

Poster Session 3 – Medicinal Chemistry

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Hypoxia-regulated faciliatative glucose transporter Glut-1 in the development of novel diagnostic and therapeutic anticancer strategies

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Hypoxic cancer cell are therapeutically resistant. This survival advantage is partially mediated by hypoxia-inducible genes expressed via the HIF-1 transcription factor and may be, to some extent, due to the overexpression of the HIF-1 regulated glucose transporter Glut-1 (Williams et al 2002). The deregulation of glucose transporter expression is related to the increased energy demand in cancer cells and is a characteristic of tumours. The overexpression of Glut-1 occurs in virtually all tumours, and predicts poor prognosis and an increased likelihood of metastasis in a variety of cancers, including carcinoma of the cervix (Airley et al 2001). It is proposed that the observed changes in tumour glucose transport may be exploited for diagnostic and therapeutic strategies. This includes the use of Glut-1 as a marker of hypoxia, with applications in the rational selection of patients for treatment with radiotherapy, as well as with hypoxia-selective chemotherapy. We have shown that Glut-1 expression correlates directly with pO_2 measurements in carcinoma of the cervix (Airley et al 2001). Here, we show that Glut-1 expression also correlates with the intracellular binding of the bioreductive hypoxia probe pimonidazole ($r = 0.536$, $P = < 0.001$, $n = 42$) in Ca cervix. So, to establish the generality of the use of Glut-1 as a marker of hypoxia, we have extended our immunohistochemical analysis into other tumour types, including tumours of the head and neck, breast, brain and paediatric neuroblastomas and rhabdomyosarcomas. We find that expression of Glut-1 varies widely, between and within tumour types. Similar variations in gene expression are also observed in panels of human tumour cell lines in-vitro (cervix, breast, lung and liver), which show variations in both constitutive and hypoxia-inducible expression of Glut-1.

We are also measuring Glut-1 expression in the NCI panel of 60 cell lines that are used in the cancer drug toxicity screening programme. We are ranking cell lines on the basis of their constitutive expression of Glut-1 and we will relate this to the toxicity profile of all the compounds that have gone through the NCI screen. We hypothesise that this COMPARE analysis will show a link between Glut-1 expression and sensitivity to certain glucose link cytotoxic molecules (e.g., glufosfamide) which are preferentially taken up by tumour cells, and certain flavones (e.g., genistein) which directly inhibit glucose transporters. A link between Glut-1 expression and cytoxicity of certain molecules may highlight candidate lead compounds in the search for novel Glut-1 dependent anti-cancer drugs.

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Effect of piperine and its analogues on melanocyte dendricity and melanogenesis

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Melanin is synthesised by melanocytes and transferred to the surrounding keratinocytes with the help of melanocyte dendrites. In vitiligo, melanocytes tend to lose their dendrites before they disappear from the lesion. We have previously reported that piperine and its analogues stimulate the proliferation of melanocytes and alter cell morphology in-vitro (Lin et al 1999; Venkatasamy et al 2001). In this study we have investigated the effect of these compounds on dendricity and melanogenesis.

For the determination of melanocyte dendricity, mouse melanocyte (melan-a) cells (1.5×10^3) were plated in a 35-mm cell culture dish and incubated for 72 h with 10 μM of each compound. At 72 h, representative fields were observed under phase contrast microscopy and cell morphology assessed by a computer aided image analyser. For melanin content determination, the cells (1.5×10^3) were plated and incubated in the same fashion. At 72 h cells were harvested and cell number determined using a haemocytometer. Melanin content was determined by solubilising with 1 M NaOH and measuring the optical density at 475 nm.

As reported earlier (Venkatasamy et al 2001), all the compounds tested increased the cell number compared to control. We have observed that the compounds also increased the number and length of dendrites. The melanin content per dish was increased but the melanin content per cell was not significantly different from control. The results are shown in Table 1.

Table 1 Effect of piperine and its analogues on mouse melanocyte dendricity and melanin content

	Mean no. of dendrites per cell	Mean length of dendrites	Mean cells no. (% control) ± s.e.m.	Total melanin content (mg) ± s.d.	Melanin content (μg/cell) ± s.d.
Control	0.65	37.63	100	16.4 ± 0.7	0.29 ± 0.03
Piperine	2.45*	67.72	156 ± 16*	24.5 ± 1.7	0.28 ± 0.04
CHP	2.83*	65.60	146 ± 16*	19.6 ± 3.5	0.24 ± 0.03
THP	2.63*	64.65	135 ± 22*	22.3 ± 4.1	0.30 ± 0.06
PPT	2.55*	67.42	141 ± 11*	21.6 ± 2.2	0.28 ± 0.02

*$P < 0.05$, vs control (unpaired *t*-test). CHP = cyclohexylamide derivative; THP = tetrahydropiperine; PPT = piperettine

To conclude, this study showed that piperine and selected analogues stimulated the proliferation and dendrite formation of mouse melan-a melanocytes in-vitro. Since the cells are in the proliferation phase, melanogenesis was not much altered.

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Synthesis of *myo*-inositol tetrakisphosphate analogues: possible treatment for the cystic fibrosis airway

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Cystic fibrosis is the most common fatal genetic disease, arising from a defective gene for salt and fluid secretion. The lungs are severely affected by bacterial infections, which typically lead to early death. Ways to enhance the chloride-channel activity should therefore be of therapeutic benefit. There is evidence to suggest that the inositol tetrakisphosphates, D-*myo*-Ins(3,4,5,6)P_4 and D-*myo*-Ins(1,4,5,6)P_4, down-regulate and stimulate chloride and water efflux, respectively. Therefore antagonists of D-*myo*-Ins(3,4,5,6)P_4 and agonists of D-*myo*-Ins(1,4,5,6)P_4 are of potential benefit to cystic fibrosis patients (Eckmann et al 1997).

It has been reported that difluoro analogues of the inositol tetrakisphosphates act as agonists of D-*myo*-Ins(1,4,5,6)P_4 (Xie et al 1998). We report here the synthesis

of some mono fluoro analogues, D-1-deoxy-1-fluoro-*myo*-Ins(3,4,5,6)P_4, D-3-deoxy-3-fluoro-*myo*-Ins(1,4,5,6)P_4, D-2-deoxy-2-fluoro-*myo*-Ins(3,4,5,6)P_4 and D-2-deoxy-2-fluoro-*myo*-Ins(1,4,5,6)P_4, of D-*myo*-Ins(3,4,5,6)P_4 and D-*myo*-Ins(1,4,5,6)P_4, as potentially better agonists.

An important intermediate, D/L-3,4,5,6-tetra-*O*-benzyl-*myo*-inositol was synthesised (Solomons et al 1998), which acted as a common starting point for synthesis of all four mono fluoro analogues. This racemic intermediate needs to be resolved to obtain the monofluoro analogues as single enantiomers. The racemate was separated by forming diastereoisomeric esters with optically active (−)-(1S, 4R)-camphanic acid chloride. Either selective recrystallisation or flash column chromatography could separate these diastereoisomers. To obtain the correct, *myo* stereochemistry overall, the second reactive centre needed to be inverted using Mitsunobu chemistry. Indeed the use of the Mitsunobu chemistry also led to a shortened synthesis for one pair of the enantiomers. Previously the use of (diethylamino)sulfur trifluoride (DAST) had been the only procedure used to introduce fluoride groups, but the use of DAST on one of the routes led solely to a ring closure reaction and an unwanted side product, which did not contain fluorine. Here the formation of a triflate, and displacement with tetrabutyl ammonium fluoride (TBAF) gave the required monofluorides. Benzyl protecting groups were removed by hydrogenation using activated palladium on charcoal as a catalyst. The phosphate groups were introduced with dibenzyl *N*,*N*-diisopropylphosphoramidite, in the presence of 1*H*-tetrazole, followed by oxidation with meta-chloroperoxybenzoic acid (mCPBA). The *P*-*O* benzyl groups will be removed by hydrogenation to give the enantiomerically pure mono fluorinated inositol tetrakisphosphates, after which biological testing will be initiated.

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Thymidine phosphorylase from *Escherichia coli*: substrates and tight-binding inhibitors

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Thymidine phosphorylase (TPase, EC 2.4.2.4) catalyses reversible phosphorolysis of pyrimidine 2'-deoxynucleosides, forming 2-deoxyribosephosphate and pyrimidine, and provides metabolic routes for anti-pyrimidine therapeutic agents, such as 5-fluorouracil and antivirals. The recent discovery that TPase is identical with platelet-derived endothelial cell growth factor (PD-ECGF) and has angiogenic activity has given major impetus to the design of strong, specific inhibitors of the human enzyme. X-ray crystal structures for TPase from *Escherichia coli* and *Bacillus stearothermophilus*, led to a calculated model for human TPase, which is some 40% sequence-similar to the *E. coli* enzyme and thence to rationalisation of the strong inhibition by 5-chloro-6-(2-imino-pyrrolidin-1-ylmethyl)-1*H*-pyrimidine-2,4-dione (TPI) of human TPase (Cole et al 1999), which TPI inhibits competitively (K_i 17 nM, Fukushima et al 2000). Kinetic patterns do not transfer well between species for TPase and we now report that towards *E. coli* TPase TPI and an imidazole analogue, 6-(2-amino-imidazol-1-ylmethyl)-5-bromo-1*H*-pyrimidine-2,4-dione), act as extremely tight binding inhibitors. We also report the optimisation of a spectrophotometric assay (Nakayama et al 1980) that we have adapted to allow 96-well assay of this enzyme and inhibition studies.

Assays of *E. coli* TPase (Sigma Chemical Co.) were conducted at 355 nm at 25°C using a 96-well Molecular Devices (Spectramax) spectrophotometer at 0.13 mM 5-nitro-2-deoxyuridine, 0.1M phosphate pH 7.4 with 45 nM TPase. 3',5'-Diacetyl-5-nitro-2'-deoxyuridine, prepared from 3',5'-diacetyl-2'-deoxyuridine by transfer nitration using triflic acid and 1-nitropyrazole, was alkaline methanolysed to 5-nitro-2'-deoxyuridine.

When percent activity was plotted against TPI concentration the apparent I50 value was proportional to the enzyme concentration indicating a process other than simple inhibition. For both TPI and the imidazolic inhibitor a plot of activity against concn inhibitor for data between 20 and 100% residual activity gave straight lines with intercepts on the concn inhibitor axis proportional to the amount of enzyme added. The simplest explanation is extremely tight (stoichiometric) binding. To assess this, a mixture of enzyme and TPI (1:1.3 molar ratio) was gel-filtered (Sephadex G-25). Activity of eluted protein was 0.52 ± 0.03% of control similarly treated in the absence of TPI. Thus, the inhibition is sufficiently tight not to be reversed by gel filtration taking place over 155 min. In this situation the intercept on the [I] axis of plots of residual activity vs [I] gives the concentration of enzyme. This was confirmed by using the imidazolic inhibitor and TPI to titrate the same stock solution of TPase: titration inhibition-data coincided for these inhibitors of different structures.

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In-vitro evaluation of a range of substituted phenyl sulphamates and the role of pK_a of the parent phenol in the inhibition of oestrone sulphatase

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It has been shown that the inhibition of the enzyme oestrone sulphatase may be a new therapeutic target in the treatment of hormone-dependent breast cancer. We have been involved in the search for potent irreversible inhibitors of this enzyme. In general, the potent inhibitors contain a sulphamate moiety. From an initial consideration of the recently proposed mechanism for the enzyme oestrone sulphatase (ES), we concluded that the potent inhibitory activity observed within compounds containing the sulphamate moiety was probably related to the stability of the anion which has been presumed to exist during the course of the ES catalysed reaction. We argued that electron-withdrawing groups would stabilise the O^-, while electron-releasing groups would destabilise the resulting ion, as such the inhibitory activity would be expected to increase and decrease respectively. Here, we report the results of our initial study into the role of pK_a and thus describe the synthesis and evaluation of some phenolic-based compounds. As a result of this work we propose a novel mechanism for the inhibition of ES by sulphamate-based compounds.

In the synthesis of the inhibitors, we used a standard literature procedure involving the reaction between aminosulphonyl chloride with the phenolic derivative. In the synthesis of the 3- and 4-substituted compounds no major problems were encountered and the compounds were obtained in good yield. However, although a number of ortho substituted compounds have been shown to undergo sulphamoylation, we could not achieve the desired ortho sulphamoylated product from the parent phenol.

The biochemical evaluation of the series of compounds was undertaken in triplicate using the previously reported method of Selcer et al (1996). The pK_a of the parent phenols were determined using a spectrophotometric method (Harwood & Moody 1989).

The results (Table 1) of the biochemical evaluation show clearly that a correlation does indeed exist between the inhibitory activity and the pK_a of the starting phenol.

That is, when the hydrolysis reaction occurs, the resulting phenoxide ion and its stability is a major factor in determining the inhibitory activity of the sulphamate containing inhibitors.

Table 1 Initial screening of four sulpahamate derivatives of phenol (n = 3)

R	IC50 (μM)	pK_a
p-Cl	1584 ± 9.7	9.5
p-Br	912 ± 5.6	9.29
m-CN	191 ± 4.3	8.54
m-NO_2	120 ± 3.9	8.28

From the results of this study, we are able to propose a new mechanism for the inhibition of ES by sulphamate containing compounds which takes into consideration the cleavage of the S-OAr bond and the irreversible inhibition through the formation of an imine moiety at the active site.

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Weak inhibitory effect of *Vochysia suraminensis* and *V. lehmanii* crude extracts on the cell proliferation of three human cancer cell lines

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The Vochysiaceae comprises approximately 200 species and 7 genera (*Callisthene*, *Erisma*, *Erismadelphus*, *Euphronia* (*Lightia*), *Qualea*, *Salvertia* and *Vochysia*), which are geographically distributed throughout tropical America and have some economical value as timbers. Although the biochemical and biological activities have not been studied very well, recently some investigations revealed that a few compounds of the genus *Vochysia* exert interesting biological properties such as cytotoxic and antiviral effects (Boudouin et al 1983; Houghton 1997). Thus, it was considered important to evaluate the anti-proliferative effect of two indigenous South American species, *Vochysia suraminensis* and *V. lehmanii*.

Ethanolic extracts were made from the bark. The effect on the cell proliferation rate was analysed by SRB in-vitro assay (Skehan et al 1990), using human cancer cell lines, non-small cancer cell (CORL23), breast adenocarcinoma (MCF7) and colon cancer (LS174T), as models for anti-proliferative effect. The growth profiles and optimum concentration of cells was determined as well as the effect of EtOH and DMSO to discard the possible interference of the solvent in the activity of the extracts.

The analysis showed slight differences between the growth profiles of the three human cancer cell lines when extracts were dissolved in the two different solvents. No remarkable inhibitory effect on the growth rate of any of the cell lines was observed. The IC50 values were over 200 $\mu g\,mL^{-1}$., this value being lower than the threshold for activity laid down by the NCI for active crude extracts from plants, which is $< 50\,\mu gmL^{-1}$.

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