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SYNTHESIS AND BIO-ACTIVITY EVALUATION OF 2-STYRYLCHROMONES

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ABSTRACT

2-Styrylchromones are very good biological active compounds, prepared by Baker-Venkataraman rearrangement through 1,3-diketones as intermediates starting from *o*-hydroxyacetophenones and cinnamic acids. The synthesized compounds are characterized by some physical properties and spectral studies like IR, NMR and LCMS. They are screened for their antimicrobial activity against *Xanthomonas campestis* & *Agrobacterium tumafeciens* bacteria and *Aspergillus niger* & *Penicillum chrysogenium* fungal strains. Among five synthetic compounds, chloro and methoxy substituted compounds exhibit very good activity.

KEYWORDS: **2-Styrylchromones, 1,3-diketones, antimicrobial activity, *Xanthomonas campestris, Aspergillus niger* etc.**

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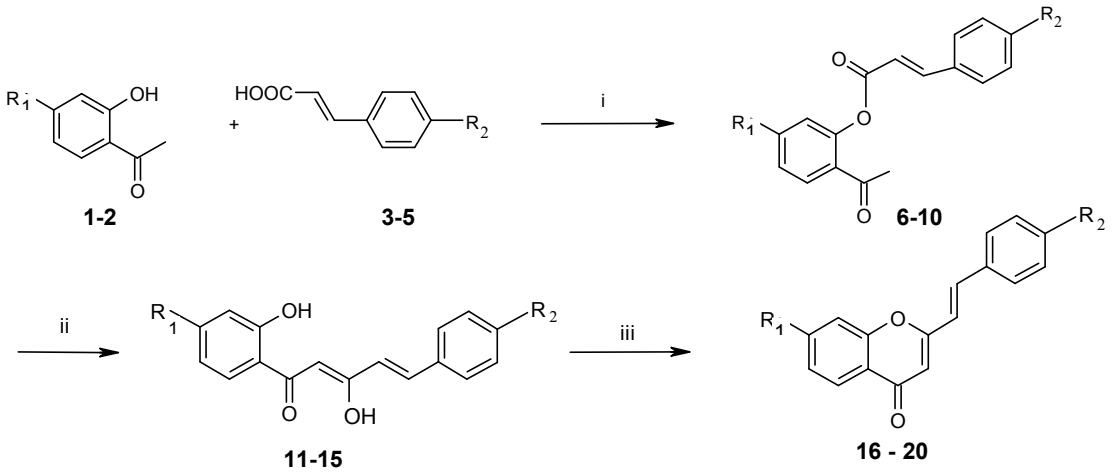
INTRODUCTION

Molecules containing the chromone structure have a wide range of biological activities[1-6] including tyrosine and protein kinase C inhibitors, antifungal, antiallergic, antiviral, antitublin, anti-hypertensive and anticancer agents, as well as being active at benzo-azepine receptors, lipoxygenase, cycloxy-genase and modulating P-glycoprotein-mediated multidrug resistance (MDR). 2-Styrylchromones are new class of flavonoids, structurally related to 2-phenylchromones and are one of the scarcest classes of natural chromones, obtained from the marine blue green algae, cryptophyte, *Chrysophaeum taylori*[7,8] in 1986 by W.H. Gerwick. The natural derivatives demonstrated cytotoxic activity against Leukemia cells[7,8] and those obtained by synthesis[9] exhibited antiallergic, anti-tumor[10], antagonism of A_3 adenosine receptor and xanthine oxidase inhibitor[11] properties. Chromones and related compounds are wide spread in the plant kingdom from algae to conifers. Chromones have been found to be active in a number of plant cycles, including growth regulation, indole acetic acid oxidation and dormancy inhibition as well as exhibiting cytokine in type behavior and stimulating oxygen up takes in plant tissues[12]. The fluro chromones, khalin has lipid alternating capability[13], while styrylchromones, hormo-thamnione has been found as potent cytotoxic agent for p388 lymphocytic leukemia and HL-60 human promyetocytic cell lines in vitro[7]. The use of chromones as antiviral[14], and anti-cancer[15] and anti-inflammatory[16] agents is very well known. Some 2-styrylchromones[17] with hydroxy and methoxy substitution showed better antioxidant activity, determined by superoxide radical scavenging (NBT) method. Recently a series of halosubstituted[18] styryl-chromones have been prepared and tested for the antioxidant activity by using DPPH(1,1-diphenyl-2-picrylhydrazyl) mehod, and among the synthesized compounds, two compounds showed strong antioxidant activity while remaining compounds in the normal range. In the view of important bio-active properties, we are inspired to synthesize five 2-styrylchro-mones in good yield from *o*-hydroxyaceto-phenones and cinnamic acids as starting materials via 1,3-diketones as intermediates in three steps[17].

EXPERIMENTAL

General: Melting points of all synthetic compounds were determined on kofler hot-stage apparatus in an open capillary tubes are uncorrected. Elemental analysis was carried out on a vario EL-III. IR spectra were recorded on a Perkin-Elmer BX1 FTIR spectrophoto-meter. ^{1}H-NMR (400 MHz), spectra were recorded on a Joel JNM λ-300 spectrometer using TMS as internal reference, the values for chemical sifts (δ) being given in ppm and coupling constants (J) in hertz (Hz). LCMS was recorded on an agilent-1100 periods LC/MSD (VL). TLC was carried out on GF_{254} silica gel plates. Acme silica gel-G and Merck silica gel (100 to 200, 60 to 120 meshes) were used for analytical TLC and Column chromatography respecti-vely. All other chemicals and solvents used were obtained from commercial sources and used as received standard procedures. In the three steps method, 2'-cinnamoyloxyacetophenon-es (**6-10**) were synthesized initially in 100% yield form the reaction of 2'-hydroxyacetophe-nones (**1**,**2**) with appropriate cinnamic acids (**3-5**) in pyridine solution using Phosphorus oxychloride as condensing agent. Baker-Venkataraman rearrangement of these compounds into 1,3-diketones (enolic form, **11**-**15**) in best yields, was carried out in pyridine solution using powered potassium hydroxide. Finally 2-styrylchromones (**16**-**20**) were obtained from 1,3-diketones (enols) as follows.



Scheme-1

For compounds 6-15

6, 11. $R_1 = R_2 = H$
7, 12. $R_1 = H$; $R_2 = Cl$
8, 13. $R_1 = H$; $R_2 = OMe$
9, 14. $R_1 = OAc$; $R_2 = H$
10, 15. $R_1 = OAc$; $R_2 = OMe$

For compound 16-20

16. $R_1 = R_2 = H$
17. $R_1 = H$; $R_2 = Cl$
18. $R_1 = H$; $R_2 = OMe$
19. $R_1 = OH$; $R_2 = H$
20. $R_1 = OH$; $R_2 = OMe$

Reagents & Conditions: (i) Pyridine; $POCl_3$, rt. 4 hrs.
(ii) Dry pyridine, KOH, rt. 1-2 hrs.
(iii) AcOH, aq.H_2SO_4, reflux, 90-95 ^oC, 2-3 hrs.

General procedure for preparation of 2-styrylchromones:
The diketone was dissolved in acetic acid in round bottom flask and add with stirring aqueous sulphuric acid. The reaction mixture was refluxed on a boiling water bath with intermittent shaking for 3 hrs. After cooling to room temperature, the reaction mixture was poured on to crushed ice with stirring and allowed the ice to melt. The chromone was filtered, washed with water until the washings were no longer acidic. The product was dried and purified by column chromatography over silica gel using Hex-EtOAc as eluent. Finally the product was recrystallized from aq. ethanol.

2-Styrylchromone (16):
Pale pink color solid, M.P:208-210^{0}C, Analysis: Calcd. for $C_{17}H_{12}O_2$: C-82.25; H-4.83 % , Found: C-82.30; H-4.75 %, IR(KBr) υ_{max}: 3070, 1632, 1607, 1585, 1437, 1246, 1029, 843, 758 cm^{-1}; ^{1}H-NMR ($CDCl_3$): δ 8.09(1H, dd, J=8.8, 2.3, H-5), 7.90-7.81(4H, m, H-2',6',7,8), 7.61(1H, d, J=16.0Hz, Hβ), 7.50-7.38(4H, m, H-3',4',5',6), 6.85(1H, d, J=16.0 Hz, Hα), 6.37(1H, s, H-3); LC-MS (ESI, negative ion mode): m/z 247.

2-(4-Chlorostyryl)chromone (17):
Cream color solid. M.P: 220-222^{0}C; Analysis: Calcd. for $C_{17}H_{11}O_2$ Cl : C-72.21; H-3.89; Cl-12.56 % , Found: C-72.19; H-3.75; Cl-12.50 %; IR(KBr) υ_{max}: 3076, 1627, 1602, 1544, 1446, 1245, 1012, 732 cm^{-1}; ^{1}H-NMR (DMSO-d_6): δ 8.19(1H, dd, J=8.9, 2.3Hz, H-5), 7.70 (1H, dd, J=8.8, 2.7 Hz, H-8), 7.58 (1H, d, J=16.0 Hz, H- β), 7.56 (2H, d, J=8.3 Hz, H-2′, 6′), 7.47-7.38 (2H, m, H-6,7), 6.98(2H, d, J=8.3 Hz, H-3′, 5′), 6.78 (1H, d, J=16.0 Hz, H-α), 6.38 (1H, s, H-3); LC-MS (ESI, negative ion mode) : m/z- 281.5 $(M-H)^-$.

2-(4-Methoxystyryl)chromone(18):
Pale yellow color solid M.P :192-194^{0}C, Analysis: Calcd. for $C_{17}H_{12}O_2$: C-77.69; H-

5.03; %; Found: C-77.62; H-5.10; %, IR(KBr) υ_{max}: 2943, 2831, 1630, 1601, 1510, 1464, 1254, 1172, 1027, 966,848, 756 cm^{-1}; ^{1}H-NMR ($CDCl_3$): 8.20 (1H, dd, J=9.0, 2.7 Hz, H-5), 7.72 (1H, dd, J=8.9, 2.3 Hz, H-8), 7.62 (1H, d, J=16.0 Hz, H-β), 7.56 (2H, d, J=8.2 Hz, H-2′, 6′), 7.52-7.40 (2H, m, H-6,7), 6.96 (2H, d, J=8.2 Hz, H-3′, 5′), 6.66 (1H, d, J=16.0 Hz, H-α), 6.34 (1H, s, H-3), 3.84 (3H, s, Ar-OCH_3); LC-MS (ESI, negative ion mode): m/z-277 $(M-H)^-$.

7-Hydroxy-2-styrylchromone (19):

Light brick red solid (250 mg, 96%), MP: 198-200 °C, Analysis: Calcd. for $C_{17}H_{12}O_3$: C-77.27; H-4.45; %, Found: C-77.22; H-4.53; %; IR(KBr) υ_{max}: 3374, 3013, 1627, 1601, 1518, 1432, 1246, 1025, 843, 752 cm^{-1}; ^{1}H-NMR (DMSO-d_6): δ 9.80(1H, br, s, Ar-OH), 7.79(1H,d,J=8.9 Hz, H-5), 7.53 (1H, d, J=16.0 Hz; H-β), 7.50(2H,d,J=8.6Hz, H-2′,6′), 6.90(1H,d,J=16.0 Hz, H-α), 6.87(1H, d, J=2.3 Hz,H-8), 6.86(1H,dd,J=8.6, 2.3 Hz-H-6), 6.78 (2H, d,J=8.6 Hz H-3′,5′), 6.29(1H,s,H-3); 3.86(3H,s.Ar-OCH_3); LC-MS (ESI, negative ion mode): m/z – 263 $(M-H)^-$.

7-Hydroxy -2-(4-methoxystyryl) chromone (20):

Brick yellow solid M.P-248-251^0 C; Analysis: Calcd. for $C_{18}H_{14}O_4$: C-73.46; H-4.76; % , Found: C-73.23; H-4.75; %, IR (KBr)υ_{max} : 3433, 2957,2835,1637,1608,1515,1466, 1237,1135,842,810,764 cm^{-1}; ^{1}H-NMR (DMSO-d_6): δ 9.78 (1H, br, s, Ar-OH), 7.83(1H,d,J=8.8 Hz, H-5), 7.55 (1H, d, J=16.0 Hz; H-β), 7.52(2H,d,J=8.6 Hz, H-2′,6′), 6.96(1H,d,J=16.0 Hz,H-α), 6.89 (1H, d, J=2.2 Hz,H-8), 6.87(1H,dd,J=8.6, 2.2 Hz-H-6), 6.83(2H,d,J=8.6 Hz, H-3′,5′), 6.23(1H,s,H-3); 3.83(3H, s.Ar-OCH_3); LCMS(ESI, Negative ion mode)**:** m/z-293$(M-H)^-$.

BIOLOGICAL ACTIVITY

ANTIBACTERIAL ACTIVITY

Determination by Agar cup method

The antibacterial activity of 2-styrylchromones was studied by agar cup method[19, 20]. Glass Petri dishes used were sterilized and nutrient broth was used as basal medium for testing bacteria. The nutrient broth medium was prepared by taking beet extract (1 gm/lit), yeast extract (2 gm/lit), peptone (5.0 gm/lit), NaCl (5 gm/lit) agar (15 gm/lit) and with pH (7.0); and plated into petri dishes, allowed to solidification. The selected Bacterial culture, single colony was inoculated in to broth medium and kept for overnight at 25 ^{0}C. The overnight Bacterial culture was spread evenly over the entire surface and left undisturbed for few minutes to percolate the culture. Wells (4 mm) were created using a sterile borer into the solidified agar medium. The selected compounds were added to each well (100 & 50 µL) at peripheral and the reference compound (streptomycin) was added at the centre. Thus the prepared plates were incubated at room temperature (at about 25 ^{0}C) for about 3-5 days. After incubation period, the plates were collected and record the inhibition zone in mm (from the margin of the well to surface of inhibition). Dimethyl sulphoxide (DMSO) was used as solvent to prepare the stock solutions (5 mg in 0.5 ml) of the compounds initially and also to maintain proper control. A control well was also placed on the test plates to compare the effect of the test samples and to nullify the effect of solvent (DMSO) respectively.

ANTIFUNGAL ACTIVITY

Determination by disc diffusion method

The antifungal activity was tested by disc diffusion method[21, 22]. The potato dextrose agar was sed as basal medium for testing fungi. The potato dextrose agar medium was prepared by taking yeast extract (3 gm/lit), peptone (10 gm/lit), dextrose (20 gm/ lit), agar (15 gm/lit), distilled water (1 lit) and with pH (6.0) and plated into petri dishes, allowed to solidification. The potato dextrose agar plates were inoculated with each fungal culture (10 days in old) by point inoculation. The filter paper discs (5mm in diameter) impregnated with 100 µL and 50 µL concentrations of the extracts were placed on test organism-seeded plates. DMSO was used to dissolve the tested compounds and was completely evaporated before application on test organism-seeded plates. The blank disk

impregnated with solvent DMSO followed by drying off, was used as negative control and Nystatin (10 µg) used as positive control. The activity was determined after 72 h of incubation at 28^0C. The diameters of the inhibition zones were measured in mm.

RESULTS AND DISCUSSIONS

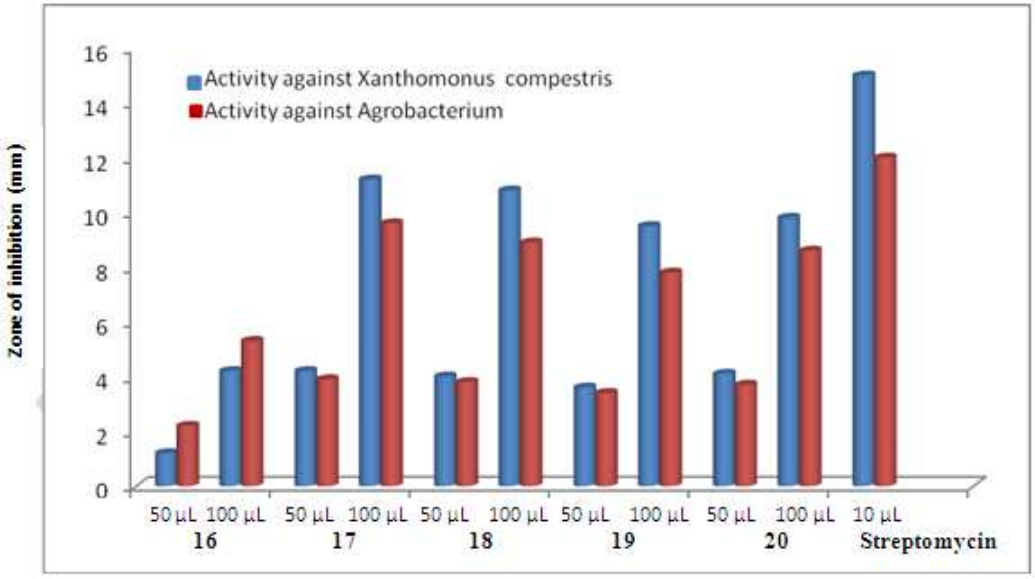
The 2-styrylchromones synthesized for present work were characterized by their physical properties and spectral studies. The IR spectrum of these compounds exhibited a band in the region 1637-1626 cm^{-1} for α, β-unsaturated carbonyl group. The aromatic system of styrylchromones was noticed in the range of 1432-1608 cm^{-1}. The absorption bands at 3374-3433 cm^{-1} showed presence of –OH groups. In the ^{1}H-NMR spectra, the characteristic signals for the H-3 was observed around δ 6.23-6.38 ppm. The resonance signal to β-H (δ 7.53-7.62 ppm) was appeared at higher frequency values than that of α-H (δ 6.96-6.66 ppm) due to the mesomeric deshielding effect of chromone ring. The Trans configuration of C_α-C_β double bond was assigned from the coupling constant values, $J_{H\alpha-H\beta}$ ≈ 16.00 Hz of all synthetic compounds. The hydroxyl protons were resonated at δ ≈ 9.80-9.78 ppm assigned to Ar–OH groups. The aromatic protons were noticed in the range of 8.20-6.78 ppm depending on the number, nature and position of substituents. The chemical shift of H-5 proton of 2-styrylchromones was strongly deshielded by the 4-keto group and appears between δ 8.20-7.79 ppm. LCMS spetra of all the 2-styrylchromones showed intense molecular ion peak in negative ion mode at respective molecular weights.

The antibacterial activity of 2-styryl-chromones (**16**-**20**) was studied *in vitro* by agar cup method at two different concen-trations against *Xanthomonas campestis* and *Agrobacterium tumafeciens* stains. The screening results indicated that all the compounds exhibited antibacterial activities against the tested bacteria. It was noticed that the 2-styrylchromones with chloro and methoxy substitution (**17**, **18** & **20**) exhibited greater inhibitory activity against bacteria compared to the remaining 2-styrylchromones (**16**&**19**). It was also observed that the 2-styrylchromone (**17**) with Cl group at 4'-position exhibited highest bacterial effect than the other 2-styrylchromones against two tested bacteria. The results of diameter of zone of inhibition (in mm) of synthetic 2-styrylchromones have been incorporated in Table-1 and fig.1

Table-1
Antibacterial activity of 2-styrylchromones

Compound	R_1	R_2	Conc. (µL)	**Zone of inhibition** (mm)	
				Xanthomonas campestris	*Agrobacterium tumafeciens*
16	H	H	50	1.2	2.2
			100	4.2	5.3
17	H	Cl	50	4.2	3.9
			100	11.2	9.6
18	H	OMe	50	4.0	3.8
			100	10.8	8.9
19	OH	H	50	3.6	3.4
			100	9.5	7.8
20	OH	OMe	50	4.1	3.7
			100	9.8	8.6
Streptomycine			10	15	12

Figure-1
Antibacterial activity of 2-styrylchromones

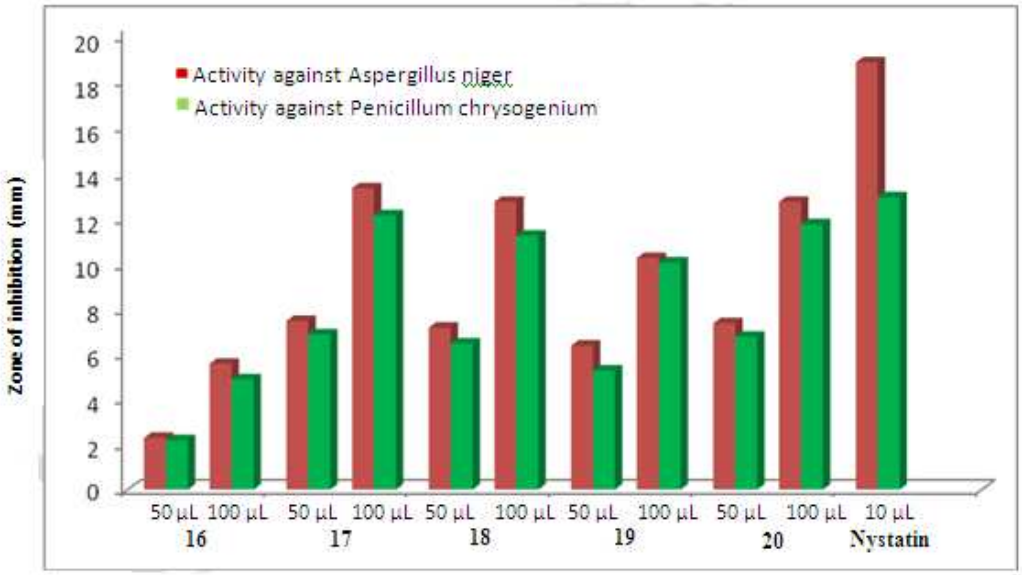


Antifungal activity of 2-styrylchromones (**16**-**20**) was studied *in vitro* at same concentrations by disc diffusion method against two fungal stains and the results indicated that all the compounds exhibited good antifungal activities against the tested fungi. It was noted that the 2-styrylchromone having chloro and methoxy groups (**17**, **18**) showed greater inhibitory activity against both fungi compared to the remaining 2-styrylchromones. Among 102 and 103; 102 exhibited some better antifungal activity than the later. From the results it was concluded that the 2-styrylchromone with methoxy & chloro groups at 4' position was responsible for the greater antifungal effects. The results of diameter of zone of inhibition (in mm) of 2-styrylchromones were incorporated in Table-2 and fig.2.

Table-2
Antifungal activity of 2-styrylchromones

Compound	**R_1**	**R_2**	**Conc. (µL)**	**Zone of inhibition** (mm) *Aspergillus niger*	*Penicillum chrysogenium*
16	H	H	50	2.3	2.2
			100	5.6	4.9
17	H	Cl	50	7.5	6.9
			100	13.4	12.2
18	H	OMe	50	7.2	6.5
			100	12.8	11.3
19	OH	H	50	6.4	5.3
			100	10.3	10.1
20	OH	OMe	50	7.4	6.8
			100	12.8	11.8
Nystatin			10	19	13

Figure-2
Antifungal activity of 2-styrylchromones



CONCLUSION

We have successfully synthesized 2-styrylchromones (16-20) in good yields and carried out their antimicrobial studies which showed good antimicrobial activity. Among synthesized five compounds, chloro and methoxy substituted compounds possessed better antimicrobial properties and also they exhibited better antifungal than antibacterial activity.

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