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Synthesis, antimicrobial activity, and in silico studies of 1,2,4-oxadiazoles from ethyl levulinate

Josefa Aqueline da Cunha Lima[a], Erick Caique Santos Costa[b], Giselle Barbosa Bezerra[a], Jadson de Farias Silva[a], Rodrigo Ribeiro Alves Caina[b], João Rufino de Freitas Filho[a], Juliano Carlo Rufino Freitas[a]

[a] *Programa de Pós-Graduação em Química, Universidade Federal Rural de Pernambuco, Recife, 52171-900, Pernambuco, Brasil.* * *akelinecunha@gmail.com*

[b] *Programa de Pós-Graduação em Ciências Naturais e Biotecnologia, Universidade Federal de Campina Grande, Cuité, 58175-000, Paraíba, Brasil.*

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Abstract

This study describes the synthesis, antimicrobial activity, and *in silico* assessment of 1,2,4-oxadiazoles from ethyl levulinate. For that, we prepared arylamidoximes and treated them with ethyl levulinate, obtaining the respective 1,2,4-oxadiazoles through a reaction sequence of *O*-acylation followed by cyclodehydration. Then, we assessed the antimicrobial activity of these compounds against *Staphylococcus aureus*, *Enterococcus faecalis*, *Escherichia coli*, *Pseudomonas aeruginosa*, and *Candida utilis* using the broth microdilution technique. We analyzed *in silico* studies using the online bioinformatics platforms SwissADME and PASS. We obtained arylamidoximes and 1,2,4-oxadiazoles in good yields. The 1,2,4-oxadiazoles showed moderate antimicrobial activity, inhibiting two microorganisms: the bacterium *P. aeruginosa* and the fungus *C. utilis.* *In silico* studies of 1,2,4-oxadiazoles have shown promising properties, such as good oral absorption, low probability of toxicity, good body distribution, and potential to develop metabolic and enzymatic activities. The investigation of the antimicrobial activity together with the *in silico* studies showed that the synthesized 1,2,4-oxadiazoles are promising structures for the development of new therapeutic agents.

Keywords: Antimicrobial activity, computational studies, heterocyclic, organic synthesis.

Síntese, atividade antimicrobiana e estudo *in silico* de 1,2,4-oxadiazóis derivados do levulinato de etila

Abstract

Este estudo descreve a síntese, avaliação da atividade antimicrobiana e *in silico* de 1,2,4-oxadiazóis derivados do levulinato de etila. Nesse sentido, arilamidoximas foram preparadas e tratadas em seguida com o levulinato de etila, fornecendo os respectivos 1,2,4-oxadiazóis através de uma sequência reacional de *O*-acilação seguida de ciclodesidratação. A avaliação da ação antimicrobiana foi realizada contra *Staphylococcus aureus*, *Enterococcus faecalis*, *Escherichia coli*, *Pseudomonas aeruginosa* e *Candida utilis* através da técnica de microdiluição em caldo. Os estudos *in sílico* foram realizados através das plataformas digitais de bioinformática SwissADME e PASS *online*. As arilamidoximas e os 1,2,4-oxadiazóis foram obtidos em bons rendimentos. Os 1,2,4-oxadiazóis apresentaram atividade antimicrobiana moderada, uma vez que apresentaram inibição contra dois microrganismos, a bactéria *P. aeruginosa* e o fungo *C. utilis*. Os estudos *in silico* dos 1,2,4-oxadiazóis demonstraram propriedades promissoras, como boa absorção via oral, baixa probabilidade de apresentarem toxicidade, boa distribuição corpórea, bem como potencial de desenvolverem atividades metabólicas e enzimáticas. A investigação da ação antimicrobiana juntamente com os estudos *in silico* demostraram que os 1,2,4-oxadiazóis sintetizados são estruturas promissoras para o desenvolvimento de novos agentes terapêuticos.

Palavras-chave: Ação antimicrobiana, estudos computacionais, heterocíclicos, síntese orgânica.

Introduction

Microbial resistance is a major public health problem worldwide, which is partly due to inappropriate and/or excessive use of antibiotic drugs (Prestinaci, Pezzotti, & Pantosti, 2015). Resistance occurs when microorganisms undergo genetic mutation through exposure to antimicrobial drugs, multiplying resistant organisms and thus hindering the treatment and cure of diseases (Prestinaci, Pezzotti & Pantosti, 2015).

A report published in 2019 by the US Centers for Disease Control and Prevention (CDC) estimates that more than 2.8 million infections by antibiotic-resistant microorganisms occur annually in the USA, resulting in more than 35,000 deaths per year (CDC, 2019). In addition, a considerable portion of commercial antimicrobials (for example, posaconazole, ampicillin, ciprofloxacin) have limited action against resistant strains of microorganisms (Cunha, Nogueira, & Aguiar, 2018).

These problems motivate the search for new sources of substances that may have antimicrobial action, stimulating investments aimed at promising compounds, such as those with the 1,2,4-oxadiazole ring in their structure. This heterocyclic nucleus has stood out for presenting several biological applications, acting as: antiasthmatic, antidiabetic, anti-inflammatory, antitumor, neuroprotective, immunosuppressive, antioxidant, and antimicrobial (Freitas *et al.*, 2012). Several studies highlight the promising antimicrobial activity of compounds containing the 1,2,4-oxadiazole ring. Examples include the research by Krishna & collaborators (2015), Freitas & collaborators (2012), Cunha, Nougueira, & Aguiar (2018), Srivastava & collaborators (2003), and Leite & collaborators (2000).

However, active compounds must undergo a rigorous process until their approval, which includes several obstacles and demands a lot of time and money. Moreover, many molecules do not reach the end of this process due to unfavorable physicochemical and/or biological characteristics that are only identified during the tests (Silva, 2016).

Thus, to circumvent the obstacles in the development of a new drug, as well as to identify the most promising molecules in the early stages of this process, researchers have developed different *in silico* tools based on computational and mathematical methods. These methods have proven effectiveness in predicting a great diversity of biological and physicochemical characteristics of the compounds under study (Papa *et al.*, 2017).

Considering this and the countless reports of the antimicrobial action of 1,2,4-oxadiazole compounds, this study describes the synthesis, antimicrobial activity, and *in silico* assessment of 1,2,4-oxadizole compounds from ethyl levulinate.

Materials and Methods

Reagents and equipment

Companies Vetec and Sigma-Aldrich provided the reagents and solvents with a degree of purity higher than or equal to 98%. We purified the solvents - hexane and ethyl acetate - by fractional distillation in a Vigreux column. We monitored the reactions by thin layer chromatography (TLC) using silica gel plates containing fluorescent indicator F_{254}, which we eluted in hexane/ethyl acetate systems and visualized using an ultraviolet lamp.

We purified the resulting products by liquid column chromatography using Merck silica gel 60 (70-230 mesh) as the stationary phase and the solvents hexane and ethyl acetate, in different proportions, as the mobile phase. To evaporate the reaction solvent, we used a rotary evaporator (Büchi Rotavapor, model R-114) connected to a vacuum pump (KNF Neuberger).

We characterized the structures of the compounds using hydrogen nuclear magnetic resonance (^{1}H NMR) and carbon nuclear magnetic resonance (^{13}C NMR) in a spectrometer (Varian Unity Plus), applying a frequency of 400 MHz for the hydrogen nucleus and 100 MHz for the carbon nucleus. For spectroscopic analyses, we used deuterated chloroform and dimethyl sulfoxide ($CDCl_3$ and DMSO-*d6*) as solvents. We calibrated the device using $Si(CH_3)_4$ (0.0 ppm) as an external reference for ^{1}H and ^{13}C NMR. We expressed the chemical shifts in δ (ppm) and the coupling constants (*J*) in hertz (Hz). For the ^{1}H NMR spectrum, we analyzed the shifts in relation to the central peak of $CDCl_3$ at 7.27; for the ^{13}C NMR spectrum, we analyzed the shifts in relation to the central peak of $CDCl_3$ at 77.0.

Procedure for the synthesis of arylamidoximes (2a-c)

We synthesized arylamidoximes (2a-c) by adapting the methodology of Andrade *et al*. (2016). For that, we added hydroxylamine hydrochloride (2.08 g; 30 mmol), sodium carbonate (1.6 g; 15 mmol), and 40 ml of distilled water at room temperature to a 125 ml round-bottom flask. Then we added 10.0 mmol of the corresponding nitrile (1a-c) dissolved in 40 ml of ethanol to the flask. We subjected the reaction mixture to an ultrasonic bath at 55 ± 5 °C for 30 minutes, verifying the completion of the reaction by TLC. After completion, we evaporated the ethanol with the aid of a rotary evaporator for subsequent extraction of the organic phase. We extracted the organic phase from the aqueous medium using ethyl acetate (3 x 25 ml) with the aid of a separating funnel. After collection, we dried the organic phase with anhydrous Na_2SO_4. Then, we filtered and removed the extraction solvent under reduced pressure. We purified arylamidoximes (2a-c) by crystallization using the chloroform/hexane system (90:10).

Benzamidoxime (2a): 1.09 g (80% yield); white solid: m.p. 74-76 °C; IR (KBr pellet): ν_{max} 3448, 3356, 3212, 3059, 2893, 2361, 1645, 1590, 1499, 1446, 1384, 1107, 926, 768, 687 cm^{-1}; ^{1}H NMR (400 MHz, DMSO-*d*$_6$) δ 9.63 (1H, s, OH), 7.69-7.67 (2H, m, $H_{Aromatic}$), 7.38-7.36 (3H, m, $H_{Aromatic}$), 5.81 (2H, s, NH_2); ^{13}C NMR (100 MHz, DMSO-*d*$_6$) δ 150.8, 133.4, 128.9, 128.1, 125.4 (Tarasenko *et al.*, 2017).

p-methylbenzamidoxime (2b): 1.35 g (90% yield); white solid: m.p. 144-146 ºC; IR (KBr pellet): ν_{max} 3500, 3367, 3049, 2916, 1664, 1588, 1418, 1391, 1099, 936, 823, 747 cm^{-1}; ^{1}H NMR (400 MHz, DMSO-*d*$_6$) δ 9.52 (1H, s, OH), 7.56 (2H, d, *J* = 8.2 Hz, $H_{Aromatic}$), 7.17 (2H, d, *J* = 8.2 Hz, $H_{Aromatic}$), 5.73 (2H, s, NH_2), 2.31 (3H, s, aryl-CH_3); ^{13}C NMR (100 MHz, DMSO-*d*$_6$) δ 150.8, 138.2, 130.5, 128.6, 125.3, 20.8 (Andrade *et al.*, 2016; Tarasenko *et al.*, 2017).

p-chlorobenzamidoxime (2c): 1.45 g (85% yield); white solid: m.p. 128-129 ºC; IR (KBr pellet): ν_{max} 3468, 3346, 3152, 3053, 2893, 2361, 1918, 1655, 1589, 1497, 1380, 1087, 920, 840, 722 cm^{-1}; ^{1}H NMR (400 MHz, DMSO-*d*$_6$) δ 9.73 (1H, s, OH), 7.69 (2H, d, *J* = 8.6 Hz, $H_{Aromatic}$), 7.43 (2H, d, *J* = 8.6 Hz, $H_{Aromatic}$), 5.86 (2H, s, NH_2); ^{13}C NMR (100 MHz, DMSO-*d*$_6$) δ 149.9, 133.4, 133.2, 128.1, 127.1

(Andrade *et al.*, 2016; Tarasenko *et al.*, 2017).

Procedure for the synthesis of 4-(3-(aryl)-1,2,4-oxadiazol-5-yl)-butan-2-one (4a-c)

We synthesized 1,2,4-oxadiazoles (4a-c) following the methodology of Freitas & collaborators (2007) with some modifications. For that, we added ethyl 4-oxopentanoate (0.111 g, 0.75 mmol), the appropriate arylamidoximes (2a-c) (1.00 mmol), and K_2CO_3 (0.12 g, 0.85 mmol) to a small glass test tube. Then, we thoroughly ground this mixture. We subjected the glass test tube with the reaction mixture to irradiation in a domestic microwave oven (100% power, 650 W) for 7 min, and then cooled it. Thereafter, we purified the crude product by chromatography on silica gel using hexane/ethyl acetate (9:1) to produce the corresponding 1,2,4-oxadiazoles (4a-c).

4-(3-(phenyl)-1,2,4-oxadiazol-5-yl)-butan-2-one (4a): 149.2 mg (92% yield); colorless liquid; ^{1}H NMR (400 MHz, $CDCl_3$) δ ppm 8.07-8.05 (*m*, 2H, $H_{Aromatic}$); 7.52-7.58 (*m*, 3H, $H_{Aromatic}$), 3.22 (*t*, 2H, *J* = 7.2 Hz, $H_{Alkylic}$); 3.09 (*t*, 2H, *J* = 7.2 Hz, $H_{Alkylic}$); 2.27 (*s*, 3H, $H_{Alkylic}$), ^{13}C NMR (100 MHz, $CDCl_3$) δ 205.2; 178.7; 168.1; 131.0; 128.7; 127.3; 126.7; 39.2; 29.7; 20.6 (Freitas *et al.*, 2007).

4-(3-(p-methylphenyl)-1,2,4-oxadiazol-5-yl)-butan-2-one (4b): 150.25 mg (87% yield); colorless liquid; ^{1}H NMR (400 MHz, $CDCl_3$) δ ppm 7.93 (*d*, 2H, *J* = 8.0 Hz, $H_{Aromatic}$); 7.28 (*d*, 2H, *J* = 7.6 Hz, $H_{Aromatic}$); 3.20 (*t*, 2H, *J* = 6.8 Hz, $H_{Alkylic}$); 3.07 (*t*, 2H, *J* = 6.4 Hz, $H_{Alkylic}$); 2.41 (*s*, 3H, $H_{Alkylic}$); 2.26 (*s*, 3H, $H_{Alkylic}$); ^{13}C NMR (100 MHz, $CDCl_3$) δ 205.3; 178.6; 168.2; 141.4; 129.4; 127.3; 123.9; 39.3; 29.8; 21.5; 20.7 (Freitas *et al.*, 2007).

4-(3-(p-chlorophenyl)-1,2,4-oxadiazol-5-yl)-butan-2-one (4c): 169.21 mg (90% yield); colorless liquid; ^{1}H NMR (400 MHz, $CDCl_3$) δ ppm 8.00 (*d*, 2H, *J* = 8.0 Hz, $H_{Aromatic}$); 7.46 (*d*, 2H, *J* = 8.8 Hz, $H_{Aromatic}$); 3.20 (*t*, 2H, *J* = 7.6 Hz, $H_{Alkylic}$); 3.07 (*t*, 2H, *J* = 6.4 Hz, $H_{Alkylic}$); 2.26 (*s*, 3H, $H_{Alkylic}$); ^{13}C NMR (100 MHz, $CDCl_3$) δ 205.1; 179.0; 167.4; 137.2; 129.1; 128.6; 125.2; 39.1; 29.8; 20.6 (Freitas *et al.*, 2007).

In vitro assay of antimicrobial activity

We assessed the *in vitro* antimicrobial activity of the synthesized 1,2,4-oxadiazoles (4a-c) against the bacteria *Staphylococcus aureus* (UFPEDA 02), *Enterococcus faecalis* (UFPEDA 138), *Escherichia coli* (UFPEDA 224), *Pseudomonas aeruginosa* (UFPEDA 416), and the fungus *Candida utilis* (UFPEDA 1009). We kept the microorganisms on nutrient agar (NA), stored at 4 °C. We used the antimicrobial drugs metronidazole and fluconazole as positive controls. We determined the values of the minimum inhibitory concentrations (MICs) according to the methodology of Espinel-Ingroff & collaborators (2005).

Computational studies

We obtained the values of consensual log $P_{o/w}$ (cLogP), molecular mass (MM), number of hydrogen bond donors (nHBD), number of hydrogen bond acceptors (nHBA), number of Lipinski rule violations, gastrointestinal absorption, blood-brain barrier (BBB) permeation, and the presence of structural alert fragments through the SwissADME online bioinformatics platform. This platform compiles the contribution of different authors in the field of medicinal chemistry and chemoinformatics to process different *in silico* analyses (Daina, Michielin, & Zoete, 2017).

We used the PASS online platform to survey the possible biological activities of compounds 4a-c, evaluating more than 3500 potential biological activities, including: pharmacological effects; mechanisms of action; toxic and adverse effects; interaction with metabolic enzymes and transporters; toxicological action for some organisms such as protozoa, microorganisms, and terrestrial and aquatic organisms that impact the environment; among other information (Oliveira, 2014).

Results and Discussion

This study initially considered a general strategy for the synthesis of 4-(3-(aryl)-1,2,4-oxadiazol-5-yl)-butan-2-ones (4a-c) (Figure 1). In this way, different arylnitriles (1a-c) reacted with hydroxylamine hydrochloride and basic salt in hydroethanolic medium under heating. The procedure led to the arylamidoximes (2a-c) with yields that ranged from 89-92%, similarly to Freitas *et al.* (2007). The next step consisted of subjecting arylamidoximes (2a-c) and ethyl 4-oxopentanoate (ethyl levulinate) to an *O*-acylation reaction followed by cyclodehydration, leading to compounds 4a-c.

It is noteworthy that the synthesis of compounds 4a-c included microwave irradiation. The application of this form of heating proved to be very efficient since other procedures require high temperatures and generate byproducts, usually hindering purification (Freitas *et al.*, 2012).

The characterization of 1,2,4-oxadiazoles 4a-c by nuclear magnetic resonance of hydrogen (^{1}H NMR) and carbon (^{13}C NMR) led to spectroscopic data that corroborate literature values (Freitas *et al.*, 2007).

The growing need for new antimicrobial agents has been driven by several factors, mainly by the frequent cases of resistant microorganisms, encouraging the constant search for molecules that can help solve these problems (Lee & Lee, 2018). Among the efforts in the search for new therapeutic agents, tests aimed at obtaining MIC values of compounds under development have stood out in the early stages of the process of discovering new drugs, providing highly reliable and safe results that help in the identification of promising compounds (Zorzi, 2013). Thus, the present study assessed the antimicrobial properties of compounds 4a-c with MIC values representing the lowest concentration capable of visually inhibiting 100% of the growth of the tested microorganism (Espinel-Ingroff *et al.*, 2005). The study included microorganisms of clinical importance, which are frequently involved in infectious conditions and in antimicrobial resistance events. These microorganisms are the bacteria *S. aureus* (UFPEDA 02), *E. faecalis* (UFPEDA 138), *E. coli* (UFPEDA 224), and *P. aeruginosa* (UFPEDA 416), and the fungus *C. utilis* (UFPEDA 1009) (Table 1).

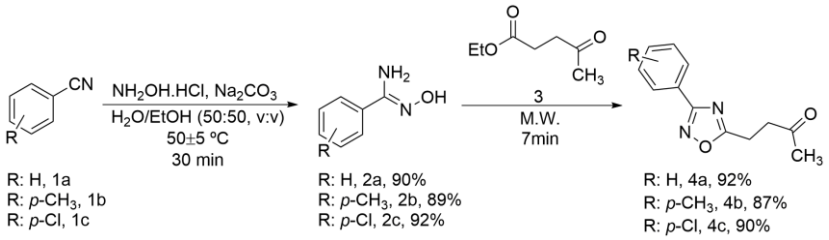

Figure 1. General strategy for the synthesis of 1,2,4-oxadiazoles 4a-c.

All strains were susceptible to the antimicrobial activity of the standards used (metronidazole for bacterial strains and fluconazole for fungal strains), attesting to the feasibility of the tests. All compounds showed antimicrobial activity (Table 1). According to Morales & collaborators (2008), MIC values indicate good antimicrobial activity when in the range of 50-500 μg mL^{-1}, moderate activity when in the range of 500-1500 μg mL^{-1}, and poor activity when above 1500 μg mL^{-1}. Thus, the antimicrobial activities of compounds 4b and 4c proved to be poor or close to the maximum limit of the moderate range for the strains. Meanwhile, compound 4a had moderate antimicrobial activity, with MIC values of 625 μg mL^{-1} for *P. aeruginosa* and 156.2 μg mL^{-1} for *C. utilis*.

Table 1. Antimicrobial activity of the compounds 4a-c and the positive controls (metronidazole and fluconazole), represented by the minimum inhibitory concentration (MIC) in μg mL^{-1}.

Compound	*S. aureus*	*E. faecalis*	*E. coli*	*P. aeruginosa*	*C. Utilis*
4a	+	+	+	625	156.2
4b	+	+	+	+	2500
4c	+	+	+	2500	1250
Metronidazole	19.5	19.5	19.5	19.5	-
Fluconazole	-	-	-	-	19.5

(+) denotes bacterial growth; (-) not evaluated

Srivastava & collaborators (2003) studied 1,2,4-oxadiazole compounds with molecular architecture similar to that of the compounds of the present study, observing moderate antimicrobial activity. However, these authors synthesized a compound with greater similarity to compound 4a, which does not contain a substituent in the aryl group, not showing any antimicrobial action against the microorganisms of their experiment. Leite & collaborators (2000) also studied antimicrobial activity using compounds similar to those of the present study and obtained satisfactory results, with MICs of 64 μg mL^{-1} and 128 μg mL^{-1} against *E. coli* and *Proteus mirabilis*, respectively. In that study, the compounds also acted against *P. aeruginosa*, however, with little effect.

Considering the low antifungal activity of 1,2,4-oxadiazole compounds with similar structure (Krishna & collaborators, 2015), the antifungal activity of compound 4a in the present study is an extremely relevant result.

The antimicrobial evaluation of compounds 4a-c showed the electronic influence of the substituents introduced in the aromatic ring. For example, the compound containing the leaving group (-Cl) showed a lower MIC in relation to the compound containing the donor group (-CH_3).

Aiming to know the theoretical pharmacokinetic, toxicological, and chemical characteristics of compounds 4a-c, the next step of the present research consisted of the computational study of these compounds using the SwissADME platform (Table 2).

Table 2. *In silico* pharmacokinetic, toxicological, and chemical properties of compounds 4a-c.

Property	Compound		
	4a	4b	4c
cLogP	2.12	2.51	2.64
MM	216.24	230.26	250.68
nHBD	0	0	0
nHBA	4	4	4
Lipinski	0	0	0
GI Absorption	High	High	High
BBB	Yes	Yes	Yes
Alert fragments	No	No	No

cLogP: consensual Log $P_{o/w}$; MM: molecular mass; nHBD: number of hydrogen bond donors; nHBA: number of hydrogen bond acceptors; Lipinski: number of Lipinski rule violations; GI Absorption: gastrointestinal absorption; BBB: blood-brain barrier.

The cLogP value corresponds to the lipophilicity of compounds 4a-c (Table 2). Before the drug interacts with its pharmacological target, it must be able to penetrate through body barriers and reach its place of action, a process driven by its pharmacokinetic characteristics. According to Barreiro & Fraga (2015), the ability of drugs to permeate through body barriers and distribute themselves throughout the body relate directly to the lipophilicity of the compound. These authors consider LogP values between 1 and 3 as the ideal lipophilicity range for a drug. Thus, compounds 4a-c showed cLogP values within the ideal lipophilicity range, which points to good pharmacokinetic characteristics.

In this sense, the evaluation of compounds 4a-c followed the Lipinski 'Rule of Five' (2004). Lipinski developed this rule from the analysis of 2,245 drugs that showed good standards of oral bioavailability, making it one of the main starting points in the development of new promising molecules. The study determines that a molecule will present a good standard of oral bioavailability when it satisfies a set of physicochemical parameters, which are: molecular mass (MM) less than 500 Daltons, partition coefficient (cLogP) less than 5, maximum of five hydrogen bond donor groups (nHBD), and maximum of ten hydrogen bond acceptor groups (nHBA) (Lipinski, 2004). All the compounds of the present study satisfy all the specifications contained in the 'Rule of Five' (Table 2), corroborating the good

pharmacokinetic potential presented by the cLogP analysis. This points to the high potential of these compounds to be well absorbed after oral administration, which is a relevant fact. This type of administration has unique benefits such as convenience, low cost, possibility of self-administration, greater adherence to treatment, and lower risk of triggering systemic infections in the user (Golan *et al.*, 2014).

Additionally, the high gastrointestinal (GI) absorption and the ability to cross the blood-brain barrier (BBB), both indicated by the SwissADME platform, are further evidence of the good pharmacokinetic characteristics of these compounds. The estimate of high gastrointestinal absorption reinforces the results of the other analyses, while the ability to cross the BBB indicates good patterns of body distribution, showing the ability of these compounds to access the central nervous system and fight diseases therein (Table 2) (Daina, Michielin, & Zoete, 2017).

Compounds 4a-c do not have alert fragments in their molecular structure, indicating a good toxicity pattern (Table 2). This analysis was performed by the SwissADME platform following Brenk & collaborators (2008), who created a list of molecular fragments known to be toxic, chemically reactive, metabolically unstable, or to be responsible for unsatisfactory pharmacokinetic standards.

Together, the results of lipophilicity, oral bioavailability, and toxicity highlight these molecules as good candidates for the development of new therapeutic agents. It is noteworthy that 80% of the molecules under research do not reach clinical studies, and 50% of them are unsuccessful due to unfavorable pharmacokinetic and toxicological properties (Silva, 2016).

Continuing the *in silico* studies, the present research included the analysis of compounds 4a-c on the online platform PASS, which addresses more than 3500 potential biological activities expressed as probability "to be active" (Pa) (Oliveira, 2014). The main activities reported by the platform for these compounds were their effects on different physiological agents such as enzymes and receptors. These reports highlight their inhibitory activity on the tyrosine kinase platelet-derived growth factor receptor (PDGFR), a receptor identified as a possible target in cancer treatment. For this receptor, the Pa values ranged from 82.3% to 85.3%.

Compounds 4a-c also showed other important activities, of which stand out the anti-inflammatory (Pa values ranging from 60.3% to 62.8%), antiprotozoal (Pa values ranging from 42.3% to 50.2%), and anxiolytic (Pa values varying from 40.0% to 47.2%).

The results corroborate different scientific studies for other oxadiazole compounds. Ranjan & collaborators (2018) explored the anti-inflammatory activity of 1,2,4-oxadiazoles, pointing out compounds more active and selective than the standard drug, ketoprofen. Chaves & collaborators (2017) reported the action of different oxadiazoles against different species of the genus *Leishmania* spp., with IC_{50} values below 11 µM for promastigote forms and below 5 µM for amastigote forms. Faizi & collaborators (2012) demonstrated the anxiolytic activity of oxadiazoles, suggesting activity on GABA receptors similar to that of drugs used for this purpose, such as benzodiazepines.

These results show how promising these compounds are, with the possibility of becoming good drugs in the future. The present research corresponds to the first steps in the process of discovering new drugs and encouraging the development of future studies on these molecules and their derivatives.

Conclusion

In summary, the present study synthesized three compounds containing the 1,2,4-oxadiazole nucleus from ethyl levulinate, in yields ranging between 87-92%. The *in vitro* evaluation of the synthesized compounds against different bacterial strains and a fungal strain demonstrated promising antimicrobial activity. The compounds inhibited the bacterium *P. aeruginosa* and the fungus *C. utilis* with moderate to high minimum inhibitory concentration (MIC) values. In addition, computational studies have shown promising properties for the three 4-(3-(aryl)-1,2,4-oxadiazol-5-yl)-butan-2-ones, indicating the possibility of these molecules being well absorbed after oral administration, with low probability of toxicity and good body distribution. Moreover, these compounds showed good metabolic and enzymatic activities, also acting as anti-inflammatory, antiprotozoal, and anxiolytic agents.

The prospecting of new bioactive molecules has stood out as one of the main initiatives to supply the need for new drugs, mainly antimicrobials. The present research points to the need for developing future studies that can help enhance the demonstrated antimicrobial activity of these compounds. Further research should also evaluate other possible activities of these molecules and their derivatives, thus contributing to the implementation of new therapeutic agents.

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