were screened at multiple substitution points for every binding mode. The interaction between reagent and ensemble conformer was explored through simulated annealing optimisation of an empirical free-energy function.
Chemical synthesis and biological testing of the designed compounds showed that the protocol was successful in both improving the activity of the compounds and pinpointing the preferred binding mode. Further studies have resulted in the discovery of NU8231 (IC_{50} = 5 μM) which shows cellular activity.

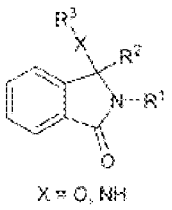

Figure 1. Isoindolinone scaffold.

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Chemical and structural studies on thioredoxin-inhibitory antitumour quinols

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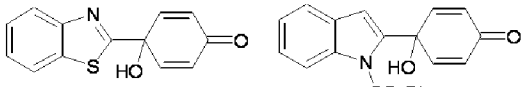
Quinols with a 6/5 heterobicyclic substituent, exemplified by the experimental antitumour agents AW 464 and BW 114, exhibit potent (low nanomolar GI_{50}) and selective activity in vitro and in vivo against certain colon and renal cell lines. Accumulated target evidence (NCI COMPARE, gene microarray, biochemical assay, mass spectrometry) strongly implicates the quinols as selective irreversible inhibitors of the 12kDa redox protein thioredoxin, a relevant anticancer drug target upregulated in certain tumours and with a multitude of intracellular functions relating to tumourigenesis (e.g. regulation of transcription factors NF-κ B, AP-1, and HIF-1α).
The lead quinol compounds are synthetically accessible, lipophilic small molecules. In the case of AW 464 (and related structures), syntheses of multigram quantities are available following a "one-pot" reaction between 2-lithiobenzothiazole and benzoquinone ketal followed by in situ deprotection. Members of the BW 114 family of compounds can be synthesised by an analogous synthetic route to the AW 464 series, or more efficiently via a palladium-catalysed Sonogashira coupling between an ortho-iodoarylsulfonylaniline and 4-ethynyl-4-hydroxycyclohexa-2,5-dienone. This latter route can be adapted towards the synthesis of more water-soluble BW 114 derivatives for potential preclinical development.
Crystal structures for the two antitumour quinols AW 464 and BW 114 have been determined. In both compounds the hydroxy group was found to interact intermolecularly with the ketone oxygen, via a water bridge in AW 464 and directly in BW 114. Michael adducts of 2× MeSH and various dithiols (including the ^{32}Cys-Gly-Pro-^{35}Cys fragment from thioredoxin) have been built computationally, leading to a thioredoxin adduct model that can accommodate both series of quinols. "Docking" studies have identified the most likely orientations of these quinols in the active site of human thioredoxin and the critical structural features contributing to recognition and potency



124 POSTER

Peptidomimetic inhibitors of Stat3: structure–activity relationships and cellular activity

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Signal transduction and activator of transcription 3 (Stat3) mediates signals from the IL-6 family of cytokines, EGF, Src etc., is constitutively activated in a variety of tumors (e.g breast, head and neck, prostate), and is a target for anti-cancer drug design [1]. Stat3 becomes activated by phosphorylation of Tyr705 and dimerizes by reciprocal interactions between SH2 domain of one molecule and the phosphotyrosine of the second. The dimer translocates to the nucleus and initiates transcription of anti-apoptotic genes resulting in cancer cell proliferation. To disrupt Stat3 activity we have embarked on the development of peptidomimetic inhibitors targeted to the SH2 domain. A lead peptide, acetyl-Y(p)LPQTV-amide (1), was found which exhibited an IC_{50} value of 150 nm [2]. SAR studies have revealed a number of important peptide-protein contacts, e.g. pY+1 backbone NH and the pY+3 Gln side chain NH_2 protons and the fact that the Leu-Pro peptide bond is *trans*. This work has lead to high affinity peptidomimetics with IC_{50} values of ca 100 nM in a fluorescence polarization assay. Pro-drug versions of one of the peptidomimetics as well as an analogue of peptide 1, when attached to the hydrophobic membrane transporting sequence AAVLLPVLLAAP, inhibit Stat3 activity in cells in culture. Stat3 translocation to the nucleus (measured by EMSA) and expression of a luciferase reporter gene were inhibited in IL-6 stimulated HepG2 and HepB3 hepatoma cells. Both inhibitors also inhibit the growth of breast carcinoma (MDA-MB231, MDA-MB468), epidermoid (A431) and multiple myeloma (MM-1) cells in culture. Thus our Stat3 inhibitors inhibit the growth of both EGFR and IL-6 pathway-dependant cells.

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125 POSTER

Synthesis, biological evaluation and structure activity relationships of a novel series of aromatic hydroxamic acids as potent HDAC inhibitors

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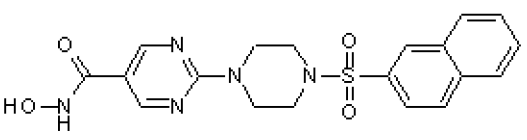
Histone deacetylases (HDACs) represent a family of enzymes that compete with histone acetyltranferases (HATs) for modification of the nucleosomal histone proteins. Histone acetylation status modulates chromatin structure and thereby regulates transcriptional activity of a subset of genes. Aberrant reduction in acetylation due to disruption of HDAC or HAT activity is associated with the development of cancer [1)]. Deregulated, sustained HDAC recruitment to the chromatin is observed in specific forms of leukaemia and lymphoma, such as APL and non Hodgkin's lymphoma [2)]. In agreement with a key role of HDAC activity in cancer, HDAC inhibitors from various structural families induce histone hyperacetylation, activate gene expression and consequently, inhibit the cell cycle, activate differentiation programmes or induce apoptosis. HDAC inhibitors have been described to exhibit potent anti-tumor activity in human xenograft animal models, suggesting that this class of compounds represents promising novel cancer therapeutic agents [3)]. We have recently described the discovery of R306465 (JNJ16241199) as a highly potent HDAC inhibitor, showing anti-proliferative activity in a wide panel of tumor cell lines of different origin and exhibiting anti-tumor activity when dosed orally in human xenograft-bearing nude mice [4)].
In order to fully explore the Structure Activity Relationship around the aryl hydroxamic acid core template, several compound libraries were generated. In this poster, the design and execution for representative libraries will be briefly described. The resulting chemical libraries were evaluated against an array of enzymatic and cellular assays, which generated a clear and consistent SAR. Representative data will be shown, complemented by ADME profiling results.



R306465 (JNJ16241199)

IC_{50} HDAC = 6 nM (HeLa nuclear extract)
IC_{50} A2780 = 30 nM (cell proliferation)

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Structure–activity relationships

126 POSTER

Development of a new series of tricyclic pyrimido-indole inhibitors targeting Aurora kinases

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The Aurora kinase family of proteins are serine/threonine kinases that regulate the processes of centrosome separation and duplication in preparation for mitotic spindle formation and chromosome separation. Aurora-A is overexpressed in several solid tumor types, including breast, ovary, prostate, pancreas and colorectal cancers and its overexpression is thought to contribute to tumor progression by increasing genomic instability and altering cell cycle checkpoints. Because of its role in the process of tumorigenesis, Aurora-A has been reported to be an attractive target for anti-cancer drug development. We have initiated a drug development program to identify specific inhibitors of Aurora kinase activity. This program is based on a combination of rational design, synthesis and screening. We have developed a novel series of potent and selective ATP-competitive Aurora kinase inhibitors utilizing tricyclic pyrimido-indole core, which is structurally distinct from other reported kinase inhibitors. Such tricyclic compounds modeled into the ATP-binding site of Aurora kinase in such a way that the tricyclic pyrimidine ring orients into the hydrophobic adenine-binding site to form backbone H-bonds with the E211, Y212 and A213 residues of the hinge region. Several leads from this series have emerged from SAR studies around 4, 6 and 7th position of pyrimido-indole moiety. A lead compound from this series, MP-235, has been shown to inhibit the Aurora kinases at nanomolar concentrations (IC50 = 90nM). This lead has been further modified to identify analogues with more potent activity and greater selectivity towards the Aurora kinases. Cell growth studies in the human pancreatic cell lines MiaPaCa-2 and Panc-1 as well as other cancer cell lines show that these novel Aurora kinase inhibitors can result in antiproliferative effects in tumor cells. (Supported by NIH Grant CA 95031-01)

127 POSTER

Integrin receptor binding and cytotoxicity of cyclopeptides and their Chlorambucil conjugates containing RGD or NGR sequence

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Introduction: The RGD peptide sequence found in most ECM component is the general recognition site for the integrin receptor family like vitronectin ($\alpha_v \beta_3$) and fibronectin ($\alpha_5 \beta_1$) receptor, however other adhesion sequences, like the NGR came into focus as well. Selective $\alpha_v \beta_3$ ligands are suitable for vasculature targeted cancer therapy and also serve as tools for targeted drug delivery into the tumor vasculature. For this purpose we have synthesized and investigated peptide derivatives (single capital letters for *L*-amino, small letters for *D*-amino acids; pF-F for *p*-fluoro-phenylalanine; pNH_2-F for *p*-amino-phenylalanine) and their Chlorambucil (Clb) conjugates.
Methods: Linear peptides were prepared by solid phase method, cyclisations were performed in solution. For fluorescent labeling 5(6)-carboxyfluorescein was used. Receptor recognizing ability of the peptide derivatives was checked in a competitive displacement assay using a ^{125}I-radiolabeled multivalent ligand for $\alpha_v \alpha_3$ integrin (RGD-protein conjugate). For *i n vitro* cytotoxicity assay HUVEC, human HBL and LND1 melanoma cells and fibroblasts were used.
Results: c(VRGDf) **1**, c(VRGDpFf) **2**, c(DapRGDf) **3**, c[Dap(Clb)RGDf] **4**, c[K(Clb)RGDf] **5**, c(VRGDpNH $_2$f) **6** show equally high affinity for $\alpha_v \beta_3$ receptor, while H-CNGRCV-NH_2 **7**, c(LNGRV) and c(LNGRv) do not bind to it, according to the radioactive displacement assay.
All cell types used in the cytotoxicity assay show different fibronectin and vitronectin receptor expression. Except for HUVEC, chlorambucil-coupled peptides show significantly less toxicity than Chlorambucil alone in all cell types tested, suggesting a compromised ability to cross the cell membrane. In addition, free peptides show by themselves some cytotoxicity to most cell types used, compound **3** being by far the most toxic to HUVEC.
Conclusions: All the cyclopeptide derivatives and their alkylating conjugates containing the RGD sequence preserve the selective $\alpha_v \beta_3$ integrin receptor binding affinity of the reference peptide **1**, while cyclopeptides with the NGR motif do not bind to this receptor. Cell adhesion kinetics on fibronectin matrix appears to be correlated with the level of expression of the corresponding receptors. The higher toxicity observed on HUVEC can be explained by a possible initiation of the apoptotic pathway.

128 POSTER

Sapphyrins: structure-activity relationships in a novel series of potential anti-cancer agents

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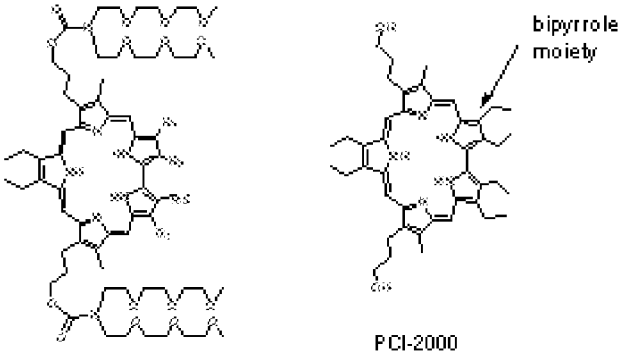
Sapphyrins are pentapyrrolic metal-free expanded porphyrins. We have previously shown that the first generation sapphyrin compound, PCI-2000, induces apoptosis in a variety of hematologic tumor cell lines. PCI-2000 triggered an apoptotic pathway as demonstrated by apoptotic morphology, annexin V binding, release of cytochrome C from mitochondria, activation of caspases 9, 8, and 3 and cleavage of the caspase 3 substrate PARP. To investigate structure activity relationships among sapphyrin derivatives, we focused on four 2nd generation derivatives, PCI-2050, PCI-2051, PCI-2052 and PCI-2053 where polyethylene glycol groups were introduced to increase their water solubility. Structurally, these four compounds differ by their alkyl substituents on the bipyrrole moiety. Treatment of Ramos cells in tissue culture with these derivatives (1 uM for 16 hrs) showed the following activity profile as assessed by annexin V binding and caspase activity: PCI-2050> PCI-2051> PCI-2052> PCI-2053.Interestingly, treatment of Ramos cells with 0.5 uM of each sapphyrin for 48 hrs showed a slightly different activity profile: PCI-2050> PCI-2052> PCI-2051> PCI-2053. Drug uptake, measured as fluorescence emission >650 nm after excitation at 488 nm correlated with drug activity (except for PCI-2053, which is not fluorescent under the experimental conditions). To explore in vivo biological activity, we treated CD-1 nude mice bearing Ramos xenografts with each of the sapphyrins (10 umol/kg). Animals were sacrificed 48 hrs after treatment and analyzed for drug uptake in the tumor using flow cytometry. The relative order of uptake into tumor cells was PCI-2050> PCI-2052> PCI-2051. Tumor cells from sapphyrin-treated animals grew less well in culture and had more apoptotic cells than those derived from control animals in proportion to the drug uptake in tumor cells. Inhibition of sapphyrin treated tumor cell growth relative to control tumor cell growth was: 91% for PCI-2050, 79% for PCI-2052, 20% for PCI-2051 and 16% for PCI-2053. PCI-2050 showed anti-tumor activity in a Ramos xenograft model with minimal toxicity when given at 10 umol/kg × 2 days. Our work demonstrates that sapphyrins induce apoptosis both in tissue culture and



PCI-2000

PCI-2050, R_1 = Et, R_2 = Me
PCI-2051, R_1 = Et, R_2 = Et
PCI-2052, R_1 = Me, R_2 = Me
PCI-2053, R_1 = H, R_2 = H