Original Research Article

Synthesis and insecticidal activity of 1,2,4-oxadiazoles derivates against*Plutellaxylostella* L. (Diamondback Moth)

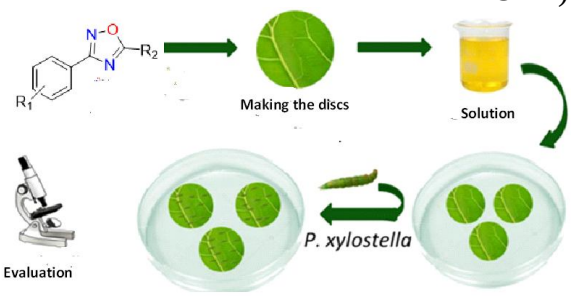
ABSTRACT

The diamondback moth (*Plutellaxylostella*) is a cosmopolitan pest known for its destruction of cruciferous plants. Commercial insecticides, such as Decis 25 EC and Azamax, are the main forms of controlling this pest in irrigated systems in the agricultural communities of the city of Garanhuns (northeastern Brazil). However, the difficulty in controlling this pest resides in its high fecundity and short lifecycle, which serve as resistance mechanisms to insecticides. Considering the immediate need to find alternative products to insecticides employed in these agricultural communities, nine 1,2,4-oxadiazoles were synthesized using an easy, mild method with high to moderate yields. The effect of 4-(3-phenyl-1,2,4-oxadiazole-5-yl)-butan-2-one (**6a**), 4-(3-*p*-tolyl-1,2,4-oxadiazol-5-yl)-butan-2-one (**6c**) and (*R,S*)-3-p-benzylphenyl-1,2,4-oxadiazole-5-yl)-ethanol (**7e**)and its precursors was assessed against third instar larvae of *Plutellaxylostella* L and the results were compared to commercial insecticides available in the communities. Compounds **6a** and **6c** exhibited the same level of toxicity but were more toxic than compound **7e**. All 1,2,4-oxadiazoles exhibited high toxicity. *P. xylostella* was less susceptible to the commercial insecticides used as the positive controls than the 1,2,4-oxadiazole derivatives. The results demonstrate that the larvae of *P. xylostella* are highly sensitive to the ring of 1,2,4-oxadiazole, which can be used as potential insect-control agent.

Keywords: 1,2,4-Oxadiazoles; insecticidal activity; diamondback moth.

Graphical Abstract

SYNTHESIS AND INSECTICIDAL ACTIVITY OF 1,2,4-OXADIAZOLES DERIVATES AGAINST*PLUTELLA XYLOSTELLA* L. (DIAMONDBACK MOTH)



The present study aimed to describe the synthesis and evaluate the insecticidal activity of 4-(3-aryl-1,2,4-oxadiazole-5-yl)-butan-2-one and (*R,S*)-3-aryl-5-(1-hydroxy-ethyl)-1,2,4-oxadiazole against *P. xylostella.*

1. INTRODUCTION

The diamondback moth (*Plutellaxylostella*) is a cosmopolitan pest known for its destruction of cruciferous plants [1]. As agricultural production has improved in irrigated farming regions in the state of Pernambuco (northeastern Brazil), this pest has become a concern to local communities, causing considerable harm to kale, lettuce, cabbage, cauliflower, broccoli, etc. [2, 3].

The intensive use of insecticides derived from pyrethrin (Decis 25 E.C.) and/or azadirachtin (Azamax) is the main form of controlling this pest in specific communities in the municipality of Garanhuns in the state of Pernambuco, Brazil. Despite the constant use of these insecticides, the control of *P. xylostella* has been difficult due to its dispersal capacity, high fecundity and short lifecycle. Moreover, this organism has demonstrated resistance mechanisms to different commercially available insecticides, including Decis 25 E.C.[2] Thus, the use of these products has been restricted due to the occurrence of a resistant *P. xylostella* population to the active ingredients and consequent increase in application and production costs.

Considering the immediate need to encounter alterative products to insecticides employed in Garanhuns, Brazil, for the control of pests of cruciferous crops, molecular structural modifications inspired by molecular models with known biological properties are a promising method acquiring new compounds with insecticidal properties, such as 1,2,4-oxadiazole derivatives, which are five-member heterocyclic compounds with two nitrogen atoms and an oxygen atom. First synthesized in 1884, the pharmacological potential of substances with a 1,2,4-oxadiazole nucleus has currently piqued the interest of different research groups [4]. Such compounds are recognized as having anti-inflammatory [5, 6], anti-infective [7], antimicrobial [8-10], and antitumor [11] properties as well as insecticidal properties against urban pests [12]and pests of medicinal interest [13]. To date, few

literature reviews have addressed the synthesis and biological study of these rings [14, 15], such as pyridine-substituted 1,2,4-oxadiazoles with insecticidal activity [16-18], antifungal [19], and herbicidal activity [20].

To minimize the high costs for the control of the diamondback moth in specific communities in the city of Garanhuns (northeastern Brazil), the present study aimed to describe the synthesis and evaluate the insecticidal activity of two 4-(3-aryl-1,2,4-oxadiazole-5-yl)-butan-2-one, one (*R,S*)-3-aryl-5-(1-hydroxy-ethyl)-1,2,4-oxadiazole and two derivative materials against *P. xylostella*. The findings were compared to the insecticides Decis 25 EC and azamax as positive controls.

2. MATERIAL AND METHODS

2.1 General consideration

All commercially available reagents were used directly without purification unless otherwise stated. All solvents used in the reactions were distilled for purity. Melting points were determined using an electrothermal digital melting point apparatus (model IA9100) and uncorrected. Infrared spectra were recorded as KBr films on a Brucker IFFS66 series Fourier transform spectrophotometer. ^{1}H and ^{13}C NMR spectra were recorded on a Bruker DPX 400 spectrometer at 400 MHz and 100 MHz, respectively, using DMSO-*d6* as the solvent and Me_4Si as the internal standard. Chemical shifts are reported in ppm. Coupling constants are reported in Hz. Thin layer chromatography (TLC) was performed using Merck Silica gel 60 F254 plates. The precise heating area in the oven was located and the experiments were repeated at least twice. Thus, the authors are confident that these experiments can be repeated by any chemist.

2.2 General procedure for esterification reaction

The appropriatecarboxylic acid (60.4 mmol), methanol (65 ml) and sulfuric acid (0.7 mL) were refluxed for 3 hours. The progress of the reaction was monitored by TLC. After the reaction, excess alcohol was removed under reduced pressure and the residue was extracted with dichloromethane (3 x 20 mL). The extract was washed with a solution of sodium hydroxide and subsequently with distilled water, dried over anhydrous sodium sulfate and vacuum concentrated to yield the crude product, which was purified by column chromatography (hexane: ethyl acetate, 9:1) to give the desired carboxylic ester. Methyl levulinate and methyl lactate were obtained with yields of 75% and 78%, respectively.

2.2.1 Synthesis of 4-(3-aryl-1,2,4-oxadiazol-5-yl)-butan-2-one(6a-c)

In a glass reactor, a mixture of appropriate arylamidoximes**5a–c** (1 mmol, 0.136g) and 1.25 mmol (0.05g) of NaOH and 2 mL DMSO were added. The mixture was homogenized and then 1,0 mmol (0.13 g of methyl levulinate **4**) was added. After homogenization of all reagents, the reaction mixture was taken to ultrasound at room temperaturefor 30 min. At the completion of the reaction, the compound was chromatographed over a silica gel column and eluted with *n*-hexane–ethyl acetate (9:1). The spectroscopic data of all synthesized compounds matched reported values [4].

2.2.2 Synthesis of (R,S)-3-aryl-1,2,4-oxadiazole-5-yl)-ethanol (7d-f)

In a glass reactor, a mixture of appropriate arylamidoximes**5d-f** (1.00 mmol, 0.136g) and 1.25 mmol (0.05g) of NaOH and 2 mL DMSO were added. The mixture was homogenized and then 1,54 mmol (0.16 g of (*R,S*)-methyl lactate **2**) was added. After homogenization of all reagents, the reaction mixture was taken to ultrasound at room temperaturefor 20 min. At the completion of the reaction, the compound was chromatographed over a silica gel column and eluted with *n*-hexane–ethyl acetate (7:3). The fractions containing the desired

compound were combined and the solvent evaporated for the acquisition of chromatographically pure (*R,*S)-3-aryl-1,2,4-oxadiazole-5-yl)-ethanol(**7d-f**).

2.2.3 Compound (R,S)-3(-o-tolyl-1,2,4-oxadiazole-5-yl)-ethanols (7d)

Semisolid, yield 65%. IR (KBr): 340; 2927; 2849; 1580; 1344; 1129; 719 cm^{-1}. ^{1}H RMN (300 MHz, $CDCl_3$): δ 7.90-7.86 (d, *J*= 12.6 Hz, 2H, H-2" and H-6"), 7.39-7.37 (d, *J*= 12.6 Hz, 2H, H-3" and H-5"), 5.19-5.12(q, *J*= 6.6 Hz, 1H, C**H**-OH); 2,82 (broad singlet, 1H, OH); 2.42 (s, 3H, CH_3-Ph), 1.71 (d, *J*= 6.6 Hz, 3H, CH_3). ^{13}C RMN (75 MHz, $CDCl_3$): δ 181.0 (C-3); 168.1 (C-5); 138.7 (C-1"); 132.1 (C-4"); 128.7 (C-3" and C-5"); 124.5 (C-2" and C-6"); 63.3 (C-OH); 21.4 (CH_3-Ph); 21.3 (CH_3).

2.2.4 Compound (R,S)-3-p-fluorophenyl-1,2,4-oxadiazole-5-yl)-ethanols (7e)

Crystals from *n*-hexane, mp: 132–133, yield 58%. IR (KBr): 3407; 2992; 2850; 1615; 1245; 1129; 850 cm^{-1}. ^{1}H RMN (300 MHz, $CDCl_3$): δ 8.17-8.01 (tt, *J*= 3.0 and *J*= 9.0 Hz, 2H, H-2" and H-6"); 7.18-7.09 (tt, *J*= 3,0 and *J* =9,0 Hz, 2H, H-3" and H-5"); 5.19-5.12 (q, *J* =6.6 Hz, 1H, C**H**-OH), 3.28 (broad singlet, 1H, OH); 1.68 (d, *J*= 6,6, 3H, CH_3). ^{13}C RMN (75 MHz, $CDCl_3$): δ 181.1 (C-3); 167.2 (C-5); 129.6 (C-1"); 129.5 (C-4"); 122.5 (C-3" and C-5"); 116.2 (C-2" and C-6"); 63.2 (C-OH); 21.3 (CH_3).

2.2.5 Compound (R,S)-3-p-benzylphenyl-1,2,4-oxadiazol-5-yl)-ethanol (7f)

Crystals from *n*-hexane, mp: 100–101°C, yield 68%. IR (KBr): 3420; 2990; 2845; 1602; 1230; 1007; 710 cm^{-1}. ^{1}H RMN (300 MHz, $CDCl_3$): δ 8.03-7.98 (tt, *J*= 3.0 Hz e *J* =9.0 Hz, 2H, H-2" 1 H-6"), 7.46-7.34(m, 5H, Ph-H); 7.08-7.04 (tt, *J*=3.0 e *J*= 9.0 Hz, 2H, H-3" e H-5"), 5.13-5.10 (q, *J*= 6.6 Hz, 1H, C**H**-OH), 3.18 (broad singlet, 1H, OH); 1.69 (d, *J* 6.6, 3H, CH_3). RMN ^{13}C (75 MHz, $CDCl_3$): δ 180.7 (C-3); 167.7 (C-5); 136.2 (C-1"); 129.1 (C-4"); 128.6 (C-3" e C-5"); 127.4 (C-2" e C-6"); 70.0 (-CH_2-); 53.2 (C-OH); 21.3 (CH_3).

2.3 Biological activity

A colony of *P. xylostella* was maintained at the Natural Insecticide Laboratory of the Agronomy Department of the Rural Federal University of Pernambuco (Brazil). All experiments were conducted at a temperature of 25 ± 0.5 °C, relative humidity of 67± 2.0% and 12-hour light/dark photoperiod.

2.3.1 Insect rearing and colony maintenance

The newly-emerged adults were sexed and placed in plastic cages with a sponge soaked in water to maintain proper humidity. A disc of filter paper (diameter: 8.0 cm) and a leaf disc of *Brassicaoleracea* (diameter: 8.0 cm) were placed on the sponge to stimulate the oviposition of *P. xylostella*. Adults were fed a 10% honey solution provided in polyurethane foam attached to a circular hole at the top of the cage. Discs of kale leaves with eggs were transferred to Petri dishes daily and remained until the hatching of the offspring. The discs and the larvae were kept in rectangular plastic containers (60 · 30 cm) with organic kale leaves as food. The larvae remained in these containers until pupation and the kale leaves were replaced daily. The pupae were collected in test tubes sealed with plastic containing polyvinyl chloride for air circulation. The pupae were kept at room temperature until the emergence of the adults, which were then transferred to new cages (60 · 30 cm).

2.3.2 Larval toxicity

The leaf disc immersion method was used to determine toxicity to larvae using the method described by Bandeira et al., [21] (2013). Discs of kale leaf (diameter: 8.0 cm) were immersed for 10 s in 50 ml of the different concentrations of the compound solutions with methanol as the solvent. The leaf discs were air dried and transferred individually to Petri dishes (diameter: 9.0 cm) containing a filter paper disc (diameter: 8.0 cm) soaked in distilled

water. Ten third-instar larvae (< 12 h old) were placed on the leaf discs. After placing the lids, the Petri dishes were wrapped in plastic wrap to avoid the escape of the larvae. Concentrations ranged from 0.04 to 0.95 mg mL^{-1} for the synthesized compounds and Decis 25 E.C. and Azamax were used as positive controls. Test solutions were prepared by diluting the synthesized compounds and positive controls (Decis 25 E.C. and azamax) in methanol. Methanol alone was used as the negative control experiment carried out under the same conditions. The experimental design was entirely randomized, with five treatments including the control and four replications per treatment. Each experiment was repeated twice. Mortality was assessed after 24 h of feeding on the treated and control leaf discs.

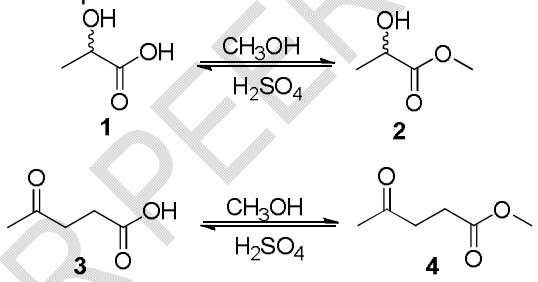
2.4 Statistical analysis

To estimate the curve slopes of the LC_{50} (lethal concentration) of each treatment, mortality data were submitted to PROBIT analysis using statistical software [22]. The concentrations were calculated based on the logarithmic series [23].

3. RESULTS AND DISCUSSION

3.1 Synthesis of 1,2,4-oxadiazole and precursors.

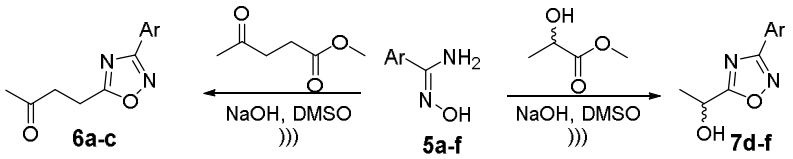
Precursors **2** and **4** were synthesized with good yields (75 and 77%, respectively) by the reaction of lactic acid or levulinic acid with methanol in the presence of H_2SO_4 as the catalyst at 70° C for 3 h (Scheme 1). The reaction was monitored by TLC. After completion, the excess alcohol was removed and the residue was extracted with dichloromethane. After being washed with sodium hydroxide and subsequently, with distilled water, the ether extract was evaporated to furnish the product.



Scheme 1. Synthesis of methyl lactate (2) and methyl levulinate (**4**).

The products were identified using spectral data (^1H and ^{13}C NMR) and all compounds were in full agreement with the proposed structure. Thearylamidoximes**5a-f**were obtained withexcellent yields(88 to 90%) following methodsdescribedin the literature [24].

The 4-[3-(aryl)-1,2,4-oxadiazol-5-yl]-butan-2-one (**6a-c**) and (*R,S*)-3-aryl-5-(1-hydroxy-ethyl)-1,2,4-oxadiazoles (**7d-f**) were synthesized using an appropriate arylamidoxime (**5a-f**), corresponding esters (**2** and **4**), sodium hydroxide and dimethyl sulfoxide mediated by ultrasound irradiation. The starting amidoximes were consumed in a short time (30 min), as evidenced by TLC (Scheme 2) [24]. Purification by liquid chromatography on a silica gel column using hexane-ethyl acetate (9:1) as eluent provided the products presumably **6a-c** and **7d-f**.



Scheme 2. Synthesis of 1,2,4-oxadiazoles **6a-c** and **7d-f**

The established reaction conditions, namely, amidoximes (1.00 equiv), esters (1.54 equiv) and potassium carbonate (1.25 equiv.) in 2 mL DMSO under ultrasonic irradiation for 30 minutes were then applied for the synthesis 1,2,4-oxadiazoles. The results are depicted in Table 1. Compounds **6a-c** and **7d-f** were obtained with moderate yields (58 to 92%).

Table 1. *Synthesis of 1,2,4-oxadiazoles* ***6a-c*** *and* ***7d-f*** *under microwave irradiation.*

Entry	*Esters*	*Amidoxime*	*Product*	*(%)[b]*
1	**4**	**5a**	**6a**	92
2	**4**	**5b**	**6b**	88
3	**4**	**5c**	**6c**	90
4	**2**	**5d**	**7d**	58
5	**2**	**5e**	**7e**	68
6	**2**	**5f**	**7f**	72

The structures of thesecompoundsweredemonstrated bytheinfrared spectraas well as[1]Hand[13]CNMR.The IR spectra of compounds **6a-c** revealed the following absorptions: 2927 cm^{-1} (aromatic ring C-H stretching), 2917 cm^{-1} (symmetric C-H stretching), 2830 cm^{-1}(asymmetric C-H stretching), 1720 cm^{-1} (C=O), 1580cm^{-1} (C=N of the five-member ring). IR absorptions at 3407 (O-H), 1634 (C=N), and 1446 cm–1 (C–O) were obtained for (*R,S*)-3-aryl-5-(1-hydroxy-ethyl)-1,2,4-oxadiazole, **7d**.

The ^{13}C NMR spectrum of **7d**showed characteristic peaks at 125.6 to 138.2 ppm, which were assigned to the aromatic carbon atoms. A peak at 21.4 ppm was attributed to aromaticmethyl. The peaks at 168.6 and 180.0 ppm were assigned to the carbon atoms of

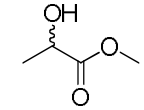
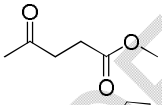
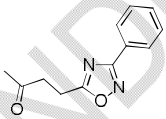
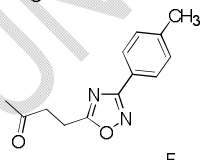
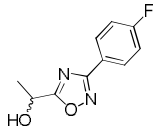
the oxadiazole ring moiety. The peaks at 22.2 and 63.2 ppm were assigned to the methylidyne and methyl carbon atoms. The ^{1}H NMR spectrum of **7d** showed characteristic signals at 8.97 (d, *J* = 8.0 Hz, 1H, H_{Aryl}) to 7.50 (m, 3H, H_{Aryl}) ppm, which were assigned to the aromatic protons. A signal at 5.16 ppm was assigned to the methylidyne proton (q, *J* = 6.8 Hz, 1H, C**H**-OH). The board singlet at 3.00 was assigned to the hydroxyl group. The singlet at 2.42 and the duplet at 1.69 ppm were assigned to the methyl substituted in the aromatic ring and methyl protons, respectively.

3.2 Biological activityof1,2,4-oxadiazóis and precursors

Table 2 displays the estimated LC_{50} for the insecticidal activity of the synthesized compounds **13a-13b** and **14b,** the positive controls Decis 25 E.C. and Azamax and the precursors methyl lactate (**2**)and methyl levulinate (**4**) of compounds **6a-6c** and **7e**, respectively. The precursors and synthesized compounds demonstrated insecticidal activity against 3rd instar *P. xylostella* larvae, as the mortality caused by these treatments was significantly higher than that found for the negative control (methanol) [21]. All 1,2,4-oxadiazoles demonstrated high toxicity and were more efficient than the respective precursors. Compounds **6a** and **6c** demonstrated the same level of toxicity and were the compounds with the greatest insecticidal activity, followed by compound **7e**.

Based on the results of the bioassays, no evident correlation was found between structure and activity for the synthesized compounds. Comparing the degrees of activity of these compounds, however, with the presence of the ring 1,2,4-oxadiazoles, independently of the replacement of the phenyl group, the three compounds tested demonstrated greater toxicity than the commercial insecticides used as the positive controls.

***Table 2**. Residual effect (LC_{50} in mg/mL) of precursors, synthesized compounds and positive controls against Plutellaxylostella.*

	Compounds/positive control	*Df*	*n*	LC_{50} (95% CI)	Slope ± SD	X^2
2		7	720	0.38 (0.37 – 0.40)	1.05±0.14	13.72
4		7	720	0.36 (0.35 – 0.39)	1.85±0.21	9.12
6a		7	720	0.28 (0.27 – 0.29)	0.95±0.11	13.87
6c		7	720	0.26 (0.25 – 0.27)	0.97±0.15	14.25
7e		7	720	0.31 (0.30 – 0.32)	2.03±0.28	11.04
	PC-1	4	720	1.11 (0.91 – 1.35)	1.19 ±0.09	6.09
	PC-2	4	720	0.42 (0.36 – 0.49)	1.51 ±0.11	5.74

n = total number of larvae tested; Df = degrees of freedom; SD = standard deviation; X^2 = chi-square test; CI = confidence interval; PC-1 = positive control Decis 25 C.E. with pyretroid as active ingredient; PC-2 = positive control Azamax with azadiractine as active ingredient.

4. CONCLUSION

The reaction between levulinic acid or lactic acid and methanol catalyzed by sulfuric acid led to the formation of different esters with good yields (75 and 78%, respectively). The synthesis of 4-[3-(aryl)-1,2,4-oxadiazole-5-yl]-butan-2-one (**6a-c**) and (*R,S*)-3-aryl-5-(1-hydroxy-ethyl)-1,2,4-oxadiazole (**7d-f**) was mediated by ultrasonic irradiation and consisted of reacting the arylamidoximes separately with esters (**2** and **4**), sodium hydroxide and DMSO. The products were obtained in moderate to excellent yields.

The present findings on the insecticidal potential of 1,2,4-oxadiazoles against the diamondback moth are promising, as the discovery of new molecules with insecticidal potential is an alternative form of the control of agricultural pests that can help avoid the selection of resistant populations, as in the case of *P. xylostella* in agricultural communities in the city of Garanhuns, state of Pernambuco, Brazil.

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