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DESIGN AND SYNTHESIS OF A NOVEL ALPHA-METHYLENE LACTONE CHEMOTHERAPEUTIC AGENT

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Abstract

Goniothalamin, a natural product isolated from the dried stem bark of Malaysian plants of the genus *Goniothalamus*, has been shown to induce apoptosis in cancer cells. The bioactivity of this molecule is thought to be due to its ability to react with thiols. One mechanism involves its reaction with glutathione, a natural antioxidant found in all cells. Using a four step synthetic sequence, a novel gamma-lactone analogue of goniothalamin has been prepared that replaces the endocylic double bond in goniothalamin's lactone core with an exocyclic double bond. It is anticipated that this alteration will allow the compound to react more rapidly with thiols and therefore increase its cytotoxicity towards cancer cells.

Keywords: Cancer, Goniothalamin, Glutathione, Methylene, Lactone

Introduction

The most common method to identify new chemotherapeutic agents is the mass screening of natural products for cytotoxicity. If promising bioactivity is observed, the compounds that compose the natural product are isolated and characterized. Chemists then use the bioactive compound's structural framework as a guide in the design and synthesis of novel chemotherapeutic agents. The National Cancer Institute estimates that approximately 60 percent of all cancer drugs are a direct result of this isolation, characterization, and manipulation process (1).

Goniothalamin **[1]** (Figure 1) was isolated from the genus *Goniothalamus*, which consists of approximately 115 species of shrubs and trees that grow in the rainforests of Asia (2). Goniothalamin, naturally isolated as the "*R*" enantiomer, was found to exhibit IC_{50} values in the low microMolar range against a variety of cancer cell lines (3). In addition to its potent bioactivity, preliminary testing has shown that goniothalamin is more cytotoxic against cancer cells than normal cell lines (4).

Goniothalamin has been shown to induce apoptosis in cancer cells by creating a redox imbalance (5). This redox imbalance, also known as oxidative stress, is the direct result of a build-up of oxygen free radicals within a cell. Oxygen-derived free radicals, for example hydroxyl radicals, are formed naturally as byproducts during oxidative cellular metabolism. These free radicals are very reactive and are therefore very toxic to the cell. To combat this natural problem, the body has built-in antioxidants. Glutathione (GSH), found in all cells, is able to neutralize free radicals, therefore maintaining a redox balance and protecting the cell from the damaging effects of oxidative stress.

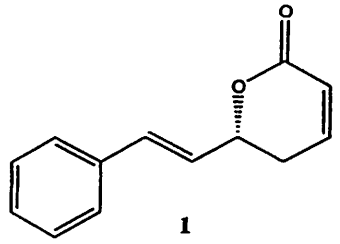


Figure 1. (*R*) Goniothalamin.

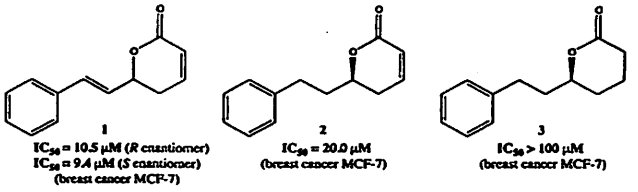


Figure 2. Synthetic Analogues of Goniothalamin.

Goniothalamin has been shown to decrease the amount of GSH found in cancer cells (5). This decrease in GSH leads to a build up of oxygen free radicals. The resulting redox imbalance induces apoptosis. It is hypothesized that goniothalamin depletes the level of antioxidants found in cancer cells by covalently bonding to GSH through a 1,4-addition reaction. This hypothesis stems from two pieces of evidence that can be found in the literature. First, it has been shown that the α,β-unsaturated carbonyl found in goniothalamin's structure is essential for its bioactivity. Synthetic analogues of goniothalamin, that do not include this double bond (compound **[3]**, Figure 2), exhibit a dramatic decrease in cytotoxicity toward cancer cells (6). Second, natural products containing α,β-unsaturated carbonyls (compounds **[4]** and **[5]**, Figure 3) have been shown to react with thiols through a 1,4-addition reaction (7,8). By forming covalent, thioether bonds with GSH, these compounds have been observed to deplete GSH levels and induce oxidative stress.

In this paper, the synthesis of a novel goniothalamin analogue is discussed. The novel racemic analogue, α-methylene lactone **[6]**, incorporates an exocyclic double bond in the structural framework of goniothalamin rather than the natural endocyclic double bond (Figure 4). The basis for this design comes from studies performed on the natural product

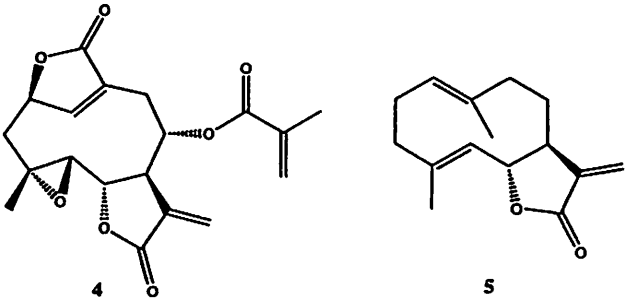


Figure 3. Elephantopin [4] and Costunolide [5].

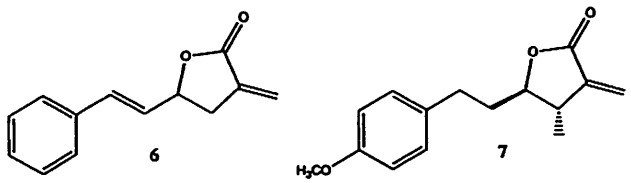


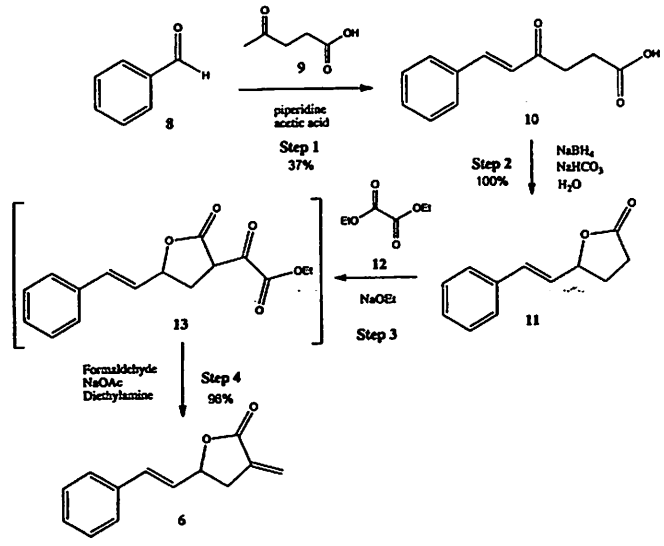
Figure 4. Novel Goniothalamin Analogue [6] and propionic acid derivative [7].

elephantopin [4] (Figure 3). The exocyclic methylene found in elephantopin was reported to react with cysteine 1000 times faster than lactones incorporating endocyclic double bonds (9). This enhanced reactivity, primarily attributed to sterics, should result in analogue [6] being much more reactive with GSH than goniothalamin.

Another indication that analogue [6] will exhibit potent cytotoxicity is propionic acid derivative [7] (Figure 4). Synthesized in 2002, this compound induced apoptosis in leukemia cell lines with an IC_{50} value of 4 μM (10). Looking at the bioactivity data reported for previously prepared derivatives of goniothalamin (compounds [2] and [3], Figure 2), it is anticipated that analogue [6], prepared in this paper, will exhibit even more potent cytotoxicity than propionic acid derivative [7]. The sequence used to synthesize analogue [6] is shown in Scheme 1.

Experimental

Column chromatography was performed on 230-400 mesh silica gel. NMR spectroscopy was recorded on a JEOL 300 MHz NMR spectrometer. Gas chromatography - mass spectrometry was carried out on an Agilent Technologies 7890A gas chromatograph. The GC column (30 m x 0.25 mm) had a 0.25 μm thick polydimethylsiloxane (PDMS) with 5% phenyl substitution stationary phase. The oven conditions were: injection port temperature = 250°C; oven starting temperature = 70°C for 5 minutes; ramp of 20°C / minute up to 250°C; the temperature was held for 20 minutes at 250°C. All starting chemicals were purchased from Sigma-Aldrich, Inc. (Atlanta, GA) and used as received.



Scheme 1. Synthesis of Novel Goniothalamin Analogue [6].

Step 1 (11)

Levulinic acid [9] (5.213 g, 0.04489 moles) was placed in a flame-dried 100 mL round-bottom flask followed by the addition of benzene (40 mL), benzaldehyde [8] (5.00 mL, 0.0449 moles), acetic acid (6.00 mL, 0.105 moles), and piperidine (2.00 mL, 0.0202 moles). The round-bottom flask was fitted with a Dean-Stark trap and subsequently heated at reflux for 54 hours. The resulting deep red solution was cooled to room temperature. Distilled water (100 mL) was added to the flask and an extraction was performed using diethyl ether (2 x 75 mL). The organic layer was dried over sodium sulfate, filtered, and concentrated on a rotary evaporator to provide a viscous, red oil. When placed on a high vacuum pump, the red oil turned into a yellowish-orange solid (9.476 g, 103.5% crude). The orange solid was recrystallized from absolute ethanol. Upon dissolving in a minimal amount of hot ethanol, the deep red solution was gradually cooled to room temperature and then placed in a freezer. After 3 hours in the freezer, a yellow crystalline solid was collected via vacuum filtration. The yellow crystals were washed with ice cold ethanol and dried using a high vacuum pump to afford pure carboxylic acid [10] (3.405 g, 37.18%). **^{1}H NMR** (300 MHz, $CDCl_3$) δ 10.2 (br s, 1H), 7.60 (d, J = 16.2 Hz, 1H), 7.58-7.52 (m, 2H), 7.44-7.36 (m, 3H), 6.76 (d, J = 16.2 Hz, 1H), 3.02 (t, J = 6.6 Hz, 2H), 2.75 (t, J = 6.6 Hz, 2H); **^{13}C NMR** δ 197.9, 178.6, 143.3, 134.4, 130.7, 129.1 (2C), 128.4 (2C), 125.8, 35.1, 28.0; **GC-MS** rt = 13.70 min; M^+ m/z = 204.

Step 2 (12)

Carboxylic acid [10] (3.332 g, 0.01627 moles) was treated with a solution of sodium bicarbonate (1.751 g, 0.02084 moles) in distilled water (70 mL). The mixture was stirred until the carboxylic acid almost completely dissolved and then sodium borohydride (1.649 g, 0.04359 moles) was added. After stirring 1.5 hours at room temperature, 3.0 M HCl was added dropwise until a pH 1 was achieved. A diethyl ether extraction (2 x 70 mL) was performed and the organic layers were combined and dried over sodium sulfate. Vacuum filtration followed by concentration on a rotary evaporator provided the desired lactone [11] as a yellow solid (3.153 g, 103.1%). As demonstrated by NMR and GC-MS analysis, further purification was not necessary (approximately 95% pure from GC-MS). The quantitative yield was taken on into the next step. If further purification is desired, the product can be recrystallized using 1:1 hexanes:ethyl acetate to afford a white crystalline product. **^{1}H NMR** (300 MHz, $CDCl_3$) δ 7.43-7.24 (m, 5H), 6.69 (d, J = 15.7 Hz, 1H), 6.20 (dd, J = 15.9, 6.6 Hz, 1H), 5.17-5.07 (m, 1H), 2.65-2.42 (m, 3H), 2.18 – 2.03 (m, 1H); **^{13}C NMR** δ 176.9, 135.8, 133.0, 128.8 (2C), 128.5, 126.8 (2C), 126.5, 80.7, 29.0, 28.6; **GC-MS** rt = 13.30 min; M^+ m/z = 188.

Step 3 (13)

Sodium metal (0.129 g, 0.00561 mol) was placed in absolute ethanol (4.0 mL) and stirred until completely dissolved. Diethyl oxalate (0.60 mL, 0.0044 mol) was added to the sodium ethoxide solution followed by the addition of lactone [11] (0.419 g, 0.00223 mol). The resultant yellow solution was stirred for 24 hours at room temperature; the solution turns a dark red color. The solvent was subsequently removed using a rotary evaporator. The remaining dark red residue was dissolved in water and diethyl ether; multiple rinses were required to dissolve the residue and get it into a separatory funnel (approximately 70 mL of water and 30 mL of diethyl

ether). The water layer was drawn off and acidified to a pH of 1 using 1.0 M HCl. The acidic solution was poured back into a separatory funnel and extracted with dichloromethane (3 x 50 mL). The combined organic layers were dried over magnesium sulfate, filtered, and concentrated under vacuum to afford the desired ethyl oxalyllactone **[13]** (0.655 g, 102%). The ethyl oxalyllactone was shown to be approximately 98% pure based on GC-MS analysis. **GC-MS** rt = 16.61 min; M^+ m/z = 288.

Step 4 (14)

Crude **[13]** (0.655 g, 0.00227 mol) was dissolved in 1,4-dioxane (4.0 mL). A catalytic amount of anhydrous sodium acetate (0.004 g, 0.00005 mol), aqueous formaldehyde solution (37 wt. % in water, 0.85 mL), and diethylamine (0.50 mL, 0.0048 mol) were subsequently added to the ethyl oxalyllactone solution. The reaction mixture was stirred at room temperature for 48 hours. The resulting dark red solution was acidified to a pH of 1 with 1.0 M HCl and extracted with ethyl acetate (2 x 50 mL). The combined organic fractions were dried over magnesium sulfate, filtered, and concentrated under vacuum to afford methylene lactone **[6]** as a light yellow solid (0.435 g, 97.5% based on the amount of lactone used in step 3). NMR and GC-MS analysis of the product illustrated that it was very pure (ranging from 85 to >98% purity based on the trial). If deemed necessary, column chromatography (1:1 hexanes:ethyl acetate) can be used to further purify the product. **^{1}H NMR** (300 MHz, $CDCl_3$) δ 7.45-7.24 (m, 5H), 6.69 (d, J = 15.9 Hz, 1H), 6.28 (dd, J = 2.7, 2.7 Hz, 1H), 6.19 (dd, J = 15.8, 6.9 Hz, 1H), 5.68 (dd, J = 2.5, 2.5 Hz, 1H), 5.18-5.08 (m, 1H), 3.22 (dddd, J = 17.0, 8.0, 2.5, 2.5 Hz, 1H), 2.80 (dddd, J = 17.0, 6.3, 2.7, 2.7 Hz, 1H); **^{13}C NMR** δ 170.1, 135.7, 134.2, 133.3, 128.8 (2C), 128.5, 126.8 (2C), 126.7, 122.5, 77.3, 34.4; **GC-MS** rt = 13.54 min, M^+ m/z = 200.

Results and Discussion

The first step in the synthetic sequence was an amine-catalyzed aldol condensation reaction between commercially available levulinic acid **[9]** and benzaldehyde **[8]**. This reaction can be run on large scale and the resulting keto-acid product **[10]** is easily purified by recrystallization. Keto-acid **[10]** was subsequently reduced using sodium borohydride. The resulting alkoxide anion immediately undergoes an intramolecular cyclization to afford lactone **[11]** in high yield. Conversion of lactone **[11]** to methylene lactone **[6]** proved to be difficult. Numerous methods have been published on performing this transformation (13-17). Three of these methods were tried. The first attempt involved an α-phosphono lactone/ Horner-Wadsworth-Emmons approach to the α-methylene lactone (15). This method, while shown to work on lactams, provided very low yields with lactone **[11]**. A second method, using ethyl formate to produce an α-formyl sodium salt intermediate, was also attempted (16). This synthetic sequence also resulted in very low yields of desired product. The third method tested involved using diethyl oxalate **[12]** to prepare an ethyloxalyl derivative. Ethyloxalyl derivatives have been prepared using both sodium hydride (14) and sodium ethoxide (13) as base. For lactone **[11]**, sodium ethoxide was found to provide much better conversion to ethyloxalyl intermediate **[13]**. The ethyloxalyl intermediate was converted into an α-methylene lactone using aqueous formaldehyde (14). Another method has also been used for this transformation (13), however aqueous formaldehyde worked so well that the other method was not attempted.

Conclusion

The desired racemic, α-methylene lactone **[6]** was prepared in four linear steps with a 36% overall yield. The elegance of this synthetic sequence comes from the limited number of purification steps that were required. Steps 2, 3, and 4 consistently exhibited greater than 95% purity based on GC-MS analysis. The simplicity of this methodology allows for facile production of large amounts of the desired α-methylene product **[6]**. Future work involves analyzing analogue **[6]** for bioactivity against cancer cell lines. If found to be active, a single enantiomer of compound **[6]** could potentially be prepared using enzymatic or asymmetric reductions of keto-acid **[10]** (12).

Acknowledgment

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