mild nitrosative stress caused by TrxR inhibition promoted breast cancer cell growth in a ER positive and intact p53 setting. On the contrary, the severe nitrosative stress caused by exposure to higher doses of CSNO and TrxR inhibition promoted growth arrest in breast cancer cells. Our results suggest that drugs modulating SNO homeostasis are potential therapeutic agents in breast cancer treatment.
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127 POSTER

Identification of CB3, a novel inhibitor of the ubiquitin-proteasome system

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Background/aim: As the ubiquitin–proteasome system (UPS) represents a promising therapeutical target we set out to identify novel UPS inhibitors with tolerable toxicity.
Material and Methods: A chemical library consisting of 10 000 compounds was screened for cytotoxicity in the colon carcinoma HCT 116 cell line using a non-clonogenic 72 hour cytotoxicity assay. Cytotoxic compounds were further investigated for UPS activity in the HEK 293 ZsGreen Proteasome Sensor cell line using an image-based screening assay. Inhibition of the proteasome led to accumulation of the fluorescent protein ZsGreen which was measured using automatic fluorescence microscopy. To validate UPS as the target and to obtain kinetic information of hit compounds, live cell monitoring was performed in the IncuCyte FLR using the ubiquitin sensor cell line MelJuSoUb^{G76V}-YFP which fluoresce when the UPS is inhibited. Microarray-based gene expression analysis was performed on hit compounds to characterize global effects after compound-treatment. To study the effect of hit compounds on normal cells, in vitro toxicity profiling was performed using a bone marrow, epithelial, liver, lymphocyte and renal toxicity assay.
Results: When screening 10 000 substances, 382 showed cytotoxic activity at 25 μM in HCT 116. Of these 382 compounds, one (CB3, Phosphoric acid, 2,3-dihydro-1,1-dioxido-3-thienyl diphenyl ester, figure 1) was identified as an inhibitor of proteasomal activity in the HEK 293 ZsGreen cells. The effect on the UPS was subsequently studied in live cell monitoring where the MelJuSoUb^{G76V}-YFP cells showed a dramatic increase in fluorescence when treated with CB3 compared to control. The global gene expression profile evoked by CB3 was similar to that of the known proteasome inhibitors MG132 and MG262. The toxicity of CB3 in the normal cell systems was relatively low compared to conventional cytotoxic compounds and was favourable compared to the approved proteasome inhibitor bortezomib. Since there are several possible targets in the UPS, detailed mechanistic studies of CB3 are ongoing.
Conclusion: CB3 is a novel inhibitor of the UPS with a promising toxicity profile and will therefore be investigated further.

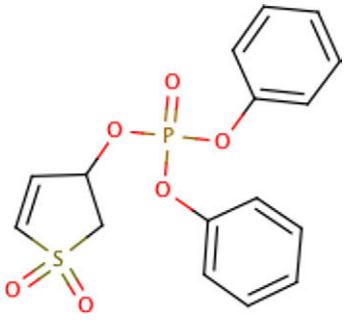

Figure 1. Structure formula of CB3

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GDC-0941 and ABT-737 cooperate to sensitize isolated mitochondria from PI3K mutant cells

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The phosphatidylinositol 3-kinase subunit PIK3 is frequently mutated in human cancers and therefore represents an interesting therapeutic target. Cancer resistance to therapies is often related to expression of anti-apoptotic Bcl-2 family members which negatively regulate mitochondrial cell death. We investigated the influence of PI3K mutations on Bcl-2 family proteins both at the cellular and mitochondrial level. We used cell lines with "knock-in" PI3K mutation (ex. human breast epithelial cells HME-1 PI3K H1047R/+) from which we purified the mitochondria to homogeneity (95%). We first characterized these mitochondria for their sensitivity to reference compounds (Calcium, t-Bid ...) on 3 parameters: swelling, $\Delta\psi$m loss and cytochrome c release. Mitochondrial preparations from wild-type and mutated HME-1 were analysed for their protein pattern in Bcl-2 family members. The PI3K mutation gives a tumoral profile to mitochondria (accumulation of anti-apoptotic Bcl-xL, Bcl-2 and decrease of pro-apoptotic Bim, Bax and Bad) and induces sensitivity to t-Bid. Cell treatment with the PI3K inhibitor GDC-0941 counteracts PI3K mutation by increasing mitochondria-associated pro-apoptotic proteins. Furthermore, these isolated mitochondria become sensitive to the Bcl-2 family inhibitor, ABT-737 suggesting an interesting cooperative effect between PI3K and Bcl-2 inhibitors. Investigations with HCT-116 PI3K +/− cells are currently under investigation to confirm the impact of PI3K mutations in cancer cells.

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ADAM17: A new therapeutic target for triple negative breast cancer?

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Background: Triple negative breast cancer (TNBC) can be defined as tumors lacking expression of ER, PR and HER2. Unlike other subgroups of patients with breast cancer, targeted therapy is currently unavailable for these patients. It has been shown that these cancers possess high levels of EGFR and thus may be dependent on EGFR signaling. Due to the fact that ADAM (a disintegrin and metalloprotease)10 and ADAM17 are involved in the proteolytic release of all EGFR ligands, inhibiting these ADAMs may be a potential therapeutic option for TNBC, either alone or in combination with EGFR-targeted agents.
Materials and Methods: We used the EGFR inhibitor, gefitinib, and an ADAM17 specific inhibitor (Wyeth), to determine their effects on TN cell lines. IC_{50} values were determined (using SoftmaxPro software) by treating cells for 5 days (1×10^3 cells/well) and measuring cell growth using acid phosphatase assay. MDA-MB-435 cells were stably transfected with ADAM17 shRNA (2 clones used) using Nucleofection technology (Lonza).
Results: In MDA-MB-435 cells, ADAM-17 silencing resulted in a decrease in the IC_{50} of gefitinib from 8.34 μM to 6.96 μM and 5.98 μM for clone 1 and clone 2, respectively (Student's paired t-test: $p = 0.004$ and $p = 0.002$), when compared with the IC_{50} for parental MDA-MB-435 cells. These findings suggest that EGFR may be involved in mediating the effects of ADAM17 on proliferation in MDA-MB-435 cells. SUM159PT cells were chosen for further investigation of the involvement of EGFR in mediating the effects of ADAM17 on *in vitro* cellular invasion and proliferation, as they express the EGFR at relatively high levels (gefitinib IC_{50}: 1.01 ± 0.35 μM). IC_{50} of the ADAM inhibitor (AI) in these cells was 6.97 ± 0.26 μM. SUM159PT cells were treated with a combination of gefitinib and AI at a ratio of 1:5, for 5 days. Selective inhibition of ADAM17 showed similar growth inhibitory effects on SUM159PT breast cancer cell lines as the EGFR inhibitor, geftinib. No synergism however, was observed using a combination of the AI and gefitinib. However adding gefitinib 72 hr following AI treatment was more effective than adding both inhibitors simultaneously, though this did not reach statistical significance.
Conclusion: ADAM17 inhibition resulted in similar growth inhibitory response to EGFR inhibition in SUM159PT TNBC cells. Due to the current issues with resistance to gefitinib, ADAM17 inhibition could be pursued as a second-line treatment.
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A role for the cholecystokinin 2 receptor (CCK-2R) in promoting cancer progression

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Background and aims: The gastrointestinal (GI) hormone, gastrin, promotes cancer progression and its down-regulation has been linked to reduced cancer stem cell numbers. Gastrin acts through the cholecystokinin 2 receptor (CCK-2R) and its biological effects are blocked by CCK-2R inhibitors. We investigated a potential role for CCK-2R in promoting survival of cancer stem cells using RNAi combined with a sensitive method to detect CCK-2R mRNA.
Materials and Methods: A panel of cancer cell-lines, including GI, glioblastoma and small cell lung cancer (SCLC), with CCK-2R-transfected