THE PENNSYLVANIA STATE UNIVERSITY
SCHREYER HONORS COLLEGE

DEPARTMENT OF LETTERS, ARTS, AND SCIENCES

EFFECT OF 1,3-THIAZOLIDIN-4-ONES ON HELA AND GIST-T1 CELL PROLIFERATION *IN VITRO*

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ABSTRACT

4-thiazolidinones have been reported as a potential drug-like molecule due to their possible anticancer and antiproliferation properties in cancer cell lines. The effects of five different novel 1,3-thiazolidin-4-ones compounds on human cervical (HeLa) and gastrointestinal stromal tumor (GIST-T1) cell lines *in vitro* were observed. The HeLa and GIST-T1 cells were grown in culture and treated with 0-400 μM concentrations for 48 hours. Cell growth was measured by absorbance and resulted in a decrease of cell proliferation; 0-100 μM showed moderate growth, whereas 200-400 μM treatment resulted in minimal to no growth. The compounds varied in substituent (F, Br, Cl) and placement (ortho, para). Results indicated a correlation between para position and decreased growth. These results agree that there are possible implications of 4-thiazolidinones possessing anticancer properties and should be further evaluated for possible mechanisms and uses.

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Chapter 1

Introduction

Cancer is a leading cause of deaths worldwide. Through preventative screenings, treatments, and research, there have been many strides in the advancement in the fight against cancer. Current research of 4-thiazolidinones considers them as possible drug-like molecules that can be designed to act as anticancer agents.[1] Each of the five 1,3-thiazolidin-4-ones observed varied in either substituent (Cl, Br, F) and/or placement on the ring. Structures are shown in Table 1.

The five novel 4-thiazolidinones were tested for antiproliferative activity on HeLa and GIST-T1 cell lines *in vitro*. HeLa, known as the first immortalized human cell line, is derived from cervical cancer tissue. Cervical cancer is the second most common cancer in women, and occurs due to the formation of cancerous lesions following persistent human papillomaviruses (HPV) infections.[2] HeLa lines have been shown to have reduced proliferation after exposure to 4-thiazolidinones in various publications.[7,8,9] Gastrointestinal stromal tumors (GIST) are a form of submucosal tumors that occur in the stomach, small intestine, and esophagus.[3]

It has been shown that 4-thiazolidiones derivatives have anticancer, antiproliferation, and cytotoxic effects on various cancerous human cell lines *in vitro*. Many of these studies have obtained data that confirmed the anticancer potency of thiazolidin-4-ones with an IC50 (half maximal inhibitory concentration) on a nanomolar level.[1,4-9] Current research also suggests that

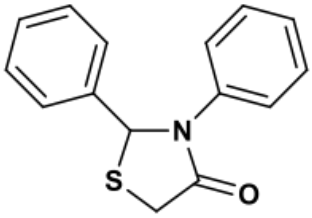
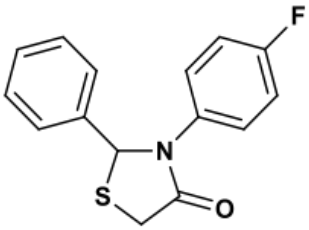
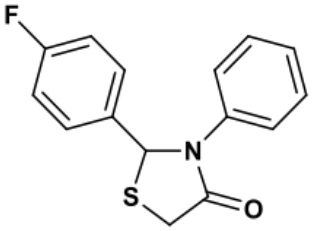
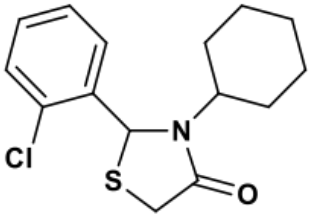
anticancer activity is associated with 4-thiazolidinones through a reactive oxygen species (ROS) buildup, and a caspase induced apoptotic pathway.[1,4-6]

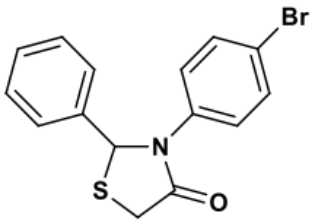
Caspase-9 belongs to a family of cysteine proteases that are implicated during apoptosis and cytokine processing. Once a cell has received an apoptotic stimulus, it will release cytochrome C from the mitochondria, that will then bind and cause a series of signals to recruit and activate Caspase-9.[10] The mechanism that induces apoptosis in cells exposed to 4-thiazolidinones is characterized by secretion of cytochrome C in the cytosol and activation of Caspase-9. Caspase-9 then activates Caspase-3 and is assumed to activate programmed cell death via a signaling cascade.[4-5,10] It has also been documented that interaction with 4-thiazolidinones increase ROS by inhibition of G_0 and G_1 cycles which inhibits cell division.[4] These data strongly support the investigation and possible role of 4-thiazolidinones in future cancer treatment.

Chapter 2

Results

Table 1. In vitro effect of compounds on HeLa human cervical, and GIST-T1 human gastrointestinal tumor lines after 48 hours (NA: non effective.)

Compound	Structure	IC_{50} Values (μM)	
		HeLa	GIST-T1
I		260	NA
II		210	140
III		290	265
IV		110	155

V		59	65

Compound V exhibited the highest degree of cytotoxic activity at low concentrations (<100 μM) in both cell lines. Compounds IV and II showed moderate cytotoxic activity at concentrations less than 250 μM. Compounds III and I required the highest concentrations (>250 μM) to display a cyctotoxic effect.

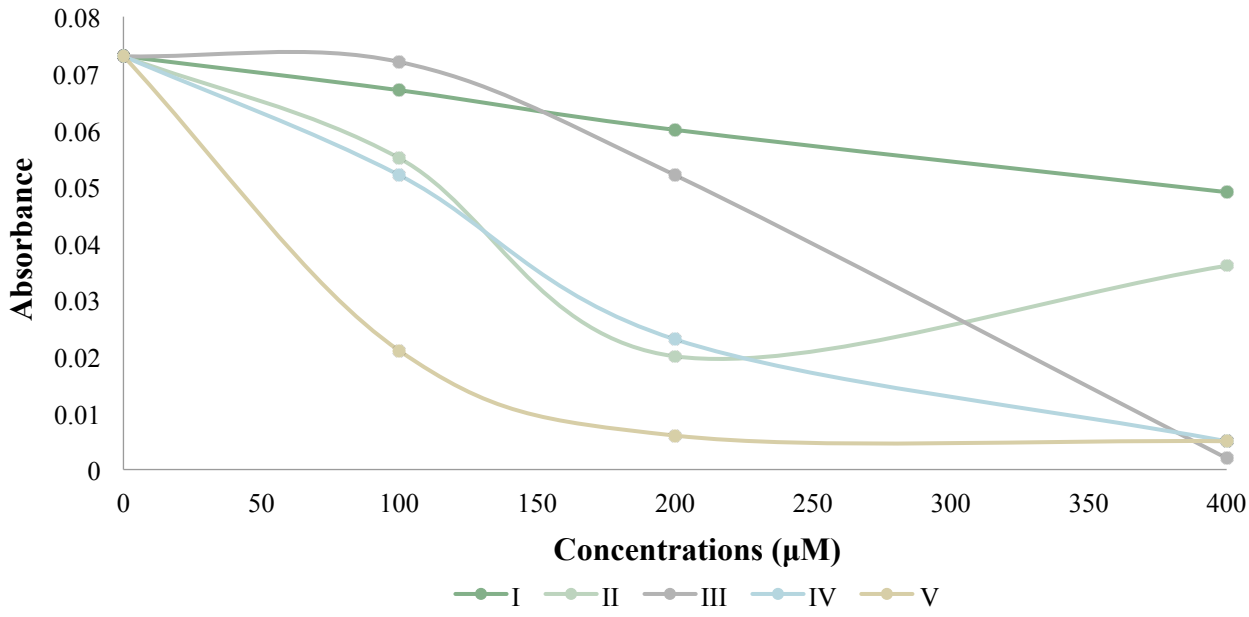


Figure 1. Absorbance of GIST-T1 cells after 48-hour exposure to 100 μM, 200 μM, and 400 μM concentrations of compounds I-V.

GIST-T1 when treated showed antiproliferation resulting in complete cell death in three out of the five compounds tested (Fig. 1). Compound V showed the greatest antiproliferative effect: A<.050 at 100 μM, A<.005 at 200-400 μM. Compound IV also displayed a strongly negative effect with an A of 0.05 at 100 μM, A<.050 at 200 μM, and A<.005 at 400 μM.

Compound III initially seemed less affective (A=0.072 at 100 μM) but began to steadily decrease at 200 μM (A=0.052), and 400 μM (A<.005). Compound II showed a maximum effect at 200 μM (A<.005). Compound I showed the least overall effect with an A of <.067 at 100-200 μM, and A<.050 at 400 μM.

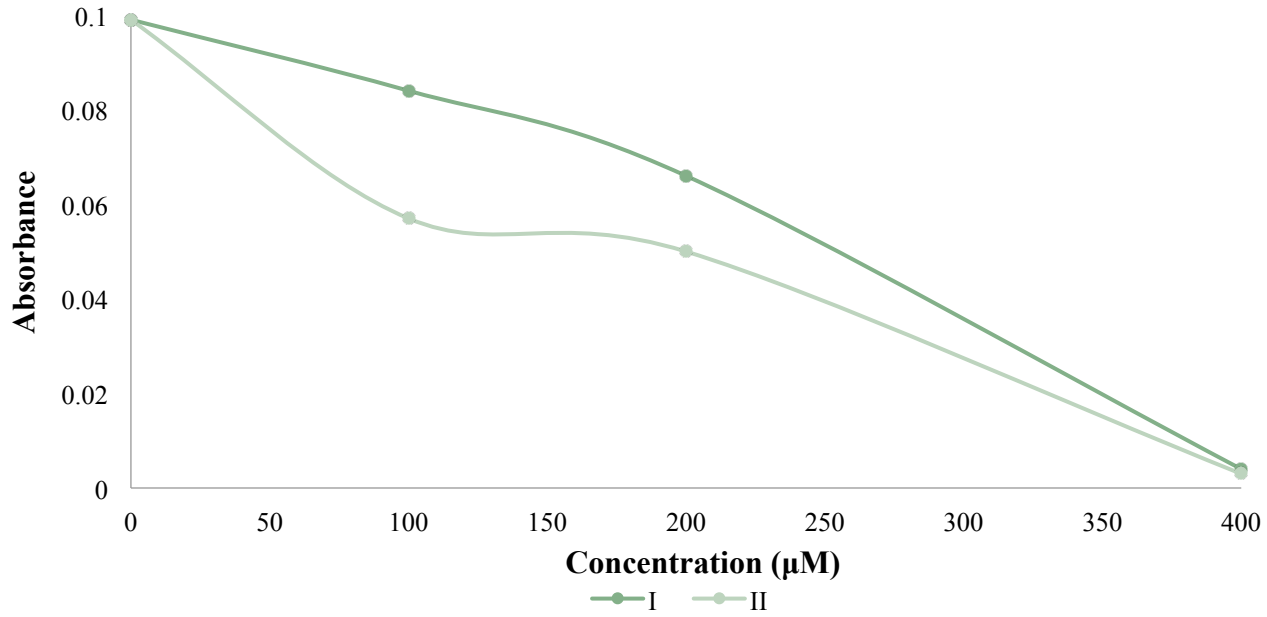


Figure 2. Absorbance of HeLa cells after 48-hour exposure to 100 μM, 200 μM, and 400 μM concentrations of compounds I and II.

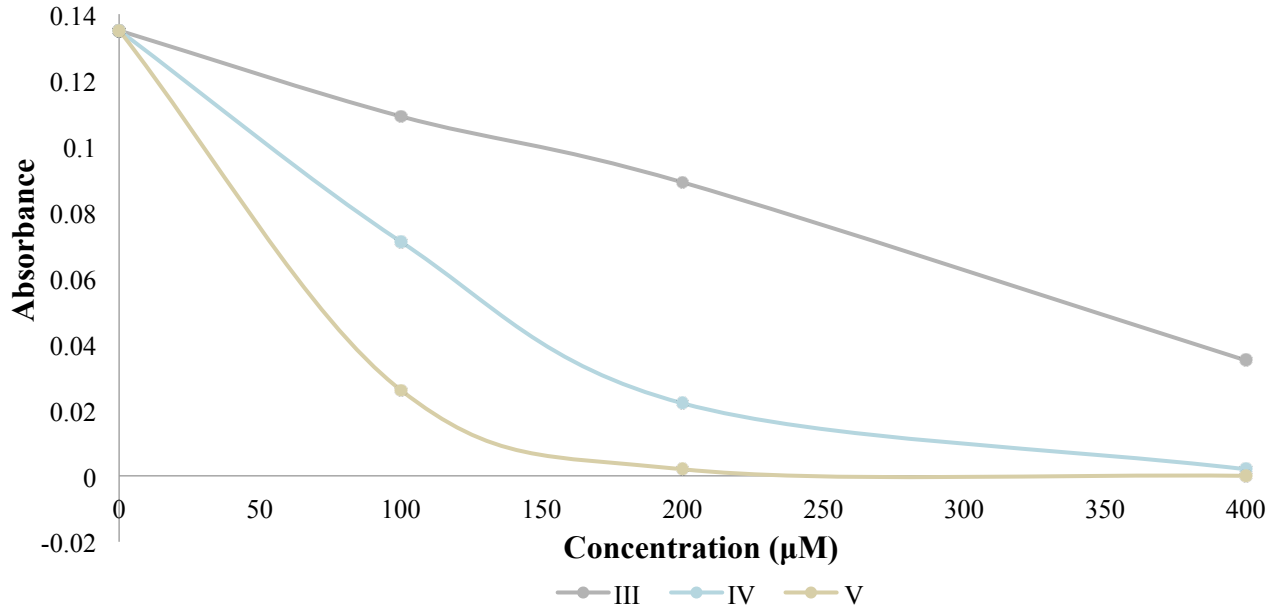


Figure 3. Absorbance of HeLa cells after 48-hour exposure to 100 µM, 200 µM, and 400 µM concentrations of compounds III-V.

Compound V also showed the most negative effect on cell proliferation in the HeLa trials: A=0.026 at 100 µM, A<.005 at 200-400 µM (Figs. 2 and 3). Compounds II and IV displayed similar results with a high level of proliferation (A>.050) at 100 µM, before exhibiting a decrease at 200 µM (A<.050), and 400 µM (A<.005). Compound I showed minimal effects at 100-200 µM (A>.050), and almost complete antiproliferation at 400 µM(A<.005). Compound III was least effective overall: A=0.109 at 100 µM, 0.089 at 200 µM, and A<.050 at 400 µM.

Chapter 3

Discussion

These data support the hypothesis that the five novel 4-thiazolidinones observed have antiproliferation properties on human cervical cancer (HeLa) and gastrointestinal stromal tumor (GIST-T1) lines *in vitro*. The mechanism for which this is achieved most likely resembles the ROS and apoptotic pathways seen in similar studies. Cytochrome C secretion observed from the cytosol activates Caspase-9 which induces programmed cell death.[1,4-6] As well as an association with a buildup of ROS, and the reduction of cell growth via inhibition of G_0 and G_1 cell cycles.[4-6]

All five novel 1,3-thiazolidin-4-ones displayed a negative effect on proliferation in both HeLa and GIST-T1 lines after 48 hours of high concentration exposure. Compound V displayed the most cytotoxic and antiproliferative effects differed with regard to a para substituted bromine compared to compound I, which lacked a substituent and resulted in the lowest cytotoxic activity in the GIST-T1 trial. Compound I also displayed the second to least antiproliferative properties in the HeLa trials. This suggests that the addition of a substituent on either ring, in either placement, increases the cytotoxic and antiproliferative effects of the compound.

Both compounds II and III have a para substituted fluorine that differed in placement based on the proximity to sulfur and oxygen on the cyclopentane. A greater distance between sulfur and increased proximity to oxygen resulted in compound II having a greater impact on proliferation at a lower concentration in both HeLa and GIST-T1 trials. Neither compound II nor III displayed a higher antiproliferative effect on either cell line in comparison to compound V. This suggests that a bromine substituent has a larger cytotoxic effect than that of fluorine.

Compounds V and II have an identical structure and substituent placement, with respect to substituent. This suggests that the substituent placement of compounds V and II is superior to that of compound III.

Compound IV had the second greatest cytotoxic and antiproliferative effect on both HeLa and GIST-T1 trials. This supports the hypothesis that the least electronegative (Br, Cl, F) substituent has a greater impact on proliferation on both HeLa and GIST-T1 lines. A comparison between compounds IV and II also suggests that the influence of the substituent is greater than the influence of placement. This further implies that compound V is superior in both placement of and substituent type to all of the five observed.

Compounds I-V had a relatively high IC_{50} in comparison to most published values of 4-thiazolidinones. An increased concentration required could possibly be correlated to the decreased length of exposure. Compounds I-V were tested after 48 hours of administration, however various literature have increased (72 hour) intervals. Future experiments will consider lengthening time of compound administration, and a decrease of concentration. We will also test for cytotoxic effect on normal cell lines, and various substituents and placement.

Chapter 4

Methods and Materials

HeLa and GIST-T1 cells were grown in Dulbecco's Modified Eagle's Medium in a 37°C incubator at 5% CO_2. We then plated 150,000 cells per well in a six well plate, and grew them for 24 hours before administering a dosage. Each dosage was either a concentration of 100 μM, 200 μM, or 400 μM. Cells were incubated for another 48 hours after administration, and then removed via trypsinazation. We quantified cell survival by measuring absorbance at 800 nm.

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