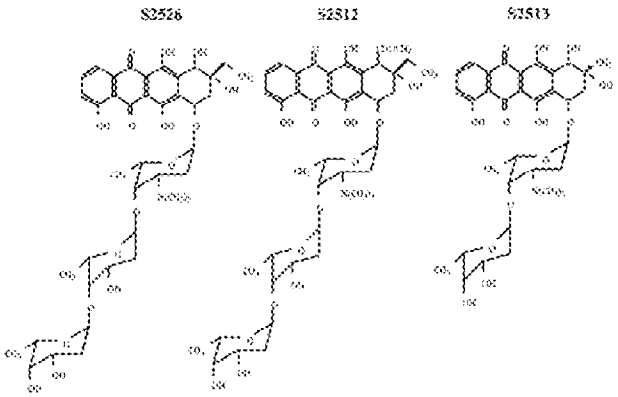

The structure-activity data of the novel compounds suggest that the 10-carbomethoxy moiety plays a role in the inhibition of topoisomerase II and arresting the cell cycle at G1. Additionally, we observed that the lack of an amino sugar residue resulted in diminished topoisomerase inhibition. Preliminary *in vitro* cytotoxicity tests revealed three compounds, S2512, S2513 and S2526, that were comparable with the clinically used anthracyclines daunorubicin, doxorubicin and aclarubicin. These drug candidates were further analysed in a broader panel of cancer cell lines. One of the compounds, S2512, showed particularly high activity against all the cell lines *in vitro*, whereas another one, S2513, was more active *in vivo* against mouse leukaemia and solid tumours.

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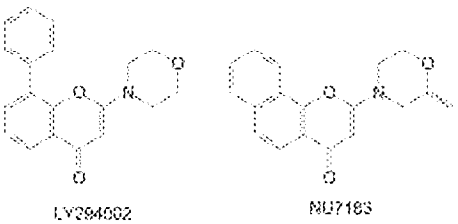
Application of combinatorial chemistry for the identification of pyran-4-one and thiopyran-4-one inhibitors of DNA-dependent protein kinase

J.J. Hollick[1], B.T. Golding[1], R.J. Griffin[1], I.R. Hardcastle[1], J.J.J. Leahy[1], N. Martin[1], L. Rigoreau[1], G.C.M. Smith[2], M.L. Stockley[2]. [1]*University of Newcastle upon Tyne, Chemistry, Newcastle upon Tyne, United Kingdom;* [2]*KuDOS Pharmaceuticals, Cambridge, United Kingdom*

DNA-dependent protein kinase (DNA-PK) detects and initiates repair of DNA double strand breaks (DSBs), and therefore inhibitors of this enzyme are potential radio- and chemo-potentiators in the treatment of cancer. Utilising the non-specific inhibitor LY294002 (DNA-PK; IC_{50} = 1.0 μM, PI-3 K; IC_{50} = 2 μM) as the structural basis for a pharmacophore mapping approach, chromen-4-ones and pyrimidoisoquinolinones have been developed, which are more potent and selective as DNA-PK inhibitors than the lead compound. Thus, the benzochromenone NU7163 has been found to exhibit excellent selectivity for DNA-PK (IC_{50} = 0.2 μM) over the related kinases ATM (IC_{50} = 100 μM) and PI-3 K (IC_{50} = 20 μM) (Griffin et al, Proc Amer Assoc Cancer Res, 43:4210, 2002). Further refinement of the pharmacophore model has resulted in the identification of pyran-4-one and thiopyran-4-one inhibitors, which retain the potency and selectivity of the analogous chromen-4-ones. These include the pyran-4-one NU7074 (DNA-PK; IC_{50} = 0.2 μM, PI-3 K; IC_{50} = 18 μM). To assist the rapid development of structure-activity relationships for these new templates, we have employed multiple parallel synthesis to prepare small chromenone and thiopyranone libraries bearing a range of substituents.



These studies have led to the identification of interesting novel compounds in both series, and have provided an insight into structural requirements for inhibitors of the PI-3 kinase family of enzymes.

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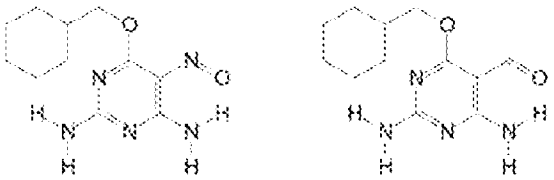
4-Alkoxy-2,6-diaminopyrimidine derivatives: inhibitors of cyclin dependent kinases 1 and 2

K.L. Sayle[1], V. Mesguishe[1], R.J. Parsons[1], J. Bentley[2], T.G. Davies[3], J.A. Endicott[4], M.E.M. Noble[5], L.Z. Wang[6], I.R. Hardcastle[7], B.T. Golding[1]. [1]*University of Newcastle Upon Tyne, Chemistry, Newcastle Upon Tyne, United Kingdom;* [2]*University of Newcastle Upon Tyne, Oncology, Newcastle Upon Tyne, United Kingdom;* [3]*University of Oxford, Molecular Biophysics and Department of Biochemistry, Oxford, United Kingdom;* [4]*University of Oxford, Molecular Biophysics and Biochemistry, Oxford, United Kingdom;* [5]*AstraZenica Pharmaceuticals, Cheshire, United Kingdom;* [6]*University of Newcastle Upon Tyne, Oncology, Newcastle Upon Tyne, United Kingdom*

The cyclin-dependent kinases (cdks) are a family of serine-threonine kinases that play a crucial role in cell cycle control. Progression of cells through the cell cycle is strictly regulated by the sequential activation and deactivation of cdks, and loss of cell cycle control, through aberrant cdk activity, leads to unrestrained proliferation. Hence, the identification of potent and selective cyclin dependent kinase inhibitors is of interest in the search for novel potential anti-cancer agents. The cdk inhibitor NU6027, 4-cyclohexylmethoxy-2,6-diamino-5-nitrosopyrimidine (IC_{50} vs. cdk1/cyclinB1 = 2.9 $\pm$ 0.1 μM and IC_{50} vs. cdk2/cyclinA3 = 2.2 $\pm$ 0.6 μM), was used as the foundation for the design of a series of 4-alkoxy-2,6-diamino-5-nitrosopyrimidine derivatives. We have successfully synthesised and evaluated a series of pyrimidines as potential inhibitors of cdks 1 and 2; and probed structure-activity relationships relative to NU6027. The introduction of simple alkoxy- or cycloalkoxy- groups at the O4-position was tolerated, with the 4-(2-methylbutoxy)-derivative (IC_{50} vs. cdk1/cyclin B1 = 12 $\pm$ 2 μM and cdk2/cyclin A3 = 13 $\pm$ 4 μM) retaining activity. Substitutions at the N6-position were not tolerated, and replacement of the 5-nitroso substituent with ketone, oxime and semicarbazone groups effectively abolished activity.



Surprisingly, NU6055 (2,6-diamino-4-cyclohexylmethoxy-pyrimidine-5-carbaldehyde), where the 5-nitroso group of NU6027 is replaced by an isosteric 5-formyl substituent, was significantly less active than the parent compound (IC_{50} vs. cdk1/cyclinB1 = 35 $\pm$ 3 μM and cdk2/cyclinA3 = 43 $\pm$ 3 μM). A comparison of the crystal structures of NU6027 and NU6055 bound to monomeric unphosphorylated CDK2, revealed differences in the binding orientations of the two inhibitors. Notably, while an intramolecular H-bond occurs between the 5-nitroso and 6-amino groups of NU6027, the corresponding interaction is not observed with the 5-formyl substituent of NU6055. Thus the parent compound, NU6027, still remains the optimal basis for future structure-activity studies for cdk inhibitors in this series.

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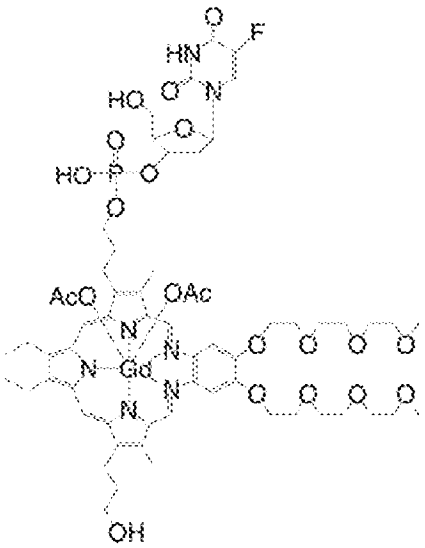
Studies on the cellular uptake and cleavage of nucleoside analogues conjugated to motexafin gadolinium

P. Lecane, D. Magda, Z. Wang, C. Lepp, C. Mani, D. Miles, R.A. Miller. *Pharmacyclics Inc., Sunnyvale, USA*

Introduction: Motexafin gadolinium (MGd, Xcytrin®) has been shown to enhance the efficacy of radiation in animal tumor models and is currently in advanced stage clinical development as an adjuvant to radiation therapy. MGd selectively localizes in tumors and has recently been demonstrated to enhance the effectiveness of the redox active drugs bleomycin and doxorubicin in preclinical models. On the basis of these data, we have investigated further possible synergy between MGd and other antineoplastic agents. One such compound, 5-fluorouracil (5-FU), is an important agent in the therapy of selected solid tumors. However, the therapeutic effect of 5-FU is limited due to the high clearance of the drug, with only 5% to 10% entering anabolism into active compounds such as the nucleoside 5-fluoro-2′-deoxyuridine (FdUrd). We have attempted to improve the biolocalization of FdUrd, and thus enhance its therapeutic index, by conjugating it with MGd through an enzymatically cleavable phosphodiester linkage.



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

M. Nunes[1], J. Kaplan[2], F. Loganzo[1], A. Zask[2], S. Ayral-Kaloustian[2], L. Greenberger[1]. [1]*Wyeth Research, Oncology, Pearl River, USA;* [2]*Wyeth Research, Chemistry, Pearl River, USA*

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 photolabeled 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

G. Zheng[1], M. Zhang[1,4], H. Li[1], D. Blessington[2], T. Busch[3], B. Chance[2], J. Glickson[1]. [1]*University of Pennsylvania, Department of Radiology, Philadelphia, USA;* [2]*University of Pennsylvania, Department of Biochemistry/Biophysics, Philadelphia, USA;* [3]*University of Pennsylvania, Department of Radiation Oncology, Philadelphia, USA;* [4]*Shanghai University, Department of Chemistry, Shanghai, China*

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 (1O2 yield: 45%) and long activation and fluorescence emission wavelengths (labs 825nm; lem 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

I. Robinson, M. Jaffar, N.S. Wind, K.J. Williams, I.J. Stratford. *University of Mancheter, School of Pharmacy and Pharmaceutical Sciences, Manchester, United Kingdom*

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