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13. SUPPLEMENTARY NOTES

Dr. Creighton passed away June 2006

14. ABSTRACT

The objective of the proposed research is to develop a novel class of prodrugs for delivering nitric oxide into multidrug resistant (MDR) tumors overexpressing the pi isoform of glutathione transferase (GSTP1-1). The MDR phenotype is due, in part, to the overexpression of the pi isozyme of glutathione (GSH)-transferase (GSTP1-1), which catalyzes the addition of GSH to electrophilic antitumor agents followed by the active export of the conjugate from tumor cells. Two recent observations suggest a novel strategy for overcoming the MDR phenotype. The first is the discovery that GSTP1-1 catalyzes the Michael addition of GSH to 2-crtonyloxymethyl-2-cyclohexenone (COMC6) causing the displacement of crotonic acid. The second is the recent finding that nitric oxide has the ability to cause tumors to undergo differentiation and apoptosis. We will take advantage of these two observations by synthesizing a new class of 2-dialkyl-oxymethyldiazene-2-cyclohexenone (DOC6) derivatives, which will undergo a GSTP1-1-catalyzed addition of GSH resulting in the displacement of dialklydiazeniumdiolate salts by a mechanism analogous to that for the GSTP-1-catalyzed displacement of crotonate from COMC6. Since the dialkyldiazeniumdiolate salts are known to spontaneously decompose to nitric oxide under neutral conditions, DOC6 should selectively deliver high concentrations of nitric oxide to MDR tumors overexpressing GSTP1-1.

15. SUBJECT TERMS

Breast Cancer, nitric oxide, multidrug resistance

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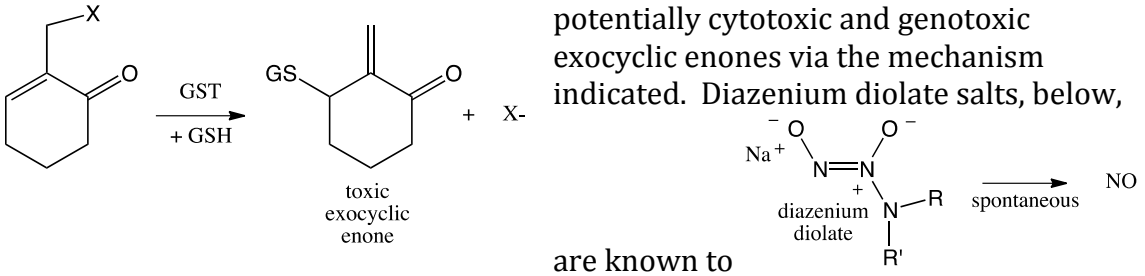
Using GSTP1-1 to Generate Nitric Oxide in MDR Breast Tumors (W81XWH-04-1-0691)
PI: Donald J. Creighton (deceased 06/2006)

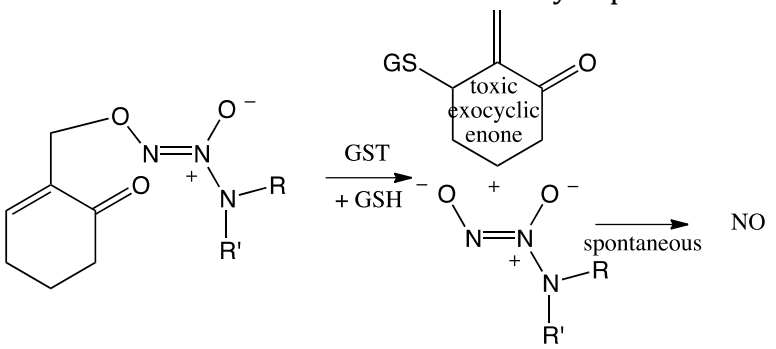
Remark to the Final Report. This report was prepared with the assistance of Diana Hamilton, Ph.D., a former post-doctoral fellow in the Creighton Lab, by Prof. James C. Fishbein, Ph.D. Professor Fishbein had familiarity with the project in years prior to Dr. Creighton's death and was assigned as group Leader and mentor to the remaining students at the time of Dr. Creighton's death.

Purpose. The overall goal of this project was to create and test the activity of a chemical entity that, when activated by drug-resistant breast cancer cells, generates two cytotoxic agents with different mechanisms of cytotoxicity – a 'two-warhead' approach.

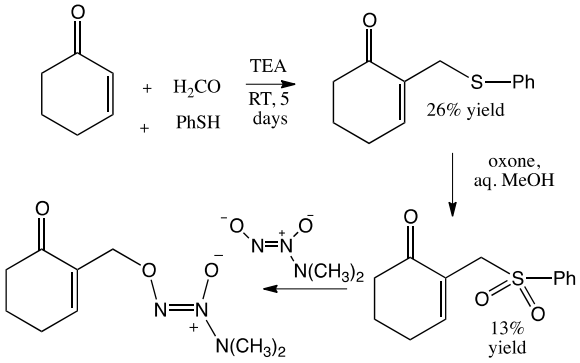
Rationale. Drug resistant breast cancer cells are protected in part through over-expression of the enzyme glutathione-S-transferase (GST). This enzyme conjugates cellular glutathione to electrophilic anti-cancer drugs, thereby inactivating them, a cytoprotective action of the cancer cell. Cyclic enones of the general structure below have been demonstrated to react with GST to generate potentially cytotoxic and genotoxic exocyclic enones via the mechanism indicated. Diazenium diolate salts, below,



are known to spontaneously decompose to generate NO which is cytotoxic and induces apoptosis at micromolar concentrations. Thus the strategy underlying the proposed project was to construct the 'two-warhead' entity depicted below that could, upon uptake by drug resistant breast cancer cells, react in a GST-catalyzed reaction to generate the exocyclic enone and a diazenium diolate that would liberate cytotoxic levels of NO, as depicted.

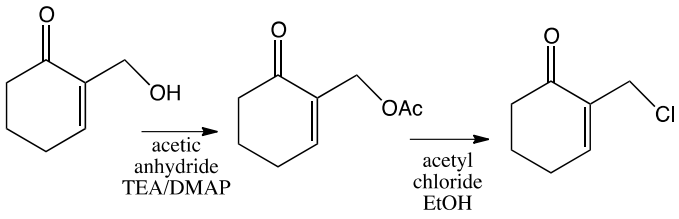


Specific Aims. A. Synthesize a series of compounds of the general structure indicated with various R and R'. B. Test for their reactivity with purified GSTP1-1 that is known to be overexpressed in multi-drug resistant breast cancers. C. Test activity of the single cyclic enone warhead in cells not overexpressing GSTP1-1 and those overexpressing GSTP1-1 to validate the GST mediated activation and finally test the two-warhead structures synthesized in Aim A.



Results. Aim A. The following synthetic scheme (left) was initially adopted.

Conditions were optimized for the first two reactions to obtain the yields indicated. While poor, the availability of the starting materials and reagents required suggested that sufficient final material might be obtainable for proof of concept. The final reaction never generated a yield of greater than 1% as an estimate from crude NMR. This reaction was attempted under a variety of solvent proportions (THF/DMSO) and temperature conditions – 0 to 50 °C. More elevated temperature led to rapid decomposition of the diazenium diolate. An alternative approach was adopted, as indicated below. From the chloride the final step anticipated was SN2 displacement by the diazenium diolate, as in the previous scheme. The chloride was indeed obtained in good yield, but the project was terminated before the final reaction was executed and optimized.



Aim B. This aim was dependent on the successful synthesis in Aim 1 and in the absence of this was not attempted.

Aim C. It was possible to test the concept of GST activation of the enone by use of the single warhead construct, the crotonate ester, below with MCF7 normal cells and MCF7 cells overexpressing GSTP1-1.m Indeed, these experiments demonstrate that overexpression of GSTP1-1 leads to substantially enhanced cytotoxicity of the crotonate ester. This observation is summarized in the resulting peer-reviewed publication in J. Med. Chem., **2005**, *48*, 6549-6552 (attached). These results were also presented in an international forum by Dr. Creighton in the Steinberg lecture – "Selective Inhibition of MCF-7 Breast Tumors Using COMC Derivatives," Uppsala University, Uppsala, Sweden, June 7, 2005 (attached).