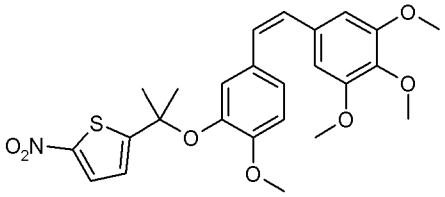
antivascular activity in the clinic, derivatives of CA4 that have a more selective antitumour activity would be advantageous.
We have designed ANG704 as a bioreductive prodrug of CA4 using the trigger-effector principle, wherein a moiety (the trigger) attached to the active drug renders the prodrug inactive until release of the drug by fragmentation, which occurs selectively under the hypoxic conditions found in solid tumours *via* an oxygen-sensitive free radical intermediate. Unlike CA4, ANG704 was itself inactive as an inhibitor of bovine brain tubulin polymerisation (CA4: IC50 2.8 μM; ANG704: no inhibition at 20 μM) and inhibition of A549 cell growth (CA4: IC50 0.25 μM; ANG704: no activity at 2 μM). Under anoxic conditions ANG704 efficiently released CA4 in the presence of supersomal cytochrome P450 reductase or homogenates of CaNT (syngeneic mouse mammary adenocarcinoma) or FaDu (human squamous cell carcinoma xenograft) tumours. The release of CA4 was inhibited in air under similar conditions. Oxygen-inhibited release of CA4 was also catalysed by lysate from A549 cells and by whole A549 cells in culture. ANG704 was stable when incubated under aerobic conditions with homogenate prepared from mouse liver, with rates of both prodrug loss and CA4 production of less than 0.01 nmol/min/mg protein at 5micromolar prodrug concentration.
ANG704 has promising *in vitro* activity as a metabolically-stable bioreductive prodrug of combretastatin A4.



ANG704

498 POSTER

Selective potentiation of the hypoxic cytotoxicity of the bioreductive drug tirapazamine

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Of the 3 mono-N-oxides examined so far by clonogenic assay of hypoxic cell suspensions, SN 29051 provided similar dose dependent potentiation of TPZ hypoxic cytotoxicity as SR 4317, while SN 29254 and SN 29047 provided greater potentiation (an extra 4 logs of killing relative to TPZ alone at a non-toxic potentiator concentration of 0.3 mM).

SN	R	X	Hypoxic TPR	Aerobic TPR	Hypoxic Cytotoxicity Ratio*
TPZ					66±10
SR 4317	H	H	2.6±0.2	0.8±0.3	240±35
29051	NH(CH2)2 N(CH3)2	H	21±3	1.3±0.3	1090±220
29047	OCH3	H	7.3±4.0	0.9±0.1	520±200
29254	NH(CH2)2 pyrrolidone	H	41±5	1.1±0.1	3145±215
29334	NH(CH2)2 N(CH3)2	6iPr	5.6±0.3	0.9±0.1	415±50
29112	NH(CH2)2 N(CH3)2	6CF3	13±6	0.9±0.2	680±190
29059	NH(CH2)2 N(CH3)2	8CH3	17±8	0.9±0.1	805±85

*Aerobic IC50/hypoxic IC50 of TPZ combined with the maximum non-toxic concentration of potentiator.

The plasma pharmacokinetics of TPZ (0.13 mmol/kg), determined from HPLC analysis of tail vein bleeds of CD-1 nu/nu mice, were not altered by co-administration of SN 29051 (0.42 mmol/kg). This combination also provided no toxicity against the retina – a hypoxic normal tissue that is damaged by TPZ at its MTD in mice. The results of HT29 xenograft excision assays, currently under progress will be reported.
Conclusions: Benzotriazine mono-*N*-oxides can selectively potentiate hypoxic cell killing by TPZ, and may improve the therapeutic utility of TPZ as a hypoxic cytotoxin in cancer treatment.

499 POSTER

N(O)-(Substituted)-b-(2),4,5-tetramethyl-3,6-dioxo-1,4-cyclohexadinen-1-propa(noate) propanoamide: bioreductive delivery systems for selective delivery of therapeutic agents into solid tumours

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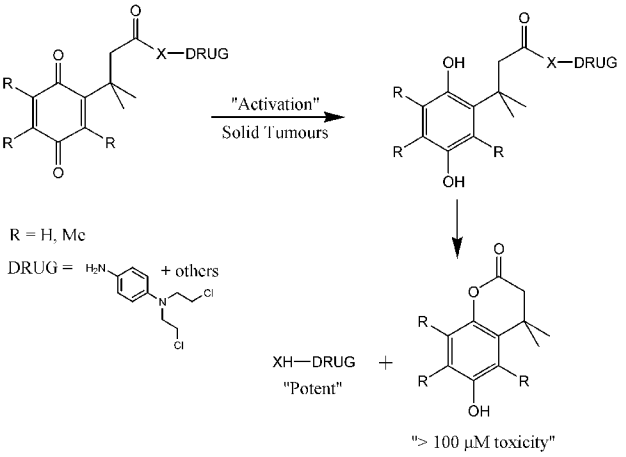
1,4-Cyclohexadiene (benzoquinone) agents have been previously developed to target the aerobic and hypoxic function of solid tumours. They are prodrugs that can either be activated under hypoxic or reductive conditions e.g. NQO1 to afford a therapeutic effect. The 1,4-cyclohexadienes synthesised here can be utilised to deliver a therapeutic entity (toxic or non-toxic) under solid tumour conditions. Upon hypoxic or reductive activation, the 1,4-cyclohexadiene product is converted to the 1,4-cyclohexadiol which spontaneously cyclises to afford the active drug (see figure).



Here we report on the synthesis of a series of 1,4-cyclohexadinenes drug conjugates (in which the drug is attached via an ester or amide link). Starting from methyl substituted 1,4-cyclohexadiols and methyl-3,3-dimethylacrylate to produce the desired lactone 6-Hydroxy-4,4,7,8-tetramethyl-1-benzopyran-2-one. Oxidation of the lactone affords the proponic acid precursor. Addition of a therapeutic agent via and ester or amide link affords the desired prodrugs. The lead cyctotoxic compound of this series has been evaluated for it toxicity in the A549 breast carcinoma cell line. The prodrug was at least 3-fold less potent (inactivated) under aerobic conditions when compared to the free drug (*N,N-bis* (2-chloroethyl)-benzene-1,4-diamine) (IC_{50} (air) prodrug: 14.04 μM IC_{50} (air) free drug 4.52 μM) Under hypoxic conditions similar toxicity was observed for the two compounds (IC_{50} (hypoxia) prodrug 6.70 μM, IC_{50} (hypoxia) free drug 4.52 μM). These results suggest that the 1,4-cyclohexadiene drug conjugates can be utilised to selectively deliver potent agents specifically and selectively into solid tumours.

500 POSTER

Bioreductive activation and in vitro cytotoxicity of MUP03/704: a novel bioreductive cytotoxic drug conjugate for solid tumours

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MUP 03/704 is a novel quinone based cytotoxic prodrug (see structure), initially designed to target NQO1 (NAD(P)H: quinone oxidoreductase 1), a cytosolic flavoprotein catalysing two-electron reductions, for its bioactivation. When reduced, the compound spontaneously breaks down to afford a potentially non-toxic lactone and the activated alkylating agent (N,N-*bis*-2-[chloroethyl]-benzene-1,4-diamine). In the absence of reduction, the prodrug is potentially non-toxic. The purpose of this study is to establish the proof of principle of MUP 03/704 cytotoxicity in cancer cell lines.
The affinity of NQO1 for MUP 03/704 as a substrate was measured using spectrophotometry. The MTT assay was used to evaluate the cytotoxicity of the prodrug in H460 lung cancer cells (high NQO1 activity) and BE colon cancer cells (no NQO1 activity). MUP 03/704 interstrand crosslinks (ICLs) induction and repair were evaluated in the same cell lines using the comet