low dosages, these compounds could stimulate the expression of some p53 pathways genes. A comprehensive study showing the differential expressions of all possible p53 pathways genes has not been reported.
Materials and Methods: The drugs were added at low dosages to isogenic p53 knock-out HCT116 colon adenocarcinoma cell lines for 16 hours. Total RNA from the cells was extracted and the expression profiles of the treated cells were compared to untreated cells by hybridization to the Illumina microarrays. Flow cytometry was performed concurrently to demonstrate cell cycle arrest and apoptosis. Genotoxic effect was determined by measuring double-stranded DNA breaks using H2AX assay. Array results were analyzed using TIGR array analysis software and interactions of differentially expressed genes were mapped using Ingenuity program. Array results were confirmed by quantitative polymerase chain reactions on selected genes.
Results: By comparing the expression profiles of the p53 isogenic cell lines, microarray analysis revealed that ActD and LMB were able to activate p53-dependent pathways at dosage of not more than 10 nM. Reduction in the number of cells in S phase was also observed at these dosages. Higher dosages of these compounds led to accumulation of cells at sub G1 phase and differential expression of genes not related to p53 pathways. The activation of p53 pathways at low dosages is similar to treatment with Nutlin. No DNA fragmentation was observed at all dosages used.
Conclusions: ActD and LMB at low dosages are able to stimulate p53-dependent pathways without a general toxic effect.

132 POSTER

SRJ09, a lead compound in anticancer drug design: in vitro, in vivo and mechanistic studies

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Background: The search for more effective and selective anticancer drugs is currently being researched actively involving the various entities of the drug discovery programme. We have shown andrographolide (AGP), a compound isolated from a local herb, *Andrographis paniculata*, to have anticancer activity *in vitro* and *in vivo*. In order to improve the antitumour properties of AGP, semisynthetic derivatives of this compound were synthesised in our laboratory, with the aim of identifying the most promising anticancer compounds and to elucidate their mechanism(s) of action.
Materials and Methods: Cell viability assays (MTT and SRB) were used to determine the *in vitro* growth inhibitory properties of compounds. Nude mice were utilised for the *in vivo* antitumour study. Flow cytometry was performed to assess cell cycle arrest and apoptosis. Western blotting was used to determine the cellular protein levels.
Results: SRJ09 (3,19-(2-bromobenzylidene)andrographolide) displayed better antitumour activity when compared with AGP and other derivatives (SRJ11 and SRJ23). In the NCI *in vitro* anticancer screen the compound showed selectivity towards melanoma, colon, renal and breast cancers. The antitumour activity of AGP, SRJ09, SRJ11 and SRJ23 was shown to be not compromised by P-glycoprotein activities in MES-SADx5 multidrug resistant cell line. The *in vivo* antitumour study showed SRJ09 delayed quadruple tumour growth by 4 days in HCT-116 colon cancer xenografted mice treated with 400 mg/kg dose (q4dx3) when compared with control. SRJ09 induced a G_1 arrest in MCF-7 breast and HCT-116 colon cancer cells and the effect was attributed to decreased CDK-4 and increased of p21 expressions without affecting the expression of cyclin D1. Apoptosis was the main mode of cell death induced by SRJ09 and was p53 and bcl-2 independent.
Conclusions: In conclusion, SRJ09 emerged as the lead anticancer agent given its ability to induce G_1 specific cell cycle arrest and apoptosis and to have *in vivo* antitumour activity. Additionally, NCI's *in silico* SOM analysis indicated this compound might have a novel molecular target. Therefore, further studies in improving the anticancer properties of SRJ09 by chemical modification will be advantageous.

133 POSTER

Discovery of potent and selective focal adhesion kinase inhibitors

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Background: Focal Adhesion Kinase (FAK) is a non-receptor tyrosine kinase that regulates multiple cell functions and it is known as a key driver of tumor cell proliferation, migration, and survival. NVP-TAE226, known as a dual inhibitor of FAK and IGF-IR with 2-phenylamino-pyrimidin-4-ylamino-benzamide scaffold, demonstrated the inhibition of growth of 4T1 murine breast tumor cells and metastasis to the lung in an orthotopic model in a dose-dependent manner. However, a potential effect on the glucose metabolism through Insulin receptor (Ins-R) kinase inhibition was suspected because of the modest selectivity of NVP-TAE226 over Ins-R kinase (approx. 8-fold). Under a particular condition in C57BL6 mice, which exhibit high sensitivity to glucose metabolism interference, an increase of insulin and glucose levels was observed at a dose of 100 mg/kg p.o..
Material and Methods: To discover FAK inhibitors with higher selectivity over Ins-R kinase than NVP-TAE226, the scaffold was modified on the basis of the structural information of FAK and Ins-R kinase. With this approach, 2-phenylamino-pyrimidin-4-ylamino-2,3-dihydro-isoindol-1-one and 2-phenylamino-pyrimidin-4-ylamino-3,4-dihydro-2*H*-isoquinolin-1-one were found to be potential scaffolds to show high selectivity not only for Ins-R kinase but also other tyrosine kinases.
Results: Among the synthesized compounds, compound **1** showed higher selectivity over Ins-R kinase than NVP-TAE226 (more than 150-fold). As a result of further optimization studies of these series, compound **2** and **3**, which exhibited more than 780-fold selectivity over Ins-R, did not show any effect on the insulin and glucose levels in the sensitive model using C57BL6 mice. Furthermore, theyshowed equivalent or more potent anti-tumor activities compared with NVP-TAE226 in the *in vivo* studies.

	IC_{50} [μmol/L]				
	FAK	CDK1	IGF-1R	Ins-R	c-Src
NVP-TAE226	0.0053	0.56	0.12	0.044	2.3
1	0.0011	2.9	0.5	0.19	7.4
2	0.0042	>10	>10	>3.3	>10
3	0.0012	>10	1.9	1.8	2.3

Conclusions: These novel classes of selective and small molecule FAK inhibitors have potential clinical applications with potent *in vivo* anti-tumor activities and high tolerability.

134 POSTER

The rational design of inhibitors of the telomere-hnRNP A1 interaction

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The heterogeneous nuclear ribonucleoparticle (hnRNP) A1 and A2 proteins are multi-functional proteins that associate with telomeres, stimulate telomerase activity, participate in mRNA transport, and are involved in pre-mRNA splicing. They have sequence-specific RNA and single-stranded DNA binding activity, via tandem RNA recognition motifs (RRM). A1 and A2 are required for the viability of transformed human cells, but are dispensable for the growth of normal cells. We undertook a rational design approach to develop small molecules capable of inhibiting binding of A1 and A2 to telomeric single stranded DNA. Based on published x-ray structures, we chose to target the core of the RRM binding pocket that interacts with the nucleotides TAG within the TTAGGG telomeric repeat. Using Biacore (TM) analysis we determined that the TAG oligo retained good affinity for A1, and using x-ray crystallography confirmed that its binding to A1 was analogous to the full telomeric repeat. Using the TAG trinucleotide as a

starting point, a multipronged analoging approach was undertaken. In the context of the natural sugar phosphate backbone, the contribution of the T position of the nucleotide mimetic was enhanced through modulation of the pKa of the pyrimidine base. At the A and G positions, a number of purine base modifications were evaluated to further increase affinity for the A1 protein. During the analoging process, computer-assisted rational design was supported by NMR spectroscopy and coupled to a stringent screening process. Biological screening consisted of an initial A1/DNA disruption assay (to determine which compounds inhibited the targeted interaction), solubility assessment by nephelometry (to exclude insoluble compounds), Biacore (TM)-based binding studies to DNA and unrelated protein targets (to eliminate undesired binding), a Biacore (TM)-based A1 binding assessment to ensure a specific interaction, then cytotoxicity testing followed by in vivo target modulation analysis to confirm biological relevance. The combination of computer-aided rational design, structural characterization, and an innovative screening strategy were critical for the identification of active and optimizable small nucleotide mimetics.

135 POSTER

Design and synthesis of BCA2 inhibitors

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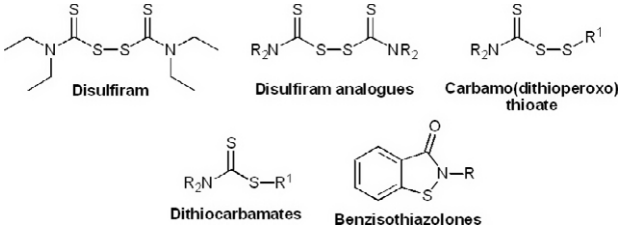
BCA2 (breast cancer-associated protein 2) is a novel RING finger E3 ubiquitin ligase that has the ability to ubiquitinate a range of other proteins. For example BCA2 was identified as a Rab7 interacting protein. It is recruited by Rab7 to mediate processes of vesicle trafficking and thereby modulating the turnover of growth factor and cell surface molecules that are important to cancer, such as EGF-R. Indeed, Burger et al. showed that BCA2 is overexpressed in 56% of 945 microarrayed invasive breast carcinomas [1].
The three dimensional structure of BCA2 has not been solved, and hence our group has built a homology model based on a related protein (EL5) having 44% residue identity. The main feature of the structure is the two zinc-chelating loops arranged in a cross brace conformation. This conformation generates a hydrophobic groove that is thought to constitute the E2 (ubiquitin conjugating enzyme) interacting surface.
The registered aldehyde dehydrogenase-inhibitory drug disulfuram inhibits BCA2 by ejecting zinc from its coordinating domain. Since rationalization of the activity of disulfiram is compromised by its poor stability and complex pharmacokinetics, a series of more stable (drug-like) zinc-affinic compounds have been synthesized. These include disulfiram analogues, carbamo(dithioperoxo)thioates, dithiocarbamates and benzisothiazolones. Initial structure–activity studies on BCA2 inhibition and cellular activity have highlighted the requirement for the disulfide bridge for optimal activity; certain disulfiram analogues were active whereas dithiocarbamates were generally inactive.



Further homology modelling on E3:E2 and E2: ubiquitin protein–protein interactions followed by virtual screening using a pharmacophore query, has identified novel molecules for synthesis. Further synthetic and BCA2 inhibitory antitumour results will be presented.

References

[1] Burger AM, Gao Y, Amemiya Y, Kahn HJ, Kitching R, Yang Y, Sun P, Narod SA, Hanna WM, Seth AK. A novel RING-type ubiquitin ligase breast cancer-associated gene 2 correlates with outcome in invasive breast cancer. Cancer Res. 2005, 65, 10401−12.

136 POSTER

Discovery of SB939, an HDAC inhibitor with a superior preclinical profile

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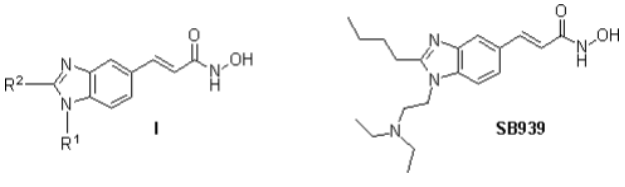
Background: Histone deacetylase (HDAC) inhibitors can significantly impact multiple processes involved in tumor progression by inducing epigenetic changes in tumor cells. SAHA (Zolinza™) has demonstrated clinical "proof-of-principle" for this class of compounds, although this and other agents currently in clinical trials have less than optimal pharmaceutical and PK properties. Our discovery program aimed to overcome these deficiencies and has identified a benzimidazole hydroxamate, SB939 (I, R^1 = 2-diethylaminoethyl, R^2 = n-butyl), a potential "best-in-class" HDAC inhibitor.
Materials and Methods: Hydroxamates (I) were synthesized according to similar protocols established for the SB639 series. A variety of side chains, R^1 and R^2, were selected to tune the drug-like properties. Enzymatic activity (IC_{50}) for HDAC1 and cell proliferation inhibition for human colon cancer cell line Colo205 were generated to establish SAR. Compounds with favorable *in vitro* pharmacology and appreciable oral PK were screened for pharmacological activity in the mouse HCT116 xenograft model. Lead candidates were further evaluated in different xenograft models with confirmation of target inhibition (histone-3 acetylation) in tumor tissues.
Results: SAR of R^1 and R^2 was established for IC_{50}. Correlations between cell and enzyme IC_{50}s with lipophilicity (logP) and between microsomal stability ($T_{1/2}$) and logP were established. Thus *in vitro* activity and metabolic stability were adjusted or tuned by using different combinations of R^1 and R^2 dramatically improving the metabolic stability of the new lead series as compared with the SB639 series. Together with excellent drug-like properties ($logD_{pH\ 7.4}$ = 2.1, solubility at pH5 >10 mg/mL), SB939 has demonstrated a superior oral PK profile to the agents currently in clinical trials. The favorable PK also translated into excellent tumor growth inhibition in animal models (e.g., HCT116, A2780, PC3, Ramos, MV4−11).
Conclusion: SB939 is a potent HDAC inhibitor, highly effective in *in vivo* tumor models, has high and dose-proportional oral bioavailability and very good ADME, safety and pharmaceutical properties. SB939 has a prolonged duration of action and is enriched in tumor tissue which may contribute to its potent anti-tumor activity. SB939 is currently being tested in phase I trials in both hematological and solid tumor patients and preliminary data show that the superior preclinical profile is translated to the clinic.



137 POSTER

Characterization of GSK1120212 a novel allosteric inhibitor of MEK1/2

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Activation of the mitogen-activated protein kinase (MAPK) pathway has been implicated in the pathogenesis and progression of cancers such as breast and melanoma. Multiple components of this signaling cascade, including BRaf and MEK, are important targets for cancer therapy and we describe here a new potent and selective inhibitor of the MEK1/2 enzymes, GSK1120212. Biochemical characterization has shown it preferentially inhibits MEK1/2 activation by $BRaf^{V600E}$ (MEK1 IC_{50} = 0.6 nM) compared to the phospho-MEK1 catalytic activity alone (IC_{50} = 10.7 nM). Similar observations were also made for c-Raf and COT activation of MEK1/2. Further investigation using phospho-mapping by LC-MS demonstrated that GSK1120212 blocks phosphorylation of S217 but not S221 on MEK1 by $BRaf^{V600E}$, showing the phosphorylation of both residues is important for downstream signaling. Against the phosphorylated MEK1 enzyme, GSK1120212 is noncompetitive vs ATP and binding is mutually exclusive with PD0325901, a MEK inhibitor already in the clinic. These data suggest that the excellent cellular activity and in vivo efficacy demonstrated by