

## Evaluation of the formation of glutathione (GSH) adducts with isothiocyanates (ITC) potentially inhibitors of human tumor cell proliferation

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### Abstract

Isothiocyanates are molecules that can interact with glutathione, a tripeptide present in high concentration in tumor cells related to the maintenance and defense of these cells. This project proposes the testing of synthesized isothiocyanates in regards to their reactivity with glutathione and performance in cell viability assays.

*Keywords: Isothiocyanates, glutathione, cellular proliferation.*

### Introduction

Isothiocyanates (ITC) constitute a class of molecules that can be found in cruciferous vegetables, being related to defense mechanisms in the plant.<sup>1</sup> The main feature of isothiocyanates is their strong electrophilicity, which turns these molecules into very good substrates in addition reactions with biological nucleophiles, such as glutathione (GSH), a tripeptide responsible for the process of detoxification of xenobiotics and oxygen radical species.<sup>2</sup>

In this project, we explore the reactivity of ITC with GSH as a mechanism of inhibiting cancer cell proliferation. The reactions of formation of ITC-GSH adducts were monitored against time by mass spectrometry (ESI-MS) and theoretically predicted using the software GaussView 5. Cytotoxicity tests were also performed using strains of cancer cells, along with healthy keratinocytes as a control.

### Results and Discussion

The six isothiocyanates tested had been previously synthesized by the preceding project in a synthetic route that involved the correspondent ketones as starting materials. Firstly, their reactivity was evaluated by calculating the LUMO energy levels of the molecules, since this orbital accepts the electrons coming from the glutathione HOMO orbitals during the adduct formation. Thus, the reactivity of ITC towards GSH would be related to low LUMO energy levels (Chart 1). It was found that even though there are some significant differences on the values of energy levels, all the ITC-GSH adducts were detected by mass spectrometry in less than 3 hours, what shows the high reactivity of these molecules.

The ITC cytotoxicity tests indicated that only the chlorine substituted ITC was effective on inducing

apoptosis of NG97 (glioma) and PANC (pancreatic) cells more intensely than in healthy cells over a narrow range. All the others were more toxic towards the keratinocytes.


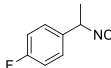
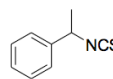
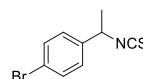
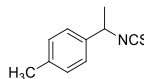
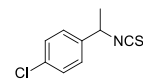
ITC	LUMO / eV	ITC	LUMO / eV
	0,31129		-0,77414
	-0,43619		-0,78312
	-0,38612		-0,83972

Chart 1. LUMO levels for the ITC synthesized.

### Conclusions

All the ITC were highly reactive towards GSH, forming the adduct ITC-GSH readily. However, this mechanism does not seem to be selective for cancer cells, seeing that almost all the ITC were highly toxic to healthy keratinocytes.

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<sup>1</sup> Bones, A. M.; Rossiter, J. T. *Physiol. Plant*, **1996**, *96*, 194-208

<sup>2</sup> Almeida, W. P.; Huber, P.C., de Fatima, A. *Quim. Nova*, **2008**, *31*, 1170-1179