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Original Article

Synthesis and Microbiology of Diesters Derived From 1, 1´-(4, 6-Dihydroxy-1, 3-Phenylene) Diethanone

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Abstract

A series of 4, 6-diacetyl-1, 3-di(substituted-phenyl carbonyloxy)benzenes or diesters (**2a-f**) were prepared and screened for in vitro antibacterial and antifungal activities. 1, 1´-(4,6-Dihydroxy-1, 3-phenylene)diethanone (**1**) was treated with different aromatic acids in dry pyridine in presence of phosphorous oxychloride to obtain the diesters (**2a-f**). The structures of the synthesized compounds were established on modern analytical techniques. The antimicrobial activity (minimum inhibitory concentration; *MIC*) of the title compounds was determined against some selected bacterial and fungal strains. The compounds showed appreciable antimicrobial activity against the tested microbes. Presence of halogen group(s) was found to increase the antimicrobial activity of the diesters.

Keywords: Resorcinol; Ester; MIC; Antimicrobial.

Introduction

There is a great interest in the discovery of new antimicrobial compounds in recent times due to the development of new strains of bacteria resistant to a variety of currently available antimicrobial treatments [1,2]. Antimicrobial resistance refers to microbes that have developed the ability to inactivate, bypass or block the inhibition or lethal mechanism of the antimicrobial agents [3]. The resorcinol derivatives are quite important compounds for both synthesis and pharmacological screening [4,5]. A variety of synthesized resorcinol derivatives have been experimentally shown to exert various important biological actions including antimicrobial activities[4-9].

In view of these points and in continuation of our work on resorcinol derivatives [6-9], it was considered worthwhile to synthesize some new diacetyl-resorcinol based diesters and to evaluate their antibacterial and antifungal activities.

Materials and Methods

Synthesis

Melting points were recorded in liquid paraffin bath using open end capillaries and are uncorrected. PMR spectra were recorded on Bruker spectropsin DPX-300 MHz in $CDCl_3$; chemical shift (ä) values are reported in parts per million (*ppm*). The splitting pattern abbreviations are as follows: s, singlet; d, doublet; dd, double doublet; m, multiplet. Mass spectra were recorded on a JEOL JMS-D 300 instrument. Elemental analyses were performed on a Perkin-Elmer 240 analyzer. Thin-layer chromatography (TLC) was carried out to observe the progress of synthesis using silica gel G as

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stationary phase and iodine chamber and UV chamber were used for locating the spots of compounds. The reaction sequence is presented in *scheme 1*.

Synthesis of 1, 1´-(4,6-Dihydroxy-1, 3-phenylene) diethanone (1)

It was prepared from resorcinol following literature method[8]. It gave a violet colour with ethanolic ferric chloride solution; positive test for phenols. Yield 72%; m.p. 184-186°C. ^{1}H NMR ($CDCl_3$, ä, ppm): 2.65 (s, 6H, 2´-$COCH_3$), 6.65 (s, 1H, H-2), 8.15 (s, 1H, H-5).

General procedure for synthesis of diesters (2a-f) [9]

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 oxychloride (0.5 mL) was added drop-wise into it. Stirring was continued for another 2h and the reaction mixture poured into ice cold water containing a little quantity of 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-f**. It did not give colour with ethanolic ferric chloride solution.

4, 6-Diacetyl-1, 3-di(2-hydroxyphenyl carbonyloxy) benzene (2a). Yield 58 %; m.p. 137–139 ºC; R*f* 0.71; ^{1}H NMR ($CDCl_3$) δ ppm: 2.61 (s, 6H, 2x –$COCH_3$), 5.86 (s, 2H, 2x –OH), 7.18 (s, 1H, H-2), 7.32–7.81 (m, 8H, 2x H-3‘,4‘,5‘,6‘), 8.32 (s, 1H, H-5); MS: m / z 434 (M^+), 435 (M^++1); Anal calc. for $C_{24}H_{18}O_8$; C 66.36, H 4.18; Found C 66.17, H 4.15.

4,6-Diacetyl-1,3-di(2-acetoxyphenyl carbonyloxy) benzene (2b). Yield 60 %; m.p. 152–154 °C; R*f* 0.74; ^{1}H NMR ($CDCl_3$) δ ppm: 2.35 (s, 6H, 2x –$COCH_3$), 2.63 (s, 6H, 2x –$COCH_3$), 7.22 (s, 1H, H-2), 7.36–7.83 (m, 8H, 2x H-3‘,4‘,5‘,6‘), 8.41 (s, 1H, H-5); MS: *m/z* 518 (M^+), 519 (M^++1); Anal calcd. for $C_{28}H_{22}O_{10}$: C, 64.86; H, 4.28; Found: C, 64.72; H, 3.97.

4,6-Diacetyl-1,3-di(4-fluorophenyl carbonyloxy) benzene (2c). Yield: 63%; m.p. 129-131°C; R*f* 0.76; ^{1}H NMR ($CDCl_3$) δ ppm: 2.59 (s, 6H, 2´-$COCH_3$), 7.16 (s, 2H, H-2), 7.24-7.51 (m, 4H, 2x H-3‘,5‘), 7.78 (d, 4H, 2x H-2‘,6‘), 8.34 (s, 1H, H-5); MS: *m/z* 438 (M^+), 439 (M^++1); Anal calcd. for $C_{24}H_{16}F_2O_6$: C, 65.76; H, 3.68. Found: C, 65.62; H, 3.55.

4 ,6-Diacetyl-1,3-di(2-bromophenyl carbonyloxy) benzene (2d). Yield: 55%; m.p. 118-119°C; R*f* 0.67; ^{1}H NMR ($CDCl_3$) δ ppm: 2.63 (s, 6H, 2x –$COCH_3$), 7.22 (s, 1H, H-2), 7.46-7.68 (m, 6H, 2x H-3‘,4‘,5‘), 7.87-8.16 (m, 2H, 2x H-6‘), 8.41 (s, 1H, H-5); MS: *m/z* 559 (M^+), 560 (M^++1), 561 (M^++2); Anal calcd. for $C_{24}H_{16}Br_2O_6$: C, 51.46; H, 2.88. Found: C, 51.23; H, 2.75.

4, 6-Diacetyl-1,3-di(4-bromophenyl carbonyloxy) benzene (3e). Yield: 58%; m.p. 140-142°C; R*f* 0.70; ^{1}H NMR ($CDCl_3$) δ ppm: 2.61 (s, 6H, 2x –$COCH_3$), 7.19 (s, 1H, H-2), 7.28 (d, 4H, 2x H-3‘,5‘), 7.85 (d, 4H, 2x H-2‘,6‘), 8.28 (s, 1H, H-5); MS: *m/z* 559 (M^+), 560 (M^++1), 561 (M^++2); Anal calcd. for $C_{24}H_{16}Br_2O_6$: C, 51.46; H, 2.88. Found: C, 51.38; H, 2.76.

4, 6-Diacetyl-1,3-di(2,4-dichlorophenyl carbonyloxy) benzene (3f). Yield: 54%; m.p. 134-136°C; R*f* 0.72; ^{1}H NMR ($CDCl_3$) δ ppm: 2.64 (s, 6H, 2x –$COCH_3$), 7.26 (s, 1H, H-2), 7.34-8.08 (m, 6H, 2x H-3‘,5‘,6‘), 8.44 (s, 1H, H-5); MS: *m/z* 539 (M^+), 540 (M^++1), 541 (M^++2); Anal calcd. for $C_{24}H_{14}Cl_4O_6$: C, 53.36; H, 2.61. Found: C, 53.22 H, 2.54.

Microbiology

The synthesized compounds were evaluated for their in vitro antimicrobial activity [10,11] against three bacterial strains and two fungal strains at a concentration of 100 μg / 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 [10]

In vitro antibacterial activity of the title compounds was determined against *Staphylococcus aureus* (ATCC-25923), *Escherichia coli* (ATCC-25922), and *Pseudomonas aeruginosa* (ATCC-27853) at a concentration of 100 mg / mL by cup plate method. 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 microbes was spread on each petridishes by a 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. Ciprofloxacin was used as standard drug for comparison. The test compound and standard drug 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 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 evaluated for their minimum inhibitory concentration (*MIC*) by turbidity method. 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 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*. Results are presented in *Table 2*.

Antifungal activity [11]

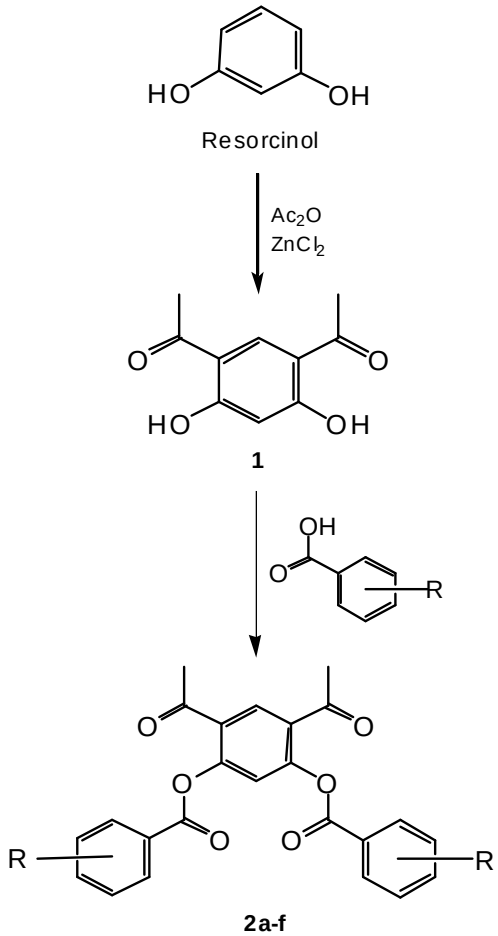
In vitro antifungal activity of the title compounds was determined against *Candida albicans* (ATCC-10231) and *Aspergillus niger* (ATCC-16404) by agar diffusion method. Sabourands agar media was prepared by dissolving peptone (1 g), D-glucose (4 g) and agar (2 g) in distilled water (100 mL) and adjusting 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 $1.6x10^4$-$6x10^4$ c.f.u. mL^{-1} and incubated for 48 h at 37°C and examined for growth. The highest dilution (lowest concentration) required to arrest the growth of fungus was regarded as *MIC* (Table 2).

Results and Discussion

Synthesis

The title compounds were synthesizes through multistep synthesis as given in **Scheme-1**. Resorcinol, the starting material, was reacted with acetic anhydride in presence of anhydrous zinc chloride to obtain 1,1´-(4,6-dihydroxy-1,3-phenylene) diethanone (**1**) [8] Compound **1** was then reacted with 2 moles of different aromatic acids in presence of phosphorous oxychloride to furnish the desired diesters (**2a-f**). The diesters did not give colour with ethanolic ferric chloride solution indicating the absence of free phenolic (-OH) group. The structures of the synthesized compounds were established on the basis of ^{1}H NMR, Mass spectral data and elemental analysis data.



(**2a**: R = 2-OH, **2b**: R = 2-$OCOCH_3$: **2c**: R= 4-F, **2d**: R= 2-Br**, 2e**: R= 4-Br, **2f**: R= 2,4-diCl)

Scheme 1: Protocol for synthesis of title Compounds (2a-f)

In general, the ^{1}H NMR spectra of the title compounds (*2a-f*) 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 phenyl rings (H-2'/3'/4'/5'/6'). 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 ±0.4% of theoretical values.

Microbiology

The title compounds (*2a-f*) were screened for their in vitro 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 the compound 2f, 4,6-diacetyl-1,3-di(2,4-dichlorophenyl carbonyloxy)benzene, exhibited good activity against *S. aureus*, *E. coli* and *C. albicans* with *MIC*-12.5 µg/mL. Similar type of activity was also shown by the compound; 4,6-diacetyl-1,3-di(4-fluorophenyl carbonyloxy)benzene (**2c**), against *S. aureus* and *E. coli* with *MIC*-12.5 µg/mL. Another compound, 4,6-diacetyl-1,3-di(4-bromophenyl carbonyloxy)benzene (**2e**), displayed significant activity against *S. aureus* with *MIC*-12.5 µg/mL. The standard drugs showed *MIC* values of 6.25 µg/mL (Table 1 & 2).

An analysis of results indicated that the title compounds 2a-f were significant in their antibacterial and antifungal actions. Presence of halogen group(s) was found to increase the antimicrobial activity of the diesters as evident by compound **2c, 2e** and 2f.

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

Compd.	Substituent (R)	Antibacterial activity[#]			Antifungal activity[#]	
		S. aureus	*E. coli*	*P. aeruginosa*	*C. albicans*	*A. niger*
2a	2-OH	-	-	-	+	-
2b	2-$OCOCH_3$	+	-	+	++	-
2c	4-F	+++	+++	+	++	+
2d	2-Br	++	++	+	+	-
2e	4-Br	+++	++	++	++	+
2f	2,4-diCl	+++	+++	+	+++	++
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*, µg/mL) of the title compounds (2a-f).

Compd.	Substituent (R)	Antibacterial activity			Antifungal activity	
		S. aureus	*E. coli*	*P. aeruginosa*	*C. albicans*	*A. niger*
2a	2-OH	>100	>100	>100	50	>100
2b	2-$OCOCH_3$	50	>100	50	25	>100
2c	4-F	12.5	12.5	50	25	50
2d	2-Br	25	25	50	50	>100
2e	4-Br	12.5	25	25	25	50
2f	2,4-diCl	12.5	12.5	50	12.5	25
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.

Conclusion

Six new diesters (**2a-f**) derived from 1,1´-(4,6-dihydroxy-1,3-phenylene)diethanone (**1**) were successfully synthesized. The antimicrobial studies showed that the synthesized compounds were having appreciable antibacterial and antifungal activities. Presence of halogen group(s) was found to increase the antimicrobial activity of the diesters. It is conceivable that further derivatization of the active compounds may result in potential antimicrobial agents.

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