A Facile Microwave Assisted Synthesis, Characterization and Antibacterial Activity of 5-Phenyl-1,3,4-Oxadiazole Derivatives for Chemotherapeutic Use

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Abstract: This paper deals with an efficient synthesis of some 2-amino-5-substituted phenyl-1,3,4-oxadiazoles by using both conventional and microwave methods for chemotherapeutic use. The obtained results confirmed that, microwave assisted technique is efficient, eco-friendly, and inexpensive method which not only gives higher yield but also reduces the reaction time significantly. The structural elucidation of the prepared compounds was carried out by the aid of IR, ^{1}H NMR, UV-Vis and mass spectral analyses. Compounds **S_a-S_f** showed antimicrobial activities against *Escherichia coli*, *Serratia marcescens, Salmonella enterica* and *Proteus vulgaris* (Gram-negative bacteria) and *Bacillus subtilis* and *Staphylococcus aureus* (Gram-positive bacteria) at low concentrations (10–1000 µg/mL).

Keywords: Microwave assisted synthesis, oxadiazoles, characterization, antibacterial activity.

1. INTRODUCTION

In recent year a lot of work has been carried out to use microwave irradiation as an alternative to conventional heating which provide higher yield and cleaner products [1-4]. 1,3,4-Oxadiazole belongs to an important class of heterocyclic compounds possess a wide spectrum of pharmacological, medicinal and biological activities. 2-Amino-1,3,4-oxadiazoles play an important role as antibacterial [5-9], anti-inflammatory [10], anticonvulsant [11], CNS stimulant [12], antimicrobial [13], insecticidal [14], anticancer [15], antiviral [16], antiparkinsonian [17], antiproliferative [18] activities. They are also used for material research for preparation of organic light emitting diode and photoluminescence polymers [19]. We reported here the preparation of some 5-phenyl-1,3,4-oxadiazole derivatives using the microwave and conventional methods for chemotherapeutic use. UV-Vis, ^{1}H NMR, IR, Elemental analysis and mass spectra as well as the biological activity of the synthesized oxadiazoles were investigated and discussed in relation to their molecular structures.

2. EXPERIMENTAL

2.1. Materials and Methods

All the aldehyde semicarbazides and anhydrous sodium acetate of AR grade and E. Merck were used in all the reactions. Melting points were determined by open capillary method using the 'Tempo' melting point apparatus and are uncorrected. The purity and homogeneity of compounds as well as completion of reaction time was checked by thin layer chromatography (TLC) using silica gel-G as adsorbent and solvent system used benzene:chloroform:methanol (5:4:1). The spots were visualized by iodine vapours after irradiation with UV light. All the compounds were purified by preparative TLC/Column chromatography. IR spectra were recorded as in KBr discs on a Perkin–Elmer (model 1430) IR spectrometer (4000-400 cm^{-1}) at the Micro-analytical unit of Tanta University. ^{1}H NMR were measured on a Bruker DMX 750 (500 MHz) FT spectrometer using d_6 DMSO as a solvent. The chemical shifts were recorded in parts per million (ppm, δ units) relative to tetramethylsilane (TMS), and coupling constant was expressed in units of hertz (Hz). UV-Vis spectra were carried out using a Cary-400 double beam recording spectrophotometer within the wavelength range 190-700 nm at room temperature. Electron spray ionization (ESI) mass spectra were taken on a Shimadzu LCMS-2010 e.v mass spectrometer at the Gakushuin University (Japan).

2.2. Preparation of 1,3,4-oxadiazoles (S_a-S_f)

2.2.1. Conventional Method

To a stirred solution of substituted semicarbazone (0.01 mole), 0.02 mole of anhydrous sodium acetate in 25 ml glacial acetic acid, and a solution of bromine (0.7 ml in 5 ml glacial acetic acid) were added drop wise at room temperature. The solution was stirred for 30 minutes, and then added to ice cold water. The precipitated product was collected by filtration and washed with water. The product was dried and recrystallised from ethanol.

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2.2.2. Microwave Method

Substituted semicarbazone (0.01 mole) and sodium acetate (0.02 mole) were dissolved in 25 ml of glacial acetic acid. The reaction mixture was transferred to conical flask fitted with guard tube. A solution of bromine (0.7 ml in 5 ml glacial acetic acid) was added drop wise at room temperature. The reaction mixture was heated for 10-20 seconds at 480 watt microwave power. The progress of reaction was monitored by TLC (silica gel–G) using benzene:chloroform:methanol (5:4:1) as solvent system. The reaction mixture was cooled at room temperature and poured on crushed ice, the product was precipitate out, filtered, washed with water, dried and recrystallised from ethanol.

Compound S_a

UV-Vis (λ_{max}, nm): 206, 275, IR (KBr cm^{-1}): 974, 1027 (C-O-C oxadiazole ring), 1652 (C=N strech), 3116-3294 (-NH_2), 2782 (Ar-CH), 917 (Ar-H), ^{1}H NMR (DMSO-d_6, δ ppm): 7.50-7.80 (m, phenyl protons), 7.37 (s, 2H, NH_2). Anal. Calcd for $C_8H_7N_3O$: C, 59.62; H, 4.38; N, 26.07. Found: C, 59.28; H, 4.15; N, 26.13.

Compound S_b

UV-Vis (λ_{max}, nm): 207, 282, IR (KBr cm^{-1}): 966, 1044 (C-O-C oxadiazole ring), 1652 (C=N strech), 3110-3295 (-NH_2), 2768 (Ar-CH), 895 (Ar-H), ^{1}H NMR (DMSO-d_6, δ ppm): 7.66-7.87 (m, phenyl protons), 7.36 (s, 2H, NH_2). Anal. Calcd for $C_8H_6BrN_3O$: C, 40.03; H, 2.52; N, 17.50. Found: C, 40.12; H, 2.33; N, 17.24.

Compound S_c

UV-Vis (λ_{max}, nm): 213, 284, IR (KBr cm^{-1}): 931, 1048 (C-O-C oxadiazole ring), 1656 (C=N stretch),3116-3291 (-NH_2), 2784 (Ar-CH), 891 (Ar-H), ^{1}H NMR (DMSO-d_6, δ ppm): 7.73-7.92 (m, phenyl protons), 7.36 (s, 2H, NH_2). Anal. Calcd for $C_8H_6ClN_3O$: C, 49.12; H, 3.09; N, 21.48. Found: C, 49.03; H, 3.01; N, 21.30.

Compound S_d

UV-Vis (λ_{max}, nm): 206, 274, IR (KBr cm^{-1}): 971, 1027 (C-O-C oxadiazole ring), 1652 (C=N stretch), 3113-3295 (-NH_2), 2780 (Ar-CH), 917 (Ar-H), ^{1}H NMR (DMSO-d_6, δ ppm): 7.51-7.77 (m, phenyl protons), 7.26 (s, 2H, NH_2). Anal. Calcd for $C_8H_6FN_3O$: C, 53.63; H, 3.38; N, 23.46. Found: C, 53.41; H, 3.22; N, 23.15.

Compound S_e

UV-Vis (λ_{max}, nm): 208, 270, IR (KBr cm^{-1}): 928, 1047 (C-O-C oxadiazole ring), 1665 (C=N stretch), 3032-3412 (-NH_2), 2780 (Ar-CH), 890 (Ar-H), ^{1}H NMR (DMSO-d_6, δ ppm): 8.22-8.49 (m, phenyl protons), 7.43 (s, 2H, NH_2). Anal. Calcd for $C_8H_6N_4O_3$: C, 46.61; H, 2.93; N, 27.18. Found: C, 46.20; H, 2.52; N, 27.01.

Compound S_f

UV-Vis (λ_{max}, nm): 207, 281, IR (KBr cm^{-1}): 971, 1022 (C-O-C oxadiazole ring), 1658 (C=N stretch), 3130-3318 (-NH_2), 2769 (Ar-CH), 832 (Ar-H), ^{1}H NMR (DMSO-d_6, δ ppm): 7.09-7.71 (m, phenyl protons), 7.05 (s, 2H, NH_2), 3.79 (S, 3H, OCH_3). Anal. Calcd for $C_9H_9N_3O_2$: C, 56.54; H, 4.74; N, 21.98. Found: C, 56.28; H, 4.33; N, 21.65.

2.3. Antimicrobial Activities

Test Microorganisms

Gram-Negative Bacteria

After Gram-staining procedure, Gram-negative cells appear pink. The Gram-negative bacteria used in this study were *Escherichia coli*, *Serratia marcescens, Salmonella enterica* and *Proteus vulgaris*.

Gram-Positive Bacteria

The thick cell wall of a Gram-positive organism retains the crystal violet dye used in the Gram-staining procedure, so the stained cells appear purple under magnification. Gram-positive bacteria used in this study were *Bacillus subtilis* and *Staphylococcus aureus*. *B. subtilis* is mostly involved in Urinary infection, wound, ulceration and septicemia. *S. aureus* is the mild stone of Gram-positive bacteria and it is a causative agent of pneumonia, meningitis and food poisoning. The tested organisms were obtained from the culture collection of Bacteriology Unit, Department of Botany, Faculty of Science, Tanta University, Egypt.

Media Used and Antimicrobial Assay

Nutrient and Mannitol salt agar media were used for growing and maintaining the tested bacteria. The antimicrobial spectrum of the synthetic compounds was determined as powdered samples by the cut-plug method on plates seeded with the tested bacteria (*E. coli, S. marcescens, S. enterica P. vulgaris* and *B. subtilis*.) on nutrient agar (which contained per liter: peptone(3 g), beef extract (5 g), NaCl (5 g) and agar (20 g) at pH 7), but *S. aureus* was seeded on Mannitol salt agar medium (which contained per liter: enzymatic digest of casein (5 g), enzymatic digest of animal tissue (5 g), beef extract (1 g), D-mannitol (10 g), NaCl (75 g), phenol red (0.025 g) and agar (15 g) at pH: 7.4). After solidification, the wells were made using cork porer and

each was filled with powdery compounds (10 mg). The plates were then incubated at 37°C for 24 h, after which the diameters of the inhibition zones were measured. Compounds which produced the highest inhibition zones were selected and assayed further at different concentrations in suspensions to quantify their inhibitory effects. Nutrient and Mannitol salt were used in activation of organisms [20].

2.4. Determination of Minimum Inhibitory Concentrations (MICs)

The minimum inhibitory concentration (MIC) was determined by agar diffusion assay using filter paper disc method. The MICs were determined for synthetic compounds against the test bacteria. It was carried out by impregnation of different concentrations of synthesized compounds (0, 10, 50, 100, 1000 µg/mL) in DMSO as a solvent and then placed on filter paper discs of the same diameter (5 mm). The agar plate dilution method was used to inoculate the bacteria in the plate. The medium was seeded with 100 µL of inoculum size $5x10^5$. The impregnated discs containing the tested samples of different concentrations were placed on the agar medium seeded with tested microorganisms. Standard antibiotic discs (Ampicillin, 5 µg/mL) and blank discs (impregnated with DMSO) were used as positive and negative control. The plates were then incubated at 37°C for 24 h to allow maximum growth of the microorganisms. The antimicrobial activities of the tested samples were determined by measuring the diameter of zone of inhibition expressed in millimeter. The inhibition zones were measured in triplicates and expressed as mean ± SD [21].

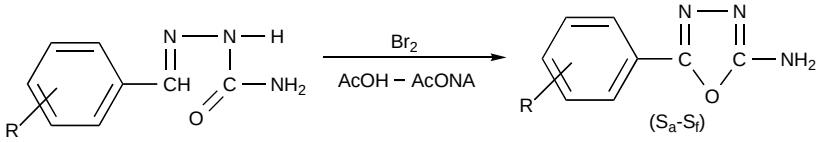
3. RESULTS AND DISCUSSION

3.1. Chemistry

The oxadiazoles (S_a-S_f) were prepared using Scheme **1**. The melting points, reaction time, yield and other physical data of synthesized oxadiazoles are given in Table **1**, while spectral data are presented in Table **2**.

The data in Table **1** confirm that there was significant difference in time taking for the preparation of oxadiazoles by conventional method which ranges from 25-30 minutes. While oxadiazoles preparation using microwave method takes only 15-20 seconds for completion of reaction. Also, there was significant difference in yield of the oxadiazoles prepared by conventional and microwave assisted synthesis. Conventional method gave poor yield of oxadiazoles ranging from 60.1-74.1%, while microwave method provide better yield ranging between 77.6-92.1%. These data revealed that microwave assisted technique is efficient, eco-friendly, and inexpensive method which not only give higher yield but also reduces the reaction time significantly.

The electronic absorption spectra of the oxadiazoles were scanned in methanol (200-500 nm) and displayed two main bands. The first band located within 206-213 nm range is attributed to the high energy π—π* transition corresponding to the ^1L_a—^1A state in the aromatic moiety. The second band at 270-284 nm range is due to n—π* transition in the CH=N group [22].



Scheme 1: Synthesis of oxadiazoles. R = H (S_a), R = 3-Br (S_b), R = 3-Cl (S_c), R = 4-F (S_d), R = 3-NO_3 (S_e) and R = 4-OCH_3 (S_f).

Table 1: Physical Characteristics of Synthesized Oxadiazoles (S_a-S_f)

S	R	Molecular formula	M.wt	M.P. (°C)	Conventional method		Microwave method	
					Time (min)	Yield (%)	Time (sec)	Yield (%)
S_a	H	$C_8H_7N_3O$	161.16	242-244	25	68.2	15	77.6
S_b	3-Br	$C_8H_6N_3OBr$	240.05	239-341	30	62.2	20	89
S_c	3-Cl	$C_8H_6N_3OCl$	195.60	153-155	30	69.1	20	77.7
S_d	4-F	$C_8H_6N_3OF$	179.15	230-232	25	60.1	15	79
S_e	3-NO_2	$C_8H_6N_4O_3$	206.16	262-264	30	74.1	20	92.1
S_f	4-OCH_3	$C_9H_9N_3O_2$	191.18	248-250	25	67.2	15	83.1

Table 2: Antimicrobial Activities (Inhibition Zones mm) Using 10 mg Powder/Well of Oxadiazole Derivatives (S_a-S_f)

Compound	Inhibition zone diameter (mm)					
	Bacteria					
	E. coli	*S. marcescens*	*S. enterica*	*P. vulgaris*	*B. subtilis*	*S. aureus*
S_a	12.3±0.6	-	12.3±0.6	-	-	13.7±0.6
S_b	13.3±0.6	10.3±0.6	17.7±2.1	12.3±0.6	11.0±0.6	16.7±0.6
S_c	-	-	20.0±2.0	-	-	-
S_d	12.3±0.6	11.7±1.2	10.7±0.6	-	-	-
S_e	13.0±0.0	15.7±0.6	21.3±0.6	15.3±0.6	-	-
S_f	-	12.3±0.6	-	-	-	-
Ampicillin	-	-	-	34.1±0.3	-	30.0±0.2

DMSO was added to different organisms as control and showed no inhibition zone.

The structures further confirmed by ^{1}H NMR where multiplet peaks for aromatic protons in the region of 7.09-8.49 ppm were observed [23]. The methoxy proton was observed as singlet at 3.79 ppm, while amino proton was observed as singlet in the region 7.26-7.43 ppm.

Formation of oxadiazoles (S_a-S_f) was confirmed by IR spectral data. The appearance of strong absorption bands at 925-975 and 1020-1050 cm^{-1} ranges are due to C-O-C linkage of the oxadiazoles ring. All compounds displayed strong bands at 1620-1665 cm^{-1} range corresponding to C=N stretching frequencies [24]. Beside the above characteristic band, all the compounds showed a doublet in the region 3100-3400 cm^{-1} corresponding to the symmetric stretching vibration of $–NH_2$ group. The stretching vibrations of the aromatic C-H groups give medium to weak bands within the 2768-2784 cm^{-1} range, while the bands appeared at 832-917 cm^{-1} are assigned to ν(Ar-H stretch).

The structure of synthesized 2,5-disubstituted-1,3,4-oxadiazoles were also confirmed by mass spectral analysis. Under EI condition, S_a and S_b showed prominent molecular ion peaks at m/z 162.21 and 241.31 corresponding to M^++1. The prominent mass peaks appeared at m/z 195.60, 179.00, 206.00 and 191.10 in the mass spectra of S_c, S_d, S_e and S_f are due to the molecular weight of the parent ion $[M]^+$. All the above data confirm the TLC and spectroscopic purity of the prepared oxadiazoles (S_a-S_f).

3.2. Biology

3.2.1. Antimicrobial Activities

The antimicrobial agents available on the market have various drawbacks such as toxicity, narrow spectrum of activity and some also exhibit drug–drug interactions. In view of the high incidence of infections in immune compromised patients, demands for new antimicrobial agents with a broad spectrum of activity and good pharmacokinetic properties have increased [25]. The synthesized Compounds **S_a-S_f** were screened for their antimicrobial activities against *Escherichia coli*, *Serratia marcescens, Salmonella enterica* and *Proteus vulgaris* as Gram-negative bacteria and *Bacillus subtilis* and *Staphylococcus aureus* as Gram-positive bacteria. The inhibition zones were measured in triplicates and the results of antimicrobial testing are reported in Table **2**. The results recorded in Table **2** showed that compound **S_b** was the most active compound against all organisms. Compounds **S_c and S_f** showed only activities against *Salmonella enteric* and *Serratia marcescens* respectively.

3.2.2. Minimum Inhibitory Concentrations (MICs)

The minimum inhibitory concentrations (MICs) of the synthesized compounds **S_a-S_f** were determined for each antimicrobial agent by using agar diffusion method. The inhibition zone was measured in triplicates in four different concentrations (10–1000 lg/mL) and the mean value ± standard deviation (SD) is recorded in Table **3**. The obtained results revealed that all compounds showed high antimicrobial activities at low concentrations (10-100 lg/mL) for all microorganisms. Also, the obtained data proved the potential usefulness of the investigated compounds **S_a-S_f** as broad spectrum antimicrobial agents and chemotherapeutic uses.

4. CONCLUSION

It can be concluded that our microwave assisted synthesis is simple, inexpensive, fast and facile in

Table 3: Minimum Inhibitory Concentrations (MICs) for Oxadiazole Derivatives (S_a-S_f)

Compound	Minimum inhibitory concentrations (MICs) (µg/mL)						
	Bacteria						
	E. coli	*S. marcescens*	*S. enterica*	*P. vulgaris*	*B. subtilis*	*S. aureus*	Mean MICs (µg/mL)
S_a	50±6.0	100±11.0	100±7.0	-	-	50±2.0	75±11
S_b	50±2.0	10±1.0	10±0.8	10±0.9	10±1.1	50±3.0	18±2
S_c	-	-	50±2.0	-	-	-	50±3
S_d	100±8.0	50±4.0	10±0.8	-	-	-	53±5
S_e	100±12.0	10±1.4	10±1.0	50±4.0			42±2
S_f	-	50±5.0	-	-	-	-	50±3
Ampicillin	-	-	-	5.0±0.1	-	5±0.1	5±0.1

The standard antibiotics was Ampicillin (MIC = 5 µg/mL).

comparison to previously reported conventional methods in reducing the heating time and improving the yield of the clean product. The newly synthesized compounds exhibit a remarkable inhibition of the growth of Gram-negative bacteria at low concentrations.

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