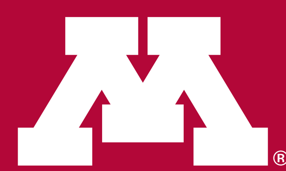

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Development of APOBEC3 Cytosine Deaminase Inhibitors

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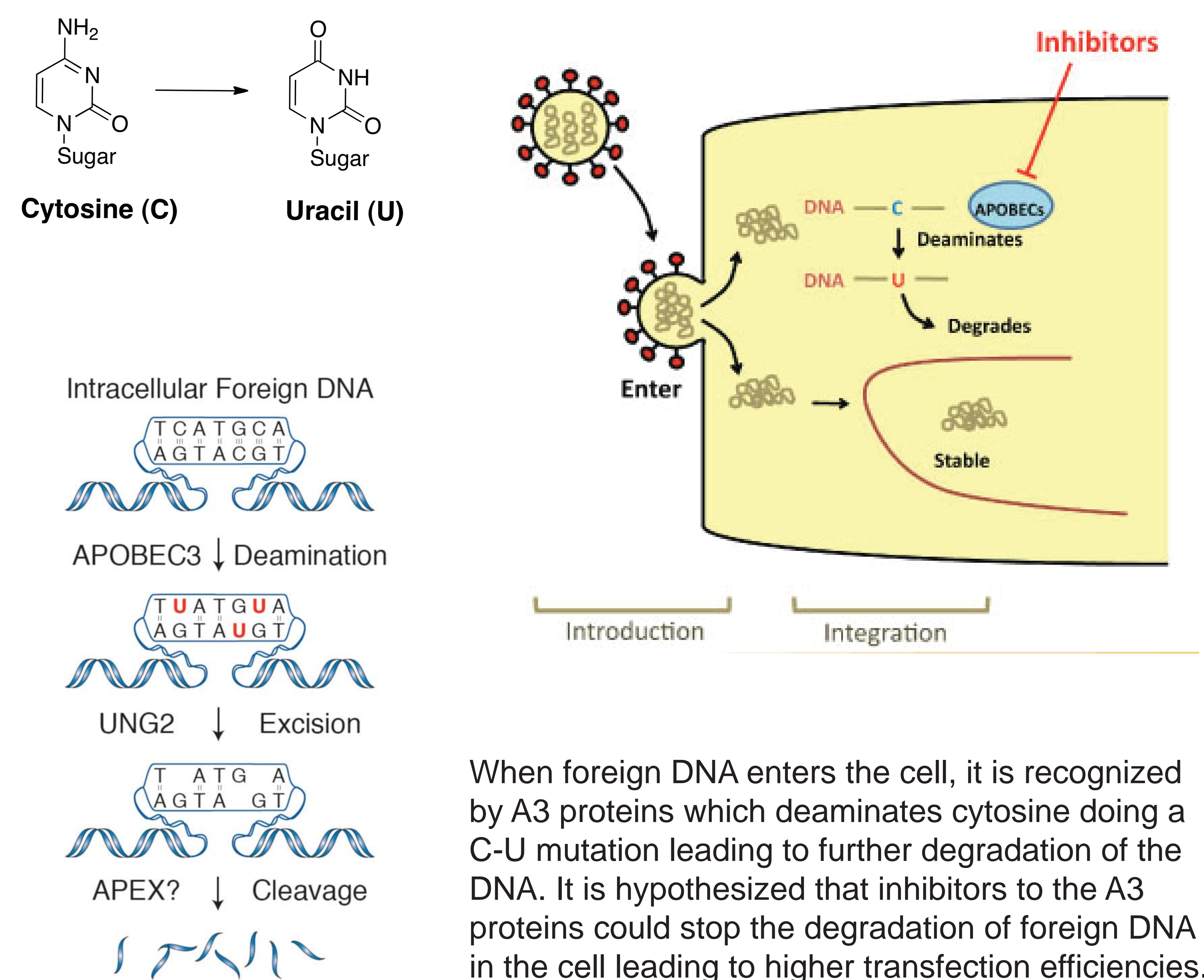
APOBEC3 Protein Deamination Mechanism:



Intracellular Foreign DNA

TCATGCA / AGTACGT

APOBEC3 Deamination

TUATGUA / AGTAUGT

UNG2 Excision

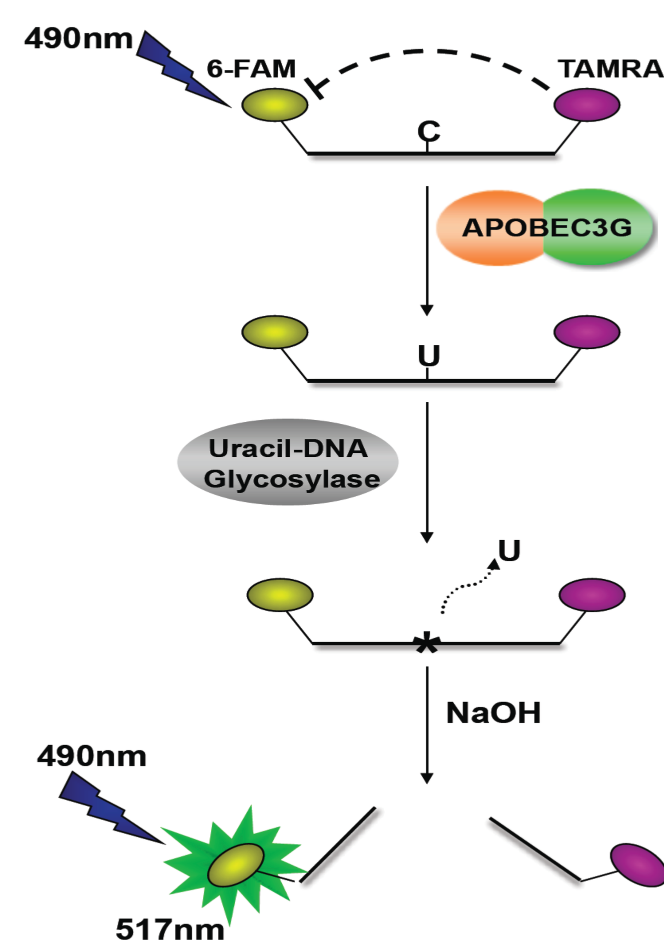
T ATG A / AGTA GT

APEX? Cleavage

When foreign DNA enters the cell, it is recognized by A3 proteins which deaminates cytosine doing a C-U mutation leading to further degradation of the DNA. It is hypothesized that inhibitors to the A3 proteins could stop the degradation of foreign DNA in the cell leading to higher transfection efficiencies.

Stenglein et al. *Nat. Struct. Mol. Biol.* **2010**, *17*, 222 (Foreign DNA Restriction)

Fluorescence Based DNA Deamination Assay:



DNA deamination assay run in high throughput screens. DNA oligonucleotide with 5' label (FAM) fluorescent and 3' fluorescence quenching molecule (TAMRA) is treated with A3G/A3A proteins, converting cytosine to uracil. Uracil-DNA-glycosylase removes uracil creating an abasic site on DNA. Hydroxide addition cleaves DNA and the 5' dye is removed from quence and fluorescent.

Li et al. *ACS Chem. Biol.*, in revision (Fluorescent Deamination Assay)

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Undergraduate Research Opportunity Program, University of Minnesota (TJG)

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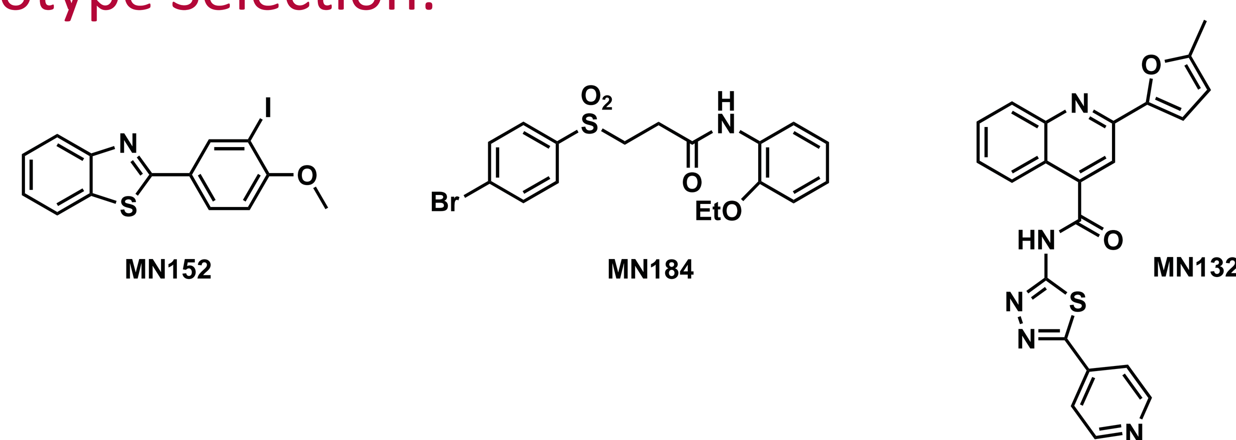
Department of Medicinal Chemistry (Start up funds to DAH)

Abstract

The APOBEC3 (A3) family of proteins degrade non-native or 'foreign' DNA in cells. We have hypothesized that blocking the enzymatic activity of A3 proteins could enhance the efficiency of foreign DNA introduction (transfection) into cells that are otherwise refractory to the process. APOBEC3 proteins degrade 'foreign' DNA by converting cytosines into uracils, which then triggers the cell to degrade the DNA due to the presence of a non-native DNA base (uracil). To identify small molecules that could inhibit A3 proteins, High Throughput Screening (HTS) was performed at the University of Minnesota and the Sanford-Burnham Medical Research Institute and over 350,000 compounds were tested for inhibition of A3A and A3G proteins. Follow-up studies by the Harris laboratory (University of Minnesota) have identifed hundreds of potential candidate molecules that can inhibit A3 activity *in vitro*. Three lead molecules from this study include MN152, MN184 and MN132. The Harki laboratory (University of Minnesota) is collaborating with the Harris laboratory to conduct detailed medicinal chemistry campaigns to optimize lead molecules for strong potency and minimal toxicity. Preliminary results from our synthesis studies of these chemotypes are presented here.

Chemotype Selection:

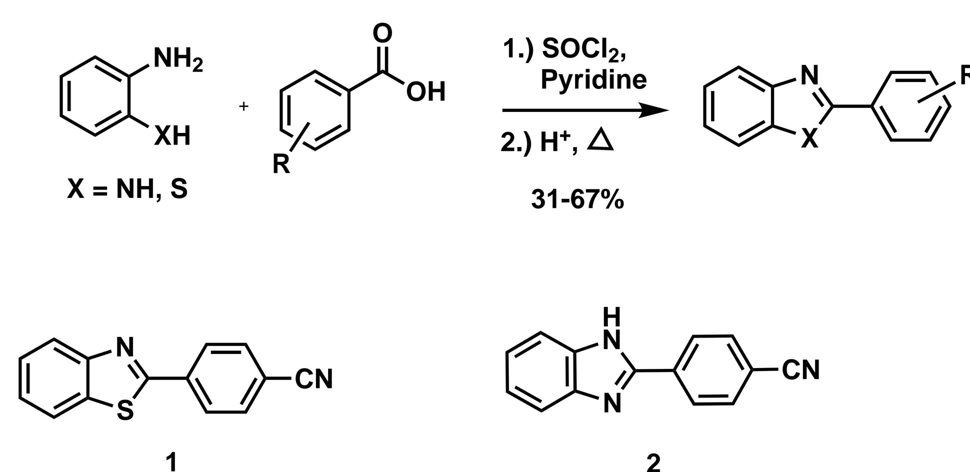


	MN152	MN184	MN132
A3A IC_{50}	0.7 uM	44 uM	2.8 uM
A3G IC_{50}	8.7 uM	9.6 uM	11 uM

IC_{50} values from the high throughput screens for MN152, MN184, and MN132.

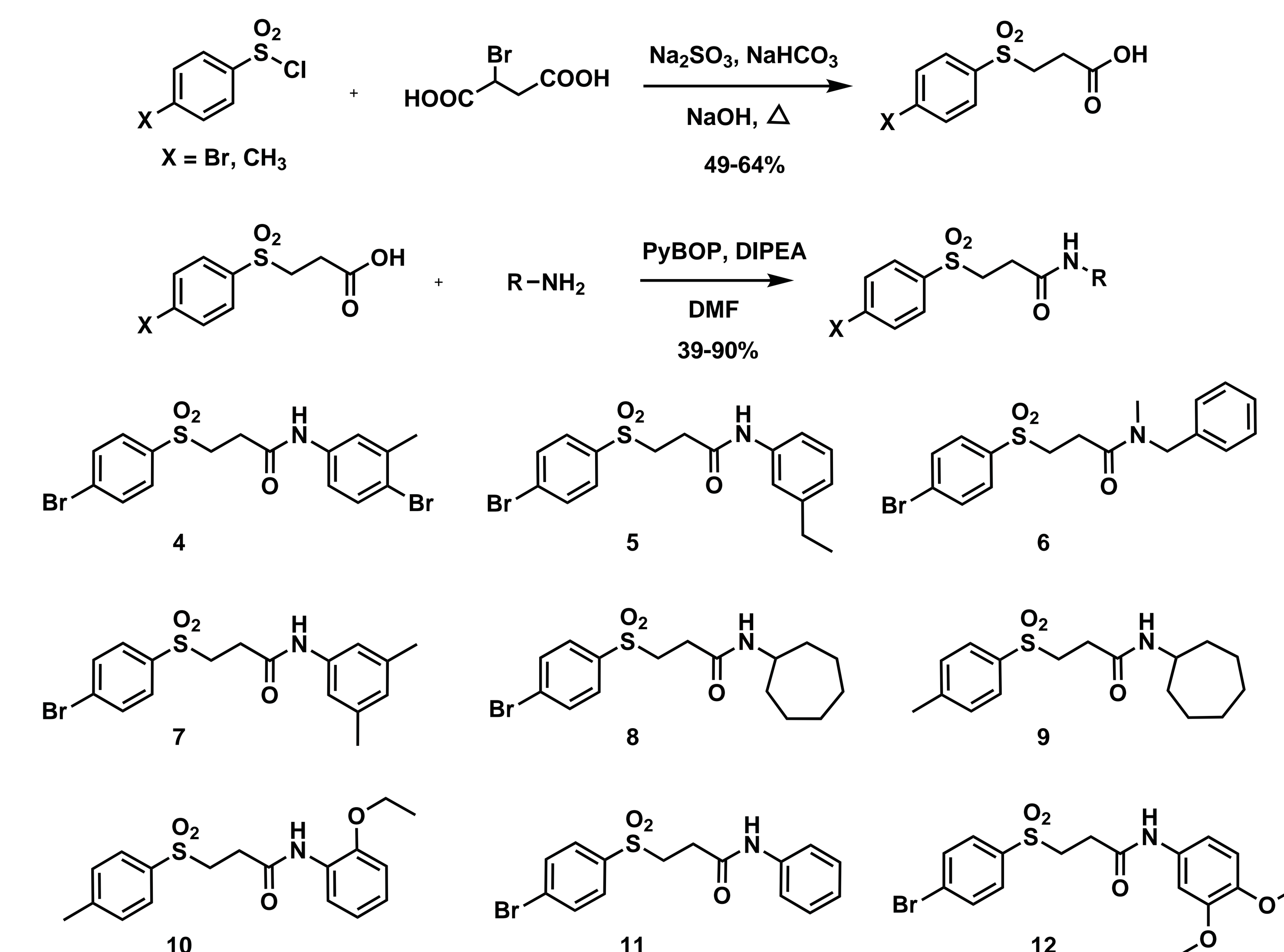
Synthesis of MN152 Analogues

Using the reaction scheme below, derivatives 1-3 of MN152 were synthesized.



Synthesis of MN184 Analogues:

Using the reaction schemes below, derivatives 4-12 of MN184 were synthesized.



Synthesis of MN132 Analogues:

Using the reaction schemes shown, derivatives 13-24 of MN132 were synthesized.