Synthesis, toxicity towards brine shrimp (Artemia salina Leach) and antimicrobial activity evaluation of 3,5-diphenylchlorinated-1,2,4-oxadiazoles

Silvio Luiz Machado¹, Luciane Vieira dos Santos², Willian Ferreira da Costa³, Benedito Prado Dias Filho³ and Maria Helena Sarragiotto² *

¹Laboratório de Síntese, Milenia Agro Ciências, Rua Pedro Antonio de Souza, 400, 86025-900, Londrina, Paraná, Brazil.
²Departamento de Química, Universidade Estadual de Maringá, Av. Colombo 5790, 87020-900, Maringá, Paraná, Brazil.
³Departamento de Análises Clínicas, Universidade Estadual de Maringá. *Author for correspondence. e-mail: mhsarragiotto@uem.br

ABSTRACT. The known oxadiazoles 3,5-bis-(phenyl)-1,2,4-oxadiazole (3a); the 3-(4-chlorophenyl)-5-phenyl-1,2,4-oxadiazole (3b); and the new 3,5-diphenylchlorinated-1,2,4-oxadiazoles 3c-e were synthesized from the reaction of benzamidoximes with an appropriated acid chloride and cyclisation of the resulting O-acylbenzamidoxime intermediate. The compounds synthesized were characterized on the basis of their IR, NMR (1D and 2D) and mass spectral data. Compounds 3a-e were evaluated for their antimicrobial activity and for their toxicity towards brine shrimp (Artemia salina Leach).

Key words: 3,5-diphenylchlorinated-1,2,4-oxadiazoles, synthesis, NMR data, antimicrobial activity, toxicity on Artemia salina Leach.

RESUMO. Síntese, toxicidade frente a Artemia salina Leach e avaliação da atividade antimicrobiana de 1,2,4-oxadiazóis-3,5-difenilclorados. Os oxadiazóis 3,5-bis-(fenil)-1,2,4-oxadiazol (3a) e 3-(4-clorofenil)-5-fenil-1,2,4-oxadiazol (3b), já descritos na literatura, e os 1,2,4-oxadiazóis-3,5-difenilclorados inéditos 3c-e, foram sintetizados pela reação de benzamidoximas com um cloreto de ácido apropriado, seguido da ciclização do intermediário O-acylbenzamidoxime resultante. Os compostos obtidos foram caracterizados com base nas análises dos dados espectroscópicos de IV, EM e RMN (uni- e bidimensionais). Os compostos 3a-e foram submetidos a bioensaios para avaliação de atividade antimicrobiana e, de toxicidade frente ao microcrustáceo Artemia salina Leach.

Palavras-chave: 1,2,4-oxadiazóis-3,5-difenilclorados, dados de RMN, atividade antimicrobiana, toxicidade em Artemia salina Leach.

Introduction

The 1,2,4-oxadiazole moiety can be found in a large number of biologically active heterocyclic compounds exhibiting pharmacological activities such as analgesic, anti-inflammatory, antimicrobial, and antiviral (Hemming, 2001). The 1,2,4-oxadiazole moieties have been used as bioisosters for esters and amides in biological studies. These unities are present in muscarinic agonists (Macor et al., 1996), serotoninergic (5-HT₃), and receptor antagonists (Swain et al., 1991; Weidner-Wells et al., 2004).

Considering the potential of this class of compounds, in order to find new biologically active oxadiazoles, we attempted to synthesize and to evaluate the toxicity and antimicrobial activity of 1,2,4-oxadiazoles containing phenylchlorinated substituents attached at C-3 and C-5. The known compounds 3,5-bis-(phenyl)-1,2,4-oxadiazole (3a); 3-(4-chlorophenyl)-5-phenyl-1,2,4-oxadiazole (3b); the new 1,2,4-oxadiazoles 3-(4-chlorophenyl)-5-(3,4-dichlorophenyl)-1,2,4-oxadiazole (3c); 3,5-bis-(3,4-dichlorophenyl)-1,2,4-oxadiazole (3d); and 3-(3,4-dichlorophenyl)-5-phenyl-1,2,4-oxadiazole (3e) were synthesized and characterized on the basis of IR, MS, ¹H NMR, ¹³C NMR/DEPT, ¹H-¹H-COSY, HMQC, and HMBC spectral data. The toxicities of the compounds 3a-e were tested by using brine shrimp (Artemia salina) bioassay. Antimicrobial activities were evaluated against the bacteria Staphylococcus aureus, Escherichia coli, Bacillus subtilis, and Pseudomonas aeruginosa, and against the fungi Candida albicans, C. krusei, C. parapsilosis, and C. tropicalis.

Material and methods

Equipments. IR spectra were recorded on a Bomem spectrophotometer model MB 100. ¹H (300 MHz), ¹³C (75.5 MHz) and 2D (COSY, HMQC, HMBC) NMR spectra were recorded in a Varian spectrometer model Mercury plus BB 300MHz, using
Compounds 3a-e were obtained from the reaction of the benzamidoximes 2a-c, in pyridine, with an appropriated acid chloride (Figure 1). To a solution of benzamidoxime (2a) (0.817 g, 6.0 mmol); 4-chlorobenzenamidoxime (2b) (1.02 g, 6.0 mmol) or 3,4-dichlorobenzenamidoxime (2c) (1.22 g, 6.0 mmol), in pyridine (10 mL), the appropriated acid chloride was added (0.02 mol). The mixture was stirred at room temperature for 4 hours. The pyridine was removed under vacuum and the solid purified by column chromatography on silica gel with mixtures of hexane/ethyl acetate as solvent.

Figure 1 Synthetic route for preparation of the 1,2,4-oxadiazoles 3a-e.

3,5-Bis-(phenyl)-1,2,4-oxadiazole (3a). 40% yield. Colorless crystals. Mp. 107-109°C (Molina et al., 1986). Characterized by comparison of its NMR data with those of literature (Poulain et al., 2001); 'H NMR (CDCl3): δ 7.47 (d, 2H, H-3 and H-5, J = 8.7 Hz), 7.61 (d, 2H, H-1 and H-6, J = 8.7 Hz). 13C NMR (CDCl3): δ 110.8 (C-1), 118.0 (CN), 129.7 (C-3 and C-5), 133.4 (C-2 and C-6), 139.6 (C-4). EI-MS (70 eV) m/z (int. rel. %): 171 (M•+, 100).

Preparation of the benzamidoximes 2a-c. To a stirred solution of commercial benzonitrile (1a) (1.23 g, 0.012 mol), 4-chlorobenzonitrile (1b) (1.64 g, 0.012 mol), or 3,4-dichlorobenzonitrile (1c) (2.05 g, 0.012 mol), in ethanol (10 mL), was added hydroxylamine hydrochloride (2.09 g, 0.03 mol) and triethyl amine (8 mL). The reaction mixture was maintained under reflux for 12 hours and the ethanol evaporated under vacuum. The mixture was poured in water (200 mL) and left to sublimation. The solid residue was purified by vacuum filtration and dried at 55°C.

Benzamidoxime (2a). 60% yield. EI-MS (70 eV) m/z (int. rel. %): 136 (M•+, 52), 103 (100).

4-Chlorobenzamidoxime (2b). 58% yield. Colorless crystals. Mp. 130-131°C; lit. 128-130°C (Ryu et al., 2001). 'H NMR (CDCl3): δ 4.86 (s, 2H, NH2), 7.37 (d, 2H, J = 8.7 Hz, H-3 and H-5), 7.57 (d, 2H, J = 8.7 Hz, H-1 and H-6). 13C NMR (CDCl3): δ 127.2 (C-3 and C-5), 128.9 (C-2 and C-6), 130.9 (C-1), 136.1 (C-4), 151.0 (NH2C=NOH). EI-MS (70 eV) m/z (int. rel. %): 170 (M•+, 90), 153 (100).

3,4-Dichlorobenzamidoxime (2c). 81% yield. Colorless crystals. Mp. 132-134°C. EI-MS (70eV) m/z (int. rel. %): 203 (M•+, 64), 186 (100).

Synthesis of the 1,2,4-oxadiazoles 3a-e.
was placed in the other side of the tank to attract the test compounds. The vials were maintained under light and after 24h the number of survivors was counted. The bioassays were carried out in triplicate and expressed with 95% confidence limit (Mc Laughlin, 1991).

### Results and discussion

#### Synthesis and characterization of the oxadiazoles 3a-e

Although several methodologies have been reported for the synthesis of 1,2,4-oxadiazoles, the reaction of an amidoxime with an acid carboxylic derivative, a carbonic acid derivative or related species remains the most commonly employed methodology for the synthesis of 1,2,4-oxadiazoles (Hemming, 2001).

In this work, 1,2,4-oxadiazoles (3a-e) were prepared by O-acylation of the benzamidoximes (2a-c) with appropriate carboxylic acid chlorides, in pyridine, and cyclisation of the resulting O-acylbenzamidoximes as shown in Scheme 1. The benzamidoximes 2a-c were available from the reaction of the nitriles 1a-c with hydroxylamine hydrochloride, in triethyl amine (Deegan et al., 1999). Nitriles 1b and 1c were obtained from the reaction of 4-chloro-benzotrichlorides or 4-chlorobenzoic acid or 3,4-dichlorobenzoic chlorides with ammonium chloride.

The IR spectra of the compounds 3a-e showed absorptions bands at 1606, 1555, 1490, 1410, 1360, 1240 and 1090 cm\(^{-1}\), consistent with the presence of an oxadiazole moiety and phenyl chlorinated groups. The IR spectra of the compounds 3a-e are consistent with the expected structures and fragmentation characteristics for aryl-1, 2, 4-oxadiazoles as shown in Table 1.

<table>
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<th>(d_2)</th>
<th>(d_3) (mult. / m Hz)</th>
<th>(d_4)</th>
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<td>126.5</td>
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<td>8.27 (d 2.0)</td>
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### Table 1. \(^{1}H\) and \(^{13}C\) NMR data (\(\delta\)CDCl\(_3\), 300 MHz) of the 1,2,4-oxadiazoles 3c-e.

[199-201°C. El-MS (70 eV) m/z (int. rel. %): 360 (M\(^{+}\), 100), 187 (31, [3,4-di-ClC\(_6\)H\(_4\)CNO]+). \(\delta\) and \(\delta\)C NMR data: See Table 1.

3-(3,4-Dichlorophenyl)-5-phenyl-1,2,4-oxadiazole (3e): 30% yield. Colorless crystals. M.p: 128-130°C. El-MS (70 eV) m/z (int. rel. %): 290 (M\(^{+}\), 77), 187 (100, [3,4-di-ClC\(_6\)H\(_4\)CNO]+). \(\delta\)H and \(\delta\)C NMR data: See Table 1.
Table 1. The chemical shifts assignments of the oxadiazoles 3a-e were based on analysis of the correlations observed in the 1H-1H-COSY, HMQCC and HMBC spectrum. From the correlations in 1H-1H-COSY spectra it was possible to confirm the connectivity between the aromatic hydrogens. The HMBC spectra showed correlations of the signals at δ 167.2 – 168.7 (C-3) and at δ 173.9 – 176.1 (C-5) with those of H-2′/H-6′ and H-2′/H-6″, respectively. From the correlations of the signals for C-1′ and C-1″ with those of aromatic hydrogens it was possible to confirm the positions of the hydrogens and, consequently, of the chlorine atoms in the aromatic rings. These data, together with the 1H-13C correlations in the HMQCC spectra permitted the chemical shifts assignments of the hydrogens and carbons, and to confirm the chlorine atoms positions in the aromatic rings for the synthesized oxadiazoles.

Biological assays

The antimicrobial bioassays showed that all compounds were inactive against the microorganisms tested with a MIC > 100 μg/mL.

The toxicities of the compounds 3a-e were tested in Artemia salina larvae. The lethal dose (LD50), which corresponds to the minimum concentration that causes 50% of the larvae mortality, was determined for all compounds. The results indicated that the oxadiazoles 3a (LD50 = 2.13 μg/mL; 1.59-2.66 μg/mL, 95% confidence limits) and 3b (LD50 = 4.57 μg/mL; 3.62-6.52 μg/mL, 95% confidence limits) exhibited significant toxicity. On the other hand, compounds 3c-e were inactive (LD50 > 500 μg/mL), indicating that the increasing of the number of chlorine atoms did not result in increasing of the toxicity. The brine shrimp assay has been used as a convenient and rapid assay to discover new cytotoxic compounds. Thus, the oxadiazoles 3a and 3b are potential cytotoxic agents and their activity on cancer cell lines will be further tested.

Conclusion

In the present work we have synthesized and evaluated the toxicity towards Artemia salina and the antimicrobial activity of 1,2,4-oxadiazoles 3a-e containing phenylchlorinated substituents attached at C-3 and C-5. The oxadiazoles 3a and 3b exhibited significant toxicity towards Artemia salina with LD50 of 2.13 and 4.57 μg/mL, respectively. The antimicrobial bioassays showed that all compounds were inactive against the microorganisms tested with a MIC > 100 μg/mL.

References


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