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Synthesis of γ-Lactam Analogues of Combretastatin A4

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Abstract

This research examines the synthesis of γ-lactam analogues of combretastatin A4 (CA4). Many anticancer drugs including CA4 lacking great specificity pose a major drawback; equal risk to non-malignant cells as cancerous cells. β-Lactam moieties have been explored by other research groups, as appropriate substitutes for the ethylene bridge in CA4 to compromise cytotoxicity to normal cells. Despite their likeness, no groups have reported successful syntheses for compounds that utilize γ-lactams in the way β-lactams have been used in this research. The integrity of the ring system in γ-lactams may prove to give more stable, less synthetically vulnerable compounds that maintain proper tubulin binding features of CA4.The synthetic route explored to access CA4 derivatives of this variety utilizes β-lactams as precursors. The N1-C4 bond in a β-lactam may be cleaved with lithium diisopropylamide (LDA) to allow a ring expansion process affording γ-lactams. To prepare the necessary β-lactam precursors, specific ketenes, generated from their respective acid chlorides, and imines were used in a Staudinger [2+2] cycloaddition. The synthesis of imines that can generate these γ-lactam derivatives via passage through the β-lactam intermediates has received much attention. To date, success has been found in preparing these imines, while attention has recently shifted towards the process of preparing the β-lactams. β-Lactam forming reactions have proven to give complex mixtures that cannot be easily identified prior to isolation. Despite this fact, the Staudinger [2+2] cycloaddition followed by the ring expansion still appears to be a viable approach to access the γ-lactam analogues of CA4, especially as encouraging results regarding the potential transformation of a doubly trimethoxy substituted imine into a β-lactam have been found.

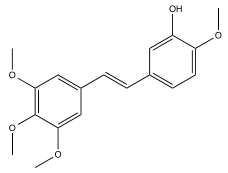
1. Introduction

1.1 Tubulin Targeting Agents and Combretastatin A4

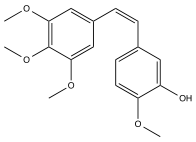
The design of drugs that prevent or discourage the proliferation of cancer cells, via the disruption of microtubule activity, has been the aim of many research groups.[1 2 3 4 5] Tubulin is a protein which composes microtubules that are a major cytoskeletal feature in eukaryotic cells. Furthermore tubulin is an α, β heterodimeric protein which harbors convenient active sites in its subunits, such as the colchicine domain.[1] α tubulin and β tubulin are two proteins coded by separate genes that spontaneously bind to form the αβ heterodimer, which arranges themselves into protofilaments which further arrange themselves into microtubules. Microtubules are essential to cell activities such as transport and, namely, growth and division.Two main classes of drugs are known to disrupt microtubule activity, both have unique sub-strategies. Paclitaxel and epothilone of the vinca alkaloid class have been found to prevent the disassembly of tubulin, while drugs such as colchicine and combretastatin A4 (CA4) prevent tubulin from polymerization. Of concern in this research is advancement of the latter variety of drugs, those like CA4 and colchicine. Drugs resembling CA4 and or colchicine are shown to bind specifically in the colchicine domain. Specifically, CA4 is shown to target colon, lung, and leukemia cancers.[6] In many drugs like colchicine and CA4,

neovasculature resistance is shown close to the maximum tolerated dose, aside from CA4 itself.[7] Because of this fact, CA4 bears great potential to suppress neovasculature while causing minimal detriment to the subject treated. The potential of CA4 is great, however, many drawbacks are present which hinder CA4 from being an effective long term fix for cancers, these have included and are not limited to low specificity, low water solubility, and low energetic stability.

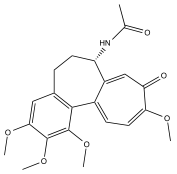
CA4 is a compound consisting of three elements; a 3,4,5-trimethoxybenzene as the A ring, and a 3-methoxy-4-hydroxybenzene as the B ring, joined by the ethylene bridge. It is critical that in CA4, the two rings be in the cis conformation as the *trans* is biologically inactive and yields no notable therapeutic properties. Ultimately CA4 stands as an ideal scaffold for synthesizing an arsenal of tubulin targeting compounds. These are those compounds which attempt to mimic the action of CA4 by salvaging the key features attributed to its binding capabilities, such as the 3,4,5-trimethoxybenzene moiety, and the coveted arrangement of the A and B rings relative to one another. The CA4 derivatives synthesized to date are numerous and growing. Colchicine and both the *cis* and *trans* conformations of CA4 are displayed below in figure 1.1.



1. (*trans*)-CA4



2. (*cis*)-CA4



3. Colchicine

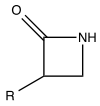
Figure 1.1 Colchicine (1), *cis* Combretastatin A4, (2), *trans* Combretastatin A4 (3)

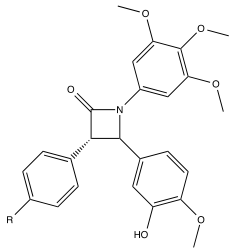
1.2 β-Lactam Analogues of Combretastatin A4

The four membered cyclic amides, β-lactams, were once almost exclusively recognized in antibiotic medications (e.g. amoxicillin, penicillin, carbapenem). It is known that, in penicillins, the β-lactam region irreversibly binds the active site of a transpeptidase enzyme (a bacterial enzyme that forms cross-links in peptidoglycan cell walls), upon interaction with a key serine residue.[8]

In recent years β-lactams have received attention for having roles in anticancer drugs related to CA4, in part, due to their safety profile.[9] In β-lactam CA4 analogues, the role of the β-lactam is not to interact with the surrounding amino acid residues in the colchicine site, in this way the use of β-lactams is unrelated to their antibiotic mechanism. However the need is great for anticancer agents with higher cytotoxic effects in cancerous cell and lower cytotoxic effects in normal cells; as β-lactams have been used in many drugs in the past without resulting in harm to normal cells they bear the potential to accommodate the need for such anticancer drugs.[10]

In CA4 a structural dilemma coincides with the need to incorporate well understood, and favorable, moieties into its structure, this issue concerns stabilization of the *cis* over the *trans* conformation which points towards replacement of the standard ethylene bridge with other structures; β-lactams fit the profile sought to achieve a structure that is more friendly to non-malignant cells while maintaining proper tubulin binding activity. As a substitute for the ethylene bridge in CA4, β-lactams have been shown to maintain tubulin binding activity precisely because they facilitate a similar spatial arrangement of the A and B as the ethylene bridge. Figure 1.2 below shows the general structures for a β-lactam and a β-lactam CA4 analogue respectively.





4. General form of β-lactam. 5. β-lactam analogue of CA4.

Figure 1.2. General structure for β-lactam (2-azetidinone) **(4),** and a CA4 β-lactam analogue **(5).**[1]

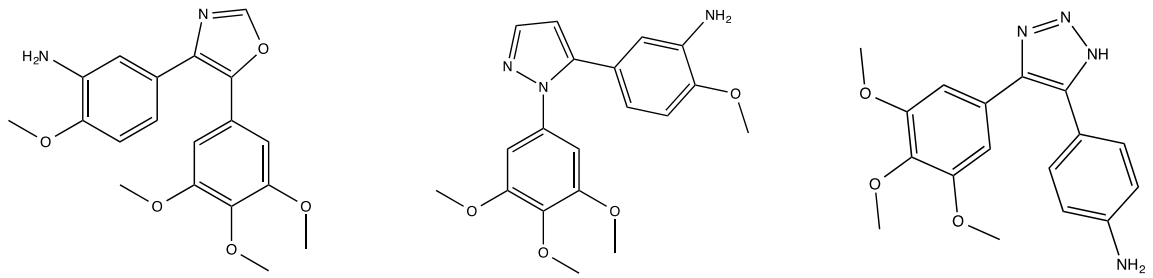
The O'Boyle group is one group that has published studies on derivatives such as **5**, including syntheses, docking studies, and assays on certain cell lines including MCF-7 breast cancer cell lines. One study of the compounds synthesized involve the use of various five membered heterocycles at the 3 position on the β-lactam, such as the thienyl group seen in **5**, and another investigates various other substituted phenyl groups attached to the 3 position of the β-lactam. In a more recent paper by O'Boyle a wider variety of β-lactam CA4 analogues were accessed via Reformatsky and Staudinger reactions. The group concluded that there was a trend for potency in small heterocyclic systems. The compounds containing the phenyl groups as the R group showed highly significant activity in human breast cancer MCF-7 and MDA-MB-231 cell lines, showing subnanomolar activity in the MCF-7 cell line.[2] The focus of this project is to expand upon previous β-lactam research by exploring syntheses of γ-lactam analogues of CA4. The approach of interest will require passage through β-lactam compounds as precursors.

1.3 γ-Lactam Analogues of Combretastatin A4

γ-Lactams are structural moieties well understood in medicine, they are attractive targets for organic chemists because of their wide presence in nature, and great breadth of biological activities.[11] Like β-lactams, γ-lactams have a good record for posing little harm to normal cells, additionally they have the potential to facilitate a similar spatial arrangement between two aryl rings as seen between N1 and C4 of a β-lactam. This means that γ-lactams have a good chance at combating cancers while causing minimal harm to the subject. This fact makes this family of compounds an ideal candidate for replacement of the ethylene bridge in CA4.

One publication separately concluded that five membered rings in place of the ethylene bridge bear a good record for maintaining both cytotoxic and antitubulin qualities, to name a few this includes imidazole, 1,3-oxazole, pyrazole, and triazole groups.[7] γ-Lactams also contain an extra carbon, which may help to secure a *cis* conformation in the CA4 γ-lactam analogue by sterically blocking the formation of the otherwise favorable *trans* conformation, and resulting in a less strained and more stable ring system providing the linkage. As a bonus, the extra available position on the γ-lactam may permit further research of different substituents that could be essential in tackling advanced pharmacokinetic issues.

Investigation of γ-lactams in anticancer agents, especially related to CA4, has been limited; the inspiration for this project is derived almost directly from the research carried out on the anticancer potential of β-lactams. Syntheses for structures that utilize γ-lactams as a substitute for the double bond seen in CA4 have not previously been established, this is despite the fact that many other types of five membered heterocyclic analogues have received attention, such as 1,3 oxazole, pyrazole, and triazole.[7] Figure 1.3 below shows the structures of these compounds in manners they have been applied.



6. 1,3 oxazole 7. Pyrazole 8. triazole

Figure 1.3. Heterocycles previously investigated as substitutes for the ethylene bridge in CA4. 1,3 oxazole, 6 pyrazole 7, and triazole 8.

Apart from anticancer agents γ-lactams and their syntheses have been investigated widely, one publication suggests a ring expansion of β-lactams to access the γ-lactams is possible by cleavage of the N1-C4 bond with a strong base. This reaction uses lithium diisopropylamide (LDA) in a THF solution at room temperature.[12] Figure 1.4 below shows the proposed mechanism. LDA does not attack the most acidic hydrogen as expected; the attack of the carbon located between the phenyl group and nitrogen by the C4 lone pair has been attributed to the anomeric effect.

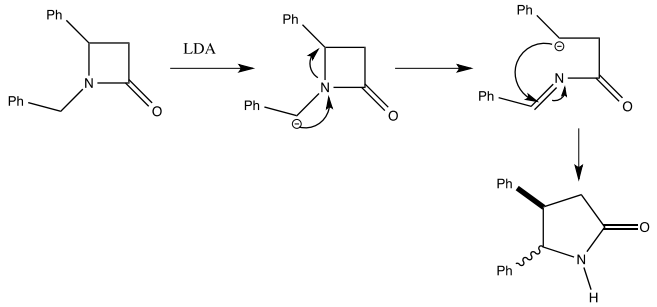


Figure 1.4. Proposed mechanism for final step, conversion of β-lactam to γ-lactam.[13]

2 Experimental

2.1 General Preparation of Imines

The appropriate benzylamine (10mmol) was added to the appropriate benzaldehyde (10mmol) in ethanol (50mL). The solution was refluxed for 3 hours under nitrogen at 78 ºC. The resulting solution was allowed to cool prior to being reduced in vacuo.[1]

2.1.1 (1a) Preparation of (N-benzyl-1-phenylmethanimine)

The general procedure for synthesis of imines was used, using benzylamine and benzaldehyde. Gained in 99% crude yield, yellow oil. ^{1}H NMR δ (400 MHz; $CDCl_3$), 4.8 (2H, s), 7.1-3 (9H, m), 7.8 (1H, m), 8.4 (1H, s).

2.1.2 (1b) Preparation of (N-(3,4,5-trimethoxybenzyl)-1-(3,4,5-trimethoxyphenyl) methanimine

The general procedure for synthesis of imines was used, using 3,4,5 trimethoxybenzylamine, and 3,4,5 trimethoxybenzaldehyde. Gained in 89% yield, off white powder. ^{1}H NMR δ (400 MHz;$CDCl_3$), 3.85 (4H,s,OMe), 3.87 (8H,s,OMe), 3.90 (4H,s,OMe), 3.92 (8H,s,OMe), 4.7 (2H,s), 6.5 (2H,s,arom.), 6.9 (2H,s,arom.), 8.2 (1H,s).

2.1.3 (1c) Preparation of N-(3,4-dimethoxybenzyl)-1-phenylmethanimine

The general procedure for synthesis of imines was used. Gained in 67% crude yield, oily opaque tan mixture. ^{1}H NMR δ (400 MHz;$CDCl_3$), 3.81 (3H,s,OMe), 3.82 (3H,s,OMe), 4.75 (2H,d), 6.85 (2H,m,arom.), 7.4 (2H,d,arom.), 7.5 (1H,t,arom.), 7.6 (1H,t,arom.), 7.9 (1H,d,arom.).

2.1.4 (1d) Preparation of N-(4-methoxybenzyl)-1-phenylmethanimine

The general procedure for synthesis of imines was used. Gained in >100% crude yield, yellow oil. Purification methods to be determined. ^{1}H NMR δ (400 MHz;CDCl3), 3.8(3H,s,OMe), 4.8(2H,s), 6.9(2H,d, arom.), 7.3(2H,d, arom.), 7.4(2H,t,arom.), 7.4(1H,t, arom.), 7.8(2H,d,arom.), 8.4(1H,s).

2.1.5 (1f) Preparation of 5-((benzylideneamino) methyl)-2-methoxyphenol

The general procedure for synthesis of imines was used. Gained in >100% crude yield, brown powder. Purification methods are to be determined.

2.2 General Preparation of β-Lactams

The appropriate imine (5mmol) and triethylamine (15mmol) were added to dry CH_2Cl_2 (50mL), the mixture was brought to reflux at 60 ºC. The appropriate acyl chloride was added drop wise to mixture via a septum. This mixture was refluxed for 3 hours, then cooled and washed with distilled water (2x50mL) then with saturated aqueous sodium bicarbonate solution (50mL). The organic layer was dried by filtration through anhydrous sodium sulfate. The organic layer was collected and reduced in vacuo.[1]

2.2.1 (2a) Preparation of 1-benzyl-3-methyl-3,4-diphenylazetidin-2-one

The general procedure for synthesis of β-lactams was used, using 1a as the imine. 2-phenylacetyl chloride was made using thionyl chloride and phenyl acetic acid, this was used as the acid chloride. A red and yellow oil was obtained, ^{1}HNMR indicated the presence of a mixture, but nothing of a β-lactam compound.

2.2.2 (2b) Preparation of 1-benzyl-3,3,4-triphenylazetidin-2-one

The general procedure for synthesis of β-lactams was used, using 1a as the imine, and diphenylacetyl chloride as the acid chloride. Column chromatography in 70% hexane/ 30% ethyl acetate was carried out over the mixture to separate it into two components. Further separation is required before evaluation of the material can take place.

2.2.3 (2c) Preparation of 3,3-diphenyl-1-(3,4,5-trimethoxybenzyl)-4-(3,4,5-trimethoxyphenyl)azetidin-2-one

The general procedure for synthesis of β-lactams was used, using 1b as the imine, and diphenylacetyl chloride as the acid chloride. A red oil was conceived and worked up. Proceeding work up, a white solid crystallized directly out of the mixture, this white solid was extracted using warm methanol and filtered on a coarse fritted funnel. The solid was collected and the rest of the solution, in methanol, was placed in the refrigerator for approximately a week and a half to separate more of the white solid from solution. The liquid in methanol was later reduced *in vacuo*, and determined to be a mixture of compounds via ^{1}HNMR. Further separation must take place to identify the individual components.

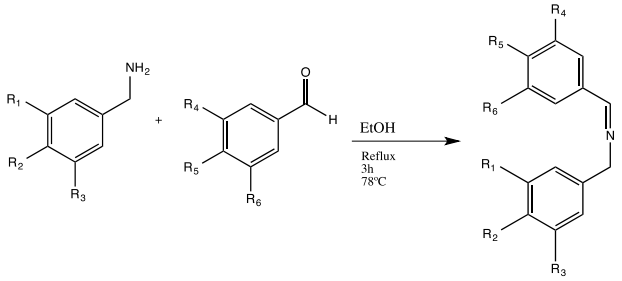
3 Results/ Discussion

The synthetic scheme to access γ-lactam analogues of CA4 consists of three steps. These steps include synthesis of imine, synthesis of β-lactam, and synthesis of γ-lactam. The success to date has been on synthesis of a variety of imines, those with an extra carbon inserted between the nitrogen and a phenyl group, which can be subjected to the

Staudinger [2+2] cycloaddition. Two attempts to synthesize β-lactams from imine precursors have been carried out. Attempts to isolate the components of the resulting mixtures of these reactions, to allow further evaluation, are currently underway.

3.1 Synthesis of Iminesrjdc nngxdgn

For the first step of this project, synthesis of the unique imines, with one extra carbon between the nitrogen and respective substituted phenyl group, was performed. This was effectively carried out as a condensation reaction, which was performed in five different scenarios and confirmed for three, in reasonable to high yields. Uniquely substituted benzaldehydes and benzylamines were the necessary reactants. **1a** and **1b** were synthesized with yields between 90-100%. The general form of the reaction is displayed in figure 3.1. **1c** was afforded a significantly lower yield than expected, of 67%.



1a: $R_1=R_2=R_3=R_4=R_5=R_6=H$
1b: $R_1=R_2=R_3=R_4=R_5=R_6=OCH_3$
1c: $R_1=R_2=OCH_3$, $R_3=H$, $R_4=R_5=R_6=H$
1d: $R_1=R_3=H$, $R_2=OCH_3$, $R_4=R_5=R_6=H$
1f: $R_1=H$, $R_2=OCH_3$ $R_3=OH$, $R4=R_5=R_6=H$

Figure 3.1. Displayed above is the general form of the first step in the synthetic scheme, the synthesis of imines.

3.2 Synthesis of β-lactam (Staudinger [2+2] Cycloaddition)

The Proposed mechanism of the formation of a β-lactam in the Staudinger [2+2] cycloaddition is shown in figure 3.2 below.[14] Imines and ketenes are both families of molecules that can act as nucleophiles or electrophiles, however in the Staudinger [2+2] cycloaddition the ketene undergoes nucleophilic attack by the imine and the following cycloaddition yields a β-lactam. The ketene is formed *in situ* as the product of the reactions between triethylamine, ethanol, and the acid chloride in use.

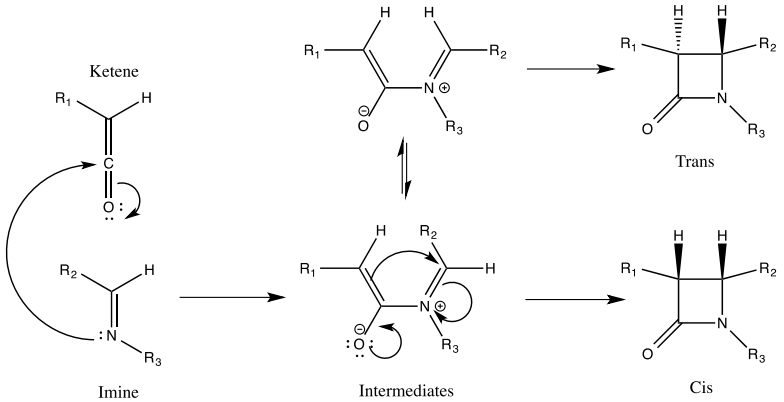
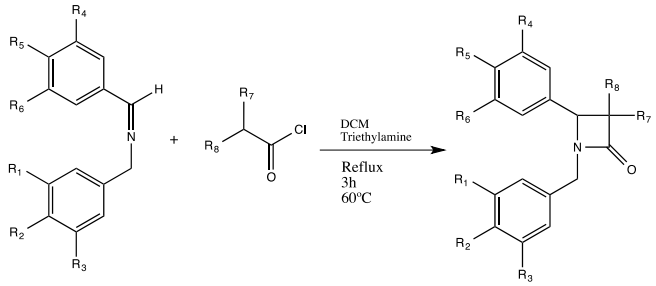


Figure 3.2. Proposed mechanism of β-lactam formation Staudinger [2+2] Cycloaddition.

The Staudinger [2+2] cycloaddition method of synthesizing β-lactams is common and reliable. This requires the purified products from step 1 to be utilized as a reactant. No β-lactams of interest have successfully been synthesized and evaluated for this project to date. Based on the proposed mechanism there is no reason to suspect that the extra carbon in the imine should interfere with the formation of the ring; Figure 3.3 below shows the general form of the reaction.



2a: $R_1=R_2=R_3=R_4=R_5=R_6=H$, $R_7=H$ $R_8=ph$
2b: $R_1=R_2=R_3=R_4=R_5=R_6=H$, $R_7=R_8=ph$
2c: $R_1=R_2=R_3=R_4=R_5=R_6=OCH_3$, $R_7=R_8=ph$

Figure 3.3. The general form of the ring opening reaction that gives rise to β-lactams is shown above.

For **2a** a mixture of yellow and red oil was obtained, ^{1}HNMR data showed that starting material **1a** was largely present, so this reaction was not observed to afford any useful product. After suspecting that the reactivity of the reactants were hindered as a result of potentially impure acid chloride, we commercially obtained these reagents. From this point forward diphenylacetyl chloride was the acid chloride used in preparing these products. The attempt to carry out the reaction forming **2b** used **1a** as the imine, multiple products were determined to be in this mixture. Efforts to isolate and identify the components of the mixture are still underway. **2c** is still being isolated and identified as is **2b** however, a white product with similar polarity and appearance to the starting imine **1b** crystallized out of solution after work-up. The ^{1}HNMR evidence for this product contradicted suspicion that this was **1b**, ^{1}HNMR does not indicate it is the β-lactam product either. Further evaluation of these compounds must take place.

4 Conclusion

The condensation reactions performed by O'Boyle, utilizing substituted anilines and benzaldehydes to synthesize imines can, in fact, be expanded to benzylamines and benzaldehydes with reasonable to high yield in four of the cases studied. This approach provided a tool box of different imines, some of which may eventually afford a γ-lactam analogue of CA4. Based on the understood mechanism of the Staudinger [2+2] cycloaddition[14], there is no reason to suspect these newly investigated imines are less reactive with their ketenes than the imines in O'Boyle's work. However, no success has been claimed in the preparation of the β-lactams.

Currently, we can claim that the approach taken to synthesize imines necessary for the proceeding steps in this project is sound. A number of imines that can be subjected to the next two steps in the overall scheme have been synthesized. The focus at this stage has shifted from the pursuit of imines, to the pursuit of the desired β-lactams. The synthesis of these β-lactams, if successful, will pave the way for the novel step of this synthetic scheme, that leading to γ-lactam CA4 analogues.

5 Acknowledgements

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