NUSANTARA BIOSCIENCE
Vol. 4, No. 3, pp. 101-104
November 2012

ISSN: 2087-3948
E-ISSN: 2087-3956
DOI: 10.13057/nusbiosci/n040302

Eco-friendly synthesis and potent antifungal activity of 2-substituted coumaran-3-ones

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Manuscript received: 16 September 2012. Revision accepted: 19 November 2012.

Abstract. *Solanki P, Shekhawat P. 2012. Eco-friendly synthesis and potent antifungal activity of 2-substituted coumaran-3-ones. Nusantara Bioscience 4: 101-104.* 3-halochromones (IIa-c and IIIa-c) have been synthesized by treating 1- (2-hydroxyphenyl)-3-methyl-1,3-propanediones (Ia-c) with bromine or sulphuryl chloride in dioxane respectively. These chromones were employed in the synthesis of 2-acetyl-coumaran-3-ones (IVa-f). These were subjected to Knoevenagel condensation to give 2-cinnamoyl coumaran-3-ones. In vitro assay and field trials of these compounds against *Fusarium oxysporum* were carried out to study the antifungal effect of target compounds. Compound Va was the most effective growth inhibitor of the pathogen, whereas Vc showed a little tendency and Vb, Vd, Ve and Vf hardly inhibit the growth.

Keywords: microwave, cyclodehydration, Knoevenagel condensation

Abstrak. *Solanki P, Shekhawat P. 2012. Sintesis ramah lingkungan dan potensi aktivitas antifungi dari 2-tersubstitusi coumaran-3-on. Nusantara Bioscience 4: 101-104.* 3-halokromon (IIa-c dan IIIa-c) telah disintesis dengan memperlakukan 1- (2-hidroksifenil)-3-metil-1,3-propanedion (Ia-c) dengan brom atau belerang klorida dalam dioksan secara berturut-turut. Kromon ini digunakan dalam sintesis 2-asetil-coumaran-3-ona (IVa-f). Lalu, dilakukan kondensasi Knoevenagel untuk menghasilkan 2-cinnamoyl coumaran-3-on. Uji in vitro dan uji coba lapangan dari senyawa-senyawa ini terhadap *Fusarium oxysporum* dilakukan untuk mempelajari efek antifungi senyawa target. Senyawa Va adalah inhibitor pertumbuhan patogen yang paling efektif, sedangkan Vc menunjukkan kecenderungan sedikit dan Vb, Vd, Ve dan Vf tidak menghambat pertumbuhan.

Kata kunci: microwave, siklodehidrasi, kondensasi Knoevenagel

INTRODUCTION

Synthons having chromone moiety are associated with various biological activities such as antibacterial (Tanaka et al. 2009), antifungal, antiallergic and diuretic (Abraham and Rotella, 2010). Substitution of halogen in these molecules enhanced the above activities, likewise 2-coumaranones, i.e. 3H-2-benzofuranones are also proved to be potential synthons for various products extended for agriculture or having physiological effects. Therefore, processes are continuously being sought which allow it to be obtained rapidly and cheaply from inexpensive commercially available products. Researchers have also synthesized coumaranone from cyclohexanone and glyoxalic acid in presence of dehydration catalyst (Vallejos et al. 1997). Process has been also described for preparation of enol lactone 2-oxocyclihexidine acetic acid and to its application to preparation of 2- coumaranone by (Carmona et al. 1998). Benzofuranone derivatives found to possess potential antipsychotic (Aranda et al. 2008), anticancer (Charrier et al. 2009; Mishra et al. 2011), peroxidase activity (Ghadami et al. 2012), cytotoxicity activity (Terasawa et al. 2001), antibacterial activity (Hadj-Esfandiari et al. 2007) and other biological activities (Li and Chen et al. 2008; Adadiran et al. 2001). With reference to observation and versatility of chromones and coumaranones, attempts have been made to synthesize the compound under microwave irradiation (Goncalo et al. 1999) with a rapid environment benign, cleaner and cheaper work up.

MATERIALS AND METHODS

Eco-friendly synthesis

All chemicals and solvents were purchased from Sigma Aldrich. Melting points were determined by open capillary methods on a 'Veego' VMP-D apparatus and are uncorrected. TLC was done using silica gel G plates using 3x8 cm (Sigma-Aldrich) and visualized in an iodine chamber. The IR spectra (KBr) were determined on "Perkin Elmer 577 spectrometer and the values are expressed in cm^{-1} and H1NMR (chemical shift in δ ppm) were recorded on Perkin Elmer R-32 and Varian XL-100A high NMR spectrophotometer using TMS as reference either in $CDCl_3$ or DMSO-d_6 as solvent. C, H and N analyses were carried out on Carlo Erba 1106 Analyser (Italy). Physical

parameters and schematic diagram of this eco-friendly synthesis can be shown in Table 1 and Figure 1.

3-bromo-2-methylchromones (IIa-c): 1- (2-hydroxy-phenyl)-3-methyl-1-propane diones (Ia-c) (10 mmol) was dissolved in dioxane (0.5 mL) and solution of pure bromine (0.5 mL) in dioxane (15 mL) was added with constant stirring. The reaction mixture was warmed and kept for 30 minutes. After cooling, the reaction mixture was diluted with water and then the crude product was filtered and crystallized using ethanol to get 75-80% yield. Ia-IR in cm^{-1}-1630 (C=O), 1490 (C=C), 1340 (γ-yrone). PMR-δ 2.44 (s, 3H, Ar-CH_3), 2.62 (s, 3H, heteroaromatic), 7.25-8.00 (m, 3H, Ar-H). UV λ_{max} 310 mm.

3-Chloro-2-methylchromones (IIIa-c): A mixture of 1- (2-Hydroxyphenyl)-3-methyl-1,3-propanediones (Ia-c) (10 mmol) and sulfuryl chloride (10 mmol) in dioxane (25 mL) was irradiated under microwave at 450 W for 45 sec with intermittent heating. It was then diluted and crude product thus obtained was crystallized from ethanol to get 70-75% yield. IIIa-IR in cm^{-1}-1640 (C=O), 1490 (C=C), 1340 (γ-pyrone). PMR-δ 2.45 (s, 3H, Ar-CH_3), 2.62 (s, 3H, heteroaromatic), 7.25-8.00 (m, 3H, Ar-H). UV λ_{max} 305 mm.

2-Acetyl coumaran-3-ones (IVa-c): A solution of 3-halochromones (IIa-c) or (IIIa-c) (1 g) in ethanol (20 mL) was treated with aq. KOH solution separately and the reaction mixture was exposed to MW for 1 minute. Cool and diluted product was acidified with HCl. The crude product thus obtained was crystallized from ethanol to get compound (IVa-c) in 50-60% yield. IVa-IR in cm^{-1}-3340 (-OH), 1625 (C=O), 1490 (C=C), 2945 (C-H). PMR - δ 2.44 (s, 3H, Ar-CH_3), 2.46 (s, 3H, $COCH_3$), 6.25 (s, 1H,CO-CH), 7.21-8.00 (m, 3H, Ar-H). UV λ_{max} 345 mm.

2-Cinnamoyl coumaran-3-ones (Va-f): A mixture of 2-acetylcoumaran-3-one (IVa-c) (10 mmol) and aromatic aldehyde (20 mmol) in ethanol (20 mL) and few drops of piperidine (0.5 mL) was exposed to MW for 1 minute with intermittent heating. After cooling the reaction mixture was diluted, filtered and crystallized from ethanol to get 65-70% yield. Va-IR in cm^{-1}-2950 (-OH),1600 (C=O). PMR-δ 2.41 (s, 3H, Ar-CH_3), 7.21-7.82 (m, 8H, Ar-H), 6.5-7.1 (dd, 2H,-CH=CH).

Table 1. Physical parameters of the eco-friendly synthesis

Sr. No.	Entry	M.F.	R	R^1	R^2	M.W. Sec	M.P. (°C)
1.	IIa	$C_{11}H_9O_2Br$	CH_3	H	-	-	132
2.	IIb	$C_{10}H_7O_2Br$	H	H	-	-	132
3.	IIc	$C_{11}H_9O_2Br$	H	CH_3	-	-	93
4.	IIIa	$C_{11}H_9O_2Cl$	CH_3	H	-	45	124
5.	IIIb	$C_{10}H_7O_2Cl$	H	H	-	40	128
6.	IIIc	$C_{11}H_{10}O_2Cl$	H	CH_3	-	45	121
7.	IVa	$C_{11}H_{10}O_3$	CH_3	H	-	60	126
8.	IVb	$C_{10}H_8O_3$	H	H	-	55	138
9.	IVc	$C_{11}H_{10}O_3$	H	CH_3	-	60	142
10.	Va	$C_{18}H_{13}O_3$	CH_3	H	H	60	119
11.	Vb	$C_{19}H_{15}O_4$	H	H	OCH_3	60	211
12.	Vc	$C_{17}H_{11}O_4$	H	H	H	60	154
13.	Vd	$C_{18}H_{13}O_4$	H	H	OCH_3	65	134
14.	Ve	$C_{18}H_{13}O_3$	H	CH_3	H	60	140
15.	Vf	$C_{19}H_{15}O_4$	H	CH_3	OCH_3	65	149

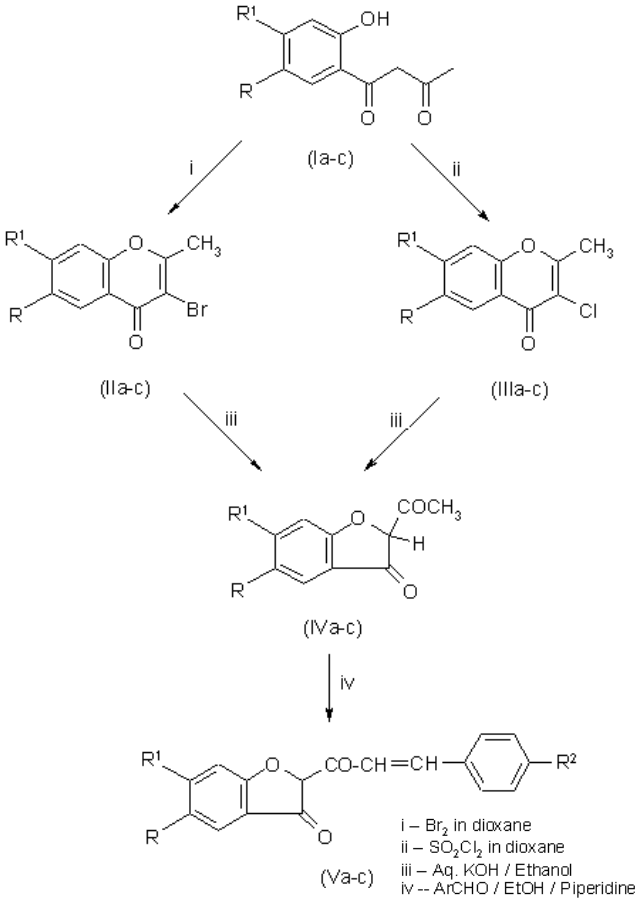

Figure 1. Scheme of the eco-friendly synthesis

In vitro assay of target compounds against *Fusarium oxysporum*

Application and utility of heterocycles in agriculture crop to eradicate the alarming diseases has drawn the attention of research scientist. The following samples were tested at the concentration noted against *Fusarium oxysporum* by using poisoned food technique (Schmitz, 1930). One week old culture of *F. oxysporum* was grown on potato dextrose agar medium (PDA) in Petri plates for assessing efficiency of newly synthesized samples. Solutions of 100 ppm concentration were taken in 250 mL conical flask containing 100 mL of sterilized and melted potato dextrose agar medium, mixed thoroughly by gentle swirling the flask and poured into a sterile Petri disc and allowed to solidify. A 8 mm culture disc pathogen is *F. oxysporum* was inoculated and the plates were incubated in an inverted position at room temperature. Inoculated PDA medium without sample served as control. Three replications were maintained. The mean radial growth of the colony was measured at 48, 72 and 96 hrs after inoculation respectively. The results were expressed as percent inhibition over control (Table 2). The percent inhibition of the growth was calculated by the formula of Vincent (1927).

$$I = (C-T)/C \times 100$$

I: Inhibition of mycelia growth, C: Growth in control, T: Growth in treatment

Table 2. Radial growth of *F. oxysporum* on PDA medium at 100 ppm concentration of samples at 48, 72 and 96 hrs of incubation; and percent growth inhibition of *F. oxysporum* at 100 ppm after 96 hours.

Sample	Mean radii growth (mm)			% Growth inhibition after 96 hrs
	48 hrs	72 hrs	96 hrs	
Va	0.00	0.00	0.00	100.00
Vb	24.33	44.66	68.83	0.72
Vc	15.83	26.50	41.83	39.66
Vd	25.33	45.16	69.50	-0.24
Ve	24.33	46.16	69.16	0.28
Vf	24.66	45.83	69.33	0.00
Control	25.66	46.33	69.33	-

Among the six samples tested, sample Va was the most effective recorded percent growth inhibition over control after 96 hrs of incubation. Compound Vc also showed 39.66% of inhibition whereas samples Vb, Vd, Ve and Vf did not inhibit the growth of *F. oxysporum*.

Control of fungal diseases in agricultural crops

The most general means of controlling plants diseases is in field, greenhouse and sometimes in storage, through the use of chemical compounds that are toxic to the pathogens, such chemical either inhibits germination, growth, and multiplication of the pathogen or outright lethal to the pathogen. In this part, the synthesized compounds Va and Vc had been tested on Bengal grams (Chickpea or Gram; *Cicer aritinum* L.) whose growth is generally retarded due to vascular cause by *F. Oxysporum* (Table 3-4).

Gram is mostly affected by wilt *F. oxysporum*. It is most important rabi crop grown on large scale in India. So in the present work, in vitro assay of compound I and IV that have shown to possess a remarkable fungicidal activity, had been selected to evaluate the antifungal activity against *F. oxysporum*, a pathogen of Bengal gram crop. Post culture method is adopted in this experiment. Studies have been carried out in triplicate to select the requisite concentration of newly synthesized fungicide for plant growth.

Design of experiment

Seven pots of the size 30x20 cm were taken for the three replication to increase the precision of the experiment. Approximately 1.5 kg soil for each pot was autoclaved for complete sterilization. A control pot C was filled up with sick soil 100 g of fungal culture had been mixed with soil in 100: 1 proportion. Three pots for each sample were filled with sick soil and labeled as P_1-P_3 and Q_1-Q_3. Pregerminated seeds of Bengal gram were procured from Krishi Vigyan Kendra Durgapur, Amravati for these trials. Aqueous solution of 100 pp, of the sample P and Q were prepared and ten seeds for each replication, soaked in the sample solution and allowed to dry. Treated seeds sowed in pot P_1-P_3 and Q_1-Q_3. Seeds soaked in distilled water were sown in control pot C. Some physical parameters like (a) percent germination (b) number of leaves per plant (c) plant height and (d) mortality had been observed and noted periodically.

Table 3. Fungicide effect of newly synthesized compound Va on *Cicer arietinum*

Periodicity days	Observations for control experiment				Replications											
					A				B				C			
	a	b	c	d	a	b	c	d	a	b	c	d	a	b	c	d
8	20	8	4	80	70	7	5	30	80	8	5	20	75	8	5	25
15	-	30	10	-	80	75	20	-	85	74	20	-	80	72	25	-
30	-	25	10	10		90	25	-	-	95	25	-	-	75	29	-
45	-	25	10	5		102	27	-	-	105	29	-	-	98	32	-
75	-	26	10	-		125	30	-	-	115	25	-	-	113	25	-
120	-	-	-	-		130	35	-	-	120	32	-	-	118	35	-

Table 4. Fungicide effect of newly synthesized compound Vc on *Cicer arietinum*.

Periodicity days	Observations for control experiment				Replications											
					A				B				C			
	a	b	c	d	a	b	c	d	a	b	c	d	a	b	c	d
8	20	8	4	80	70	6	4	30	92	8	5	28	65	6	5	-
15	-	30	10	-	75	62	20	25	68	65	22	32	70	70	25	-
30	-	25	10	10	-	85	26	-	-	80	25	-	-	80	30	-
45	-	25	10	5	-	98	26	-	-	95	27	-	-	92	33	-
75	-	26	10	-	-	115	24	-	-	120	29	-	-	118	35	-
120	-	-	-	-	-	133	23	-	-	130	30	-	-	125	35	-

Note: a-% Germination, b-No. of leaves per plant, c-Plant height, d-Mortality

RESULTS AND DISCUSSION

Cyclodehydration of substituted 1, 3-propanedione (Ia-c) with bromine in dioxane gave 3-bromo-2-methyl chromones (IIa-c), which is characterized by γ pyrone nucleus. IR of IIa shows characteristic peak at 1340 cm^{-1} (γ-pyrone) and disappearance of phenolic-OH singlet of 1,3-propane-diones. PMR spectra also show prominent signals at 2.44 (s, Ar-CH_3), 2.63 (s, Ar-CH_3 heteroaromatic) and 7.25-8.00 (m, 3H, Ar-H) whereas signals of keto-enol tautomers of 1, 3-dione get disappeared due to cyclodehydration. Similarly, 1,3-propanedione (Ia-c) reacts with sulfuryl chloride in dioxane under microwave at 450 W for 45 sec gave 3-chloro-2-methylchromones (IIIa-c) in 80% yield. IR and PMR showed presence of γ-pyrone nucleus and absence of signals of phenolic-OH and keto-enol tautomerism.

2-Acetylcoumaranones (IVa-c) have been synthesized from a solution of 3-halochromones (IIa-c) or (IIIa-c) in ethanol in aqueous alkaline medium under microwave exposure for one minute. PMR signal at 6.25 δ is identified as (-COCH) coumaran proton and an acetyl signal at 2.46 δ. In IR spectra frequency for γ-pyrone (1340 cm^{-1}) get disappeared, a signal at 3340 cm^{-1} (-OH) also confirm the structure (IVa-c). These compounds (IVa-c) are subjected to condensation with aromatic aldehyde (0.02 mol) in

ethanolic condition in presence of piperidine under microwave radiation. The absence of acetyl signal and appearance of-COCH=CH-dd at 7.19-7.25 range confirms the presence of cinnamoyl group and a singlet at 9.18 δ for-OH group. IR frequency at 2950 cm^{-1} (-OH) and 1600 (>C=O) also correlates with the structure as 2-cinnamoylcoumaran-3-ones (Va-f).

A remarkable antifungal activity was shown by compound Va and Vc. Fungicidal activity of these compounds was found to be excellent in different trials in vitro assay against. *F. oxysporum*. Compound Va had shown to have better fungicidal effect as compared to compound Vc. In control experiments rate of mortality is found to be very high due to sick soil, which diminished in remarkable extent in three trials where the seeds were treated with synthesized compounds. Thus synthesized heterocycles are found to possess potential antifungal activity

CONCLUSION

Microwave-assisted organic synthesis has attracted attention in recent years due to enhanced reaction rates, higher yields, improved purity, ease of work up after reaction and eco-friendly reaction conditions compared to the conventional methods. Some of the synthesized compounds possess potent antifungal activities against *Fusarium oxysporum*. Compound Va was the most effective growth inhibitor of the pathogen, whereas Vc showed a little tendency and Vb, Vd, Ve and Vf hardly inhibit the growth. Candidly, these are few prototype trials taken to root out the overwhelming situation prevailing in biosphere. This work will definitely provide access to organic chemists synthesizing everlasting number of compounds of high potent which may serve mankind.

ACKNOWLEDGEMENTS

The author expresses their sincere thanks to the Principal of Vidya Bharti Mahavidyalaya, Amravati, Maharashtra, India and the Director of Krishi Vigyan Kendra, Durgapur Badnera, Maharashtra, India for providing necessary laboratory facilities.

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