Importantly, a highly orally available analogue of **MI-63** regresses tumors in multiple xenograft models of human cancer with wild-type p53 at dose-schedules that cause no toxicity to mice.
Conclusions: Our data show that **MI-63** and its potent analogues represent a class of highly promising new anticancer agents and warrant clinical evaluations for the treatment of many different types of human cancers.

165 POSTER

Furan ring-opened 17-hydroxywortmannin analogs as phosphoinositide 3-kinase inhibitors active in human tumor xenograft models

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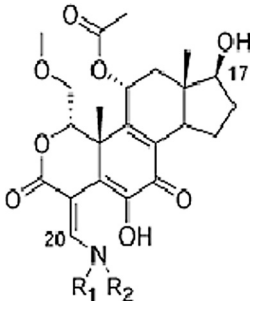
Phosphoinositide 3-kinase (PI3K) is an important target for cancer chemotherapy due to the deregulation of its signaling pathway in a wide spectrum of human tumors. Wortmannin and its analog, 17-hydroxywortmannin (17-HWT), are potent PI3K inhibitors whose therapeutic use has been impeded by inherent defects such as instability and toxicity. Secondary amines react with 17-HWT at the C-20 position to generate furan ring-opened analogs, as shown in the structural formula, with improved properties such as increased aqueous solubility, greater stability, and less toxicity, as evidenced by a higher therapeutic index. 17-HWT is available by the stereoselective sodium borohydride reduction of the natural product wortmannin, available from fermentation. Ring-opened analogs were tested versus the PI3K enzyme (alpha isoform) and in tumor cells deficient in the tumor suppressor gene PTEN (e.g. LNCap). The most potent analogs were then evaluated in human tumor xenograft models in the nude mouse. A variety of different amines (and other nucleophiles) were used containing varying lipophilic and polar groups. Structure activity relationships among these resulting analogs will be presented. In conclusion, ring opening of 17-HWT with secondary amines gives compounds with improved properties (e.g. better stability and reduced toxicity) that are active in human tumor xenograft models in the nude mouse.



166 POSTER

ADME and PK/PD attributes of SB939, a potent orally active HDAC inhibitor

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Histone deacetylase (HDAC) inhibitors are an emerging class of therapeutic agents that induce tumor cell cytostasis, differentiation and apoptosis in various hematological and solid malignancies. They are believed to exert their anti-tumor activity through chromatin remodeling and gene expression modulation that affect cell cycle and survival pathways. In the literature, there is very little information on the ADME attributes of the HDAC inhibitors and their pharmacokinetics in preclinical species. In our HDAC program, *in silico*, *in vitro* and *in vivo* ADME (Absorption, Distribution, Metabolism and Elimination) studies are incorporated in the screening cascade with a view to discovering compounds with optimal ADME properties. These ADME results provided insight to the medicinal chemists in the lead optimization process and led to the identification of SB939 as a promising drug candidate. SB939 exhibits high metabolic stability in human liver microsomes with no major inhibition or induction of major drug metabolizing cytochrome P450 isozymes. The compound has good aqueous solubility and shows high cell permeation in the Caco2 screen. The pharmacokinetic parameters of SB939 were better than the other HDAC inhibitors in clinical trials. In a preclinical tumor model, the superior pharmacokinetic parameters of SB939 correlated well with effects on a pharmacodynamic marker (H-3 acetylation) as well as anti-tumor efficacy.

167 POSTER

A pyrazyne scaffold for the generation of novel inhibitors of B-RAF

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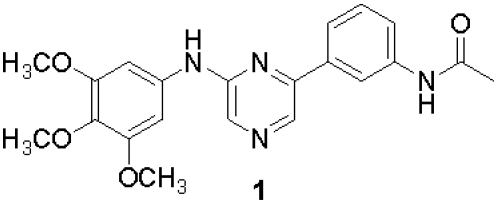
B-RAF is a serine/threonine kinase mutated in 7% of cancers, with a incidence of 70% of melanomas. Mutated B-RAF is oncogenic and expression of the activated mutant V600E leads to increased proliferation and survival of malignant cells. As a result of a Biofocus high-thoughput screen of a 23,000 compound chemical library, 2-(3,4,5-trimethoxy-phenylamino)-6-(3-acetamidophenyl)pyrazine **1** was identified in as a B-RAF inhibitor (IC_{50}(B-RAF) = 3.5 μM). Medicinal chemistry around the trimethoxy phenyl ring identified several sub-micromolar B-RAF inhibitors, for example the 5-oxazolylphenyl analogue **2** (IC_{50}(B-RAF) = 800 nM). The compounds were screened for cellular activity: phospho-ERK inhibition and antiproliferative effects. Valuable SAR data were obtained for the development of this novel class of pyrazine-based compounds as B-RAF inhibitors.



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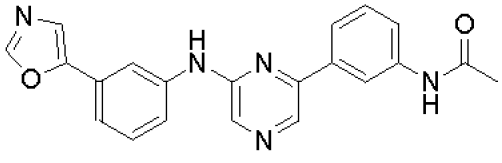

2

168 POSTER

Design, synthesis and evaluation of novel, selective carbonic anhydrase IX inhibitors as anti-cancer agents

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Carbonic anhydrases (CA's) are a family of Zn-containing isozymes. CA's catalyse the hydration of carbon dioxide to proton and bicarbonate and are primarily involved in pH homeostasis. Of the 15 different isozymes known to date, CAIX has been identified as being tumour-specific, where it is over-expressed in many solid tumours (Zavada, et al, 1992). The role of CAIX in tumour has been attributed to poor prognosis and increased metastasis, which may be due, in part, to the increased acidification of the extra-cellular milieu. It is proposed that selectively inhibiting CAIX decreases the metastastic incidents and increases the uptake of conventional anticancer agents specifically into solid tumours (Stubbs, et al, 2000).
Here, we report synthesis and enzymatic evaluation of a select series of non-toxic, but potent CAIX inhibitors. Initially, a 3D homology model of CAIX was constructed (48% sequence identity) using the Swiss model protocol (Peitsch, et al, 1993). The compounds were then designed based on the skeletal structure of known potent CA inhibitors and on the active site geometry. The synthesised compounds were evaluated using a HTS screen against purified human CAI, CAII and CAIX protein (René, et al, 2005).
Many of compounds synthesised showed good inhibition towards the CAIX protein (micro-molar range). Two compounds, in particular, 2-nitro-N,N-bis(2-chloroethyl)benzenesulfonamide (IC50 [CAI: 776 μM]; [CAII:

854 μM]; [CAIX: 0.0791 μM]), and 3-nitro-N,N-bis(2-chloroethyl)benzenesulfonamide (IC50 [CAI: 317 μM], [CAII: 568 μM], [CAIX: 0.0872 μM]) showed approximately 10,000-fold increase in selectivity towards the CAIX (when compared to CAI and CAII). This enhancement of selectivity may be due to increased binding of the compounds to the active site residues of CAIX (such as Thr193, Thr194 and Zn). The lead compounds are currently being evaluated in combination with cytotoxic anticancer agents in variety of tumour models.

Drug resistance and modifiers

169 POSTER

Sensitisation of neuroblastoma tumours to chemotherapy by use of a novel class of MRP1 small molecule inhibitor

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Neuroblastoma is the most common solid tumour in children under five. Despite recent advances in chemotherapy that have improved the survival rates of other cancers, outcomes for children with this disease remain poor. Aggressive drug refractory tumours often display amplification of the *MYCN* oncogene. Previous results in our laboratory have implicated expression of the Multidrug Resistance-associated Protein (*MRP1*) gene as a powerful prognostic marker in neuroblastoma and also provided evidence that MYCN regulates expression of *MRP1* (*NEJM*, 334:231–8, 1996; *Oncogene*, 23:753−62, 2004; *J Clin Oncol*, 24:1546−53, 2006). To investigate the role of MRP1 *in vivo*, *MYCN* transgenic mice that develop neuroblastoma were crossed with *MRP1* knockout mice to yield tumours either wild type or null for the *MRP1* gene. Engraftment of these tumours into Balb/c *nu*/*nu* mice and treatment with chemotherapy demonstrated that knocking out *MRP1* leads to significantly increased sensitivity to MRP1 substrate drugs. Since clinically relevant modifiers of MRP1 are scarce, we devised a unique cell-based readout system in order to identify small molecule inhibitors of MRP1. The readout system utilized a p53-responsive reporter as a sensor of drug accumulation in cells and was used to screen a 2300 compound small molecule library that had been enriched for modulators of multidrug transporters (*PNAS* 98:14078−83, 2001). Following primary and secondary screening, we have identified several candidate MRP1 inhibitor molecules encompassing four distinct structural classes. These molecules were found to greatly sensitize tumour cells overexpressing MRP1, beyond the levels of known transporter modulators. One compound, 4H10, was investigated *in vivo* for its ability to sensitise tumours to conventional chemotherapy. 4H10, in combination with vincristine (VCR) or etoposide, increased the sensitivity of neuroblastoma tumours to these conventional chemotherapeutic agents in *MYCN* transgenic mice. Similar sensitisation was also observed with human neuroblastoma cells xenografted into nude mice. Initial biodistribution experiments suggest 4H10 increases retention of VCR in the tumour compared to other tissues. This study has therefore identified a novel class of MRP1 small molecule inhibitor that has the potential to be used in the treatment of neuroblastoma.

171 POSTER

Inhibition of the drug-resistant T315I mutant of BCR-Abl

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BCR-Abl is responsible for the proliferation of leukemic cells in the majority of patients with chronic myelogenous leukemia (CML). Imatinib, which inhibits BCR-Abl, is commonly used to treat patients with BCR-Abl positive disease. However imatinib-resistant mutations of BCR-Abl have been widely observed clinically, including the T315I "gate keeper" mutation. It has been shown that this mutant is also resistant to all second-generation BCR-Abl inhibitors, including dasatinib and AMN107.
EXEL-2280 (XL228) is a potent small molecule inhibitor of BCR-Abl which exhibits low nanomolar IC50 values for inhibition of wild-type and imatinib-resistant mutant forms, including T315I and E255K, in biochemical assays. EXEL-2280 also potently inhibits BCR-Abl autophosphorylation and subsequent activation of CrkL and STAT5 in cells expressing wild-type or T315I mutant forms of BCR-Abl. To assess the pharmacodynamic effects of EXEL-2280 on BCR-Abl, K562 cells expressing wild-type BCR-Abl, or BaF3 cells stably transfected with the T315I mutant of BCR-Abl, were implanted into SCID mice to form solid tumors. In both tumor models, a single oral dose of EXEL-2280 inhibited phosphorylation of BCR-Abl and its downstream effectors, CrkL and STAT5, while imatinib only inhibited the wild-type form of BCR-Abl.
These results indicate that EXEL-2280 potently inhibits wild type and drug resistant forms of BCR-Abl in vivo, and provide a rational basis for the clinical development of this agent for the treatment of CML in patients resistant to imatinib and to second-generation inhibitors (i.e. dasatinib) due to the T315I mutation.

172 POSTER

The adhesion molecule L1CAM mediates chemoresistance in pancreatic carcinoma cells

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Background: Pancreatic ductal adenocarcinoma (PDAC) is one of the most malignant diseases characterized by rapid tumor progression and profound chemoresistance. We recently reported that induction of a chemoresistant phenotype in the PDAC cell line PT45-P1 by long term chemotherapy involves an increased IL1b-dependent secretion of nitric oxide (NO) that in turn accounts for efficient caspase inhibition. In the present study we elucidated the involvement of L1CAM, an adhesion molecule previously found in other tumor diseases, in an IL1b- and NO-dependent chemoresistance.
Material and Methods: L1CAM knock down was performed by using Stealth siRNA. L1CAM expression was analysed by westernblot. Caspase activation and apoptosis induction was determined by a luminiscent caspase-3/-7 assay. iNOS expression was analysed by Real-time PCR and NO secretion in cell culture supernatants was determined by NO assay based on Griess reaction. L1CAM expression in human pancreatic carcinoma specimens was analysed by immunohistochemistry.
Results: Chemoresistant PT45-P1res cells, but not chemosensitive parental PT45-P1 cells, express high levels of L1CAM in an ILb-dependent fashion. L1CAM knock-down in PT45-P1res cells efficiently reduced iNOS expression and NO secretion and led to an increase of anti cancer-drug induced caspase activation, an effect reversed by the NO donor SNAP. Interestingly, L1CAM ectodomain shedding, i.e. by ADAM10, as reported for other L1CAM related activities, seems to be dispensable for anti-apoptotic protection by L1CAM. Neither the shedded L1CAM ectodomain was detected in chemoresistant L1CAM expressing PT45-P1 cells nor did the administration of various metalloproteinase inhibitors affect L1CAM-dependent chemoresistance. Immunohistochemical analysis revealed L1CAM expression in pancreatic cancer specimens supporting a potential role of L1CAM in the malignancy of this tumor. These findings substantiate our understanding of the molecular mechanisms leading to chemoresistance in PDAC cells and indicate for the first time an important role of L1CAM in this scenario. Furthermore, L1CAM might also be of importance for invasion and metastasis of pancreatic carcinoma cells, a role which has to be defined yet. Taking all these findings into account, L1CAM represents an interesting therapeutic target to overcome chemoresistance and to concomitantly interfere with the process of metastasis.

173 POSTER

NFkB is a critical mediator of BRCA1 induced chemoresistance

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Our group has previously reported using multiple cell line model systems that BRCA1 mediates resistance to a range of DNA damaging agents including etoposide, cisplatin and bleomycin. In agreement with these preclinical models a number of clinical retrospective studies have shown that BRCA1 mutant tumours are more sensitive to DNA damaging chemotherapy compared to tumours from matched non-carrier patients, and that BRCA1 mutation carriers have a worse overall survival if they do not receive adjuvant chemotherapy. NFκB is a well-documented mediator of chemoresistance in response to DNA damaging agents. The aim of this study is to evaluate the role of NFkB in mediating BRCA1 dependent chemoresistance. We examined the activation of NFκB by electromobility shift assay (EMSA) in BRCA1 deficient HCC-1937 cells that had been reconstituted with BRCA1 (HCC-BR) or empty vector (HCC-EV), following treatment with etoposide. There was a clear activation of NFκB in HCC-BR cells treated with etoposide compared to HCC-EV cells. Similarly we demonstrated that siRNA mediated inhibition of endogenous BRCA1 in T47D cells abrogated etoposide induced activation of NFκB as shown by EMSA and luciferase assays, where luciferase was placed under the control of an NFκB promoter. To investigate the consequences of NFκB activation on cell survival, NFκB was inhibited using either siRNA against the p65 subunit of NFκB, or with the chemical inhibitor BAY-7082. Treatment with either of these inhibitors caused a significant reduction