Synthesis and cytotoxicity of Pyrazoles derivatives from ketenedithioacetals

Gabriel R. Antunes (G)[1*], Cecília S. Santos (G),[2] Gabriela F. M. Lopes (G),[2] Silmara N. Andrade,[2] Fernando P. Varotti (PQ),[2] Diego P. Sangi (PQ)[1]

[1]Instituto de Ciências Exatas, Universidade Federal Fluminense, Volta Redonda - RJ; [2] Centro de Ciências da Saúde, Universidade Federal de São João Del-Rei, Divinópolis - MG. *gabrielrodriguesantunes@id.uff.br

RESUMO

Ketene dithioacetals were used to synthesize a series of novel 3-methylthiopyrazole derivatives via vinylic substitution followed by intramolecular cyclization. The compounds were evaluated for their *in vitro* cytotoxicity against MDA-MB-231 human breast adenocarcinoma cell line, A549 human lung carcinoma cell line, TOV-21g human ovarian adenocarcinoma cell line, WI-26VA4 human lung fibroblast cell line, and THP-1 human acute monocytic leukemia cell line. Among the eight synthesized compounds, compound 4 exhibited selective cytotoxicity with an IC_{50} of 4.2 ± 3 μM against THP-1 cells, and no significant activity against non-tumoral WI-26VA4 fibroblasts. These results identify compound 4 as a *hit compound* and support its potential as a promising scaffold for the development of antitumor agents.

Palavras-chave: Pyrazoles, Synthesis, Cytotoxicity.

Introdução

Cancer remains one of the leading causes of mortality worldwide, with breast, lung, and ovarian cancers, as well as acute and chronic myeloid leukemias, representing major therapeutic challenges due to their high incidence, aggressive clinical course, and frequent development of resistance to standard therapies. Preclinical studies employing human cancer cell lines—including MDA-MB-231 (triple-negative breast cancer), TOV-21G (ovarian carcinoma), A549 (lung carcinoma), WI-26VA4 (non-malignant lung fibroblasts), THP-1 (acute myeloid leukemia)—are essential for evaluating the cytotoxicity, selectivity, and mechanisms of action of new compounds. These models recapitulate key features of solid tumors and hematologic malignancies, providing a robust platform for screening and comparative analysis. Despite advances in targeted therapies and immunotherapy, conventional treatment regimens continue to face limitations such as systemic toxicity, low selectivity, and tumor relapse. In this context, the investigation of synthetic heterocyclic compounds was undertaken as a strategy to identify more effective and selective antineoplastic agents with potential activity against these clinically relevant cancer types.

Pyrazoles constitute a class of heterocyclic compounds recognized as privileged structures for a wide range of biological applications being associated with diverse activities on multiple biological targets. Celecoxib is an example of pyrazole that exhibits both anti-inflammatory and antidiabetic properties, while penthiopyrad is an effective antifungal agent. There is considerable interest in pyrazole derivatives for potential antitumor activity. Notably, ruxolitinib acts as an antitumor JAK1/2 inhibitor, and crizotinib inhibits ALK and ROS1 kinases, with the pyrazole nucleus playing a critical role in the interaction between the kinase and the drug (Figure 1).

Due to their importance, numerous methods have been developed for the synthesis of pyrazoles. Ketenedithioacetals are interesting building blocks for the synthesis of various heterocycles. Their structure features two methylthio groups, which act as good leaving groups, as well as additional electron-withdrawing substituents attached to the double bond. This combination effectively polarizes the π-system, thereby facilitating nucleophilic attack on the electrophilic carbon atoms bonded to sulfur. In this work, we employed polarized ketenedithioacetals as electrophiles for [3+2] cyclization, to obtain 3-methylthio pyrazole derivatives, using different hydrazines as nucleophiles.

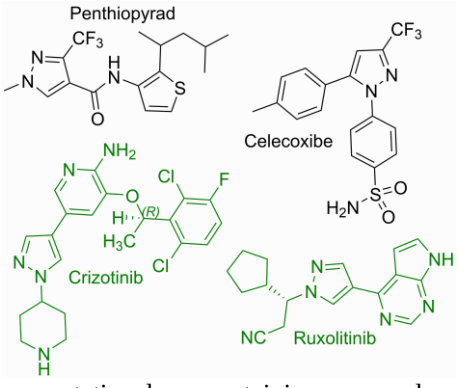

Figure 1. Representative drugs containing a pyrazole nucleus.

Experimental

General procedure for the synthesis of pyrazole derivatives

First, we prepared the ketenedithioacetals through a one-pot, three-step synthesis. In this reaction, DMF was used as the solvent and K_2CO_3 as the base to deprotonate compounds containing hydrogens α to electron-withdrawing groups. Subsequently, carbon disulfide was added and underwent nucleophilic attack by the generated carbanion. The resulting intermediate salt was then

methylated with methyl iodide. The product was isolated after work-up by the addition of water followed by filtration.

After isolation of the ketenedithioacetals, they were subjected to cyclization reactions as follows. Specifically, 1 mmol of the prepared starting materials was dissolved in 5 mL of ethanol, and 1 mmol of a hydrazine derivative was added. The reaction mixture was stirred and heated at 80 °C under reflux for 4 to 24 hours.

General procedure for the synthesis of pyrazole derivatives

Human cells were grown in RPMI-1640 medium supplemented with 10% fetal bovine serum (FBS) and gentamicin (100 µg/mL). Cultures were maintained at 37°C in a humidified atmosphere with 5% CO_2.

Cell viability was assessed through the MTT (3-[4,5-dimethylthiazol-2-yl]-2,5-diphenyl-tetrazolium bromide). For the assay, 100 µL of complete medium containing 1×10^4 cells was added to each well of a 96-well tissue culture microtiter plate. Incubation occurred at 37°C in a humidified 5% CO_2 incubator for 24 hours prior to experimentation. Post medium removal, 100 µL of fresh medium, along with test compounds at concentrations ranging from 0.01 to 100 µM, was added to each well and incubated at 37°C for 48 hours. All test compounds were pre-solubilized in dimethyl sulfoxide (DMSO) to prepare a stock solution. It is important to note that the final DMSO concentration used in the treatment was strictly controlled at all stages of the experiment (≤ 0.2%), ensuring that its effect did not interfere with the results obtained.

Subsequently, medium replacement with 100 µL of MTT solution (0.5 mg/mL) per well occurred, followed by an additional 3-hour incubation under previous conditions. 100 µL of DMSO was introduced into each well to dissolve formazan crystals. Absorbance (Abs) at 550 nm was measured using a microplate reader (Spectramax M5e, Molecular Devices, Sunnyvale, CA, USA). Percentage of growth inhibition was calculated using the formula [1-(Abs of treated/Abs of control)]×100. All experiments were performed in triplicate, and results were expressed as mean IC50 values. IC_{50} values were calculated using OriginPro 8.0 software (OriginLab Corporation, Northampton, MA, USA).

Resultados e Discussão

Three hydrazines were used as nucleophiles, phenylhydrazine, hydrazine hydrate, and methylhydrazine to react with three different ketenedithioacetals. As a result, eight 3-methylthiopyrazole derivatives were obtained, with yields ranging from 42% to 91% (Figure 2).

The synthesized 3-methylthiopyrazole derivatives were evaluated for cytotoxic activity against the selected cancer cell lines. Notably, several compounds exhibited promising activity, with IC_{50} valuesin the low micromolar range, for example, GSCB116 showed an IC_{50} of 4.2 ± 3 µM, while GSCB118 and GSCB112 displayed IC_{50} values of 10.30 ± 6 µM and 25.8 ± 13.3 µM, respectively. These results indicate selective and potent cytotoxic effects, supporting the relevance of this scaffold for further investigation (Table 1).

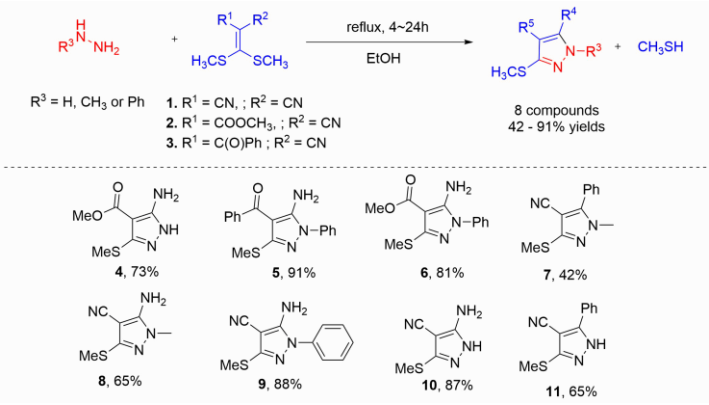

Figure 2. Procedure and obtained pyrazoles

Compound	IC50 (µM)				
	MDA-MB-231	**A549**	**TOV-21g**	**WI-26VA4**	**THP-1**
4	>100	>100	>100	>100	>100
5	**25,8±13,3**	>100	>100	>100	>100
6	>100	>100	>100	>100	>100
7	>100	>100	>100	>100	**31,2±19**
8	>100	>100	>100	>100	>100
9	**77,2±32,0**	>100	>100	>100	**4,2±3**
10	>100	>100	>100	>100	>100
11	>100	>100	>100	>100	**10,3±6**

Table 1. *In vitro* cytotoxicity IC_{50} values obtained against MDA-MB231, A549, TOV-21g, WI-26VA4 and THP-1 cells.

Conclusões

By applying a vinylic substitution on polarized ketenedithioacetals followed by intramolecular cyclization, eight novel 3-methylthiopyrazole derivatives were synthesized in yields ranging from 42% to 91%. In vitro cytotoxicity assays against MDA-MB-231, A549, TOV-21G, WI-26VA4, and THP-1 cell lines revealed selective activity for some compounds. Notably, GSCB116 exhibited an IC_{50} of 4.2 µM against THP-1 cells, without significant toxicity in non-tumorigenic lung fibroblasts. These findings suggest that compound 4 can be classified as a *hit compound* and represents a promising starting point for the development of more potent and selective antitumor agents.

Agradecimentos

To the Fluminense Federal University

Referências

1. Jung, N.; Stanek, B.; Graßle, S.; Nieger, M.; Brase, S. Organic Letters, v. 16, n. 4, p. 1112–1115, 2014

2. Sivaramakarthikeyan, R. et al. Biomedicines, v. 10, n. 5, p. 1124, 2022.

3. Baliza, L. R. S. P. et al. Chemical Biology & Drug Design, 2024.