

Studies on Diesters Derived from Diacetyl Resorcinol

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Abstract: A series of diesters (**2a-d**) were synthesized from diacetyl resorcinol (**1**) and studied for their antimicrobial activities. In the first step, resorcinol was utilized to prepare diacetyl resorcinol (**1**), which was then reacted with substituted aryl acids to furnish the title compounds (**2a-d**). The structures of the synthesized compounds were established on the basis of data obtained from ¹H-NMR, Mass and elemental analysis. The antimicrobial activity (minimum inhibitory concentration; MIC) of the compounds (**2a-d**) was determined against different bacterial and fungal strains.

Keywords: Resorcinol, ester, cup-plate method, antibacterial, antifungal.

1. INTRODUCTION

Microbial resistance to antimicrobial drugs refers to the microbes that have developed the ability to inactivate, bypass or block the inhibition or lethal mechanism of the antimicrobial agents¹⁻⁴. The demand of new antimicrobial agents is increasing due to bacterial resistance to a number of antibiotics^{3,4}. Several classes of compounds have been explored to develop potential antibacterial agents. Among these classes, resorcinol derivatives seem promising class owing to their importance in synthesis and biological screening including antimicrobial screening⁵⁻⁹. Resorcinol derived heterocycles possess variety of pharmacological activities such as antitumor and antiproliferative, anti-inflammatory, anticoagulant, antioxidant and antimicrobial actions⁵⁻¹².

In view of these observations and in continuation of our work on resorcinol derivatives⁹⁻¹² it was considered worthwhile to study some new diesters; (1-(2,4-dimethoxy-5-[3-(substituted-phenyl)acryloyl]phenyl)-4-(substituted-phenyl)but-2-en-1-ones, as antibacterial and antifungal agents.

2. MATERIALS AND METHODS

2.1 Chemistry

Melting points are uncorrected, and were recorded in liquid paraffin bath using open end capillaries. ¹H-NMR spectra were recorded on Bruker spectropin DPX-300 MHz in CDCl₃; chemical shift (δ) values are reported in parts per million (ppm). The splitting pattern abbreviations are as follows: s, singlet; d, doublet; m, multiplet. Mass spectra was recorded on JEOL JMS-D 300 instrument. Elemental analyses were performed on a Perkin-Elmer 240 analyzer and were found in the range of ±0.4% for each element analyzed (C,H,N). Thin-layer chromatography (TLC) was carried out to monitor the reactions using silica gel G as stationary phase and iodine chamber and UV lamp were used to visualize the spots. The reaction sequence is presented in **Scheme 1**.

Synthesis of diacetyl resorcinol (**1**)

It was prepared from resorcinol following literature method¹⁰. It gave a violet colour with ethanolic ferric chloride solution; positive test for phenols. Yield 72%; m.p. 184-186°C. ¹H NMR (CDCl₃, δ, ppm): 2.65 (s, 6H, 2×-COCH₃), 6.65 (s, 1H, H-2), 8.15 (s, 1H, H-5).

General procedure for synthesis of diesters (**2a-d**)¹¹

To a solution of **1** (2 mmol; 0.388gm) in dry pyridine (10 mL) was added a solution of aromatic acid (4 mmol) in dry pyridine (5 mL). The contents were stirred for a few minutes and then phosphorous

oxy-chloride (0.5 mL) was added dropwise into it. Stirring was continued for another 2h and the reaction mixture was poured into ice cold water containing HCl. A solid mass separated out which was filtered, washed with water and dried. It was crystallized from methanol: dichloromethane mixture to furnish TLC pure compounds **2a-d**. It did not give color with ethanolic ferric chloride solution showing absence of phenolic (-OH) group.

4,6-Diacetyl-1,3-di(2-furyl carbonyloxy)benzene (2a). Yield 68 %; m.p. 182–184 °C; R_f 0.75; ^1H NMR (CDCl_3) δ ppm: 2.62 (s, 6H, 2x $-\text{COCH}_3$), 6.71–7.28 (m, 6H, 2x furyl ring), 7.32 (s, 1H, H-2), 8.24 (s, 1H, H-5); MS: m/z 382 (M^+); $\text{C}_{20}\text{H}_{14}\text{O}_8$; C 62.83, H 3.69; Found C 62.64, H 3.61.

4,6-Diacetyl-1,3-di(2-benzoyl-phenyl carbonyloxy)benzene (2b). Yield 53 %; m.p. 168–170 °C; R_f 0.69; ^1H NMR (CDCl_3) δ ppm: 2.63 (s, 6H, 2x $-\text{COCH}_3$), 6.86–7.72 (m, 19H, 2x *o*-benzoyl-phenyl protons + 1H, H-2), 8.35 (s, 1H, H-5); MS: m/z 610 (M^+). Anal calcd. for $\text{C}_{38}\text{H}_{26}\text{O}_8$: C, 74.75; H, 4.29. Found: C, 74.61; H, 4.35.

4,6-Diacetyl-1,3-di(2-naphthoxy-methyl carbonyloxy)benzene (2c). Yield: 60%; m.p. 152–153 °C; R_f 0.78; ^1H NMR (CDCl_3) δ ppm: 2.61 (s, 6H, 2x $-\text{COCH}_3$), 4.61 (s, 4H, 2x $-\text{OCH}_2$), 7.16–8.25 (m, 15H, 2x naphthyl protons + 1H, H-2), 8.46 (s, 1H, H-5); MS: m/z 562 (M^+); Anal calcd. for $\text{C}_{34}\text{H}_{26}\text{O}_8$: C, 72.59; H, 4.66. Found: C, 72.38 H, 4.43.

4,6-Diacetyl-1,3-di(2-naphthyl carbonyloxy)benzene (2d). Yield: 52%; m.p. 140–142 °C; ^1H NMR (CDCl_3) δ ppm: 2.57 (s, 6H, 2x $-\text{COCH}_3$), 7.24 (s, 1H, H-2), 7.21–8.19 (m, 14H, 2x naphthyl + 1H, H-2), 8.38 (s, 1H, H-5); MS: m/z 502 (M^+); Anal calcd. for $\text{C}_{32}\text{H}_{22}\text{O}_6$: C, 76.48; H, 4.41. Found: C, 76.18 H, 4.25.

2.2 Microbiology

The synthesized compounds (**2a-d**) were evaluated for their *in vitro* antimicrobial activity^{13,14} against three bacterial strains and two fungal strains at a concentration of 100 $\mu\text{g/mL}$ by cup plate method. Compounds inhibiting growth of one or more of the test microorganisms were further tested for their minimum inhibitory concentration (MIC).

Antibacterial activity

The compounds were screened for their *in vitro* antibacterial activity¹³ against *Staphylococcus aureus* (ATCC-25923), *Escherichia coli* (ATCC-25922), and *Pseudomonas aeruginosa* (ATCC-27853) bacterial strains at a concentration of 100 $\mu\text{g/mL}$ by cup plate method. Ciprofloxacin was used as standard drug for comparison. Freshly prepared liquid agar medium (25 mL/petridish) was poured into each petridishes and the plates were dried by placing in an incubator at 37 °C for 1 h. Then standardized culture of microorganism was spread on each petridishes by L-shaped spreader. Wells (6 mm) were made using an agar punch and, each well was labeled accordingly. A control (solvent) was also included in the test. The test compound and standard drug solutions (100 $\mu\text{g/mL}$) were made in dimethylsulfoxide (DMSO) and added in each well separately and petridishes kept aseptically for 1h for diffusion of the sample. After the completion of diffusion, all the petridishes were kept for incubation at 37 °C for 24 h and then diameter of the zone of inhibition was measured in mm (Table 1).

Compounds inhibiting growth of one or more of the test microorganisms were further tested for their minimum inhibitory concentration (MIC) by turbidity method. A solution of the compounds (100 $\mu\text{g/mL}$) was prepared in DMSO and a series of doubling dilutions prepared with sterile pipettes. To each of a series of sterile test tubes a standard volume of nutrient broth medium was added. A control tube containing no antimicrobial agent was included. The inoculum consisting of an overnight broth culture of microorganisms was added to separate tubes. The tubes were incubated at 37° for 24 h and examined for turbidity. The highest dilution (lowest concentration) required to arrest the growth of bacteria was regarded as MIC (Table 2).

Antifungal activity

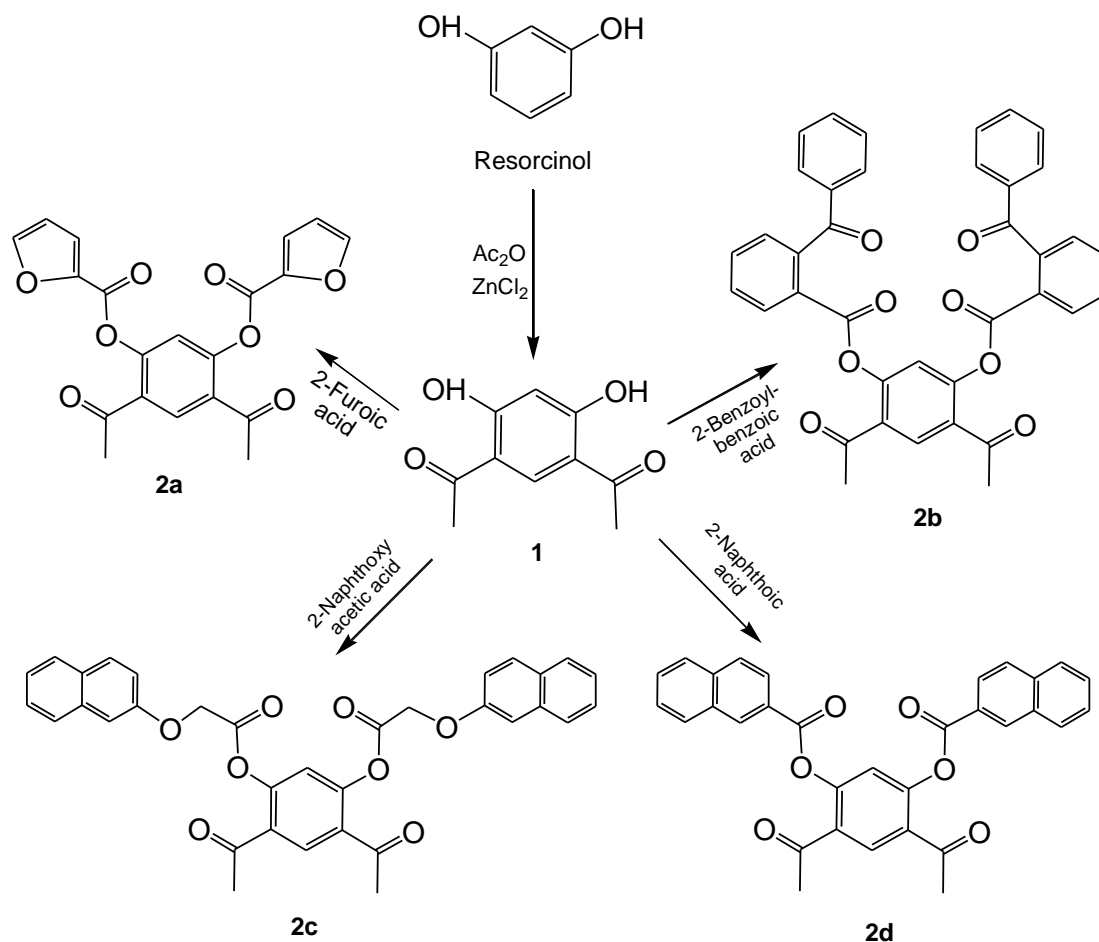
Antifungal activity of the synthesized compounds was determined against *Candida albicans* (ATCC-10231) and *Aspergillus niger* (ATCC-16404) by agar diffusion method¹⁴. Sabourands agar media was prepared by dissolving peptone (1 g), D-glucose (4 g) and agar (2 g) in distilled water (100 mL) and adjusting pH to 5.7. Normal saline was used to make a suspension of spore of fungal strain for

lawning. A loopful of particular fungal strain was transferred to 3 mL saline to get a suspension of corresponding species. Agar media (20 mL) was poured into each petridish and the plates were dried by placing in an incubator at 37°C for 1 h. Wells were made using an agar punch and, each well was labeled accordingly. A control was also prepared in triplicate and maintained at 37°C for 3-4 days. The test compounds and standard drug (Griseofulvin) solutions (100 µg/mL) were made in dimethylsulfoxide (DMSO) and added in each well separately and petridishes kept aseptically for 1h for diffusion of the sample. After the completion of diffusion, all the petridishes were kept for incubation at 37°C for 3-4 days and then diameter of the zone of inhibition was measured in mm (**Table 1**). Compounds inhibiting growth of one or more of the fungal strains were further tested for their minimum inhibitory concentration (*MIC*). A solution of the compounds (100 µg/mL) was prepared in DMSO and a series of doubling dilutions prepared with sterile pipettes. To each of a series of sterile test tubes a standard volume of nutrient broth medium was added. A control tube containing no antimicrobial agent was included. The tubes were inoculated with approximately 1.6×10^4 - 6×10^4 c.f.u. mL⁻¹ and incubated for 48 h at 37°C and examined for growth. The lowest concentration (highest dilution) required to arrest the growth of fungus was regarded as *MIC* (**Table 2**).

3. RESULTS AND DISCUSSION

3.1 Chemistry

The steps involved in the synthesis of title compounds are given in **Scheme-1**. The starting material, resorcinol, was treated with acetic anhydride in presence of anhydrous zinc chloride to get diacetyl resorcinol (**1**)¹⁰. Compound **1** was then condensed with different aromatic acids in presence of phosphorous oxychloride in dry pyridine to obtain diesters¹¹ (**2a-d**). They did not give colour with ethanolic ferric chloride solution indicating the absence of free phenolic (-OH) group. The structures of the synthesized compounds were supported by ¹H NMR, Mass spectral data and elemental analysis results.



Scheme 1: Protocol for synthesis of title compounds (**2a-d**).

The ^1H NMR spectrum of diacetyl resorcinol (**1**)¹⁰ showed a singlet at δ 2.65, which could be accounted for six protons of two acetyl groups. The ring protons, H-2 and H-5, gave singlet at δ 6.65 and 8.15, respectively.

The ^1H NMR spectra of the title compounds (**2a-d**) revealed the presence of two acetyl groups as singlet at around δ 2.6. Resorcinol ring protons, H-2 and H-5, appeared as two singlet at around δ 7.2 and δ 8.4, respectively. Other signals were observed at appropriate δ values integrating for the protons of two substituted aryl rings. The mass spectra of diesters showed the presence of molecular ion peak in reasonable intensities.

Elemental analyses values of the synthesized compounds were found within $\pm 0.4\%$ of theoretical values.

3.2 Antimicrobial activity

The title compounds (**2a-d**) were screened for their antibacterial activity against *Staphylococcus aureus* (ATCC-25923), *Escherichia coli* (ATCC-25922) and *Pseudomonas aeruginosa* (ATCC-27853) bacterial species, and antifungal activity against *Candida albicans* (ATCC-10231) and *Aspergillus niger* (ATCC-16404). The antimicrobial screening data showed that one compound; 4,6-diacetyl-1,3-di(2-benzoyl-phenyl carbonyloxy)benzene (**2b**), showed good activity against *S. aureus* and *E. coli* with MIC-12.5 $\mu\text{g/mL}$. Similar type of activity was shown by 4,6-diacetyl-1,3-di(2-furyl carbonyloxy)benzene (**2a**) against *S. aureus* and *C. albicans* with MIC-12.5 $\mu\text{g/mL}$. The standard drugs showed MIC values of 6.25 $\mu\text{g/mL}$ (Table 1 & 2).

Table 1. Preliminary antibacterial and antifungal activities of the title compounds (**2a-d**).

Compd.	Antibacterial activity [#]			Antifungal activity [#]	
	<i>S. aureus</i>	<i>E. coli</i>	<i>P. aeruginosa</i>	<i>C. albicans</i>	<i>A. niger</i>
2a	+++	+	-	+++	-
2b	+++	+++	+	++	+
2c	+	-	+	+	+
2d	-	-	-	+	-
Standard-1 [†]	++++	++++	++++	nt	nt
Standard-2 [†]	nt	nt	Nt	++++	++++

[#]Zone of inhibition: - = < 5 mm (insignificant or no activity), + = 5-9 mm (weak activity), ++ = 10-14 mm (moderate activity), +++ = 15-20 mm (good activity), ++++ = > 20 mm (excellent activity).

[†]Standard-1 = Ciprofloxacin, Standard-2 = Griseofulvin, nt = not tested.

Table 2. Antibacterial and antifungal activities (MIC, $\mu\text{g/mL}$) of the title compounds (**2a-d**).

Compd.	Antibacterial activity [#]			Antifungal activity [#]	
	<i>S. aureus</i>	<i>E. coli</i>	<i>P. aeruginosa</i>	<i>C. albicans</i>	<i>A. niger</i>
2a	12.5	50	>100	12.5	>100
2b	12.5	12.5	50	25	50
2c	50	>100	50	50	50
2d	>100	>100	>100	50	>100
Standard-1 [†]	6.25	6.25	6.25	nt	nt
Standard-2 [†]	nt	nt	Nt	6.25	6.25

nt = not tested; [†]Standard-1 = Ciprofloxacin, Standard-2 = Griseofulvin.

4. CONCLUSION

Four new diesters (**2a-d**) were synthesized from diacetyl resorcinol (**1**). The antimicrobial screening results indicated that the title compounds were having appreciable antibacterial and antifungal activities. Two compounds; 4,6-diacetyl-1,3-di(2-furyl carbonyloxy)benzene (**2a**) and 4,6-diacetyl-1,3-di(2-benzoyl-phenyl carbonyloxy)benzene (**2b**), showed significant antimicrobial action against the tested microbes.

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