SYNTHESIS, STRUCTURAL CHARACTERIZATION, AND PRELIMINARY ANTICANCER ACTIVITY OF 3-(4-SUBSTITUTED ARYL)-1,2,4-OXADIAZOL-5-YL PROPIONIC ACYL HYDRAZIDES

José Maurício dos Santos Filho[1]; Luan C. G. Ramos[2]; Savio M. Pinheiro[2]; Gardenia C. G. Militão[3]

*[1]Laboratório de Planejamento e Síntese Aplicados à Química Medicinal (**SintMed**®)-Departamento de Engenharia Química-UFPE-PE; [2]Departamento de Química-UFPB-PB; [3]Departamento de Fisiologia e Farmacologia, Centro de Biociências-UFPE-PE*

Keywords: Acyl hydrazides, 1,2,4-Oxadiazole, Anticancer activity

Introduction

Organic acid hydrazides include the vast group of organic derivatives of hydrazine containing the active functional group (-C(=O)-NH-NH_2), which can be easily accessed from carboxylic acids and hydrazine. They are an important starting material in organic chemistry as they present great interest in the synthesis of hetero-aliphatic and heterocyclic compounds, such as oxadiazoles, triazoles, Schiff bases, pyrazoles, thiazoles, thiosemicarbazides, and semicarbazides [Majumdar *et al.* 2014], as seen in Figure 1.

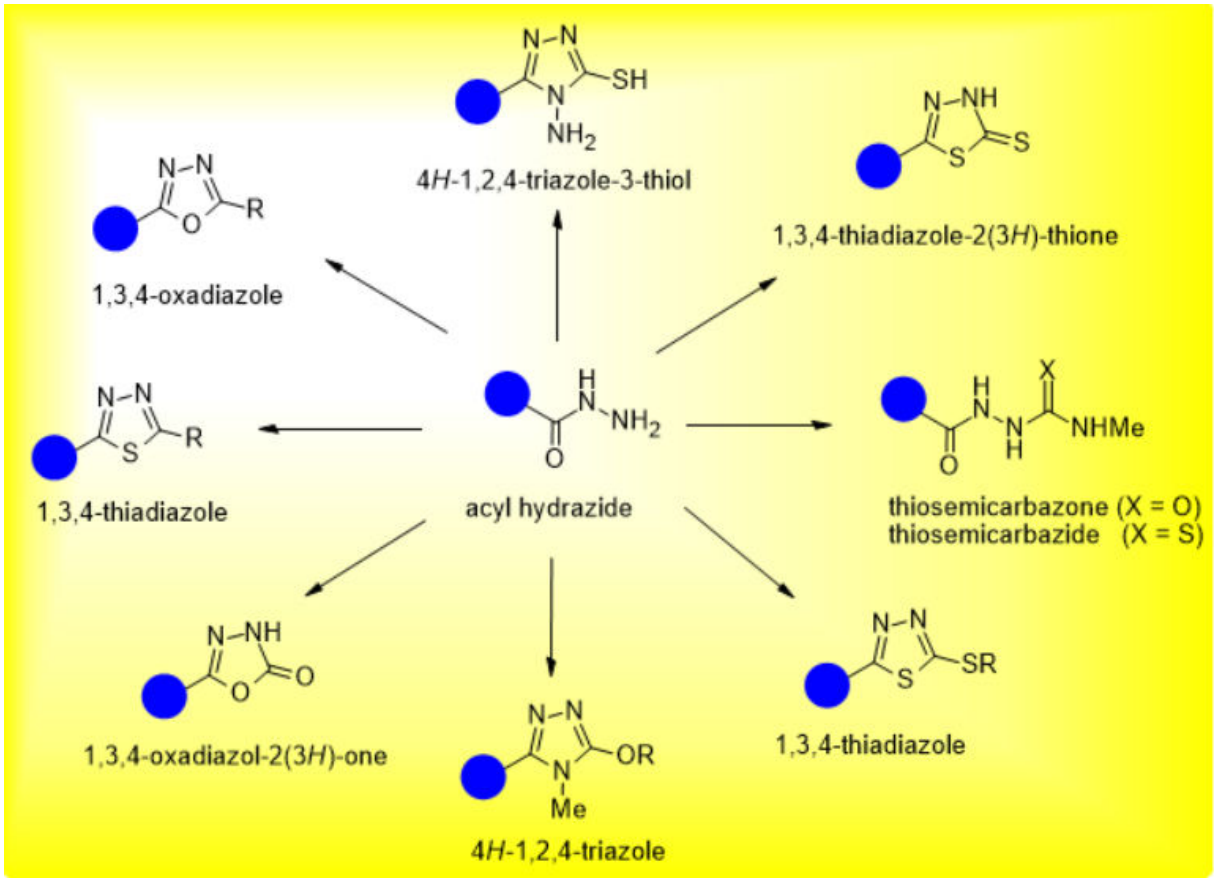


Figure 1. Heterocompompounds from acyl hydrazides.

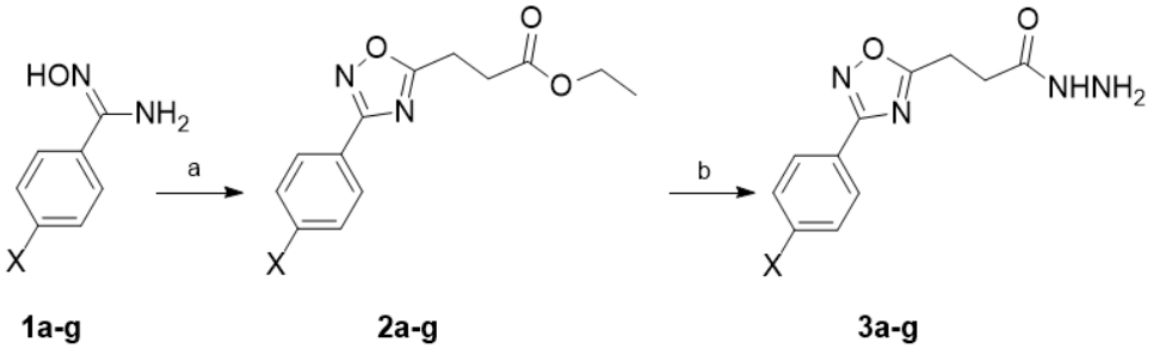
The acyl hydrazide moiety is an important functional group in medicinal chemistry since the availability of protons to donation aids in its pharmaceutical importance. The remedial possibilities of acid hydrazides gained *momentum* after the innovation of isonicotinic acid hydrazide (INH), the first acyl hydrazide with clinical application against tuberculosis. The remarkable clinical value of INH stimulated the study of other heterocyclic hydrazide-bearing monocyclic nuclei like furan, pyrrole, thiophene, and dicyclic nuclei like quinoline and isoquinoline. In recent years, hydrazides have received major attention due to their biological activities such as antifungal, antitumor, antimicrobial, antiglycation, antituberculosis, and inhibitory effects against influenza virus, HIV, and aspartic protease, among others [Khan *et al.* 2022].

Due to that biological versatility, our group decided to exploit the potential anticancer activity of acyl hydrazide linked to 1,2, 4-oxadiazole ring, since cancer is one of the main causes of death worldwide. According to the International Agency for Research on Cancer (IARC), it was estimated that there were close to 20 million new cases of cancer in the year 2022 alongside 9.7 million deaths from cancer. These statistics suggest that approximately one in five men or women develop cancer in a lifetime, whereas around one in nine men and one in 12 women die from it [Bray *et al.* 2024]. Most of the anticancer drugs currently available are chemotherapeutic agents with high cytotoxic activity and numerous side effects. The lack of selectivity of action is still a major problem in the treatment of neoplasms [Arora *et al.* 2014]. Therefore, there is a need to search for new therapeutic agents using molecular modification, in special molecular hybridization strategy, merging two or more pharmacophoric groups to obtain new bioactive compounds.

As it is well known, heterocyclic compounds, *i.e.*, 5-membered and 6-membered rings, or fused ring systems, play an imperative role in the finding and progress of new drug molecules with the highest potency and lower toxicity. In this context, oxadiazoles are small five-membered heterocycles, composed of two carbon, one oxygen, and two nitrogen atoms, which attracted a lot of interest in medicinal chemistry [Davinder *et al*. 2019]. In a previous work of our group, a series of 1,2,4-oxadiazole-acyl hydrazides was synthesized as key intermediates in the synthesis of thiosemicarbazides and 1,3,4-oxadiazoles [dos Santos Filho *et al.* 2009], providing a structural model for further structural modifications and investigation on biological activity. This strategy was retaken in this work, exploiting the potential anticancer activity of novel aryl-1,2,4-oxadiazole acyl hydrazides with an ethylene spacer, leading to seven novel 1,2,4-oxadiazole propionic acyl hydrazides as potential anticancer agents.

Material and Methods

The 1,2,4-oxadiazole propionic acyl hydrazide **3a-g** were prepared in two steps from the amidoximes **1a-g**, previously obtained at ***SintMed***® [dos Santos Filho *et al.* 2009]. First, a cyclocondensation reaction between amidoximes and ethyl 4-chloro-4-oxobutyrate in the presence of base in dry tetrahydrofuran (THF) [Maingot *et al.* 2010], as depicted in Scheme 1, led to pure 1,2,4-oxadiazole propionic ethyl esters **2a-g**, which were refluxed in ethanol with hydrazine hydrate to afford the title compounds **3a-g**, according to the general procedures below.



1a-g **2a-g** **3a-g**

X = H (a), CH_3 (b), F (c), Cl (d), Br (e), NO_2 (f), OCH_3 (g)

Reagents and conditions: a) $ClCO(CH_2)_2CO_2CH_2CH_3$, DIPEA, TBAF, dry THF, reflux, 4.5 h; b) NH_2NH_2 55%, EtOH, 70-75n °C, 2 h.

Scheme 1. Synthetic route for 1,2,4-oxadiazole propionic acyl hydrazides **3a-g.**

General procedure for 1,2,4-oxadiazol propionic ethyl esters **2a-g**. In a round-bottom flask, amidoxime (1 eq), *N.N*-diisopropylethylamine (DIPEA) (1.1 eq), and THF (5 mL) were added. Then the ethyl 4-chloro-4-oxobutyrate (1.1 eq.) was added dropwise. The resulting mixture was stirred at reflux. After the appropriate reaction time, tetrabutylammonium fluoride (TBFA) was added and the resulting mixture was refluxed overnight. The reaction was monitored by TLC. The solvent was removed under reduced pressure and the crude product was dissolved in dichloromethane. The mixture in CH_2Cl_2 was washed 3 times with HCl 1M. Organic layers were dried over $MgSO_4$ and filtered before evaporation in vacuo.

Preparation of 1,2,4-oxadiazole propionic acyl hydrazides **3a-g**. A solution of esters **2a-g** (1 eq.) in ethanol was mixed with hydrazine hydrate (2 eq.) and refluxed for 6 h until the reaction's completion. After cooling, the products precipitated and were filtered to afford the products as white solids, whose yields, melting points, and infrared data are given in Table 1. Melting points were determined on a Gallenkamp capillary apparatus and were uncorrected. Infrared spectra were recorded using KBr discs on a Perkin-Elmer Paragon 500 FT-IR spectrometer.

Compounds 1,2,4-oxadiazole propionic acyl hydrazide **3a-g** were screened for their cytotoxicity against cancer cell lines HEP-2 (human laryngeal cancer), HL60 (acute promyelocytic leukemia) and NCI H-292 (human lung carcinoma), using the 3-(4,5-dimethyl-2-thiazolyl)-2,5-diphenyl-2H-tetrazolium bromide (MTT) reduction assay after 72 h incubation. For all experiments, cells were plated in 96-well plates. After 24 h the compounds were diluted (25 µg/mL) in a medium with 0.5% dimethylsulfoxide (DMSO), and tumor cells were screened in triplicate at three different experiments. Doxorubicin (DOX) (0.01–5 µg/mL) was used as the positive control. The percentage of cell growth inhibition (mean and standard deviation) after 72 h treatment with compounds at a single concentration of 25 µg/mL was calculated. Only compounds that presented at least 75% growth inhibition at three cell lines were considered active for determining the IC_{50} values [Berridge *et al.* 1996]. GraphPad Prism version 5.0 was used to perform all analyses (GraphPad Software).

Results and Discussion

The synthesis of compounds 1,2,4-oxadiazole propionic hydrazide **3a-g** was successfully carried out according to the proposed methodology in good yields. The targeted products were isolated as pure solids after recrystallization from EtOH and characterized through their TLC analyses, melting points, and infrared (IR) spectroscopic data as observed in Table 1. Characteristic bands for NH (3330-3230 cm^{-1}) and C=O (1680-1640 cm^{-1}) were observed, indicating the structural identity of the obtained products. The general yields ranged

from 73 to 81% after one recrystallization, which is an excellent outcome, avoiding tedious and expensive purification steps.

Table 1. Characterization data for the derivatives 1,2,4-oxadiazole acyl propionic hydrazides **3a-g**

Comp.	X	Yield (%)	M.p. (°C)	IR (cm^{-1})
3a	H	73	137-139	3299 (NH), 3034 (ArCH), 2932 (Aliphatic CH), 1653 (C=O), 1522 (C=N)
3b	CH_3	79	138-140	3299, 3275 (NH), 3164, 3048 (ArCH), 2918 (Aliphatic CH), 1644 (C=O), 1589 (C=N)
3c	F	76	138-140	3314, 3218 (NH), 3044 (ArCH), 2940 (Aliphatic CH), 1668 (C=O), 1626 (C=N)
3d	Cl	78	167-168	3291 (NH), 3164, 3044 (Ar CH), 1644 (C=O), 1586 (C=N)
3e	Br	75	167-169	3294, 3193, 3167 (NH), 3046 (ArCH), 2940 (Aliphatic CH), 1644 (C=O), 1585 (C=N)
3f	NO_2	81	183-185	3309 (NH), 3088, 3024 (ArCH), 2932 (Aliphtic CH), 1650 (C=O), 1572 (C=N)
3g	OCH_3	80	171-172	3293, 3197, 3170 (NH), 3045 (ArCH), 2939 (Aliphatic CH), 1645 (C=O), 1590 (C=N)

The preparation of the 1,2,4-oxadiazole propionic acyl hydrazides **3a-g** has brought about a set of molecules, which were expected to disclose in vitro antitumor activity. Biological data are given in Table 2.

Table 2. Cell growth inhibition (%) and standard deviation (SD) for 1,2,4-oxadiazole acyl propionic hydrazides **3a-g**

Comp.	X	NCI H-292 (%)±SD	HL60 (%)±SD	HEP-2 (%)±SD
3a	H	0±0	15.2±4,1	25.5±7,1
3b	CH_3	0±0	24.8±3,9	29.3±9,1
3c	F	0±0	18.6±5,4	33.6±11,5
3d	Cl	43.9±2,9	24.0±0,05	61.2±2,7
3e	Br	46.8±2,6	20.8±3,2	77.6±5,7
3f	NO_2	34.1±5,2	25.4±1,4	32.2±2,0
3g	OCH_3	36.1±4,5	0±0	16.5±2,2
Dox		83.9±0.21	96.0±1.0	87.6±5.18

However, the outcomes observed in Table 2 were disappointing, with values of inhibition corresponding to inactivity for the NCI H-292 and HL60. On the other hand, the results arising for the HEP-2 cell line were much more promising with compound **3e** disclosing inhibition of 77.6%, the best result in the series. In a closer look at Table 2, it can be observed that the other moderate inhibition outcome for **3d** involves the chlorine substituent Since this work is a preliminary approach, it points to the need for the expansion of this investigation toward more derivatives with more substituent diversity to establish greater comparison parameters, which can put in evidence how such groups affect the biological responses and the structural planning of new molecules for further studies.

Conclusions

Herein, a successful strategy for synthesizing novel 1,2,4-oxadiazole propionic acyl hydrazides **3a-g** was described and seven novel compounds were isolated and characterized. Preliminary antitumor screening was carried out, bringing to light some structural aspects affecting the biological response. These outcomes suggest the need for further studies involving other substitutions that can elucidate how more complex structural features can influence the anticancer activity of this class of compounds.

Acknowledgments

The authors are grateful to Mrs. Eliete de Fátima V. B. N. da Silva and the Analytical Centre of Fundamental Chemistry Department, Universidade Federal de Pernambuco, for the NMR, and IV experiments.

References

Arora, S.; Agarwal, S.; Singhal, S. Anticancer Activities of Thiosemicarbazides/Thiosemicarbazones: A Review. **Int. J. Pharm. Pharm. Sci.**, 6 (9), 34-41, 2014.

Bray, F.; Laversanne, M.; Sung, H.; Ferlay, J.; Siegel, R. L.; Soerjomataram, I.; Jemal, A. Global cancer statistics 2022: GLOBOCAN estimates of incidence and mortality worldwide for 36 cancers in 185 countries. **CA: A Cancer J. Clin.**, 74 (3), 229-263, 2024.

Berridge, M. V.; Tan, A. S.; McCoy, K. D.; Wang, R. The Biochemical and Cellular Basis of Cell Proliferation Assays that Use Tetrazolium Salts. **Biochemica**, 4: 14-19, 1996.

Davinder, K.; Virender, K.; Rakesh, M.; Gajendra, S. Oxadiazole-an Important Bioactive Scaffold for Drug Discovery and Development Process against HIV and Cancer-A Review. **Curr. Bioact. Compd.**, 15, 271-279, 2019.

dos Santos Filho, J. M.; de Lima, J. G.; Leite, L. F. C. C. Synthesis, Characterization, and Anti-Inflammatory Evaluation of 1,2,4-Oxadiazoles Combined with Thiosemicarbazide and 1,3,4-Oxadiazole Moieties. **J. Heterocyclic Chem.**, 46, 722-727, 2009.

Khan, F. A.; Yaqoob, S.; Ali, S.; Tanveer, N.; Wang, Y.; Ashraf, S.; Hasan, K. A.; Khalifa, S. A. M.; Shou, Q.; Ul-Haq, Z.; Jiang, Z. H.; El-Seedi, H. R. Designing Functionally Substituted Pyridine-Carbohydrazides for Potent Antibacterial and Devouring Antifungal Effect on Multidrug Resistant (MDR) Strains. **Molecules,** 28 (1), 212-, 2022.

Maingot, L; Leroux, F; Landry, V; Dumont, J; Nagase, H; Villoutreix, B; Sperandio, O; Deprez-Poulain, R; Deprez, B. New non-hydroxamic ADAMTS-5 inhibitors based on the 1,2,4-triazole-3-thiol scaffold. **Bioorg Med Chem Lett.,** 20 (21), 6213-6216, 2010.

Majumdar, P.; Pati, A.; Patra, M.; Behera, R. K.; Behera, A. K. Acid Hydrazides, Potent Reagents for Synthesis of Oxygen-, Nitrogen-, and/or Sulfur-Containing Heterocyclic Rings. **Chem. Rev.,** 114 (5), 2942-2977, 2014.