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94th Annual Meeting 2003

April 5-9, 2003 • Metro Toronto Convention Centre
Toronto, Ontario, Canada

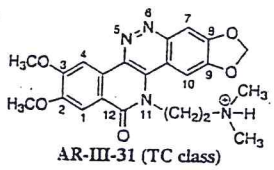
Proceedings

Volume 44
March 2003

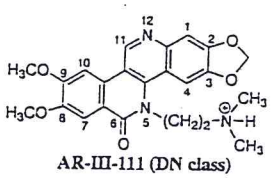
ers in otherwise identical analogs by studying their interaction with wt- and mutant human topoisomerase II alpha. Two mutations, L169I and R162Q displayed differential sensitivity towards closely related analogs, suggesting that the linker region in these compounds plays a highly specific role in protein drug interaction. The finding that the L169I mutation, which likely represents a subtle structural change, was sufficient to confer resistance further emphases the importance of this region of the protein for bisdioxopiperazine inhibition of topoisomerase II. When comparing the sensitivity profiles of different bisdioxopiperazines against wt and mutant proteins to that of mitindomide, we observed a spectrum of sensitivity closely resembling that of ICRF-154, a bisdioxopiperazine with no linker substituents.

#450 Dibenzo[*c,h*]1,6-napthyridines and triazachrysenes: Structure-activity studies of their topoisomerase I poisoning, DNA binding, and antitumor properties. Daniel S. Pilch, Hsing-Yin Liu, John E. Kerrigan, Jin-Ming Yang, Christopher M. Barbieri, Tsai-Kun Li, Angela Liu, Leroy F. Liu, Alex L. Ruchelman, Sudhir K. Singh, Edmond J. LaVoie, and Yuan-Chin Tsai. *UMDNJ-Robert Wood Johnson Medical School, Piscataway, NJ, The Cancer Institute of New Jersey, New Brunswick, NJ, and Rutgers University, Piscataway, NJ.*

Dibenzo[*c,h*]1,6-napthyridines (DNs) and triazachrysenes (TCs) are two new families of heterocyclic compounds that selectively poison human topoisomerase I (TOP1). The TOP1 poisoning activities and DNA binding properties of five structurally related representatives of the TC and DN classes of compounds have been evaluated. Suicide substrate experiments reveal that these compounds act by inhibiting the religation step of the TOP1 catalytic reaction. Viscometric studies reveal that all five compounds bind to duplex DNA via an intercalative mode of interaction. However, a comparison of the relative DNA binding affinities and TOP1 poisoning efficacies of these compounds suggests that DNA binding alone is not sufficient to impart potent TOP1 poisoning activity. In this connection, computer modeling studies indicate that these compounds stabilize the TOP1-DNA cleavable complex through an array of interactions with both the DNA and the enzyme. Two of the compounds studied (AR-III-31 and AR-III-111, see structures provided) exhibit TOP1 poisoning activities and cytotoxicities versus RPMI 8402 and P388 cells that exceed those of the clinical drugs topotecan and irinotecan (CPT-11). In addition, P388/CPT45 cells, which do not contain any detectable TOP1, are 100-fold more resistant to AR-III-31 and AR-III-111 than P388 cells, an observation consistent with TOP1 being the cytotoxic target of these compounds. The antitumor activity of AR-III-111 was evaluated in athymic nude mice with the human tumor xenograft MDA-MB-435, a non-estrogen responsive breast tumor cell line. Significantly, administration of AR-III-111 either orally or by IP injection induced similar extents of tumor regression to irinotecan, *but at one-tenth the dose (2.0 versus 20 mg/kg)*. In the aggregate, these observations highlight the potential of the TC and DN classes of compounds as effective antineoplastic agents.



AR-III-31 (TC class)



AR-III-111 (DN class)

#451 Novel anthrapyrazoles: In vitro activity against squamous cell carcinoma of the head and neck and breast carcinoma and mechanism of action. Denny Lin, Kim K. Larson, Xing Wu, Hallie Taylor, Frank S. Guziec, Jr., Brian B. Hasinoff, Paul D. Kerr, and Asher Begleiter. *University of Manitoba, Winnipeg, Manitoba, Canada, Southwestern University, Georgetown, TX, and Manitoba Institute of Cell Biology, Winnipeg, Manitoba, Canada.*

Background: Anthrapyrazoles have been investigated as cancer chemotherapeutic agents. The mechanism of action of these compounds is thought to involve inhibition of DNA topoisomerase II. By altering the molecular structures of these drugs, enhanced activity against cancer cells may result. However, these alterations may change the mechanism by which these drugs work. Objectives: A structure-activity study was designed to determine the in vitro potency of seven novel anthrapyrazoles against head and neck squamous cell carcinoma and breast carcinoma cells. Inhibition of topoisomerase II as a mechanism of action was also investigated. Methods: The MTT cell growth inhibition assay was used to generate dose-response curves for each drug. All seven drugs were tested for activity against head and neck squamous cell carcinoma cells and two of the drugs were tested for activity against breast carcinoma cells. From these curves, 50% growth inhibitory concentrations (IC_{50}) were calculated. Inhibition of catalytic topoisomerase II activity by each compound was measured using a fluorometric DNA decatentation assay and corresponding IC_{50}'s were calculated. Cell growth inhibition and topoisomerase II inhibition activities of the compounds were then correlated. Results: All seven drugs inhibited growth of the head and neck squamous cell carcino[illegible]s for the seven drugs ranged from 1.2 to 66.2 μM. All seven drug[illegible]oisomerase II, with the IC_{50}'s ranging from 4.1 to 48.4 μM. For both assays, the most potent anthrapyrazoles were those bearing a tertiary amine side chain. Changing the position of the chloro- group on the anthrapyrazole skeleton had opposite effects on cell growth inhibition compared with topoisomerase II inhibition. Growth inhibitory activities in breast carcinoma cells were similar to those obtained in head and neck squamous cell carcinoma cells. Statistical analysis showed no apparent correlation between inhibition of cell growth and inhibition of topoisomerase II activity. Conclusions: While these novel anthrapyrazoles showed activity against head and neck squamous cell carcinoma and breast carcinoma cells, those compounds bearing a tertiary amine side chain possessed the greatest potency. Activities were similar in the two tumor cell lines. The lack of correlation between inhibition of cell growth and of topoisomerase II activities suggests that inhibition of topoisomerase II may not be the primary mechanism of action for these drugs. (Supported by the National Cancer Institute of Canada and the Canadian Institutes of Health Research).

#452 Pyrrolobenzodiazepine-polyamide libraries: Synthesis and DNA binding selectivity. Geoff Wells, Thomas Gale, Alison Hardy, Anzu Hanaguchi, John A. Hartley, Garry P. Wilkinson, Paul M. Loadman, Terry Jenkins, Philip W. Howard, and David E. Thurston. *CRUK Gene Targeted Drug Design Research Group, The School of Pharmacy, London, UK, CRUK Drug-DNA Interactions Research Group, Department of Oncology, University College London, London, UK, CRUK Cancer Research Unit, University of Bradford, Bradford, UK, and Yorkshire Cancer Research Laboratory of Drug Design, University of Bradford, Bradford, UK.*

The ability to modify gene transcription by inhibiting the activity of specific transcription factors or blocking transcription in the coding region of a gene has potential applications in the treatment of cancer. A sufficient number of base pairs must be recognised in order to obtain a specific effect. At the molecular level the minor-groove binding pyrrolobenzodiazepines (PBDs) recognise and covalently bind to Pu-G-Pu sequences, whereas heterocyclic polyamides (HPAs) interact reversibly with a variety of sequences depending on the nature of the heterocycle and the binding stoichiometry. The aim of this work is to synthesize hybrid molecules that combine the recognition characteristics of both molecules. Solution phase peptide coupling methods have been used to synthesise a series of PBD-HPA hybrids with polyamide components (N-methylpyrroles) of varying length with a long-term view of studying the effect of polyamide length on DNA binding-site size and selectivity, binding affinity, cellular penetration, and *in vitro* cytotoxicity. GW6 consists of a PBD attached to three N-methylpyrrole heterocycles. Footprinting studies have shown that the molecule binds to a GATAATC sequence suggesting that it possesses DNA-recognition properties characteristic of both the PBD and HPA portions of the molecule. Using fluorescence microscopy we have shown that, unlike Dervan hairpin polyamides that bind to a similar number of base pairs, GW6 penetrates the nucleus of cultured MCF-7 cells. It also has *in vitro* cytotoxic properties with a mean GI_{50} of < 10 nM in the NCI's 60 cell line screen. Synthesis of the PBD-HPA conjugates has been successfully transferred to solid phase methodology using a variety of heterocyclic building blocks, and has been extended to the preparation of PBD-heterocycle combinatorial libraries. These libraries have been synthesised in conjunction with a peptide coding strand to enable identification of an individual DNA-binding molecule. A 100,000-member library has been screened against a number of rhodamine labelled gene fragments including one taken from *bcr-abl* and a number of hits have been obtained. Edman degradation of the Tag sequences has revealed the structures of 10 lead molecules which are currently being re-synthesized for further biological evaluation.

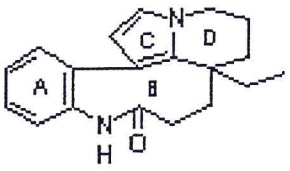
#453 Cytotoxic and apoptotic effects of new substituted resveratrol analogues in sensitive and resistant leukemia cells. Manlio Tolomeo, Marinella Roberti, Daniele Simoni, Lucia Crosta, Stefania Grimaudo, Riccardo Rodanin, Riccardo Baruchello, Daniela Pizzirani, and Nicola Gebbia. *Divisione di Ematologia e Servizio AIDS. Palermo, Italy, Dipartimento di Scienze Farmaceutiche, Ferrara, Italy, Consorzio di Ricerca sul Rischio Biologico in Agricoltura (Co.Ri.Bi.A.), Palermo, Italy, Divisione di Ematologia, Palermo, Italy, Dipartimento di Scienze Farmaceutiche, Ferrara, Italy, and Dipartimento di Scienze Farmaceutiche, Bologna, Italy.*

Resveratrol, a phytoalexin present in grapes, has recently been suggested as a potential cancer chemopreventive agent based on its striking inhibitory effects on cellular events associated with cancer initiation, promotion, and progression. The interesting anticancer activity, associated with its simple structure suggests that in principle resveratrol can be a lead molecule for the discovery of new chemoterapeutic agents. In this context efforts have recently been devoted to the detailed study of structure-activity relationships (SAR) of substituted stilbene derivatives of this type. Recently resveratrol has also been shown to induce apoptosis in different cancer cell lines. We recently started a study aimed to evaluate the apoptotic activity of a novel class of stilbene compounds structurally related to vitamin A, and some derivatives

were found endowed with potent apoptotic activity in both normal and MDR cell lines, we believed that it would be further informative to deeply explore other classes of related stilbenes as a logical starting point in the quest of novel anticancer chemotherapeutic agents. On the bases of this premises we prepared various resveratrol like derivatives with the aim to find compounds endowed with potent apoptotic activity against tumor cells. A variety of substituents were introduced in position 3 and 4 (OH, NH2, OCH3). The replacement of 3,5-hydroxy groups with methoxy functions was also investigated in this series together with the determination of the effect of double bond isomerization (Z and E geometry). All the synthesized compounds were tested in vitro for the ability to induce cell growth inhibition and apoptosis in several sensitive, MDR and apoptotic-resistant leukemia cells. Two compounds characterized by the introduction of methoxy functions showed an activity comparable to that of daunorubicin and greater than that showed by etoposide, citarabine. They also prove to be potent apoptosis inducing agents active in multidrug resistant (MDR) cell lines, and in cells resistant to the apoptotic effects of several chemoterapeutic drugs, including cis-platinum, 5-fluoruracil and citarabine.

#454 Biomimetic alkaloid synthesis and SAR study of racemic Rhazinilam analogues. Guangli Yang, Ernest Hamel, and William G. Bornmann. *Memorial Sloan-Kettering Cancer Center, New York, NY and National Cancer Institute at Frederick, Federick, MD.*

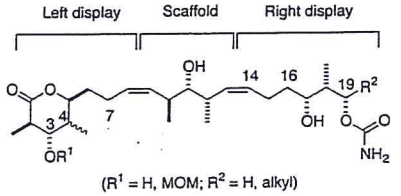
(-)-Rhazinilam has been reported to have a unique activity of profile. This antimitotic compound induces spiralization of tublin such as that observed for Vinblastine and also inhibits the cold-induced disassembly of microtubules as that described for Taxol. In the present study, novel racemic rhazinilam derivatives by modification of the D-ring size and the substituents on the juncture of D and B rings have been prepared from aldehydes and indoloazepine. X-ray structure study of three different D-ring size rhazinilam analogues is reported. Structure-activity relationship studies suggest that changing the ring size of the D-ring or moving the ethyl group is not desirable, but that the ethyl group can be replaced with other substituents.



Racemic Rhazinilam

#455 Synthesis and biological evaluation of simplified discodermolide analogs. Raghavan Balachandran, Nakyen Choy, Youseung Shin, Phu Qui Nguyen, Charitha Madiraju, Kenneth A. Giuliano, Dennis P. Curran, and Billy W. Day. *University of Pittsburgh, Pittsburgh, PA and Cellomics, Inc., Pittsburgh, PA.*

The potent microtubule stabilizing agent discodermolide is a novel compound currently undergoing clinical evaluation. Both the scarcity and synthetic complexity of this marine sponge-derived natural product have emphasized the need for analog synthesis. A series of 4-epi-7-dehydroxy-14,16-didemethyl analogs were synthesized and screened for biological activity. Like discodermolide, one of the analogs, NC2-86, showed a low critical temperature for hypernucleation of bovine brain tubulin assembly, blocked at low temperature the disassembly of tubulin polymer, and efficiently competed for the paclitaxel binding site. Antiproliferative potencies of NC2-86 were in the high nanomolar range against human breast, prostate and ovarian cancer cell lines, including paclitaxel-resistant cells. Combinations of NC2-86 with discodermolide or paclitaxel were synergistic in blocking cell proliferation when data were examined by the Chou and Talalay method. High content multiparameter fluorescent cell profiling, an automated cell imaging approach, showed that both discodermolide and NC2-86 stabilized microtubules as well as activated kinases associated with early mitotic events and stress pathway activation, albeit with different potencies. Implications for structure-activity relationships will be presented. Supported by NIH grant CA78039.



(R^1 = H, MOM; R^2 = H, alkyl)

#456 Discovery and SAR of gambogic acid as a potent apoptosis-inducing natural product. Sui Xiong Cai, Han-Zhong Zhang, John Drewe, Ben Tseng, and Shailaja Kasibhatla. *Maxim Pharmaceuticals, San Diego, CA.*

Apoptosis is a normal process of development and tissue homeostasis. While excessive apoptosis can result in organ failure and neurodegenerative diseases, insufficient apoptosis is a hallmark of cancer. Therefore, the discovery of novel inducers of apoptosis could lead to the development of new anticancer agents. Herein we report the discovery of gambogic acid, a natural product isolated from gamboge, as a novel inducer of apoptosis in cancer cells through our caspase and cell-based HTS assay. EC50 values, as measured by caspase activation, are at the low to sub-micromolar range for different cells. Gambogic acid also has sub-micromolar or better potency (GI50) against most cancer cell lines tested in the growth inhibition assay. In order to understand the SAR and improve the chemical and pharmacological properties of gambogic acid, we have designed and synthesized many derivatives. These include the modification of the carboxylic acid in the 30 position, the hydroxy in the 6-position, and the carbon-carbon double bond in the 9,10-positions. Derivatives of gambogic acid have been found to be active in vivo in mouse xenograft models. We will report in detail the isolation, chemistry, SAR and in vitro characterization of gambogic acid.

#457 Prostate cancer inhibitors isolated from *Rumex acetosella* (Sheep Sorrel) and quantified in two commercial herbal products: Essiac™ and Flor-Essence™. Andy Eberding, Dong Sheng Ming, G.H. Neil Towers, Sherwin Xie, and Emma Guns. *The Prostate Center at Vancouver General Hospital, Vancouver, British Columbia, Canada and University of British Columbia, Department of Botany, Vancouver, British Columbia, Canada.*

Two commonly used alternative cancer treatments, Essiac™ and Flor-Essence™, both include a common herbal ingredient, *Rumex acetosella* (Sheep Sorrel). We have isolated luteolin, luteolin-7-O-glucoside and gallic acid from *Rumex acetosella* using sequential organic fractionation. Luteolin, luteolin-7-O-glucoside and gallic acid were characterized using ^{1}H-Nuclear Magnetic Resonance (NMR), ^{13}C-NMR, mass spectroscopy and by comparing the spectral data with corresponding reported values from the literature. Luteolin is known to inhibit normal and malignant prostate cell growth and proliferation both *in vitro* and *in vivo*. Luteolin-7-O-glucoside is a derivative of luteolin that is glucosolated at the 7-hydroxyl of the root aglycon. Gallic acid has recently been shown to significantly inhibit proliferation and induce apoptosis in LNCaP prostate cancer cells. High Performance Liquid Chromatography (HPLC) was used to quantify the amount of luteolin in aqueous extracts of Essiac™ and in Flor-Essence™ at 0.78 and 1.56-mg/100-mL, respectively. Luteolin-7-O-glucoside was similarly quantified in Essiac™ and in Flor-Essence™ extracts at 1.00 and 5.63-mg/100-mL, respectively. Crystal violet cytotoxicity assays, using LNCaP and PC-3 prostate cancer cell lines, of both extracts and the quantified compounds revealed the effects of luteolin to be greater than that of luteolin-7-O-glucoside, sheep sorrel aqueous extract or Essiac™ aqueous extract, in that rank order. Luteolin, luteolin-7-O-glucoside and gallic acid are three pharmacologically active agents, as shown in this work or in the literature, which may provide a therapeutic effect in the treatment of prostate and other cancers. It is of considerable interest that these specific compounds are components of Essiac™ and Flor-Essence™, two popular alternative cancer treatments. The combined action of the three compounds in a single extract may result in an efficacious prostate cancer therapy.

#458 Mithramycin SK, a novel aureolic acid-type antitumor compound generated by combinatorial biosynthesis, shows an improved therapeutic index compared to mithramycin in *in vitro* antitumor and toxicity assays. Lily L. Remsing, Ana M. Gonzalez, Uwe Rix, Alfredo F. Braña, Carmen Mendez, Jose A. Salas, and Jürgen Rohr. *University of Kentucky, Lexington, KY and Universidad de Oviedo, Oviedo, Spain.*

In attempts to investigate possibilities to generate novel, therapeutically improved aureolic acid-type antitumor drugs through combinatorial biosynthesis, we have been focusing on modifying the post-polyketide synthase (post-PKS) tailoring steps of the mithramycin biosynthesis by *Streptomyces argillaceus* ATCC 12956 (see also abstract: L.L Remsing, H. Bahadori et al.). In this context, we recently focused on ketoreductase (KR) encoding genes, which appeared to be promising to gain initial insights in structure-activity-relationships (SAR) regarding the pentyl side chain attached at C-3 of the drug, since a KR step within this side chain was proposed to occur as the last step of the mithramycin biosynthesis. Insertional inactivation of *mtmW*, a gene located ca. 8 kb downstream of the mithramycin PKS genes, yielded an *S. argillaceus* mutant, which accumulated two new mithramycin analogues, demycarosyl-mithramycin SK and mithramycin SK (Mtm SK), the latter being the major product. Subsequent structure elucidation confirmed that MtmW is indeed the enzyme, which catalyzes the ketoreduction step affecting the 3-side chain in mithramycin biosynthesis. Surprisingly, the structures of mithra-