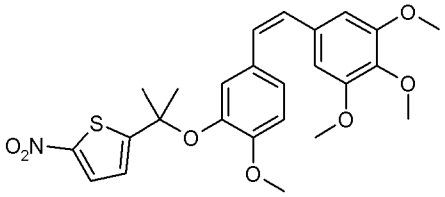
antivascular activity in the clinic, derivatives of CA4 that have a more selective antitumour activity would be advantageous.
We have designed ANG704 as a bioreductive prodrug of CA4 using the trigger-effector principle, wherein a moiety (the trigger) attached to the active drug renders the prodrug inactive 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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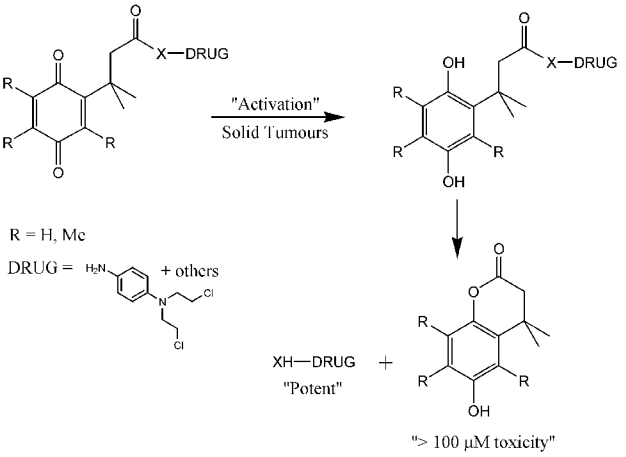
1,4-Cyclohexadiene (benzoquinone) agents have been previously developed to target the aerobic and hypoxic function of solid tumours. They are prodrugs that can either be activated under hypoxic or reductive conditions e.g. NQO1 to afford a therapeutic effect. The 1,4-cyclohexadienes synthesised here 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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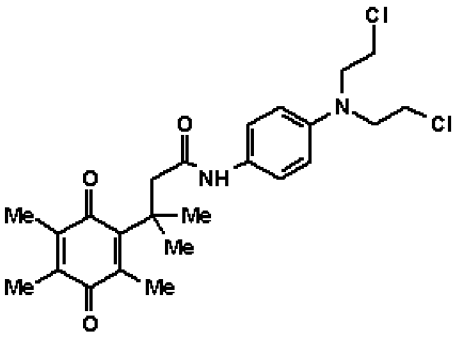
MUP 03/704 is a novel quinone based cytotoxic prodrug (see structure), initially designed to target NQO1 (NAD(P)H: quinone oxidoreductase 1), a cytosolic flavoprotein catalysing two-electron reductions, for its bioactivation. When reduced, the compound spontaneously breaks down to afford a potentially 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

assay. MUP 03/704 exhibited a Km of approximately 940 μM and a Vmax of 5.77 mmol/min/mg of NQO1. A 5-fold difference in IC_{50} was obtained between the two cell lines, with 20 μM in H460 cells and >100 μM in BE cells, after 1h exposure to the drug. Analysis of DNA ICLs in cell lines showed differences both in terms of extend of DNA damage induced and repaired. At a dose of 20 μM, 10% more ICLs were obtained in H460 cells compared to BE cells, with respectively around 40% and 30% of DNA crosslinked. But, after just 6h of recovery, BE cells repaired approximately 78% of the damage whereas H460 cells repaired only around 45%. In addition, treating H460 cells with flavone-8-acetic acid (a known inhibitor of NQO1) prior and during the drug treatment did not significantly reduce either the drug cytotoxicity or the ICLs formation.



MUP 03/704 chemical structure.

In conclusion, the results of this study indicate MUP 03/704 could be effectively reduced by NQO1 in cell free system and induced formation of ICLs in cancer cells. The results in interstrand crosslinks induction and repair may explain the variation in cell line sensitivity to the drug. In addition, other reductases, such as cytochrome P450 reductase could be activating the prodrug. More studies will be carried out to further characterise its pharmacological features and to investigate its bioactivation mechanisms.

Topoisomerase I inhibitors

501 POSTER

Prospective UGT1A1 genotyping in a phase I study of safety and pharmacokinetics of liposome encapsulated SN-38 (LE-SN38)

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Polymorphisms in the promoter region of the hepatic enzyme UGT1A1 are associated with increased risk of irinotecan (CPT-11) toxicity. These variant alleles affect expression of this enzyme, which glucuronidates SN-38, the active moiety of CPT-11. This Phase I study is assessing safety and pharmacokinetics of liposomal SN-38 (LE-SN38) in patients with advanced cancer who have failed prior therapies. To establish safe dose levels for patients with and without the common *UGT1A1*28* variant allele, patients are stratified prospectively according to their *UGT1A1* genotype, defined by the number of TA repeats in the $A(TA)_nTAA$ promoter sequence. Strata consist of homozygous wild-type (6/6), homozygous variant (7/7), and heterozygous (6/7) patients, who are expected to have normal, low, and intermediate levels of glucuronidation activity, respectively. LE-SN38 is infused intravenously over 90 minutes every 21 days until disease progression or unacceptable toxicity occurs. Dose escalation is planned with separate patient cohorts receiving 2.5 to 90 mg/m^2 of LE-SN38. As of May 2004, genotype frequencies of 152 screened patients were 43% homozygous wild-type, 44% heterozygous, 11% homozygous variant, and 2% other; 58 of these patients were enrolled in the study. Dose escalation has reached 40 mg/m^2 for the wild-type and heterozygous strata, and 20 mg/m^2 for the homozygous variant stratum. Best response has been stable disease for up to 15 treatment cycles. Pharmacokinetic (PK) data indicate that drug exposure is greatest in homozygous variant patients, where the rate of conversion to SN-38 glucuronide is greatly reduced. PK differences between the wild-type and heterozygous strata are less pronounced. At a dose of 40 mg/m^2 LE-SN38, preliminary mean AUC_{0-8} values for plasma SN-38 in the latter groups were 3223 and 6498 ng·hr/mL, respectively, exceeding the value of 1120 ng·hr/mL reported for the approved CPT-11 dose of 350 mg/m^2. Severe diarrhea, which can occur with CPT-11 treatment, has not been observed. However, neutropenia appears to be dose limiting, with 2 wild-type patients experiencing dose-limiting toxicity at 40 mg/m^2. One of these 2 patients was heavily pre-treated with 9 prior chemotherapeutic regimens. To bring the best possible dose into Phase II second and third line patient populations, the study has been amended to continue dose escalation in the wild-type and heterozygous strata by enrolling only minimally pre-treated patients (⩽3 prior regimens). Dose escalation and accrual also continues in the homozygous variant stratum. Greater drug exposure observed in homozygous variant patients suggests that prospective genotyping is warranted to prevent overdosing of these patients. It remains to be determined whether wild-type and heterozygous patients will exhibit clinically significant differences in safety profiles that would require differential dosing for these patients.

502 POSTER

SUMO conjugation and proteolysis regulate cell sensitivity to DNA topoisomerase I poisons

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Camptothecin (CPT) reversibly stabilizes a covalent DNA toposiomerase I (Top1)-DNA complex, which is converted into lethal lesions during S phase. Despite intense investigation, cellular processes required for the repair of CPT-induced DNA lesions remain poorly characterized. To address this, a yeast genetic screen was used to define genes that protect cells from self-poisoning *top1* mutants: Top1T722A mimics CPT by inhibiting DNA religation, while Top1N726H exhibits increased rates of DNA cleavage. A *UBC9* mutant (*ubc9P123L*) was isolated with enhanced sensitivity to Top1 poisons and DNA damaging agents at 35ºC. *UBC9* encodes a highly conserved E2 enzyme that conjugates the ubiquitin-like protein SUMO to lysine residues in substrate proteins. Sumoylation alters protein activity, subcellular localization and/or complex formation. SUMO is also recycled by the Ulp1 and Ulp2 proteases. In *ubc9P123L*, a Pro123 to Lys substitution reduces SUMO conjugates at 35ºC. This suggests a higher threshold of Ubc9 activity is required to maintain cell viability in the presence of genotoxic agents. Supporting this model, *ubc9P123L* complemented the essential function of the Ulp2 protease at 35ºC, but not cell hypersensitivity to hydroxyurea (HU) and Top1 poisons. Further, overexpression of human *UBC9* restored the viability of yeast strains deleted for *UBC9*, yet did not restore *ubc9P123L* cell resistance to Top1 poisons or HU. In human Ubc9, Pro123 lies in a loop over the catalytic cysteine, and is immediately N-terminal to residues implicated in substrate binding. As a Pro123 to Ala mutation had no effect on Ubc9 activity, structural perturbations in Ubc9P123L seem unlikely. Rather chimeric human-yeast Ubc9 enzymes indicate substrate binding and/or E3 ligase interactions are critical determinants of Ubc9 function in yeast. This is consistent with our identification of the *Siz1* E3 ligase as a dosage suppressor of *ubc9P123L* cell sensitivity to Top1 poisons. In contrast, cells deleted for *ULP2* (*ulp2Δ*) were extremely sensitive to Top1 protein levels. *ulp2Δ*, *top1Δ* cells were hypersensitive to HU at all temperatures and rapidly acquired compensatory mutations, allowing growth at 35ºC. HU sensitivity at 26–30ºC and the genetic instability were suppressed in wild-type *TOP1*, *ulp2Δ* strains. Yet, these cells did not tolerate increased levels of Top1. Thus, diverse effects on SUMO conjugation, induced by defects in Ubc9 or SUMO proteases, alter the cytotoxic consequences of Top1 activity.

503 POSTER

First results of diflomotecan, a new topoisomerase 1 inhibitor, as oral soft-gel capsules in a phase I dose escalation study in patients with advanced malignant solid tumours

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Diflomotecan is a new generation topo 1 inhibitor, being an E-ring modified camptothecin analogue. It was tested versus irinotecan and topotecan in xenograft models, both as oral and intravenous (iv) administration. In terms of both tumour growth inhibition and survival time, diflomotecan was more active than irinotecan and topotecan in most models. As oral bioavailability of diflomotecan in the preclinical setting was high (65%), it entered in a phase I dose escalation study.
A total of 18 patients were enrolled, 10 men and 8 women. The median age was 56 years (33–70), and the median WHO PS 1 (0–1). Patients