initially bound ^{131}I activity up to 24 h compared to that for ^{125}I. In SK-N-SHsst2 cells, the specific internalized radioactivity of [^{131}I]**5** was 2- to 4-fold higher than that of [^{125}I]GluTOCA. It was 6- to 7-fold higher than that of [^{125}I]**7** suggesting that, contrary to observations above, NET may have some role in its uptake (OIBG has no significant affinity to NET). In summary, while [^{131}I]MIBG-Octreotate demonstrated higher internalized radioactivity in SK-N-SHsst2 cells *in vitro* compared to [^{125}I]Glu-TOCA and [^{125}I]OIBG-Octreotate, its uptake was lower than that of [^{125}I]MIBG. We plan to modify the linker between MIBG and octreotate in order to facilitate the interaction of this hybrid molecule with both tumor associated targets present on NB cells.

116 POSTER

Inhibition of p53-MDM2 pathway by novel boronic-chalcones

A. Modzelewska[1], A. Geetha[2], M. Ghosh[3], C. Pettit[1], N.E. Davidson[1], T. Holak[3], P. Huang[2], S.R. Khan[1]. [1]*The Johns Hopkins Medical Institutions, Oncology, Baltimore, USA;* [2]*The University of Texas M.D. Anderson Cancer Center, Molecular Pathology, Houston, USA;* [3]*Max Planck Institute for Biochemistry, Biochemistry, Munich, Germany*

The p53 tumor-suppressor pathway is inactivated in a majority of human cancers. Although the p53 gene is frequently deleted or mutated in many human malignancies, a substantial percentage of tumors also express intact p53 and overexpression of MDM2 is commonly observed. The oncoprotein MDM2 negatively regulates p53 function by binding to this protein to enhance proteolytic degradation, hence destroying the cell cycle checkpoint and allowing the progression of damaged cells. This P53/MDM2 interaction has been implicated as a possible mechanism for cancer development in several tumors including human sarcomas. Thus disruption of p53-MDM2 interaction with synthetic compounds should stabilize p53 in the nucleus and offer a novel therapeutic potential for cancer therapy. A series of boronic-chalcones have been investigated as possible MDM2 antagonists. The goal of the current studies is to build upon the paradigm of the boronic-chalcone analogs to identify more effective and selective agents can be found. We have successfully designed and synthesized boronic-chalcone derivatives that inhibit growth of human breast and colorectal cancer cell lines with IC_{50} values from 1 to 5 μM. The cytotoxic effect of these compounds was measured by multiple analyses including MTT assay, annexin-V reactivity, and colony formation assay. Both apoptosis analysis and colony formation assay in p53 isogenic cells showed that the p53+/+ colon cancer cells are more sensitive to the active boronic-chalcones than the p53−/− colon cancer cells. We have shown by multidimensional NMR spectroscopy that boronic-chalcone derivatives are MDM2 inhibitors that bind to a subsite of the p53-binding cleft of human MDM2. Structure-activity relationship studies and molecular modeling studies of this new class of compounds are underway. Upon the identification of the most active compounds, these cytotoxic agents will be tested for their potency and selectivity for tumor cells. The lead compounds will then be tested in vivo models of human breast and colon cancer. These studies will serve to identify the best candidate that will subsequently test in clinical trials as treatment for breast and colon cancer.

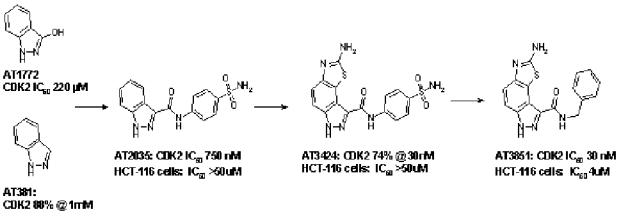
117 POSTER

Identification of novel cyclin dependent kinase 1/2 inhibitors using fragment based high-throughput X-ray crystallography and structure based drug design

P.G. Wyatt[1], V. Berdini[2], M.G. Carr[1], J.E. Curry[3], D.J. Davis[3], M. O'Reilly[4], G. Saxty[1], M.S. Squires[3], A.J. Woodhead[1], A.J.-A. Woolford[1]. [1]*Astex Technology, Medicinal Chemistry, Cambridge, UK;* [2]*Astex Technology, Computational Chemistry, Cambridge, UK;* [3]*Astex Technology, Biology, Cambridge, UK;* [4]*Astex Technology, Protein Structure, Cambridge, UK*

The poster describes the use of high-throughput X-ray crystallography and fragment-based drug discovery (Astex's Pyramid™ technology) to develop a number of lead series with potent cyclin-dependent kinase 1 and 2 (CDK-1, CDK-2) inhibitory activity and antiproliferative activity against cancer cell lines.

Astex has developed an integrated crystallography-based approach, which allows the detection of high efficiency binding molecular fragments and their subsequent optimization using structure-based drug design into potent novel drug candidates. Soaking apo-crystals of CDK-2 with cocktails of low molecular weight compounds identified a number of start points for chemistry programmes. Optimisation of one of these start points, using X-ray structures of synthesized molecules, allowed the rapid identification of compounds with potent CDK activity. Further improvements in the initial leads have identified compounds with both potent CDK and single figure nanomolar anti-proliferative activity.



These lead molecules were characterised in a range of cell-based assays, demonstrating their anti-proliferative effect resulted from a specific cell cycle arrest and tumour cell death by apoptosis. The mechanism of action of this inhibition was confirmed by monitoring the phosphorylation of downstream substrates.

Furthermore the compounds were shown to exhibit negligible toxicity towards non-proliferating fibroblast cells, and were equipotent in cells lacking p53 or expressing PgP.

The in vivo pharmacokinetic and xenograft activity of this series of compounds will be described in the accompanying poster.

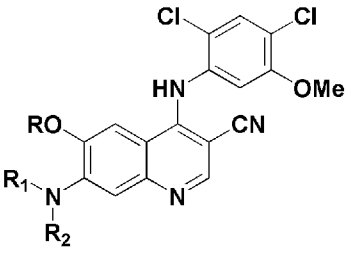
In conclusion using Astex's Pyramid™ technology a number of potent CDK1/2 inhibitors have been identified with potent anti-tumour activity.

118 POSTER

Substituted 7-amino-4-anilino-6-alkoxy-3-quinolinecarbonitriles as Src kinase inhibitors

H. Tsou[1], E. Overbeek-Klumpers[1], W. Hallett[1], J. Golas[2], F. Boschelli[2]. [1]*Wyeth Research, Chemical and Screening Sciences, Pearl River, NY, USA;* [2]*Wyeth Research, Oncology, Pearl River, NY, USA*

As a prototype for non-receptor tyrosine kinases and proto-oncogenes, Src plays an important role in the signal transduction pathways that regulate several cellular functions such as proliferation, differentiation, migration, and angiogenesis. Activation and over-expression of Src have been implicated in cancer, osteoporosis and stroke. Therefore, inhibition of Src kinase could prove effective in the treatment of these diseases. Earlier, a Wyeth team reported 7-alkoxy, 7-alkenyl, 7-alkynyl, and 7-phenyl-4-anilino-3-quinolinecarbonitriles as potent Src kinase inhibitors. In this paper, we report a series of substituted 7-amino-4-anilino-3-quinolinecarbonitriles. Some of them are low nanomolar inhibitors of Src kinase, and their SAR will be discussed. Methods for introducing substituents with various chain lengths on the 7-amino group will also be presented.



119 POSTER

A novel strategy to inhibit Stat3 for human cancer therapy

N. Jing, Y. Li, W. Sha, W. Xiong, D. Tweardy. *Baylor College of Medicine, Medicine, USA*

Background: Stat3 has been suggested as a critical mediator of oncogenic signaling in the development and progression of human cancers and is active in prostate cancers (82%), breast cancers (69%), head and neck cancers (HNSCC) (>90%), nasopharygeal carcinoma (71%) as well as in many other cancers. Several Stat3 regulated genes, such as Bcl-x and Mcl-1, play important roles in cancer progression. Despite a strong rationale for targeting Stat3 for the treatment of human cancers, current chemotherapeutic approaches have not yet incorporated this strategy. We propose a novel strategy to inhibit Stat3, which should be useful in the development of novel cancer therapeutic approaches.

Methods: To design a novel inhibitor of Stat3, we employed several procedures: (1) structure-based drug design and optimization based upon our newly established model of drug/Stat3 complex and a structure-activity relationship (SAR) between inhibitors and Stat3, (2) chemical synthesis,

(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*

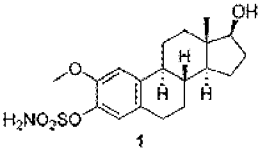
We are studying interaction of cisplatin analogs with DNA nucleobases. A series of novel platinum (IV) nucleobase monoadducts of the type [Pt^{IV}(DACH)*trans*-(X) $_2$LCl]NO_3 (where DACH = *trans*-1*R*,2*R*-diaminocyclohexane, L = adenine, guanine, and 9-ethylguanine and X = acetato ligand) have been 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.



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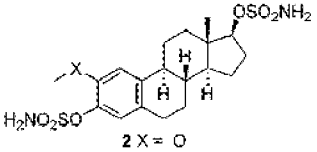

2 X = O
3 X = CH_2
4 X = S

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