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Microwave Assisted Efficient One-pot Synthesis, Characterization of Organophosphorus Based Hydrazone Derivatives under Solvent-FreeConditions and Their Antimicrobial Activity

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Abstract: Rapid and efficient solvent-free one-pot synthesis of dialkylamino alkyl -1- (4-bromobenzylidene) phosphonohydrazone derivatives 2(a-e) by the condensation reaction of *N,N*-dialkylamino alkylphosphorohydrazides 1(a-e) with *p*-bromobenzaldehyde using silica under microwave irradiation is described. The one-pot synthesis on solid inorganic support provides the products in good yields. The structural features of the synthesized compounds were characterized by IR, ^{1}H-NMR, ^{13}C-NMR, Mass and elemental analysis. The newly synthesized compounds are screened for antimicrobial activity. The results showed that some of these compounds were showed activity against all the tested bacteria. Thus, microwave heating technology in drug synthesis should be promoted in order to reducing the cost of basic drugs and chemicals.

Key words: Antimicrobial activity · Microwave irradiation · Phosphonohydrazones · Silica · 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 remains 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 drugs and pharmaceuticals 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 activities [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 the presence of polystyrene sulfonic acid in aqueous medium using microwaves [16], only microwaves [17], acidic alumina [18], ultrasound irradiation in aqueous medium [19]. The syntheses of phosphorohydrazones were reported to have several drawbacks such as use of carcinogenic solvent, long reaction time and formation of several by-products. Therefore, we report here a new method for the preparation of phosphonohydrazones. Silica supported microwave irradiation technique was found capable to producing high yields of phosphonohydrazones by condensation of phosphonohydrazides with aromatic aldehydes under mild conditions. The method have advantages, such as ease of execution and work-ups, fast rate of reactions, higher yields, solvents-less reaction conditions and low cost. Chemically synthesized compounds such as hydrazones were used for treating bacterial infections. Therefore these compounds were synthesized under this aim and evaluated against two Gram- positive strains (*Staphylococcus aureus* and *Bacillus sublitis)* and two Gram-negative strains (*Escherichia coli* and *Pseudomonas aeruginosa).*

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On the other hand microwave assisted organic reactions have emerged as a new 'lead' in organic synthesis with important advantages like highly accelerated rate of reaction along with improvement in yield and quality of products [20]. Microwave assisted organic synthesis (MAOS) continues to affect synthetic chemistry significantly by enabling rapid, reproducible and scaleable chemistry development. The use of microwave irradiation is an established tool in organic synthesis for achieving better selectivity, rate enhancement and reduction of thermal degradation byproducts, thus keeping in view the advantages of these techniques and immense biological importance of phosphonohydrazones. Thus, it was felt worthwhile to study the reaction under microwave irradiation and to screen the target compounds for antimicrobial activities.

Experimental: All the chemicals and solvents were obtained from E-Merck (Darmstadt, Germany) and were used without further purification. All Melting points are uncorrected and were taken with an Electrothermal melting point apparatus (Electrothermal Eng. Ltd, Essex, UK). IR spectra were obtained on a Shimadzu Dr-8031 instrument. The ^{1}H- ^{13}C-NMR spectra of the synthesized compounds were measured in $CDCl_3$ 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. 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 in iodine vapor.

General Procedure for the Preparation of the Compounds 2(a-e): To phosphonohydrazides (0.01M) taken in conical flask, silica gel grounded (1gm) and *p*-bromobenzaldehyde (0.01M) were added and mixed well. The prepared mixture was subjected to microwave irradiation at 180 W at 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 phosphonohydrazides spot on the TLC, the solvent was evaporated in vacuum and the product was purified by column chromatography.

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

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

***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_3), 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 ($CDCl_3$): δ 0.94 (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);

Tble 2:Results of antimicrobial activity of the tested compounds.

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

Key to symbols [23]
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)
According to 23th Reference.

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.

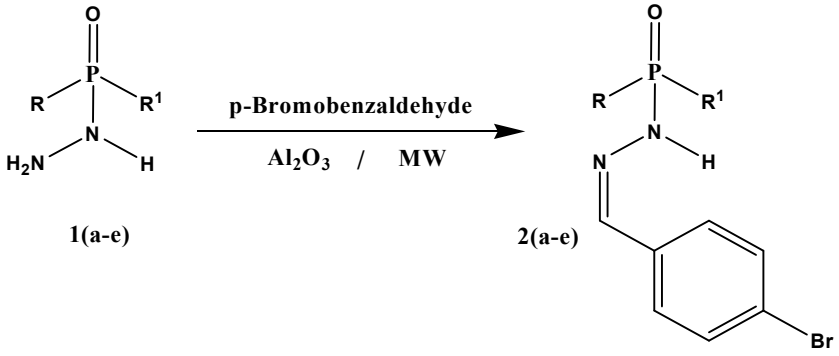
Screening for Antibacterial Activity: The antimicrobial activities were determined using disc diffusion method [21] by measuring the zone of inhibition in mm. All newly synthesized compounds i.e. 2(a-e) were screened *in vitro* for their antibacterial activity against two Gram- positive strains (*Staphylococcus aureus* and *Bacillus sublitis)* and two Gram-negative strains (*Escherichia coli* and *Pseudomonas aeruginosa)* at concentration of 500 µg/ml. Ciprofloxin (10 µg/disc) was used as a standard drug for antibacterial screening. All synthesized compounds exhibited sufficient antibacterial activities. Each experiment was done in triplicate and the average reading was taken. The results are tabulated in Table 2.

RESULTS AND DISCUSSION

Chemistry: We now report the synthesis of *N,N*-dialkyl alkyl-1-(4-bromo benzylidene) phosphonohydrazones 2(a-e) that were prepared from *N,N*-dialkylamino alkylphosphorohydrazides 1(a-e) under MW and solvent-free conditions in short reaction times (Scheme 1). Therefore, initially, we prepared *N,N*-dialkylamino alkylphosphorohydrazides 1(a-e) by reported method [22]. In order to determine the optimum conditions for the synthesis of Organophosphorus based hydrazone derivatives, variations in molar ratios of reagents and the irradiation time and power level of microwave set-up were investigated. After some experimentation, we found a set of conditions that generally provides products in good yield. 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.

Estimated Place of Scheme1
Estimated Place of Table 1

Antimicrobial Activity of Compounds 2(a-e): To check the biological activity of the compounds, the series of 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. From the data presented in Table 2, the preliminary screening results for the compounds 2(a-e) established that compound 2c inhibited strongly the growth of *S.aureus B.subtilis* and moderately *E.coli and P.aeruginosa* growth while 2e inhibited strongly, moderately and slightly the growth of *S.aureus, B.subtilis* and *P.aeruginosa* respectively. Compound 2a and 2d inhibited moderately while 2b slightly the growth of *S.aureus* and *B.subtilis.* Estimated place of TABLE 2



Scheme 1: Preparation rout of the compounds

CONCLUSION

In conclusion, we have synthesized a series phosphonohydrazones 2(a-e) using rapid, efficient and solvent-free, one-pot reaction with excellent yields under microwave irradiation. The main advantage of this method is that the reactions were found to be clean and had operational simplicity. Amongst the synthesized compounds evaluated 2(a-e), compound 2c was found to exhibit excellent activity against all the tested bacteria. Biological evaluation of these derivatives may furnish some other important applications that could be useful for the searching of new antimicrobial molecules from synthesis methods.

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