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Synthesis, characterization, and antimicrobial evaluation of novel 1,2,4-oxadiazoles derived from trans-3,4-(methylenedioxy)-cinnamic acid

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Abstract

Compounds containing heterocyclic ring systems are of great importance both medicinally and industrially. Five-membered 1,2,4-oxadiazole heterocycles have received considerable attention because of their unique bioisosteric properties and unusual wide spectrum of biological activities. In this study, a series of 2-(3-aryl-1,2,4-oxadiazol-5-yl)-*trans*-3,4-(methylenedioxy)-cinnamyl derivatives was synthesized and characterized, and *in vitro* experimental models were used to evaluate their antimicrobial activity. Synthesis, which involved microwave irradiation for 5 min, provided moderate yields of 1,2,4-oxadiazole (34–50%). Infrared (IR) *and nuclear magnetic resonance (*^{1}H NMR and ^{13}C NMR) spectroscopy were used to determine the structures of 1,2,4-oxadiazole. The disk diffusion method was used to test the antibacterial activity of the novel 1,2,4-oxadiazole derivatives against Gram-positive (*Staphylococcus aureus*, *Enterococcus faecalis*, *and Bacillus subtilis*) and Gram-negative (*Escherichia coli and Klebsiella pneumoniae*) bacteria. The derivatives, 2-(3-*m*-toluyl-1,2,4-oxadiazol-5-yl)-3,4-(methylenedioxy)-cinnamyl and 2-(3-pyrimidyl-1,2,4-oxadiazol-5-yl)-3,4-(methylenedioxy)-cinnamyl exhibited a minimum inhibitory concentration (MIC) of 19.5 μg mL^{-1} against *S. aureus*, and is four-fold more potent than the standard metronidazole (MIC =78 μg mL^{-1}).

Keywords: 1,2,4-oxadiazole; cinnamic acid; amidoxime; antibacterial.

Síntese, caracterização e avaliação antimicrobiana de 1,2,4-oxadiazóis derivados do ácido trans-3,4-(metilenodioxi)-cinâmico

Resumo

Os compostos que contêm sistemas de anéis heterocíclicos são de grande importância tanto na medicina quanto na indústria. O anel heterocíclico de 1,2,4-oxadiazol de cinco membros tem recebido atenção considerável por causa de suas propriedades bioisostéricas únicas e um espectro excepcionalmente amplo de atividades biológicas. Este estudo teve como objetivo a síntese, a caracterização e a avaliação da atividade antimicrobiana de uma série de 2-(3-aril-1,2,4-oxadiazol-5-il)-*trans*-3,4-(metilenodioxi)-cinamila usando modelos experimentais *in vitro*. A síntese dos 1,2,4-oxadiazóis foi desenvolvida, com duração de 5 min, usando irradiação de micro-ondas fornecendo os compostos em rendimentos moderados (34-50%). Suas estruturas foram determinadas usando espectroscopia de IV (Infravermelho), Resonância magnética nuclear de hidrogênio (RMN ^{1}H) () eRessonancia magnética nuclear de carbono 13 (RMN ^{13}C). As atividades antibacterianas dos novos derivados de 1,3,4-oxadiazóis foram testado contra Gram positivo (*Bacillus subtilis, Enterococcus faecalis e Staphylococcus aureus*) e Gram negativo (*Escherichia coli* e *Klebsiella pneumoniae*) bactérias usando o método de difusão em disco. Os 2-(3-*m*-toluil-1,2,4-oxadiazol-5-il)-*3,4*-(metilenodioxi)-cinamila e o 2-(3-pirimidil-1,2,4-oxadiazol-5-il)-*3,4*-(metilenodioxi)- cinamila apresentaram resultados contra *S. aureus*, com valor de CIM de 19,5 μg mL^{-1} quatro vezes mais potente que o metronidazol padrão (CIM=78 μg mL^{-1}).

Palavras-chave: 1,2,4-Oxadiazol; ácido cinâmico; amidoxime; antibacterina.

Introduction

Heterocyclic structures are important of constructing numerous organic molecules geared towards various applications. Furthermore, there have been significant advances in the biological applications of widely studied five-membered heterocyclic compounds (Biernacki *et al*., 2020), such as oxadiazoles, thiadiazoles, triazoles, and tetrazoles (Aliabadi, 2016, Puzanov *et al*., 2021 Vinaya, Chandrashekara & Shivaramu, 2019).

Several biological functions, such as anticancer, (Maftei, *et al*., 2013; Kumar *et al*., 2011; Moniot *et al*., 2017), anti-inflammatory (Bezerra *et al*., 2005; Gobec *et al*., 2015; Bora *et al*., 2014), anticonvulsant (Mohammadi-Khanaposhtani *et al*., 2016), antibacterial (Baral *et al*., 2019), antifungal (Sortino *et al*., 2008), antiviral (Chernyshov *et al*., 2022), antioxidant Gobec *et al*., 2015), analgesic (Farooqui *et al*., 2009), anti-insomnia (Brotschi *et al*., 2019), antiparasitic (Haugwitz *et al*., 1985), antidepressant, anti-edema, and anti-Alzheimer (Biernacki *et al*., 2020) activity have been attributed to compounds with the 1,2,4-oxadiazole ring. Additionally, 1,2,4-oxadiazoles exhibit pesticidal activity, such as insecticidal (Liu *et al*., 2017), antifungal (Yang *et al*., 2021), and herbicidal activity (Ölmez & Waseer, 2020).

Cinnamic acid, which constitutes the essential oils of several botanical species, is an aromatic fatty acid containing a phenyl ring with an acrylic acid substituent. The alkene group of the acrylic acid substituent commonly exhibits *trans* isomerism (Yilmaz *et al*., 2018; Rodrigues *et al*., 2019).

Segundo *et al*. (2020) demonstrated that the alkene bond in cinnamic acid is modifiable. Such modifications in a variety of compounds are useful for enhancing the efficacy of the aforementioned pesticidal activities and bioactivities. Several studies have also reported the pharmacological potential of natural and synthetic cinnamic acid derivatives (Debnath *et al*., 2003; Sova, 2012; De Vita *et al*., 2014; Zhang *et al*., 2015). In cinnamic acid, modification of some substitutions in the aromatic ring, such as the -N-C-O- sequence, can possibly generate derivatives having antibacterial and antibiofilm activity because these modifications may enable the derivatives to react with the nucleophilic centers of microbial targets (Parrino *et al*., 2021). Because of these possibilities, studies of the antimicrobial activity of 1,2,4-oxadiazoles from cinnamic acid are important for expanding our knowledge of the biological activity of 1,2,4-oxadiazoles.

In this study, we aim to synthesize, characterize, and use *in vitro* experimental models to evaluate the antimicrobial activity of a series of 2-(3-aryl-1,2,4-oxadiazol-5-yl)-*trans*-3,4-(methylenedioxy)-cinnamyl compounds.

Materials and Methods

Reagents and solvents

The reagents and solvents used in the study were commercially obtained from Sigma Aldrich, Merck, Kinetics, Dynamics, and Vetec with P.A. Solvents such as *n*-hexane (C_6H_{12}) and ethyl acetate ($C_4H_8O_2$) were initially purified by fractional distillation in a Vigreux column. Anhydrous calcium sulfate was added as a drying agent in dichloromethane. For thin-layer chromatography (TLC), silica gel plates, which contained the fluorescent indicator F_{254} (Merck), were used as the solid phase; hexane/ethyl acetate systems were used as the mobile phase. The plates were visualized with an ultraviolet lamp and were developed in 254 nm and 365 nm ultraviolet irradiation chambers using a sulfuric acid:ethanol (5:95) mixture and iodine vapor. In the chromatographic columns, silica gel 60 (Merck, 70-230 mesh) was used as the stationary phase.

Equipment

The ^{1}H and ^{13}C NMR spectra were obtained with Varian Unity Plus instruments (300 or 400 MHz for hydrogen and 75 or 100 MHz for carbon-13). $CDCl_3$ was used as the solvent or to acquire the residual solvent signal, which served as an internal reference. The chemical shift values (δ) are expressed in parts per million (ppm) and the coupling constants (*J*) in hertz (Hz). A Varian Model 640 FT-IR spectrometer was used to generate the IR spectra. A BioSan PFM II device was used to determine the melting points, which were left uncorrected. Microwave-assisted synthesis was performed with a branded CEM device to determine the SP. A rotary evaporator (Fisatom Rotavapor, model 802) connected to a vacuum pump was used to evaporate the reaction solvent.

Synthesis of ethyl-trans-3,4-(methylenedioxy)-cinnamate (Voisin-Chiret et al., 2007)

In a round-bottom flask, *trans*-3,4-(methylenedioxy)-cinnamic acid (1.0 g) was added to ethanol (50 ml). Concentrated H_2SO_4 (2 ml) at 0 °C was then slowly added to the flask. The reaction mixture was then heated under reflux for 4 h. Thin layer chromatography (TLC) with a mobile phase of hexane/ethyl acetate (7:3) was used to monitor the reaction.

The reaction mixture was concentrated *in vacuo*, and Na_2CO_3 (1 mol/L) was then added until the pH reached ≥ 8, forming a precipitate. In a separatory funnel, the alkaline mixture was extracted with ethyl acetate (3 × 20 ml), and the collected organic phase was washed slowly with Na_2CO_3 (10 ml; 1 mol/L). The organic phase was then dried with anhydrous Na_2SO_3, filtered, and rotary evaporated to obtain a white solid.

Characterization: white solid, 97% yield, melting point (mp): 65 °C; IR (KBr, ν/cm^{-1}): 1703 (C=O), 1624 (C=C), 1496 (Ar), 1455 (Ar), 1256 (OCH_2O), 1174 (OCH_2O); ^{1}H NMR (300 MHz, $CDCl_3$) δ 7.58 (d, *J* = 15.9 Hz, 1H), 7.02 (d, *J* = 1.7 Hz, 1H), 7.00 (dd, *J* = 8.0 and 1.7 Hz, 1H), 6.80 (d, *J* = 7.9 Hz, 1H), 6.25 (d, *J* = 15.9 Hz, 1H), 6.08–5.93 (m, 2H), 4.25 (q, *J* = 7,1 Hz, 2H), 1.32 (t, *J* = 7.1 Hz, 3H); ^{13}C NMR (75 MHz, $CDCl_3$) δ 167.2, 149.50, 148.28, 144.21, 128.87, 124.30, 116.19, 108.48, 106.44, 101.48, 77.40, 60.34, 14.30.

Synthesis of arylamidoximes

An Erlenmeyer flask was charged with 20 mmol of the

nitriles (*p*-toluoylnitrile (C_8H_7N), *m*-t toluoylnitrile (C_8H_7N), phenylnitrile (C_7H_5N), 4-pyridinonitrile ($C_6H_4N_2$), *p*-bromonitrile (C_7H_4NBr), and *p*-chloronitrile (C_7H_4NCl), which were separately dissolved in 100 mL of ethyl alcohol. In another Erlenmeyer flask, hydroxylamine hydrochloride (30 mmol; $NH_2OH.HCl$) and sodium carbonate (CaCO3; 15 mmol) were dissolved in water (10 ml) and stirred for approximately 10 min at room temperature. The hydroxylamine hydrochloride and sodium carbonate mixture was then added to the nitrile solution, which was stirred continuously at 25 °C for 24 h until the nitrile disappeared, which was evidenced by TLC with a chloroform:hexane system (1:1) as the mobile phase. After the reaction was complete, a rotary evaporator was used to evaporate the ethanol to subsequently extract the organic phase. A separatory funnel was used to separate the aqueous phase from the organic phase, which was further extracted with ethyl acetate (2 × 10 ml). After collection, the organic phase was dried over anhydrous Na_2SO_4. The extracted solvent was then filtered and removed under reduced pressure. The crude product was then recrystallized from chloroform to produce pure arylamidoximes in moderate to good yields (52–99%). The IR, ^1H, and ^{13}C NMR data are consistent with the data reported by Andrade *et al*. (2016).

Synthesis of novel 1,2,4-oxadiazoles

In a glass reactor, the mixture of ester 2 (1 mmol), arylamidoximes (1.3 mmol), K_2CO_3 (1.5 mmol) and DMF (5 drops) was subjected to microwave irradiation (150 W, 180 °C, and maximum pressure of 3 bar) for 2 min. TLC with chloroform as the mobile phase was used to monitor the reaction. After the reaction was complete, water (20 ml) was added to the reaction mixture, and a separatory funnel was used to separate the organic phase, which was extracted with chloroform (3 × 10 ml). The resultant products were subsequently evaporated. TLC with silica gel and hexane/ethyl acetate (99:1) was used to purify the crude products to yield the desired 1,2,4-oxadiazoles.

2-(3-Fenil-1,2,4-oxadiazol-5-il)-*3,4*-(metilenodioxi)-cinamila (6a): White solid, 50% yield, mp: 142–144 °C; IR (KBr, ν/cm^{-1}): 1651 (C=C), 1539 (C=N), 1492 (Ar), 1442 (Ar), 1250 (OCH_2O), 1345 (C-O); ^1H NMR (300 MHz, $CDCl_3$): δ 8.14−8.11 (m, 2H), 7.80 (d, 1H, *J* = 16.0 Hz), 7.54 (m, 3H), 7.13 (s, 1H), 7.10 (d, 1H, *J* = 8.1 Hz), 6.89 (d, 1H, *J* = 16.0 Hz), 6.87 (d, 1H, *J* = 7.8 Hz), 6.05 (s, 2H); ^{13}C NMR (75 MHz, $CDCl_3$): δ 175.4, 168.6, 149.8, 148.5, 142.3, 131.1, 128.9, 128.8, 127.4, 127.0, 124.5, 108.7, 108.2, 106.6, 101.7.

2-(3-*p*-Toluil-1,2,4-oxadiazol-5-il)-*3,4*-(metilenodioxi)-cinamila (6b): White solid; 46% yield; mp: 135–137 °C, IR (KBr, ν/cm^{-1}): 1638 (C=C), 1558 (C=N), 1502 (Ar), 1460 (Ar), 1255 (OCH_2O), 1035 (C-O); RMN ^1H (300 MHz, $CDCl_3$) δ 7.97–7.93 (m, 1H), 7.90 (s, 1H), 7.78 (d, *J* = 16.3 Hz, 1H), 7.38 (t, *J* = 7.5 Hz, 1H), 7.32 (s, 1H), 7.10 (d, *J* = 1.5 Hz, 1H), 7.08 (dd, *J* = 8.0, 1.6 Hz, 1H), 6.88 (d, *J* = 15.0 Hz, 1H), 6.84 (d, *J* = 1.6 Hz, 1H), 6.02 (s, 1H), 2.43 (s, 3H); ^{13}C NMR (100 MHz, $CDCl_3$) δ 175.29, 168.70, 149.80, 148.50, 142.24, 138.58, 131.84, 128.88, 128.71, 127.94, 126.81, 124.50, 124.42, 108.67, 108.19, 106.17, 101.64, 21.30.

2-(3-*m*-Toluil-1,2,4-oxadiazol-5-il)-*3,4*-(metilenodioxi)-cinamila (6c): White solid; 46% yield; mp: 137–138 °C, IR (KBr, ν/cm^{-1}): 1639 (C=C), 1557 (C=N), 1504 (Ar), 1461 (Ar), 1254 (OCH_2O), 1033 (C-O); ^1H NMR (400 MHz, $CDCl_3$): δ 7.95 (s, 1H), 7.93 (d, 1H, *J* = 8.0 Hz), 7.80 (d, 1H, *J* = 16.4 Hz), 7.39 (t, 1H, *J* = 7.6 Hz), 7.33 (d, 1H, *J* = 6.8 Hz), 7.12 (s, 1H), 7.1 (d, 1H, *J* = 8.8 Hz), 6.89 (d, 1H, *J* =16.0 Hz), 6.87 (d, 1H, *J* = 7.2 Hz), 6.05 (s, 2H), 2.45 (s, 3H); ^{13}C NMR (100 MHz, $CDCl_3$): δ 175.3, 168.7, 149.8, 148.5, 142.3, 138.6, 131.9, 128.9, 128.7, 127.9, 126.8, 124.5, 124.4, 108.7, 108.2, 106.2, 101.7, 21.3.

2-(3-Pirimidil-1,2,4-oxadiazol-5-il)-*3,4*-(metilenodioxi)-cinamila (6d): White solid; 34% yield; mp: 200 °C; IR (KBr, ν/cm^{-1}): 1644 (C=C), 1560 (C=N), 1504 (Ar), 1450 (Ar), 1270 (OCH_2O), 1040 (C-O); ^1H NMR (300 MHz, $CDCl_3$) δ 8.79 (d, *J* = 5.0 Hz, 2H), 7.99 (d, *J* = 5.2 Hz, 1H), 7.81 (d, *J* = 16.3 Hz, 1H), 7.12 (s, 1H), 7.08 (s, 1H), 6.88 (s, 1H), 6.87 (d, *J* = 16.0 Hz, 1H), 6.04 (s, 2H); ^{13}C NMR (100 MHz, $CDCl_3$) δ 176.21, 167.05, 150.47, 150.09, 148.59, 143.14, 134.63, 128.63, 124.73, 121.30, 108.74, 107.62, 106.21, 101.74.

2-(3-*p*-Bromofenil-1,2,4-oxadiazol-5-il)-*3,4*-(metilenodioxi)-cinamila (6e): White solid; yield 46%; mp: 178–180 °C; IR (KBr, ν/cm^{-1}): 1649 (C=C), 1538 (C=N), 1500 (Ar), 1455 (Ar), 1261 (OCH_2O), 1039 (C-O); ^1H NMR (400 MHz, $CDCl_3$) δ 7.99 (d, *J* = 8.2 Hz, 2H), 7.78 (d, *J* = 16.3 Hz, 1H), 7.63 (d, *J* = 8.2 Hz, 2H), 7.11 (s, 1H), 7.09 (d, *J* = 8.2 Hz, 1H), 6.88 (d, *J* = 15.2 Hz, 1H), 6.85 (d, *J* = 2.8 Hz, 1H), 6.04 (s, 2H); ^{13}C NMR (100 MHz, $CDCl_3$) δ 175.62, 167.92, 149.94, 148.56, 142.61, 132.10, 128.91, 128.80, 125.97, 125.62, 124.56, 108.72, 107.96, 106.21, 101.69.

2-(3-*p*-Clorofenil-1,2,4-oxadiazol-5-il)-*3,4*-(metilenodioxi)-cinamila (6f): White solid; 41% yield; mp: 187–188 °C; IR (KBr, ν/cm^{-1}): 1636 (C=C), 1572 (C=N), 1504 (Ar), 1453 (Ar), 1266 (OCH_2O), 1039 (C-O); ^1H NMR (400 MHz, $CDCl_3$) δ 8.06 (d, *J* = 7.5 Hz, 2H), 7.79 (d, *J* = 16.2 Hz, 1H), 7.47 (d, *J* = 7.7 Hz, 2H), 7.12 (s, 1H), 7.08 (s, 1H), 6.87 (d, *J* = 15.2 Hz 1H), 6.85 (s, 1H), 6.04 (s, 2H); ^{13}C NMR (101 MHz, $CDCl_3$) δ 175.60, 167.83, 149.93, 148.56, 142.60, 137.22, 129.14, 128.72, 125.52, 124.55, 108.72, 107.99, 106.21, 101.70.

In vitro assay of antimicrobial activity

To assess the antimicrobial activity of the cinnamic acid derivatives, the microdilution technique was used to determine the minimum inhibitory concentrations (MICs) in a Multiplate™ 96-well. The test was performed according to the Clinical and Laboratory Standards Institute (CLSI, 2010) and the use of the Multiplate™ 96-well depended on the microorganisms tested. Saubouraud (Sab) liquid culture medium was used for filamentous and yeast fungi, and Mueller–Hinton (MH) broth was used for Gram-positive and Gram-negative bacteria. Different microorganisms from the Department of Antibiotics of the Federal University of Pernambuco were used, namely three Gram-positive bacteria (*Staphylococcus aureus* (UFPEDA 02/ATTCC 6538), *Bacillus subtilis* (UFPEDA 86), and *Enterococcus faecalis* (UFPEDA 138)), and one Gram-negative bacterium (*Escherichia coli* (UFPEDA 224/ATCC 25922)). Additionally, metronidazole and fluconazole were used as positive controls. The methodology of Espinel-Ingroff *et al*.

(2005) was used to determine the minimum inhibitory concentrations (MICs). All samples were analyzed in triplicate. The microplates were cultured at 37 °C for 18–24 h for bacteria and at 30 °C for 48–72 h for fungi.

Results and Discussion

The modified method of Voisin-Chiret *et al*. (2007) was used for synthesizing ethyl-*trans*-3,4-methylenedioxy-cinnamate 2 (Figure 1). The synthesis involved reacting *trans*-3,4-methylenedioxy-cinnamic acid 1 with ethanol and sulfuric acid.

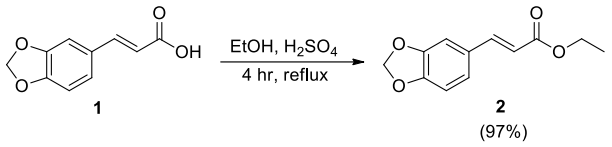


Figure 1. Synthesis of ethyl-*trans*-3,4-methylenedioxy-cinnamate.

IR, ^{1}H NMR, and ^{13}C NMR spectroscopy were used to confirm the structure of ethyl-*trans*-3,4-methylenedioxy-cinnamate. The IR spectrum shows the symmetrical and asymmetrical stretching of the CH_2 of the 1,3-benzodioxol group at 2990 cm^{-1}, the CO stretching of the methylenedioxyl group at 927 cm^{-1}, and the stretching of C=O in the conjugated esters at 1702 cm^{-1}.

The ^{1}H NMR spectrum of compound 2 shows a singlet with a 2H integral at 6.0 ppm, which was assigned to the CH_2 of the methylenedioxyl ring. The 3H integral at 6.80 ppm has a doublet multiplicity at 7.02 ppm and a double doublet at 7.00 ppm with J = 1.67 and 8.03, respectively, and was assigned to the aromatic hydrogens. Two doublets at 7.58 and 6.25 ppm with a coupling constant of ^{3}J = 15.93 are associated with the *E* stereoisomerism of the alkene hydrogens in the cinnamate double bond. Additionally, the spectrum shows a quartet at 4.23 ppm associated with the O-CH_2 group and a triplet at 1.32 ppm associated with the hydrogens of the methyl group.

The ^{13}C NMR spectrum of the compound shows a signal at 14.30 ppm, which corresponds to methyl carbons (CH_3). The signal at 60.34 ppm corresponds to the methylene of the O-CH_2 group in compound 2. The signal at 101.48 ppm was assigned to the CH_2 of the methylenedioxyl group, whereas those at 144 and 116.19 ppm were assigned to the vinyl carbons. The signal at 167.12 ppm was assigned to the carbonyl carbon of the ester. The signals at 149.5–106.4 ppm were assigned to the aromatic carbons, confirming the structure of the compound. These data agree with those of Voisin-Chiret *et al*. (2007) for the esterification of a similar compound.

Subsequently, six arylamixoximes were synthesized from the reaction of arylnitriles with hydroxylamine hydrochloride and sodium carbonate (Figure 2).

Nitriles with electron-withdrawing groups on the phenyl ring resulted in good yields of the respective amidoximes (5d, 5e, and 5f). Additionally, when a heteroaromatic nitrile (3d) was used, the corresponding amidoxime (5d) was obtained in 84% yield after 12 h.

Because arylamidoximes are widely described in the literature, only IR spectroscopy was performed for their characterization. The IR spectra of compounds 5a–f show that the characteristic band at 2240 cm^{-1} for nitriles, which is associated with the stretching of the C≡N bond, was absent. The bands at 3359–3453 cm^{-1}, 3200 cm^{-1}, and 1650 cm^{-1}, corresponding to the symmetrical and asymmetric stretching of the NH_2, OH, and C=N groups, respectively, confirm the formation of the six arylamidoximes. The spectroscopic data of the compounds are congruent with those of Lima *et al*. (2020) and Andrade *et al*. (2016).

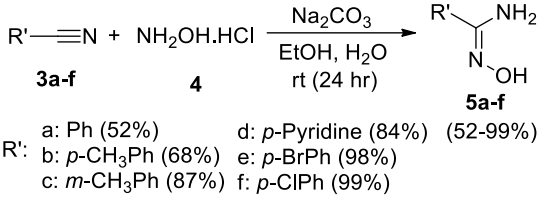


Figure 2. Synthesis of the different arylamidoximes (5a-f).

The proposed mechanism for the synthesis of arylamidoximes consists of an initial nucleophilic attack of hydroxylamine on arylnitrile (Figure 3). This reaction can be promoted by an electron pair from either the O (route A) or N atom (route B) of hydroxylamine, resulting in intermediates III and I, respectively. Routes A and B are in equilibrium; however, in route B, the equilibrium is more shifted towards the reactants because of the lower stability of this species when compared to intermediate III. Intermediate III can accept an electron pair from the N atom bonded to the O atom to under intramolecular rearrangement, forming a three-membered ring, intermediate II. Intermediate III is in equilibrium with intermediate I.

Once intermediate I is formed, it can undergo intramolecular rearrangement followed by allylic rearrangement to yield intermediate IV, which leads to the desired arylamidoxime. Additionally, depending on the amount of hydroxylamine in the reaction mixture and the stability of the intermediate III generated *in situ*, a parallel reaction can lead to the formation of an undesired by-product, that is, arylamide, because the bound proton can be abstracted from intermediate V by the O atom of the NH_2O^- ion and transferred to the N atom (Figure 4). This mechanistic proposal was initially elaborated by Srivastava *et al*. (2009) after an extensive study of amidoximes.

The 2-(3-aryl-1,2,4-oxadiazol-5-yl)-*trans*-3,4-methylene dioxy)-cinnamyl derivatives (6a–f) were synthesized under microwave irradiation, and involved reacting different aryamidoximes (5a–f) and ethyl-*trans*-3,4-methylenedioxycinnamate 2 with a base (K_2CO_3) in dimethylformamide (DMF; Figure 4).

Note that electron-donating groups on the aromatic ring, such as the methyl group at C-6b(-6c), favor the reaction, leading to a better yield. Comparatively, electron-removing groups disfavor the reaction, as evidenced by the lower yields of compounds 6d–f.

Thus, electron-withdrawing groups, such as chlorine and bromine, result in slightly lower yields compared to electron-donating groups.

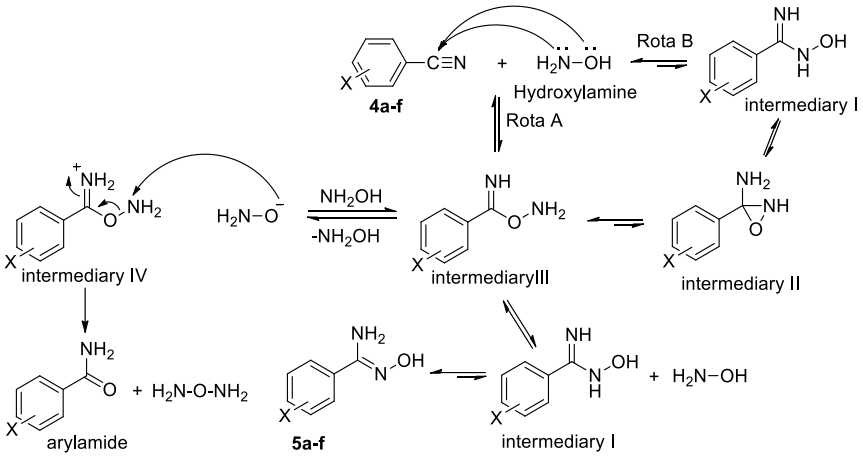


Figure 3. Mechanistic proposal for the synthesis of arylamidoximes.

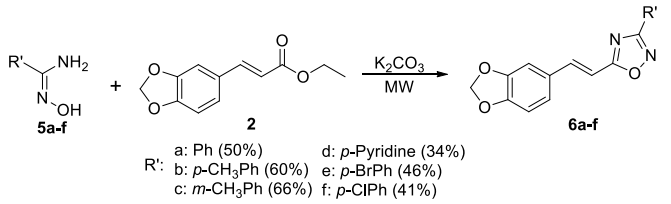


Figure 4. Synthesis of 2-(3-aryl-1,2,4-oxadiazol-5-yl)-*trans*-3,4-(methylenedioxy)-cinnamyl.

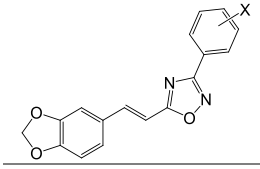
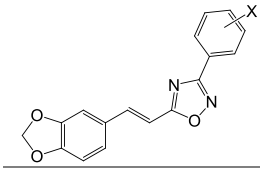
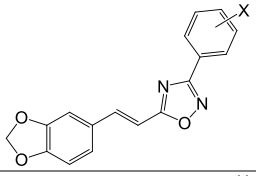
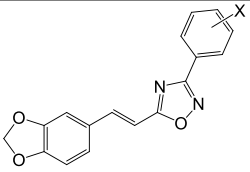
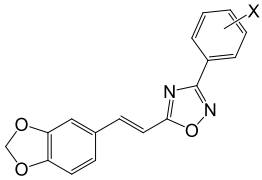
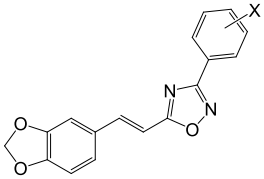
This result is obtained because the nucleophilicity of arylamidoximes is reduced when electron-withdrawing groups are directly attached to the aromatic ring. Table 1 summarizes the experimental data for synthesized and characterize compounds (6a–f).

All the ^{1}H NMR spectra of the 2-(3-aryl-1,2,4-oxadiazol-5-yl)-*trans*-3,4-(methylenedioxy)-cinnamyl derivatives show a singlet with a 2H integral at 6.04 ppm, which was assigned to the methylene hydrogens of the methylenedioxy group. The 3H integral between 7.48–8.85 ppm was assigned to the aromatic hydrogens. The vinylic hydrogens in cinnamate at 6.90 and 7.81 ppm with a coupling constant J = 15.95–16.26 Hz) are characteristic of *E* stereoisomers. The ^{13}C NMR spectrum shows that the carbons of the oxadiazole nucleus have signals at 175.4 and 168.6 ppm. The signals at 142.3 and 108.7 ppm were assigned to the alkene carbons, which display *E* stereoisomerism. The signals at 149.9–106.2 ppm were assigned to the aromatic carbons. The signal at 101.7 ppm was assigned to the carbons of the methylenedioxy group. The characteristic signals of the starting material, such as the carbonyl ester signal at 167.12 ppm and that of the O-CH_2 group at 60.34 ppm, were not observed, which confirm the proposed structure.

Figure 5 shows the proposed reaction mechanism for the formation of 1,2,4-oxadiazoles. Initially, the acidic proton from compounds 3a–f is removed with a suitable base, creating an anion on the oxygen atom.

The ester carbonyl then undergoes nucleophilic attack by the oxygen of amidoxime to furnish unstable tetrahedral species I with subsequent loss of an alcohol to give II.

Table 1. Experimental data for the synthesis of 2-(3-aryl-1,2,4-oxadiazol-5-yl)-*trans*-3,4-(methylenedioxy)-cinnamyl.

Compound	Code	Yield (%)*	Melting point (ºC)
	6a (X = H)	62	143-144
	6b (X = *p*-CH_3)	60	135-136
	6c (X = *m*-CH_3)	66	136-137
	6d (X = py)**	34	200
	6e (X = *p*-Br)	46	179-180
	6f (X = *p*-Cl)	41	187-188

*After chromatographic purification. **Pyrimidyl.

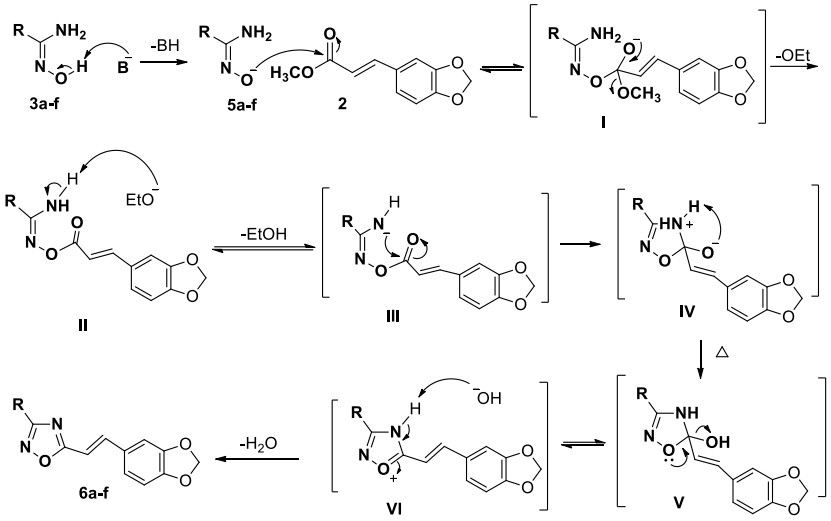


Figure 5. Plausible mechanism for the formation of 2-(3-aryl-1,2,4-oxadiazol-5-yl)-*trans*-3,4-(methylenedioxy)-cinnamyl.

Subsequently, compound II is converted to III by proton abstraction and cleavage of the N-H bond (with the electron pair migrating to the nitrogen atom) to yield compound II. This intermediate is then cyclized to provide IV, which further undergoes dehydration upon heating (intermediates V and VI) to produce the desired 1,2,4-oxadiazole.

Among the evaulated compounds, 6c and 6d showed antimicrobial activity against the tested bacteria (Table 2).

Table 2. Minimum inhibitory concentration of 2-(3-aryl-1,2,4-oxadiazol-5-yl)-*trans*-3,4-(methylenedioxy)-cinnamyl against *S. aureus, B. subtilis, E faecalis,* and *E. coli*

Compound	Bacteria				
	S. aureus	*B. Subtilis*	*E. coli*	*K. pneumoniae*	*E. faecalis*
2	2500	2500	1250	1250	2500
6c	19.5	(-)	625	2500	2500
6d	19.5	2500	2500	19.5	2500
6f	2500	>2500	2500	>2500	2500
Met*	78	>2500	78.1	625	156.2

(-) test not performed. *Metronidazole.

These 1,2,4-oxadiazoles (6c and 6d) were the most active with the same MIC value of 19.5 μg mL^{-1} against *Staphylococcus aureus*, inhibiting it at a concentration lower than the standard. Compound 6d also showed a MIC of 19.5 μg mL^{-1} for *Klebsiella pneumoniae*, a result superior to that obtined with the standard, metronidazole. Compound 6c showed moderate activity against *Escherichia coli* with a MIC of 625 μg mL^{-1}.

Compounds 6c and 6d were more active (19.5 μg mL^{-1}) than the standard metronidazole (78 μg mL^{-1}). Based on the previous studies by Ibrahim *et al*. (2021), this inhibitory activity is likely attributable to the 1,2,4-oxadiazolyl group. In their study, Ibrahim *et al*. (2021) synthesized a series of 1,2,4-oxadiazole-chacones and demonstrated that some compounds showed antibacterial potential, proving that they had a broad spectrum of activity against the Gram-positive and Gram-negative organisms tested. Additionally, Ibrahim *et al*. (2021) observed that the tested compounds had a MIC of 3.12 μM, representing twice the activity of the standard, ciprofloxacin (MIC = 6.25 μM). According to Morales *et al*. (2008), MIC values indicate good antimicrobial activity within the range of 50–500 μg mL^{-1}, moderate activity within the range of 500–1500 μg mL^{-1}, and weak activity above 1500 μg mL^{-1}.

Conclusion

Six novel arylamidoximes were synthesized with satisfactory yields. Of these compounds, pyridineami doxime, 4-chlorobenzamidoxime, and 4-bromobenza midoxime had the highest yields. Additionally, the esterification of trans-3,4-methylenedioxy-cinnamic acid provided ethyl-trans-3,4-methylenedioxy-cinnamate ester in a yield excelent. Furthermore, novel 2-(3-aryl-1,2,4-oxadiazol-5-yl)-trans-3,4-(methylenedioxy)-cinnamyl deriva tives were synthesized from different arylamidoximes and ethyl-trans-3,4-methylenedioxy cinnamate under mild conditions in satisfactory yields.

Although the antimicrobial activity of ethyl-trans-3,4-methylenedioxy-cinnamate and 2-(3-aryl-1,2,4-oxadiazol-5-yl)-trans-3,4-(methylenedioxy)-cinnamyl was evaluated, the other derivatives, namely 2-(3-m-toluyl-1,2,4-oxadiazol-5-yl)-3,4-(methylenedioxy)-cinnamyl and 2-(3-pyrimidyl-1,2, 4-oxadiazol-5-yl)-3,4-(methylenedioxy)-cinnamyl showed notable antibacterial activity against *S. aureus*, making them four times more potent than the standard, metronidazole. Future research should also evaluate other possible activities of these compounds and their derivatives as likely therapeutic agents.

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