Synthesis and cytotoxicity of new 1,3-diazepines derivatives

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RESUMO (Times New Roman, tam 12)

Ketene dithioacetals were used to synthesize a small class of new 2-substituted 1,3-diazepine and studied 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, and the WI-26VA4 human lung fibroblast cell line. The five 1,3-diazepinecompounds didn´t show *in vitro* cytotoxic activity against human cell lines.

Keywords: Ketene dithioacetals, diazepines, Synthesis, Cytotoxicity.

Introduction

Diazepines are a fascinating class of heterocyclic compounds due to their significant applications in the biological sciences. Benzodiazepines were the first heterocyclic compounds to be recognized as privileged structures, and other 1,2, 1,5 and 1,4-diazepine derivatives have also exhibited notable biological activities (1-4).

The 1,3-diazepine scaffold is similarly regarded as a privileged structure in medicinal chemistry. This moiety is present in numerous biologically active compounds, including natural products, and serves as a framework for designing molecules with diverse biological activities, such as anticancer agents (e.g., pentostatin), antibiotics (e.g., avibactam), and HIV protease inhibitors (e.g., DMP323). Despite the extensive documentation on the synthesis of 1,3-diazepines, 1,3-diazepan-2-ylidenes remain virtually unexplored (5-12).

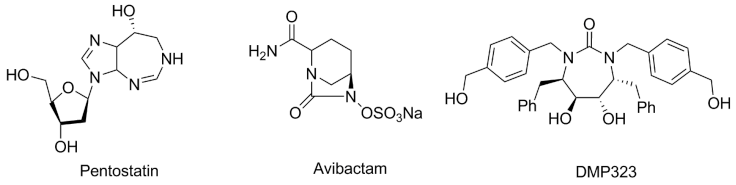

Figure 1. Examples of drugs containing the 1,3-diazepine moiety.

Ketene dithioacetals are valuable building blocks in organic synthesis. These compounds readily undergo double vinylic substitution, enabling the single-step formation of heterocyclic compounds with exocyclic double bonds (13,14). Motivated by the potential biological activity and materials science applications of these structures, ketene dithioacetals were employed to synthesize novel 1,3-diazepan-2-ylidenes and evaluate their citotoxicity against human cells.

Experimental

General procedure for the synthesis of 1,3-diazepines

In a glass suitable for microwave reactions, polarized dithioacetal (**1-6**) (1 mmol) and 1,4-diaminobutane (1 mmol) in ethanol (3 mL) were added. It was irradiated with microwaves for 60 minutes, maintaining a temperature at 110°C and constant stirring.

In the end of the reaction, the solvent was evaporated and the products described below were obtained in pure form after chromatography.

The data from the infrared spectroscopy, mass spectrometry and nuclear magnetic resonance spectra of hydrogen (^{1}H NMR) and carbon (^{13}C NMR) that were used to identify the synthesized compounds are as follows.

Procedure to cytotoxicity evaluation

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 IC_{50} values. IC_{50} values were calculated using OriginPro 8.0 software (OriginLab Corporation, Northampton, MA, USA).

Results and Discussion

Using ketene dithioacetals (**1-6**), we did a microwave assisted synthesis using 1,4-diaminobutane as nucleophile, in order to synthesize 2-substituted 1,3-diazepines, and evaluated their cytotoxicity properties. Five 2-substituted 1,3-diazepines (**7-12**) were produced with yields varying in the range of 43-95%.

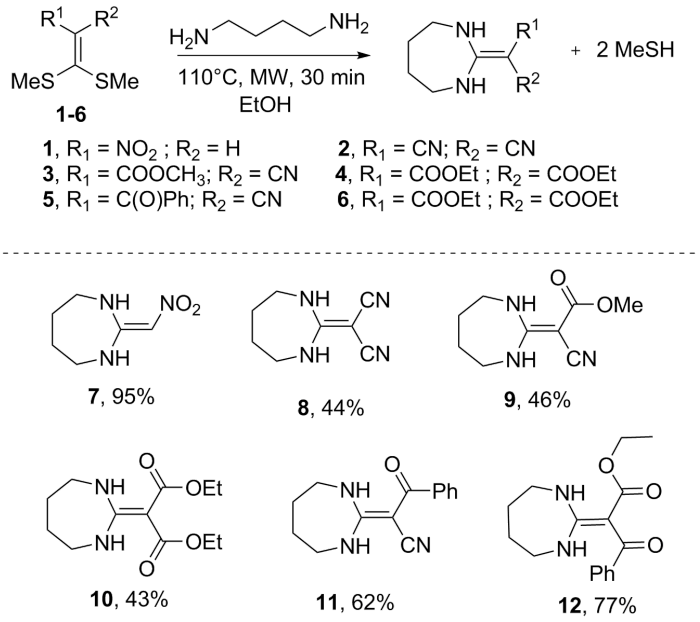

Figure 2. Synthesis of 1,3-diazepines (**7-12**).

In an attempt to determine the cytotoxicity of the compounds, we tested them against four human cell lines. None of the tested compounds exhibited cytotoxicity against the cell lines. The data are presented in Table 1.

Table 1 - *In vitro* cytotoxicity IC_{50} values obtained against MDA-MB-231, A549, TOV-21g and WI-26VA4 cell lines.

Compounds	IC_{50} (µM)			
	MDA-MB-231	A549	TOV-21g	WI-26VA4
7	>100	>100	>100	>100
8	>100	>100	>100	>100
9	>100	>100	>100	>100
10	>100	>100	>100	>100
11	nd	nd	nd	nd
12	nd	nd	nd	nd

[a] nd = Not determined

Conclusions

Appling the double vinylic substitution in ketene dithioacetals, we synthesize five unpublished 2-substituted 1,3-diazepines with 43 - 95 % of yield. The synthesized compounds didn´t show *in vitro* cytotoxic activity against MDA-MB-231, A549, TOV-21g and WI-26VA4 cell lines.

Acknowledgments

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