**Template for Preparation of a Proposal – Key Elements**

**Descriptive Title**: Chiral Cyclopropylcarbinyl DNA Crosslinkers as Antitumor Agents

**Example from the Literature**:

Bifunctional nitrogen mustard agents like mechlorethamine induce cytotoxic DNA crosslinks



Reference(s):

1. Wang, Q.-Q.; Rowshan, A. B.; Daya, V. W.; Bowman-James, K. “Sulfur, oxygen, and nitrogen mustards: stability and reactivity” *Org. Biomol. Chem*. **2012**,*10*, 8786-8793

2. R.; Williams, R. M. “DNA Cross-Linking Agents as Antitumor Drugs “ *Chem. Rev.* **1998**, *98*, 2723-2796

**Simple modification** that constitutes a new idea: replace aziridinium intermediate with cyclopropylcarbinyl cation intermediate

Cyclopropyl rings also facilitiate SN1 reactions at neighboring centers, forming stabilized cyclopropylcarbinyl cations. Correctly positioning two leaving groups on a cyclopropylcarbinyl ring should facilitate crosslinking of DNA strands.



Reference(s):

Caserio, M. C.; Graham, W. H.; Roberts, J. D. “Small-ring compounds—XXIX A reinvestigation of the solvolysis of cyclopropylcarbinyl chloride in aqueous ethanol. Isomerization of cyclopropylcarbinol” *Tetrahedron* **1960**, *11*, 171-182.

**Utility of the new idea**:

• Alternative cytotoxicity profile relative to nitrogen mustards

• Potential match of alkylator C2v symmetry relative to chirality of DNA

Reference(s):

1. Brookes P.; Lawley PD. "The reaction of mono- and di-functional alkylating agents with nucleic acids". *Biochem. J.* **1961**, *80,* 496–503.

2. Saha, P.; Debnath, C.; Bérubé G. “Steroid-linked nitrogen mustards as potential anticancer therapeutics: a review.” *J. Steroid Biochem. Mol Biol.* **2013**, *37*, 271-300.

**Review of the area of utility that justifies the proposal idea**:

Nitrogen mustards were first employed as chemical warfare agents but were soon recognized as antitumor compounds. Mechlorethamine is the most primitive of this class of drugs and is still used clinically. The potent cytotoxicity of nitrogen mustards like mechlorethamine results from the ability of a compact molecular architecture to form crosslinks between opposing DNA strands through sequential formation of strained aziridinium intermediates. Much of the toxicity associated with nitrogen mustards is related to single-strand alkylation, without cross-linking, and alkylation of proteins. By matching the chirality of the the cyclopropane and funcitonalizing the geminal dimethyl groups, better selectivity might be achieved.

Reference(s):

Einhorn J. “Nitrogen mustard: the origin of chemotherapy for cancer.” *Int. J. Radiat. Oncol. Biol. Phys.* **1985**, *11*, 1375-8.

Povirk, L. F.; Shuker, D. E. “DNA damage and mutagenesis induced by nitrogen mustards.” *Mutat Res.* **1994**, *318*, 205-26.

**What chemical or biological reactions or techniques will be needed to test the idea?**

1. Synthesize both enantiomers of the compound

2. Quantify DNA crosslinking versus hydrolysis reactions with double-stranded DNA in phosphate buffered saline at 37 °C

3. Quantify DNA crosslinking versus protein alkylation with double-stranded DNA in PBS at 37 °C in the presence of a protein (bovine serum albumin).

4. Compare cytotoxicity of both enantiomers versus control using against NIH 3T3 cells using LDH assay.

Reference(s):

Osler, J. D.; Unsworth, W. P.; Taylor, R. J. K. “The Cope rearrangement of gem-dimethyl substituted divinylcyclopropanes” *Org. Biomol. Chem*. **2013**, *11*, 7587 – 7594.

Bauer, G. B.; Povirk, L. F. “A Specificity and Kinetics of Interstrand and Intrastrand Bifunctional Alkylation by Nitrogen Mustards at a G-G-C Sequence” *Nuc. Acids Res.* **1997** 25, 1211-1218.

**What is the most likely reason the idea will fail?**

• Too hydrophobic for solubility

• Insufficient selectivity for anionic DNA

Reference(s):

White, L. P. “Influence of pH on the Toxicity of Nitrogen Mustard” *Science* **1960**, *131*, 1041-1043.

**Suggest a proposed backup plan**

A cationic quaternary ammonium group or related could be appended to the gem dimethyl

A cationic quaternary ammonium group could be used as a leaving group instead of chloride.

Reference(s):

O’Cash, G. O.; Joyner, F. B.; “Crystallinity promoters” US Patent 3408341, 1968.