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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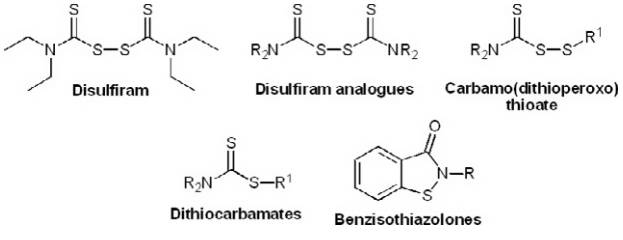
BCA2 (breast cancer-associated protein 2) is a novel RING finger E3 ubiquitin ligase that has the ability to ubiquitinate a range of other proteins. For example BCA2 was identified as a Rab7 interacting protein. It is recruited by Rab7 to mediate processes of vesicle trafficking and thereby modulating 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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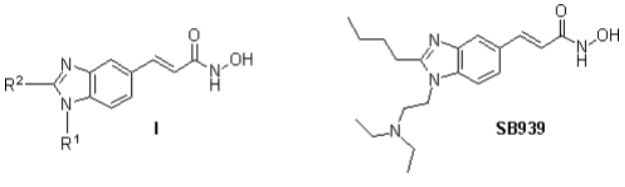
Background: Histone deacetylase (HDAC) inhibitors can significantly impact multiple processes involved in tumor progression by inducing epigenetic changes in tumor cells. SAHA (Zolinza™) has demonstrated clinical "proof-of-principle" for this class of compounds, although this and other agents currently 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

GSK1120212 is a result of the potent and selective inhibition of MEK1/2, and support its advancement for the treatment of cancer in humans.

138 POSTER

Design, synthesis, biochemical and biological evaluations of novel and potent small-molecule inhibitors of STAT3

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Constitutive activation of the Signal Transducers and Activators of Transcription 3 (STAT3) is frequently detected in human cancer specimens from patients with advanced diseases and cancer cell lines, but not in normal epithelial cells. Persistent activation of STAT3 signaling has been demonstrated to directly contribute to oncogenesis by stimulating cell proliferation and preventing apoptosis in human cancer cells. STAT3 activation may not only provide a growth advantage, allowing accumulation of tumor cells, but also confer resistance to conventional therapies that rely on apoptotic machinery to eliminate tumor cells. STAT3 represents an important and specific molecular target for designing an entirely new molecularly targeted therapy for human cancer with constitutively active STAT3 with potentially low toxicity to the normal cells without constitutive STAT3 signaling.
STAT3 is recruited from cytosol and makes specific interactions through its SH2 domain with different cytokine receptor with phosphotyrosine docking sites on the receptors. STAT3 then becomes phosphorylated on a carbonyl terminal tyrosine (Tyr705). Tyrosine physphorylation of STAT3 causes it to dimerize and translocate to the nucleus and bind to specific promoter sequences on its target genes. Dimerization of STAT3 is a decisive event for its activation. Thereby, blocking the dimerization of STAT3 using a small molecule antagonist is a very attractive therapeutic approach for developing a molecularly targeted therapy for the treatment of human cancer in which STAT3 is constitutively activated. Herein, we wish to report the design, synthesis, biochemical and biological evaluations of novel and potent small-molecule inhibitors of STAT3. Our most potent inhibitors bind to Stat3 with low nanomolar affinities and display excellent selectivity over Stat1 and Stat5. These compounds are excellent biochemical and pharmacological tools to further elucidate the role of Stat3 in cancer and promising lead compounds for the development of potent and specific Stat3 inhibitors for the treatment of human cancer.

139 POSTER

Enhanced drug delivery to brain tumors with a new paclitaxel-peptide conjugate

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Background: The main limiting factor in the treatment of brain tumors or metastasis is the low accessibility of the central nervous system (CNS) to drugs due to the blood–brain barrier. In the present study, the utilization of a new strategy based on a peptidic vector (Angiopep) capable of delivering drugs into the CNS in non-invasive manner was evaluated. Paclitaxel which accumulation into the CNS is hindered due to the P-glycoprotein efflux pump, was conjugated to the peptidic vector. The in-vitro and in-vivo properties of this conjugate (ANG1005) were characterized using different approaches.
Material and Methods: The sensitivity of a panel of cancer cell lines to ANG1005 was evaluated in vitro. The pharmacokinetic behavior of ANG1005 in plasma after IP, IV injection or IV infusion and its toxicity were determined in vivo on healthy Nude rats. The antitumor activity of ANG1005 was evaluated by MRI in a model of Nude rats bearing NCI H460 lung tumor implanted in the brain.
Results: Among all tumor cell lines tested in vitro, ANG1005 displayed an IC50 (concentration inducing a 50% cell death) in the nanomolar range for the NCI H460 and U 87 MG cell lines. These IC50 were of the same order of magnitude than for paclitaxel. Toxicity experiments showed that the maximal total treatment dose (MTTD) using a Q3Dx5 schedule was 6 mg/kg/inj when ANG1005 was injected IV. With the same schedule, IV infusion enabled to increase treatment doses as the MTTD reached 15 mg/kg. Pharmacokinetic studies indicated that maximal ANG1005 plasma concentrations were similar after a single IV injection at 11.25 mg/kg or an IV infusion at 15 mg/kg. However, the area under the time-concentration curve (AUC) was slightly higher for rats receiving ANG1005 via IV infusion as compared to rats dosed via IV injection. After a single IP injection at 75 mg/kg, ANG1005 plasma concentrations and AUC remained lower. A preliminary in vivo experiment was performed in a model of Nude rats bearing NCI H460 tumors. Magnetic resonance imaging revealed a reduction of tumor growth early after the start of treatments for rats treated IP with ANG1005 at 75 mg/kg as compared to rats receiving the vehicle or paclitaxel.
Conclusions: These results demonstrate that ANG1005 delivers paclitaxel into the CNS and enhances its activity in an aggressive model. ANG1005 is currently under evaluation in phase I clinical trials for the treatment of glioma and brain metastases in human.

140 POSTER

Macrolactone based inhibitors of Heat Shock Protein 90

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Heat shock protein 90 (Hsp90) is an ATP-dependent molecular chaperone for many overexpresed or mutant oncogenic proteins. This enzyme has become an attractive target for chemotherapeutic agents, since its inhibition will disrupt multiple cancer causing pathways simultaneously and hence may address the six hallmarks of cancer. Radicicol (**1**) is a potent natural product inhibitor of Hsp90 in vitro, but does not have any substantial in vivo activity. It has been suggested that this is due to the reactivity and metabolic lability of both the enone and epoxide functionalities. To address this problem, a series of macrocyclic lactones based on radicicol **1** was made in our laboratory. This led to the discovery of compound NP-261 (**2**) which lacks the unwanted functionality present in radicicol **1** but retains nanomolar biochemical activity.
In order to further improve the cellular activity of NP261 **2**, a second generation of analogues was designed **3**–**4**. It was reasoned that cellular activity might be enhanced by increasing the molecular rigidity of the macrocyclic ring. Our strategy was to investigate a number of key analogues which set to achieve this goal. This included a series of triazoles with varying ring sizes **3**, macrolactams and altering the substituents on the macrocyclic ring **4**. We report the synthetic challenges and biological evaluation of these new analogues.

141 POSTER

Design and synthesis of novel indole derivatives as selective apoptosis-inducers

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Evasion of apoptosis is one of the hallmarks of cancer [1]. Although the apoptotic pathway contributes to the cytotoxic effect of most cancer chemotherapeutics, selective induction of apoptosis in cancer cells would confer advantages over conventional therapy in terms of efficacy, toxicity and drug resistance.
Different small libraries of novel indole-based heterocyclic systems were designed to act as selective pro-apoptotic agents in cancer cells. Twenty-two compounds of the 5-(2-indolyl)-3-substituted-1,2,4-oxadiazole class were designed, based on a previously reported series of selective proapoptotic 3,5-diaryl-1,2,4-oxadiazoles [2]. The new compounds were prepared from the corresponding indole-2-carboxylate ester and different amidoximes in moderate yields with simple reaction workup. Another library of ten compounds of indole-based 3,5-disubstituted isoxazoles with different indole orientations was prepared by dipolar cycloaddition between terminal alkynes and aldoximes in excellent yields. A third series was designed to act as Bcl-2 inhibitors, based on a series flexible heteroarotenoids [3] with urea or thiourea linkers reported to affect the level of antiapoptotic Bcl-2 proteins in cancer cell lines and have selective apoptosis-inducing activity. Docking of these Flex-Hets at the Bcl-2 surface pocket showed good interaction suggesting the possibility of acting as Bcl-2 inhibitors, but the presence of a deep hydrophobic groove that is not utilized by these Flex-Hets suggested that incorporation of a larger side chain could result in better inhibitors. Further docking studies have revealed possibilities for extension of the Flex-Hets structure to probe further binding interactions with the Bcl-2 domain potentially leading to more potent pro-apoptotic molecules.
Examination of the in vitro cytotoxic effect of the newly prepared compounds of the 1,2,4-oxadiazole series on a panel of human cancer cell lines showed that the COLO 320 (colon) and MIA PaCa-2 (pancreas) were the most chemosensitive cell lines with IC50 mean values in the micromolar range. Moreover, potency and efficacy of compound 21 (5.7 μM and 75.9%, respectively) on the poorly differentiated pancreatic cancer cell line MIA PaCa-2 were almost superimposable to those observed for 5-fluorouracil.
Different novel series of indole-based compounds were designed and synthesized to act as selective pro-apoptotic agents with different molecular