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Original Article

Studies on 1-(2, 4-Dimethoxy-5-[3-(Substituted-Phenyl)Acryloyl]Phenyl)-4-(Substituted-Phenyl)But-2-En-1-Ones

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Abstract

A series of 1-(2, 4-dimethoxy-5-[3-(substituted-phenyl)acryloyl]phenyl)-4-(substituted-phenyl)but-2-en-1-ones (**3a-e**) were synthesized and evaluated for their antimicrobial actions. Resorcinol was used as starting material for the preparation of 1, 1´-(4, 6-dihydroxy-1, 3-phenylene) diethanone (**1**), which then treated with dimethylsulphate to obtain 1-(5-acetyl-2, 4-dimethoxyphenyl)-1-ethanone (**2**). Compound (**2**) was reacted with substituted aromatic aldehydes in ethanol in presence of KOH to furnish the title compounds (**3a-e**). 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. Two compounds, **3d** and **3e**, showed good antimicrobial activity against *S. aureus* and *E. coli*.

Keywords: Chalcone; MIC; Antibacterial; Antifungal.

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Introduction

The incidences of bacterial and fungal infections are increasing day by day, and the problem is further complicated due to increasing microbial resistance to a number of available antimicrobial drugs [1,3]. Different factors like HIV-infection, cancer, immuno-compromised host, immunosuppressive therapy, age, filthy place, etc. contribute to already existing problem. Therefore, more effective antimicrobial agents with broad spectrum of activity are required to combat the situation.

Among different compounds that have been explored for developing antimicrobial agents, chalcones have played an important role [4]. Chalcones have attracted considerable attention due to their important biological actions including antimicrobial action [5,6]. Further, chalcone derivatives have special place in natural as well as in synthetic chemistry because this system is a frequently encountered structural motif in a number of pharmacologically important compounds [5-8]. Resorcinol has been chemically explored to obtain a variety of compounds of potential pharmaceutical interest [9,10]. In view of these points and in continuation of our work on bischalcones [10-12] it was considered worthwhile to synthesize some new resorcinol derivatives; (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 spectropsin 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 [10]. 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).

Synthesis of 1-(5-acetyl-2,4-dimethoxyphenyl)-1-ethanone (2) [11]

A mixture of 1,1´-(4,6-dimethyl-1,3-phenylene)diethanone (**1**) (2.5 mmol), dimethylsulphate (5 mmol) and anhydrous potassium carbonate (11.25 g) in dry acetone (100 mL) was refluxed for 6 h. The contents were then filtered, concentrated to a small volume and poured onto crushed ice. A solid mass separated out which was filtered, washed with water, dried and then crystallized from methanol: dichloromethane mixture to give shiny needles of **3** (it did not give violet colour with ethanolic ferric chloride solution; negative test for phenols). Yield 74%, m.p. 164-166°C. ^{1}H NMR ($CDCl_3$) δ 2.61 (s, 6H, 2´-$COCH_3$), 3.93 (s, 6H, 2´-OCH_3), 6.34 (s, 1H, H-2), 8.37 (s, 1H, H-5). MS: *m/z* 222 (M^+), 207, 177, 175, 149. Anal calcd. for $C_{12}H_{14}O_4$: C, 64.85; H, 6.35. Found: C, 64.71; H, 6.22.

General procedure for synthesis of 1-(2, 4-dimethoxy-5-[3-(substituted-phenyl)acryloyl]phenyl)-4-(substituted-phenyl)but-2-en-1-ones (3a-e) [11]

A mixture of compound **2** (5 mmol) in ethanol (20 mL), an aromatic aldehyde (10 mmol) and a solution of potassium hydroxide (3 g) in distilled water (5 mL) was stirred for 2 h 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 **3a-e** (It gave a red colour with conc. sulphuric acid; positive test for chalcones, and no colour with ethanolic ferric chloride solution; negative test for phenols).

1-(2,4-Dimethoxy-5-[3-(3-methylphenyl) acryloyl] phenyl)-4-(3-methylphenyl)but-2-en-1-one (3a). Yield: 51%, m.p. 196-198°C. ^{1}H NMR ($CDCl_3$) *d* 2.41 (s, 6H, 2´-CH_3), 3.93 (s, 6H, 2´-OCH_3), 6.41 (s, 1H, H-3'), 7.35 (d, 2H, 2xH-a), 7.47 (m, 2H, 2xH-5), 7.72 (dd, 2H, 2xH-4), 7.97 (d, 2H, 2xH-b), 8.14 (dd, 2H, 2xH-6), 8.19 (s, 1H, H-6'), 8.32 (s, 2H, 2xH-2). MS: *m/z* 426 (M^+). Anal calcd. for $C_{28}H_{26}O_4$: C, 78.85; H, 6.14. Found: C, 78.74; H, 5.96.

1-(2,4-Dimethoxy-5-[3-(4-hydroxyphenyl) acryloyl]phenyl)-4-(4-hydroxyphenyl)but-2-en-1-one (3b). Yield: 55%, m.p. 232-233°C. ^{1}H NMR ($CDCl_3$) *d* 3.96 (s, 6H, 2´-OCH_3), 6.48 (s, 1H, H-3'), 6.92 (d, 4H, 2´H-3,5), 7.28 (d, 2H, 2xH-a), 7.53 (d, 4H,2´H-2,6), 7.79 (d, 2H, 2xH-b), 8.22 (s, 1H, H-6'). MS: *m/z* 430 (M^+). Anal calcd. for $C_{26}H_{22}O_6$: C, 72.55; H, 5.15. Found: C, 72.42; H, 5.23.

1-(2,4-Dimethoxy-5-[3-(2-nitrophenyl) acryloyl] phenyl) -4-(2-nitrophenyl)but-2-en-1-one (3c). Yield: 60%, m.p. 216-218°C. ^{1}H NMR ($CDCl_3$) *d* 3.97 (s, 6H, 2´-OCH_3), 6.53 (s, 1H, H-3'), 7.08-7.31 (m, 4H, 2´H-3,5), 7.43-7.56 (m, 6H, 2xH-4,6,a), 7.86 (d, 2H, 2xH-b), 8.14 (s, 1H, H-6'). MS: *m/z* 488 (M^+). Anal calcd. for $C_{26}H_{20}N_2O_8$: C, 63.93; H, 4.13; N, 5.74. Found: C, 64.11; H, 3.86; N, 5.62.

1-(2,4-Dimethoxy-5-[3-(3-chlorophenyl) acryloyl] phenyl)-4-(3-chlorophenyl)but-2-en-1-one (3d). Yield: 62%, m.p. 200-202°C. ^{1}H NMR ($CDCl_3$) *d* 3.96 (s, 6H, 2´-OCH_3), 6.51 (s, 1H, H-3'), 7.24 (d, 2H, 2xH-a), 7.55 (m, 2H, 2xH-5), 7.89 (dd, 2H, 2xH-4), 8.01 (d, 2H, 2xH-b), 8.17 (s, 1H, H-6'), 8.26 (dd, 2H, 2xH-6), 8.41 (s, 2H, 2xH-2). MS: *m/z* 466 (M^+), 467 (M^++1). Anal calcd. for $C_{26}H_{20}Cl_2O_4$: C, 66.82; H, 4.31. Found: C, 66.65; H, 4.27.

1-(2,4-Dimethoxy-5-[3-(2,4-dichlorophenyl) acryloyl] phenyl)-4-(2,4-chlorophenyl)but-2-en-1-one (3e). Yield: 56%, m.p. 186-188°C. ^{1}H NMR ($CDCl_3$) *d* 3.93 (s, 6H, 2´-OCH_3), 6.48 (s, 1H, H-3'), 7.21-7.58 (m, 8H, 2xH-3,5,6 + 2xH-), 7.83 (d, 2H, 2xH-b), 8.16 (s, 1H, H-6'). MS: *m/z* 550 (M^+), 551 (M^++1), 552 (M^++2). Anal calcd. for $C_{27}H_{20}Cl_4O_4$: C, 58.93; H, 3.66. Found: C, 58.65 H, 3.52.

Antimicrobial activity

The synthesized compounds were evaluated for their 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 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 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 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 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.

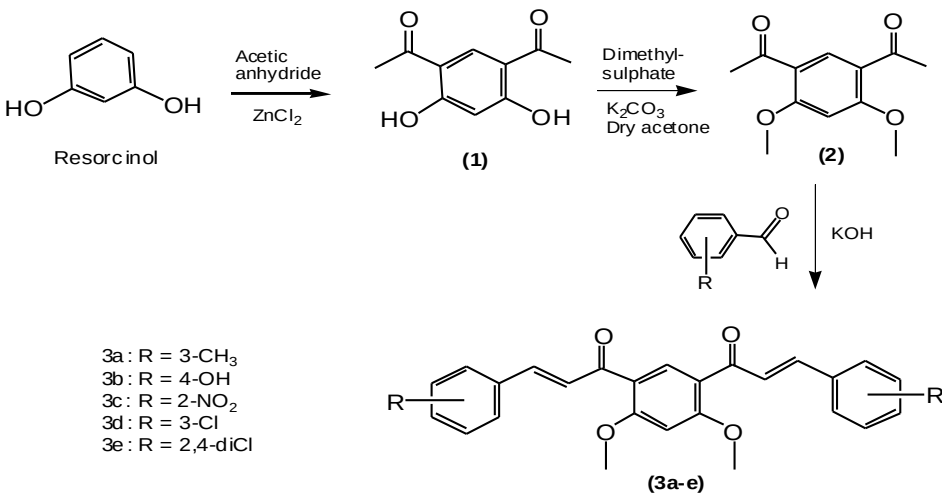
Antifungal activity

In vitro 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 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 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, 1,1´-(4,6-dihydroxy-1,3-phenylene)diethanone (**1**), prepared from resorcinol [10], was treated with dimethylsulphate in dry acetone in presence of anhydrous potassium carbonate to get 1,1´-(4,6-dimethyl-1,3-phenylene) diethanone (**2**) [11], which gave negative ferric chloride test showing the absence of phenolic-hydroxyl group. Compound (**2**) was then condensed with different aromatic aldehydes in presence of potassium hydroxide following Claisen-Schmidt reaction conditions to furnish 5 new bischalcones (**3a-e**). These compounds gave a red colour with conc. sulphuric acid; positive test for chalcones, and no colour with ethanolic ferric chloride solution; negative test for phenolic-hydroxyl group. The structures of the synthesized compounds were further supported by ^{1}H NMR, Mass spectral data and elemental analysis results.



Scheme 1: protocol for synthesis of title compounds (3a-e).

The ^{1}H NMR spectrum of 1, 1´-(4,6-dimethyl-1, 3-phenylene)diethanone (**2**) [11] showed a singlet at *ä* 2.61, which could be accounted for six protons of two acetyl groups. The two methoxyl groups appeared as singlet at δ 3.93. The ring protons, H-2 and H-5, gave singlet at δ 6.34 and 8.37, respectively. The mass spectrum of the compound (**2**) showed molecular ion peak at *m/z* 222.

The ^{1}H NMR spectra of the title compounds (3a-e) revealed the presence of two methoxyl groups as singlet at around δ 3.9, and two -CH=CH- groups as two doublets at around δ 7.3 and δ7.9 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 8.1, respectively. Other signals were observed at appropriate δ values integrating for the protons of two substituted phenyl rings. The mass spectra of bischalcones showed the presence of molecular ion peak in reasonable intensities. In case of compounds having phenyl rings with chloro-substituents (**3d** & **3e**), the molecular ion peak or their fragments having chloro-group appeared as cluster of peaks. Elemental analyses values of the synthesized compounds were found within ±0.4% of theoretical values.

Antimicrobial activity

The title compounds (3a-e) 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 two compounds, 1-(2,4-dimethoxy-5-[3-(3-chloroyphenyl) acryloyl]phenyl)-4-(3-chlorophenyl)but-2-en-1-one 3d and 1-(2,4-dimethoxy-5-[3-(2,4-dichlorophenyl) acryloyl]phenyl) -4-(2,4-chlorophenyl) but-2-en-1-one **3e**, exhibited good activity against *S. aureus* & *E. coli* with *MIC*-12.5 ìg / mL. Rest of the compounds showed moderate to low antimicrobial activities. The standard drugs showed *MIC* values of 6.25 ìg / mL (Table 1 & 2).

An analysis of results indicated that the title compounds 3a-e were appreciable in their antibacterial and antifungal actions. Presence of chloro group(s) found to increase the antimicrobial activity of the bischalcones.

Table 1: Preliminary antibacterial and antifungal activities of the title compounds (3a-e).

Compd.	Substituent (R)	Antibacterial activity#			Antifungal activity#	
		S. aureus	*E. coli*	*P. aeruginosa*	*C. albicans*	*A. nige*
3a	3-Methyl	+	-	-	+	-
3b	4-Hydroxy	++	+	++	++	-
3c	2-Nitro	+	-	+	+	+
3d	3-Chloro	+++	+++	+	++	-
3e	2,4-Dichloro	+++	+++	++	++	+
	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*, mg/mL) of the title compounds (**3a-e**).

Compd.	Substituent (R)	Antibacterial activity			Antifungal activity	
		S. aureus	*E. coli*	*P. aeruginosa*	*C. albicans*	*A. niger*
3a	3-Methyl	50	>100	>100	>100	>100
3b	4-Hydroxy	25	50	25	25	>100
3c	2-Nitro	50	>100	50	50	50
3d	3-Chloro	12.5	12.5	50	25	>100
3e	2,4-Dichloro	12.5	12.5	25	25	50
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

A series of 1-(2,4-dimethoxy-5-[3-(substituted-phenyl)acryloyl]phenyl)-4-(substituted-phenyl)but-2-en-1-ones (**3a-e**) were successfully synthesized starting from resorcinol. The antimicrobial studies showed that the synthesized compounds were having significant antibacterial and antifungal activities. Presence of chloro group(s) increased the antimicrobial activity of the bischalcones. These derivatives may be further explored to develop potential antimicrobial agents.

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