Characterization and Quantification of Specific DNA Damage

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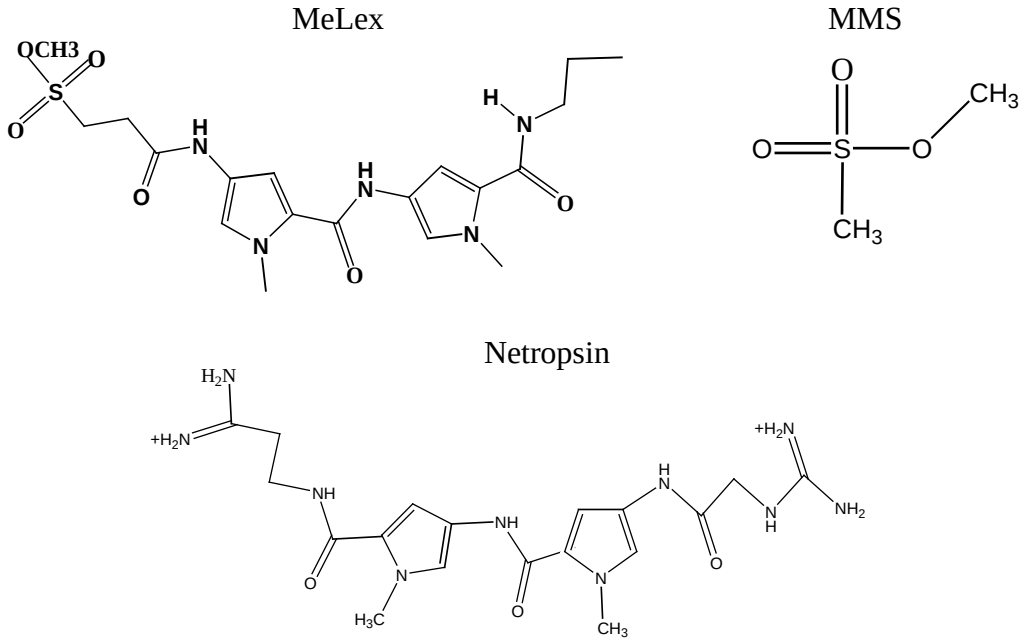
Our overall goal is to develop compounds that trigger cell death and not mutation by selectively damaging DNA at only those sites that lead to this specific outcome. My project was to characterize the damage inflicted by the compounds and quantify the damage through high performance liquid chromatography (HPLC) methods to assess the ability of these compounds to be specific in the damage they cause and their level of selectivity.

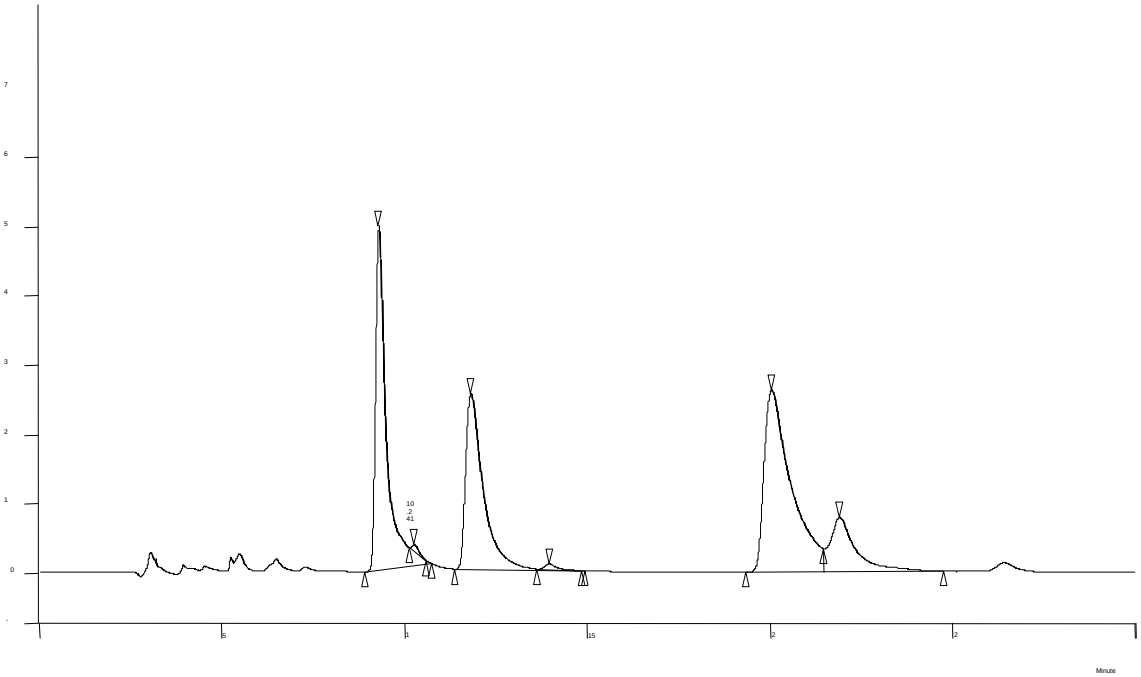
I exposed DNA for twenty-four hours to two damaging compounds, MeLex and MMS (Figure 1). MeLex is a large compound shown to damage in the adenine-thymine (A/T) rich regions of DNA. MMS, on the other hand, is much smaller and can cause damage randomly on DNA. DNA was also exposed to an inhibitor compound, netropsin (Figure 1), which binds strongly in A/T rich regions. This was done in order to determine its ability to inhibit the damaged caused by the compounds being tested.

Figure 1: Chemical structures of compounds used in this study.



The damaged components were released from DNA by heating the DNA at 90º C for 20 minutes. The remaining undamaged DNA was precipitated from the solution with cold hydrochloric acid. The solution with the dissolved damaged components of DNA was injected into the HPLC in order to determine the amount of specific DNA damage the compounds inflicted. The HPLC separated each of the damaged components, which were detected and quantitated by UV. A typical HPLC output is shown in figure 2. Each different peak shows a different damaged DNA component. The area under each peak is proportional to the amount of damage. This ratio of the different components shows the selectivity of the damaging compounds.

Figure 2



The results obtained are summarized in the table below. These results show that the molecules made in our lab, Me Lex, is highly selective in causing the kind of DNA damage that leads only to cell death and not to mutation. MMS is more random in the damage it causes, and does not give a high proportion of the kind of damage that leads to cell death. The inhibitor netropsin inhibits damage by Me Lex but has little effect on MMS, as expected.

compound	conc (μM)	Netropsin conc (μM)	**adduct level (μmol/mol DNA)**		
			3-MeA	**3-MeG**	**7-MeG**
MMS	5000		716±21	66±5	4370±233
	500		91±8	not detected	592±143
MMS with Netropsin	5000	100	473±18	38±7	4134±220
	500	100	71±1	not detected	757±15
Me-Lex	250		8827±489	105±20	1094±63
	100		4913±151	66±4	385±9
Me-Lex with Netropsin	250	100	641±18	31±3	983±37
	100	100	108±4	18	349±12

Table 1. Results obtained

I completed these studies in December 2004 and presented these results in a seminar in the Department of Chemistry and Biochemistry and at the South Eastern Regional Conference of the American Chemical Society held in Raleigh in November 2004. Working on this project has been an invaluable experience for me and has motivated me to go to graduate school. I am extremely grateful to the UNCW Undergraduate Research Committee for awarding me this fellowship and encouraging undergraduate research.