SYNTHESIS, STRUCTURAL CHARACTERIZATION, AND PRELIMINARY ANTICANCER ACTIVITY OF NOVEL ARYL-1,2,4-OXADIAZOLE-*N*-CYCLOHEXYL THIOSEMICARBAZIDES

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Introduction

Cancer is one of the main causes of death worldwide. The International Agency for Research on Cancer (IARC) estimated that there were close to 20 million new cases of cancer in the year 2022 alongside 9.7 million deaths from cancer. The estimates 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 [Torre *et al.* 2016; Bray *et al.* 2024]. Antineoplastic drugs can be divided into inhibitors of mitotic pathways and/or DNA replication and inhibitors of molecular targets involved in tumor progression. The ability of some compounds to chelate metal ions has now been recognized as a major factor in their antiproliferative effects. For example, the complexation of thiosemicarbazides and their derivated thiosemicarbazones with iron ions turned out to be of key importance for antitumor activity, causing oxidative damage and inhibiting ribonucleotide reductase [Kalinowski *et al.* 2009]. Several anticancer mechanisms of action have been proposed for the new connections; for example, the destruction of the tyrosyl radical, inhibition of synthesized DNA, inhibition of Topo I/II, and mobilization of intracellular Fe. Additionally, thiosemicarbazides can form complexes with Cu (II) ions, leading to more effective drugs against prostate cancer than the ligands themselves. Most of the anticancer drugs currently available are chemotherapeutic agents with high cytotoxic activity with 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 in order to obtain new bioactive compounds.

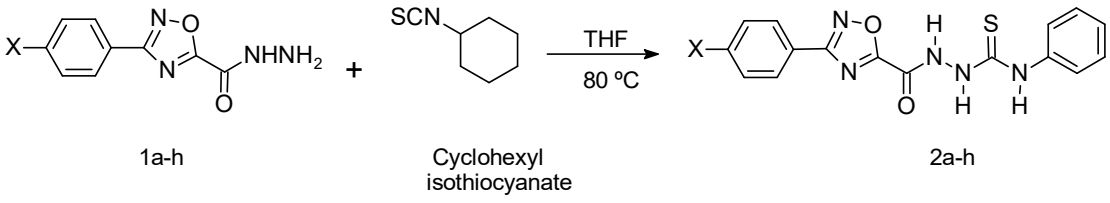
Thiosemicarbazide is an important structural motif that has the potential to display chemical functionality in biologically active molecules. Optimization of this structure can result in groundbreaking discovery of a new class of anticancer agents. Structural Activity Relationship (SAR) studies showed that a large number of thiosemicarbazides of heterocyclic compounds have low π-electron density at the side chain part and the ring heteroatom should be reasonably a good electron pair donor to transition metals to form coordination compounds. Thiosemicarbazides display antiproliferative activity on different tumor cell lines and have been a common feature of all compounds with carcinogenic potency. Thiosemicarbazides and their derivatives display interesting biological activities, including anticancer, antimicrobial, anti-HIV, antiviral, insecticidal, anti-sclerotic, anti-oxidant, and antiparasitic activities [Arora *et al.* 2014].

Similarly, 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-thiosemicarbazide hybrids was synthesized and evaluated for their

anti-inflammatory activities [dos Santos Filho *et al.* 2009]. This strategy was retaken in this work, exploiting the potential anticancer activity of novel aryl-1,2,4-oxadiazole-*N*-cyclohexyl thiosemicarbazides.

Material and Methods

The 1,2,4-oxadiazole hydrazides **1a-h** were already described by our group and can be achieved in three easy and efficient steps [dos Santos Filho *et al.* 2009]. They provide access to the designed thiosemicarbazide derivatives **2a-h** by reaction with cyclohexyl isothiocyanate in a polar protic solvent under inert atmosphere and refluxing (Scheme 1), according to the general procedure below.



X: H (a), CH_3 (b), F (c), Cl (d), Br (e), CH_3O (f), NO_2 (g), OH (h)

Scheme 1. Synthesis of hybrids 1,2,4-oxadiazole cyclohexyl thiosemicarbazides **2a-h**

Preparation of aryl-1,2,4-oxadiazole-*N*-cyclohexyl thiosemicarbazides **2a-h**: A solution of hydrazides **1a-h** (0.5 mmol) in 2.5 mL THF was heated at 80 ºC, then cyclohexyl isothiocyanate (0.5 mmol, 0.068 g) was added, and the reaction was allowed to proceed at reflux under inert atmosphere for 5 h before cooling to room temperature. The solvent was removed in vacuo and the crude products were recrystallized from CH_2Cl_2/petroleum ether (30–60 ºC) to afford, after filtration, the thiosemicarbazides **2a-h**, 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.

Hybrid 1,2,4-oxadiazole thiosemicarbazides **2a-h** were tested for their cytotoxicity against cancer cell lines HEP-2 (human laryngeal cancer), HT29 (human colon adenocarcinoma) 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 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]. All flow cytometry results were expressed as the mean ± standard deviation compared to the negative control using ANOVA followed by Dunnett's multiple comparison test ($p < 0.05$). GraphPad Prism version 5.0 was used to perform all analyses (GraphPad Software).

Results and Discussion

The synthesis of hybrid 1,2,4-oxadiazole-thiosemicarbazides **2a-h** was carried out according to the proposed methodology, and the reaction progression was monitored by TLC until total consumption of 1,2,4-oxadiazole hydrazides **1a-h** was observed. The targeted products were isolated as pure solids after recrystallization from CH_2Cl_2/petroleum ether 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}), C=O (1700-1680 cm^{-1}),

and C=S (1220-1280 cm^{-1}) were observed, indicating the structural identity of the obtained products. The general yields ranged from good to moderate after one recrystallization step, which is an excellent outcome, avoiding tedious and expensive purification steps.

Table 1. Characterization data for the derivatives aryl-1,2,4-oxadiazole-*N*-cyclohexyl thiosemicarbazides **2a-h**.

Comp.	X	Yield (%)	M.p. (°C)	IR (cm^{-1})
2a	H	68	186-188	3321, 3237 (NH), 3055 (Ar CH), 2928 (Aliphatic CH), 1679 (C=O), 1540 (C=C), 1255 (C=S)
2b	CH_3	75	204-206	3331, 3219 (NH), 3050 (Ar CH), 2930 (Aliphatic CH), 1688 (C=O), 1534 (C=C), 1220 (C=S)
2c	F	51	176-178	3326, 3234 (NH), 3160 (Ar CH), 2934 (Aliphatic CH), 1715 (C=O), 1534 (C=C), 1235 (C=S)
2d	Cl	70	191-192	3326, 3221 (NH), 3060 (Ar CH), 2934 (Aliphatic CH), 1683 (C=O), 1540 (C=C), 1238 (C=S)
2e	Br	75	192-194	3327, 3220 (NH), 3057 (Ar CH), 2934 (Aliphatic CH), 1682 (C=O), 1537 (C=C), 1238 (C=S)
2f	OCH_3	75	187-188	3304, 3139 (NH), 3045 (Ar CH), 2934 (Aliphatic CH), 1721 (C=O), 1552 (C=C), 1251 (C=S)
2g	NO_2	75	198-200	3332, 3277 (NH), 3040 (Ar CH), 2936 (Aliphatic CH), 1724 (C=O), 1527 (C=C), 1273 (C=S)
2h	OH	86	186-188	3395 (OH), 3362, 3282 (NH), 3050 (Ar CH), 2935 (Aliphatic CH), 1706 (C=O), 1539 (C=C), 1224 (C=S)

The synthesis of the 1,2,4-oxadiazole thiosemicarbazides **2a-h** has brought about a set of molecules with two recognized pharmacophoric groups, which were expected to disclose good biological results.

Table 2-Cell growth inhibition (%) and standard deviation (SD) for 1,2,4-oxadiazole thiosemicarbazides **2a-h**

Comp.	X	NCI H-292 (%)±SD	HT29 (%)±SD	HEP-2 (%)±SD
2a	H	13,7±6,2	0±0	14,9±4,5
2b	CH_3	19,7±1,5	6,4±1,4	36,6±1,5
2c	F	13,5±5,1	39,6±11,4	75,6±7,8
2d	Cl	0±0	24,9±8,8	53,6±8,1
2e	Br	0±0	0±0	30,7±1,3
2f	OCH_3	12,6±1,8	0±0	16,2±1,1
2g	NO_2	2,8±1,5	4,7±7,4	31,7±3,2
2h	OH	0±0	0±0	9,9±2,7
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 HT29. On the other hand, the results arising for the HEP-2 cell line were much more promising with compound **2c** disclosing inhibition of 75,6%, the best result in the series. In a closer look at Table 2, it can be observed that the best inhibition values involve substituents with higher electron-withdrawing effects, namely, chlorine, bromine, and nitro. Since this work is a preliminary approach, it points to the need for the expansion of this investigation towards more *N*-substituents other than cyclohexyl, 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 the synthesis of novel structural hybrids of aryl-1,2,4-oxadiazole-*N*-cyclohexyl thiosemicarbazides was described and eight 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 *N*-substitutions that can elucidate how more complex structural features can influence the anticancer activity of this class of compounds.

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