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N'-BENZYLIDENE-*N*-(THIAZOLYL)ACETOHYDRAZIDE DERIVATIVES: SYNTHESIS AND ANTIMICROBIAL ACTIVITY EVALUATION

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ABSTRACT. A series of new *N'*-benzylidene-*N*-(thiazolyl)acetohydrazide derivatives was obtained by the acetylation of 2-(2-benzylidenehydrazinyl)thiazole derivatives using acetic anhydride. The antimicrobial activity of the new and parent compounds was screened against Gram-positive and Gram-negative bacteria using agar well diffusion method. 4-Methyl-2-[2-(4-hidroxibenzylidene)-hydrazinyl)-thiazole was identified as the most efficient, with a broad activity spectrum against both Gram positive and Gram negative bacteria.

Key words*: acetohydrazide, thiazole, antimicrobial*

INTRODUCTION

The screening of antibacterial activity of novel synthetic compounds keep on as an evolving research enquiry for the development of effective and safe antimicrobial agents. Antibiotics resistance developed by bacteria became a significant concern in health, medical and environmental area, due to the fact that it may turn out to be a major threat for individuals with poor immune systems. For instance, the diabetic foot ulcer complicated by bacterial infections is in 50% of the cases responsible for reducing the life quality of the patients, amputations and morbidity [1].

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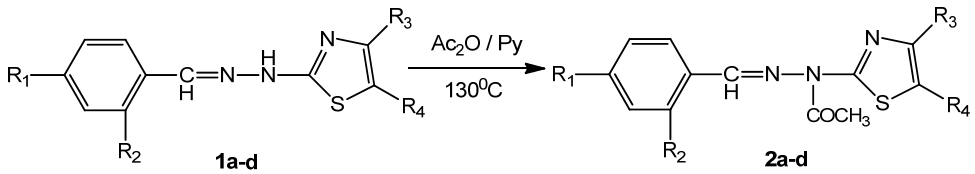
1,3-Thiazole heterocyclic unit appeared in the structure of several synthetic hydrazine derivatives with diverse biological activity, *e.g*. anti-inflamatory [2], antimicrobial [3-7], anticonvulsant [8], anti-tumor [9], antifungal [10,11], or antioxidant representatives [12,13]. Continuing our studies devoted to the chemical synthesis of new thiazole derivatives [14-19], in this work we report four new *N'*-benzylidene-*N*-(thiazolyl)acetohydrazide derivatives. The antimicrobial activity screening against several Gram positive and Gram negative bacteria strains reported in this study included both the newly synthesized (thiazolyl)acetohydrazide derivatives and the parent 2-(2-benzylidenehydrazinyl)thiazole derivatives which were previously synthesized by Hantzsch condensation of arylidene-thiosemicarbazones with α-halogenocarbonyl derivatives [14]

RESULTS AND DISCUSSIONS

The acylation of 2-(2-benzylidenehydrazinyl)thiazole derivatives **1a-d** with acetic anhydride in the presence of catalytic amounts of pyridine afforded the new *N'*-benzylidene-*N*-(thiazolyl)acetohydrazide derivatives **2a-d** in high yields (scheme 1). The structure of the new compounds was unambiguously assigned based on spectroscopic data and elemental analysis.



1, 2	a	b	c	d
R_1	H	H	Cl	OH
R_2	H	H	Cl	H
R_3	C_6H_5	CH_3	CH_3	CH_3
R_4	H	$COCH_3$	$COCH_3$	H

Scheme 1. Synthesis of *N'*-benzylidene-*N*-(thiazolyl)acetohydrazide derivatives

The antimicrobial activity of **1a-d** and **2a-d** was tested *in vitro* against 20 bacterial strains isolated from human venous leg ulcers secretions samples, from patients admitted to Emergency Clinical Hospital Cluj County. In Table 1 are summarized the values of the inhibition zones determined by agar well diffusion

method. A structure-activity correlation between the *in vitro* antimicrobial activity and the structural modifications associated to the substitution pattern of the phenyl ring (unsubstituted, *o,p*-dihalogenated, *p*-hydoxilated), or the thiazole ring (4-phenyl, 4-methyl, 5-acetyl) can be observed.

Table 1. Antimicrobial activity of **1a-d** and **2a-d** determined by diffusion method – inhibition zones (in mm)

Micro org. strain	Mean of the inhibition zone diameter [mm]								
	1a	**2a**	**1b**	**2b**	**1c**	**2c**	**1d**	**2d**	**Positive control[h]**
1[a]	6.10	6.21	6.13	0	0	0	**17.18**	0	0
2[a]	6.12	8.23	12.51	10.14	0	0	**11.48**	0	27.16
3[a]	0	0	0	0	0	0	**13.52**	0	25.02
4[a]	8.12	6.21	7.63	7.31	0	0	**19.72**	0	14.77
5[a]	6.14	0	0	6.32	0	0	**14.93**	11.69	0
6[a]	0	0	6.45	6.12	0	0	**12.34**	0	9.66
7[a]	6.85	7.01	6.04	6.95	0	0	**15.35**	14.12	0
8[a]	6.10	6.13	6.15	6.21	0	0	**18.01**	0	9.81
9[a]	6.01	6.02	6.05	6.46	0	0	**18.13**	0	7.35
10[a]	6.89	6.21	7.03	6.75	0	0	**15.64**	0	11.97
11[b]	6.45	6.09	6.08	6.01	0	0	**19.24**	0	16.63
12[b]	6.12	6.78	8.34	8.02	0	0	**16.35**	0	15.57
13[c]	8.25	8.86	9.34	9.21	0	0	**11.44**	0	0
14[c]	6.04	7.64	8.05	8.56	0	0	**20.91**	0	18.12
15[d]	7.86	7.03	11.56	14.31	0	7.44	**15.54**	8,32	16.52
16[d]	7.86	6.28	8.12	9.43	0	0	**14.97**	0	14.33
17[d]	7.24	7.01	6.98	7.21	0	0	**15.88**	0	21.24
18[e]	7.08	6.03	6.87	7.21	0	0	**20.48**	0	27.10
19[f]	6.74	7.15	6.98	7.12	0	0	**8.24**	0	0
20[g]	7.21	8.31	7.28	7.25	0	0	**8.34**	0	0

[a]Microorganisms strains: [a]*Staphylococus aureus;* [b]*Staphylococus epidermidis*; [c]*Trueperella pyogenes;* [d]*Bacillus licheniformis;* [e]*Pediococcus pentosaceus,* [f]*Enterococcus faecium;* [g]*Pseudomonas aeruginosa.* [h] *Amoxicilin* 30 μg

DMSO did not produced any inhibition zone.

Inhibition zone diameter larger than 7mm indicates susceptibility of the microorganism to the tested compounds.

Moderate susceptibilities of the microorganisms to compounds containing a 2-substituted thiazole ring (**1a, 2a)** as well as 2,3-disubstituted thiazole ring **1b, 2b** can be observed in table 1, with the exception of a noticeable activity of **1b, 2b** against *S. aureus* strain 2 and *B. licheniformis* strain 15. In

these cases, the structural modification introduced by acetylation of the hydrazide unit did not cause a major modification of the antimicrobial activity in comparison to the parent compounds.

According to our experimental results, all the tested microorganisms proved to be resistant to the *o,p*-chlorinated derivative **1c** (which did not produce any inhibition zone) as well as to its acetyl derivative **2c,** with one exception for *B. licheniformis* strain 15 which displayed *in vitro* susceptibility.

As it may be seen from Table 1, all the tested microorganism strains appeared highly susceptible to the hydroxy derivative **1d**, which produced the largest growth-inhibition zones (8.24-19.72 mm)**;** in many cases **1d** proved to be more efficient than the standard antibiotic Amoxicillin which was used in our experiments as a positive control. Microorganism strains *5, 13, 19, 20* proved to be resistant to amoxicillin, appeared largely susceptible to **1d**.

The acetylation of the hydrazide unit of **1d** caused the suppression of antimicrobial activity in relation to the majority of the tested microorganisms (only *S aureus* stains 5, 7 and *B. licheniformis strain 15* appeared susceptible to **2d**).

The structural modifications brought by different substitution patterns of the tested compounds also contributed to the variation of their polarity and distribution on the Mueller Hinton agar, thus affecting the *in vitro* experimental results. The formation of hydrogen bonds between *p*-hydroxyphenyl derivative and agar may had favorize the increase of the susceptibility results recorded in the case of **1d.**

CONCLUSIONS

The acylation of 2-(2-benzylidenehydrazinyl)thiazole derivatives can be conveniently performed using acetic anhydride in the presence of catalytic amounts of pyridine.

The antimicrobial activity of the new *N'*-benzylidene-*N*-(thiazolyl) acetohydrazide derivatives and their parent 2-(2-benzylidenehydrazinyl)thiazole derivatives tested *in vitro* by agar well diffusion method against 20 bacterial strains isolated from human samples indicated 4-methyl-2-[2-(4-hidroxibenzylidene)-hydrazinyl)-thiazole **1d** as the most efficient, with a broad activity spectrum against both Gram positive and Gram negative bacteria. All the microorganisms tested proved to be resistant to 5-acetyl-4-methyl-2-[2-(2,4-dichlorobenzylidene)-hydrazinyl)-thiazole **1c**. The acetylation of the hydrazide unit mainly suppressed or reduced the antimicrobial activity of the 2-(2-benzylidenehydrazinyl)thiazole derivatives.

EXPERIMENTAL

Melting points were determined on open glass capillaries using an Electrothermal IA 9000 digital melting point apparatus. The mass spectra were recorded using a Varian MAT-311A. The ^{1}H-NMR spectra were recorded with Bruker WM-400 spectrometer in the $CDCl_3$. The quantitative elemental analyses were recorded using an Vario EL analyser.

Chemical Syntesis

2-(2-Benzylidenehydrazinyl)thiazole derivatives **1a-d** were prepared according to our previously reported procedure [14].

General procedure of acetylation of 2-(2-Benzylidenehydrazinyl)thiazole derivatives

Hydrazinothiazole (**1a-d**) (2 mmol) was treated with acetic anhydride (2 ml) and catalytic amounts of pyridine. The resulting mixture was heated at reflux for 5 minutes and further concentrated under reduced pressure. The product was precipitated by adding ethanol. The obtained solid was recrystallized from ethanol.

***N'*-benzylidene-*N*-(4-phenylthiazol-2-yl)acetohydrazide 2a**

Brown crystals, yield 2.4 g, 75%; m.p. 152-153 °C; MS (EI) *m*/*z*: 321 (M^+); 279; 176; 134; 77; 43 (100%);

Calcd. for: $C_{18}H_{15}N_3OS$, C, 67.27; H, 4.70; N 13.07; Found: C, 67.32; H, 4.75; N, 13.05; ^{1}H-NMR (400MHz, $CDCl_3$) δ ppm: 2.67 (s, 3H, CH_3), 6.85 (s, 1H, Th-CH), 7.41-7.49 (m, 6H, ArH), 7.74 -7.9 (m, 4H, ArH), 8.95 (s, 1H, CH=N).

***N*-(5-acetyl-4-methylthiazol-2-yl)-*N'*-benzylideneacetohydrazide 2b** Yellow crystals; yield 2.26 g, 76%; m.p. 134-135 °C; MS (EI) *m*/*z*: 301 (M^+); 258; 224; 197; 104; 77; 43 (100%); ^{1}H-NMR (400MHz, $CDCl_3$) δ ppm: 2.26 (s, 3H, CH_3), 2.56 (s, 3H, CH_3), 2.68 (s, 3H, CH_3), 7.48-7.54 (m, 3H, ArH), 7.82 (d, ^{3}J=8.2 Hz, 2H), 9.19 (s, 1H, CH=N). Calcd. for: $C_{15}H_{15}N_3O_2S$, C, 59.78; H, 5.02; N 13.94; Found: C, 60.32; H, 5.10; N, 13.96;

***N*-(5-acetyl-4-methylthiazol-2-yl)-*N'*-(2,4-dichlorobenzylidene)acetohydrazide 2c**

White-yellow crystals; yield 2.69 g, 73%, m.p. 207 °C, MS (EI) *m*/*z*: 369/371 (M^+\M^{+2}); 327; 292; 198; 183; 156; 141; 71; 43 (100%); ^{1}H-NMR (400MHz, $CDCl_3$) δ ppm: 2.44 (s, 3H, CH_3), 2.61(s, 3H, CH_3), 2.75 (s, 3H,

CH_3), 7.52 (d, ^3J=8.1 Hz, 1H, ArH), 7.71 (s, 1H, ArH), 7.98 (d, ^3J=8.1 Hz, 1H, ArH), 9.24 (s, 1H, CH=N). Calcd. for: $C_{15}H_{13}Cl_2N_3O_2S$, C, 48.66; H, 3.54; N 11.35; Found: C, 48.72; H, 3.61; N, 11.56;

***N'*-(4-hydroxybenzylidene)-*N*-(4-methylthiazol-2-yl)acetohydrazide 2d**: White crystals; yield 1.96 g, 72%, m.p. 105-106 °C, MS (EI) *m*/*z*: 275 (M^+); 233; 156; 114; 106; 43 (100%); ^{1}H-NMR (400MHz, $CDCl_3$) δ ppm: 2.24 (s, 3H, CH_3), 2.68 (s, 3H, CH_3), 4.96 (bb, 1H, OH), 6.33 (s, 1H, Th-CH), 6.92 (d, 2H, ^3J = 8.1 Hz), 7.58 (d, 2H, ^3J = 8.1 Hz), 7.97 (s, 1H, CH=N); Calcd. for: $C_{13}H_{13}N_3O_2S$, C, 56.71; H, 4.76; N 15.26; Found: C, 56.82; H, 4.83; N, 15.28;

Antimicrobial test

Agar well diffusion method

The in vitro antimicrobial activity of **1a-d** and **2a-d** was conducted by the routine agar well-diffusion method, similarly to the procedure used in disk-diffusion method according to the Clinical and Laboratory Standards Institute (CLSI) CLSI-M02-A10 (2009) as described by Markey et co.,[20] with some modification depending on the tested products.

In this study, a total of 20 bacterial strains collected from patients admitted to Emergency Clinical Hospital Cluj County were isolated from human venous leg ulcers secretions samples as follows: ten strains of *Staphylococus aureus*, two strains of *Staphylococus epidermidis*, two strains of *Trueperella pyogenes*, three strains of *Bacillus licheniformis*, one strain of *Pediococcus pentosaceus*, one strain of *Enterococcus faecium* and one strain of *Pseudomonas aeruginosa* strains. Characterization of the pathogens was based on the classical phenotype: morphological, cultural and biochemical methods and for identification an automated microbiology system VITEK 2 compact, bioMérieux was used.

The bacterial strains were inoculated separately on nutrient agar plate (Merck, Germany) and incubated at 37±2^{0}C for 24 hours.

Than a standardized inoculum of the tested microorganism with an optical density adjusted to a 0.5 McFarland turbidity standard (approximately 10^6UFC/ml) in a sterile saline solution were prepared. The Mueller Hinton agar plates (Merck, Germany) were than inoculated by spreading a volume of 500 µl the microbial suspension over the entire agar surface. After the plates dried at 35°C for 15-20 minutes seven radially hole with a diameter of 5 mm were punched aseptically and a volume of 25 µL of the synthetic compounds solution in DMSO at 100 mM concentration were disposed into each well. As a negative and positive control DMSO (Dimethyl Sulfoxide) and Amoxicillin 30 µg/ml

were used. The Petri dishes were incubated in an aerobic atmosphere at 37±2^{0}C for 48 hours. All the procedures were carried out in duplicates, then the diameter of the inhibition zones (in mm) was measured with electronic caliper with digital screen.

The rights of the patients regarding the confidentiality of personal information were respected in agreement to Helsinki declaration of Ethical Principles for Medical Research Involving Human Subjects.

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