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Synthesis, antioxidant and antimicrobial activity of three new 2-styrylchromones and their analogues

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ABSTRACT

2-styrylchromones have structural similarities with flavonoids, particularly those belonging to the class of flavones. Three new 2-styrylchromones and their two analogues have been prepared from β–diketones in acidic medium. The structures of all synthesized compounds were confirmed by spectral studies like FT-IR, ^{1}H-NMR, ^{13}C-NMR and Mass Spectroscopy. The antioxidant and antimicrobial activities of synthesized compounds were determined by superoxide free radical (NBT) method and Agar-cup method respectively. Among the five synthesized compounds, ***16***, ***17*** *show good antioxidant activity whereas* ***16***, ***15*** *show better antimicrobial activity.*

Key words: 2-Styrylchromones, β–diketones, spectral studies, antioxidant activity, antimicrobial activity etc.

INTRODUCTION

2-Styrylchromones are new class of flavonoids, structurally related to flavones (2-phenylchromones) and are one of the scarcest classes of natural chromones. Hormothamnione-**1** and 6-desmethoxyhormothamnione-**2** shown in **fig.-1** are the first and the best of our knowledge and are the only naturally occurring styrylchromones. These compounds have been isolated from the marine blue green algae, cryptophyte, chrysophaeum taylori [1,2] in 1986 by W.H.Gerwick. Before and after the isolation of natural 2-styrylchromones, several analogues of these compounds have been synthesized and tested in different biological system. The natural derivatives demonstrated cytotoxic activity against Leukemia cells[1,2] and those obtained by synthesis [3] exhibited anti-allergic, anti-tumor[4], antagonism of A_3 adenosine receptor and xanthine oxidase inhibitor [5] properties.

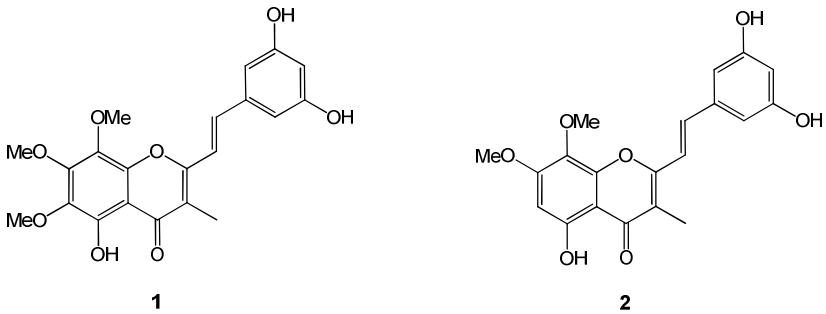


Fig.1 Natural 2-styrylchromones

2-Styrylchromones have been deeply studied and have shown to possess innumerable biological activities [6-11] from which the anti-oxidant [12] properties are the best described. Considering the structural similarities, some of these properties are likely to be shared with 2-styrylchromones, although it needs to be experimentally confirmed.

The 2-Styrylchromone derivatives are characterized by the attachment of a styryl group to the C_2 position of the chromone structure.

Both the natural and synthetic compounds possess considerable biological activities [13, 14-17]. Several 2-styrylchromones-6-carboxylic acid displayed anti-allergic activity [3] when administrated orally. Hormothamnione showed potent cytotoxicity against P 388 hypocitic Leukaemia and HL-60 human promyelotic Leukaemia cells [18, 19], while 6-desmethoxy-hormothamnione and synthetic acids exhibited anti-tumor activity [3] against 9 Kb and colon 38 tumor cells. Recently, 2-styrylchromonols and 2-styrylfuranochromones have been described as A_3 adenosine receptor antagonists.

Various biological activities of 2-styrylchromones inspired to step into this new class of organic compounds. Presently, three new 2-styrylchromones along with their two analogues have been successfully synthesized from β–diketones. Our research group have been actively involved especially in the synthesis of 2-styrylchromones for the last few years.

MATERIALS AND METHODS

The structures of all the synthesized compounds are confirmed by various spectral studies. FT-IR spectra were recorded on a Perkin-Elmer BX1 FTIR spectrophotometer. ^{1}H-NMR (400MHz) and ^{13}C-NMR (400 MHz) spectra were recorded on a Brucker-400 spectrometer using TMS as internal reference and the values for chemical sifts (δ) are given in ppm and coupling constants (J) in hertz (Hz). LCMS was recorded on an agilent-1100 periods LC/MSD (VL). Elemental analysis was performed on a vario EL-III. The TLC was carried out on Merck silica plates. Column chromatography was performed with Merck silica gel of 60-120, 100-200 mesh. Melting points were determined on a Kofler hot-stage apparatus in an open capillary tube. All the other chemicals and solvents used were obtained from commercial sources and used as received standard procedures.

3. EXPERIMENTAL PROCEDURE

General procedure for the synthesis of 2-Styrylchromones (13-17):

B-diketones (**8-12**) were synthesized starting from substituted *o*-hydroxyacetopheno- nes and cinnamic acids [20, 21]. Three new 2-styrylchromones (**13-15**) and their two analogues (**16**&**17**) have been synthesized from β–diketones (**Scheme-1**). The required quantity of β–diketones (1.5 mmol) was dissolved in acetic acid (2.5 mL) in R.B. flask and added 0.1mL of diI.H_2SO_4 with shaking. The reaction mixture was allowed to reflux for 2-3 hours. After cooling, the reaction mixture was poured into crushed ice with stirring and filtered off, washed with water until the washings were no more acidic in nature. The product was dried and then purified by column chromatography with 4:1 hexane: ethyl acetate using 60-120 mesh silica gel as an adsorbent. The physical properties are listed in the table-1.

Table 1: Physical properties of synthesized compounds

Compound	R_1	R_2	M.P (°C)	Yield (%)
13	6-OH	4'-OMe	257-259	84
14	6-OMe	4'-Me	160-162	87
15	6-OMe	4'-OMe	167-169	89
16	6-OH	4'-OH	269-271	82
17	6-OMe	4'-OH	258-260	86

4. BIOLOGICAL ACTIVITY

4.1. ANTIOXIDANT ACTIVITY

Superoxide free radical scavenging activity was determined. The superoxide free radical scavenging activity of 2-styrylchromones was determined by the NBT method [22, 23]. The reaction mixture contained EDTA (6.6 µM), NaCN (3 µg), riboflavin (2 µM), NBT (50 µM), various concentrations of the test drug in ethanol and a phosphate buffer (58 mM, pH 7.8) in a final volume of 3 mL. Optical density was measured at 560 nm. The test tubes were uniformly illuminated with an incandescent lamp for 15 min, after which the optical density was measured again at 560 nm. The per cent inhibition of superoxide radical generation was measured by comparing mean absorbance values of the control and those of the test substances. IC_{50} values were obtained from the plot drawn of concentration in µg versus percentage inhibition and were converted into µM. All the tests were run in triplicate and averaged.

4.2. ANTIMICROBIAL ACTIVITY

4.2a Determination of Antibacterial activity by Agar cup method

The antibacterial activity of 2-styrylchromones was studied by agar cup method [24, 25]. Sterilized glass Petri dishes were used and potato dextrose agar was used as basal medium for test bacteria. The saboroudes broth medium was prepared by taking peptone (1.0 g) and dextrose (4.0 g) in warm distilled water (100 mL). The selected bacteria

culture, single colony was inoculated in to broth medium and kept for incubation for overnight at 25 ^{o}C. The saboroudes agar medium was prepared by taking peptone (1.0 g), dextrose (4.0 g) and agar (2.0 g) in warm distilled water (100 mL) and plated into Petri dishes, allowed to solidification. The overnight bacteria 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).

4.2b Determination of Antifungal activity by Disc diffusion method

The antifungal activity was tested by disc diffusion method [26, 27]. The potato dextrose agar was seed 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 ^{o}C. The diameters of the inhibition zones were measured in mm.

RESULTS AND DISCUSSION

Three new 2-styrylchromones and their two analogues have been prepared in good yield from diketones (**8-12**) according to the sequence shown in **scheme-1**. For this, the dietones were synthesized starting from *o*-hydroxyacetophenones (**3-4**) and cinnamic acids (**5-7**) by treating with phosphorusoxychloride in pyridine solution, followed by treatment with powdered KOH in dry pyridine. The cyclo dehydration of 8-12 followed by hydrolysis of protecting (acetyl) groups was achieved by refluxing in water bath in the presence of acetic acid containing few drops of H_2SO_4 into the desired 2-styrylchromones (**13-17**).

In the ^{1}H-NMR spectra of synthesized compounds, the aromatic protons resonate in the region δ 7.71-6.80 whereas the proton at 3rd position resonates at δ 6.37-6.30 owing to shielding affect. The signals for phenolic hydroxyl groups were observed in the region at δ 9.97-9.52. The methoxy substituent on the aromatic ring displayed signals at 3.98-3.83 ppm as singlets. The *trans* configuration of α and β protons of the styryl group was conformed from the large J values of vicinal protons ($J_{\alpha H-\beta H}$) at around δ 16.00 ppm. The resonances assigned to H-β (δ 7.59-7.29) appear at higher chemical shift value than H-α (δ7.00-6.90) due to the conjugation of C_{α}-C_{β} double bond with carbonyl group. The chemical shift of H-8 proton was strongly deshielded and appears at δ 7.70-7.09 ppm.

The FT-IR spectrum reveals the presence of various functional groups like Ar-OH, -OMe, -Me, -C=O, Ar-C=C etc. by displaying absorption bands at 3389-3349, 2987-2922, 1639-1629 and 1480-1437 respectively. The LCMS spectra of all the 2-styrylchromones showed intense molecular ion peak in respective mass spectra.

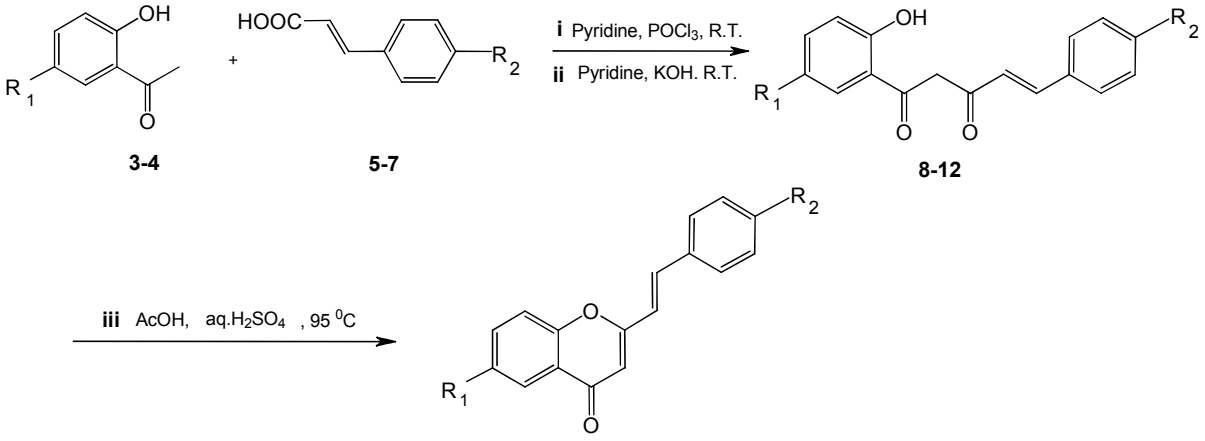
Physical and Spectral data of synthesized compounds

(13): 6-Hydroxy-2-(4-methoxystyryl)chromone: Brick red color solid: Yield 84% (369 mg). M.P. 257-259 °C, Analysis found: C, 73.43; H, 4.74%. Calcd. for $C_{l8}H_{14}O_4$: C, 73.46; H, 4.76%; **FT-IR(cm^{-1}):** 3349, 2932, 1635, 1618, 1466, 1250, 1169.; **^{1}H-NMR (DMSO-d$_6$):** δ 9.81 (1H, br, s, Ar-OH), 7.68 (1H, d, J=8.6 Hz, H-8), 7.57 (1H, d, J=16.3 Hz, H-β), 7.41 (2H, d, J=8.4 Hz, H-2',6'), 7.29 (2H, m, H-5,7), 6.93 (1H, d, J=16.3 Hz, H-α), 6.81 (2H, d, J=8.4 Hz, H-3',5'), 6.37 (1H, s, H-3), 3.98 (3H, s, OCH_3).; **LC-MS (ESI, negative ion mode):** m/z: 293 $(M-H)^{-}$.

(14): 6-Methoxy-2-(4-methylstyryl)chromone: Orange yellow color solid: Yield 87% (380 mg). M.P. 160-162 °C, Analysis found: C, 77.99; H, 5.50%. Calcd. for $C_{19}H_{16}O_3$: C, 78.06; H, 5.52%.; **FT-IR(cm^{-1})::** 1632, 1615, 1468, 1252, 1165.; **^{1}H-NMR (DMSO-d$_6$):** δ 7.60 (2H, d, J=8.8 Hz, H-2',6'), 7.29 (1H, d, J=16.0 Hz, H-β), 7.24 (2H, d, J=8.3 Hz, H-3',5'), 7.09-7.00 (3H, m, H-5,7,8), 6.90(1H, d, J=16.0 Hz, H-α), 6.35 (1H, s, H-3), 3.89(3H, s, OCH_3), 2.3(3H, s, CH_3).; **LC-MS (ESI, negative ion mode):** m/z: 292 $(M+H)^{+}$.

(15): 6-Methoxy-2-(4-methoxystyryl)chromone: Greenish yellow color solid: Yield 89% (409 mg). M.P. 167-169 °C Analysis found: C, 73.96; H, 5.20%. Clad. for $C_{19}H_{16}O_4$: C, 74.01; H, 5.23%.; **FT-IR(cm^{-1}):** 2987, 2878, 1629,1609,1567,1437, 1245, 1160.; **^{1}H-NMR (DMSO-d$_6$):** δ 7.71 (1H, d, J=8.8 Hz, H-8), 7.59 (1H, d, J=16.0 Hz, H-β), 7.50 (2H, d, J=8.3 Hz, H-2',6'), 7.40-7.34 (2H, m, H-5,7), 7.00(1H, d, J=16.0 Hz, H-α), 6.81 (2H, d, J=8.3 Hz, H-3',5'), 6.32 (1H, s, H-3), 3.95(3H, s, OCH_3), 3.83(3H, s, OCH_3).; **LC-MS (ESI, negative ion mode):** m/z: 308 $(M+H)^+$.

Scheme for synthesis of compounds



3-4 **5-7** **8-12**

13-17

Scheme-1

(16): 6-Hydroxy-2-(4-hydroxystyryl)chromone: Reddish brown color solid: Yield 82% (344 mg). M.P. 269-273 °C Analysis found: C, 72.83; H, 4.32%. Calcd. for $C_{17}H_{12}O_4$: C, 72.86; H, 4.29%.;**FT-IR(cm^{-1}):** 3355, 1630, 1612, 1570, 1473, 1250, 1169.; **^{1}H-NMR (DMSO-d$_6$):** δ 9.52 (2H, br, s, 2 x Ar-OH), 7.65 (1H, d, J=8.8 Hz, H-8), 7.50 (1H, d, J=16.0 Hz, H-β), 7.39 (2H, d, J=8.3 Hz, H-2',6'), 7.28-7.21 (2H, m, H-5,7), 6.98 (1H, d, J=16.0 Hz, H-α), 6.80 (2H, d, J=8.3 Hz, H-3',5'), 6.30 (1H, s, H-3).; **LC-MS (ESI, negative ion mode):** m/z: 279 $(M-H)^-$.

(17): 6-Methoxy-2-(4-hydroxystyryl)chromone: Yellowish orange color solid: Yield 86% (378 mg). M.P. 258-260 ^{0}C Analysis found: C, 73.42; H, 4.73%. Clad. for $C_{l8}H_{14}O_4$: C, 73.46; H, 4.76%; **FT-IR(cm^{-1}):** 3389, 2922, 2879, 1639, 1607, 1565, 1480, 1374, 1242, 1168.; **^{1}H-NMR (DMSO-d$_6$):** δ 9.97(1H, br, s, Ar-OH), 7.67(1H, d, J=9.0 Hz, H-8), 7.59 (1H, d, J=16.0 Hz, H-β), 7.49 (2H, d, J=8.2 Hz, H-2',6'), 7.32-7.25 (2H, m, H-5,7), 6.98 (1H, d, J=16.0Hz, H-α), 6.81 (2H, d, J=8.2 Hz, H-3',5'), 6.37 (1H, s, H-3), 3.90 (3H, s, Ar-OCH3).; **LC-MS (ESI, negative ion mode):** m/z: 293 $(M-H)^-$.

Generally, two different mechanisms are used to study the antioxidant activity of the compounds, namely, superoxide scavenging and 1,1-diphenyl-2-picrylhydrazyl (DPPH) radical scavenging activities. But superoxide radical scavenging (NBT) method was used in the present research. In this, superoxide radicals are generated *in vitro* by non-enzymatic system and determined spectrophotometrically (560 nm) by following the Nitro Blue Tetrazolium (NBT) photo reduction method of McCord and Fridovich [22, 23].

The antioxidant activity of the compounds was expressed as the 50% inhibitory concentration (IC_{50}) that was measured from the plot drawn concentration (µM) verses percentages of inhibition and the results are shown in the table-2.

Table 2: Superoxide radical scavenging activity of 2-styrylchromones

Compound	R_1	R_2	IC50 µM
13	6-OH	4'-OMe	268
14	6-OMe	4'-Me	287
15	6-OMe	4'-OMe	275
16	6-OH	4'-OH	234
17	6-OMe	4'-OH	243
BHA	-	-	966
BHT	-	-	381
Vitamin-C	-	-	852
Vitamin-E	-	-	726

From the table-2 data and literature, it is very clear that 2-styrylchromones, having phenolic hydroxyl groups, exhibit very good antioxidant activity than the commercially available antioxidants, namely vitamin C (IC_{50} = 852 µM), vitamin E (IC_{50} = 726 µM), BHA (IC_{50} = 966 µM) and BHT (IC_{50} = 381 µM) (Table-2). The study revealed that the antioxidant activity of 2-styrylchromones increases with the increase in the number of phenolic hydroxyl groups. For example, the compound **16** having more phenolic hydroxyl groups exhibited more activity than the compounds **13, 17** & **14, 15** containing one or no hydroxyl groups. This is due to the stability of aroxyl radical by delocalization of pi-electron, hence the compound containing the phenolic -OH group in particular position can release the –OH proton more readily and act as good antioxidant [28]. It is quite interesting that the compounds having same number of hydroxyl and methoxyl groups exhibited variation in their activity due to difference in the position of substituents. The position of hydroxyl groups has immense influence on the activity. For example, 2-styrylchromone **17** with hydroxyl group at 4' position & methoxy group at 6^{th} position showed better activity than the compound **13** having hydroxyl group at 6^{th} position & methoxy group at 4' position. An introduction of methoxy groups did not show any significant change in the activity. Hence, the number and position of hydroxyl groups play a pivotal role in the antioxidant activity of 2-styrylchromones.

The antimicrobial activity, i.e. antibacterial and antifungal activity of 2-styrylchromones **13-17**, was studied *in vitro* by agar cup & disc diffusion methods respectively against two bacterial strains (*Xanthomonas campesrtis* & *Agrobacterium tumafeciens)* and two fungal strains (*Xanthomonas Campestris* & *Agrobacterium Tumafeciens)* at two different concentrations. The screening results indicated that all the compounds exhibited moderate to good antimicrobial activities against tested strains. It was noticed that the 2-styrylchromones with only hydroxyl substitution, **16** exhibited good inhibitory activity against bacteria, whereas the compounds with only methoxy and/or methyl substitution **14** & **15** showed less antibacterial activity, but the antifungal activity of 15 is more than the remaining compounds. It was observed that the 2-styrylchromones **13** & **17** with variation in the position of hydroxyl and methoxy groups showed some variation in their antimicrobial activities. Hence, these results revealed that the inhibitory activity of synthesized compounds against tested strains depends upon not only on the nature of substituents but also on their relative positions. From the structure-activity analysis, it is very clear that methoxy groups are responsible for decrease the antibacterial activity [29, 30] and enhance the fungal activity of compounds. The results of diameter of zone of inhibition (in mm) of synthetic 2-styrylchromones have been incorporated in table-3.

Table 3: Antimicrobial activity of synthetic 2-styrylchromones

Compound	R_1	R_2	Conc. (µL)	Zone of Inhibition in mm			
				Xanthomonas campestris	*Agrobacterium tumafeciens*	*Aspergillus Niger*	*Penicillum Chrysogenium*
13	6-OH	4'-OMe	50 100	3.9 7.7	3.6 7.2	5.9 11.2	5.7 10.8
14	6-OMe	4'-Me	50 100	3.4 6.7	3.2 6.1	5.4 10.8	5.1 10.6
15	6-OMe	4'-OMe	50 100	3.6 7.1	3.5 6.9	6.7 11.9	6.3 11.7
16	6-OH	4'-OH	50 100	5.7 11.3	5.2 11.0	5.5 11.0	5.3 10.5
17	6-OMe	4'-OH	50 100	4.6 9.2	4.3 9.0	5.7 11.1	5.2 10.7
Strepto-mycine	-	-	10	15	12		
Nystatin	-	-	10			19	13

CONCLUSION

In continuation of our research work on 2-styrylchromones, we have successfully synthesized three new 2-styrylchromones (**13-15**) in good yields along with their two analogues (**16** & **17**). All the synthesized compounds were characterized by spectral data and tested for their antioxidant and antimicrobial activities. The compounds showed good antioxidant activity and moderate antimicrobial activity. Among five synthesized compounds, hydroxy substituted compounds possessed better activities.

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