pediatric malignancies. It is composed of 35 pediatric oncology clinical centres in five European countries for early clinical trials and of 9 laboratories for preclinical evaluation of targeted anti-cancer compounds in pediatric cancer models. The aim of the preclinical ITCC biology program is to prioritize compounds for clinical development on the basis of in vitro/in vivo activity and relevance of biological targets. PM02734 is depsipeptide produced by chemical synthesis, which has in vitro growth-inhibitory properties against several adult tumor types in the low micromolar range, and low nanomolar range for prostate cancer. PM02734 is currently being evaluated in two phase I clinical trials with different dosing schedules. The objective of our study was to assess the in vitro efficacy of PM02734 in pediatric tumour models.
Material and Methods: The in vitro cytotoxicity of PM02734 was screened by the MTS-assay on a panel of 24 pediatric tumor cell lines, composed of 4 cell lines for each of the following tumor types: Ewing sarcoma, acute lymphocytic leukemia, medulloblastoma, neuroblastoma, osteosarcoma, and rhabdomyosarcoma. Cells were exposed for 72 h to PM02734 concentrations ranging from 1.26 pmol/l to 12.6 μmol/l. Experiments were performed thrice and in triplicate. GI50 was considered as parameter of growth inhibition, whereas LC50 represents cytotoxicity.
Results: PM02734 significantly though moderately reduced the growth and cell viability of all cell lines in a dose-dependent manner. The most sensitive lines were within osteosarcoma and rhabdomyosarcoma with some cell lines showing GI50s below 1 μmol/l. The LC50 values ranged from 3.0 to 15.4 μM. The mean±SD LC50 values were 10.2±3.0 μM in Ewing sarcoma, 11.9±1.3 μM in ALL, 10.9±3.7 μM in medulloblastoma, 11.0±0.8 μM in neuroblastoma, 10.9±5.5 μM in osteosarcoma and 9.6±2.0 μM in rhabdomyosarcoma, respectively.
Conclusions: PM02734 is cytostatic and cytotoxic against pediatric tumor cell lines in vitro at micromolar concentrations, with osteosarcoma and rhabdomyosarcomas being the most sensitive cell lines.

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In vitro and in vivo antitumor activity of novel aureolic acid analogues generated by metabolic engineering of the biosynthetic pathways in *Streptomyces argillaceus* and *Streptomyces griseus* subsp. *griseus*

A. Malek[1], L.E. Núñez[2], N. Menéndez[2], F. Morís[2], G.M. Carbone[1], J. Rohr[3], C. Méndez[4], J.A. Salas[4], C.V. Catapano[1]. [1]*Oncology Institute of Southern Switzerland, Experimental Oncology, Bellinzona, Switzerland;* [2]*EntreChem SL, Oncology, Oviedo, Spain;* [3]*University of Kentucky, Pharmaceutical Sciences, Lexington, USA;* [4]*Universidad de Oviedo, Biologia Funcional and IUOPA, Oviedo, Spain*

Background: Aureolic acids, like mithramycin (MTM) and chromomycin (CMM), are bacterial natural glycosylated polyketides that interact in a non-intercalative manner with DNA at GC-rich sites, inhibit binding of the GC-rich DNA binding Sp1 transcription factor, and have potent antitumor activity. MTM and CMM are interesting leads for discovery of new compounds that might be active against tumors in which Sp1 is overexpressed or overactive. Genetic engineering of the aureolic acid metabolic pathway in the producer strains *S. argillaceus* and *S. griseus* subsp. *griseus* can yield derivatives with modified polyketide-derived or deoxysugar side chains that might have improved anti-Sp1 activity and better pharmacological and toxicological properties.
Methods: MTM and CMM derivatives were produced by targeted inactivation of key genes in the producer strains and purified by HPLC. Biological activity of the new compounds was assessed in vitro in a panel of human cancer cell lines and normal cells using cell proliferation and viability assays (e.g., MTT and clonogenic assays). In vivo antitumor activity was assessed in subcutaneously implanted human tumor xenografts in nude mice following i.v. injections of the compounds using different doses and schedules of administration. Toxicity and pharmacokinetics was evaluated in CD1 mice.
Results: In vitro assays identified MTM and CMM analogues with potency comparable or superior to the parent compounds. New analogues (i.e., CMM-SK, CMM-SDK and DMC-A3, MTM SK and MTM-SDK) inhibited cancer cell growth and viability with IC50 $\leqslant$25 nM. Other derivatives (e.g., DDAC-A3, PC-A4, PC-A4C and PC-A3) exhibited antiproliferative activity only at >10-fold higher concentrations (IC50, $\geqslant$250–500 nM). Active analogues were generally less toxic in vitro to normal fibroblasts than cancer cells, suggesting an improved therapeutic index compared to the parent compounds. Selected compounds (i.e., MTM-SK and MTM-SDK) were tested in human tumor xenografts in nude mice and induced delayed tumor growth or tumor regression in different tumor models.
Conclusions: Metabolic engineering of the biosynthetic pathway of aureolic acids is a powerful approach to generate new "unnatural" compounds with diverse structures and improved properties. Using this approach we have identified MTM and CMM analogues with promising activity in a variety of in vitro and in vivo models exhibiting antitumor activity and low toxicity. These new analogues might be very effective agents to treat cancer and other conditions with abnormal activity of Sp1 and GC-rich DNA binding transcription factors.

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New clerodane diterpenes from *Casearia capitellata* as potential antitumour agents

J. Stanslas[1], G. Bagalkotkar[1], S.C. Tang[2], A.S. Hamzah[3], K. Shaari[4], N.H. Lajis[4], M.S. Saad[5]. [1]*Universiti Putra Malaysia, Faculty of Medicine and Health Sciences/Institute of Bioscience, Serdang, Malaysia;* [2]*Universiti Putra Malaysia, Faculty of Medicine and Health Sciences, Serdang, Malaysia;* [3]*Universiti Technology MARA, Faculty of Applied Science, Shah Alam, Malaysia;* [4]*Universiti Putra Malaysia, Institute of Bioscience, Serdang, Malaysia;* [5]*Universiti Putra Malaysia, Faculty of Agriculture, Serdang, Malaysia*

Background: *Casearia capitellata*, a medicinal herb was investigated for its anticancer phytochemicals owing to the fact that an extract of the plant displayed potent cytotoxicity against *in vitro* tumour cell lines.
Materials and Methods: Silica column chromatography was used to isolate and purify bioactive compounds from the crude extracts. Various spectroscopic techniques (^{1}H/^{13}C NMR, FT-IR, LC-MS, HRMS) were used to elucidate the structures of the isolated compounds. MTT cytotoxicity assay was performed to assess the *in vitro* growth inhibitory properties of extracts and compounds.
Results: Cytotoxicity-guided fractionation of sequentially extracted dichloromethane, ethyl acetate and methanol extracts of *C. capitellata* resulted in the isolation of one pentacyclic terpenoid 7α-acetoxy-hop-12(13)-en-11-one (**1**), three coumarin derivatives, 5-methoxy-7-hydroxycoumarin (**2**), 5-methoxy-7-β-D-glucopyranosylcoumarin (**3**), 5,7-dimethoxycoumarin (**4**), and two new clerodane diterpenes, casearine-A (**5**), and casearine-B (**6**). The isolation of **5** and **6** has never been reported from natural products before, whereas the isolation of **1**, **2**, **3**, and **4** is the first report from this genus. The isolated compounds were tested for cytotoxic effect against breast (MCF-7), lung (NCI-H460) and prostate (DU-145) cancer cell lines. Clerodane diterpenes **5** and **6** exhibited strong antitumour activity against MCF-7 and DU-145 cell lines with IC_{50} values ranging 2.0–4.2 microM. The compounds also exhibited cytotoxic activity against NCI-H460 cells with the IC_{50} values of 27.2 and 16.9 microM, respectively.
Conclusions: Compounds **5** and **6** were more selective towards breast and prostate cancer cells as compared with lung cancer cells. Therefore, these compounds are potential lead molecules for future antitumour studies to discover prospective clinical candidates for the treatment of breast and prostate cancers.

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New antitumour agents from *Phyllanthus pulcher*, a tropical medicinal plant

J. Stanslas[1], G. Bagalkotkar[2], S.C. Tang[2], A.S. Hamzah[3], K. Shaari[4], N.H. Lajis[4], M.S. Saad[5]. [1]*Universiti Putra Malaysia, Faculty of Medicine and Health Sciences/Institute of Bioscience, Serdang, Malaysia;* [2]*Universiti Putra Malaysia, Faculty of Medicine and Health Sciences, Serdang, Malaysia;* [3]*Universiti Technology MARA, Faculty of Applied Science, Shah Alam, Malaysia;* [4]*Universiti Putra Malaysia, Institute of Bioscience, Serdang, Malaysia;* [5]*Universiti Putra Malaysia, Faculty of Agriculture, Serdang, Malaysia*

Background: In a previous study to identify local herbs with *in vitro* antitumour properties, *Phyllanthus pulcher* was found to display a remarkable cytotoxic activity against various tumour cell lines. In this study the bioactive compounds were isolated and purified from the dried aerial parts and roots of the plant.
Materials and Methods: The plant parts were sequentially extracted with dichloromethane (DCM) and methanol (MeOH). Silica column chromatography was used to isolate and purify the bioactive compounds. Various spectroscopic techniques (1H/13C NMR, FT-IR, LC-MS, HRMS) were used to elucidate the structures of the compounds. The extracts and compounds were tested for cytotoxic effect against three human tumour cell lines representing tumours of the breast (MCF-7), lung (NCI-H460) and prostate (DU-145) using MTT assay.
Results: The DCM extract of the aerial parts exhibited potent cytotoxic activity as compared with the MeOH extract. Stigmast-5-en-3-ol-oleate (**1**), diisobutyl adipate (**2**), β-sitosterol (**3**), 7-tridecanone (**4**), sitosterol-3-O-β-D-glucopyranoside (**5**), a new coumarin derivative, 3,4-dihydroxy-5-methoxy-3′,4′,5′-trihydroxyoxepino-chromene-2-one (**6**) and a new diterpene lactone, phyllanthal-A (**7**) were isolated from the DCM fraction. Investigation on the active DCM extract of *P. pulcher* roots resulted in the isolation of two new pentacyclic triterpenes, 12(13)-dehydro-3α-acetoxyolean-28-oic acid (**8**) and lupanol acetate (**9**) and three