Background

Mitomycin C

A brief history of Mitomycin C

- Discovered in the early 1950’s and the subject of extensive research.
- Still used today to treat hypoxic cancers, such as breast, colorectal, gastric, bladder, lung, head, neck, and non-small cell lung cancers.
- Has harmful side effects resulting from oxygen radicals.

C7 Ethyl aziridinomitosene

Similarities of MCs and AZMs

- Have same basic 6-5-5-3 ring structure including the unique aziridine ring for which they are named.
- Both create DNA monoadducts and interstrand cross links (ICLs) resulting in programmed cell death.
- ICLs are what give these compounds their cancer fighting ability.

What is an interstrand cross link?

- MC or AZMs bind to DNA and prevent replication.
- Triggers apoptosis.
- Only occur at 5’CG base pairs.
- Efficient: 1 ICL per 20,000 base pairs results in cell death.

The mechanisms of MC vs AZMs

- MC reduction and cross link.
- AZM cross link.

- MC must be reduced before it can form an interstrand cross link.
- Harmful oxygen radicals can form from resulting intermediate.
- AZMs do not require reduction step to form cross link DNA.

Abstract

This project aims to synthesize a C7-ethyl modified aziridinomitosene (AZM) for future studies of its anticancer and antibiotic activity. The origin of the project stems from the toxic anti-cancer drug mitomycin C (MC), which is the structural model for C7-ethyl analog. Previous studies involving related AZMs and MC indicate that the source of the AZMs’ anti-cancer properties originates from their ability to form interstrand DNA crosslinks that lead to programmed cell death. The C7-ethyl analog is fabricated by means of a twenty step sequence that proves challenging as a result of early formation of a sensitive aziridine ring. Thus far, the synthesis has progressed to the methyl addition to the aziridine ring. The methylated aziridine product is the immediate precursor the addition of the iodide, after which the tertbutyl silane protecting group will be removed. Ultimately, this synthesis will produce a compound that will be tested for anticancer and antibiotic activity and, ideally, a more potent analog will be derived.

Figure 1. Effect of thiol on the percentage of DNA ICLs in various AZMs

Potency of AZM variants against Lukemia cancer cells

In isolated DNA the AZM with two nucleophlic binding sites yielded the highest percentage of crosslinks. The AZM with a single nucleophlic binding site also had considerable ICL yields.

In contrast to experiments with purified DNA, the monosubstituted AZM is more potent than the unsubstituted AZM in cellular trials.

Why synthesize a C7 ethyl AZM?

Answer: success of the methyl AZMs against cancer cells!

Predictions as to why the methylated AZM worked better than other AZMs

- More hindered than the AZM with two nucleophilic sites.
- More hydrophobic; greasy (easier to get past the cell membrane).

We are synthesizing the ethyl for similar reasons

- Easier passive transport across the cell membrane.
- A longer carbon chain should further increase membrane permeability.
- Increased steric hindrance will mitigate reactivity even more than in methyl AZMs.

Synthesis of C7 ethyl AZM

Future work

- Complete final steps of the synthesis.
- Cyclize remaining rings and add carbamate group.
- Pass on for biological analysis.

Acknowledgements

The project described was supported by NSF REU Grant No. CHE-1005159. The support of Dr. Don Warner, Dr. Eric Brown, and labmates: Ryan Reeves, Pete Barns, Jennifer Tanimoto, Kayla Johnson, Chloe Lombard, Chris Mallory, and the BSU 2013 chemistry REU students is greatly appreciated. I would also like to thank the National Science Foundation, National Institutes of Health, Boise State University, and Mount Holyoke College for their kindness and support. Finally I would like to say it’s not easy to love a scientist. Our work testers on the line between profession and obsession and can consume us, so I would like to give a special thanks to my family and friends for supporting my research efforts and loving me, even when work was all I could think or talk about.

Methyl addition to the aziridine ring

Signature peaks identified by means of chemical shift and integration on NMR.

- a: oxazole -6.8ppm
- b: aziridine methyl -2.1ppm
- c: dimethyl -0.01ppm
- d: tertbutyl -0.9ppm
- e: alkene hydrogens -6.2
- F: single alkene hydrogen -5.9

Progress to date

- More antibiotic activity and, ideally, a more potent analog will be derived.
- In contrast to experiments with purified DNA, the AZM with two nucleophilic binding sites yielded the highest percentage of crosslinks. The AZM with a single nucleophilic binding site also had considerable ICL yields.
- In isolated DNA the AZM with two nucleophilic binding sites yielded the highest percentage of crosslinks. The AZM with a single nucleophilic binding site also had considerable ICL yields.

The mechanisms of MC vs AZMs

MC reduction and cross link

AZM Cross link

MC must be reduced before it can form an interstrand cross link.
- Harmful oxygen radicals can form from resulting intermediate.
- AZMs do not require reduction step to cross link DNA.

The project described was supported by NSF REU Grant No. CHE-1005159. The support of Dr. Don Warner, Dr. Eric Brown, and labmates: Ryan Reeves, Pete Barns, Jennifer Tanimoto, Kayla Johnson, Chloe Lombard, Chris Mallory, and the BSU 2013 chemistry REU students is greatly appreciated. I would also like to thank the National Science Foundation, National Institutes of Health, Boise State University, and Mount Holyoke College for their kindness and support. Finally I would like to say it’s not easy to love a scientist. Our work testers on the line between profession and obsession and can consume us, so I would like to give a special thanks to my family and friends for supporting my research efforts and loving me, even when work was all I could think or talk about.

Synthesis of C7 ethyl AZM

Abstract

This project aims to synthesize a C7-ethyl modified aziridinomitosene (AZM) for future studies of its anticancer and antibiotic activity. The origin of the project stems from the toxic anti-cancer drug mitomycin C (MC), which is the structural model for C7-ethyl analog. Previous studies involving related AZMs and MC indicate that the source of the AZMs’ anti-cancer properties originates from their ability to form interstrand DNA crosslinks that lead to programmed cell death. The C7-ethyl analog is fabricated by means of a twenty step sequence that proves challenging as a result of early formation of a sensitive aziridine ring. Thus far, the synthesis has progressed to the methyl addition to the aziridine ring. The methylated aziridine product is the immediate precursor the addition of the iodide, after which the tertbutyl silane protecting group will be removed. Ultimately, this synthesis will produce a compound that will be tested for anticancer and antibiotic activity and, ideally, a more potent analog will be derived.

Figure 1. Effect of thiol on the percentage of DNA ICLs in various AZMs

Potency of AZM variants against Lukemia cancer cells

In isolated DNA the AZM with two nucleophlic binding sites yielded the highest percentage of crosslinks. The AZM with a single nucleophlic binding site also had considerable ICL yields.

In contrast to experiments with purified DNA, the monosubstituted AZM is more potent than the unsubstituted AZM in cellular trials.

Why synthesize a C7 ethyl AZM?

Answer: success of the methyl AZMs against cancer cells!

Predictions as to why the methylated AZM worked better than other AZMs

- More hindered than the AZM with two nucleophilic sites.
- More hydrophobic; greasy (easier to get past the cell membrane).

We are synthesizing the ethyl for similar reasons

- Easier passive transport across the cell membrane.
- A longer carbon chain should further increase membrane permeability.
- Increased steric hindrance will mitigate reactivity even more than in methyl AZMs.

Synthesis of C7 ethyl AZM

Future work

- Complete final steps of the synthesis.
- Cyclize remaining rings and add carbamate group.
- Pass on for biological analysis.

Acknowledgements

The project described was supported by NSF REU Grant No. CHE-1005159. The support of Dr. Don Warner, Dr. Eric Brown, and labmates: Ryan Reeves, Pete Barns, Jennifer Tanimoto, Kayla Johnson, Chloe Lombard, Chris Mallory, and the BSU 2013 chemistry REU students is greatly appreciated. I would also like to thank the National Science Foundation, National Institutes of Health, Boise State University, and Mount Holyoke College for their kindness and support. Finally I would like to say it’s not easy to love a scientist. Our work testers on the line between profession and obsession and can consume us, so I would like to give a special thanks to my family and friends for supporting my research efforts and loving me, even when work was all I could think or talk about.