Drug screening

440

Cytotoxic indolequinones as NQO1-directed tumour-specific bioreductive prodrugs

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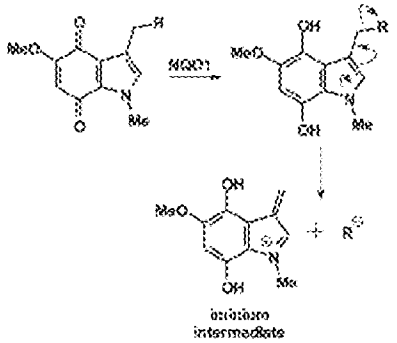
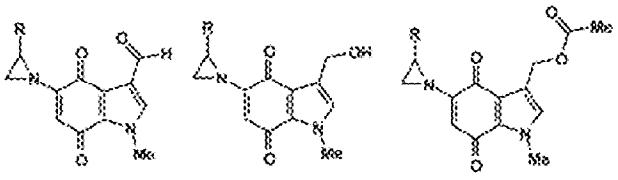
Introduction: The synthetic series of indoloquinone bioreductive alkylating agents were originally developed to target the hypoxic fraction of solid tumours. They are somewhat similar in structure to the naturally occurring anti-tumour antibiotic mitomycin C (MMC).



They are catalysed by reductive enzymes, such as DT-diaphorase (NQO1), which is present or over-expressed in many solid tumours. The catalytic process results in the formation of the iminium intermediate (via a C-3 elimination process), which is one of the main alkylating species that is responsible for their cytotoxic effects. Aims We report the synthesis of novel indolequinoes with varying substituents at the C-3 and C-5 positions of the indole ring. The compounds were evaluated as bioreductively-activated cytotoxins in a clonal *in vitro* model in order to investigate the structure-activity relationship for the NQO1 under aerobic conditions. Materials and methods The T47D (parental) human breast tumour cell line has low levels of endogenous NQO1. The parental was transfected with mammalian expression vectors encoding the cDNA for human NQO1. Stable clones were isolated and characterised as NQ-1 (300-fold over-expression of DTD). The synthetic indolequinone analogues were evaluated for their cytotoxicity against the parental and the NQ-1 clonal cell lines under both aerobic (and hypoxic) conditions. The compounds were compared against MMC. The compounds were also evaluated for specificity to NQO1 in a cell-free system. Results and conclusion Overall, the lead compound 3-[(acetoxy)methyl]-5-aziridinyl-1-methylindole-4,7-dione (C-1) was significantly more potent than MMC in all the cell lines under aerobic conditions (see Table).



Drug	T47D	IC50(air) (μM)	IC50 (N2) (μM)	DTD specificity
MMC	Wt	2.3 ± 2.0	0.75 ± 0.22	–
	NQ-1	2.7 ± 0.13	1.01 ± 0.36	–
A1	Wt	1.33 ± 0.47	3.5 ± 2.5	
	DT-1	1.62 ± 0.37	2.54 ± 1.67	75.53
A2	Wt	1.89 ± 1.11	14.0 ± 7.5	
	DT-1	3.7 ± 1.8	27.8 ± 3.8	39.23
	DT-1	0.18 ± 0.13	0.025 ± 0.015	42.11
B2	Wt	54.8 ± 17.0	2.46 ± 2.4	
	DT-1	0.66 ± 0.30	0.418 ± 0.193	12.81
C1	Wt	0.50 ± 0.18	0.055 ± 0.036	
	Dt-1	0.00087 ± 0.004	0.0016 ± 0.0014	–
C2	Wt	3.67 ± 1.49	0.35 ± 0.064	
C2	Dt-1	0.0059 ± 0.0055	0.0020 ± 0.0013	–

The lead compound was more toxic in the NQ-1 cell line than the parental under aerobic conditions suggesting that they are excellent substrates for NQO1 (Table). In the absence of the 3-acetyl substituent the potency is markedly reduced this may be due to the reduced rate of formation of the reactive intermediate species. Thus, novel and potent indolequinones can be designed to selectively target the aerobic fraction of DTD-rich tumours.

441

In vivo chamber angiogenesis assay: A simple growth factor-induced angiogenesis assay for pre-clinical screening of anti-angiogenic compounds

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Efficient *in vitro* and *in vivo* angiogenesis assays, to assess and compare anti-angiogenic activity are a prerequisite for the discovery and characterization of anti-angiogenic targets. Here we describe an optimized Matrigel plug assay based on subcutaneous implanted chambers and two fast and reproducible measuring techniques. Plexiglas ring/nylon net filter-chambers (0.2 ml) containing growth factor-reduced Matrigel and 300 ng basic fibroblast growth factor (bFGF) were subcutaneously implanted into the right flank of rats. Chamber angiogenesis was scored on day 10 post-implantation by computer image analysis of the chamber, and by optical density reading at 415 nm of a PBS solution of the chamber content. bFGF significantly induced chamber angiogenesis. Histological examination confirmed that numerous functional blood vessels with red blood cells within the lumen were present in the bFGF-stimulated chambers. The anti-angiogenic positive control compound TNP-470 (10 mg/kg/d) completely inhibited bFGF-induced angiogenesis ($p<0.0001$, Mann Whitney U-test). In contrast, the immuno-inhibitory compounds cyclosporin A (15 mg/kg/d), indomethacin (1 mg/kg/d), and prednisolone (5 mg/kg/d) showed no anti-angiogenic activity, indicating that the bFGF-induced angiogenesis was driven by bFGF and not by an inflammatory response or a foreign body reaction. We conclude that the present chamber angiogenesis assay is a very useful method for evaluation of the effects of anti-angiogenic compounds.

442

A high-throughput fluorescent anisotropy screen to identify small molecules that inhibit AP-1 binding to DNA

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B-ZIP proteins are dimers that bind to sequence-specific DNA and regulate gene expression. These include the FOS|JUN heterodimer and C/EBP homodimer that have been implicated in regulating cell growth and apoptosis. Thus these proteins are potential therapeutic targets for cancer therapy. After B-ZIP protein binding to fluorescein labeled DNA, the tumbling rate of the DNA dramatically slows and this can be spectroscopically monitored using fluorescent anisotropy. We are using this assay in a high-throughput mode using 384 well plates to identify small molecules that disrupt B-ZIP|DNA complexes. We are running the assay using four different B-ZIP dimers (AP-1, CREB, C/EBP, PAR) bound to double-stranded sequence-specific fluorescein labeled oligonucleotides. This parallel approach quickly identifies compounds that non-specifically inhibit the B-ZIP|DNA complex by interacting with DNA. We will present the data from the screening of a variety of compounds including the Developmental Therapeutics Program's "Diversity Set" of 2000 chemically diverse compounds and the 'Training Set" of 230 compounds which includes representatives of standard anticancer drug classes and selected compounds with well-defined biological modes of action. Testing of these libraries has allowed characterization of assay reproducibility and performance.