



Structure of MGd-FdUrd conjugate.

**Methods:** Compound stability was assessed by reversed phase HPLC on an HP1050 system. Flow cytometry was performed using a BD FACSCalibur instrument. Cell proliferation was evaluated in 96-well format using a formazan reduction (MTT) assay.

**Results:** Uptake of MGd and MGd-FdUrd conjugate into the A549 human lung carcinoma cell line was quantified by flow cytometry. Based on the median fluorescence >650 nm, there was approximately 40% uptake of the conjugate relative to MGd. Intracellular enzymatic cleavage of the phosphodiester linker joining the nucleoside and MGd moieties was demonstrated by HPLC analysis of cell pellets and extracellular treatment medium. Moreover, an anti-nucleoside antibody-based flow cytometric assay determined that the nucleoside portion of a similar conjugate containing 5-bromo-2'-deoxyuridine (BrdU) was incorporated into DNA. MGd-FdUrd conjugate cleavage and stability in human serum was addressed. The majority of the compound was uncleaved after 24hr incubation in serum and displayed comparable stability to that of MGd. In addition, preliminary data using MTT indicate that MGd-FdUrd and FdUrd inhibit cell proliferation to a similar degree.

**Conclusions:** The phosphodiester linkage between MGd and FdUrd is stable in human serum. The MGd-FdUrd conjugate is taken up by A549 cells, and is released in active form under intracellular conditions. This approach may allow the targeted delivery of nucleoside analogues to tumors.

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### Two photoaffinity analogs of HTI-286, a synthetic analog of hemiasterlin, interact with alpha-tubulin

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HTI-286 is a synthetic analog of hemiasterlin, a naturally occurring tripeptide derived from marine sponges. The molecule depolymerizes microtubules, is a poor substrate for MDR1, and overcomes paclitaxel-resistance in human tumor xenograft models. Phase I trials with HTI-286 are in progress. Two tritium-labeled benzophenone analogs of HTI-286 were synthesized and their interaction with purified tubulin was investigated. Each analog had a benzophenone-reactive group in a distinctly different region of the molecule. It was found that both analogs specifically and solely photo-labeled alpha-tubulin. Photolabeling was inhibited by unlabeled photoaffinity analog, HTI-286, vinblastine, and another peptide-like anti-microtubule agent, dolastatin-10. However, similar concentrations of paclitaxel and colchicine were found to either enhance binding of the photoprobe to tubulin or have no effect; the result depended on the temperature of the reaction. To identify the binding site(s), alpha-tubulin bound to photoprobe was subjected to sequential formic acid and LysC digestions. A 16-kDa formic acid fragment was found to contain the radiolabel and is predicted to be the C-terminal fragment of alpha-tubulin. Following both formic acid and LysC digestions, a 3-kDa peptide was obtained. The identification of this peptide and the amino acid site of interaction are in progress. Our studies support the previous proposal that HTI-286 and other peptide-like anti-microtubule

agents have similar binding domains and these regions overlap with the Vinca-binding site previously speculated to be in beta-tubulin. However, this data is the first to suggest that a tubulin-binding peptide may interact with alpha-tubulin and is consistent with mutations in alpha-tubulin that have been already reported in HTI-286-resistant cells.

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### Molecular beacon based photosensitizers for imaging guided cancer therapy

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Near-infrared (NIR) dyes are presently attracting considerable interest as fluorescence probes for detection of cancer and as photosensitizers for cancer treatment by photodynamic therapy (PDT). Since tissue is relatively transparent to NIR light, NIR active fluorescence imaging (NIRF) and PDT are capable of detecting and treating, respectively, subsurface tumors, including breast cancer. Stable bacteriochlorophyll (BChl) analogs derived from *R. Sphaeroides* are excellent NIR dyes for NIRF and PDT because of because of their favorable photophysical properties (102% yield: 45%) and long activation and fluorescence emission wavelengths (λ<sub>abs</sub> 825nm; λ<sub>em</sub> 840nm). A current limitation of both NIRF and PDT modalities is their lack of sufficient tumor-to-tissue contrast due to the nonspecific nature of delivering the dye to the tumor, which has led to false negatives for NIRF and a limited therapeutic window for PDT. Hence, agents targeting "cancer signatures," i.e. molecules that accumulate selectively in cancer cells, are particularly attractive. We are currently focused on two of such signatures: the tumor-specific mRNAs and the LDL receptor (LDLR) overexpressed in certain tumors. Our first approach is to develop BChl based molecular beacons (hairpin antisense oligonucleotides) so that the dye would be activated only in cancer cells when the beacon hairpin hybridizes to the target mRNA. This will unfold the hairpin and these agents will light up (by emitting fluorescence) and destroy (by producing reactive oxygen species) the cancer cells, while leaving normal cells undetectable and unharmed. In the second approach, BChl cholesteryl oleate conjugates are synthesized and reconstituted into the LDL lipid core. Imaging studies showed that such LDL beacons were selectively internalized by LDLR overexpressing tumors both *in vitro* and *in vivo*. By targeting to these cancer signatures, our goal is to significantly improve the tumor-to-normal tissue ratio of NIRF guided PDT for subsurface cancers.

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### Indolequinone carbamate prodrugs of mustards as hypoxia-selective cytotoxins

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**Introduction:** The indolequinone class of bioreductive drugs have been developed whereby the p-quinonoid prodrug is reduced under hypoxic condi-

