Synthesis, characterization and physiological activity of some novel isoxazoles

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Abstract. Hushare VJ, Rajput PR, Malpani MO, Ghodile NG. 2012. Synthesis, characterization and physiological activity of some novel isoxazoles. Nusantara Bioscience 4: 81-85. A series of chlorosubstituted 4-arylisoxazoles have been synthesized by refluxing chlorosubstituted-3-aryllavones and 3-alkoylchromone with hydroxylamine hydrochloride in dioxane medium containing 0.5 mL piperidine. Chlorosubstituted-3-aryllavones and chlorosubstituted-3-alkoylchromone were prepared by refluxing them separately with iodine crystal in ethanol. Initially chlorosubstituted-3-aryllavones and 3-alkoylchromone were prepared by the interaction of different aromatic and aliphatic aldehydes with 1-(2-hydroxy-3,5-dichlorophenyl)-3-phenyl-1,3-propanedione. Constitutions of synthesized compounds were confirmed on the basis of elemental analysis, molecular weight determination, UV-Visible, I.R., and 1H-NMR spectral data. The titled compounds were evaluated for their growth promoting activity on some flowering plants viz. Papaver rhoeas, Calendula officinalis, Gladiola tristis, Gaillardia aristata, Dionthus chinensis, and Iberis sp. (candytuft). The results indicate that applied plants had higher shoots and more number of leaves.

Keywords: chlorosubstituted 3-aryllavones, 3-alkoylchromone, 3-aryllavones, 3-alkoylchromone, isoxazoles.

Abstrak. Hushare VJ, Rajput PR, Malpani MO, Ghodile NG. 2012. Sintesis, karakterisasi dan aktivitas fisiologis beberapa isoxazoles baru. Nusantara Bioscience 4: 81-85. Serangkaian chlorosubstituted-4-arylisoxazoles telah disintesis dengan mencampur chlorosubstituted-3-aryllavones dan 3-alkoylchromone dengan hidroksilamin hidroklorida dalam medium dioksan yang mengandung 0.5 mL piperidine. Chlorosubstituted-3-aryllavones dan chlorosubstituted-3-alkoylchromone dibuat dengan cara mencampur keduanya secara terpisah dengan yodium kristal dalam etanol. Awalnya, chlorosubstituted-3-aryllavones dan 3-alkoylchromone dibuat melalui interaksi antara aldehida aromatik dan alifatik yang berbeda dengan 1-(2-hidroksi-3,5-Dikhlorofenil)-3-fenil-1,3-propanedione. Senyawa yang terbentuk diuji berdasarkan analisis unsur, berat molekul, serta data spektrum UV-Vis, IR dan 1H-NMR. Senyawa tersebut diuji berdasarkan aktivitas fisiologisnya dalam mendorong pertumbuhan beberapa tanaman berbunga, yaitu: Papaver rhoeas, Calendula officinalis, Gladiola tristis, Gaillardia aristata, Dionthus chinensis, dan Iberis sp. (candytuft). Hasilnya, tanaman yang diaplikasikan dengan senyawa tersebut lebih tinggi dan jumlah daunnya lebih banyak.

Kata kunci: chlorosubstituted 3-aryllavones, 3-alkoylchromone, 3-aryllavones, 3-alkoylchromone, isoxazoles.

INTRODUCTION

Heterocyclic compounds are widely distributed in nature and are essential for the sustainable life on earth (Joule and George 1978). The azoles containing one oxygen and one nitrogen atom at 1, 2-position are designated as isoxazoles. Isoxazole derivatives have been found to possess a broad spectrum of biological activities such as anti-inflammatory (Balvi and Anzaldi et al. 2005), antibacterial (Magar et al. 2011), anticonvulsant (Muthukumar et al. 2011), antiobiotic (Doyle et al. 1961), anti-tubercular (Haripara et al. 2004) anxiolytic (Wagner et al. 2004), antiepileptic (Schwate and Willmre 2001), PPAR-δ (Epple et al. 2006), agonists, acetylcholine-esterase (Manelungu et al. 2004) and erosive inhibitor (Gupta et al. 2005), antifungal (Flores et al. 2006), antitumor (Ramana and Reddy 2011), analgesic (Osman et al. 2012), chemotherapy (Hamada and Sharshira 2011), antiarthritic (Patterson et al. 1992), antiviral (Goda et al. 2003), anesthetic, anticancer, hypolipidemic (Coutoulis-Arroyopoulou et al. 2006), antiarrhythmic (Inoue et al. 1991), insect antiinfedent (Son et al. 2006), glycogen phosphorylaseinhibitor (Benfita et al. 2006), antipsychotic (Markourtz et al. 1999), anticoagulant (Qan et al. 1999), acetylene esterase inhibitor (Manelungu et al. 2004), CNS active (Griesbeck et al. 2011), isoxazole derivatives controlled Botryis cinerea on cucumbers (Shionogi and Co. Ltd. 1983) and thrombosis (Padmavaithi et al. 2000). One of the isoxazole derivatives was found to have antiviral properties against herpes type-2 virus (Sterling Drug Inc 1982), whereas some isoxazole derivatives also reported having corrosion inhibitor properties for fuels and lubricants (Marzinzik and Felder 1997).
The newly synthesized compounds successfully screened for their growth promoting impact on some flowering plants, viz. *Papaver rhoeas*, *Calendula officinalis*, *Gladiolus tristis*, *Gaillardia aristata*, *Dianthus chinensis*, and *Iberis* sp.

**MATERIALS AND METHODS**

This research includes synthesis, characterization and physiological activity test of some novel isoxazoles on some flowering plants. The melting points of all synthesized compounds were recorded using hot paraffin bath and are uncorrected. Chemicals used were of A.R. Grade. $^1$H NMR spectra using acetone. I.R. spectra were recorded on Perkin-Elmer spectrophotometer in the range 4000-400 cm$^{-1}$ in nujol mull and as KBr pellets. UV-VIS spectra were recorded in nujol medium.

**RESULTS AND DISCUSSION**

**Synthesis of new isoxazoles**

Synthesis pathway of novel compounds of isoxazoles is shown in Figure 1.

**Synthesis of 2-benzoyloxy-3, 5-dichloroacetophenone (3a), 2-Hydroxy-3, 5-dichloroacetophenone (2a)** (0.04 mol) and benzoyl chlorides (0.05 mol) were dissolved in 10% NaOH (30 mL). The reaction mixture was stirred for about half an hour. The products thus separated was filtered, washed with sodium bicarbonate (10%) washing and then again with water. The solid product thus separated was crystallized from ethanol to get the compound m.p. 64°C.

**Synthesis of 1-(2′-hydroxy-3′, 5′-dichlorophenyl)-3-phenyl-1,3-propanedione (4a) 2-Benzoyl-3,5-dichloroacetophenone (3a)** (0.05 mol) was dissolved in dry pyridine (40 mL). The solution was warmed up to 60°C and pulverized KOH (15 g) was added to it slowly with constant stirring. Then it was kept overnight and acidified by adding ice cold HCl (10%). The brownish yellow solid product thus separated was filtered, washed with sodium bicarbonate solution (10%) and finally again with water. It was then crystallized from ethanol to get the compound (4a) m.p. 110°C.

**Synthesis of 3-benzoyl-2-(4′-nitrophenyl) -6, 8-dichloroflavone (5a)**. A mixture of 1-(2’-hydroxy-3’,5’-dichlorophenyl)-3-phenyl-1,3-propanedione (4a) (0.01 mol) and p-nitrobenzaldehyde (0.02 mol) was refluxed in dioxane (25 mL) containing 0.5 mL piperidine for 15 -20 min. After cooling the reaction mixture was acidified with dil. HCl (20%). The product thus separated was crystallized from ethanol to get the compound (5a). Similarly 3-benzoyl-2-(4′-chlorophenyl)-6,8-dichloroflavone (5b) and 3-benzoyl-2-buty1-6,8-dichlorochromone (5c) were synthesized separately from the compound (4a) by using p-chlorobenzaldehyde and valeraldehyde respectively.

**Synthesis of 3-benzoyl-2-(4′-nitrophenyl)-6, 8-dichloroflavone (6a)** 3-Benzoyl-2-(4′-nitrophenyl)-6, 8-dichloroflavone (5a) (0.01 mol) was refluxed for about 10 minutes with crystal of iodine in ethanol (20 mL). After cooling the reaction mixture was diluted with water. The solid product thus separated was filtered, washed with sodium bicarbonate solution and then with water. Finally, it was crystallized from ethanol to get the compound (6a). Similarly compounds 3-benzoyl-2-(4′-chlorophenyl)-6, 8-dichloroflavone (6b) and 3-benzoyl-2-buty1-6, 8-dichlorochromone (6c) were synthesized separately from the compounds (5b) and (5c) respectively.

**Synthesis of 3-(2′-hydroxy-3′, 5′-dichlorophenyl)-4-benzoyl-5-(4′-nitrophenyl)-isoxazole (7a)** A mixture of 3-benzoyl-2-(4′-nitrophenyl)-6,8-dichloroflavone (6a) (0.01 mol) and hydroxylamine hydrochloride (0.02 mol) was refluxed in dioxane (20 mL) containing 0.5 mL piperidine for two hours. After cooling the reaction mixture was diluted with water. The product thus separated was filtered crystallized from ethanol to get the compound (7a). Similarly other compounds 3-(2′-hydroxy-3′,5′-dichlorophenyl)-4-benzoyl-5-(4′-chlorophenyl)-isoxazole (7b) and 3-(2′-hydroxy-3′,5′-dichlorophenyl)-4-benzoyl-5-buty1-isoxazole (7c) were synthesized separately from the compounds (6b) and (6c) respectively.

**Characterization of titled compounds**

The newly synthesized compounds were characterized on the basis of elemental analysis, molecular determination, U.V., I.R., N.M.R. spectral analysis

**Compound (5a)** Yield 75%, M.P. 90°C: Elemental analysis for C$_2$H$_3$O$_2$Cl$_2$: Found C = 59.68, H = 2.86, N = 3.16. Calculated C = 59.72, H = 2.94, N = 3.16%)

**Compound (5b)** Yield 70%, M.P. 120°C: Elemental analysis for C$_2$H$_3$O$_2$Cl$_2$: Found C = 61.10, H = 2.96., Calculated C = 61.18, H = 3.01, %. UV spectrum (EtOH), $\lambda_{max}$ -364 nm (n $\rightarrow$ π*) IR (cm$^{-1}$) 3050.5 (C=H stretching in Ar) 1611.4 (C=O), 1437.9 (C-N=O), 1278 (-C-O), 1182.6 (-C-O) 717.7 cm$^{-1}$ (-C-Cl). $^1$H NMR spectrum (400 MHz, CDCl3), δ ppm 6.75 (1H, d, -CH-CH) 7.39 (1H, d, -CH-CH), 6.875 -8.712 (11H, m, Ar-H) (Silverstein et al. 1991).

**Compound (5c)** Yield 70%, M.P. 120°C: Elemental analysis for C$_2$H$_3$O$_2$Cl$_2$: Found C = 61.10, H = 2.96., Calculated C = 61.18, H = 3.01, %. UV spectrum (EtOH), $\lambda_{max}$ -363 nm (n $\rightarrow$ π*) IR (cm$^{-1}$) 3068.7 (C=H stretching in Ar) 1612.6 (C=O), 1427.7 (C=H in Ar), 1086.5 (C-O), 764.6 cm$^{-1}$ (-C-Cl). $^1$H NMR spectrum (400 MHz, CDCl3), δ ppm 6.76 (1H, d, -CH-CH) 6.79 (1H, d, -CH-CH), 6.92-8.315 (11H, m, Ar-H).

**Compound (5d)** Yield 75%, M.P. 155°C: Elemental analysis for C$_2$H$_3$O$_2$Cl$_2$: Found C = 63.54, H = 4.50, Calculated C = 63.66, H = 4.77, %. UV spectrum (EtOH), $\lambda_{max}$ -363 nm (n $\rightarrow$ π*) IR (cm$^{-1}$) 3068.7 (C=H stretching in Ar) 2866.4 (C=H stretching in Ar), 2866.4 (C=H in -CH$_2$), 1689.5 (C=O), 1605.8 (C=H stretching in Ar) 1547.7 (-CH$_2$ Bending), 1286.3 (C =O in ether), 705.4 cm$^{-1}$ (-C-Cl). $^1$H NMR spectrum (400 MHz, CDCl3), δ ppm 0.90 (3H, t, -CH$_2$-CH$_3$) 1.25 (2H, m, CH$_2$-CH$_2$-CH$_2$) 1.50 (2H, t, -CH$_2$-CH$_2$-CH$_2$) 2.65 (2H, t, CH$_2$-CH$_2$-CH$_2$) 6.88 (1H, d, C-H), 6.93 (1H, d, C-H) 7.00 -8.225 (7H, m, Ar-H).

**Compound (6a)** Yield 80%, M.P. 164°C: Elemental analysis for C$_2$H$_3$O$_2$Cl$_2$: Found C = 59.00, H = 2.30, N = 3.00, Calculated, C = 60.00, H = 2.50, N = 3.18, %.


Compound (6b)- Yield 75%, M.P. 168°C. Elemental analysis for C22H12O3Cl: Found C = 61.14, H = 2.20. Calculated C = 61.18, H = 2.56. UV spectrum (EtOH), λmax - 345 nm (n → π*) IR (cm⁻¹), 2917.8 (C-H stretching in Ar), 1639.5 (C=O), 1091.1 (C-O in ether), 768.2 cm⁻¹ (C-Cl). ¹H NMR spectrum (400 MHz, CDCl3), δppm 6.848-8.319 (11H, Ar-H).

Compound (6c)- Yield 80%, M.P. 162°C. Elemental analysis for C23H13O3Cl: Found C = 63.60, H = 4.10. Calculated C = 64.00, H = 4.27, %. UV spectrum (EtOH), λmax 301nm (n → π*), IR (cm⁻¹) 2942.5 (C-H bending), 2851.7 (CH str. in alkane), 1637.9 (C=O), 1431.7 (C-H bending). ¹H NMR spectrum (400 MHz, CDCl3), δppm 6.915-8.129 (11H, Ar-H).

Compound (7a)- Yield 80%, M.P. 148°C. Elemental analysis for C22H12O3N2Cl2: Found C = 57.80, H = 2.40, N = 6.00. Calculated C = 58.02, H = 2.64, N = 6.15 %.

Compound (7b)- Yield 85%, M.P. 160°C. Elemental analysis for C22H12O3NCl3: Found C = 59.15, H = 2.50, N = 3.00. Calculated C = 59.39, H = 2.69, N = 3.14 %. UV spectrum (EtOH), λmax 312nm, IR (cm⁻¹) 1623 (C=O), 1604.1 (C=N), 1464.1 (C=N), 1275.1 (C-N), 763 cm⁻¹ (C-Cl). ¹H NMR spectrum (400 MHz, acetone), δppm 6.915-8.129 (11H, Ar-H).

Compound (7c)- Yield 80%, M.P. 165°C. Elemental analysis for C22H12O3N2Cl2: Found C = 58.02, H = 2.64, N = 6.15 %. UV spectrum (EtOH), λmax 301nm, IR (cm⁻¹) 3021 (C=O), 2917.8 (C-H bending), 1639.5 (C=O), 1091.1 (C-O in ether), 768.2 cm⁻¹ (C-Cl). ¹H NMR spectrum (400 MHz, acetone), δppm 6.915-8.129 (11H, Ar-H).

Figure 1. Synthesis of novel compounds of isoxazoles

Where R = C₆H₅-N=O₂, C₆H₅-Cl, (CH₂)₃-CH₃
Growth promoting activities on some flowering plants
The experimental set up of the study was divided into seed treatment and field experiment. (i) Seed treatment: with a view to safeguard dormant seed’s potential from harmful external agencies, the seed of the test plants were treated by test compounds before sowing. (ii) Field experiment: pre-germinated quality seeds of P. rheas, C. officinalis, G. tristis, G. aristata, D. chinensis, and Iberis sp. were procured from genuine agricultural agencies.

The beds of black cotton soil, 2.5 x 2.5m size were prepared on an open field. The sowing of seeds of all six flowering plants under examination was done in beds and earthen pots separately by conventional methods and irrigated as and when required. The plants from each bed and pot were divided into two groups, i.e., A and B which were designated as ‘control’ and ‘treated’ group plants respectively. The plants from group B were sprayed with the solution of test compounds at fortnightly intervals. The field experiments were conducted to compare the treated plants of group B with untreated plants of controlled group A. In this context, the observation was recorded on 15, 30, 45, 60, 75 and 90 days after sowing; corresponding to early vegetative, late vegetative, flowering, pod filling and pod maturation stages, with special reference to number of leaves and height of shoots. The results of field’s experiments with test compounds are tabulated in Table 1.

Data presented in Table 1 clearly indicates that shoot heights of P. rheas and C. officinalis, G. tristis, G. aristata, D. chinensis, and Iberis sp. up to 45 days did not show any significant increase. However, the shoot heights increased to a remarkable extent from 60 days onwards after sowing, and it continued with the same drift up to 90 days in almost all the treated plants as compared to the plants of control group. This trend of substantial increase in shoot height geared up in P. rheas and C. officinalis right from the second week of the experiment. It has been found that control group plants overshadowed this trend in the third observation only in the plants P. rheas and C. officinalis treated with compound 4a.

Table 1. Activity of the test compounds (4a), (5a), (6a), (7a)

<table>
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<tr>
<th>Periodicity of the observation (in days)</th>
<th>Poppy (P. rheas)</th>
<th>Calendula (C. officinalis)</th>
<th>Gladiolus (G. tristis)</th>
<th>Gaillardia (G. aristata)</th>
<th>Pink (D. chinensis)</th>
<th>Candytuft (Iberis sp)</th>
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Activity of the test compound 1-(2’-hydroxy-3’, 5’-dichlorophenyl)-3-phenyl-1, 3-propanedione (4a)
15 3 3 9 10 1 2 1 9 4 2 1 1 1 4 4 3 4 5 2 2 4 4 3 3 9 10 30 7 5 15 14 4 7 18 18 6 5 2 2 6 8 15 8 5 4 10 8 6 4 15 20 45 10 10 20 25 8 10 30 30 10 9 4 2 8 15 30 20 7 8 15 16 8 7 45 40 60 12 12 22 30 10 16 55 55 15 15 6 3 12 18 50 38 10 12 25 29 12 11 60 70 75 16 17 30 47 13 20 80 85 20 20 6 4 16 20 70 60 13 16 34 41 15 15 100 105 90 20 23 50 78 15 21 100 115 22 21 6 4 20 21 105 95 18 20 50 63 18 19 130 150

Activity of the test compound 3-benzoyl-2-(4’-nitrophenyl)-6, 8-dichloroflavone (5a)
15 3 3 9 7 2 2 9 12 2 2 1 1 4 2 4 5 2 2 4 2 3 4 9 10 30 7 6 15 10 4 5 18 28 6 5 2 2 6 5 15 8 5 4 10 6 6 8 15 27 45 10 12 20 20 8 11 30 40 10 9 4 2 8 8 30 20 7 7 15 12 8 12 45 62 60 12 18 22 25 10 15 55 60 15 16 5 5 12 10 50 38 10 9 25 18 12 15 60 100 75 16 22 30 38 13 20 80 100 20 21 6 5 16 12 70 60 13 12 34 26 15 19 100 145 90 20 26 50 48 15 22 100 130 22 25 6 5 20 18 105 80 18 17 50 45 18 24 130 205

Activity of the test compound 3-benzoyl-2-(4’-nitrophenyl)-6, 8-dichloroflavone (6a)
15 3 3 9 8 2 3 9 14 2 2 1 1 4 4 4 6 2 3 4 5 3 3 9 10 30 7 7 15 15 4 6 18 30 6 5 2 2 6 6 15 18 5 7 10 12 6 7 15 18 45 10 12 20 38 8 10 30 50 10 11 4 3 8 10 30 27 7 10 15 18 8 12 45 56 60 12 16 22 50 10 14 55 70 15 15 5 5 12 14 50 50 10 15 25 29 12 15 60 98 75 16 20 30 60 13 18 80 90 20 21 6 5 16 18 70 80 13 20 34 45 15 19 100 135 90 20 24 50 100 15 20 100 120 22 23 6 5 20 21 105 140 18 21 50 70 18 23 130 200

Activity of the test compound 3-(2’-hydroxy-3’, 5’-dichlorophenyl)-4-benzoyl-5-(4’-nitrophenyl) isoxazole (7a)
15 3 2 9 7 2 9 10 2 3 1 1 4 3 4 6 2 2 4 3 3 4 9 10 30 7 4 15 12 4 5 18 22 6 10 2 3 6 6 15 14 5 4 10 15 6 6 15 30 45 10 8 20 20 8 8 30 48 10 12 4 3 8 10 30 40 7 8 15 25 8 11 45 50 60 12 14 22 45 10 13 55 77 15 16 5 5 12 15 50 60 10 12 25 49 12 18 60 120 75 16 18 30 60 13 17 80 135 20 20 6 6 16 19 70 90 13 16 34 76 15 21 100 172 90 20 24 50 95 15 20 160 100 22 23 6 7 20 23 105 135 18 20 50 120 18 23 130 210

Note: C = control, T = treated
The treatment of compound 5a showed very positive results in the increase of shoot height in all tested plants as compared to control group plants except in the D. chinensis plant that has been mentioned in the 2nd observation. The treatment of compound 6a promoted shoot height of test plants to a great extent except in P. rheas whereas this plant started responding to the treatment of title compound 6a after 6th week onward only. The same trend was retained by the test plants on treatment with the title compound 7a.

The data about the foliage of the test plants depicted in Table 1 revealed that there was a remarkable increase in the number of leaves in treated plants as compared to control group plants. However, in the first phase of the experiment, there was no significant difference between treated and control group plants of P. rheas, and C. officinalis. But the fifth week onwards the foliage density gradually increases and it shoots up in the later phase of the study to a considerable extent as compared to the control group plants.

CONCLUSION

The synthesized compounds were screened for their growth promoting activity on some flowering plants viz: Papaver rheas, Calendula officinalis, Gladiola tristis, Gaillardia aristata, Dianthus chinensis and Iberis sp. The efforts have been made to examine and analyze the morphology of treated plants. When the comparison of morphological characters was made between those of treated and control groups plants, it was interesting to note that all the plants exhibited significant shoot growth and considerable increase in the number of leaves as compared to those of untreated ones.

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