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Antitumoral activity of a polyisoprenylated benzophenone (CLU-502) isolated from Clusia sp

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We have characterized a polyisoprenylated benzophenone isolated from *Clusia rosea*, collected in Florida, USA. This novel substance, called CLU-502, was isolated from methanolic extracts of the plant using RP-HPLC coupled to a PDA-detector. In the SRB proliferation assay, CLU-502 demonstrated a potent cytotoxic activity in a panel of human tumor cell lines such as neuroblastoma and leukemia. Both, wild-type cell lines and sub-lines resistant to doxorubicin, cis-platin, etoposide, SN38, raltitrexel or 5-fluorouracil were highly sensitive to CLU-502. Interestingly, normal fibroblasts were more resistant to this compound than cancer cells. CLU-502 inhibits the unwinding activity of topoisomerase I in a relaxation of pBR322 plasmid, as well as the decatenation of kinetoplasmids by human topoisomerase II. In addition, CLU-502 induce a dose-dependent inhibition of telomerase activity in vitro. A dose-dependent effect on the phosphorylation of ERK1/2, the cytosolic downstream enzymes of the MAP-kinase pathway, was also detected in Western blot analyses. The interaction of CLU-502 with PD98059, a specific inhibitor of ERK1/2 phosphorylation, resulted in a synergistic cytotoxic effect when both drugs were administered to tumor cells simultaneously. As the result of the exposure of cell cultures to CLU-502, a cell cycle arrest of the G_1 phase through the induction of $p21^{CIP}$ was detected. CLU-502 induces a down-regulation of N-myc proto-oncogen in LAN-1 wild type and etoposide resistant, Pgp positive neuroblastoma cell line. Apoptosis/DNA-damage in tumor cells was observed in agarose gels after the exposure of CLU-502 at different concentrations. Initial toxicological studies in a nude mouse model revealed that treatment with CLU-502 was well tolerated up to 100 mg/kg. Finally, preliminary studies had shown that CLU-502 is active in vivo in a neuroblastoma and colon carcinoma nude-mice models.

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Novel mTOR Inhibitors with improved pharmacological properties over rapamycin

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The mammalian target of rapamycin (mTOR), a downstream protein kinase of the phosphatidylinositol 3-kinase (PI3K)/Akt (protein kinase B) signaling pathway that mediates cell survival and proliferation, is a prime strategic target for anticancer drug development. Rapamycin is a highly specific inhibitor of mTOR and its analogues are the only agents in clinical development that target the PI3K/Akt pathway.

In spite of various semi-synthetic modifications of rapamycin, the structural changes produced have been limited to only a few positions around the macrolactone ring, for example, the hydroxyls at C40 and C28 as well as those at C16 and C27 after dealkylation. More limited chemistry has been applied at other functionalities such as the ketone at C9 and the conjugated triene moiety. These limitations inherent to synthetic modification of rapamycin are further exemplified by the fact that most rapamycin analogues under clinical development, CCI-779, RAD001 and ABT-579 are all modified at the C40 hydroxyl group outside FKBP and mTOR binding domains. As a consequence they are likely to share similar profiles with rapamycin. For example, CCI-779 shares the same tumor inhibition profile with rapamycin and the Pearson correlation coefficient of the in vitro antiproliferative activities and potencies of the two agents across the 60-cell-line screen is 0.86.

We report here the use of engineered biosynthesis approach to explore wider structural space of rapamycin and the discovery of novel rapamycin analogues with improved pharmacological properties over rapamycin. The challenges of using biosynthetic engineering for the modification of rapamycin include the size and complexity of the rapamycin biosynthetic gene cluster (which has made heterologous expression approaches difficult) and the genetic intractability of the rapamycin producing organism, *Streptomyces hygroscopicus* NRRL5491. Examples of the genetic modification of this organism are extremely limited.

Deletion of a portion of the rapamycin biosynthetic gene cluster, using conjugative methods for DNA transfer to *S. hygroscopics* followed by targeted recombination, provided a rapamycin non-producing mutant, MG2−10. The deleted genes included those responsible for all of the oxidative (at C9, C16 and C27) and methylation (at the C16, C27 and C39 hydroxyl groups) modifications of the first enzyme free intermediate of rapamycin biosynthesis. In addition, it was discovered that one of these genes, *rapK*, is essential for the production of the first enzyme free biosynthetic intermediate, as it is required for the production of (or regulation of the production of) the starter unit of the rapamycin polyketide synthase. Another of the genes, *rapL*, is required for the production of the pipecolic acid unit incorporated into rapamycin; we have shown previously that deletion of this gene and exogenous feeding of related amino acids provided access to novel rapalogues.

Complementation of *S. hygroscopics* MG2−10 with gene cassettes carrying combinations of the deleted genes provided access to a library of rapalogues bearing altered oxidation and alkylation patterns (24 possible combinations). The feeding of exogenous carboxylic acids (starter unit analogues) or amino acids (pipecolic acid analogues) to specific mutants provided an orthogonal approach for increasing diversity through mutasynthesis of the engineered strains. The presentation will provide further details of the experiments described herein and of the novel rapalogues generated by these methods.

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Parthenolide sensitizes ultraviolet (UV) B-induced apoptosis via PKC but independent of AKT

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Parthenolide is the principal sesquiterpene lactone in feverfew (*Tanacetum parthenium*) with proven anti-inflammatory property. We have previously reported that parthenolide possesses strong anti-cancer activity in UVB-induced skin cancer using SKH-1 hairless mice. In order to further understand the mechanism(s) involved in the anti-cancer activity of parthenolide, we investigated the role of protein kinase B (AKT) and protein kinase C (PKCs) in the sensitization activity of parthenolide on UVB-induced apoptosis. Parthenolide pre-treatment vastly sensitizes JB6 cells to UVB-induced apoptosis. Although parthenolide inhibits the UVB-induced phosphorylation of AKT at Thr308 site (but not at Ser473 site), transient transfection of a constitutively active AKT plasmid does not affect this sensitization, indicating that AKT activation is not directly involved in cell death induced by parthenolide-UVB. On the other hand, several subtypes of PKCs have been reported to be involved in UVB-induced signaling cascade with both pro- and anti-apoptotic activities. Here we focused on 2 novel PKCs (the pro-apoptotic PKCδ and the anti-apoptotic PKCη) and the anti-apoptotic atypical PKCζ. UVB induces the translocations of these PKCs from the cytosol to membrane, an indication of their activations. Parthenolide pre-treatment enhances the translocation of PKCδ, and in contrary, inhibits the translocations of PKCη and ζ. Similar results were also detected when the kinase activities of these PKCs were tested. Moreover, pre-treatment with a specific PKCδ inhibitor Rotterlin completely diminishes the sensitization effect of parthenolide on UVB-induced apoptosis. In conclusion, we demonstrated that parthenolide sensitizes UVB-induced apoptosis via a PKC-dependent pathway but independent of AKT.

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Real time imaging of cancer therapeutic effects of a natural product turmeric in cell culture and animal models

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Introduction: The goal was to visualize the anti-cancer effect of a natural product, curcumin, a major component of turmeric normally eaten by humans. We report that curcumin has a fluorescence characteristic that can be detected under visible light using appropriate filters. To accomplish the goal we utilized our powerful light-based imaging system where incorporation of curcumin into the cancer cells and apoptosis were visualized in cell culture and in animal experiments.

Materials and method: Curcumin (diferuloylmrthane) was purified from fresh turmeric roots. Human origin ovarian cancer cells (Skov3 and Ovcar-3) cervical cancer cells (HeLa) were cultured in DMEM medium containing non-essential amino acids, and 2mM L-glutamine with 10% FBS. The cells were incubated at 37C in a humidified atmosphere containing 95% air and 5%CO2. Cancer cells were harvested by trypsin/ EDTA 24 hrs followed by plating in triplicates wells in 24 well plates. Cells were allowed to grow for 24h in DMEM supplemented with 2mM L-glutamine with 10% FBS. Curcumin (50 ug/well) was added to each cell line in triplicate wells. In additional triplicate wells, no curcumin was added for controls. At defined times (2h, 5h, 8h and 24 h for HeLa and SKOV3 and 24h, 48h, 72h, and 96h for Ovcar3), cells were washed twice with PBS. Wells were imaged with a Leica DMIRE 2 fluorescent microscope and Spot RT color camera. Cell viability was determined by trypan blue exclusion and plates were imaged again. For in vivo studies, each type of cancer cells (1×106 cells/mouse)

was implanted intraperitoneally. Live images were collected immediately. Next day curcumin (200 ug/ mouse) was injected ip. Images were collected after 2nd and 4th days with fluorescent stereomicroscope. Upon necropsy peritoneal wash was collected for culture and further examination to assess the cell death relative to the imaging data.
Results: In vitro results showed all treated cancer cells displayed green fluorescence. Viability test showed 100% cells were dead that were GFP-positive. In vivo images showed curcumin incorporation into the implanted cancer cells that were was imaged with GFP filter. On day 2 more GFP signal was detected than 4, since cells were killed by curcumin.
Conclusions: Therapeutic effect of curcumin was detected by fluorescent stereomicroscopy in a non-invasive way. This natural product can be used as a preventive medicine against cancer.

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A new antitumor compound, ECO-04601: preclinical evaluation and in vivo efficacy in glioma

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ECO-04601 is a structurally novel farnesylated diazepinone (MW 462) discovered using Ecopia's genomic platform through analysis of actinomycete loci encoding bioactive compounds. ECO-04601 is being developed as an antitumor agent as it shows suitable pharmaceutical properties, including in vivo efficacy, low toxicity, rapid absorption and bioavailability in target tissues. We have recently shown that ECO-04601 strongly inhibits proliferation of several human cancer cell lines in vitro, including low and high-grade human glioma cells (IC50 = 1 to 8 microM). To demonstrate in vivo efficacy, nude mice were inoculated with rat C6 glioma cells (5 millions/ml) either subcutaneously (6/group), or orthotopically in the caudate putamen (10/group). Daily treatment (10 to 30 mg/kg, i.p.) was initiated 24 hrs following glioma cell inoculation. When tumors cells were implanted subcutaneously, treatment with ECO-04601 resulted in a significant decrease of the tumor volume by 60%. In the orthotopic model, mice were treated daily with ECO-04601 until spontaneous death. Initial results indicate efficacy of ECO-04601 in this model as we observed a seven-day increase in the median survival of treated mice as compared to the vehicle-treated group. No significant loss of body weight was observed during the chronic treatment regimen of tumor-bearing mice suggesting a favorable toxicity profile of the compound that has been further confirmed by acute and subchronic administration of ECO-04601 in healthy animals. Female CD-1 mice tolerated single intravenous doses of 100 mg/kg of ECO-04601 and repeated subcutaneous or oral doses of 225 mg/kg. Preliminary pharmacokinetic experiments suggest rapid absorption and tissue distribution of the compound following administration by various routes of administration. These data highlight the promising therapeutic potential of ECO-04601 in aggressive tumors like gliomas.

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In vitro and in vivo characterizations of naturally occurring BBIs in reversal of p-gp mediated multidrug resistance

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Background: Multidrug resistance (MDR) is one of the major obstacles limiting the efficacy of cancer chemotherapy. The overexpression of the membrane associated P-glycoprotein (P-gp), which acts as an energy-dependent drug efflux pump, is believed to play a critical role in MDR. A promising strategy to conquer drug resistance is to develop functional MDR modulators that can specifically inhibit the P-gp activity. Through screening a series of natural products, we have recently identified six bisbenzylisoquinoline alkaloids (BBIs) that possess potent activity to reverse P-gp-mediated drug resistance. In this study, we characterized and evaluated the ability of these newly identified MDR Modifiers in reversal of P-gp-mediated drug resistance caused by different antineoplastic agents.
Materials and Methods: T wo human MDR cells (MCF-7/adr and KBv200) and their drug-sensitive parental cells were used for *in vitro* evaluation and characterization. The *in vivo* activity of these promising BBIs was evaluated through establishment of xenograft tumor models.
Results: In vitro assays indicated that these natural BBIs showed potent activities to restore sensitivity of resistant tumor cells, such as MCF-7/adr and KBv200 cells, to many antitumor drugs including doxorubicin, vincristine and paclitaxel. Further analyses by measurement of radioactive [^{3}H]-vincristine and [^{3}H]-paclitaxel indicated that these BBIs increased intracellular drug accumulation in MDR cells, but had little effect on drug-sensitive cells. Through establishment of xenograft models bearing the intrinsically resistant KBv200 tumors, we also tested one of these compounds (FF0019) and demonstrated that this class of naturally occurring MDR modifiers could also significantly potentiate the antitumor activity of VCR and paclitaxel *in vivo*.

Table 1. Effect of BBIs on reversing MDR[a]

		Fold shift of Dox IC_{50}^b		Fold shift of VCR IC_{50}^c	
		MCF-7/ADR	MCF-7	KBv200	KB
FF0011	5 μM	22.1±6.6	1.2±0.7	12.8±1.5	1.3±0.8
	2.5 μM	19.7±1.9	1.1±0.2	9.5±1.9	1.0±0.2
	1.25 μM	7.6±2.2	0.9±0.3	4.1±1.1	1.0±0.3
	0.625 μM	2.3±1.7	0.9±0.2	1.8±0.2	0.9±0.4
FF0012	5 μM	24.5±3.8	1.1±0.4	17.1±3.5	1.2±0.2
	2.5 μM	17.1±2.3	1.0±0.2	9.4±1.7	1.0±0.1
	1.25 μM	7.5±1.9	1.0±0.4	6.1±2.5	1.0±0.2
	0.625 μM	3.0±0.5	1.4±0.4	3.4±2.9	1.1±0.5
FF0014	5 μM	35.0±7.7	1.9±0.3	18.0±6.4	1.4±0.6
	2.5 μM	13.3±4.8	1.4±0.4	9.4±3.6	1.3±0.4
	1.25 μM	6.9±1.6	1.3±0.1	6.1±2.2	1.0±0.1
	0.625 μM	3.2±0.9	O.9±0.3	27±0.5	0.8±0.4
FF0015	5 μM	43.9±15.0	1.1±0.2	21.9±3.5	1.8±0.6
	2.5 μM	32.0±7.2	0.9±0.4	12.9±5.0	1.3±0.5
	1.25 μM	10.3±7.3	1.0±0.3	7.2±4.0	1.3±0.3
	0.625 μM	5.3±2.0	1.1±0.4	3.9±1.6	1.1±0.2
FF0018	5 μM	42.7±6.8	1.3±0.6	20.1±4.6	1.6±0.5
	2.5 μM	22.9±5.4	1.3±0.4	12.9±3.8	1.1±0.3
	1.25 μM	7.7±2.2	1.0±0.3	5.8±2.3	1.1±0.4
	0.625 μM	3.6±1.1	1.0±0.3	2.8±1.1	1.0±0.4
FF0019	5 μM	49.0±7.9	1.4±0.4	23.0±5.6	1.5±0.6
	2.5 μM	29.4±5.8	1.0±0.3	15.6±6.6	1.3±0.4
	1.25 μM	12.3±3.9	1.0±0.2	8.9±2.5	1.1±0.5
	0.625 μM	3.4±0.7	0.9±0.2	3.2±0.9	0.9±0.2
VRP	5 μM	7.6±3.4	1.0±0.3	6.4±1.4	1.1±0.4

[a]This table is based on three separate experiments and presented as mean±SEM.
[b]The IC_{50} of Dox for MCF-7/adr and MCF-7 cells in the absence of BBIs are 16.712 μM and 0.1718 μM, respectively.
[c]The IC_{50} of VCR on KBv200 and KB cells in the absence of BBIs are 0.054 μM and 4.437 μM, respectively.

Conclusions: These results suggested that the mechanism of these compounds to reverse MDR was associated with the increase in the intracellular drug accumulation through inhibiting the activity of P-gp. These compounds may possess great promising in being developed into novel anticancer drugs as modifiers of MDR (Supported by NIH Grants CA82440 and CA92280).

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Bioavailability and pharmacokinetic study of the novel oral C-Seco taxane derivative IDN 5390 in mice

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Background. IDN5390 is the prototype of C-Seco taxanes, a new class of semi-synthetic taxoids. C-seco taxanes are characterized by an opening of C-ring by cleavage of C7-C8 bond. It was selected from a screening of new molecules with antiangiogenic and antimetastatic properties. It has shown high antitumor activity and good tolerability against a variety of human tumor xenografts including ovarian, colon ca and glioblastoma either sensitive or resistant to paclitaxel. The therapeutic advantages of IDN 5390 over paclitaxel were evident when the drug was administered by protracted oral-treatment schedules.
Aims: To characterize the pharmacokinetic of IDN 5390 after single and repeated administration in mice, we have determined the bioavailability, tissue distribution, faecal and urinary excretion and the *in vitro* (hepatic microsomes) and *in vivo* metabolism.
Materials and Methods. The study was carried out in CDF1 female mice treated with single intravenous and oral doses of 60, 90 and 120 mg/Kg or, for one week with protracted oral daily exposure of 90 mg/Kg. Blood, urine, faeces and tissue samples were taken at different time points. IDN 5390 were determined by HPLC with UV detection in plasma and tissues and by HPLC/MS/MS in urine, faeces and microsomes.