other known compounds including 3α-acetoxy-25-hydroxyolean-12-en-28-oic acid (**10**), glochidone (**11**) and glochidonol (**12**). Among the cytotoxic compounds, **1**, **5**, **10** and **12** were selective toward MCF-7 cells (IC_{50} 17.1–69.2 microM), whereas compound **7** was more active against DU-145 cells (IC_{50} 20.5 microM).
Conclusions: Ability of some of the compounds in exhibiting selective growth inhibition of breast and prostate cancer cells suggests these agents maybe beneficial in the treatment of human breast and prostate cancers.

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Chlamydocin, a HDAC inhibitor identified by Compare analyses in a cellular screen

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Background: Inhibitors of histone deacetylases (HDAC) were shown to be potent anti-proliferative and pro-apoptotic agents. At Oncotest we have developed a cellular profiling screen in which new compounds are being tested in a standard cell line panel consisting of 36 cell lines from all major solid tumor types with a subsequently Compare Analyses. In the search for novel HDAC inhibitors we have screened a collection of 2000 pure compounds derived from natural products.
Methods: 4,000 to 10,000 adherent cells were seeded in 96 well plates, compounds were added at 5 different concentrations one day later and left over for 4 days. The read-out of the assay is propidium iodide-based fluorescence, which is a measure of viable cell number. Based on IC_{50} and IC_{70} values tumor selectivity of test compounds were analysed. In the Compare Analysis the IC_{50} and IC_{70} pattern of the new compounds are compared with the corresponding patterns of about 100 agents with known mechanism of action using Spearman Correlations.
Results: The known HDAC inhibitors show distinct IC_{50} and IC_{70} activity profile in the Oncotest 36 cell line panel. Concentration-dependent antitumor activity was detected for the 5 structurally diverse HDAC inhibitors Depsipeptide (mean IC_{70} = 0.009 μM), M344 (1.7 μM), SAHA (3.9 μM), acetyldinaline (22 μM) and SBHA (61 μM). The benzamide analog acetyldinaline and M344, as well as the three hydroxamic acids M344, SAHA and SBHA showed similar activity patterns. We used this cellular activity pattern to screen pure natural compounds isolated from bacteria and fungi. Amoung 2000 compounds tested, Chlamydocin showed the closest match with HDAC inhibitors. Chlamydocin was originally isolated from the fungus Diheterospora chlamydosporia. Chemically it belongs to a family of hydrophobic cyclic tetrapeptides. Potent anticancer activity was reported in-vitro. Compare analysis revealed significant correlations of Chlamydocin to M344, SAHA, SBHA and acetyldinaline, the spearman rho ranked between 0.75 and 0.61. Chlamydocin was potent with a mean IC_{70} of 0.018 μg/ml. It showed selective activity in 3/4 prostate, in 3/5 NSCLC, 2/3 ovarian cancer cell lines as well as in 2/5 melanomas.
Conclusion: In conclusion, the evaluation of 5 structurally diverse HDAC inhibitors revealed closely related activity profiles in a panel of 36 cell lines. In the Oncotest cell line screen Chlamydocin was found to be highly potent and selective, and that it act as an HDAC inhibitor a property which was published by Scheper et al (JPET 304:881, 2003). This finding demonstrates that our cellular screen with the subsequent Compare Analysis is able to identify inhibitors against targets of high interest for cancer therapy.

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Screening for the inhibitor against filopodia protrusion

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Background: Filopodia, a rod-like cell membrane protrusion, is a morphological marker of metastatic tumor cells. On the other hand, the use of small molecular inhibitors has potential benefit to dissect the underlying cellular processes in cancer. In this study, we attempted to obtain the inhibitor against filopodia protrusion as analytical tool of metastatic tumor cells.
Material and Methods: Filopodia protrusion: filopodia was induced by Epidermal Growth Factor (EGF) stimulus in human epidermis carcinoma A431 cells. After 30 minutes, filopodia protrusion was observed under microscopy. Screening source or compounds were treated 30 minutes prior to the EGF stimulus. Intracellular ATP: cellular ATP levels were quantitated by ATP assay kit (Sigma). Metabolome analysis: metabolites in cells were collected by methanol extraction. Amounts of each metabolite were quantitatively analyzed by CE-MS system. Glucose uptake: cells were treated with 2-[^{3}H]deoxyglucose, washed and lysed. Radioactivity was counted by Tri-Carb (Perkin-Elmer). And following compounds are additionally used: rotenone, antimycins, and oligomycins.
Results: In the course of screening, we found that glucopiericidin A (GPA) strongly inhibited filopodia in combination with the inhibitors of mitochondrial respiratory chain complexes (MRCIs). Under this condition, we also found that cellular ATP levels were dramatically decreased. Since the process of actin polymerization in filopodia depends on the ATP-energy, it is likely that the decrease of cellular ATP levels caused the inhibition of filopodia protrusion in cells co-treated with GPA and MRCIs. On the other hand, it is well known that inhibition of both glycolysis and mitochondrial oxidative phosphorylation processes results in marked decrease in ATP levels. Thus, we hypothesized that GPA would be a glycolysis inhibitor. To examine this possibility, we conducted metabolome analysis and found that cellular levels of lactate and pyruvate were decreased by the treatment with GPA. Moreover, we found that GPA inhibited cellular incorporation of glucose, indicating that GPA inhibits glycolysis. Meanwhile, malignant tumor cells located within solid tumors possess higher glycolytic capacity because tumors in this region are distant from blood vessels and lack of oxygen, and thereby, mitochondria respiration is limited. This forces them to activate glycolysis to survive. Therefore, we examined whether GPA affects tumor cell viability when mitochondria is suppressed by MRCI. As a result, GPA synergistically induced cell death in A431 cells with MRCI. Therefore, it is likely that GPA, an inhibitor of glycolysis would be effective against the viability in tumor cells.
Conclusions: We identified GPA as a glycolysis inhibitor and suggested that GPA would be a potential candidate for cancer chemotherapy.

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Fusicoccin derivative (ISIR-005) suppresses anchorage-independent growth of cancer cells through anoikis activation

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Cotylenin A, which has a diterpenoid tricarbocyclic skeleton, was isolated as a plant growth regulator, and has been shown to affect several physiological processes of higher plants and to have differentiation-inducing activity in several myeloid leukemia cell lines. Cotylenin A also affected the differentiation of leukemic cells that were freshly isolated from acute myeloid leukemia patients in primary culture. Injection of the human promyelocytic leukemia cell line NB4 into mice with severe combined immunodeficiency resulted in the death of all mice due to leukemia. Administration of cotylenin A significantly prolonged the survival of mice inoculated with retinoid-sensitive and -resistant NB4 cells without no appreciable adverse effects. Combined treatment with interferon-alpha and cotylenin A significantly inhibited the growth of human lung cancer cells as xenografts without apparent adverse effects. These results suggest that cotylenin A is useful in therapy for leukemia and some other malignancies. However, cotylenin A is difficult to apply to clinical study, since the supply is very limited and it has an epoxide-ring. For clinical application, in the present study, we aimed to synthesize various derivatives from fusicoccins, which are closely related to cotylenin A and are able to be supplied in a large amount as metabolites of phytopathogenic fungus (and examined their differentiation-inducing effects). Although natural fusicoccins did not induce differentiation of myelomonocytic leukemia cells, we synthesized several fusicoccin derivatives with differentiation-inducing activity and without epoxide-ring, based on the structure–activity relationship of cotylenin derivatives. We found some effective derivatives and ISIR-005 was the most potent at inducing differentiation of leukemia cells. Although a low concentration of ISIR-005 hardly affected cell proliferation of lung carcinoma A549 cells, it effectively inhibits anchorage-independent growth and migration of the cells. The drug restored the sensitivity of cancer cells to anoikis. Enhanced anoikis appears to be mediated in part by modulated function of Bcl-2 family proteins.

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Leucinostatins suppress prostate cancer cell growth through the tumour-stromal cell interactions

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The tumor-stromal cell interactions play an important role in the growth and metastasis of tumors through diffusible factors and cell-cell adhesion. Modulation of the tumor-stromal cell interactions could result in the suppression of tumor growth and metastasis. We have therefore been studying the tumor-stromal cell interactions of prostate cancer and searching for the modulators of the interactions. We designed a coculture system of prostate cancer cells and prostate stromal cells (PrSC) and we recently found that IGF-I secreted from PrSC regulates the growth of prostate cancer. The small molecules that inhibit the growth of prostate cancer cells in coculture with PrSC will become new type anticancer