(i.p. 1 mg/kg, twice a week) in a rat chondrosarcoma model. The therapeutic efficiency of RAD001 and of the combination RAD001/adryamicin was evaluated using chondrosarcoma volume evolution (MRI), tumor necrosis percentage, tumor MVD quantification analysis between the treated and control groups.
We showed that in comparison to adriamycin, RAD001 significantly inhibited tumor growth progression (tumor doubling time of 33 days and 7 days respectively for RAD001 treated-tumors and control ($p < 0.01$)) and that targeting mTOR along with chemotherapy treatment did not exhibited additive antitumor effects in vivo when compared with RAD001 as single agent (adriamycin, RAD001 and RAD001+Adriamycin induced respectively tumor inhibition rate of: 43%; 74% ($p < 0.01$); 52% ($p < 0.05$)(. No histological differences or treatment-induced necrosis could be observed between the different groups. RAD001 inhibited the phosphorylation of mTOR and S6 and did not alter the activation of Akt.
Taken together, our preclinical data indicate that RAD001 alone has a beneficial effect *in vivo* on chondrosarcoma tumor progression and may be effective as single agent in treating chondrosarcoma patients.

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Novel small-molecule inhibitors of Interleukin-6 (IL-6) signalling

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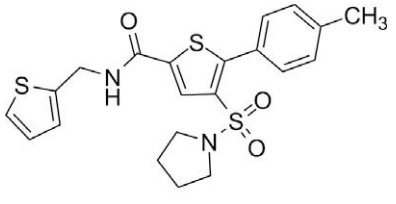
Interleukin-6 (IL-6) is a pro-inflammatory cytokine that plays a key role in the pathophysiology of several cancers. It binds to the IL-6 receptor (IL-6R), inducing dimerisation of the gp130 complex, and initiating signalling through the STAT3 pathway. For example, its over-expression is implicated in the pathology of multiple myeloma, renal cell, prostate, cervical and breast carcinomas, and the discovery of IL-6 inhibitory agents could lead to new classes of anti-cancer drugs.
As part of a drug discovery programme aimed at identifying small-molecule inhibitors of this pathway, a library of arylsulphonamidyl thiophene amides has been prepared through an efficient 4-step synthetic route. Library members were evaluated in a standard MTS assay to evaluate cell viability in STAT3-dependent MDA-MB-231 breast cancer cells, in which STAT3 signalling is IL-6 stimulated. In parallel, the compounds were also evaluated in STAT3-null A4 cells as a control. These experiments identified the "hit" arylsulphonamidyl thiophene amide RH06 (Figure 1) which had apparent selective inhibitory activity at the low micromolar level, with the ability to reduce the viable cells in the RH06-treated MDA-MB-231 line by ~40% compared to control. Furthermore, the high percentage of viable cells remaining after carrying out a trypan blue exclusion assay in the same cell lines indicated that RH06 is cytostatic rather that cytotoxic. Next, a luciferase reporter assay was used to evaluate the selectivity of RH06 towards the STAT3 promoter in HeLa cells using a SV40 promoter as control. The STAT3 promoter-luciferase cells were treated with Oncostatin M as an IL-6 mimic to activate STAT3 signalling *via* the IL-6/gp130 receptor. In these experiments RH06 showed demonstrated selective inhibition of STAT3 transcriptional activity with an EC_{50} in the 1 μM range. Finally, Western Blot studies of the effect on RH06 on STAT3 protein levels in MBA-MD-231, Hela and CT26 cells showed that it inhibits STAT3 phosphorylation which may account for its overall mechanism of action at the cellular level.



RH06

Figure 1: Structure of RH06

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The dual PI3K/mTOR blocker NVP-BEZ235 sensitizes cancer cells against irreversible ErbB inhibitors

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Epidermal growth factor (EGF) receptor-related antigens (EGFR, ErbB1−4, HER1−4) represent emerging drug targets in oncology. However, resistance against ErbB-targeting drugs occurs frequently in cancer patients. Drug-resistant cells may exhibit drug-refractory phosphatidylinositol 3-kinase (PI3K) or mitogen-activated protein kinase (MAPK) signaling, but the relative impact and contribution of these two downstream pathways to drug resistance are still controversially discussed. We examined the effects of the two very potent, irreversibly binding ErbB receptor tyrosine kinase inhibitors (RTKIs) pelitinib (EKB-569) and canertinib (CI-1033) on PI3K- and MAPK activity in ErbB RTKI-sensitive and ErbB RTKI-resistant breast and ovarian cancer cells. Western blot analysis revealed that ErbB phosphorylation was abrogated by the inhibitors in both drug-sensitive and drug-resistant cells, whereas AKT- and GSK3b phosphorylation were drug-dependently downregulated only in drug-sensitive cells. ErbB RTKI sensitivity did not correlate with expression of wildtype PTEN or PIK3CA, nor was it associated with drug-dependent silencing of ERK1,2 in the breast and ovarian cancer cell lines examined. Moreover, exogenous AKT, but not MEK, significantly induced drug resistance. Our data demonstrate that blocking AKT phosphorylation is essential and sufficient, whereas abrogation of ERK phosphorylation is not required for ErbB RTKI anticancer efficacy. AKT phosphorylation may thus be a useful biomarker of ErbB RTKI sensitivity in breast and ovarian cancer cells. Supported by 'Medical Scientific Fund of the Mayor of the City of Vienna' (#08037) and 'Initiative Krebsforschung' Medical Univ. Vienna.

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Discovery and characterization of PI3Kbeta isoform-selective inhibitors

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Background: Abnormal PI3K pathway activation plays a major role in cancer, as a result of either RTK activation and/or somatic mutations of major components of the pathway, including activating point mutations and amplification of the *PIK3CA* gene, as well as loss of negative regulatory proteins such as PTEN. Most of the ATP-competitive PI3K inhibitors currently in clinical development inhibit all class I PI3K isoforms: however, several recent reports support the development of isoform-specific inhibitors. In particular, while PI3Kα specific inhibitors are predicted to inhibit growth of tumors with PIK3CA mutations, PTEN-deficient tumors have been shown to depend on PI3Kβ. In addition, isoform specific PI3K inhibitors may exhibit better safety profiles compared to pan-selective PI3K inhibitors, and thus be more suitable to combine with other targeted or cytotoxic therapies.
Methods: ATP-competitive inhibitors with selectivity for PI3Kβ were identified via high-throughput screening and optimized using a structure-based design approach. Biochemical activities against the different PI3K isoforms were measured using a HTRF assay. Compound effects on AKT phosphorylation were measured in different cell lines using western blotting or Meso Scale Discovery multi-array techniques.
Results: PI3Kβ-selective inhibitors with biochemical IC50 values below 100 nM and good selectivity over other PI3K isoforms and a diverse panel of protein and lipid kinases were identified. Cellular assays demonstrate that PI3Kβ compounds potently inhibit phosphorylation of AKT (cellular IC50s <300 nM) in *PTEN*-deficient tumor cell lines. Consistent with its biochemical selectivity profile, the most PI3Kβ-selective inhibitors were inactive on AKT phosphorylation in PI3Kα-activated tumor cells. *In vivo* pharmacodynamic analyses following oral administration of these isoform-selective inhibitors to mice bearing xenografted tumors demonstrated dose-dependent inhibition of phosphorylation of PI3K downstream effectors at well-tolerated doses.
Conclusions: Novel PI3Kβ-selective small molecular mass inhibitors were identified and characterized *in vitro* and *in vivo* for PI3K pathway modulation in the context of different activating genetic abnormalities. Cellular selectivity for *PTEN*-deficient tumor cells versus PI3Kα-activated tumor cells was demonstrated. These preclinical results support the development of isoform-selective PI3Kβ inhibitors for the treatment of cancer patients harboring tumors with *PTEN*-deficient specific genotypes.