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Microwave assisted synthesis and antimicrobial evaluation of phosphonohydrazone derivatives

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Rapid and efficient solvent-free one-pot synthesis of dialkylamino alkyl-1-(4-bromobenzylidene) phosphonohydrazone derivatives by the condensation reaction of *N, N*-dialkylamino alkylphosphorohydrazides with p-bromobenzaldehyde using silica under microwave irradiation is described. The structural features of the synthesized compounds were characterized by IR, ^{1}H-NMR, ^{13}C-NMR, mass spectroscopy and elemental analysis. The newly synthesized compounds were screened for antimicrobial activity against two Gram-positive strains (*Staphylococcus aureus* and *Bacillus sublitis)* and two Gram-negative strains (*Escherichia coli* and *Pseudomonas aeruginosa)*. The results showed that some of these compounds were active against all tested bacteria.

Keywords: Antimicrobial activity, Microwave irradiation, Phosphonohydrazones, Solvent-free conditions.

INTRODUCTION

Infectious diseases are one of the leading causes of death worldwide. During the past few decades, new infectious diseases have appeared and old ones previously thought to be controlled have reemerged [1] and thus, despite of many significant developments in the antimicrobial therapy, many problems remain to be solved for most of antimicrobial drugs available [2]. Hence, discovery of novel antimicrobial agents with better pharmacological profile is still highly desirable. Hydrazones have drug and pharmaceutical properties. These compounds are widely used as anti-inflammatory [3], anticancer [4], analgesic [5], anticonvulsant [6], antituberculous [7], antiproliferative [8], antitumor [9,10], anti-HIV [11], antimycobacterial [12] and antimicrobial agents [13]. Moreover, hydrazones are used to synthesize indoles [14], 4-thiazolidin-4-ones [3], azetidines [15]. Generally, these compounds are synthesized by the condensation reaction of substituted hydrazines/hydrazides with aldehydes and ketones in organic solvents [3]. These are also synthesized by the reaction of hydrazide and carbonyl compounds in presence of polystyrene sulfonic acid in aqueous medium using microwaves [16], only microwaves [17], acidic alumina [18], ultrasound irradiation in aqueous medium [19]. There are reports for the synthesis of phosphonohydrazones, which have several drawbacks such as use of carcinogenic solvents, long reaction time and formation of several by-products. Therefore, we have reported a new method for the preparation of phosphonohydrazones. It was found that silica supported microwave irradiation technique is capable of producing high yields of phosphonohydrazones by condensation of phosphonohydrazides with aromatic aldehydes under mild conditions. The method has advantages, such as ease of execution and work-up, fast rate of reactions, high yields, solvent-free reaction conditions and low cost. Chemically synthesized compounds like hydrazones could be prospectful for treating bacterial infections. Therefore, these compounds were synthesized and evaluated against two Gram-positive strains (*Staphylococcus aureus* and *Bacillus sublitis)* and two Gram-negative strains (*Escherichia coli* and *Pseudomonas aeruginosa).*

EXPERIMENTAL

All chemicals and solvents were obtained from E. Merck (Darmstadt, Germany), and were used without further purification. All melting points were taken with an Electrothermal melting point apparatus (Electrothermal Eng. Ltd, Essex, UK) and were uncorrected. IR spectra were recorded in KBr on a Shimadzu Dr-8031 instrument. ^{1}H- and ^{13}C-NMR spectra of the synthesized compounds were measured in a $CDCl_3$ solution and TMS as the internal standard using a Varian Mercury 400 instrument at 400 and 75 MHz, respectively. All chemical shifts were reported as δ (ppm) values.

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The mass spectra were recorded on a LCQ ion trap mass spectrometer (Thermo Fisher. San Jose, CA, USA), equipped with an EI source. Elemental analyses were carried out using a Perkin-Elmer, CHN elemental analyzer model 2400 and were within ± 0.4% of the theoretical values. The purity of the newly synthesized compounds was checked by TLC on plates (Merck) and spots were visualized by exposing the dry plates to iodine vapor.

General procedure for the preparation of the compounds 2(a-e):

To phosphonohydrazides (0.01M) taken in a conical flask, ground silica gel (1 g) and p-bromobenzaldehyde (0.01M) were added and mixed well. The mixture was subjected to microwave irradiation at 180 W for the appropriate time (Table 1). After complete conversion, as indicated by TLC, the mixture was extracted with petroleum ether (3×50 ml) and washed with water (3×50 ml). After the disappearance of the phosphonohydrazide spot on the TLC, the solvent was evaporated in vacuum and the product was purified by column chromatography.

N,N-dibutyl amino isopropyl-1-(4-bromobenzylidene) phosphonohydrazone (**2a**)

IR (KBr, cm^{-1}): 3366(NH), 2988(C_6H_5), 2892(C-H), 1621(C=N), 1556(C=C), 1451(C-N), 1233(P=O), 1173(P-N-N), 1089,1152 (P-N-C), 811 (C-Cl), 692 (P-C); ^{1}H-NMR ($CDCl_3$): δ 0.85(t, J = 12.75 Hz, 6H, CH_3), 1.05 (dd, J = 7.51 Hz, 6H, CH_3), 1.11 (dd, J = 7.51 Hz, 6H, CH_3), 1.26 (m, J = 8.31 Hz, 4H, CH_2), 1.56 (m, J = 8.31 Hz, 4H,CH_2), 2.41 (m, J^{P-H} = 20.61 Hz,1H, CH), 3.06 (m, J = 8.51 Hz, 4H ,CH_2), 6.91 (d, J^{P-H} = 23.41 Hz, 1H ,NH), 7.51 (d, 2H, Ar-H), 7.58(d, 2H, Ar-H), 8.41 (m, 1H, CH); ^{13}C-NMR ($CDCl_3$): δ 11.50 (CH_3), 11.5 (CH_3), 14.1 (CH_3), 19.39 (CH_2), 21.84 (CH), 33.71 (CH_2), 125.4 (C-Br), 130-132 (Ar-C), 154.70 (C=N-NH); MS(m/z): 417 ($M+H^+$). Anal. Calcd for $C_{18}H_{31}BrN_3OP$: C, 51.94; H, 7.45; N, 10.10. Found: C, 51.97; H, 7.42; N, 10.10.

N,N-dibutyl amino phenyl-1-(4-bromobenzylidene) phosphonohydrazone **(2b**)

IR (KBr, cm^{-1}): 3340(NH), 2979 (C_6H_5), 2908 (C-H), 1617 (C=N), 1561 (C=C), 1458 (C-N), 1247 (P=O), 1181 (P-N-N), 1086, 1160 (P-N-C), 822 (C-Cl), 704(P-C); ^{1}H-NMR ($CDCl_3$): δ 0.92 (t,*J*= 8.66 Hz, 6H,CH_3),1.16 (m, *J*=7.46 Hz,4H, CH_2),1.56(m, *J*=7.46 Hz, 4H, CH_2), 2.75(m, *J*=8.16 Hz, 4H, CH_2), 7.10(d, J^{P-H} =23.46 Hz, 1H, NH), 7.26-7.86(m, *J*=7.64 Hz,9H, Ar-H), 8.55 (s, 1H,N=CH); ^{13}CNMR ($CDCl_3$) :δ 10.26 (CH_3), 14.53 (CH_2), 21.45 (CH_3), 31.21 (CH_2), 42.44 (CH_2), 125.4 (C-Br), 128-132 (Ar-C), 154.76 (C=N-NH); MS (m/z): 451($M+H^+$). Anal. Calcd. For $C_{21}H_{29}BrN_3OP$: C, 56.05; H, 6.44; N, 9.33. Found: C, 56.10; H, 6.44; N 9.35.

N,N-diisopropyl amino isopropyl-1-(4-bromobenzylidene) phosphonohydrazone **(2c)**

IR (KBr, cm^{-1}): 3366 (NH), 3010 (C_6H_5), 2909 (C-H), 1615 (C=N), 1535(C=C), 1432(C-N), 1246(P=O), 1175(P-N-N), 1083, 1151 (P-N-C), 815 (C-Cl), 692 (P-C); ^{1}H-NMR ($CDCl_3$):δ0.94 (t, J = 9.79 Hz, 6H, CH_3), 1.06 (dd, J = 7.56 Hz, 6H, CH_3), 1.12 (dd, J= 8.32 Hz,6H,CH_3), 1.33 (m, J=9.43 Hz,4H,CH_2), 2.36 (m, J^{P-H}=22.76 Hz,1H,CH), 3.06 (m, J = 8.36 Hz, 4H, CH_2), 6.35 (d, J^{P-H}=19.65 Hz, 1H ,NH), 7,79 (d, 2H, Ar-H), 7,88(d, 2H, Ar-H), 8.41(m, 1H, CH); ^{13}C-NMR ($CDCl_3$): δ 10.14 (CH_3), 15.62 (CH_3), 16.16 (CH_3), 21.25 (CH_2), 31.05 (CH), 42.54 (CH_2), 125.4 (C-Br), 127-130 (Ar-C), 154.78 (C=N-NH); MS (m/z): 389 ($M+H^+$). Anal. Calcd for $C_{16}H_{27}BrN_3OP$: C, 49.50; H, 6.95; N, 10.82. Found: C, 49.51; H, 6.93; N, 10.80.

N,N-diisopropyl amino phenyl-1-(4-bromobenzylidene) phosphonohydrazone **(2d)**

IR (KBr, cm^{-1}): 3388 (NH), 2955 (C_6H_5), 2880 (C-H), 1614 (C=N), 1546 (C=C), 1454 (C-N), 1237 (P=O), 1175 (P-N-N), 1070, 1154 (P-N-C),809 (C-Cl), 694 (P-C), ^{1}H-NMR ($CDCl_3$): δ 0.82 (t, *J*=7.53 Hz,6H,CH_3), 1.46 (m, *J*=7.46 Hz, 4H,CH_2), 2.73 (m, *J*=7.43Hz, 4H, CH_2), 6.53 (d, J^{P-H} =23.23, 1H,NH), 7.30-7.66 (m, *J*=6.95Hz, 9H, Ar-H), 8.54 (s,1H,CH); ^{13}C-NMR ($CDCl_3$): δ 10.46 (CH_3), 22.35 (CH_2), 46.23(CH_2), 125.5 (C-Br), 126-133 (Ar-C), 154.71 (C=N-NH); MS (m/z): 423 ($M+H^+$). Anal. Calcd for $C_{19}H_{25}BrN_3OP$: C, 54.05; H, 5.92. N, 9.95. Found: C, 54.15; H, 5.95; N 9.98.

N,N-dibutyl amino isopropyl-1-(4-chlorobenzylidene) phosphonohydrazone **(2e)**

IR (KBr, cm^{-1}): 3352 (NH), 2980 (C_6H_5), 2901 (C-H), 1615(C=N), 1559(C=C), 1466(C-N), 1232(P=O), 1155 (P-N-N), 1078, 1154 (P-N-C), 816 (C-Cl), 721 (P-C); ^{1}H-NMR ($CDCl_3$): δ 0.71 (d, *J*=7.55 Hz, 3H, CH_3), 0.88(d, *J*=7.42 Hz, 6H, CH_3), 1.06 (dd, J=7.91 Hz, 6H, CH_3), 1.49 (m, *J*=7.95 Hz, 2H, CH), 2.38 (m, J^{P-H}=23.72 Hz,1H, CH), 2.80 (m, *J*=7.57 Hz, 4H, CH_2), 8.11 (d, =24.57 Hz, 1H, NH),7.34-7.84 (m,4H, Ar-H), 8.51 (s, 1H, N=CH);

^{13}C NMR ($CDCl_3$): δ 10.21 (CH_3), 14.76 (CH_3), 16.45 (CH_3), 16.66 (CH), 31.35 (CH_2), 33.10 (CH), 125.5 (C-Br), 128-133 (Ar-C), 154.76 (C=N-NH); MS(m/z): 417 ($M+H^+$). Anal. Calcd. For $C_{18}H_{31}BrN_3OP$: C, 51.94; H, 7.45; N, 10.10. Found: C, 51.96; H, 7.48; N, 10.10.

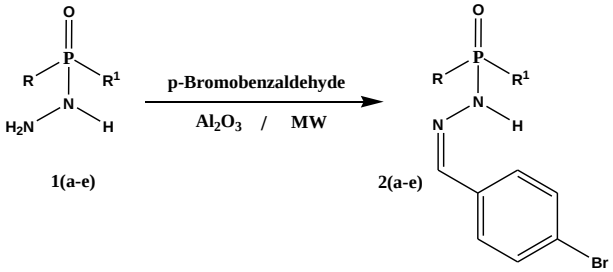
Screening for antibacterial activity

The antimicrobial activities were determined using the disc diffusion method [20] by measuring the zone of inhibition in mm. All newly synthesized compounds **2(a-e)** were screened *in vitro* for their antibacterial activity against two Gram-positive strains (*Staphylococcus aureus* and *Bacillus sublitis)* and two Gram-negative strains (*Escherichia coli* and *Pseudomonas aeruginosa)* at a concentration of 500 µg/ml. Ciprofloxin (10 µg/disc) was used as a standard drug for antibacterial screening. All synthesized compounds exhibited satisfactory antibacterial activities. Each experiment was done in triplicate and the average reading was taken.

RESULTS AND DISCUSSION

Chemistry

We now report the synthesis of *N,N*-dialkyl alkyl-1-(4-bromo benzylidene) phosphono-hydrazones **2(a-e)** from *N, N*-dialkylamino alkylphosphorohydrazides **1(a-e)** under MW and solvent-free conditions in short reaction times (Scheme). Initially, we prepared *N,N*-dialkylamino alkylphosphorohydrazides **1(a-e)** by a reported method [21]. In order to determine the optimum conditions for the synthesis of organophosphorus based hydrazone derivatives, variations in molar ratios of reagents, irradiation time and power level of the microwave set-up were investigated. After some experimentation, we found a set of conditions that generally provides products in good yields. In this regard, several reactions of *N,N*-dialkylamino alkylphosphorohydrazides with p-bromobenzaldehyde were performed under different conditions. These reactions were monitored by TLC. The synthesized compounds were identified on the basis of IR, ^{1}H-NMR, ^{13}C-NMR, mass spectra and elemental analysis.



Scheme. Preparation route of the compounds

Table 1. Physical data of the newly synthesized compounds 2(a-e)

Entry	R	R^1	Reaction Time (min)	m. p ºC	Yield (%)
2a	$^iC_3H_7$	$N(C_4H_9)_2$	4.0	144	77
2b	C_6H_5	$N(C_4H_9)_2$	5.0	165	80
2c	$^iC_3H_7$	$N(C_3H_7)_2$	4.0	137	85
2d	C_6H_5	$N(C_3H_7)_2$	5.0	162	75
2e	$^iC_3H_7$	$N(^iC_4H_9)_2$	5.0	160	76

Antimicrobial activity of compounds 2(a-e)

To check the biological activity of the compounds, the compounds **2(a-e)** were screened for *in vitro* antimicrobial activity against a variety of bacteria, two Gram- positive strains (*Staphylococcus aureus* and *Bacillus sublitis)* and two Gram-negative strains (*Escherichia coli* and *Pseudomonas aeruginosa)*. All compounds were assayed for antibacterial activity. The preliminary screening results for the compounds **2(a-e)** (Table 2) revealed that compound **2c** showed activity against all tested bacteria while **2e** was active against three bacteria (except *E. coli*). Compound **2a** and **2d** showed antimicrobial activity towards *S. aureus* and *B. subtilis* while compound **2b** showed slight activity against *S. aureus, B. subtilis.*

Table 2: Results for the antimicrobial activity of the tested compounds

Compound No.	Zone of inhibition in mm				
	Antibacterial activity				
	S. aureus		*B. subtilis*	*E. coli*	*P.aeruginosa*
2a	++	++	-		-
2b	+	+	-		-
2c	+++	+++	++		++
2d	++	++	-		-
2e	+++	++	-		+
Ciprofloxacin	+++	+++	+++		+++

Key to symbols: Highly active = +++ (inhibition zone > 12 mm); Moderately active = ++ (inhibition zone 9 – 12 mm); Slightly active = + (inhibition zone 6 – 9 mm); Inactive = - (inhibition zone < 6 mm)

CONCLUSION

In conclusion, we have synthesized a series of phosphonohydrazones **2(a-e)** by a rapid, efficient, solvent-free, one-pot procedure with excellent yields under microwave irradiation. The main advantage of this method is the clean and easy work-up. Among the synthesized compounds **2(a-e**), compound **2c** showed excellent activity against all tested bacteria. Biological evaluation of these derivatives may furnish some other important applications.

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МИКРОВЪЛНОВА СИНТЕЗА И АНТИМИКРОБНА АКТИВНОСТ НА ПРОИЗВОДНИ НА ФОСФОНОХИДРАЗОНА

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(Резюме)

Описана е бърза и ефективна синтеза без разтворител на диалкил-амино алкил-1-(4-брообензилиден) фосфохидразонови производни чрез кондензация *N,N*- диалкил-амино-алкил-фосфорохидразиди с р-бромобензалдехид в присъствие на силициев диоксид при микровълново облъчване. Структурата на синтезираните съединения а охарактеризирани чрез IR, ^{1}H-NMR, ^{13}C-NMR, мас-спектроскопия и елементен анализ. Новите съединения са скринирани за антимикробна активност срещи два Грам-положителни щама (*Staphylococcus aureus* и *Bacillus sublitis)* и два Грам-отрицателни щама (*Escherichia coli* и *Pseudomonas aeruginosa).* Резултатите показват, че някои от тези съединения са активни спрямо всички изпитани бактерии.