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Original Article

Studies on 1,1´-(4,6-dihydroxy-1,3-phenylene)diethanone based Diesters

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ABSTRACT

A series of diesters (**2a-f**) were synthesized from 1,1´-(4,6-dihydroxy-1,3-phenylene)diethanone (**1**) and evaluated for their antimicrobial actions. Resorcinol was used as a starting material for the preparation of 1,1´-(4,6-dihydroxy-1,3-phenylene)diethanone (**1**), which was then reacted with substituted aryl acids to furnish the title compounds (**2a-f**). The structures of the synthesized compounds were confirmed on the basis of ^{1}H-NMR, Mass and elemental analysis results. The antimicrobial activity (minimum inhibitory concentration; *MIC*) of the title compounds was determined against some selected bacterial and fungal strains. One compound, **2c**, emerged as lead compound with good antimicrobial activity against *S. aureus*, *E. coli* and *C. albicans*.

Key words: Diacetyl resorcinol, ester, antibacterial, antifungal.

INTRODUCTION

The demand of new antimicrobial agents is high due to the development of new strains of bacteria resistant to a variety of currently available antibiotic treatments [1]. 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 agents [2-4]. A number of chemical compounds have been explored to develop promising antimicrobial compounds; and among them resorcinol derivatives have emerged as important compounds for both synthesis and biological screening including antimicrobial screening [5-9]. Resorcinol based compounds have been reported to possess important pharmacological activities including anticoagulant, antitumor and antiproliferative, antiinflammatory, antioxidative as well as antmicrobial activities [5-12].

In view of these observations and in continuation of our work on resorcinol based compounds [9-12] it was considered worthwhile to study few new diesters derived from resorcinol; (1-(2,4-dimethoxy-5-[3-(substituted-phenyl)acryloyl]phenyl)-4-(substituted-phenyl)but-2-en-1-ones, as antimicrobial agents.

MATERIALS AND METHODS

Chemistry: Melting points are uncorrected, and were recorded in liquid paraffin bath using open end capillaries. ^{1}H-NMR spectra were recorded on Bruker spectropspin DPX-300 MHz in $CDCl_3$; chemical shift (δ) 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 spectroscopic analyses for compounds were performed on a JEOL JMS-D 300 instrument. Elemental analyses were performed on a Perkin-Elmer 240 analyzer and were in range of ±0.4% for each element analyzed (C,H,N). Thin-layer chromatography was carried out to monitor the reactions using silica gel G as stationary phase and iodine chamber and UV lamp were used for visualization of TLC spots. The reaction involved in synthesis is given in **scheme 1**.

*Synthesis of 1,1´-(4,6-Dihydroxy-1,3-phenylene) diethanone (**1**):* It was prepared from resorcinol following literature method [11]. It gave a violet

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colour with ethanolic ferric chloride solution; positive test for phenols. Yield 72%; m.p. 184-186°C. ^{1}H NMR ($CDCl_3$, δ, 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**)* [12]*:* 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 dropwise into it. Stirring was continued for another 2h and the reaction mixture 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-f**. It did not give color with ethanolic ferric chloride solution showing absence of phenolic (-OH) group.

*4,6-Diacetyl-1,3-di(3-methoxyphenyl carbonyloxy) benzene (**2a**):* Yield 62 %; m.p. 141–142 ºC; R*f* 0.73; ^{1}H NMR ($CDCl_3$) δ ppm: 2.63 (s, 6H, 2x –$COCH_3$), 3.89 (s, 6H, 2x–OCH_3), 7.19 (s, 1H, H-2), 7.23–7.44 (m, 6H, 2x H-4`,5`,6`), 7.47–7.58 (m, 2H, 2x H-2`), 8.19 (s, 1H, H-5); MS: m/z 462 (M^+), 463 (M^++1); $C_{26}H_{22}O_8$; C 67.53, H 4.80; Found C 67.41, H 4.65.

*4,6-Diacetyl-1,3-di(4-aminoyphenyl carbonyloxy) benzene (**2b**):* Yield 51 %; m.p. 133–135 °C; R*f* 0.78; ^{1}H NMR ($CDCl_3$) δ ppm: 2.58 (s, 6H, 2x –$COCH_3$), 5.21 (s, 2H, 2x –NH_2), 7.23 (d, 4H, *J* = 7.6 Hz, 2x H-3`,5`), 7.34 (s, 1H, H-2), 7.82 (d, 4H, *J* = 7.8 Hz, 2x H-2`,6`), 8.53 (s, 1H, H-5); MS: *m/z* 432 (M^+), 433 (M^++1). Anal calcd. for $C_{24}H_{20}N_2O_6$: C, 66.66; H, 4.66; N, 6.48. Found: C, 66.48; H, 4.72; N, 6.30.

*4,6-Diacetyl-1,3-di(4-hydroxy-3-methoxyphenyl carbonyloxy)benzene (**2c**)*: Yield: 62%; m.p. 167-169°C; R*f* 0.69; ^{1}H NMR ($CDCl_3$) δ ppm: 2.61 (s, 6H, 2×-$COCH_3$), 3.93 (s, 6H, 2×-OCH_3), 5.78 (s, 2H, 2x –OH), 6.98 (d, 2H, 2x H-6`), 7.13 (d, 2H, 2x H-5`), 7.21 (s, 2H, H-2), 7.26 (s, 2H, 2x H-2`), 8.28 (s, 1H, H-5); MS: *m/z* 494 (M^+), 495 (M^++1); Anal calcd. for $C_{26}H_{22}O_{10}$: C, 63.16; H, 4.48. Found: C, 62.88; H, 4.35.

*4,6-Diacetyl-1,3-di(phenylmethylcarbonyloxy) benzene (**2d**):* Yield: 68%; m.p. 134-135°C; R*f* 0.72; ^{1}H NMR ($CDCl_3$) δ ppm: 2.60 (s, 6H, 2x –$COCH_3$), 4.21 (s, 4H, 2x -CH_2), 7.18 (s, 1H, H-2), 7.43-7.58 (m, 6H, 2x H-3`,4`,5`), 7.75-7.96 (m, 4H, 2x H-2`,6`), 8.29 (s, 1H, H-5); MS: *m/z* 430 (M^+), 431 (M^++1); Anal calcd. for $C_{26}H_{22}O_6$: C, 72.55; H, 5.15. Found: C, 72.38; H, 5.10.

*4,6-Diacetyl-1,3-di(phenyloxymethylcarbonyloxy) benzene (**3e**):* Yield: 71%; m.p. 158-160°C; R*f* 0.67; ^{1}H NMR ($CDCl_3$) δ ppm: 2.65 (s, 6H, 2x –$COCH_3$), 4.67 (s, 4H, 2x -OCH_2), 7.22 (s, 1H, H-2), 7.49-7.64 (m, 6H, 2x H-3`,4`,5`), 7.76-8.18 (m, 4H, 2x H-2`,6`), 8.36 (s, 1H, H-5); MS: *m/z* 462 (M^+), 463 (M^++1); Anal calcd. for $C_{26}H_{22}O_8$: C, 67.53; H, 4.80. Found: C, 67.28 H, 4.96.

*4,6-Diacetyl-1,3-di(1-naphthyloxymethyl carbonyloxy) benzene (**3f**):* Yield: 56%; m.p. 137-139°C; ^{1}H NMR ($CDCl_3$) δ ppm: 2.58 (s, 6H, 2x –$COCH_3$), 4.62 (s, 4H, 2x -OCH_2), 7.24 (s, 1H, H-2), 7.38-8.23 (m, 14H, naphthyl, 2x H-2`,3`,4`,5`,6`,7`), 8.51 (s, 1H, H-5); MS: *m/z* 562 (M^+), 563 (M^++1); Anal calcd. for $C_{34}H_{26}O_8$: C, 72.59; H, 4.66. Found: C, 72.45 H, 4.28.

Antimicrobial activity

The synthesized compounds were evaluated for their *in vitro* antimicrobial activity [13,14] 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: The compounds were screened for their *in vitro* antibacterial activity [13] against *Staphylococcus aureus* (ATCC-25923), *Escherichia coli* (ATCC-25922), and *Pseudomonas aeruginosa* (ATCC-27853) bacterial strains at a concentration of 100 µg/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 1h. 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 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 24h 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 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 24h 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: Antifungal activity of the synthesized compounds was determined against *Candida albicans* (ATCC-10231) and *Aspergillus niger* (ATCC-16404) by agar diffusion method [14]. 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 1h. 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 lowest concentration (highest dilution) required to arrest the growth of fungus was regarded as *MIC*. Results are presented in **Table 2**.

RESULTS AND DISCUSSION

Chemistry: The protocol for synthesis of title compounds is presented in **Scheme 1**. The starting material, resorcinol, was treated with acetic anhydride in presence of anhydrous zinc chloride to get 1,1´-(4,6-dihydroxy-1,3-phenylene)diethanone (**1**). Compound **1** was then condensed with different aromatic acids in presence of phosphorous oxychloride in dry pyridine to furnish diesters (**2a-f**). 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 further supported by ^{1}H NMR, Mass spectral data and elemental analysis results.

The ^{1}H NMR spectrum of 1,1´-(4,6-dihydroxy-1,3-phenylene)diethanone (**1**) [11] 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 ^{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. 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.

Antimicrobial activity: The title compounds (2a-f) 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(4-hydroxy-3-methoxyphenyl carbonyloxy)benzene (2c), showed good activity against *S. aureus*, *E. coli* and *C. albicans* with *MIC*-12.5 µg/mL. Similar type of activity was shown by the compound 2f against *S. aureus* with *MIC*-12.5 µg/mL. Rest of the compounds showed moderate to low antimicrobial activities. The standard drugs showed *MIC* values of 6.25 µg/mL (**Table 1** & **2**).

CONCLUSION

A series of diesters (**2a-f**) derived from 1,1´-(4,6-dihydroxy-1,3-phenylene)diethanone (**1**) were successfully synthesized starting from resorcinol. The antimicrobial screening results indicated that the synthesized compounds were having significant antibacterial and antifungal activities. 4,6-Diacetyl-1,3-di(4-hydroxy-3-methoxyphenyl carbonyloxy) benzene (**2c**) emerged as lead compound with significant antimicrobial action against *S. aureus*, *E. coli* and *C. albicans* with *MIC*-12.5 µg/mL. It may be further stated that further exploration of the lead compound could possibly result in potential antimicrobial agents.

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

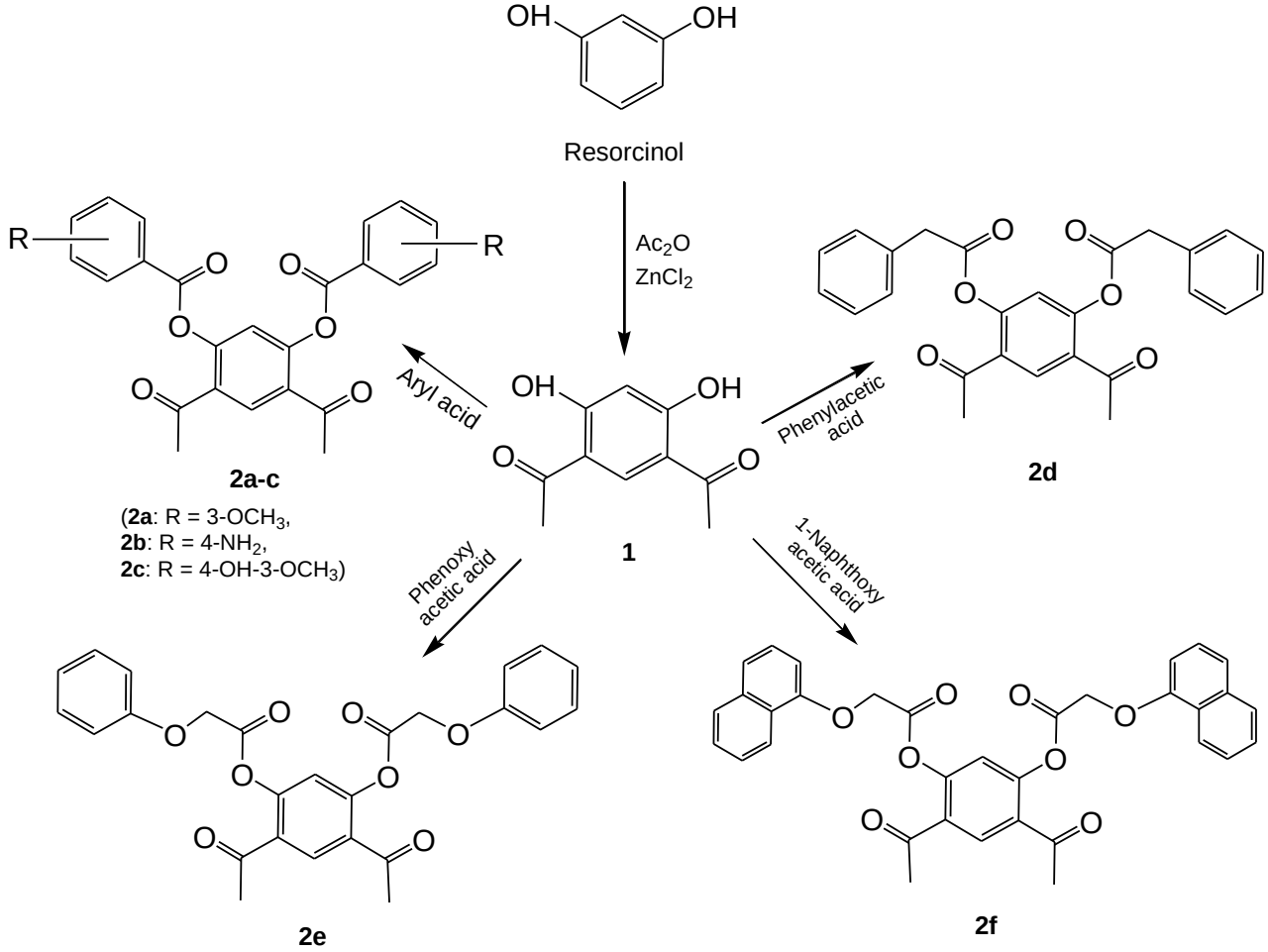
Compd.	Antibacterial activity[#]			Antifungal activity[#]	
	S. aureus	***E. coli***	***P. aeruginosa***	***C. albicans***	***A. niger***
2a	+	+	-	+	-
2b	++	+	+	++	+
2c	+++	+++	++	+++	++
2d	-	-	+	+	-
2e	+	-	+	-	-
2f	+++	++	+	++	++
Standard-1[†]	++++	++++	++++	nt	nt
Standard-2[†]	nt	nt	nt	++++	++++

[#]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.	Antibacterial activity[#]			Antifungal activity[#]	
	S. aureus	***E. coli***	***P. aeruginosa***	***C. albicans***	***A. niger***
2a	50	50	>100	50	>100
2b	25	50	50	25	50
2c	12.5	12.5	25	12.5	25
2d	>100	>100	>100	50	>100
2e	50	>100	50	>100	>100
2f	12.5	25	50	25	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.



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

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