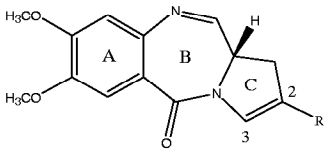
404

Structure-Activity-Relationship (SAR) study of novel C2/C3-unsaturated C2-aryl-substituted pyrrolobenzodiazepine monomer antitumour agents

N. Cooper[1], A.M. Burger[2], C.S. Matthews[1], P.W. Howard[1], D.E. Thurston[1]. [1]*The School of Pharmacy, University of London, CRUK gene targeted drug design research group;* [2]*University of Freiburg, Tumour Biology Center, Freiburg, Germany*

The pyrrolobenzodiazepines (PBDs) are low molecular weight antitumour antibiotics derived from Streptomyces species. They exert their antitumour effects by covalently binding to guanine bases in the minor groove of DNA in a sequence selective manner. Although a large variation in C-ring unsaturation patterns and A- and C-ring substitution is found in nature, we have synthesised the first examples of C2/C3-unsaturated C2-aryl-substituted variants which show differential *in vitro* cytotoxicity in tumour cell lines and promising *in vivo* antitumour activity in a number of human tumour xenografts including melanoma, ovarian and renal models. One such molecule, DRH-417, has been selected for further development by the EORTC. The aim of this study is to synthesise and evaluate a number of PBDs with structurally diverse C2-aryl moieties so that SAR techniques can be used to define the role of steric, electronic and partition coefficient parameters in order to optimise both *in vitro* and *in vivo* activity.



R = aryl functionality

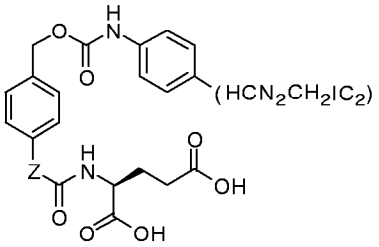
The synthetic route employed to produce these uniquely-substituted PBD monomers is novel and based on the use of the Suzuki-Miyaura coupling reaction to introduce the C2-aryl substituents. This has allowed us to take advantage of the extensive range of commercially available boronic acids to maximise the diversity of the C2-aryl moiety. For most analogues the synthetic route is robust, provides reasonable yields and should be easily scaleable for compounds selected for further development. These new PBD monomers have been screened in >30 cancer cell lines including several from the NCI's 60-cell line panel. The data demonstrate that, as a class, the compounds evaluated to date have potent anti-proliferative activities with selectivity towards melanoma, ovarian, lung and renal cell lines (mean IC_{50} values ranging from 1 to 27 nM). However, some analogues are remarkably active in melanoma (MALME3M and SKMEL28) and cisplatin-resistant ovarian (SKOV3) cell lines with IC_{50} values in the low nanomolar (i.e. 1-10) and sub-nanomolar (e.g. ~0.04) region, respectively. Preliminary human tumour xenograft experiments have demonstrated antitumour activity for some analogues in ovarian, melanoma and renal models. The results of the SAR study will be presented, and the likely influence of the Hansch-type parameters of the C2-substituent on both *in vitro* and *in vivo* biological activity described.

405

Comparisons between the behaviour of prodrugs and pro-prodrugs in Gene-directed enzyme prodrug therapy (GDEPT) with carboxypeptidase G2 (CPG2)

D. Niculescu-Duvaz[1], I. Niculescu-Duvaz[1], F. Friedlos[1], J. Martin[1], I. Scanlon[1], L. Davies[1], R. Marais[2], C.J. Springer[1]. [1]*Institute of Cancer Research, Cancer Research UK Centre for Cancer Therapeutics, Sutton;* [2]*Institute of Cancer Research, Cancer Research UK Centre for Cell and Molecular Bi, London, United Kingdom*

A large number of prodrugs have been designed to be cleaved by carboxypeptidase G2 (CPG2). The accumulated data shows that at least two factors are important in determining the activity of these prodrugs: the potency of the released drug and the kinetics of the activation step. The main limitation in the design of these prodrugs is that nitrogen mustards are the only cytotoxic moieties than can be used, due to the steric requirements for the activation process by CPG2. This obviously limits the mechanism of action of the released drugs. In order to overcome this issue the synthesis of prodrugs using self-immolative linkers was investigated and a number of pro-prodrugs were obtained. Comparative studies of their physico-chemical and biological parameters (kinetics, logP, IC_{50} in cells with and without expressed CPG2, IC_{50} of the released drug) with respect to the model prodrugs were performed.



Z = NH, O.

The linkers alone showed kinetics comparable to that of the model prodrugs. However, the pro-prodrugs showed slower kinetics. Despite this, pro-prodrugs derived from aromatic nitrogen mustards and anthracyclines achieved differentials of 33 and 23 fold respectively in colorectal carcinoma LS174T cells. This indicates that successful self-immolative prodrugs can be designed and synthesised for use in GDEPT.

406

Hydrazones derived from monosubstitued 2-acetylpyridines and 2-hydrazino-1-methylbenzimidazole: Synthesis and biological studies

J. Easmon[1], S. Fürtinger[1], G. Lackner[1], E. Magreiter[2], J. Hofmann[2], G. Heinisch[1], G. Puerstinger[1]. [1]*University of Innsbruck, Department of Pharmaceutical Chemistry, Innsbruck, Austria;* [2]*University of Innsbruck, Institute of Medical Chemistry and Biochemistry, Innsbruck, Austria*

In the course of the development of novel hydrazones as potential antitumor agents, we have found that 1-methyl-2-benzimidazolyl hydrazone derived from 2-acetylpyridine (compound EPH 116) exhibits potent cytotoxic activity (IC_{50} = 0.004-0.018 μM) *in vitro* against a pannel of human tumor cell lines (Easmon et al., Int. J. Cancer (2001) 94, 89-96). EPH 116 was also found to be a potent inducer of apoptosis in Burkitt's lymphoma cells compared to camptothecin. Furthermore, EPH 116 inhibited the growth of CXF 280 colon tumor xenografts in nude mice in a dose dependent manner. In view of this promising antitumor activity we have synthesized several analogues of EPH 116 in which various positions of the 2-acetylpyridine ring is substituted by electron withdrawing or donating groups. The antiproliferative activities of these agents were studied in a pannel of human tumor cell lines (Burkitt's lymphoma, Hela cervix carcinoma, HT-29 colon carcinoma, hydroxyurea-resistant and multidrug resistant KB cell lines). The activities were compared to that of EPH 116. The following conclusions could be drawn: i) All the compounds are potent inhibitors of the proliferation of Burkitt's lymphoma cells (IC_{50} = 0.001-2.02 μM). ii) 2-Acetylpyridines bearing electron donating substituents are highly cytotoxic to HeLa (IC_{50} = 0.0003-0.183 μM) and HT-29 (IC_{50} = 0.003-0.14 μM) cells compared to those bearing electron withdrawing groups (IC_{50} = 0.165-17.56 μM). iii) The 4-methyl- (EPH 349) and 5-methyl- (EPH 350) 2-acetylpyridine derived hydrazones turned out to be the most potent compounds especially against HeLa (IC_{50} = 0.0004 μM) and HT-29 (IC_{50} = 0.003 μM) cell proliferation. In Burkitt's lymphoma cells, two-fold IC50-concentrations of two novel hydrazones derived from 2-acetyl-6-phenylpyridine (EPH 355) and 2-acetyl-4-dimethylaminopyridine (EPH 362) induced 60 and 81 % apoptosis respectively. On the contrary, two-fold IC50-concentrations of hydroxyurea and camptothecin induced 6.5 and 10 % of apoptosis respectively. The synthesis and structure-activity relationships of this class of novel antitumor agents will be presented. Financial support was provided by the Austrian Science Foundation (FWF), project No. P12384-MOB.

407

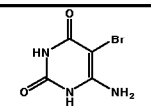
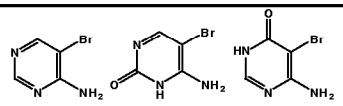
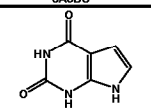
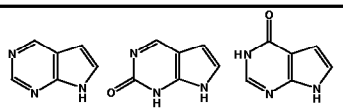
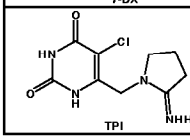
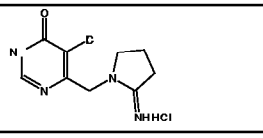
Design and synthesis of prodrugs of thymidine phosphorylase inhibitors for xanthine oxidase biotransformation

P. Reigan, E. Chinje, K.T. Douglas, S. Freeman, A. Gbaj, I.J. Stratford. *University of Manchester, School of Pharmacy and Pharmaceutical Sciences, Manchester, United Kingdom*

Xanthine oxidase (XO; EC 1.1.3.22) catalyses the oxidation of hypoxanthine and xanthine to uric acid. Hypoxia regulates XO activity at both the pre- and post-translational level. High levels of XO have been found in human colon (HT29, SW620, LOVO), bladder (RT112) and mammary (HB4a) cancer cell lines. XO activity is increased in human colorectal, prostate and brain tumours. XO prodrug activation has previously been utilised to increase the bioavailability and solubility of acyclovir and 2'-F-ara-ddI. Thymidine phos-

phorylase (TP; PD-ECGF; EC 2.4.2.4) catalyses the reversible phosphorolysis of thymidine to thymine and 2-deoxy-a-D-ribose-1-phosphate, which is dephosphorylated to give 2-deoxyribose, an angiogenic factor. TP is elevated in several hypoxic tumours, promoting both angiogenesis and metastasis, and suppressing apoptosis. Inhibitors of TP are therefore of significant interest in cancer chemotherapy. TP inhibitors are attractive targets for the XO prodrug strategy, since both enzymes are regulated by hypoxia and expressed in the cytoplasmic regions in mammary and colorectal tumours. The prodrugs synthesised were deoxygenated analogs of the known TP inhibitors, 6-amino-5-bromouracil (6A5BU), 7-deazaxanthine (7-DX) and 5-chloro-6-[1-(2-imino-pyrrolidinyl)methyl]uracil hydrochloride (TPI) (Table 1). These prodrugs were designed to exploit the oxidative hydroxylation reaction catalysed by XO.

Table 1. TP inhibitors and XO prodrugs synthesised and evaluated for TP inhibition and XO activation

TP INHIBITOR	XO PRODRUGS
 6A5BU	
 7-DX	
 TPI	

The 6A5BU prodrugs were synthesised by bromination of the appropriate 6-aminopyrimidines. The 7H-pyrrolo[2,3-d]pyrimidine prodrugs of 7-DX were obtained by cyclisation of 4-aminoprimidinyl-acetaldehydes or by Stille coupling of the relevant 5-bromo-6-aminopyrimidine. Coupling of 5-chloro-6-chloromethylpyrimidin-4-one with 2-iminopyrrolidine afforded the desired prodrug of TPI. The inhibition of recombinant E. coli TP by these prodrugs and inhibitors was determined using a spectrophotomeric assay. The XO prodrugs displayed no inhibition of TP, whereas the TP inhibitors showed the expected activity. The biotransformation of these prodrugs to the desired TP inhibitor has been evaluated using bovine XO by UV assay. In utilising the XO prodrug approach it is hoped that the prodrugs synthesised may display improved bioavailability with tumour and hypoxic specificity.

408

D-ring modified steroids as potent oestrone sulphatase inhibitors

D.S. Fischer[1], L.W.L. Woo[1], J.C. Fenton[2], A. Purohit[2], 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, St. Mary's Hospital, London, UK*

Oestrogens, and to a lesser extent androgens, play a major role in the promotion and development of hormone-dependent breast cancers. Regulation and control of the level of active steroids can be achieved via the inhibition of one or several enzymes of steroidogenesis. The enzyme oestrone sulphatase (STS), which converts oestrone sulphate to oestrone, is now considered as a key therapeutic target for depleting oestrogenic stimulation to tumours. Several potent steroidal inhibitors of STS have been reported, of which oestrone-3-O-sulphamate (EMATE) is the benchmark inhibitor. It irreversibly inhibits the enzyme in a time- and concentration-dependent manner but was found to be oestrogenic *in vivo*. In the search for new potent inhibitors of STS that are devoid of oestrogenicity, we synthesised a number of D-ring modified derivatives of EMATE. A methodology for ring expansion was developed where the D-ring of oestrone was cleaved, via a haloform reaction, to a dicarboxylic acid derivative and that was then closed by thermal condensation with urea. The N-atom of the resulting piperidinedione moiety is designed to act as a versatile anchor for the introduction of a variety of side-chains through alkylation.

Upon *in vitro* biological evaluation of the analogues, compounds 2 and 3 have been identified as the two most potent inhibitors in the series. Their

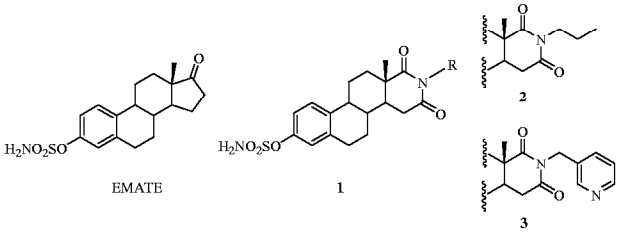


Figure. Structures of EMATE and its piperidinedione analogue **1**. The two most potent compounds are **2** and **3**.

IC_{50}s obtained from a placental microsomes preparation were both found to be 1 nM which is about 18-fold lower than that of EMATE. Unlike EMATE, these compounds are non-oestrogenic since they did not stimulate uterine growth in ovariectomised female Wistar rats at an oral dose of 10mg/kg/day administered over a period of 5 days. The crystal structure of 2 has also been determined which will provide structural information for this series of EMATE analogues. This work therefore represents a new strategy for the design of potent, orally active and non-oestrogenic oestrone sulphatase inhibitors that are structurally distinctive from previously known active compounds. This work is funded by Sterix Ltd. LWLW, BVLP, AP and MR are stockholders of Sterix.

409

Complex pattern of molecular targets for antineoplastic pteridines

D. Marko, M. Kemény, S. Meiers, K. Merz, G. Eisenbrand. *University of Kaiserslautern, Department of Chemistry, Division of Food Chemistry, Germany*

7-Benzylamino-6-chloro-2-piperazino-4-pyrrolidino-pteridine (E481) is a potent inhibitor of cAMP-specific phosphodiesterase (PDE4), inhibiting the growth of tumor cell lines in the low micromolar range. Structure-activity studies indicated that in addition to PDE4-inhibition, other cellular effects might be of relevance for the growth inhibitory activity of pteridines. The mitogen-activated protein kinase (MAPK) cascade plays an important role in the regulation of cell proliferation. We therefore investigated the influence of substituted pteridines on the MAPK cascade. The potency of the compounds to inhibit the tyrosine kinase activity of the epidermal growth factor receptor (EGFR) was studied using EGFR isolated from A431. The tyrosine kinase activity was determined by ELISA. The known EGFR inhibitor tyrphostin AG 1478 was used as a positive control. We found that the potency of E481 to inhibit EGFR kinase (IC_{50} = 48 μM) is several orders of magnitude weaker than PDE4 inhibition (IC_{50} = 16 nM). Structure-activity studies showed that modulation of the piperazino residue in position 2 of the pteridine ring system strongly affects EGFR inhibitory properties. A 2-(2-aminoethylamino)-substituent was found to strongly increase the inhibition of the EGFR. Concomitantly, PDE4 inhibitory properties were nearly eliminated. In contrast, the 6-dechloroanalogue did not show EGFR inhibitory potency, whereas PDE4 inhibitory properties were retained. We furthermore investigated the consequence of EGFR or PDE4 inhibition on downstream signaling pathways, such as the MAPK cascade, known to be crucial for cell proliferation. One of the nuclear substrates of MAP kinase is the transcription factor Elk-1. We transiently transfected A431 cells with a luciferase reporter gene plasmid whose expression depends on the phosphorylation of a GAL4-Elk1 fusion protein. For substituted pteridines, we found that not only EGFR inhibitors result in a reduction of Elk-1 phosphorylation, but also effective PDE4 inhibitors, lacking EGFR inhibitory properties. The results indicate, that the transmission of the mitogenic signal, measured as phosphorylation of Elk-1, is substantially influenced by crosstalks between the MAPK cascade and other cellular pathways like for example the cAMP pathway, which is potently affected by several members of this class of compounds.