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Antimicrobial activity of 5-arylidene aromatic derivatives of hydantoin. Part 2☆

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Abstract

Various 5-chloroarylidene-2-amino substituted derivatives of imidazoline-4-one were synthesized and evaluated for their activity in vitro against *Mycobacterium tuberculosis* and other type strains of bacteria and fungi. 2-Chloro- and 2,4-dichlorobenzylidene substituted hydantoins exhibited antimycobacterial effect. The most potent compounds **3i**, **3j**, **3o**, **3q** and **3s** were classified for further tests. The antimitotic effect of the investigated hydantoins was also examined. © 2002 Elsevier Science S.A. All rights reserved.

Keywords: Arylidene hydantoins; Antimycobacterial activity; Antibacterial activity; Antimitotic activity

1. Introduction

Antimicrobial activity was stated among hydantoins possessing aromatic or heterocyclic substituents at imidazolone nitrogen e.g. *N*-acyl and 5-arylidene derivatives of hydantoin and 2-thiohydantoin [1–3]. Antifungal activity was shown for compounds with *N*-aromatic acyl substituents with 5-aromatic, arylidene or without substituents derivatives [4–8]. Antifungal and antimicrobial activity was found for the arylidene, arylhydrazone and aromatic substituted derivatives (with)out substituents on nitrogen atoms [9–14].

As a result of the analysis of the above presented literature data and continuing our studies on the biological activity of hydantoin derivatives [15–18], we have synthesized and examined the new series of 5-chloroarylidene-2-amino substituted derivatives of hydantoin. Their antimicrobial effect on the set of type strains of microorganisms as well as the antimycobacterial activity was investigated.

The antimitotic effect of the obtained hydantoins was also examined.

For each compound the octanol–water coefficient (log *P* combined) and distribution coefficient (log *D*) were calculated with PALLAS program [19].

2. Chemistry

The starting 5-(2-chlorobenzylidene)-, 5-(2,6-dichlorobenzylidene)- and 5-(2,4-dichloro-benzylidene)-2-thiohydantoins were prepared according to the literature procedure [20]. To prepare target compounds 5-chlorobenzylidene-2-thiohydantoins **1** were treated with methyl iodide (Scheme 1). Obtained methylthio derivatives **2** were reacted with 10% excess of amines possessing (un)substituted aromatic residue to give with good yields solid products **3a**–**q** (Table 1). Last reactions were carried out in toluene for the benzylamine derivatives (**3f**–**h**, **3n**) or in acetic acid for aniline derivatives (**3a**–**e**, **3i**–**m**, **3o**–**q**), with 10% excess of the appropriate amine. Target compounds were recrystallized from acetic acid, methanol or DMF.

The purity of all obtained compounds was checked by thin-layer chromatography. The structures were

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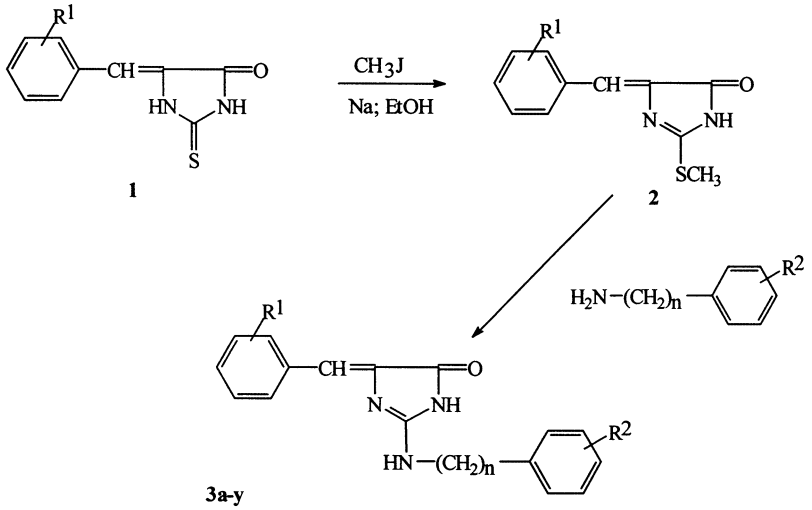

Scheme 1. Synthesis of 5-arylidene hydantoin derivatives **3a**–**y**.

confirmed by elemental and spectral analyzes (IR, ^{1}H NMR).

The present work is the continuation of our studies on structure—antimicrobial activity relationships among 5-arylidene hydantoin derivatives. Some of the compounds with structure **3** (**3r**–**y**) mentioned for comparison in this work were synthesized and examined previously [18].

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 the BACTEC 460 radiometric system [21] (Table 2).

Log *P* combined and log *D* values prediction of the obtained compounds taken with PALLAS program are presented in Table 1. The compounds show insignificant acid–basic properties and pH hardly influences on log *D* values, so that log *P* = log *D* (at pH 7.00) for all investigated compounds.

In the tested group, 2-chloro- and 2,4-dichloro benzylidene compounds (**3i**–**y**) showed better effect against *M. tuberculosis* than 2,6-dichloro group (**3a**–**h**). The occurrence of an additional chlorine atom in the *ortho*-position in a benzylidene residue reduced activity, while the influence of additional *para*-chloro substituent on the antitubercular effect is more difficult to specify. On the basis of the obtained screening results, we can state that in each series among all aniline derivatives compounds substituted with 4-chloro-aniline residue (**3a**, **3i**, **3o**) possessed the highest antimycobacterial activity. On the whole, with growing distance between the imidazolone and aromatic ring the activity seems to decrease. The most potent compounds **3i**, **3j**, **3o**, **3q** and **3s** were classified to further tests.

Log *D* values calculated for 2-chloro compounds (**3o**–**y**) at pH 7.00 varied from 3.23 to 4.97; for 2,4-dichloro from 4.07 to 5.76. For 2,6-dichloro derivatives log *D* varied from 3.95 to 5.68. The comparison of the lipophilic properties indicates that values of log *P* for the benzylamine structures (**3f**–**h**, **3n**, **3t**–**y**) are lower than values of log *P* for the aniline derivatives, what seems to be correlated with microbiological effects. However, no exact relation between log *P* and mycobacterial activity of the tested compounds was found.

Table 1
Arylidene hydantoins

Comp.	R^1	R^2	*n*	Log *P*	Log *D*[a]
3a	2,6-diCl	4-Cl	0	4.90	4.90
3b	2,6-diCl	2,4-diCl	0	5.68	5.68
3c	2,6-diCl	3-Cl	0	4.96	4.96
3d	2,6-diCl	2-Cl	0	4.89	4.89
3e	2,6-diCl	H	0	4.18	4.18
3f	2,6-diCl	4-Cl	1	4.72	4.72
3g	2,6-diCl	4-F	1	4.14	4.14
3h	2,6-diCl	4-OCH3	1	3.95	3.95
3i	2,4-diCl	4-Cl	0	4.98	4.98
3j	2,4-diCl	2,4-diCl	0	5.76	5.76
3k	2,4-diCl	3-Cl	0	5.04	5.04
3l	2,4-diCl	2-Cl	0	4.97	4.97
3m	2,4-diCl	H	0	4.25	4.25
3n	2,4-diCl	H	1	4.07	4.07
3o	2-Cl	4-Cl	0	4.19	4.19
3p	2-Cl	2,4-diCl	0	4.97	4.97
3q	2-Cl	3-Cl	0	4.24	4.24
3r	2-Cl	2-Cl	0	4.18	4.18
3s	2-Cl	H	0	3.46	3.46
3t	2-Cl	H	1	3.28	3.28
3u	2-Cl	4-Cl	1	4.00	4.00
3w	2-Cl	4-F	1	3.43	3.43
3y	2-Cl	4-OCH3	1	3.23	3.23

[a] Log *D* calculated for pH 7.0.

Table 2
Antibacterial activity against *M. tuberculosis* H37Rv

Comp. 2,6-dichloro	MIC (μg/mL)	Inhibition [a] (%)	Comp. 2,4-dichloro	MIC (μg/mL)	Inhibition [a] (%)	Comp. 2-chloro	MIC (μg/mL)	Inhibition [a] (%)
3a	>12.5	17	**3i**	<12.5	100	**3o**	<12.5	99
3b	>12.5	4	**3j**	<12.5	96	**3p**	>12.5	37
3c	>12.5	0	**3k**	>6.25	48	**3q**	>12.5	91
3d	>6.25	0	**3l**	>6.25	13	**3r**	>12.5	79 [b]
3e	>6.25	1	**3m**	>6.25	47	**3s**	>12.5	93
3f	>12.5	0	**3n**	>12.5	46	**3t**	>12.5	20 [b]
3g	>12.5	5				**3u**	>12.5	45 [b]
3h	>12.5	0				**3w**	>6.25	10
						3y	>6.25	8

[a] MIC Rifampin = 0.25 μg/mL (98% inhibition).
[b] MIC Rifampin = 0.5 μg/mL (100% inhibition).

Table 3
Minimum inhibitory concentration (MIC) μg/mL

Comp.	*M. catarrhalis*	*H. influenzae*	*M. luteus*	*S. aureus*	*B. cereus*	*S. pneumoniae*
3b				78		78
3c	20					156
3f	78	39				78
3g	78	78				78
3h						156
3i			156			
3o	39					
3p	39				156	
3q	78		78			
3s		156				
3u	156					
Gentamycin [a]	0.075	1.25	0.15	0.0045	0.018	
Nalidixic acid [b]	39	0.62			625	

[a] MIC (μg/mL) estimated for gentamycin.
[b] MIC (μg/mL) assigned for nalidixic acid.

The obtained compounds were also investigated against type strains of microorganisms listed in Section 4.2.2. For active compounds the minimum inhibitory concentration (MIC) values were determined using disc diffusion methods by NCCLS procedures [22,23]. Results of these tests are collected in Table 3.

Obtained compounds were mainly active against *Moraxella catarrhalis* and *Streptococcus pneumoniae*. Some of them inhibited growth of *Haemophilus influenzae* (**3f**, **3g**), *Micrococcus luteus* (**3i**, **3q**), *Staphylococcus aureus* (**3b**) and *Bacillus cereus* (**3p**). 2,6-Dichlorobenzylidene derivative **3c** showed high activity against *M. catarrhalis* with MIC = 20 μg/mL lower than MIC of nalidixic acid (39 μg/mL). The activity against *S. pneumoniae* has been shown only by 2,6-dichloroarylidene derivatives (**3b**, **3c**, **3f**–**h**). The 2,4-dichloro substituted compounds (except **3i**) were almost totally devoid of antimicrobial effect (MIC > 156 μg/mL). We also observed that the lack of any substituent in amine residue (**3e**, **3m**, **3n**, **3s** and **3t**) reduced antibacterial activity against tested strains.

No compound has shown antifungal effect against *Candida albicans*.

Obtained compounds were assayed on cdc2 kinase and cdc25 phosphatase tests for their antimitotic activity according to the European Organization for Research and Treatment of Cancer (EORTC) program [24–26].

All tested compounds were (according to EORTC program procedure) inactive in these tests ($IC_{50} > 10$ μM).

4. Experimental

4.1. Chemistry

Melting points (uncorrected) were determined on Mel-Temp melting point apparatus (Laboratory Devices Inc., USA). Thin-layer chromatography was performed with Merck silica gel GF_{254} aluminum sheets, using the following developing system:

chloroform–acetone 1:1. Spots were detected by their absorption under UV light. Elemental analyzes agree with theoretical values within ±0.40%, unless otherwise stated.

IR spectra were recorded with Fourier transform infrared spectrometer FT/IR-410 (Jasco Co., Japan) using KBr discs. The following abbreviations were used: br (broad), s (sharp). ^{1}H NMR spectra of compounds were determined in DMSO-d_6 solution (Varian Mercury 300 MHz with TMS as an internal standard. All chemical shifts are quoted in δ values.

The predictions of log *P* combined and log *D* values were determined with the PALLAS program [19].

Compounds **1** were prepared according to the literature procedure [20].

4.1.1. General procedure for synthesis of compounds *3a–q*

To the stirred solution of sodium (0.04 mol) in 200 mL of ethanol, arylidene-2-tiohydantoin (**1**) (0.04 mol) and methyl iodide (0.04 mol) were added. After stirring at room temperature (r.t.) for 30 min the solid of **2** was filtered off, washed with water and dried. Methylthio derivatives **2** were found to be analytically pure Table 4.

A mixture containing 5 mmol of **2**, 5.5 mmol of amine in 30 mL of toluene (for compounds **3f–h**, **3n**) or acetic acid (**3a–e**, **3i–m**, **3o–q**) was refluxed for 9 h, and then allowed to cool. The product was isolated by suction and recrystallized from acetic acid (**3d**, **3l**, **3o–q**), DMF with addition of H_2O (**3a–e**, **3i–m**) or methanol (**3f-h**, **3n**).

3a: ^{1}H NMR δ (ppm) = 6.33 (s, 1H, CH=), 7.32–7.43 (m, 4H, H-2″, H-3″, H-5″, H-6″), 7.49–7.54 (m, 3H, H-3′, H-4′, H-5′), 10.05 (br.s, 1H, $NH_{aniline}$), 10.48 (br.s, 1H, 3-NH). *Anal.* $C_{16}H_{10}ON_3Cl_3$ (C, H, N).

3b: ^{1}H NMR δ (ppm) = 6.16 (s, 1H, CH=), 7.10 (br.s, 1H, H-6″), 7.26–7.38 (m, 3H, H-3′, H-5′, H-5″), 7.47 (s, 1H, H-3″), 7.50–7.53 (m, 1H, H-4′), 9.84 (br.s, 1H, $NH_{aniline}$), 11.26 (br.s, 1H, 3-NH). *Anal.* $C_{16}H_9ON_3Cl_4$ (C, H, N).

3c: ^{1}H NMR δ (ppm) = 6.28 (br.s, CH=), 6.39 (br.s, CH=), 7.08 (s, 1H, H-6″), 7.25–7.55 (m, 5H, H-3′, H-4′, H-5′, H-4″, H-5″), 7.50 (s, 1H, H-2″), 9.97 (br.s, $NH_{aniline}$), 10.30 (br.s, 1-NH), 11.02 (br.s, 3-NH). *Anal.* $C_{16}H_{10}ON_3Cl_3$ (C, H, N).

3d: ^{1}H NMR δ (ppm) = 6.14 (s, 1H, CH=), 7.01 (t, J = 8 Hz, 1H, H-4″), 7.23 (t, J = 7.3 Hz, 1H, H-5″), 7.30–7.48 (m, 5H, H-3′, H-4′, H-5′, H-3″, H-6″), 9.82 (br.s, 1H, $NH_{aniline}$), 10.16 (br.s, 1H, 3-NH). *Anal.* $C_{16}H_{10}ON_3Cl_3$ (C, H, N).

3e: ^{1}H NMR δ (ppm) = 6.31 (s, 1H, CH=), 7.04 (t, J = 8 Hz, 1H, H-4′), 7.29–7.38 (m, 4H, H-3′, H-5′, H-3″, H-5″), 7.43–7.53 (m, 3H, H-2″, H-4″, H-6″), 9.94 (br.s, 2H, $NH_{aniline}$, 3-NH). *Anal.* $C_{16}H_{11}ON_3Cl_2$ (C, H, N).

3f: ^{1}H NMR δ (ppm) = 4.39 (s, CH_2), 4.54 (d, J = 5.4 Hz, 2H, CH_2), 6.19 (s, 1H, CH=), 7.31–7.55 (m, 7H, H_{aromat}), 8.09 (t, N*H*–CH_2), 9.20 (br.s, 1-NH), 9.99 (br.s, 1-NH), 10.13 (br.s, 3-NH), 10.99 (br.s, 3-NH). *Anal.* $C_{17}H_{12}ON_3Cl_3$ (C, H, N).

3g: ^{1}H NMR δ (ppm) = 4.39 (s, CH_2), 4.53 (d, J = 5.7 Hz, 2H, CH_2), 6.19 (s, 1H, CH=), 7.09–7.55 (m, 7H, H_{aromat}), 8.08 (t, J = 5.5 Hz, N*H*–CH_2), 9.16 (br.s, 1-NH), 9.95 (br.s, 1-NH), 10.14 (br.s, 3-NH), 10.89 (br.s, 3-NH). *Anal.* $C_{17}H_{12}ON_3Cl_2F$ (C, H, N).

3h: ^{1}H NMR δ (ppm) = 4.31 (s, CH_2), 4.43 (d, J = 12.6 Hz, 2H, CH_2), 6.15 (s, 1H, CH=), 6.88 (d, J = 8.2

Table 4
Physical and IR data of compounds **3a–q**

Comp.	Yield (%)	m.p. °C (solvent)	TLC	Molecular formula	IR (cm^{-1})
3a	84	301–303 (DMF + water)	0.65	$C_{16}H_{10}ON_3Cl_3$	1640 (ArCH=), 1688, 1694 (C=O), 3192 (N–H)
3b	43	289–291 (DMF + water)	0.86	$C_{16}H_9ON_3Cl_4$	1640 (ArCH=), 1688, 1704 (C=O), 3196, 3336 (N–H)
3c	72	305–306 (DMF + water)	0.70	$C_{16}H_{10}ON_3Cl_3$	1646 (ArCH=), 1693 (C=O), 3196 (N–H)
3d	51	267–269 (DMF + water, CH_3COOH)	0.85	$C_{16}H_{10}ON_3Cl_3$	1610 (ArCH=), 1703 (C=O), 3231, 3428 (N–H)
3e	74	298–300 (DMF + water)	0.47	$C_{16}H_{11}ON_3Cl_2$	1639 (ArCH=), 1704 (C=O), 3365 (N–H)
3f	88	237–239 (methanol)	0.16	$C_{17}H_{12}ON_3Cl_3$	1696, 1706 (C=O), 3308 (N–H)
3g	90	239–240 (methanol)	0.23	$C_{17}H_{12}ON_3Cl_2F$	1648 (ArCH=), 1694, 1704 (C=O), 3320 (N–H)
3h	70	221–223 (methanol)	0.30	$C_{18}H_{15}O_2N_3Cl_2$	1710 (C=O), 3309 (N–H)
3i	63	291–293 (DMF + water)	0.16	$C_{16}H_{10}ON_3Cl_3$	1656 (ArCH=), 1714 (C=O), 3149, 3353 (N–H)
3j	55	304–309 (DMF + water)	0.67	$C_{16}H_9ON_3Cl_4$	1650 (ArCH=), 1683, 1729 (C=O), 3176 (N–H)
3k	67	296–297 (DMF + water)	0.22	$C_{16}H_{10}ON_3Cl_3$	1654 (ArCH=), 1716 (C=O), 3276, 3446, 3542 (N–H)
3l	86	276–277 (DMF, CH_3COOH)	0.77	$C_{16}H_{10}ON_3Cl_3$	1651 (ArCH=), 1724 (C=O), 3338, 3426 (N–H)
3m	55	304–306 (DMF + water)	0.42	$C_{16}H_{11}ON_3Cl_2$	1641 (ArCH=), 1699 (C=O), 3347 (N–H)
3n	83	219–221 (methanol)	0.24	$C_{17}H_{13}ON_3Cl_2$	1662 (ArCH=), 1718 (C=O), 3083 (N–H)
3o	89	297–299 (acetic acid)	0.23	$C_{16}H_{11}ON_3Cl_2$	1656 (ArCH=), 1686, 1696 (C=O), 3164, 3348 (N–H)
3p	74	279–281 (acetic acid)	0.67	$C_{16}H_{10}ON_3Cl_3$	1644 (ArCH=), 1680 (C=O), 3324 (N–H)
3q	85	292–295 (acetic acid)	0.18	$C_{16}H_{11}ON_3Cl_2$	1640 (ArCH=), 1678, 1688 (C=O), 3172 (N–H)

Hz, 2H, H-3″, H-5″), 7.22–7.53 (m, 5H, H-3′, H-4′, H-5′, H-2″, H-6″), 7.98 (br.s, N*H*–CH_2), 9.16 (br.s, 1-NH), 9.84 (br.s, 3-NH), 10.10 (br.s, 3-NH). *Anal.* $C_{17}H_{13}ON_3Cl_2$ (C, H, N).

3i: ^{1}H NMR δ (ppm) = 6.70 (s, 1H, CH=), 7.42–7.66 (m, 4H, H-3′, H-5′, H-2″, H-6″), 7.77 (d, J = 8.7 Hz, 2H, H-3″, H-5″), 8.80 (d, J = 9.2 Hz, 1H, H-6′), 10.10–11.20 (br.s, 2H, $NH_{aniline}$, 3-NH). *Anal.* $C_{16}H_{10}ON_3Cl_3$ (C, H, N).

3j: ^{1}H NMR δ (ppm) = 6.59 (s, 1H, CH=), 7.38–7.47 (m, 4H, H-3′, H-5′, H-5″, H-6″), 7.76 (br.s, 1H, H-3″), 8.25 (br.s, 1H, H-6′), 10.24 (br.s, 2H, $NH_{aniline}$, 3-NH). *Anal.* $C_{16}H_9ON_3Cl_4$ (C, H, N).

3k: ^{1}H NMR δ (ppm) = 6.72 (s, 1H, CH=), 7.14 (d, J = 8 Hz, 1H, H-6″), 7.36–7.46 (m, 2H, H-4″, H-5″), 7.59 (d, J = 7.5 Hz, 1H, H-5′), 7.66 (s, 1H, H-3′), 8.06 (s, 1H, H-2″), 8.81 (d, J = 8 Hz, 1H, H-6′), 10.38 (br.s, 1H, $NH_{aniline}$), 11.00 (br.s, 1H, 3-NH). *Anal.* $C_{16}H_{10}ON_3Cl_3$ (C, H, N).

3l: ^{1}H NMR δ (ppm) = 6.61 (s, 1H, CH=), 7.15 (t, J = 7.4 Hz, 1H, H-4″), 7.38 (t, J = 7.4 Hz, 1H, H-5″), 7.46–7.52 (m, 2H, H-5′, H-6″), 7.65 (s, 1H, H-3′), 7.94 (br.s, 1H, H-3″), 8.58 (br.s, 1H, H-6′), 10.97 (br.s, 2H, $NH_{aniline}$, 3-NH). *Anal.* $C_{16}H_{10}ON_3Cl_3$ (C, H, N).

3m: ^{1}H NMR δ (ppm) = 6.68 (s, 1H, CH=), 7.10 (t, J = 7.3 Hz, 1H, H-4″), 7.38 (t, J = 7.8 Hz, 2H, H-3″, H-5″), 7.52 (d, J = 8.6 Hz, 1H, H-5′), 7.64 (s, 1H, H-3′), 7.74 (d, J = 8 Hz, 2H, H-2″, H-6″), 8.85 (br.s, 1H, H-6′), 10.17 (br.s, 1H, $NH_{aniline}$), 10.80 (br.s, 1H, 3-NH). *Anal.* $C_{16}H_{11}ON_3Cl_2$ (C, H, N).

3n: ^{1}H NMR δ (ppm) = 4.57 (s, 2H, CH_2), 6.51 (s, 1H, CH=), 7.22–7.47 (m, 6H, H-5′, H-2″, H-3″, H-4″, H-5″, H-6″), 7.59 (s, 1H, H-3′), 8.32 (br.s, 1H, N*H*–CH_2), 8.90 (d, J = 8.1 Hz, 1H, H-6′), 11.03 (br.s, 1H, 3-NH). *Anal.* $C_{17}H_{13}ON_3Cl_2$ (C, H, N).

3o: ^{1}H NMR δ (ppm) = 6.81 (s, 1H, CH=), 7.27–7.52 (m, 5H, H-3′, H-4′, H-5′, H-3″, H-5″), 7.80 (d, J = 8.7 Hz, 2H, H-2″, H-6″), 8.80 (d, J = 7.6 Hz, 1H, H-6′), 10.25 (br.s, 1H, 1-NH), 10.98 (br.s, 1H, 3-NH). *Anal.* $C_{16}H_{11}ON_3Cl_2$ (C, H, N).

3p: ^{1}H NMR δ (ppm) = 6.63 (s, 1H, CH=), 7.26–7.52 (m, 6H, H-3′, H-4′, H-5′, H-3″, H-5″, H-6″), 8.30 (br.s, 1H, H-6′), 10.20 (br.s, 1H, $NH_{aniline}$), 10.74 (br.s, 1H, 3-NH). *Anal.* $C_{16}H_{11}ON_3Cl_2$ (C, H, N).

3q: ^{1}H NMR δ (ppm) = 6.83 (s, 1H, CH=), 7.14 (d, J = 6.8 Hz, 1H, H-6″), 7.27–7.43 (m, 5H, H-3′, H-4′, H-5′, H-4″, H-5″), 8.19 (s, 1H, H-2″), 8.83 (d, J = 6.9 Hz, 1H, H-6′), 10.34 (br.s, 1H, $NH_{aniline}$), 11.05 (br.s, 1H, 3-NH). *Anal.* $C_{16}H_{11}ON_3Cl_2$ (C, H, N).

In the ^{1}H NMR spectra of the compounds **3f–h** double signals assigned to 1-NH and 3-NH protons are observed. The splits are probably connected with the phenomenon of coexistence of two (or more) tautomeric forms. The discussion of dynamic nature of the described compounds is going to be presented in the separate article [27].

4.2. Biological test procedures

4.2.1. In vitro evaluation of antimycobacterial activity against *M. tuberculosis H37Rv*

Primary screening was conducted at 12.5 or 6.25 μg/mL against *M. tuberculosis* H37Rv (ATCC 27294; American Type Culture Collection, Rockville, MD) in BACTEC 12B medium using the BACTEC 460-radiometric system [21]. Compounds demonstrating at least 90% inhibition were retested 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.

4.2.2. Antimicrobial activity

The activity of the obtained compounds was investigated, using disc diffusion method [22,23], against type strains of microorganisms as follows: *S. aureus* (ATCC 25923), *S. pneumoniae* (ATCC 49619), *Enterococcus faecalis* (ATCC 29212), *Pseudomonas aeruginosa* (ATCC 27853), *Escherichia coli* (ATCC 25922), *B. cereus* (ATCC 11778), *M. catarrhalis* (CBM 5), *M. luteus* (CBM 4), *Streptococcus pyogenes* (CBM 7), *H. influenzae* (CBM 15) and yeast fungi: *C. albicans* (CBM 26). As test medium for aerobic bacteria Mueller–Hinton agar (Difco Laboratories USA) was used (for *H. influenzae* Mueller–Hinton agar supplemented with hematin and yeast extract). Tests for fungi were performed on Yeast Nitrogen Base medium (Difco). Gentamycin and nalidixic acid were used as positive drug controls.

The compounds studied were solubilized using DMSO. The basic concentration was 10 000 μg/mL. From such a solution a series of dilutions with concentrations ranging from 5 to 10 000 μg/mL were prepared (for reference substances the concentrations ranged from 0.0045 to 10 000 μg/mL). Minimum inhibitory concentration (MIC) values of the compounds were determined with reference to standard microorganism. The corresponding solution (20 μl) with a different concentration was put on the sterile paper disc (9 mm of diameter). Discs were placed on the solid medium with suspension of a tested microorganism at 0.9% NaCl. The MIC breakpoints were determined: after 24 h at 37 °C for bacteria and after 48 h at 28 °C for fungi.

All antimicrobial potency tests, the medium and microorganisms suspensions were performed according to NCCLS procedures. In each assay the control of both microorganism culture sterility and standard microorganism growth was performed. It was found that DMSO showed neither antibacterial nor fungicidal activity.

4.2.3. Antimitotic activity

The first screening test uses the $p34^{cdc2}$/$cyclinB^{cdc13}$ protein kinase, affinity-purified on $p9^{CKShs}$-sepharose beads. The enzyme activity is assayed, in the presence of potential inhibitors, using histone H1 and ^{32}P-labelled ATP [24,25].

The second screening test uses a highly purified human recombinant glutathione-*S*-transferaze/cdc25 fusion protein assayed colorimetrically for *p*-nitrophenylphosphate phosphatase activity in microtitration plates [26].

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