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Article

Synthesis, Herbicidal, Fungicidal and Insecticidal Evaluation of 3-(Dichlorophenyl)-isocoumarins and (±)-3-(Dichlorophenyl)-3,4-dihydroisocoumarins

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Este é o primeiro relato mostrando que 3-(diclorofenil)isocumarinas e (±)-3,4-(dihidroisocumarinas) são inibidores do crescimento de plantas e fungos. 3-Diclorofenil-isocumarinas foram sintetizadas pela condensação de ácido homoftálico com cloretos de diclorobenzoíla. A hidrólise alcalina destas isocumarinas formou ceto ácidos. 3-(Diclorofenil)-3,4-dihidroisocumarinas racêmicas foram obtidas pela redução de ceto ácidos a hidróxi-ácidos racêmicos, seguida pela ciclodehidratação, usando anidrido acético. As atividades herbicida, fungicida e inseticida dos compostos sintetizados foram avaliadas. Alguns dos compostos sintetizados mostraram excelentes atividades fungicida e herbicida, mas nenhum deles apresentou efeito inseticida nos insetos usados nos testes. Este estudo sugere que isocumarinas e compostos relacionados podem ser usados como guias para o planejamento de herbicidas e fungicidas bioativos.

This is the first report showing that 3-(dichlorophenyl)isocoumarins and (±)-3,4-dihydroisocoumarins are plant and plant fungus growth inhibitors. 3-Dichlorophenylisocoumarins were synthesized by condensation of homophthalic acid with dichlorobenzoyl chlorides. The alkaline hydrolysis of these isocoumarins afforded keto acids. Racemic 3-(Dichlorophenyl)-3,4-dihydroisocoumarins were obtained by reduction of keto acids to racemic hydroxy acids**,** followed by cyclodehydration using acetic anhydride. The herbicidal, fungicidal and insecticidal activities of the synthesized compounds have been evaluated. Some of the synthesized compounds show excellent herbicidal and fungicidal activities but none of the synthesized compounds presented any insecticidal effects on the test insects. The findings of this study suggest that isocoumarins and related compounds may serve as lead compounds towards the design of bioactive herbicides and fungicides.

Keywords: 3-(dichlorophenyl)isocoumarins, (±)-3-(dichlorophenyl)-3,4- dihydroisocoumarin, herbicide, fungicide, insecticide

Introduction

Weeds compete with crops for sunshine, water, nutrients, and physical space and are thus capable of greatly influencing the growth of crops and undermining both crop quality and yield. Also, many weeds are the harbor or nest of pathogens, viruses, and pests, which may result in the occurrence and spread of plant diseases and insect pests in crops. Herbicides, as the main weed control tool, play a very important role in modern agriculture. Since the discovery of the herbicide 2,4-dichlorophenoxyacetic acid (2,4-D) by Zimerman and Hitchcock, the agrochemical industry has successfully developed as wide array of herbicides with various chemical structures and modes of action.[1] However, an inevitable problem associated with the use of herbicides is the occurrence of herbicide-resistant weeds.[2] For example, the widespread use of herbicides, such as chlorsulfuron, atrazine, diclofop-methyl, and paraquat, has caused herbicide resistance in many weeds. Therefore, it is necessary to develop efficient herbicides with novel structures and modes of action to overcome the resistance of weeds.

Over the last two decades, there has been a dramatic increase in the rate of superficial and invasive fungal infections.[3-7] Modern agriculture relies on effective control of fungal diseases to increase crop yield and quality and consequently increase crop value.[8] No single fungicide can be used for all disease situations and the widespread

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use of fungicides can select for fungicide resistant pathogens.[9] Therefore, there is need for safer and more cost-effective fungicides, which are easier to use and provide better performance against resistant pathogens.

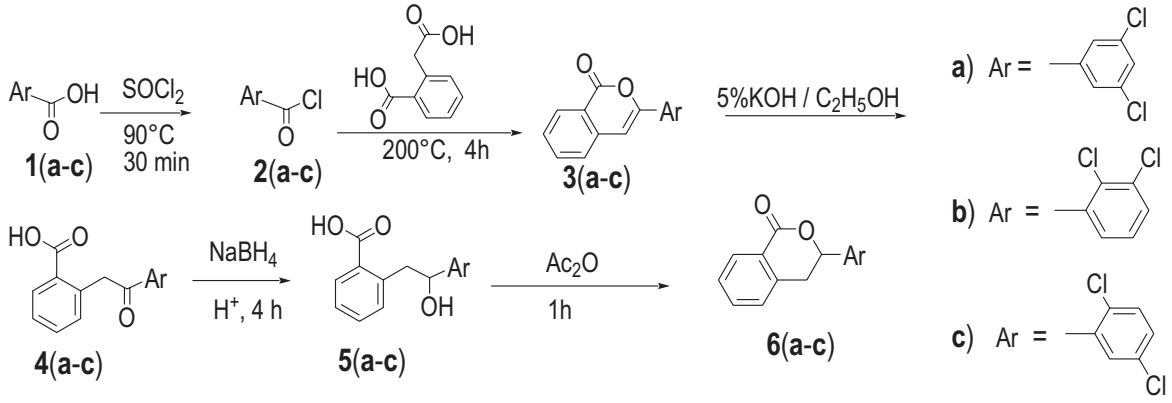
Isocoumarins and 3,4-dihydroisocoumarins are the secondary metabolites of a wide variety of fungi, lichens, molds, bacteria, higher plants and insects. The majority of isocoumarins have been isolated from various species of the fungal genera *Artemisia, Aspergillus, Ceratocystis, Fusarium, Penicillum,* and *Streptomyces* etc. A number of them are constituents of a few higher plant families. Naturally occurring isocoumarins containing halogens have been seldom reported. Examples of naturally occurring isocoumarins containing fluorine are not known yet. However, a few examples of naturally occurring chlorine- and bromine-containing isocoumarins have been reported. Laresenb,[10] has isolate chlorine-containing metabolite dichlorodiaportin from the cheese-associated cultures of *Penicillium nalgiovense*. 4-Chloro-3-[(4′-fluorophenyl) methoxy]isocoumarin has been found,[11] to be quite an effective inhibitor for human Q31 granzyme A, murine and human granzyme A, isolated from cytotoxic T lymphocytes. This isocoumarin derivative has also been found,[12] to be useful in the treatment of emphysema as serine protease inhibitor. 6-(2′-Chloro-4′-trifluoromethylphenoxy)-3,4-dihydroisocoumarin has been used,[13] as a herbicide, which almost totally controlled the growth of *Schinochloa crusgall, Sinapis alba* and other weeds. 7-Amino-4-chloro-3-(2′-bromoethoxy)isocoumarin has been synthesized,[14] and evaluated as a potent inhibitor of human leuko elastase and several blood coagulation enzymes.[15] 7-Amino-3-(2′-bromopropoxy)-4-chloroisocoumarin and 7-Amino-3-(3′-bromopropoxy)-4-chloroisocoumarin have been patented,[16] as an ascapain inhibitor in the inhibition and treatment of neurodegeneration.

In continuation of our previous studies,[17-19] and biological activities associated with chloro-substituted isocoumarins prompted us to synthesize some new 3-(dichlorophenyl) isocoumarins and their conversion to the corresponding (±)-3-(dichlorophenyl)-3,4-dihydroisocoumarins in order to check their herbicidal, fungicidal and insecticidal activities. The aim of our study was to investigate the importance of these compounds in agriculture. The general synthetic scheme is shown as follows.

Results and Discussion

Synthesis

Condensation of the acid chloride with homophthalic acid is useful for the preparation of 3-substituted isocoumarins.[17-19] A short and efficient synthesis of 3-(dichloropheny) isocoumarins **3**(**a**-**c**) by using this method and their conversion into the corresponding racemic 3-(dichlorophenyl)-3,4-dihydroisocoumarins **6**(**a**-**c**) were achieved and the herbicidal, fungicidal and insecticidal activities of the compounds were examined. Dichlorobenzoic acids **1**(**a**-**c**) were converted into their respective acid chlorides **2**(**a**-**c**) by reaction with thionyl chloride. Direct condensation of the acid chlorides **2**(**a**-**c**) with homophthalic acid at 200 °C afforded the 3-(dichlorophenyl)isocoumarins **3**(**a**-**c**), which were purified by column chromatography (eluant was pet. ether that corresponds to the fraction with a boiling range of 40-80 °C) and showed a single spot on TLC. These isocoumarins **3**(**a**-**c**) exhibited a characteristic 1H-singlet at δ 6.95, 6.89 and 7.01 ppm respectively for the C_4-H in the ^{1}H NMR. The aromatic hydrogens appeared in the expected region, *i.e.* 7.29-8.33 ppm . In the IR spectra of isocoumarins **3**(**a**-**c**), the lactone carbonyl absorptions were observed at 1708, 1704 and 1703 cm^{-1} respectively. The molecular ion peak in the mass spectrum of the isocoumarins **3**(**a**-**c**) was observed at *m/z* 290. The alkaline hydrolysis of isocoumarins **3**(**a**-**c**) afforded the



Scheme 1. The synthesis of the target compounds.

2-(dichlorobenzoylmethyl)benzoic acids **4**(**a-c**). In the IR spectra of the keto acids **4**(**a-c**), the absorptions for the ketone carbonyl were observed at 1712, 1707 and 1700 cm^{-1} respectively. The keto acid **4a** showed a 2H singlet at d 4.62 ppm for the methylene protons at C-1′. The molecular ion peak for the keto acid **4a** was observed at *m/z* 308 (M^+, 1.8%), along with peaks at 310 (M^++2, 1.1%) and 312 (M^++4, 0.2%) due to two isotopes of chlorine. In the reversible reaction, isocoumarins **3**(**a-c**) were obtained upon refluxing the keto acids **4**(**a-c**) with acetic anhydride. The melting points, IR, ^{1}H NMR and MS of these compounds are same as already synthesised isocoumarins **3**(**a-c**). Sodium borohydride reduction of keto acids **4**(**a-c**) afforded the corresponding racemic hydroxyl acids **5**(**a-c**), which were cyclodehydrated with acetic anhydride to produce (±)-3-dichlorophenyl-3,4-dihydroisocoumarins **6**(**a-c**) which exhibited the carbonyl absorptions at 1708, 1704 and 1703 cm^{-1} in the IR spectra. The signals related to hydrogen's H-3 and H-4 for compounds **6**(**a-c**) correspond to a typical ABX spin system. Thus, each of the C_4-H peaks showed a doublet of doublets at δ 3.09 and 3.29 ppm and another doublet of doublets observed at δ 5.93 ppm due to the C_3-H proton. The mass spectrum of **6a** showed molecular ion peaks at *m/z* 292.20 (11.3%) (M^+), 294.00 (9.2%) (M+2) and 296.00 (3%) (M+4) due to the two isotopes of chlorine (^{35}Cl and ^{37}Cl). Almost the same pattern was found with the (±)-3-(dichlorophenyl)-3,4-dihydro-isocoumarins **6**(**b-c**).

Biological activity

Herbicidal assays

From the biological assay results in Table 1, which summarize the herbicidal activity of the target compounds, some showed an excellent herbicidal activity in the pre-emergence treatment (soil treatment) than post-emergence (foliar treatment). Compounds **1b**, **3b**, **4**(**b-c**) and **5**(**a-c**) exhibited considerable inhibitory activity against *Brassica campestris* as compared to *Amaranthus retroflexus L, Echinochloa crus-galli* and *Digitaria sanguinalis(L.)Scop* in pre-emergence (soil treatment). Even the inhibitory activity of compounds **3b** and **5a** reaches 95% at 750 g ha^{-1}. The inhibitory activity of compound **3b** has also been measured at a concentration of 375, 188 and 94 g ha^{-1}. It has been found that with a decrease in concentration *per* hectare, the activity decreases. The other synthesized compounds also show little herbicidal activity. Although we have no information about the mode of action of these compounds, further research on the modification of their structure and the mode of action is in progress.

Fungicidal assays

The isocoumarins, dihydroisocoumarins and related compounds were tested for their fungicidal bioassay.[21] The results are reported as linear growth inhibition (LGI) against some plant pathogens (e.g *Fusarium oxysporum, Cercospora arachidicola, Alternaria solani, Gibberella zeae, Physalospora piricola and Phoma asparagi*

Table 1. Herbicide activity (% inhibition) of the synthesized compounds **1**(**a-c**), **3**(**a-c**), **4**(**a-c**), **5**(**a-c**) and **6**(**a-c**)

Compounds	Density / (g ha^{-1})	*Amaranthus retroflexus L*		*Brassica campestris*		*Echinochloa crusgalli*		*Digitaria sanguinalis(L.)Scop*	
		Soil treatment	Foliar spray	Soil treatment	Foliar spray	Soil treatment	Foliar spray	Soil treatment	Foliar spray
1a	750	0	0	19.1	12.6	7.9	17.7	0	0
1b	750	0	20.3	75.2	21.1	26.4	0	0	0
1c	750	0	0	29.2	35.5	14.4	17.6	3.3	0
3a	750	0	0	0	26.55	0	20.6	0	0
3b	750	15.9	15.1	94.9	47.9	0	0	0	0
3b	375	10.1	0	73.0	30.4	0	0	0	0
3b	188	0	0	33.7	19.7	0	0	0	0
3b	94	0	0	1.69	1.74	0	0	0	0
3c	750	0	0	1.5	8.8	5.7	10.3	0	0
4a	750	0	0	23.2	2.9	18.4	17.9	0	0
4b	750	0	0	88.5	88.2	20.9	10.3	0	0
4c	750	0	0	67.9	33.3	19.6	0.4	0	0
5a	750	13.0	0	94.3	35.5	23.7	0	11.1	22.0
5b	750	1.4	0	79.7	33.1	19.0	14.1	15.1	0
5c	750	0	0	78.2	35.2	0	10.7	0	0
6a	750	0	0	0	19.4	5.6	0	1.3	0
6b	750	0	0	0	52.8	0	0	0	21.1
6c	750	0	0	19.8	0	7.7	14.5	0	0
Clor-sulfuron	750	100	100	100	100	100	100	100	100

From the biological assay results in Table 2, which summarize the fungicidal activity of the target compounds **1**(**a-c**), **3**(**a-c**), **4**(**a-c**), **5**(**a-c**) and **6**(**a-c**). The compounds **1**(**a-c**), **5b** and **6c** exhibited considerable inhibitory activity against *Alternaria solani, Gibberella zeae* and *Physalospora piricola* Even the inhibitory activity of compounds **1a** and **1b** reaches 100% at 500 μg mL^{-1}. The other synthesized compounds also show fungicidal activity, but that activity is not too good.

Insecticidal assays

All the synthesized compounds were also tested for insecticidal activity but none of the synthesized compounds presented any insecticidal effects on the test insects.

Conclusions

In this paper, we report the synthesis, herbicidal, fungicidal and insecticidal evaluation of 3-(dichlorophenyl)isocoumarins and (±)-3-(dichlorophenyl)-3,4-dihydroisocoumarins. The results show the potentiality of some compounds, particularly **1b**, **3b**, **4**(**b-c**) and 5(**a-c**) exhibited considerable herbicidal activity against *Brassica campestris* while compounds **1**(**a-c**), **5b** and **6c** exhibited considerable fungicidal activity against *Alternaria solani, Gibberella zeae* and *Physalospora piricola.* The finding of this research suggests that these isocoumarins and related compounds may be used as potent bioactive herbicides and fungicides and in future to enhance the herbicidal and fungicidal potential for agriculture.

Experimental

The dichlorobenzoic acids were purchased from Aldrich and used without further purification. All reagents and solvents were commercially available and used as supplied. The petroleum ether used corresponds to the fraction with a boiling range of 40-80 °C. The melting points of the compounds were determined in open capillaries using a Gallenkemp melting point apparatus and are uncorrected. The infrared spectra were recorded on a Hitachi model 270-50 spectrophotometer as KBr disks or as neat liquids. 1H-NMR (300 MHz) spectra were recorded on a Bruker AM-400 as a$CDCl_3$ solution using TMS as an internal standard, while the EIMS were recorded on a MAT-112-S machine.

*General procedure for 3-(dichlorophenyl)isocoumarins **3(a-c)***

A mixture of dichlorobenzoic acid **1**(**a-c**) (53.0 mmol) and thionyl chloride (63.0 mmol) was heated for 30 min in the presence of a drop of DMF under reflux. Completion of the reaction was determined by the stoppage of gas evolution. Removal of the excess of thionyl chloride under reduced pressure to afforded the dichlorobenzoyl chlorides **2**(**a-c**).

A mixture of homophthalic acid (11.3 mmol) and the dichlorobenzoyl chloride (50.0 mmol) **2**(**a-c**) was heated at 200 °C under reflux for 4 hours. The mixture was dissolved in ethyl acetate and an aqueous solution of sodium carbonate was added in order to remove the unreacted homophthalic acid. The organic layer

Table 2. Fungicidal activity (% inhibition) of the compounds **1**(**a-c**), **3**(**a-c**), **4**(**a-c**), **5**(**a-c**) and **6**(**a-c**)

Compounds	Concentration / (μg mL^{-1})	*Fusarium oxysporum*	*Cercospora arachidicola*	*Alternaria solani*	*Gibberella zeae*	*Physalospora piricola*	*Phoma asparagi*
1a	50	8	13.6	100	11.6	57.5	0
1a	20	0	0	20	0	24.1	0
1b	50	0	4.6	100	11.6	41.3	0
1b	20	0	0	0	0	16.7	0
1c	50	0	0	54.5	11.6	42.5	0
3a	50	2.3	4.6	36.4	23.2	47.5	0
3b	50	2.29	0	0	5.8	48.8	0
3c	50	0	10	27.3	0	19.2	0
4a	50	0	20	24.2	26.5	38.5	23.5
4b	50	0	4.6	22.7	5.8	25	0
4c	50	0	10	18.2	0	26.9	0
5a	50	5.1	0	9.1	14.5	43.8	0
5b	50	0	10	9.1	0	21.2	0
5c	50	8	0	50	23.2	48.8	0
6a	50	8	0	22.7	5.8	43.8	0
6b	50	19.4	18.2	31.8	40.6	41.3	0
6c	50	0	60	21.2	11.8	53.9	35.5
Amistar	50	100	100	100	100	100	100

was separated, concentrated and chromatographed on silica gel using petroleum ether (40-80 °C) as eluent to afford 3-(dichlorphenyl)isocoumarins **3**(**a**-**c**) as solid, which was further purified by recrystallization from methanol.

*3-(3′,5′-Dichlorophenyl)isocoumarin (**3a**)*

Yield: 81%; mp 208-210 °C; IR ν_{max} / cm^{-1}: 3156(C-H), 2935 (Ar-H), 1708 (C=O), 1590 (C=C arom), 1099 (C-Cl). EI-MS *m/z* (rel. int.): 290 (M^+, 100), 292 (M^++2, 67.5), 294 (M^++4, 13); ^{1}H NMR (300 MHz, $CDCl_3$): δ 6.95 (1H , s , H-4), 7.39 (1H, t, *J* 1.2 Hz, H-4′), 7.50 (1H, m, H-7), 7.53 (1H, m, H-6), 7.55 (1H, dd, *J* 2.2, 7.6 Hz, H-5), 7.75 (2H, t, *J* 1.2 Hz, H-2′ and 6′), 8.31 (1H,dd, *J* 1.9, 7.9 Hz, H-8); Elemental analysis Found: C 61.83, H 2.73, Cl 24.30; Calculated: C 62.07, H 2.76, Cl 24.48.

*3-(2′,3′-Dichlorophenyl)isocoumarin (**3b**)*

Yield: 80%; mp 180-182 °C; IR ν_{max} / cm^{-1}: 3152 (C-H), 2931(Ar-H), 1710 (C=O), 1593 (C=C arom), 1096 (C-Cl). EI-MS *m/z* (rel. int.): 290 (M^+, 28), 292 (M^++2, 17.5), 294 (M^++4, 3.7); ^{1}H NMR (300 MHz, $CDCl_3$): δ 6.89 (1H, s, H-4), 7.29 (1H, dd, *J* 1.9, 7.9 Hz, H-4′), 7.33 (2H, t, *J* 7.8 Hz, H-5′), 7.50 (2H, t, *J* 7.8 Hz, H-6′), 7.54 (1H, m, H-7), 7.56 (1H, dd, *J* 2.2, 8.0 Hz, H-5), 7.74 (1H, m, H-6), 8.33 (1H, dd, *J* 2.1, 7.9 Hz, H-8); Elemental analysis Found: C 61.99, H 2.69, Cl 24.33; Calculated: C 62.07,H 2.76, Cl 24.48.

*3-(2′,5′-Dichlorophenyl)isocoumarin (**3c**)*

Yield: 81%; mp 187-188 °C; IR ν_{max} / cm^{-1}: 3155(C-H), 2935(Ar-H), 1710 (C=O), 1593 (C=C arom), 1093 (C-Cl). EI-MS *m/z* (rel. int.): 290 (M^+, 100), 292 (M^++2, 69.6), 294 (M^++4,13.7); ^{1}H NMR (300 MHz, $CDCl_3$): δ 7.01(1H, s, H-4), 7.32 (1H, dd, *J* 2.47,8.56 Hz, H-4'), 7.41 (1H, d, *J* 8.56 Hz, H-3'), 7.50 (1H, dd, *J* 2.1, 7.80 Hz, H-5), 7.56 (1H, m, H-7), 7.72 (1H, d, *J* 2.02 Hz, H-6'), 7.75 (1H, m, H-6), 8.32 (1H, dd, *J 2.7,* 7.9 Hz, H-8); Elemental analysis Found: C 61.89, H 2.71, Cl 24.40; Calculated: C 62.07, H 2.76,Cl 24.48.

*General procedure for 2-(dichlorobenzoylmethyl) benzoic acid **4(a-c)***

A solution of isocoumarins **3**(**a**-**c**) in ethanol (50 mL) and 5% potassium hydroxide (100 mL) were refluxed for 4 h. Ethanol was removed from the reaction mixture by distillation. Ice cold water (20 mL) was added and the reaction mixture was acidified with hydrochloric acid. The reaction mixtures were then extracted with dichloromethane (3 × 20 mL). The extracts were dried (Na_2SO_4) and evaporated to yield crude solids **4**(**a**-**c**), which were recrystallized from methanol.

*2-(3′,5′-Dichlorobenzoylmethyl) benzoic acid (**4a**)*

Yield: 81%; mp 187-188 °C; IR ν_{max} / cm^{-1}: 3300-3250 (-OH), 3155 (C-H), 2935 (Ar-H), 1710 (C=O), 1593 (C=C arom), 1093 (C-Cl): EI-MS *m/z* (rel. int.): 308 (M^+, 100), 310 (M^++2, 65.6), 312 (M^++4,11.7); ^{1}H NMR (300 MHz, $CDCl_3$): δ 4.62 (2H, s, H-1′), 7.18 (1H, dd, *J* 1.56, 7.99 Hz, H-3), 7.24 (1H, m, H-4), 7.52 (1H, m, H-5), 7.56 (1H, dd, *J* 2.41 Hz, H-4″)7.94 (2H, d, *J* 1.76 Hz, H-2″ and 6″), 8.15 (1H, dd, *J* 1.2, 7.9 Hz, H-6). Elemental Analysis Found: C 58.23, H 3.28, Cl 25.60; Calculated: C 58.44, H 3.25, Cl 25.26.

*2-(2′,3′-Dichlorobenzoylmethyl)benzoic acid (**4b**)*

Yield: 77%; mp 160-162 °C; IR ν_{max} / cm^{-1}: 3300-3250 (-OH), 3151 (C-H), 2935 (Ar-H), 1712 (C=O), 1590 (C=C arom), 1091 (C-Cl): EI-MS *m/z* (rel. int.): 308 (M^+, 97), 310 (M^++2, 55), 312 (M^++4,13); ^{1}H NMR (300 MHz, $CDCl_3$): δ 4.64 (1H, s, H-1'), 7.06 (1H, t, *J* 7.9Hz, H-5″), 7.20 (1H, dd, *J* 1.56, 7.99 Hz, H-3), 7.29 (1H, m, H-4), 7.42 (1H, t, *J* 8.17Hz, H-4″), 7.63 (1H, m, H-5), 7.81 (1H, dd, *J* 1.43, 7.78 Hz, H-6″), 8.02 (1H, dd, *J* 2.23, 8.01 Hz, H-6); Elemental Analysis Found: C 58.30,H 3.23, Cl 25.46; Calculated: C 58.44,H 3.25, Cl 25.26.

*2-(2′,5′-dichlorobenzoylmethyl) benzoic acid (**4c**)*

Yield: 71%; mp 140-142 °C; IR ν_{max} /cm^{-1}: 3300-3250 (-OH), 3157 (C-H), 2933 (Ar-H), 1711 (C=O), 1590 (C=C arom), 1094 (C-Cl): EI-MS *m/z* (rel. int.): 308 (M^+, 100), 310 (M^++2, 79), 312 (M^++4,19); ^{1}H NMR (300 MHz, $CDCl_3$): δ 4.63 (1H, s, H-1′), 7.32 (1H, m, H-4), 7.34 (1H, dd, *J* 1.99, 7.9 Hz, H-3), 7.41 (1H, d, *J* 8.9 Hz, H-3″), 7.44 (1H, d, *J* 8.8 Hz, H-4′), 7.58 (1H, m, H-5), 7.97 (1H, d, *J* 1.78 Hz, H-6″), 8.17 (1H, dd, *J* 1.67, 7.9 Hz, H-6) ; Elemental Analysis Found: C 58.33,H 3.20, Cl 25.25; Calculated: C 58.44,H 3.25, Cl 25.26.

*General procedure for the preparation of (±)-3-(dichlorophenyl)-3,4-dihydroisocoumarins **6(a-c)***

To a solution of the keto acids **4**(**a**-**c**) (2.07 mmol) dissolved in 1% potassium hydroxide solution (25 mL), and sodium borohydride (0.25 g) was added. The reaction mixture was then stirred for 1 h at room temperature. After acidification with hydrochloric acid, the reaction

mixture was extracted with ethyl acetate (2 × 50 mL). The usual workup gave the hydroxy acids **5**(**a**-**c**), which were purified by recrystallization by ethyl acetate. The hydroxyl acids **5**(**a**-**c**) were dissolved in acetic anhydride (1 mL) and heated under reflux for 2 h. The reaction mixture was cooled, water (25 mL) was added and the reaction mixture was stirred overnight. The crystals that deposited were collected by filtration and the filtrates were extracted with dichloromethane (2 × 20 mL). The solvent was removed under reduced pressure and the crude dihydroisocoumarins **6**(**a**-**c**) were purified by column chromatography on silica gel using petroleum ether (40-80 °C) as an eluent.

*2-[2′-Hydroxy-2′-(3″, 5″-dichlorophenyl)ethyl] benzoic acid (**5a**)*

Yield: 77%, mp 138-140 °C; IR ν_{max} / cm^{-1}: 3300-3250 (-OH), 3157 (C-H), 2933 (Ar-H), 1711 (C=O), 1590 (C=C arom), 1094 (C-Cl): EI-MS *m/z* (rel. int.): 310 (M^+, 100), 312 (M^++2, 79), 314 (M^++4,19); ^{1}H NMR (300 MHz, $CDCl_3$): δ 2.25 (1H, dd, *J* 8.22, 15.6 Hz, H-1′b), 2.60 (1H, dd, *J* 6.24, 15.6 Hz, H-1′a), 4.10(1H, dd, *J* 7.12, 14.20 Hz, H-2'), 7.05 (3H, t, *J* 1.76 Hz, H-2″, 4″ and 6″), 7.57 (1H, m, H-5), 7.73 (1H, dd, *J* 1.78, 8.9 Hz, H-3), 7.78 (1H, m, H-4), 8.16 (1H, dd, *J* 1.77, 7.9 Hz, H-6); Elemental Analysis Found: C 57.91, H 3.88, Cl 15.41; Calculated: C 58.06, H 3.87, Cl 15.17.

*2-[2′-Hydroxy-2′-(2″, 3″-dichlorophenyl)ethyl] benzoic acid (**5b**)*

Yield: 70 %; mp 150-152 °C; IR ν_{max} / cm^{-1}: 3300-3250 (-OH), 3159 (C-H), 2936 (Ar-H), 1713 (C=O), 1593 (C=C arom), 1094 (C-Cl): EI-MS *m/z* (rel. int.): 310 (M^+, 86), 312 (M^++2, 65), 314 (M^++4,11); ^{1}H NMR (300 MHz, $CDCl_3$): δ 2.22 (1H, dd, *J* 8.22, 15.6 Hz, H-1'b), 2.63 (1H, dd, *J* 6.24, 15.6 Hz, H-1'a), 4.17(1H, dd, *J* 7.12, 14.20 Hz, H-2'), 7.55 (1H, dd, *J* 1.76, 8.1 Hz, H-4″), 7.59 (1H, t, *J* 7.72 Hz, H-5″), 7.63 (1H, dd, *J* 1.76, 7.78 Hz, H-6″), 7.57 (1H, m, H-5), 7.73 (1H, dd, *J* 1.87, 8.9 Hz, H-3), 7.78 (1H, m, H-4), 8.13 (1H, dd, *J* 2.1, 7.9 Hz, H-6) ; Elemental Analysis Found: C 58.12, H 3.77 , Cl 15.11; Calculated: C 58.06, H 3.87, Cl 15.17).

*2-[2′-Hydroxy-2′-(2″, 5″-dichlorophenyl)ethyl] benzoic acid (**5c**)*

Yield: 72 %; mp 172-174 °C; IR ν_{max} / cm^{-1}: 3300-3250 (-OH), 3153 (C-H), 2936 (Ar-H), 1716 (C=O), 1593 (C=C arom), 1099 (C-Cl): EI-MS *m/z* (rel. int.): 310 (M^+, 100), 312 (M^++2, 75), 314 (M^++4,18); ^{1}H NMR (300 MHz, $CDCl_3$): δ 2.21 (1H, dd, *J* 8.22, 15.6 Hz, H-1'b), 2.61 (1H, dd, *J* 6.24, 15.6 Hz, H-1'a), 4.14(1H, dd, *J*7.12, 14.20 Hz, H-2'), 7.23 (1H, d, *J* 8.9 Hz, H-3″), 7.24 (1H, dd, *J* 1.56, 7.8 Hz, H-4″), 7.27 (1H, d, *J* 1.0 Hz, H-6″), 7.42 (1H, m, H-5), 7.73 (1H, dd, *J* 2.3, 8.9 Hz, H-3), 7.78 (1H, m, H-4), 8.15 (1H, dd, *J* 1.87, 7.79 Hz, H-6); Elemental Analysis Found: C 57.99, H 3.58, Cl 15.21; Calculated: C 58.06, H 3.87, Cl 15.17.

*(±)-3-(3′,5′-Dichlorophenyl)-3,4-dihydroisocoumarin (**6a**)*

Yield: 73 %; mp 184-186 °C; IR ν_{max} / cm^{-1}: 3153 (C-H), 2935 (Ar-H), 1708 (C=O), 1593 (C=C arom), 1099 (C-Cl): EI-MS *m/z* (rel. int.): 292 (M^+, 11.3), 294 (M^++2, 9.2), 296 (M^++4, 3); ^{1}H NMR (300 MHz, $CDCl_3$): δ 3.09 (1H, dd, *J*12.10, 16.25 Hz, H-4b), 3.29 (1H, dd, *J* 2.90,16.36 Hz, H-4a), 5.93 (1H, dd, *J* 2.90, 12.03 Hz, H-3), 7.30 (2H, d, *J* 2.6 Hz, H-2' and 6'), 7.44 (1H, d, *J* 2.3 Hz, H-4'), 7.48 (1H, m, H-7), 7.58 (1H, dd, *J* 2.24, 7.60 Hz, H-5), 7.66 (1H, m, H-6), 8.15 (1H, dd, *J* 1.34, 7.6 Hz, H-8); Elemental Analysis Found: C 61.44, H 3.44, Cl 10.95; Calculated: C 61.64, H 3.08, Cl 10.96.

*(±)-3-(2′,3′-Dichlorophenyl)-3,4-dihydroisocoumarin (**6b**)*

Yield: 77 %; mp 136-138 °C; IR ν_{max} / cm^{-1}: 3151 (C-H), 2939 (Ar-H), 1710 (C=O), 1597 (C=C arom), 1097 (C-Cl): EI-MS *m/z* (rel. int.): 292 (M^+, 57.5), 294 (M^++2, 18), 296 (M^++4, 13); ^{1}H NMR (300 MHz, $CDCl_3$): δ 3.10 (1H, dd, *J* 12.11, 16.20 Hz, H-4b), 3.27(1H, dd, *J* 2.90, 16.38 Hz, H-4a), 5.93 (1H, dd, *J* 2.86, 12.02 Hz, H-3), 7.29 (1H, dd, *J* 2.6, 7.4 Hz, H-6'), 7.30 (1H, t, *J* 5.91 Hz, H-5'), 7.32 (1H, dd, *J* 1.76, 7.9Hz, H-4'), 7.45 (1H, m, H-7), 7.58 (1H, m, H-6), 7.66 (1H, dd, *J* 2.6, 7.73 Hz, H-5), 8.15 (1H, dd, *J* 2.6, 7.61 Hz, H-8); Elemental Analysis Found: C 61.54, H 3.04, Cl 10.99; Calculated: C 61.64, H 3.08, Cl 10.96.

*(±)-3-(2′,5′-Dichlorophenyl)-3,4-dihydroisocoumarin (**6c**)*

Yield: 77 %; mp 121-124 °C; IR ν_{max} / cm^{-1}: 3154 (C-H), 2933 (Ar-H), 1712 (C=O), 1598 (C=C arom), 1097 (C-Cl): EI-MS *m/z* (rel. int.): 292 (M^+, 35), 294 (M^++2, 21), 296 (M^++4, 11); ^{1}H NMR (300 MHz, $CDCl_3$): δ 3.09 (1H, dd, *J* 12.10, 16.25 Hz, H-4b), 3.29 (1H, dd, *J* 2.90, 1 6.36 Hz, H-4a), 5.93 (1H, dd, *J* 2.90, 12.03 Hz, H-3), 7.24 (1H, dd, *J* 7.6 Hz, H-3′), 7.30 (1H, dd, *J* 2.6 Hz, H-6'), 7.44 (1H, dd, *J* 2.3, 8.1 Hz, H-4'), 7.48 (1H, m, H-7), 7.58 (1H, m, H-6), 7.66 (1H, dd, *J* 1.24,7.78 Hz, H-5), 8.15 (1H, *J* 1.23, 7.6 Hz, H-8). Elemental Analysis Found: C 61.44, H 3.44, Cl 10.95; Calculated: C 61.64, H 3.08, Cl 10.96.

Herbicidal assays[20]

Compound with certain concentration was dissolved in acetone or DMF and diluted with water, and the solutions

was added to certain amount of soil as soil treatment in order that the concentration of test compound was 750 kg ha^{-1}, after this, the weed seeds were geminated, each pot contains 20-30 seeds and each experiment triplicated. The plant was cultured in the green house at 25-27 °C. After 4 weeks of growth, the fresh weight was measured and the inhibition percentage was calculated according to the corresponding control. For foliate spray, after the growth of weed for 2 weeks, 750 kg ha^{-1} of solution was sprayed, and the weeds were cultured for another 2 weeks, again fresh weight was measured and the inhibition percentage was calculated according to the corresponding control. The weeds used for this experiment were *Brassica campestris, Echinochloa crusgalli, Amaranthus retroflexus L* and *Digitaria. sanguinalis(L.)Scop.*

Fungicidal assays

Using fungi growth inhibition method for fungicide activity determination as described by Fan .[21] Compound with 500 μg mL^{-1} of concentration was dissolved in water by 0.1mL of assistance of DMF and then 500 μg mL^{-1} of compound in agar plate was prepared, the fungi was inoculated and cultured in the culture tank at 24-26 °C, the diameter of fungi spread was measured two days later, growth inhibition was calculated by corresponding control.

Insecticidal assays

Weighing 10 mg sample into a 50 mL of glass beaker, then 20 mL of acetone was added, the maize leaf was dipped in the sample solution for 5 seconds, and the leaf was put in a petri dish with 10 cm of diameter to evaporate all solvents. Nine pieces of maize leaves were cut short and put into the petri dish of 10 cm diameter containing 10 *Mythimna separata* with 4 instars, the death rate of insect was detected 24 h and 96 h later experiment. For *Culex pipiens pallens* larva experiment, the 4 instars of insect was dipped into the water solution for 24 h, the death rate of insect was detected. None of the compound shows insecticidal activity.

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