growth kinetics and 48−72 hours for functional assays, allows analysis of several parameters simultaneously. U87MG and MDA MB 231 show normal growth kinetics which are inhibited in a dose-dependent manner using 17-AAG. Both models also showed significant migration and invasion mirroring their in-vivo behaviour. Furthermore, MDA MB 231 MTS migration and invasion was inhibited with sub GI50 doses of 17-AAG. Direct comparison of cell viability (CellTiterGlo assay) following drug treatment in 2D and 3D showed that both tumour models are more resistant to 17-AAG in 3D. A method to analyse protein expression was optimised using the MDA MB 231 MTS and characteristic client depletion following HSP90 inhibition was demonstrated.
Conclusions: We provide evidence that the MTS model and derived functional assays are modifiable for a relatively high-throughput format. The reproducibility and simplicity of the assays make them attractive options for drug discovery projects potentially increasing the strength of prediction of in vivo activity.

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Novel Mcl-1 inhibitors for pancreatic cancer therapy

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The anti-apoptotic myeloid cell leukemia protein Mcl-1, a member of the Bcl-2 family proteins, has emerged as a promising therapeutic target. It was demonstrated that Mcl-1 is an important survival factor for pancreatic cancer cells; its down-regulation with siRNA for example, enhances the induction of apoptosis, chemosensitivity and radiosensitivity of pancreatic cancer cells. Therefore targeting Mcl-1 to overcome apoptosis resistance is an important strategy for the development of new drugs to treat pancreatic cancer.
Through high throughput screening approach we have identified several promising lead compounds which bind to the BH3 binding site in Mcl-1 selectively over Bcl-2 and Bcl-xL, and disrupt interactions between Mcl-1/Bid BH3 peptide and Mcl-1/Bax protein. We have synthesized several analogues and established initial structure–activity relationships. The novel synthetic analogue E288 is the most potent compound with K_i = 400 nM, 10 times more potent than the identified hits. NMR spectroscopy demonstrates that E288 binds to the same BH3 domain of Mcl-1 as the Bim BH3 peptide and antagonizes Mcl-1, inhibiting cell growth and inducing apoptosis in pancreatic cancer cells with high Mcl-1 levels (BxPC-3 and Panc-1) in a time and dose-dependent manner. By using murine embryonic fibroblasts (MEFs), wild type and deficient in both Bax and Bak (double knock out), it was demonstrated that the cytotoxic activity and induction of apoptosis by several analogues, depend on Bax and/or Bak, suggesting that they function as BH3 mimetics. Furthermore, the observed induction of apoptosis was Mcl-1 dependent demonstrated through applying siRNA approach, where the transient suppression of Mcl-1 abrogated E288 mediated apoptosis in both BxPC-3 and Panc-1 cell lines.
Collectively, these findings provide good promise for further chemical modifications of this compound and further optimization toward developing a new class of anticancer drugs, Mcl-1 inhibitors.

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Combination drug screening at the NCI

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A hurdle in selection of combinations to test in clinical trials is the complexity of signaling networks, feedback loops, and incomplete knowledge about how these are affected by the myriad genetic and epigenetic variations present in cancer. As a pragmatic approach to overcoming the challenges in choosing drug combinations, we have recently initiated an *in vitro* combination drug screen that accomodates testing of rationally designed choices, but also allows for serendipity. The screen utilizes 3 cell lines, chosen from the NCI-60 panel for diversity of their molecular characteristics. The non-small cell lung cancer cell line A549 is near triploid, with mutations in CDKN2A, KRAS and STK11. HCT-116, a colon cancer cell line has a nearly normal 2N karyotype, with microsatellite instability, and mutations in BRCA2, CDKN2A, CTNNB1, FGFR2, KRAS, MLH1 and PIK3CA. The final line in the screen is the prostate cancer cell line PC-3, which is a near tetraploid with many chromosomal rearrangements, and is mutant for TP53 and PTEN. Agents being considered for combination trials (test agents) are assayed in each of these 3 cell lines against a panel of well characterized agents (modifier agents), including recently approved kinase inhibitors and conventional cytotoxic agents. Cells are exposed to drugs for 3 days at 3 concentrations of both the “test” agent and each modifier agent, yielding a 3×3 concentration matrix. A Wilcoxon statistic is used to test the hypothesis that the growth inhibitory activity of the combination is better than that expected if the 2 single agents are independent and additive. The screen has identified a number of promising combinations, including some that would not have been predicted.

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Role of the epithelial phenotype in the sensitivity of pancreatic and breast cancer cell lines to Irvalec; in vitro synergism of the combination with gemcitabine

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Background: Irvalec is a novel marine-derived cyclic peptide belonging to the Kahalalide family of compounds, currently in phase II clinical trials. Epithelial–mesenchymal transition (EMT) is a biological process defining progression from polarised epithelial phenotype to a mesenchymal phenotype, which is distinguished by fibroblast-like features. We have studied the role of EMT markers in the sensitivity to Irvalec in a panel of pancreatic and breast cancer cell lines. Furthermore, we analyzed the combination of Irvalec with gemcitabine, the most widely used chemotherapeutic drug in pancreatic cancer, in the pancreatic cancer cell lines.
Material and Methods: Six pancreatic (BxPC-3, HPAC, AsPC-1, CFPAC-1, PANC-1, MIAPaCa-2) and five breast cancer cell lines (SK-BR-3, BT-474, MDA-MB-468, MCF7, MDA-MB-231) were obtained from the ATCC. Cell viability was measured by a cristal violet assay after treatment for 72 h. Protein expression levels of different EMT markers (E-cadherin, β-catenin, snail, twist-1, slug and vimentin) were analyzed by immunohistochemistry, immunocytochemistry and western blot. The combination of Irvalec and gemcitabine was analyzed using the median effect method of Chou and Thalalay using Calcusyn software program.
Results: All cell lines were tested with Irvalec. IC50 ranges were 0.06−8.7 uM and 0.1−6.5 uM for the pancreatic and breast cancer cell lines, respectively. The most sensitive Irvalec cell lines exhibited an epithelial phenotype (high E-cadherin, low vimentin and high twist-1 expression), whereas the mesenchymal phenotype was observed in the least sensitive cell lines. The potential synergism of the combination of Irvalec with gemcitabine was also evaluated in the panel of six pancreatic cancer cell lines, after treatment for 72 h with the different drugs, as single agents or in combination. The combination of Irvalec and gemcitabine had a synergistic effect at high doses (IC90 concentrations) in all pancreatic cancer cell lines tested, whereas at IC70 concentrations synergism was observed in the three most sensitive cell lines (CI values = 0.79, 081 and 0.85 for BxPC-3, CFPAC-1 and AsPC-1, respectively).
Conclusions: Sensitivity of pancreatic and breast cancer cell lines to Irvalec positively correlates with an epithelial phenotype. The *in vitro* synergism of the combination of Irvalec and gemcitabine provide a rationale for further development of this combination.

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Lithocholic acid competitively inhibits EphA2–ephrinA1 binding: pharmacological and structural considerations

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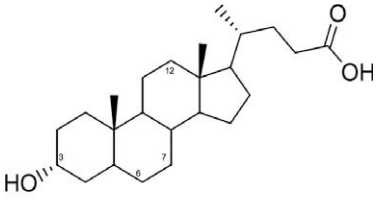
Background: Eph–ephrin system plays a central role during multiple morphogenetic processes and recent data suggest that, in a large variety of human cancers, up-regulated expression and/or de-regulated function of Eph–ephrin system may promote tumorigenesis and the development of a more aggressive and metastatic tumour phenotype.
In particular EphA2 upregulation is correlated with tumour stage and progression and expression of EphA2 in non-transformed cells induces malignant transformation and confers tumorigenic potential. Based on these evidences our aim is to develop small molecules able to modulate EphA2–ephrinA1 activity.

Materials and Methods: In order to find ligands for EphA2–ephrinA1 interaction we have set and performed an ELISA screening assay testing several natural and synthetic bioactive compounds.
Results: Binding studies identified lithocholic acid (LCA) as a competitive and reversible ligand of EphA2–ephrinA1 system in the order of 10 micromolar. Functional studies on HT29 colon cancer cells, naturally expressing EphA2, revealed that LCA dose-dependently antagonized EphA2 phosphorylation induced by ephrinA1-Fc.
In order to clarify the modality of interaction between LCA and EphA2 kinase we tested other naturally occurring bile acids and we synthesized a series of LCA derivatives exploring the positions 6,7,12 and the opposite ends of the molecule represented by the hydroxil group in position 3 and by the carboxyl moiety.
LCA derivatives resulted particularly sensitive to the modulation of the cyclopenta[a]perhydro phenanthrene scaffold. Indeed, the introduction of hydroxyl group in position 7 or 12, always produced inactive compounds, exemplified by the naturally occurring cholic, deoxycholic and chenodeoxycholic acid. Similarly, the introduction of a 6- or 7- keto group resulted detrimental for the binding affinity. The oxydation of the alpha hydroxyl group in position 3 as well as it acetylation led to compounds still able to interfere with EphA2–ephrinA1 system. Esterification or conjugation with taurine of the carboxylic group at the opposite end gave inactive compounds.
Conclusion: This study identified lithocholic acid as a competitive and reversible antagonist of EphA2 receptor. Moreover, it provided the basis for a rational chemical development of molecules targeting this new emerging system in the oncology field based on docking and molecular dynamics.



lithocholic acid (LCA)

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Identification of NPD3483 as a unique cell division inhibitor via the cell morphology-based screen

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Background: Recently, cell phenotypic profiling induced by chemical entities has been developed and recognized as an effective initial step in a small molecule probe- and drug-discovery. However, these approaches require many cellular profiling and specialized bioinfomatics techniques. The comprehensive analyses are too expensive, complicated and time-consuming to select a small number of "interesting" compounds from a large library. On the other hand, the tumor cellular shape undergoes often dynamic change specifically related to the mode of drug action. It prompted us to construct a simple cell morphology database, compiling various cellular phenotypes induced by authentic compounds, which may enable us to discover unique bioactive substances with approximately defined mechanism of action.
Material and Methods: We have examined the effect of 60 well-characterized drugs on cell-shape change in 3 mammalian cell lines (HeLa, tsNRK, and tsFT210 cells) in a time- and dose-dependent manner. Then we investigated the relationship between the induced morphological changes and the mode of action of a drug, allowing to easily discriminate the phenotypes induced by diverse types of drugs, such as cytoskeleton-interfering agents, and the inhibitors of HSP, HDAC and of macromolecular synthesis.
Results: We carried out the screening based on the morphological changes of cancer cells by using chemical libraries deposited in the RIKEN Natural Products Depository, NPDepo. In the course of the screening, we found the carboline derivative, NPD3483, inducing the cell-shape change similar to that of mitotic kinesin Eg5 inhibitors. This compound blocked cell division by affecting the formation of mitotic spindle; however the monoastral phenotype induced by well-known kinesin inhibitors was not observed. These data suggests that NPD3483 exerts its activity through a distinct mode of action from other Eg5 inhibitors.
Conclusions: To discover small drug like molecules, we constructed the cell morphology database, then explored chemical library and finally identified the NPD3483 as a unique cell division inhibitor. Our new screening system classifies well the mode of action of test compounds; moreover, it may provide a new insight into the regulatory mechanisms of the dividing cancer cells.

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High throughput screening of potential anti-cancer agents in primary cell culture using an ATP based luminescence assay

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Background: The development of anticancer agents commonly relies on screening potential drugs against cell lines. However, these show significant differences in their behaviour from tumor-derived cells in primary cell culture or xenografts. Nevertheless, cell lines continue to be used because of their convenience and utility for high throughput screens. We have developed a method which allows high throughput screening of anticancer agents in primary cell cultures.
Materials and Methods: In this study, 5605 plant extracts were each screened in 3 recurrent ovarian tumors. Cells were isolated from fresh tumor samples. These were incubated with 12.5μg/ml drug compound for 5 days in 384 well polypropylene plates (352 compounds per plate) before being lysed and ATP levels measured by luciferin-luciferase assay.
Results: There were 113 positive hits for sensitivity, where greater than 80% inhibition was achieved in all 3 tumor samples. These positive hits were then tested further for luciferase inhibition to rule out any compounds inhibiting the assay's detection system. Thirty-three of the 113 positive hits were excluded on this basis leaving 80 potential compounds to which these tumours were sensitive. Of the 80 hits obtained, 29 had previously been identified as active in a breast cancer cell line (ZR75) screen. Twenty showed toxicity against a fibroblast cell line (HS27) and were excluded from further investigation, leaving 60 extracts with activity of which 13 were previously positive in the breast cancer cell line screen.
Conclusions: This study shows a novel screening approach for large libraries of potential anti-cancer compounds which allows large numbers of agents to be tested against tumor-derived cells where previously the cell numbers required for such studies could not be achieved without use of cell lines. Of those agents selected, 47 were previously unknown from the cell line screen.

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Antitumor activity of NPD723, a novel potent cell differentiation-inducing agent in leukemic cells

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Introduction: Differentiation therapy is one of the promising strategies for treatment of leukemia. Here, we report the biological and antitumor activities of furocoumarin derivative NPD723, which is a newly identified cell differentiation-inducing compound from our chemical library, RIKEN Natural Products Depository (NPDepo).
Methods: Nitroblue tetrazolium reduction assay was used for the screening of cell differentiation-inducing compounds in HL-60 human leukemic cells. The induction of cell differentiation was evaluated by flow cytofluorimetry, RT-PCR, and western blot analyses. The anticancer activity *in vivo* was evaluated in mice xenografted with A549 human lung carcinoma. NPD723 was administered intraperitoneally at 10, 20, or 40 mg/kg on a weekly schedule of qdx5 for 2 weeks, and then tumor volume and body weight change were measured.
Results: Among 6,464 compounds tested, we found furocoumarin derivative NPD723 as the most potent compound. The activity of NPD723 was stronger than that of known differentiation-inducing agents, all-*trans*-retinoic acid (ATRA) and 1α,25-dihydroxy-vitamin D_3 (1α,25$(OH)_2D_3$); the EC_{50} values of NPD723, ATRA, and 1α,25$(OH)_2D_3$ were 0.8, 1.8, and 186.4 nM, respectively. Flow cytometric analysis showed that NPD723 induced the expression of CD11b and CD14, membrane markers of differentiation. NPD723, unlike ATRA or 1α,25$(OH)_2D_3$, did not induce the expression of retinoic acid receptor-β (RARβ) and vitamin D_3-24-hydroxylase, as determined by RT-PCR. These results suggest that NPD723 induces myeloid differentiation of HL-60 cells through a mechanism different from ATRA and 1α,25$(OH)_2D_3$. In addition, NPD723 strongly inhibited cell proliferation of various human cancer cell lines; IC_{50} values were 3.8 nM for HL-60 cells, 5.9 nM for A549 cells, and 9.3 nM for human prostate carcinoma DU145 cells. In the A549 xenograft model, NPD723 significantly inhibited tumor growth without significant loss of body weight.
Conclusions: NPD723 potently induced myeloid differentiation of HL-60 cells, and inhibited tumor growth *in vitro* and *in vivo*. These results suggest that NPD723 might be a novel anticancer agent active against leukemia and lung carcinoma.