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

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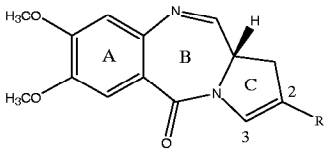
The pyrrolobenzodiazepines (PBDs) are low molecular weight antitumour antibiotics derived from Streptomyces species. They exert their antitumour effects by covalently binding to guanine bases in the minor groove of DNA in a sequence selective manner. Although a large variation in C-ring unsaturation 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.

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

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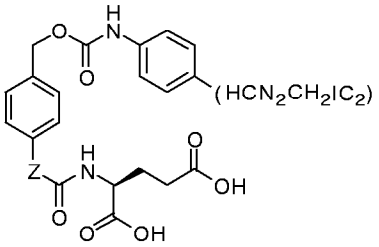
A large number of prodrugs have been designed to be cleaved by carboxypeptidase G2 (CPG2). The accumulated data shows that at least two factors are important in determining the activity of these prodrugs: the potency of the released drug and the kinetics of the activation step. The main limitation in 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.

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

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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.

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

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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-