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Synthesis and Antimicrobial Activity of (3-Formyl-4-hydroxybenzyl)triphenylphosphonium Chloride Acylhydrazones

M. R. Milenković[a], V. Živković-Radovanović[a], and L. Andjelković[b,*]

[a] *Faculty of Chemistry, University of Belgrade, Belgrade, 11000 Serbia*
[b] *University of Belgrade, Institute of Chemistry, Technology and Metallurgy, Department of Chemistry, Belgrade, 11000 Serbia*
**e-mail: ljubica@chem.bg.ac.rs*

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Abstract—In this study, four novel quaternary phosphonium acylhydrazones, derivatives of (3-formyl-4-hydroxybenzyl)triphenylphosphonium chloride, have been synthesized and their structures elucidated from IR and NMR spectra, and elemental analysis. All synthesized compounds have been tested for their antimicrobial activity, and acylhydrazones have demonstrated selective activity against Gram-positive bacteria strains.

Keywords: delocalized lipophilic cations, hydrazones, antimicrobial activity, triphenylphosphonium ions

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INTRODUCTION

Rapidly developed microbial resistance to different commonly used antimicrobial drugs became an increasing public health threat [1–3]. For instance, Gram-positive bacterial pathogen, *Staphylococcus aureus*, responsible for community-acquired and hospital-associated infections, has an extraordinary ability to develop resistance to the antibiotics it has been exposed [4, 5]. Therefore, due to occurrence of new pathogens and growing antibiotic resistance, intensive search for new therapeutic antibiotics' alternatives is of high priority.

Compounds containing delocalized lipophilic cations, such as quaternary ammonium and phosphonium ions, have been widely used as antiseptics and disinfectants [6–13]. Recently developed mitochondria-targeted antioxidants, conjugates of ubiquinone (MitoQ) and plastoquinone (SkQ1) with triphenylphosphonium moiety, exhibited the pronounced antimicrobial activity against strains of *Bacillus subtilis* [14–17]. So far Gram-positive bacteria have not developed resistance to SkQ1 and alkyl-triphenylphosphonium compounds.

Hydrazones make an important class of compounds used in development of new drugs due to their wide range of biological activities such as antimicrobial, anticonvulsant, analgesic, anti-inflammatory, antiplatelet, antitubercular, and antitumor [18, 19]. Due to excellent antimicrobial activity of delocalized lipophilic cations, their conjugates with acylhydrazones could lead to new efficient antimicrobial drugs.

Herein, we present triphenylphosphonium-containing acylhydrazone derivatives that could act as alternative antibacterial agents. No reports on antimicrobial activity of such compounds have been found in literature. Therefore, this paper presents the preliminary study of synthesis, characterization and antimicrobial activity of cationic triphenylphosphonium-containing acylhydrazones.

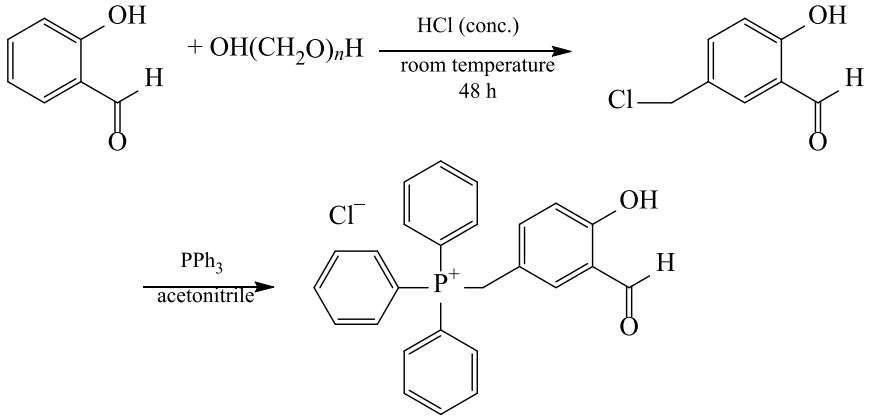
RESULTS AND DISCUSSION

(3-Formyl-4-hydroxybenzyl)triphenylphosphonium chloride was obtained in the reaction of triphenylphosphine with 5-chloromethyl salicylaldehyde according to the previously reported procedure (Scheme 1) [20].

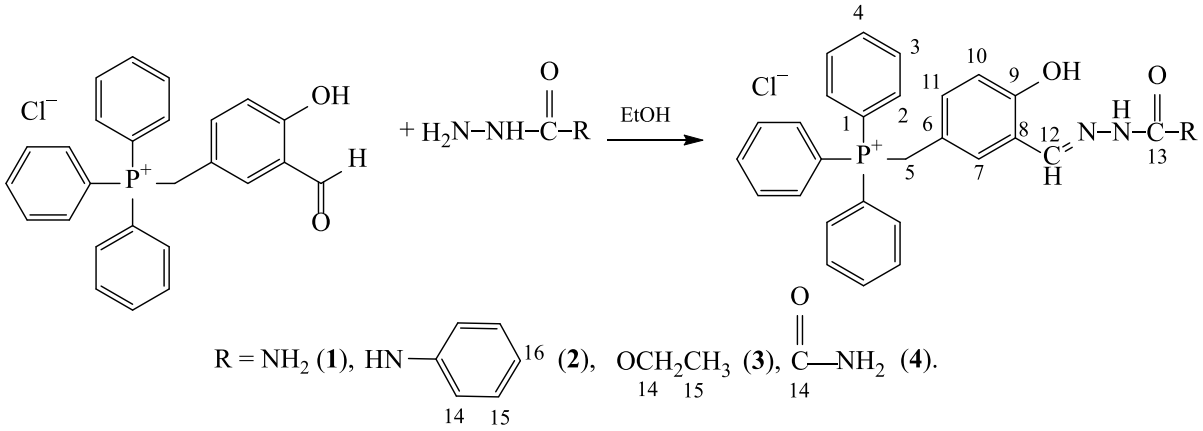
Acylhydrazones of (3-formyl-4-hydroxybenzyl)-triphenylphosphonium chloride were synthesized in the reaction of (3-formyl-4-hydroxybenzyl)triphenylphosphonium chloride with semicarbazide hydrochloride, 4-phenylsemicarbazide hydrochloride, ethyl carbazate, and oxamic acid hydrazide (Scheme 2).

In IR spectra of the products **1**–**4** (Table 1) the characteristic bands assigned to vibrations of the carbonyl, azomethine, phenol groups, and C–P bond of triphenylphosphonium were recorded. In ^{1}H NMR spectra of the products **1**–**4** the signal of aldehyde proton at 10.14 ppm was not recorded [20]. Instead, signal of the azomethine group proton was observed in the spectra

Scheme 1. Synthesis of (3-formyl-4-hydroxybenzyl)triphenylphosphonium chloride.



Scheme 2. Synthesis of (3-formyl-4-hydroxybenzyl)triphenylphosphonium chloride acylhydrazones **1**–**4**.



of compounds **1**–**4**. ^{13}C NMR spectra of compounds **1**–**4** also confirmed their structures.

Antimicrobial activity. In applied concentration of 1×10^{-3} mol/L, the tested compounds were not active against *Candida albicans* and Gram-negative *Escherichia coli*. Sensitivity of tested bacterial strains was different (Table 2) and depended mostly on the bacterial wall structure. All investigated compounds were active against Gram-positive bacteria, particularly *Micrococcus lysodeikticus*. The compound **3** demonstrated the highest antimicrobial activity probably due to more active hydrolysis of the ester functional group under physiological conditions.

EXPERIMENTAL

The reagents were obtained from Sigma-Aldrich and Acros Organics. Melting points were determined on an Electrothermal Melting Point Apparatus and are uncorrected. IR spectra were recorded on a Nicolet 6700 FT-IR spectrophotometer using the ATR technique. ^{1}H

Table 1. Characteristic IR spectral bands recorded for acylhydrazones **1**–**4**

Band	ν, cm^{-1}			
	1	**2**	**3**	**4**
C=O	1677	1709	1718	1680 and 1719
C=N	1590	1616	1617	1608
$C–O_{(phenol)}$	1273	1279	1284	1278
C–P	1438	1440	1439	1441
O–H	3569	3433	3565	3190 [overlapped with $\nu(N–H)_{amide}$]
$N–H_{(amide)}$	3400, 3185	3195	–	3372, 3190
$N–H_{(hydrazide)}$	3297	3249	3260	3228
$C–O_{(ester)}$	–	–	1251	–

Table 2. Antimicrobial activity of compounds **1–4**

Compound no.	Zone of inhibition, mm		
	Staphylococcus aureus	*Bacillus subtilis*	*Micrococcus lysodeikticus*
1	12.0	11.0	20.0
2	21.0	22.0	21.0
3	25.0	20.5	35.0
4	19.0	16.5	24.0

and ^{13}C, and 2D NMR (COSY, HSQC) spectra were measured on a Bruker Avance 500 spectrometer at room temperature using DMSO-d_6 as a solvent and TMS as an internal standard. Elemental analyses were performed on an ELEMENTARVario ELIII CHNSO analyzer.

General procedure for synthesis of (3-formyl-4-hydroxybenzyl)triphenylphosphonium chloride acylhydrazones 1–4. (3-Formyl-4-hydroxybenzyl)-triphenylphosphonium chloride (0.5 mmol) and the appropriate acylhydrazide (semicarbazide hydrochloride, 4-phenylsemicarbazide hydrochloride, ethyl carbazate, or oxamic acid hydrazide) (0.5 mmol) were dissolved in 25 mL of ethanol. In case of oxamic acid hydrazide and ethyl carbazate the reaction mixtures were acidified by adding one drop of conc. HCl. The reaction mixture was refluxed for 3 h then stored for few days at room temperature to allow the solvent to evaporate. Thus obtained precipitate of the corresponding product was filtered off. NMR spectra of compounds **1**–**4** were measured without further purification.

(*E*)-{3-[(2-Carbamoylhydrazono)methyl]-4-hydroxybenzyl}triphenylphosphonium chloride (1). White solid, yield 71%, mp 252–254°C. ^1H NMR spectrum, δ, ppm (numbering of atoms according to Scheme 2): 4.95–4.98 d (2H, J = 15.00 Hz, C^5H), 6.14 br. s (2H, NH_2), 6.71–6.73 d (1H, J = 10.00 Hz, $C^{10}H$), 6.79–6.81 d (1H, J = 10.00 Hz, $C^{11}H$), 7.24 s (1H, C^7H), 7.63–7.67 m (6H, C^2H), 7.69–7.73 m (6H, C^3H), 7.85–7.88 m (3H, C^4H), 7.97 s (1H, $C^{12}H$), 10.26 s (1H, OH), 10.40 s (1H, $NH_{hydrazide}$). ^{13}C NMR spectrum, δ_C, ppm (numbering of atoms according to Scheme 2): 26.97–27.34 d (J_{PC} = 46.25 Hz, C^5), 116.01 s (C^{10}), 117.08–117.76 d (J_{PC} = 85.00 Hz, C^1), 117.31–117.38 d (J_{PC} = 8.75 Hz, C^6), 120.56 s (C^8), 127.85 s (C^7), 129.63–129.73 d (J_{PC} = 12.50 Hz, C^3), 132.25 s (C^{11}), 133.54–133.62 d (J_{PC} = 10.00 Hz, C^2), 134.72 s (C^4), 135.75 s (C^{12}), 155.33 s (C^9), 156.10 s (C^{13}). Found, %: C 66.21; H 5.23; N 8.56. $C_{27}H_{25}ClN_3O_2P$. Calculated, %: C 66.19; H 5.14; N 8.58. M 490.

(*E*)-(4-Hydroxy-3-{[2-(phenylcarbamoyl)hydrazono]methyl}benzyl)triphenylphosphonium chloride (2). Light brown crystals, yield 75%, mp 224–226°C. ^1H NMR spectrum, δ, ppm: 4.86–4.89 d (2H, J = 15.00 Hz, C^5H), 6.61–6.62 d (1H, J = 5.00 Hz, $C^{10}H$), 6.67–6.70 d (1H, J = 15.00 Hz, $C^{11}H$), 6.92–6.95 t (1H, J = 5.00 Hz, $C^{17}H$), 7.20–7.23 t (2H, J = 5.00 Hz, $C^{16}H$), 7.29 s (1H, C^7H), 7.43–7.44 d (2H, J = 5.00 Hz, $C^{15}H$), 7.53–7.60 m (12H, C^2H, C^3H), 7.69–7.72 m (3H, C^4H), 7.96 s (1H, NH), 8.40 s (1H, $C^{12}H$), 10.47 s (1H, OH), 10.59 s (1H, $NH_{hydrazide}$). ^{13}C NMR spectrum, δ_C, ppm: 27.92–28.30 d (J_{PC} = 47.50 Hz, C^5), 116.97 s (C^{10}), 117.95–118.63 d (J_{PC} = 85.00 Hz, C^1), 118.19–118.25 d (J_{PC} = 7.50 Hz, C^6), 119.97 s (C^8), 121.03 s (C^{17}), 123.38 s (C^{16}), 129.35 s (C^{15}), 129.96 s (C^7), 130.57–130.66 d (J_{PC} = 11.25 Hz, C^3), 133.33 s (C^{11}), 134.47–134.55 d (J_{PC} = 10.00 Hz, C^2), 135.63 s (C^4), 138.33 s (C^{12}), 139.17 s (C^{14}), 153.24 s (C^9), 156.49 s (C^{13}). Found, %: C 70.11; H 5.32; N 7.40. $C_{33}H_{29}ClN_3O_2P$. Calculated, %: C 70.02; H 5.16; N 7.42. M 566.

(*E*)-(3-{[2-(Ethoxycarbonyl)hydrazono]methyl}-4-hydroxybenzyl)triphenylphosphonium chloride (3). White crystals, yield 62%, mp 252–254°C. ^1H NMR spectrum, δ, ppm: 1.19–1.22 t (3H, J = 10.00 Hz, $C^{15}H$), 4.10–4.14 q (2H, J = 10.00 Hz, $C^{14}H$), 4.97–4.99 d (2H, J = 10.00 Hz, C^5H), 6.70–6.72 d (1H, J = 10.00 Hz, $C^{10}H$), 6.78–6.80 d (1H, J = 10.00 Hz, $C^{11}H$), 7.04 s (1H, C^7H), 7.60–7.65 m (6H, C^2H), 7.69–7.73 m (6H, C^3H),7.85–7.88 m (3H, C^4H), 7.98 s (1H, $C^{12}H$), 10.94 s (1H, OH), 11.23 br. s (1H, $NH_{hydrazide}$). ^{13}C NMR spectrum, δ_C, ppm: 14.85 s (C^{15}), 27.65–28.03 d (J_{PC} = 47.50 Hz, C^5), 61.39 s (C^{14}), 117.04 s (C^{10}), 117.74–118.41 d (J_{PC} = 83.75 Hz, C^1), 118.29–118.37 d (J_{PC} = 10.00 Hz, C^6), 119.90 s (C^8), 130.49–130.59 d (J_{PC} = 12.50 Hz, C^3), 131.04 s (C^7), 133.25 s (C^{11}), 134.26–134.34 d (J_{PC} = 10.00 Hz, C^2), 135.55 s (C^4), 143.15 s (C^{12}), 153.78 s (C^9), 156.87 s (C^{13}). Found, %: C 67.13; H 5.47; N 5.41. $C_{29}H_{28}ClN_2O_3P$. Calculated, %: C 67.12; H 5.44; N 5.40. M 519.

(*E*)-(3-{[2-(2-Amino-2-oxoacetyl)hydrazono]-methyl}-4-hydroxybenzyl)triphenylphosphonium chloride (4). White solid, yield 69%, mp 258–260°C. ^1H

NMR spectrum, δ, ppm: 5.13–5.17 d (2H, J = 20.00 Hz, C^5H), 6.86 s (2H, $C^{10}H$, $C^{11}H$), 7.17 s (1H, C^7H), 7.63–7.68 m (6H, C^3H), 7.68–7.73 m (6H, C^2H), 7.84–7.88 m (3H, C^4H), 7.97 s (1H, NH_2), 8.25 s (1H, NH_2), 8.54 s (1H, $C^{12}H$), 11.12 s (1H, OH), 12.24 s (1H, $NH_{hydrazide}$). ^{13}C NMR spectrum, δ_C, ppm: 27.66–28.12 d (J_{PC} = 57.50 Hz, C^5), 117.87–118.72 d (J = 106.25 Hz, C^1), 118.46–118.55 d (J = 11.25 Hz, C^6), 131.31–131.37 d (J_{PC} = 7.50 Hz, C^7), 119.79–119.82 d (J_{PC} = 3.75 Hz, C^8), 117.33–117.35 d (J_{PC} = 2.50 Hz, C^{10}), 130.44–130.57 d (J = 16.25 Hz, C^3), 134.03–134.08 d (J_{PC} = 6.25 Hz, C^{11}), 134.45–134.55 d (J = 12.50 Hz, C^2), 135.48 s (C^4),149.03 s (C^{12}), 157.01 s (C^9), 157.80 s (C^{14}), 162.02 s (C^{13}). Found, %: C 65.02; H 4.95; N 8.10. $C_{28}H_{25}ClN_3O_3P$. Calculated, %: C 64.93; H 4.87; N 8.11. M 518.

Antimicrobial activity. Antimicrobial tests were performed against one standard fungal and four bacterial strains using agar well diffusion assay. Bacteria, Gram-negative *Escherichia coli* ATCC 25922 and Gram-positive *Staphylococcus aureus* ATCC 6853, *Bacillus subtilis* ATCC 6633, and *Micrococcus lysodeikticus* ATCC 4698, were cultivated on nutrient agar slants of LAB 8 and yeast *Candida albicans* ATCC 24433 on a maltose agar slant of LAB 37 (Lab M, Bury, United Kingdom), respectively. The bacteria were thermostated at 37°C for 24 h, while *Candida albicans* was thermostated at 28°C for 48 h. Each grown culture was suspended in 5 mL of sterile physiological solution (8 g NaCl/L) and 0.5 mL of the suspension was carefully mixed with 10 mL of the cooled molten agar medium in a Petri dish. In the solidified inoculated agar plates, the diameter 10 mm holes were made with a sterile cork borer and 100 μL aliquots of the investigated solutions were introduced therein. After three hours at room temperature (to permit diffusion before intensive microbial growth), the agar plates were thermostated as described. The obtained zones of inhibition (bactericidal and bacteriostatic) were measured. Equimolar stock solutions of the investigated compounds (1×10^{-2} mol/L) were prepared in DMSO and before the application were diluted 10 times with sterile deionized water.

CONCLUSIONS

A simple and easily processed condensation reaction of (3-formyl-4-hydroxybenzyl)triphenylphosphonium chloride with acylhydrazides (semicarbazide hydrochloride, 4-phenylsemicarbazide hydrochloride, ethyl carbazate, and oxamic acid hydrazide) has led to formation of the corresponding quaternary triphenylphosphonium hydrazones in high yields. All synthesized compounds have been characterized by supporting each other IR, and ^1H and ^{13}C NMR spectra. The products have demonstrated selective activity against Gram-positive bacteria, especially on *Micrococcus lysodeikticus*, and (*E*)-{3-[{2-(ethoxycarbonyl)hydrazono}methyl]-4-hydroxybenzyl}triphenylphosphonium chloride containing ester functional group in hydrazide fragment of the molecule has been determined as the most active one. The present study is the first report of antimicrobial activity of (3-formyl-4-hydroxybenzyl)triphenylphosphonium hydrazones which provides useful information for design of new triphenylphosphonium derivatives with the desired biological properties. The studied triphenylphosphonium acylhydrazones can be considered as the promising ligands for synthesis of potential biologically active metal complexes.

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CONFLICT OF INTEREST

No conflict of interest was declared by the authors.

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