410

A novel tumor targeting telomerase inhibitor depletes cellular atp and inhibits human prostate tumor xenograft growth in nude mouse and enhances the radiosensitivity of cultured human prostate and brain tumor cells

H. Basu[1], S. Bhattacharya[1], A. Sarkar[1], Y. Wang[1], A. Valasinas[1], S. Brummel[2], G. Wilding[2], Y. Maxuitenko[3], B. Frydman[1]. [1] *SLIL Biomedical Corp., Drug Development, Madison WI, USA;* [2] *University of Wisconsin, Medicine, Madison WI, USA;* [3] *Southern Research, Cancer Therapeutics and Immunology, Birmingham AL, USA*

The enzyme telomerase replicates telomere regions of chromosomes during cell division. We have developed several telomerase inhibitors that are specifically taken up by malignant cells and are cytotoxic against several cultured human tumor cell lines. Hypoxic cells present in solid tumors are at a disadvantage with regard to ATP production because of the low rate of oxidative phosphorylation under hypoxia. These cells are also more radioresistant than their normoxic counterparts. We have developed a class of cyclic polycations that can catalyze ATP hydrolysis. Most of these compounds deplete cellular ATP level and show marked cytotoxicity against cultured human tumor cells, particularly under hypoxia. We have covalently attached a telomerase inhibitor with one cyclic polyamine analog to produce a novel anti-neoplastic agent that can be orally administered to animals without any observable systemic toxicity up to 800 mg/kg once a week for four weeks. This agent is cytotoxic against several cultured human cell lines as determined by a colony forming efficiency assay. When given orally, it accumulates specifically in the human tumor cell xenografted in animals. An oral dose of 500-600 mg/kg once a week for three to seven weeks markedly inhibits DU-145 human prostate tumor xenograft growth in nude mouse. The growth remains completely arrested during the course of therapy to up to ten days after the end of therapy and only a very slow tumor growth rate was observed from 10 days to at least up to 30 days after the end of therapy. This agent also acts as a strong radiosensitizer against cultured human prostate and brain tumor cells and shows promise both as a potential chemotherapeutic agent as well as a radiosensitizer.

Acknowledgement: Partially funded by DOD projects DAMD17-98-1-8504 and DAMD17-01-1-0008.

411

Structure-based design and optimisation of substituted 2-phenylamino-4-(thiazol-5-yl)-pyrimidine CDK inhibitors

P.M. Fischer[1], S. Wang[1], R. Clarke[1], W. Jackson[1], C.E. Lyon[1], S. McClue[1], C. McInnes[1], R. Westwood[1], M. Walkinshaw[2], D.P. Lane[1]. [1] *Cyclacel Limited, Dundee, United Kingdom;* [2] *The University of Edinburgh, Bio Sc - ICMB, Edinburgh, United Kingdom*

A group of 4-heteroaryl-2-phenylamino-pyrimidine compounds acting as ATP-antagonistic CDK inhibitors were discovered through virtual screening based on the crystal structure of CDK2 and through structure-activity relationship (SAR) studies starting from the original screening hits (Proc. AACR 2002, 43, 4202). Until recently optimisation was performed predominantly against CDK2 in the expectation that inhibition of CDK2/cyclin E and CDK2/cyclin A would offer the best way of re-regulating altered cell cycle control in neoplasias. Very potent analogues with appreciable *in vitro* and *in vivo* anti-proliferative tumour properties were obtained as a result. More recently we have started designing, synthesising, and evaluating analogues with improved physicochemical properties, with the aim of enhancing bioavailability. Our approach has been to use the CDK2 binding modes of our lead compounds in complex crystal structures in order to design a new generation of more water-soluble analogues containing substituents capable of additional polar interactions with CDK2. We shall present our latest findings on the *in vitro* and *in vivo* activity of such compounds, including cellular mode-of-action studies and anti-tumour activity in mouse xenograft models following oral test compound administration. It has recently become clear that CDKs are not exclusively involved in cell cycle regulation; certain CDK family members instead influence transcription (CDKs 7, 8, and 9) and neuronal and secretory cell function (CDK5). In light of these new understandings in CDK biology we have extended the SARs of our 2-phenylamino-4-(thiazol-5-yl)-pyrimidine analogues to an expanded CDK assay panel, including CDK1/cyclin B, CDK2/cyclin A or E, CDK4/cyclin D1, CDK7/cyclin H, and CDK9/cyclin K or T1. Apart from apparently selective pan-CDK inhibitors, we have to date identified compounds with modest selectivity against CDK2 and CDK9, respectively. The CDK inhibition matrix SAR will be presented and the implications for the potential application of selective CDK inhibitors in different therapeutic settings will be discussed.

412

Development of an anti-angiogenic targeted toxin against glioblastoma multiforme

W.A. Hall[1,2], E. Rustamzadeh[1], H. Liu[2], D.A. Vallera[3]. [1] *Neurosurgery,* [2] *Radiology,* [3] *Radiation Oncology, University of Minnesota, Minneapolis, USA*

Introduction: The prognosis for patients with glioblastoma treated with conventional therapies remains poor. The recent development of targeted toxin therapies that are delivered directly into brain tumors has shown promise in early clinical trials.

Methods: A recombinant fusion protein called DTAT (Diphtheria Toxin/ Amino Terminal fragment) that recognized the urokinase-type plasminogen activator receptor (uPAR) was expressed in and purified from E. coli. The fusion protein was synthesized in order to simultaneously target the tumor neovasculature and glioblastoma cells for future intratumoral administration that would bypass problems associated with systemic toxicity. The hybrid molecule was created using the non-internalizing amino terminal fragment (ATF) of urokinase for binding, the catalytic portion of diphtheria toxin (DT) for killing, and the translocation-enhancing region (TER) of DT for cell internalization.

Results: DTAT, the final 57 kDa protein was highly selective for human glioblastoma *in vitro*, killing the uPAR-expressing U87 and U118 lines with an IC_{50} value of less than 1 nM, but not negative control cell lines Daudi or SKBR3. *In vitro* studies showed that DTAT was highly selective in its ability to kill human endothelial cells in the form of HUVEC. *In vivo*, DTAT caused the complete regression of small subcutaneous U118 tumors in nude mice when administered at 20 ug/day given on a 5 day schedule every other day. Analysis of serum enzyme levels showed no elevations in BUN levels indicating a lack of kidney effect, but did register a significant, albeit non-life-threatening elevation in ALT levels. In an *in vivo* mouse model of intracerebral human U87 glioblastoma, 83% of tumors responded to DTAT (60% completely, 23% partially) as determined by MRI. A statistically significant prolongation in animal survival was also seen in these animals.

Conclusions: These findings indicate that attachment of the TER of DT can render the ATF, which ordinarily does not internalize and is not cytotoxic, a selective and potent clinical alternative for treating chemotherapy or radiation-resistant glioblastoma. Our results indicate that DTAT holds promise in the treatment of human glioblastoma.

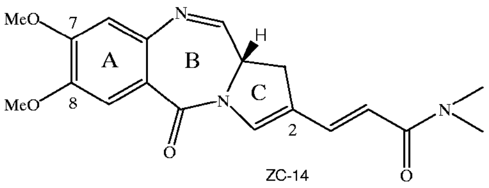
413

A concise synthetic route to C2-endo/exo-unsaturated pyrrolo[2,1-c][1,4]benzodiazepines(PBDs)with potent *in vitro* cytotoxicity

Z. Chen, S.J. Gregson, P.W. Howard, D.E. Thurston. *The School of Pharmacy, University of London, CRUK Gene Targeted Drug Design Research Group, United Kingdom*

There is currently a resurgence of interest in the pyrrolobenzodiazepine (PBD) antitumour antibiotics,the first member of which (anthramycin) was discovered by Hoffman La Roche in the 1960s. Although anthramycin and some closely related naturally-occurring PBDs were evaluated at this time in preliminary clinical trials, no significant antitumour activity was observed,and anthramycin itself showed a marked cardiotoxicity. Since this time, Hurley and co-workers have elucidated the cause of the cardiotoxicity (i.e. the presence of a C9-hydroxyl moiety), and this feature can now be designed out. Recent interest in taking the PBD dimer SJG-136 into the clinic by both Cancer Research UK and the NCI (USA) has prompted us to develop novel PBD monomers suitable for clinical evaluation. Based on recently-gained knowledge of SAR,we are interested in developing new chemical approaches to the synthesis of PBD monomers with varying degrees of C-ring unsaturation and varying substitution patterns in both the A and C-rings. In particular, recent studies have shown that C2-endo/exo-unsaturation in the C-ring and electron donating groups in the A-ring are important for optimal biological potency. We have thus developed new synthetic strategies to produce molecules of this type. The approach described here involves the application of a palladium-mediated Heck coupling reaction to introduce different side chains based on N,N-dimethylacrylamide at the C2 position of the pyrrolo C-ring. For example,this has been used to synthesize the novel 7,8-dimethoxy C2-acrylamido substituted PBD ZC-14. Use of the versatile Heck reaction at a late stage in the synthesis provides access to a wide range of analogues of this type. The same approach has been used to develop a route to the total synthesis of porothramycin, a well-known naturally occurring PBD.

ZC-14 was produced in 9 steps from commercially available veratric acid.The E-configuration of the C2-acrylamide side chain was established



ZC-14

through NMR coupling constants.ZC-14 is remarkably cytotoxic in a number of cell lines with,for example,GI50 values of between 9 and 52 nanomolar in MALME3M, HCT116,SKOV3,SKMEL28,H460 and MCF7 cell lines. The complete synthesis and full *in vitro* evaluation of ZC-14 will be described and compared to a series of closely related analogues.DNA footprinting studies are currently underway to elucidate the effect of the extended C2-acrylamido side chain on sequence-selectivity,and these results will be described.

414

Design and optimization of topoisomerase poison conjugates of triple helix-forming oligonucleotides for sequence-specific DNA cleavage

P.B. Arimondo[1], D. Guianvarc'h[1], C. Bailly[2], A. Boutorine[1], J.S. Sun[1], M. Kuwahara[3], S. Hecht[3], C. Giovannangeli[1], T. Garestier[1], C. Hélène[1]. [1] *Muséum National d'Histoire Naturelle, Laboratoire de Biophysique-CNRS UMR8646, Paris, France;* [2] *COL, IRCL, INSERM UR524;* [3] *University of Virginia, Department of Chemistry, Charlottesville, USA*

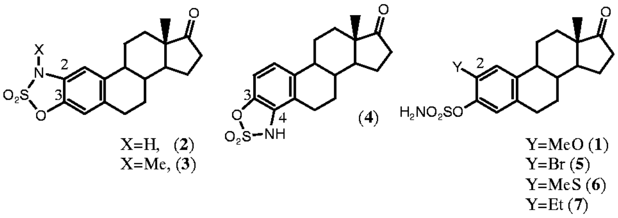
To achieve a sequence-specific DNA cleavage by topoisomerase I, derivatives of the antitumor drug camptothecin have been covalently linked to sequence-specific DNA ligands, such as triplex-forming oligonucleotides (TFO) and hairpin polyamides (MGB), that bind in a sequence-specific manner to the major and minor groove of double-helical DNA, respectively. The binding of the DNA ligand moiety of the conjugate at the target sequence positions the drug selectively at the target site, thereby stimulating topoisomerase I-mediated DNA cleavage at this site. In a continuous effort to optimize this strategy, a broad set of conjugates consisting of (i) 16-20 bases long oligonucleotides, (ii) hairpin polyamides of different length, (iii) alkyl linkers of variable length, and (iv) camptothecin derivatives substituted on the A or B quinoline ring, were designed and synthesized. Analysis of the cleavage sites at nucleotide-resolution reveals that the specificity and efficacy of cleavage depends markedly on the length of both the triple-helical structure and the linker between the oligonucleotide and the poison. Even though the CPT moiety is brought from the major groove side of DNA by the TFO and from the minor groove side by the MGB, both approaches provide a suitable route to guide a cytotoxic agent to a selected sequence in DNA. The optimized hybrid molecules induced strong and highly specific cleavage. Such rationally designed camptothecin conjugates could provide useful antitumor drugs directed selectively against genes bearing the targeted binding site. In addition, they represent a powerful tool to probe the molecular interactions in the DNA/topoisomerase I complex.

415

A-ring Analogues of Oestrone 3-O-Sulphamate as Potent Steroid Sulphatase Inhibitors and Potential Anti-Cancer Agents

L.W.L. Woo[1], M.P. Leese[1], B. Leblond[1], A. Purohit[2], L. Wood[3], G. Packham[3], J. Robinson[1], N. Vicker[1], M.J. Reed[2], B.V.L. Potter[1]. [1] *University of Bath, Pharmacy and Pharmacology, Bath;* [2] *Endocrinology and Metabolic Medicine, Faculty of Medicine, Imperial College;* [3] *CRC Wessex Medical Oncology Unit, Centre Block, Level F, Southampton General Hospital, Southampton, UK*

There is strong evidence to suggest that a concomitant inhibition of steroid sulphatase (STS), which converts oestrone (E1) sulphate to E1 and also dehydroepiandrosterone (DHEA) sulphate to DHEA, will further attenuate oestrogenic stimulation to hormone-dependent cancers. E1-3-O-sulphamate (EMATE) was the first potent, orally active, irreversible STS inhibitor developed by us but, unexpectedly, it was found to be also highly oestrogenic. Hence, the challenge has been to design non-oestrogenic STS inhibitors with comparable, or even superior, potency. Whilst there has been success in achieving this goal through the development of the non-steroidal coumarin sulphamates (notably 667COUMATE),1 we have also shown in a series of A-ring modified analogues of EMATE that 2-MeO-EMATE (1) is a highly potent STS inhibitor like EMATE, but in contrast, is devoid of oestrogenicity.2 Here, we further investigate the effects of A-ring modification of EMATE by preparing the 5-ring analogues of EMATE (2 - 4) and a series of 2-substituted EMATE (F, Cl, Br, I, CN, MeS and Et). The most potent STS inhibitor here is 2-Br-EMATE (5) whose IC_{50} of 1.7 nM from a placental microsomes preparation is some 11-fold lower than that of EMATE. Preliminary results from a luciferase reporter gene-expression assay have shown that (5) did not induce oestrogenic activity up to a concentration of 100 nM (c.f. 10 nM for EMATE) indicating that (5) is less oestrogenic than EMATE. A homology model of the human STS has been built from the crystal structure of ASA and the human STS sequence in order to understand the relationship between the binding of these compounds to the active site and their STS inhibitory activities. Although 2-MeS- (6) (IC_{50} = 120 nM) and 2-Et-EMATE (7) (IC_{50} = 820 nM) were found to be less potent than EMATE as STS inhibitors *in vitro*, like (1), these compounds have been shown to induce apoptosis (presumably via tubulin disruptions) in a MCF-7 cells proliferation assay (42%, 53% and 51% inhibition at 1 mM for 1, 6 and 7 respectively). Hence, 1, 6 and 7 are antiproliferative agents with potential applications for treating both hormone dependent- and hormone independent cancers.



X=H, (2)
X=Me, (3)

(4)

Y=MeO (1)
Y=Br (5)
Y=MeS (6)
Y=Et (7)

This work is funded by Sterix Ltd. LWLW, BVLP, AP and MR are stockholders of Sterix.

References

[1] Woo et.al. (2000) Chem. & Bio. 7: 773.
[2] Purohit et.al. (1998) J. Steroid Biochem. Mol. Bio., 64: 269.

416

Novel Antineoplastic Complexes of Bismuth(III), Cerium(III) and Lanthanum(III)

M.A. Jakupec[1], V. Arion[1], W. Berger[2], S. Wild[3], H. Zorbas[4], B.K. Keppler[1]. [1] *Inst. of Inorganic Chemistry, Vienna University, Wien, Austria;* [2] *Institute for Cancer Research, Vienna University, Wien, Austria;* [3] *Max Planck Institute of Biochemistry, Martinsried, Germany;* [4] *Institute of Biochemistry, Ludwig-Maximilian University Munich, München, Germany*

In a search for new lead structures for development of non-platinum antineoplastic metallopharmaceuticals we have identified acetatobis[1-(azepanyl)-4-(2-pyridyl)-2,3-diazapenta-1,3-dien-1-thiolato-N',N3,S]bismuth(III) (KP1255), trans-[aquachlorobis(1,10-phenantroline)cerium(III)] dichloride (KP776) and [tris(1,10-phenantroline)lanthanum(III)] trithiocyanate (KP772) as potent agents among series of related complexes, which have been synthesized in our laboratory.

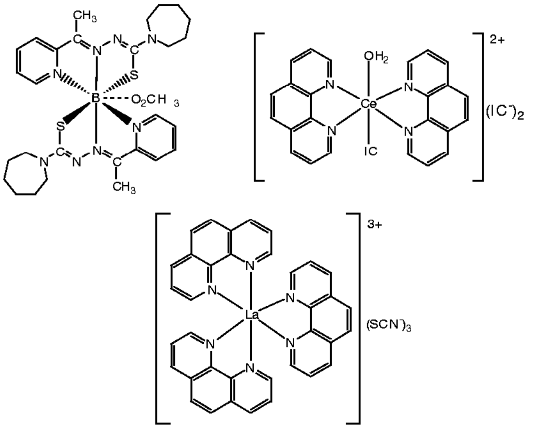


Figure 1. Structures of the bismuth complex KP1255 (top left), the cerium complex KP776 (top right) and the lanthanum complex KP772 (bottom).

Unlike platinum drugs the bismuth complex KP1255 displays rather flat dose-response curves with mean IC_{50} values in the low nanomolar range