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THE DESIGN AND SYNTHESIS OF NOVEL GONIOTHALAMIN ANALOGUES

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Abstract

Every two minutes a woman in the United States is diagnosed with breast cancer. In recent years, one method to identify potential chemotherapeutic agents has been the mass screening of natural products for cytotoxicity. One compound discovered in this manner was goniothalamin. Goniothalamin was isolated from the dried stem bark of the plant *Goniothalamus sesuipedalis* and exhibits cell specific anticancer activity against breast cancer. Goniothalamin has been extensively studied and a large number of synthetic analogues have been prepared in an attempt to determine the structural features necessary for bioactivity. These studies have focused primarily on the manipulation of goniothalamin's styryl substituent. The focus of this research is on the lactone core of goniothalamin. Analogues have been prepared that replace the lactone ring with a lactam. It is anticipated that alteration of the lactam nitrogen substituent will potentially lead to analogues with better bioavailability and reactivity than the natural product.

Keywords: Cancer, Goniothalamin, Lactam

Introduction

Goniothalamin [**1**], shown in Figure 1, was isolated from the dried stem bark of the plant *Goniothalamus sesquipedalis* (1). This natural product has been shown to have anticancer activity and exhibits an IC_{50} value of 10.5 μM against the breast cancer cell line MCF-7 (2). The mechanism through which goniothalamin induces cytotoxicity is through programmed cell death, specifically apoptosis (3-5). Goniothalamin is known to trigger the caspase cascade, which is an integral part of apoptosis. This mechanism makes goniothalamin cell specific (3-5).

Due to its anticancer activity and limited availability from natural sources, 0.16-1.2% yield from *Goniothalamus* bark,(1) goniothalamin has received considerable attention as a synthetic target over the past twenty-five years. The first synthesis of goniothalamin was published in 1979. This synthesis confirmed the natural product's absolute configuration as being "*R*" (6). Since then, a variety of new enantioselective syntheses of goniothalamin have been reported and the biological activity of the compound has been extensively studied and discussed (2,7). A large number of synthetic analogues of goniothalamin have also been prepared in an attempt to determine the structural features of the molecule necessary for bioactivity (2,7,8). These structure/activity relationship studies have focused primarily on the manipulation of the styryl substituent. Analogues have also been prepared that have removed the double bond in the lactone ring and a slight decrease in bioactivity was observed (2). To date, however, no investigations have been performed on the importance of the lactone ring in the bioactivity of goniothalamin.

In this paper, the racemic synthesis of three analogues of goniothalamin will be described. The analogues, shown in Figure 2, will address the importance of the lactone ring by replacing the lactone core of goniothalamin with a lactam ring system. There are three potential benefits to incorporating nitrogen into the goniothalamin structure. First, a lactam has much greater metabolic stability than a lactone. This should lead to an increase in the drug's bioavailability (9). Second, the lactam nitrogen provides a means to

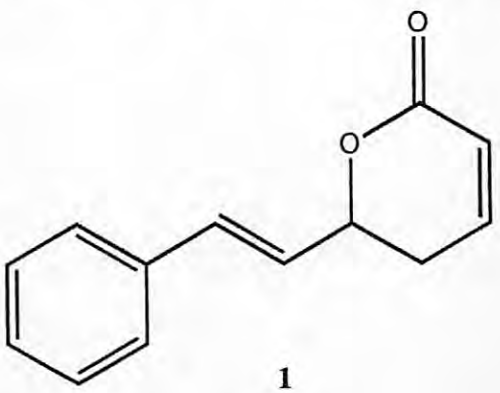

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Figure 1. Goniothalamin.

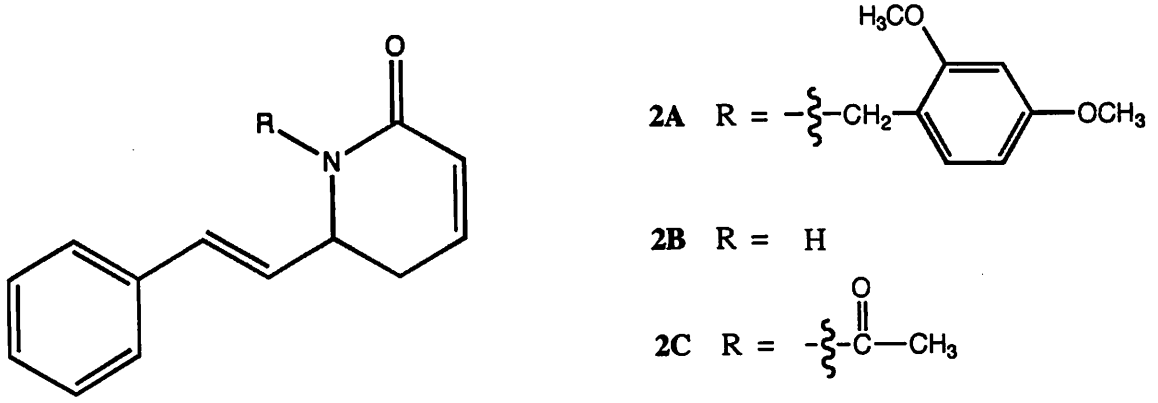
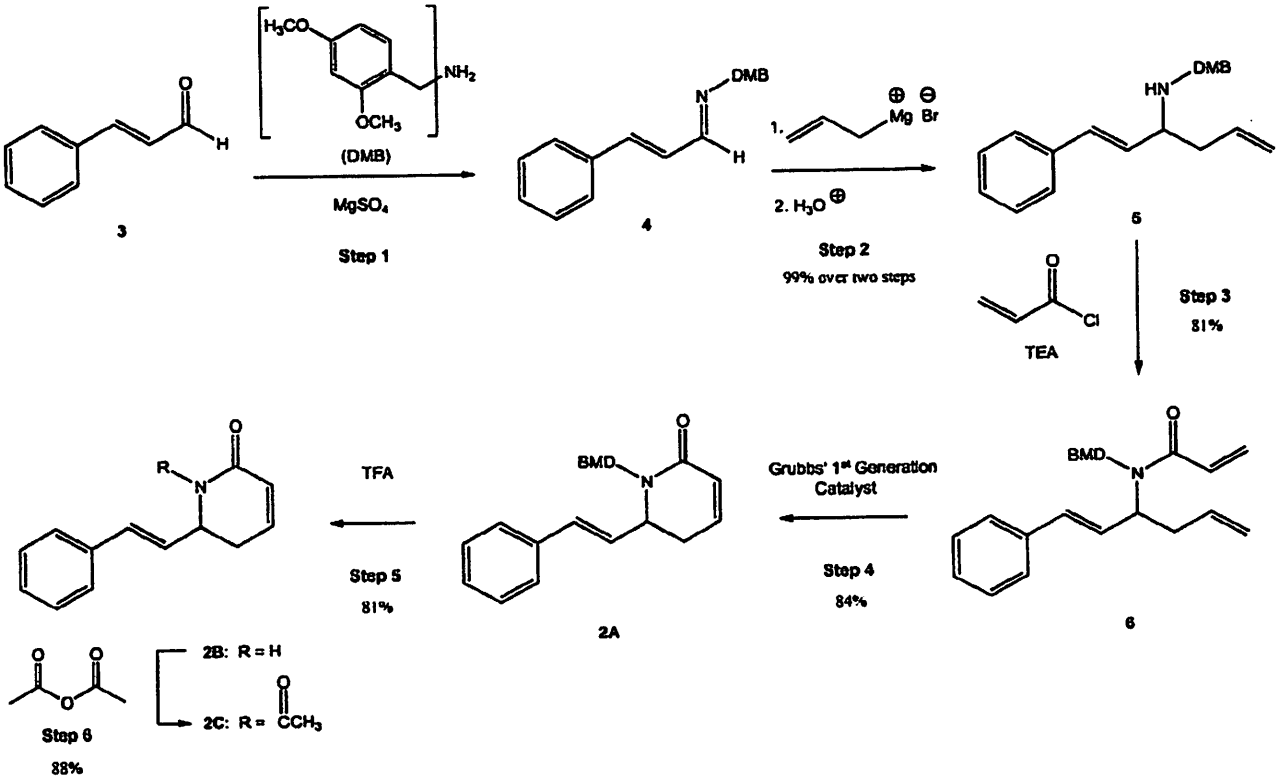


Figure 2. Novel goniothalaminanalogues.

control the drug's solubility. The compound's solubility can be controlled by incorporating lipophilic or hydrophilic substituents onto the nitrogen (10). A third benefit of the nitrogen substituents is that they can be used to alter the electronics of the ring system (11). Addition of different groups on the nitrogen, for example electron withdrawing substituents, should allow for the reactivity of the ring to be manipulated. Because of the electron withdrawing ability of the N-acetyl substituent, it is anticipated that derivative **[2C]** will exhibit reactivity more similar to the natural product lactone structure than analogues **[2A]** and **[2B]**.

The synthetic sequence used to prepare goniothalamin analogues **[2A-2C]** is shown below in Scheme 1. Lactam **[2A]** was synthesized in four steps from commercially available *trans*-cinnamaldehyde (3) and 2,4-dimethoxybenzylamine. The key step in the sequence was a Grubbs ring-closing metathesis reaction to construct the lactam ring system. Once prepared, lactam **[2A]** was converted into analogues **[2B]** and **[2C]** via established procedures (12,13).



Scheme 1. Synthetic sequence used to prepare goniothalamin analogues.

Experimental

Column chromatography was performed on 230-400 mesh silica gel. NMR spectra (^{1}H NMR at 300 MHz and ^{13}C NMR at 75 MHz) were recorded on a Jeol 300 MHz NMR spectrometer with $CDCl_3$ solvent. All starting chemicals were purchased from Aldrich and used as received.

Steps 1 and 2: Secondary Amine [5]

Trans-cinnamaldehyde (1.00 mL, 7.94 mmoles) and dry magnesium sulfate (7.65 g, 63.5 mmoles) were placed in diethyl ether (50 mL) and stirred at 0 °C (14). 2,4-Dimethoxybenzylamine (1.25 mL, 8.34 mmoles) was added to the suspension and the resulting mixture was stirred at 0 °C for two hours. After two hours, filtration and concentration of the mixture provided a residue that was dissolved in tetrahydrofuran (35 mL) and cooled to -40 °C (15). The resulting solution was treated with allylmagnesium bromide in ether (16 mL, 1M solution) and stirred overnight (18 hours) at room temperature. The mixture was then quenched with saturated ammonium chloride, extracted with diethyl ether, and concentrated under vacuum. The light yellow oil was purified via flash chromatography (ethyl acetate) to provide the desired secondary amine **[5]** as a clear oil (2.53 g, 99%): **^{1}H NMR** (300 MHz) δ 7.45-7.20 (m, 5H), 7.11 (d, J = 8.2 Hz, 1H), 6.51 (d, J = 15.9 Hz, 1H), 6.46 (d, J = 2.2 Hz, 1H), 6.43 (dd, J = 8.2, 2.2 Hz, 1H), 6.12 (dd, J = 15.8, 7.9 Hz, 1H), 5.84-5.67 (m, 1H), 5.17-5.04 (m, 2H), 3.85 (d, J = 13.3 Hz, 1H), 3.80 (s, 6H), 3.63 (d, J = 13.3 Hz, 1H), 3.25 (dt, J = 7.4, 7.4 Hz, 1H), 2.38-2.27 (m, 2H); **^{13}C NMR** δ 160.2, 158.9, 137.3, 135.3, 132.9, 131.2, 130.7, 128.6 (2C), 127.4, 126.4 (2C), 120.8, 117.5, 103.7, 98.7, 59.1, 55.4, 55.3, 46.8, 40.7.

Step 3: Amide [6]

Secondary amine **[5]** (2.53 g, 7.83 mmoles) and triethylamine (3.27 mL, 23.5 mmoles) were dissolved in THF (50 mL) and placed in an ice bath (14). Acryloyl chloride (1.27 mL, 15.6 mmoles) was added dropwise and the resulting mixture was stirred overnight (18 hours) at room temperature. The reaction was quenched with saturated ammonium chloride, extracted with diethyl ether, and concentrated under vacuum. Purification by column chromatography (50:50 ethyl acetate:hexanes) afforded amide **[6]** as a yellow oil (2.41 g, 81%): **^{1}H NMR** (300 MHz) δ 7.29 -7.17 (m, 5H), 7.04 (d, J = 8.3 Hz, 1H), 6.46 (d, J = 16.2 Hz, 1H), 6.43-6.38 (m, 2 H), 6.18 (dd, J = 15.9, 7.4 Hz, 1H), 5.84-5.64 (m, 2H), 5.60 (dd, J = 6.9, 5.5 Hz, 1H), 5.27-5.17 (m, 1H), 5.13-4.99 (m, 2H), 4.47 (s, 2H), 3.78 (m, 1H), 3.77 (s, 6H), 2.63-2.42 (m, 2H); **^{13}C NMR** δ 167.2, 160.3, 157.5, 137.0, 135.0, 132.5, 128.9, 128.5 (2C), 128.4, 128.2, 128.0, 127.6, 126.5 (2C), 118.5, 117.4, 103.8, 98.4, 57.0, 55.5, 55.3, 43.5, 36.9.

Step 4: DMB-Protected Lactam [2A]

Grubbs' ruthenium catalyst (0.214 g, 0.252 mmoles) in dicholoromethane (17 mL) was slowly added dropwise to a boiling solution of amide **[6]** (0.964 g, 2.52mmoles) in CH_2Cl_2 (400 mL) (14). The resulting mixture was stirred for two hours at reflux and then cooled to room temperature. DMSO (0.93 mL) was then added, and the solution was stirred overnight (18 hours). The crude product was concentrated under vacuum and purification by column chromatography (50:50 ethyl acetate:hexanes) provided the desired lactam **[2A]** as a purple solid (1.19 g, 84%): **^{1}H NMR** (300 MHz) δ 7.35-7.21 (m, 6H), 6.50-6.35 (m, 4H), 6.20 (dd, J = 15.8, 7.1 Hz, 1H), 6.06 (dd, J = 9.9, 2.5 Hz, 1H), 5.12 (d, J = 14.9 Hz, 1H), 4.20-4.12 (m, 1H), 4.05 (d, J = 14.9 Hz, 1H), 3.79 (s, 3H), 3.77 (s, 3H), 2.74 (dddd, J = 17.8, 7.4, 2.7, 2.7 Hz, 1H), 2.31 (ddd, J = 17.8, 6.0, 1.7 Hz, 1H); **^{13}C NMR** δ 164.2, 160.3, 158.7, 136.8, 136.3, 131.8, 130.5, 128.7 (2C), 128.0, 127.1, 126.5 (2C), 125.4, 118.4, 104.3, 98.5, 56.8, 55.5, 55.4, 42.3, 30.1.

Step 5: Lactam [2B]

Trifluoroacetic acid (3.2 mL) was added to the dimethoxybenzyl-protected lactam **[2A]** (0.732 g, 2.05 mmoles) in a flame-dried round bottom flask equipped with a reflux condenser (12). The solution was immediately heated to 70°C for 10 minutes and the resulting solution was then concentrated under vacuum. Purification by column chromatography (ethyl acetate) afforded the deprotected lactam **[2B]** as a white solid (0.543 g, 81%): **^{1}H NMR** (300 MHz) δ 7.38-7.20 (m, 5H), 6.65 (br s, 1H), 6.55 (d, J = 15.9 Hz, 1H), 6.57-6.49 (m, 1H), 6.15 (dd, J = 15.8, 7.3 Hz, 1H), 5.92 (ddd, J = 9.9, 3.7, 1.9 Hz, 1H), 4.26 (dt, J = 7.7, 7.7 Hz, 1H), 2.56-2.43 (m, 1H), 2.31 (dddd, J = 17.7, 9.2, 3.6, 2.5 Hz, 1H); **^{13}C NMR** δ 166.7, 140.6, 136.1, 132.3, 128.7 (2C), 128.3, 128.2, 126.7 (2C), 124.4, 53.3, 30.3.

Step 6: N-Acetyl Lactam [2C]

DMAP (0.37 mL, 0.30 mmoles), triethylamine

(0.042 mL, 0.30 mmoles), acetic anhydride (0.047 mL, 0.50 mmoles), and acetonitrile (2 mL) were added to lactam **[2B]** (0.050g, 0.25 mmoles) (13). The reaction was heated at reflux for 5.5 hours. An extraction was performed using diethyl ether and brine. Purification by column chromatography (ethyl acetate) provided the desired *N*-acetyl lactam **[2C]** as a light yellow solid (0.0623 g, 88%): **^{1}H NMR** (300 MHz) δ 7.34-7.18 (m, 5H), 6.81-6.73 (m, 1H), 6.47 (dd, J = 15.8, 1.1 Hz, 1H), 6.17 (dd, J = 15.9, 6.3 Hz, 1H), 6.03 (dd, J = 9.8, 2.5 Hz, 1H), 5.65-5.57 (m, 1H), 2.89-2.77 (m, 1H), 2.59 (s, 3H), 2.58-2.46 (m, 1H); ^{13}C NMR δ 172.9, 165.0, 142.5, 136.2, 132.2, 128.6 (2C), 128.0, 126.7, 126.6 (2C), 125.9, 52.1, 29.8, 27.6.

Results and Discussion

Compounds **[2A]**, **[2B]**, and **[2C]** were prepared using a linear, six step, synthetic sequence featuring a Grubbs ring-closing olefin metathesis reaction. The initial starting materials were commercially available *trans*-cinnamaldehyde and 2,4-dimethoxybenzylamine. The dimethoxybenzylamine was chosen as a starting material after careful investigation of the literature. Wiemer and co-workers found that in a very similar system, a nitrogen protected with a *para*-methoxybenzyl group could not be deprotected under typical conditions (14). There was literature precedence, however, that showed that an *N*-dimethoxybenzyl protecting group could be removed under acidic conditions (12). Because of these references, a fairly uncommon, 2,4-dimethoxybenzyl protecting group was chosen for the synthesis. Upon treatment with 2,4-dimethoxybenzylamine, *trans*-cinnamaldehyde afforded crude imine **[4]** which was immediately treated with allylmagnesium bromide. Purification of the resulting reaction mixture afforded secondary amine **[5]** in 99% yield over the two steps. Treatment of amine **[5]** with acryloyl chloride and triethylamine provided the desired amide **[6]** as a yellow oil. At this point, an intramolecular olefin metathesis reaction was carried out using Grubbs' first generation ruthenium catalyst. The two terminal double bonds in amide **[6]** were closed to form the first target, lactam **[2A]**, in good yield. The dimethoxybenzyl protected lactam was then treated with trifluoroacetic acid to afford the unprotected lactam **[2B]** in 81% yield. Initial treatment of lactam **[2B]** with acetyl chloride and triethylamine provided poor conversion to the *N*-acetyl lactam **[2C]**. Large amounts of starting material were observed after extensive reaction times. A procedure was then found in the literature which demonstrated that acetic anhydride was much more reactive than acetyl chloride when used with 4-dimethylaminopyridine (DMAP) (13). Subsequent use of these reaction conditions provided the desired product **[2C]** in 88% yield. The entire six step sequence was accomplished in a 48% overall yield, resulting in the synthesis of three goniothalamin analogues with potential for chemotherapeutic activity.

Conclusion

With all three goniothalamin derivatives in hand, the racemic compounds will be assayed for bioactivity against breast cancer cell lines. Lactam ring systems have been shown to exhibit potent cytotoxicity in other natural products, (16,17) so there is the potential that these analogues will be just as active as goniothalamin itself. If this is the case, as discussed previously, these lactam analogues will be superior to goniothalamin. They will have increased stability, and by modifying the nitrogen substituents, chemists will have the potential to manipulate both the compound's bioavalibilty and reactivity.

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