(3) assays of candidate drug activity, including electrophoretic mobility shift assay (EMSA), RNAse protection assay (RPA), and assays of cellular apoptosis and toxicity, and (4) confirmation of anti-tumor in nude mouse xenograft models.
Results: (1) G-quartet forming oligonucleotides (GQ-ODN), named T40214 and T40231, were developed as potent agents, which specifically inhibit Stat3 DNA-binding activity in several human cancer cell lines, such as hepatoma (HepG2), prostate (PC-3), breast (MDA-MB-468), and head and neck (167, B4B8, 1968) cancer cells, holding promise for the systemic treatment of many forms of human cancer. (2) We have constructed a model of GQ-ODN/Stat3 complex and established a structure-activity relationship (SAR) between GQ-ODN and Stat3 dimer for drug design and screening. (3). We have also developed a novel and effective intracellular delivery system for GQ-ODNs. This delivery system greatly increased the delivery efficiency and drug activity of GQ-ODNs within cells. Also this system was capable of delivering G-quartet inhibitors into tissues and tumors in xenograft animal models. (4) Our *in vivo* data demonstrated that T40214 and T40231 suppressed the growth of prostate and breast tumors *in vivo* by inhibiting Stat3 activation, resulting in a dramatic increase in apoptosis of tumor cells. The mean size of the breast tumor xenografts of placebo-treated mice increased from 11 fold over 18 days while the mean sizes of both T40214 and T40231-treated mice remained unchanged ($p<0.001$). The mean size of the prostate tumor xenografts of placebo-treated mice increased from 9 fold over 10 days while mean sizes of both T40214 and T40231-treated mice were only increased by 2.2 and 4 fold, respectively ($p<0.05$). The results also demonstrated that the mean levels of phosphorylated Stat3 (p-Stat3), Bcl-x_L and Bcl-2 were decreased by 9, 4.3 and 10-fold, respectively, and caspase 3 cleavage products increased 3-fold in the tumors from drug-treated animals compared to tumors from placebo-treated mice. The percentage of apoptotic cells was increased nearly 8-fold in the tumors of drug-treated mice (83.6±1.0%) compared to the tumors of placebo-treated mice (11.2±10.1%).
Conclusion: GQ-ODNs as novel anti-cancer agents specifically inhibited Stat3 activation among other STAT protein members and suppressed the expression of Stat3–regulated anti-apoptotic genes, such as *Bcl-x_L*, *Bcl-2* and *Mcl-1*. GQ-ODN also suppressed the growth of human tumors where Stat3 is activated and significantly increased apoptosis of tumor cells. Therefore, GQ-ODNs as a new class of potent anti-cancer agents hold promise for the systemic treatment of many forms of human cancer.

120 POSTER

Synthesis and cytoxicity studies of platinum nucleobase adducts

A.R. Khokhar, M.S. Ali, Z.H. Siddik. *University of Texas, M.D. Anderson Cancer Center, Experimental Therapeutics, Houston, USA*

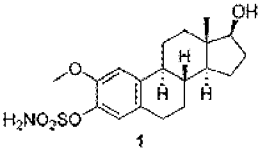
We are studying interaction of cisplatin analogs with DNA nucleobases. A series of novel platinum (IV) nucleobase monoadducts of the type [Pt^{IV}(DACH)*trans*-(X) $_2$LCl]NO_3 (where DACH = *trans*-1*R*,2*R*-diaminocyclohexane, L = adenine, guanine, and 9-ethylguanine and X = acetato ligand) have been synthesized and characterized by elemental analysis and by NMR spectroscopic technique. The crystal structure of the model nucleobase complex [Pt^{IV}(*trans*-1*R*,2*R*-diaminocyclohexane) *trans*-(acetate)$_2$(9-ethgua)Cl]$NO_3 \cdot H_2O$ was determined using a single crystal X-ray diffraction method. The complex crystallized in the monoclinic space group $P2_1/c$, with a = 10.446(2) Å, b = 22.906(5) Å, c = 10.978(2) Å, Z = 4, and R = 0.0569, based upon the total of 11570 collected reflections. In this complex, platinum had a slightly distorted octahedron geometry owing to the presence of a geometrically strained five-member ring. The two adjacent corners of the platinum plane were occupied by the two amino nitrogen of DACH, whereas, the other two equatorial positions occupied by chloride ion and 9-ethylguanine. The remaining two axial positions were occupied by the oxygen atoms of acetato ligands. The DACH ring was in a chair configuration. An intricate network of intermolecular hydrogen bonds held the crystal lattice together. Such DACH-Pt-DNA adducts have good in vitro cytotoxic activity against the cisplatin-sensitive human cancer ovarian A2780 cell line (IC50 = 1–8 μM). Interestingly, a substituted nucleobase (9-ethylguanine) adduct was over 6-fold more potent than regular adducts. The cross-resistance factor against the 44-fold cisplatin-resistant 2780CP/clone 16 cells was about 3–9; thus, the cytotoxicity of adducts was indicative of low potency, but the resistance factors were also substantially low. These results suggest that DNA adducts of DACH-Pt are cytotoxic with low cross-resistance. (Supported by NCI CA 77332 and CA 82361)

121 POSTER

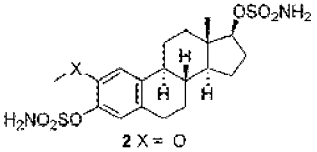
Synthesis, anti-proliferative and anti-angiogenic effects of sulfamoylated 2-methoxyestradiol analogues

M.P. Leese[1], K. Gaukroger[1], J.J. Robinson[1], J.M. Day[2], S.P. Newman[2], S. Chander[2], A. Purohit[2], M.J. Reed[2], B.V.L. Potter[1]. [1]*University of Bath, Department of Pharmacy and Pharmacology, Bath, UK;* [2]*Imperial College, Endocrinology and Metabolic Medicine, London, UK*

The ability of 2-methoxyestradiol (2-MeOE2), an endogenous estrogen metabolite, to inhibit both the proliferation of human cancer cells and angiogenesis is well established. Sulfamoylated derivatives of 2-MeOE2, such as 2-methoxy-3-*O*-sulfamoyl estradiol **1** (2-MeOE2MATE), display enhanced activity and, in contrast to 2-MeOE2, cause an irreversible cell cycle arrest. In this study we report on the structure activity relationships of the family of *mono*- and *bis*-sulfamoylated 2-substituted estradiols as anti-proliferative agents. Efficient multi-step chemical syntheses of these compounds have been developed allowing a determination of synergistic effects of 2-, 3- and 17-substituents. To rationalize the activities observed in this series we have applied computational modelling techniques to identify the likely site of interaction of these molecules with tubulin. Novel compounds were evaluated against the proliferation of human breast (MCF-7) and ovarian (A2780) cancer cells *in vitro*. Optimal anti-proliferative activity in the simple estradiol-3-*O*-sulfamate series was afforded by the 2-methoxy, 2-ethyl and 2-methyl sulfanyl functions. The bioisosteric nature of these 2-substituents is illustrated by the anti-proliferative activities observed for 2-methoxy-, 2-ethyl- and 2-methyl sulfanyl-estradiol-3,17-*O*, *O*-*bis* sulfamates **2**–**4** which caused 50% growth inhibition in A2780 ovarian cancer cells at concentrations of 0.24, 0.26 and 0.23mM respectively. Subsequent experiments on the bioisosteric replacement of the 17-sulfamate group delivered several further active series which caused 50% growth inhibition (A2780) at concentrations as low as 0.04 μM. Further evaluation of these compounds showed that these compounds show highly promising anti-angiogenic activity. Compound **2** inhibited HUVEC proliferation at 0.33 μM, cord formation at 0.06 μM and chemotaxis at 0.36 μM in *in vitro* studies. Results obtained in the NCI hollow fibre assay and Lewis Lung model, as well as in *in vivo* models of angiogenesis underline the therapeutic potential of sulfamoylated 2-methoxyestradiol analogues as drug candidates with a multi-targeted mode of action.



1



2 X = O
3 X = CH_2
4 X = S

122 POSTER

Identification of inhibitors of the MDM2–p53 interaction using a virtual screening approach with multiple binding modes

I.R. Hardcastle[1], S.U. Ahmed[1], S. Guyenne[1], C. Hutton[1], P. Kallblad[2], S. Kemp[1], J. Lunec[1], R. Reid[1], K. Saravanan[1], H.M.G. Willems[2]. [1]*University of Newcastle upon Tyne, Northern Institute for Cancer Research, Newcastle upon Tyne, UK;* [2]*DeNovo Pharmaceuticals, Cambridge, UK*

Rational structure-based molecular design can be used to improve lead compounds. This requires that the structure of the intermolecular complex formed between the target protein and the lead compound is known. Ligand-protein docking studies can be used to overcome a lack of experimental structural data. Whilst it is critical for any following experiment that the correct binding mode is selected, it can be difficult to distinguish a single docking solution as preferred. Failure to identify the correct binding mode will spoil subsequent design efforts.
The impact of considering multiple binding modes from docking studies has previously been statistically quantified. We made use of the approach in a virtual screen of reagents on a lead scaffold (Figure 1) known to inhibit the protein-protein interaction between MDM2 and p53. The aim was two-fold: first, to improve the affinity and drug-likeness of the compounds through the use of substituents with an increased level of functionality; second, to narrow down the number of putative binding modes by introducing stronger directionality in the interaction.
A small number of geometrically diverse and high-scoring binding modes were selected from a large pool of docking solutions of the lead compound. The scaffold was extracted from the selected solutions and reagents

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.

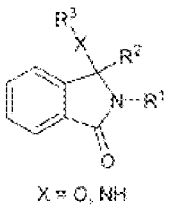


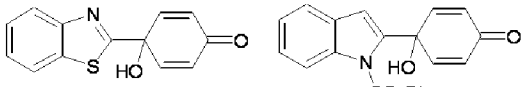
Figure 1. Isoindolinone scaffold.

123 POSTER

Chemical and structural studies on thioredoxin-inhibitory antitumour quinols

A.D. Westwell[1], A.J. McCarroll[1], J.M. Berry[1], R. Ren[2], D.L. Rathbone[2], P.R. Lowe[2], C.H. Schwalbe[1], M. Burke[1], C.A. Laughton[1], M.F.G. Stevens[1]. *[1]Centre for Biomolecular Sciences, School of Pharmacy, Nottingham, UK; [2]Aston University, Aston Pharmacy School, Birmingham, UK*

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

J.S. McMurray[1], P.K. Mandal[1], D.R. Coleman[1], Z. Ren[1], X. Chen[2], W. Liao[2], N.J. Donato[3]. *[1]The University of Texas M.D. Anderson Cancer Center, Neuro-Oncology, Houston, TX, USA; [2]The University of Texas M.D. Anderson Cancer Center, Biochemistry and Molecular Biology, Houston, TX, USA; [3]The University of Texas M.D. Anderson Cancer Center, Bioimmunotherapy, Houston, TX, USA*

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.

References

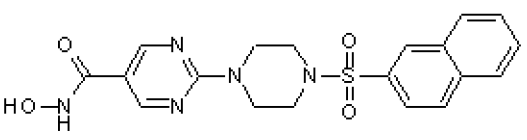
[1] Yu, H.; Jove, R. *Nature Rev. Cancer.* **2004**, *4*, 97.
[2] Ren, Z. Cabell, L.A., Schaefer T.S., McMurray, J.S *Biorg. Med. Chem. Lett* **2003** *13*, 633

125 POSTER

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

K. Van Emelen[1], J. Arts[2], P. Angibaud[5], H. De Winter[3], S. Van Brandt[1], M. Verdonck[1], J. Van Dun[2], T. Geerts[2], W. Floren[2], J. Van Gompel[4]. *[1]Johnson&Johnson PRD, Medicinal Chemistry, Beerse, Belgium; [2]Johnson&Johnson PRD, Oncology Discovery Research, Beerse, Belgium; [3]Johnson&Johnson PRD, Molecular Informatics, Beerse, Belgium; [4]Johnson&Johnson PRD, ADME/Tox, Beerse, Belgium; [5]Johnson&Johnson PRD, Medicinal Chemistry, Val De Reuil, France*

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)