12.65 (s, 1H, N_3–H); IR (KBr): ν 3203 (NH), 1727 (C_4=O), 1653 (ArCH=), 1487, 1464, 1351, 1182, 1084, 891, 811, 751 cm^{-1}. *Anal*. ($C_{10}H_6Cl_2N_2OS$): C, H, N.

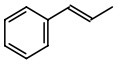
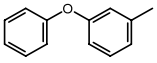
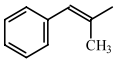
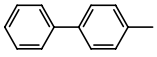
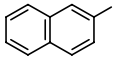
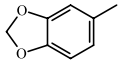
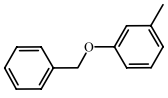
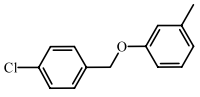
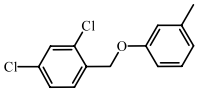
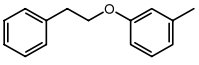
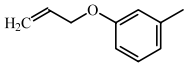
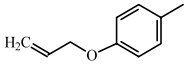
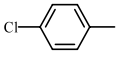
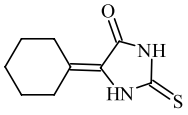
*4.1.2.2. Z-5-(3,5-Difluorobenzylidene)-2-thiohydantoin (**14**).* Obtained as described in Ref. [9], the reaction mixture was refluxed for 2.0 h, m.p. 278–280 °C (yellow crystals), yield 97% raw product was analytically pure; R_f (A) 0.54; R_f (B) 0.89; ^{1}H NMR (300 MHz): δ 6.44 (s, 1H, ArCH=), 7.19–7.26 (m, 1H, H-4′), 7.48 (d, $J = 6.87$ Hz, 2H, H-2′, H-6′), 12.29 (s, 1H, N_1–H), 12.47 (s, 1H, N_3–H); IR (KBr): ν 3203 (NH), 1725 (C_4=O), 1649 (ArCH=), 1585, 1486, 1249, 1177, 991, 959, 857 cm^{-1}. *Anal*. ($C_{10}H_6F_2N_2OS$): C, H, N.

*4.1.2.3. Z-5-(4-Ethoxybenzylidene)-2-thiohydantoin (**21**).* Obtained as described in Ref. [9], the reaction mixture was refluxed for 5 min, m.p. 238–241 °C (yellow crystals from AcOH), yield 83%; R_f (A) 0.42; R_f (B) 0.84; ^{1}H NMR (300 MHz): δ 1.33 (t, $J = 6.87$ Hz, 3H, CH_3), 4.07 (q, $J = 6.87$ Hz, 2H, CH_2O), 6.44 (s, 1H, ArCH=), 6.94 (d, $J = 8.51$ Hz, 2H, H-3′, H-5′), 7.70 (d, $J = 8.79$ Hz, 2H, H-2′, H-6′), 12.05 (s, 1H, N_1–H), 12.23 (s, 1H, N_3–H); IR (KBr): ν 3282, 1725 (C_4=O), 1640 (ArCH=), 1598, 1479, 1256, 1180, 826, 719 cm^{-1}. *Anal*. ($C_{12}H_{12}N_2O_2S$): C, H, N.

*4.1.2.4. Z-5-(4-Propoxybenzylidene)-2-thiohydantoin (**22**).* Obtained as described in Ref. [9], the reaction mixture was refluxed for 2.0 h, m.p. 228–230 °C (yellow crystals from AcOH), yield 78%, raw product was analytically pure; R_f (A) 0.47; R_f (B) 0.86; ^{1}H NMR (300 MHz): δ 0.97 (t, $J = 7.42$ Hz, 3H, CH_3), 1.67–1.79 (m, 2H, CH_3CH_2), 3.98 (t, $J = 6.32$ Hz, 2H, CH_2O), 6.45 (s, 1H, ArCH=), 6.95 (d, $J = 8.52$ Hz, 2H, H-3′, H-5′), 7.71 (d, $J = 8.80$ Hz, 2H, H-2′, H-6′), 12.04 (s, 1H, N_1–

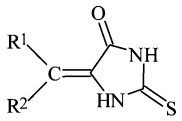
Table 2
Antimyconbacterial activity of arylidene hydantoins

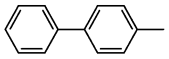
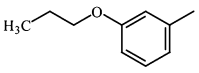
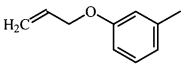
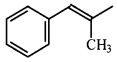
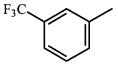
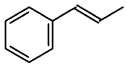
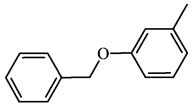
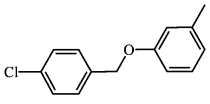


Comp.	R^1	R^2	% Inhibition*,**	log P_{comb}	log $D_{6.5}$
33	H		94	0.79	0.76
34	H		78	1.93	1.91
35	H		95	1.27	1.25
36	H		97	2.10	2.07
37	H		16	1.36	1.33
38	H		53	1.36	1.33
39	H		0	-0.03	-0.06
40	H		94	1.81	1.78
41	H		95	2.54	2.51
42	H		84	3.32	3.29
43	H		93	2.27	2.24
44	H		93	0.82	0.79
45	H		93	0.72	0.69
46	CH_3		0	0.62	0.6
47	CH_3		0	1.26	1.24
48			0	0.46	0.46

* % Inhibition at 6.25 μg/ml of the compound; ** MIC RIF: 0.015-0.125 μg/ml; MIC INH: 0.025-0.05 μg/ml

Table 3
MIC and cytotoxicity values of the selected compounds



Comp.	R^1	R^2	% Inhibition at 6.25 μg/ml of the compound	MIC vs.$H_{37}Rv$ (μg/ml)	IC50 (μg/ml)	SI (IC50/MIC)
36	H		97	0.78	6.7	8.6[a]
22	H		95	1.56	4.5	2.9[b]
44	H		93	3.13	6.7	2.1[a]
35	H		95	3.13	*	*
26	H		92	6.25	>10	>1.6[b]
33	H		94	6.25	8.6	1.4[b]
40	H		94	6.25	>5	>0.8[a]
41	H		95	6.25	>5	>0.8[a]

[a] MIC RIF ≤ 0.0075 μg/ml; IC50 INH > 1000, IC50 RIF = 72.4, IC50 DMSO = 0.0114

[b] MIC RIF ≤ 0.0075 μg/ml; IC50 INH > 1000, IC50 RIF = 96, IC50 DMSO = 0.0111

* Insoluble in tissue culture medium, unable to test IC_{50}.

H), 12.26 (s, 1H, N_3–H); IR (KBr): *v* 1724 (C_4=O), 1651 (ArCH=), 1597, 1475, 1257, 1174, 959, 822, 539 cm^{-1}. *Anal*. ($C_{13}H_{14}N_2O_2S$): C, H, N.

*4.1.2.5. Z-5-(3-Trifluoromethylbenzylidene)-2-thiohydantoin (**25**).* Obtained as described in Ref. [9], the reaction mixture was refluxed for 2.0 h, m.p. 213–216 °C (cream coloured crystals from AcOH), yield 87%; R_f (A) 0.41; R_f (B) 0.87; ^1H NMR (300 MHz): δ 6.51 (s, 1H, ArCH=), 7.58–7.70 (m, 2H, H-4′, H-5′), 8.00 (d, $J = 7.50$ Hz, 1H, H-6′), 8.08 (s, 1H, H-2′), 12.33 (br.s, 2H, N_1–H, N_3–H); IR (KBr): *v* 3443, 3247 (N–H), 2849 (CH_2), 1728 (C_4=O), 1653 (ArCH=), 1503, 1332, 1194, 1125, 912, 692 cm^{-1}. *Anal*. ($C_{11}H_7F_3N_2OS$): C, H, N.

*4.1.2.6. Z-5-(2,5-Dimethylbenzylidene)-2-thiohydantoin (**28**).* Obtained as described in Ref. [9], the reaction

mixture was refluxed for 1.0 h, m.p. 255–256 °C (yellow crystals from AcOH), yield 90%, raw product was analytically pure; R_f (A) 0.52; R_f (B) 0.82; ^{1}H NMR (300 MHz): δ 2.29 (s, 3H, $C_{5'}$–CH_3), 2.30 (s, 3H, $C_{2'}$–CH_3), 6.52 (s, 1H, ArCH=), 7.06 (d, $J = 7.69$ Hz, 1H, H-3′), 7.12 (d, $J = 7.69$ Hz, 1H, H-4′), 7.43 (s, 1H, H-6′), 12.15 (s, 1H, N_1–H), 12.33 (s, 1H, N_3–H); IR (KBr): ν 3134 (N–H), 1722 (C_4=O), 1655 (ArCH=), 1518, 1376, 1221, 1101, 968, 803, 654 cm^{-1}. *Anal*. ($C_{12}H_{12}N_2OS$): C, H, N.

*4.1.2.7. Z-5-(4-Methoxy-2,5-dimethylbenzylidene)-2-thiohydantoin (**29**).* Obtained as described in Ref. [9], the reaction mixture was refluxed for 0.5 h, m.p. 292–293 °C (orange crystals from AcOH), yield 81%, raw product was analytically pure; R_f (A) 0.49; R_f (B) 0.84; ^{1}H NMR (300 MHz): δ 2.14 (s, 3H, $C_{5'}$–CH_3), 2.34 (s, 3H, $C_{2'}$–CH_3), 3.80 (s, 3H, OCH_3), 6.51 (s, 1H, ArCH=), 6.85 (s, 1H, H-3′), 7.48 (s, 1H, H-6′), 12.09 (s, 1H, N_1–H), 12.25 (s, 1H, N_3–H); IR (KBr): ν 1718 (C_4=O), 1637 (ArCH=), 1597, 1491, 1378, 1258, 1089, 964, 857, 686 cm^{-1}. *Anal*. ($C_{13}H_{14}N_2O_2S$): C, H, N.

*4.1.2.8. Z-5-(α-Methyl-trans-cinnamylidene)-2-thiohydantoin (**35**).* Obtained as described in Ref. [9], the reaction mixture was refluxed for 2.0 h, m.p. 238–240 °C (green yellow crystals from AcOH), yield 58%; R_f (A) 0.44; R_f (B) 0.82; ^{1}H NMR (300 MHz): δ 2.19 (s, 3H, CH_3), 6.23 (s, 1H, ArCH=), 7.00 (s, 1H, CH=), 7.26–7.33 (m, 1H, H-4′), 7.39 (d, $J = 4.67$ Hz, 4H, H-2′, H-3′, H-5′, H-6′), 11.72 (s, 1H, N_1–H), 12.27 (s, 1H, N_3–H); IR (KBr): ν 1723 (C_4=O), 1628 (ArCH=), 1583, 1494, 1372, 1189, 969, 915, 752, 701 cm^{-1}. *Anal*. ($C_{13}H_{12}N_2OS$): C, H, N.

*4.1.2.9. Z-5-(4-Phenylbenzylidene)-2-thiohydantoin (**36**).* Obtained as described in Ref. [9], the reaction mixture was refluxed for 0.25 h, m.p. 221–224 °C (yellow crystals from AcOH), yield 70%; R_f (A) 0.46; R_f (B) 0.86; ^{1}H NMR (300 MHz): δ 6.52 (s, 1H, ArCH=), 7.36–7.41 (m, 1H, H-4″), 7.48 (def. t, 2H, H-3″, H-5″), 7.72 (def. d, 4H, H-3′, H-5′, H-2″, H-6″), 7.84 (d, $J = 8.52$ Hz, 2H, H-2′, H-6′), 12.20 (s, 1H, N_1–H), 12.37 (s, 1H, N_3–H); IR (KBr): ν 3253 (N–H), 1720 (C_4=O), 1648 (ArCH=), 1475, 1337, 1171, 1092, 764, 693 cm^{-1}. *Anal*. ($C_{16}H_{12}N_2OS$): C, H, N.

*4.1.2.10. Z-5-(1-Naphthylidene)-2-thiohydantoin (**37**).* Obtained as described in Ref. [9], the reaction mixture was refluxed for 0.25 h, m.p. 290–292 °C (lemon coloured crystals from AcOH), yield 69%; R_f (A) 0.46; R_f (B) 0.87; ^{1}H NMR (300 MHz): δ 7.06 (s, 1H, ArCH=), 7.53–7.64 (m, 3H, H-3′, H-6′, H-7′), 7.79 (d, $J = 7.14$ Hz, 1H, H-2′), 7.90–7.99 (m, 2H, H-4′, H-5′), 8.06 (d, $J = 8.79$ Hz, 1H, H-8′), 12.22 (s, 1H, N_1–H), 12.39 (s, 1H, N_3–H); IR (KBr): ν 3129 (N–H), 1721 (C_4=O), 1640 (ArCH=), 1492, 1373, 1339, 1231, 1194, 965, 804, 781, 644 cm^{-1}. *Anal*. ($C_{14}H_{10}N_2OS$): C, H, N.

*4.1.2.11. Z-5-(2-Naphthylidene)-2-thiohydantoin (**38**).* Obtained as described in Ref. [9], the reaction mixture was refluxed for 1.0 h, m.p. 271–272 °C (lemon coloured crystals from AcOH), yield 86%; R_f (A) 0.45; R_f (B) 0.86; ^{1}H NMR (300 MHz): δ 6.62 (s, 1H, ArCH=), 7.52–7.56 (m, 2H, H-6′, H-7′), 7.77 (dd, $J =$ 1.65, 8.65 Hz, 1H, H-3′), 7.89–7.98 (m, 3H, H-4′, H-5′, H-8′), 8.37 (s, 1H, H-1′), 12.30 (s, 1H, N_1–H), 12.41 (s, 1H, N_3–H); IR (KBr): ν 3130 (N–H), 1722 (C_4=O), 1641 (ArCH=), 1493, 1373, 1339, 1231, 1194, 965, 804, 781, 644cm^{-1}. *Anal*. ($C_{14}H_{10}N_2OS$): C, H, N.

*4.1.2.12. Z-5-[3,4-(Methylenedioxy)benzylidene]-2-thiohydantoin (**39**).* Obtained as described in Ref. [9], the reaction mixture was refluxed for 0.5 h, m.p. 298–300 °C (yellow crystals from AcOH), yield 80%; R_f (A) 0.38; R_f (B) 0.83; ^{1}H NMR (300 MHz): δ 6.08 (s, 2H, CH_2O), 6.42 (s, 1H, ArCH=), 6.95 (d, $J = 8.24$ Hz, 1H, H-5′), 7.24 (dd, $J = 1.10$, 7.44 Hz, 1H, H-6′), 7.43 (d, $J = 1.10$ Hz, 1H, H-2′), 12.06 (s, 1H, N_1–H), 12.28 (s, 1H, N_3–H); IR (KBr): ν 1717 (C_4=O), 1649 (ArCH=), 1595, 1488, 1344, 1190, 1106, 1033, 968, 922, 799 cm^{-1}. *Anal*. ($C_{11}H_8N_2O_3S$): C, H, N.

*4.1.2.13. Z-5-[(3-Benzyloxy)benzylidene]-2-thiohydantoin (**40**).* The mixture of 12.2 g (0.1 mol) 3-hydroxybenzaldehyde, 17.1 (0.1 mol) benzylbromide and 6.9 g (0.05 mol) of anhydrous K_2CO_3 in 100 ml of EtOH was refluxed for 4.0 h. After cooling the solid was filtered off, the filtrate was evaporated to the oily residue, dissolved in CH_2Cl_2 and washed three times with 2% NaOH solution till the disappearance of the starting aldehyde [checked with tlc R_f (C) 0.08]. The product R_f (C) 0.45 was recrystallized from EtOH, m.p. 55–56 °C; yield 75% and used as starting material in the synthesis of **40** with the method described in Ref. [9]; the reaction mixture was refluxed for 2.0 h, m.p. 212–215 °C (yellow crystals from AcOH), yield 74%; R_f (A) 0.41; R_f (B) 0.88; ^{1}H NMR (200 MHz): δ 5.17 (s, 2H, CH_2O), 6.46 (s, 1H, ArCH=), 7.01–7.06 (m, 1H, H-5′), 7.31–7.39 (m, 5H, C_6H_5), 7.40–7.50 (m, 3H, H-2′, H-4′, H-6′), 12.24 (br.s, 1H, N_1–H), 12.32 (br.s, 1H, N_3–H); IR (KBr): ν 3436 (N–H), 3248 (N–H), 1720 (C_4=O), 1648 (ArCH=), 1476, 1232, 1052, 896, 776, 684, 544 cm^{-1}. *Anal*. ($C_{17}H_{14}N_2O_2S$): C, H, N.

*4.1.2.14. Z-5-[3-(4-Chlorobenzyloxy)benzylidene]-2-thiohydantoin (**41**).* Obtained as described for **40**, m.p. 220–222 °C (lemon coloured crystals from AcOH), yield 86%; raw product was analytically pure; R_f (A) 0.41; R_f (B) 0.88; ^{1}H NMR (200 MHz): δ 5.17 (s, 2H, CH_2O), 6.44 (s, 1H, ArCH=), 7.00–7.05 (m, 1H, H-5′), 7.32–7.36 (m, 3H, H-2′, H-4′, H-6′), 7.43–7.53, (m, 4H,

H-2″, H-3″, H-5″, H-6″), 12.28 (s, 2H, N_1–H, N_3–H); IR (KBr): *v* 3432 (N–H), 2876 (CH_2), 1722 (C_4=O), 1644 (ArCH=), 1352, 1306, 1232, 1088, 1012, 668 cm^{-1}. *Anal*. ($C_{17}H_{13}ClN_2O_2S$): C, H, N.

*4.1.2.15. Z-5-[3-(2-Phenylethoxy)benzylidene]-2-thiohydantoin (**43**).* Obtained as described for **40**, m.p. 159–161 °C (yellow crystals from AcOH), yield 45%; R_f (A) 0.45; R_f (B) 0.88; ^{1}H NMR (200 MHz): δ 3.05 (t, $J = 6.86$ Hz, 2H, $C_6H_5CH_2$), 4.26 (t, $J = 6.88$ Hz, 2H, CH_2O), 6.46 (s, 1H, ArCH=), 6.92–6.98 (m, 1H, H-5′), 7.22–7.36 (m, 8H, C_6H_5, H-2′, H-4′, H-6′), 12.29 (br.s, 2H, N_1–H, N_3–H); IR (KBr): *v* 3164 (N–H), 2856 (CH_2), 1722 (C_4=O), 1640 (ArCH=), 1572, 1480, 1364, 1240, 900, 764, 702, 500 cm^{-1}. *Anal*. ($C_{18}H_{16}N_2O_2S$): C, H, N.

*4.1.2.16. Z-5-[(3-Allyloxy)benzylidene]-2-thiohydantoin (**44**).* Obtained as described for **40**, m.p. 184–187 °C (yellow crystals from AcOH), yield 69%; R_f (A) 0.42; R_f (B) 0.88; ^{1}H NMR (200 MHz): δ 4.59 (s, 2H, OCH_2), 5.25 (d, $J = 10.71$ Hz, 1H, H_c), 5.40 (d, $J =$ 17.00 Hz, 1H, H_b), 5.97–6.10 (m, 1H, H_a), 6.91 (t, $J =$ 8.52 Hz, 1H, H-5′), 7.02 (s, 1H, ArCH=), 7.07 (def. t, 1H, H-4′), 7.27–7.32 (m, 1H, H-2′), 7.58 (d, $J = 15.65$ Hz, 0.5H, H-6′), 8.40 (d, $J = 16.20$ Hz, 0.5H, H-6′), 11.88 (s, 0.5H, N1–H), 12.13 (s, 1.5H, N_3–H, N_1–H), 12.18 (s, 0.5H, N_3–H); IR (KBr): *v* 3115 (NH), 2869 (CH_2), 1707 (C_4=O), 1606 (ArCH=), 1513, 1208, 947, 770, 685 cm^{-1}. *Anal*. ($C_{13}H_{12}N_2O_2S$): C, H, N.

*4.1.2.17. Z-5-[(4-Allyloxy)benzylidene]-2-thiohydantoin (**45**).* Obtained as described for **40**, m.p. 219–221 °C (yellow crystals from AcOH), yield 80%; R_f (A) 0.28; R_f (B) 0.77; ^{1}H NMR (200 MHz): δ 4.61 (d, $J = 5.21$ Hz, 2H, CH_2O), 5.27 (d, $J = 10.50$ Hz, 1H, H_c), 5.39 (dd, $J = 17.27$, 1.46 Hz, 1H, H_b), 5.97–6.06 (m, 1H, H_a), 6.46 (s, 1H, ArCH=), 6.98 (d, $J = 8.64$ Hz, 2H, H-3′, H-5′), 7.72 (d, $J = 8.69$ Hz, 2H, H-2′, H-6′), 12.09 (s, 1H, N_1–H), 12.26 (s, 1H, N_3–H); IR (KBr): *v* 3231, 3124 (N–H), 2920, 2854, 2831 (C–H), 1724 (C_4=O), 1643 (ArCH=), 1598, 1514, 1486, 1375, 1252, 1177, 963, 825, 543, 494 cm^{-1}. *Anal*. ($C_{13}H_{12}N_2O_2S \cdot 0.5CH_3COOH$): C, H, N.

4.2. Microbiology

4.2.1. In vitro evaluation of antimycobacterial activity [15]

Primary screening was conducted at 6.25 μg/ml against *M. tuberculosis* H_{37}Rv (ATCC 27294; American Type Culture Collection, Rockville, MD) in BACTEC 12B medium using the Microplate Alamar Blue Assay (MABA). Compounds demonstrating at least 90% inhibition in the primary screen were re-tested at lower concentrations against *M. tuberculosis* H37Rv to determine the actual MIC in the 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 considered as active and classified to further tests.

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