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Studies on 1,1´-(4,6-dihydroxy-1,3-phenylene)diethanone based bischalcones and flavones

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ABSTRACT

*Resorcinol based bis-chalcones (**2a-d**) and flavones (**3a-d**) were synthesized and evaluated for their antimicrobial activity. Bis-chalcones (**2a-d**) were prepared by condensing 1,1´-(4,6-dihydroxy-1,3-phenylene)diethanone (**1**) with appropriate aryl-aldehydes, oxidative cyclization of bis-chalcones in presence of iodine gave corresponding flavones (**3a-d**). The synthesized compounds were evaluated for their antimicrobial actions against some selected bacterial and fungal strains. One compound, 1-{2,4-dihydroxy-5-[3-(2,4-dichlorophenyl)-2-propenoyl]phenyl}-3-(2,4-dichlorophenyl)-2-propen-1-one (**2d**), emerged as lead compound with significant antimicrobial activities.*

Keywords: Chalcones, flavones, antibacterial, antifungal

INTRODUCTION

The increasing incidence of resistance to currently available majority of antimicrobial agents is becoming a major concern [1-3]. In recent years, the incidence of bacterial and fungal infections is increasing at an alarming rate due to increased incidences of HIV-infection, tuberculosis, cancer, accidents, etc. Different factors like immunosuppressive therapies, invasive procedures, mucosal barriers, toxicity, high cost and age further contribute to the problem [1-3]. These points clearly indicate the need of more effective antimicrobial agents with a broad spectrum of activity.

Several compounds have been researched for developing potential antimicrobial agents. A number of natural and synthetic flavonoidal derivatives-chalcones, flavanones and flavones have been reported to have significant antimicrobial as well as antifungal activities [4-11]. Flavonoids acquire a special place in natural chemistry and in heterocyclic chemistry due to their wide pharmaceutical and pharmacological applications [6-12]. Flavonoidal ring system is also a frequently encountered structural motif in many pharmacologically important compounds [12-18]. Flavonoids derived from resorcinol (1,3-benzenediol) have been reported to show significant antimicrobial activities [14-18]. In view of these points and in continuation of our work on flavonoids [15-18], it was considered worthwhile to study some new resorcinol based chalcones and flavones for their antimicrobial actions.

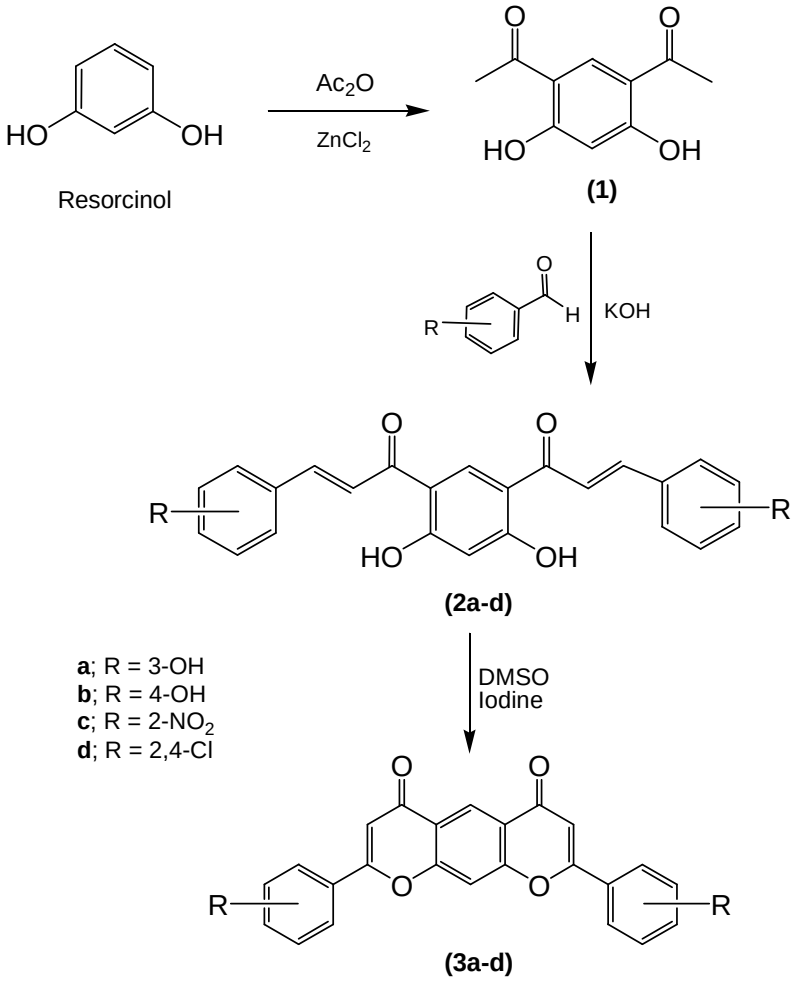
MATERIALS AND METHODS

Synthesis

Melting points were recorded in liquid paraffin bath using open end capillaries and are uncorrected. ^{1}H-NMR spectra were recorded on Bruker spectropsin DPX-300 MHz in $CDCl_3$; chemical shift, δ, values are reported in parts per million (*ppm*) downfield from tetramethylsilane (TMS), which was used as internal standard. The splitting pattern abbreviations are as follows: s, singlet; d, doublet; m, multiplet. Mass spectra were recorded on a JEOL JMS-D 300 instrument fitted with a JMS 2000 data system at 70 eV. Elemental analyses were performed on a

Perkin-Elmer 240 analyzer and the values were found within ±0.4% of theoretical values. Thin-layer chromatography was carried out to monitor the reactions using silica gel G as stationary phase.

Synthesis of 1,1´-(4,6-Dihydroxy-1,3-phenylene)diethanone (**1**): It was prepared from resorcinol following literature method [17]. 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).



Scheme 1: Protocol for synthesis of title compounds (**2a-d** & **3a-d**).

General method for the synthesis of Bis-chalcones (**2a-d)** [18]: A mixture of **2** (5 mmol) in ethanol (20 mL), arylaldehyde (10 mmol) and a solution of potassium hydroxide (3 g) in distilled water (5 mL) was stirred for 2h at room temperature and then left overnight. It was poured into cold water and acidified with HCl, a solid mass separated out which was filtered, washed with water, sodium bicarbonate solution (2% w/v in water) and again with water. It was crystallized to give **2a-d**. It gave a violet color with alcoholic ferric chloride solution and a red color with conc. sulphuric acid.

3-(3-Hydroxyphenyl)-1-{5-[3-(3-hydroxyphenyl)-2-propenoyl]-2,4-dihydroxyphenyl}-2-propen-1-one (2a): Yield 60%; m.p. 218-220°C. ^{1}H NMR ($CDCl_3$, δ, ppm): 6.59 (s, 1H, H-3'), 7.12-7.75 (m, 8H, 2×H-2,4,5,6), 7.79 (d, 2H, *J*= 15.6 Hz, 2xH-α), 8.13 (d, 2H, *J*= 15.6 Hz, 2xH-β), 8.48 (s, 1H, H-6'). MS (*m/z*): 402 (M^+). Analysis: ($C_{24}H_{18}O_6$), calcd.: C 71.64, H 4.51%. found: C 71.38, H 4.63%.

3-(4-Hydroxyphenyl)-1-{5-[3-(4-hydroxyphenyl)-2-propenoyl]-2,4-dihydroxyphenyl}-2-propen-1-one (2b): Yield 61%; m.p. 192-194°C. ^{1}H NMR ($CDCl_3$, δ, ppm): 6.57 (s, 1H, H-3'), 7.12 (d, 4H, *J*= 8.1 Hz, 2×H-3,5), 7.41 (d, 2H, *J*= 15.6 Hz, 2xH-α), 7.66 (d, 4H, *J*= 8.1 Hz, 2×H-2,6), 8.02 (d, 2H, *J*= 15.9 Hz, 2xH-β), 8.54 (s, 1H, H-6'). MS (*m/z*): 402 (M^+). Analysis: ($C_{24}H_{18}O_6$), calcd.: C 71.64, H 4.51%. found: C 71.45, H 4.48%.

1-{2,4-Dihydroxy-5-[3-(2-nitrophenyl)-2-propenoyl]phenyl}-3-(2-nitrophenyl)-2-propen-1-one (2c): Yield 66%; m.p. 208-210°C. ^{1}H NMR ($CDCl_3$, δ, ppm): 6.56 (s, 1H, H-3'), 7.19-7.32 (m, 4H, 2×H-3,5), 7.43-7.65 (m, 4H, 2xH-4,6), 7.79 (d, 2H, *J*= 15.3 Hz, 2xH-α), 8.22 (d, 2H, *J*= 15.6 Hz, 2xH-β), 8.56 (s, 1H, H-6'). MS (*m/z*): 460 (M^+). Analysis: ($C_{24}H_{16}N_2O_8$),; calcd.: C 62.61; H 3.50, N 6.08%. found: C 62.48, H 3.62, N 5.95%.

1-{2,4-Dihydroxy-5-[3-(2,4-dichlorophenyl)-2-propenoyl]phenyl}-3-(2,4-dichlorophenyl)-2-propen-1-one (2d): Yield 58%; m.p. 179-180°C. ^{1}H NMR ($CDCl_3$, δ, ppm): 6.62 (s, 1H, H-3'), 7.39-7.67 (m, 6H, 2×H-3,5,6), 7.83 (d, 2H, *J*= 15.6 Hz, 2xH-α), 8.17 (d, 2H, *J*= 15.6 Hz, 2xH-β), 8.51 (s, 1H, H-6'). MS (*m/z*): 506 (M^+), . Analysis: ($C_{24}H_{14}Cl_4O_4$), calcd.: C 56.72, H 2.78%. found: C 56.58, H 2.66%.

General method for the synthesis of Flavones (3a-d) [18]: To a solution of compound **2a** (200 mg) in dimethylsulphoxide (5 mL), 2 crystals of iodine were added. The contents were refluxed for 30 min, cooled to room temperature and poured into ice cold water. A solid mass separated out which was filtered, washed with water, sodium thiosulphate solution (2%w/v in water) and again with water. After drying it was crystallized from methanol: dichloromethane mixture to give TLC pure **3a-d** (It did not give colour with ethanolic ferric chloride solution).

2,8-bis(3-Hydroxyphenyl)-4*H*,6*H*-pyrano[3,2-*g*]chromene-4,6-dione (3a): Yield 42%; m.p. 172-174°C. ^{1}H NMR ($CDCl_3$, δ, ppm): 6.88 (s, 2H, 2×H-3), 7.41 (s, 1H, H-8), 7.45-7.93 (m, 8H, 2x *m*-hydroxyphenyl), 9.22 (s, 1H, H-5). MS (*m/z*): 398 (M^+). Analysis: ($C_{24}H_{14}O_6$), calcd.: C 72.36, H 3.54%. found: C 72.18, H 3.39%.

2,8-bis(4-Hydroxyphenyl)-4*H*,6*H*-pyrano[3,2-*g*]chromene-4,6-dione (3b): Yield 46%; m.p. 185-187°C. ^{1}H NMR ($CDCl_3$, δ, ppm): 6.86 (s, 2H, 2×H-3), 7.35 (s, 1H, H-8), 7.38-7.81 (m, 8H, 2x *p*-hydroxyphenyl), 9.28 (s, 1H, H-5). MS (*m/z*): 398 (M^+). Analysis: ($C_{24}H_{14}O_6$), calcd.: C 72.36, H 3.54%. found: C 72.24, H 3.42%.

2,8- bis(2-Nitrophenyl)-4*H*,6*H*-pyrano[3,2-*g*]chromene-4,6-dione (3c): Yield 48%; m.p. 238-240°C. ^{1}H NMR ($CDCl_3$, δ, ppm): 6.95 (s, 2H, 2×H-3), 7.37 (s, 1H, H-8), 7.41-7.88 (m, 8H, 2x *o*-nitrophenyl), 9.32 (s, 1H, H-5). MS (*m/z*): 456 (M^+). Analysis: ($C_{24}H_{12}N_2O_8$), calcd.: C 63.17, H 2.65, N 6.14%. found: C 63.10, H 2.58, N 6.06%.

2,8- bis(2,4-Dichlorophenyl)-4*H*,6*H*-pyrano[3,2-*g*]chromene-4,6-dione (3d): Yield 52%; m.p. 224-225°C. ^{1}H NMR ($CDCl_3$, δ, ppm): 6.89 (s, 2H, 2×H-3), 7.44 (s, 1H, H-8), 7.46-7.81 (m, 6H, 2x dichlorophenyl), 9.23 (s, 1H, H-5). MS (*m/z*): 504 (M^+). Analysis: ($C_{24}H_{10}Cl_4O_4$), calcd.: C 57.18, H 2.00%. found: C, 57.02; H, 2.14%.

ANTIMICROBIAL ACTIVITY

The synthesized compounds were evaluated for their antimicrobial activity [19,20] against three bacterial strains and two fungal strains.

Antibacterial activity

The compounds were screened for their antibacterial activity against *Staphylococcus aureus* (ATCC-29737), *Escherichia coli* (ATCC-25922), and *Pseudomonas aeruginosa* (ATCC-27853) bacterial strains at a concentration of 100 µg/mL by cup plate method [19]. Compounds inhibiting growth of one or more of the above microorganisms were again tested for their minimum inhibitory concentration values (*MIC*). Ciprofloxacin was used as standard drug for comparison. The *MICs* were determined by broth dilution technique. A solution of the compounds was prepared in dimethylformamide (DMF) and a series of doubling dilutions prepared. 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 also included. The inoculum consisting of an overnight broth culture of microorganisms was added to separate tubes. The tubes were incubated at 37° for 24 hours and examined for turbidity. The highest dilution (lowest concentration) required to stop the growth of bacteria was regarded as *MIC*.

Antifungal activity

Antifungal activity of the synthesized compounds was determined against *Candida albicans* (ATCC-10231) and *Aspergillus niger* (ATCC-16404) agar diffusion method [20]. Sabourand 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. Excess of suspension was decanted 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 antifungal activity of the compounds was compared with the standard drug; Griseofulvin. The nutrient broth, which contained logarithmic serially two fold diluted amount of test compound and controls was inoculated with approximately $1.6x10^4$-$6x10^4$ c.f.u. mL^{-1}. The cultures were incubated for 48 h at 37°C and the growth was monitored. The lowest concentration (highest dilution) required to arrest the growth of fungus was regarded as minimum inhibitory concentration (*MIC*).

RESULTS AND DISCUSSION

Synthesis

Reaction sequence followed for the preparation of title compounds is presented in **Scheme-1**. In the first step, resorcinol was treated [17] with acetic anhydride in presence of anhydrous zinc chloride to obtain 1,1´-(4,6-dihydroxy-1,3-phenylene)diethanone (**1**). Then, compound **1** was condensed with different aryl-aldehydes in presence of potassium hydroxide following Claisen-Schmidt reaction conditions [18] to obtain four bis-chalcones (**2a-d**). Finally, bis-chalcones were cyclized in presence of iodine in DMSO to furnish corresponding flavones (**3a-d**). The structures assigned to the compounds were supported by ^{1}H NMR, Mass spectral and microanalysis data.

In general, the ^{1}H NMR spectra of bis-chalcones (**2a-d**) showed the presence of two -CH=CH- groups as two doublets at δ 7.7 and δ 8.2 as two doublets integrating for two CH-α and two CH-β protons, respectively. Chalcone ring protons H-3` & H-6`appeared as singlet at δ 6.5 and δ 8.5, respectively. Other signals were observed at appropriate δ values integrating for the protons of two phenyl rings. These compounds gave positive ferric chloride test showing the presence of hydroxyl group. Mass spectral of bis-chalcones showed the presence of molecular ion peaks in reasonable intensities. Oxidative cyclization of bis-chalcones into flavones **3a-d** was carried out using DMSO/I_2 reagent. In ^{1}H NMR spectra of flavones **3a-d** showed the presence of three singlet at δ 6.8, δ 7.4, and δ 9.2 integrating for 2xH-3, H-8 and H-5 of flavone ring, respectively. Mass spectral of flavones showed the presence of molecular ion peaks in reasonable intensities. The spectral data together with negative ferric chloride test confirmed the cyclization of bis-chalcones to flavones.

Antibacterial and antifungal activity

All the synthesized compounds 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 compounds **3d** exhibited good activity against *S. aureus*, *P. aeruginosa* and *C. albicans* with *MIC* 12.5 µg/mL and appreciable activity against *E. coli* and *A. niger* with *MIC*-25 µg/mL. Similar type of activity was shown by the compound **3c** against *C. albicans* with *MIC*-12.5 µg/mL. Results are presented in **Table 1** & **2**.

Table 1: Preliminary in vitro antibacterial and antifungal activities of the title compounds (2a-d & 3a-d)

Compd.	-R	Antibacterial activity[#]			Antifungal activity[#]	
		S. aureus	*E. coli*	*P. aeruginosa*	*C. albicans*	*A. niger*
2a	3-OH	+	-	-	+	-
2b	4-OH	-	+	-	-	-
2c	2-NO_2	++	+	+	++	+
2d	2,4-Cl	+	+	+	++	++
3a	3-OH	-	-	-	+	+
3b	4-OH	-	-	-	+	-
3c	2-NO_2	++	++	+	+++	++
3d	2,4-Cl	+++	++	+++	+++	++
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: In vitro antibacterial and antifungal activities (*MIC*, µg/mL) of the title compounds (2a-d & 3a-d)

Compd.	-R	Antibacterial activity[#]			Antifungal activity[#]	
		S. aureus	*E. coli*	*P. aeruginosa*	*C. albicans*	*A. niger*
2a	3-OH	50	>100	>100	50	>100
2b	4-OH	>100	50	>100	>100	>100
2c	2-NO_2	25	50	50	25	50
2d	2,4-Cl	50	25	25	25	25
3a	3-OH	>100	>100	>100	50	50
3b	4-OH	>100	>100	>100	50	>100
3c	2-NO_2	25	25	50	12.5	25
3d	2,4-Cl	12.5	25	12.5	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.

An analysis of results indicated that the flavones (**3a-d**) were good in their antibacterial and antifungal actions, while bis-chalcones (**2a-d**) were appreciable in their antimicrobial activity. The synthesized compounds were slightly

better in their antifungal actions. Oxidative cyclization of chalcones resulted in compounds (flavones) with improved antimicrobial actions.

CONCLUSION

A series of bis-chalcones (**2a-d**) and their corresponding flavones (**3a-d**) were successfully synthesized and their structures were established on the basis of modern analytical techniques. The antimicrobial studies showed that the synthesized compounds were having significant antibacterial and antifungal activities. 2,8-bis(2,4-Dichlorophenyl)-4*H*,6*H*-pyrano[3,2-*g*]chromene-4,6-dione (**3c**) emerged as lead compound among the synthesized compounds. Presence of electron withdrawing group(s) increased the antimicrobial activity. Further modification of the lead may result in better antimicrobial agents.

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