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Synthesis and cytotoxicity of pyranonaphthoquinone natural product analogues under bioreductive conditions

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ABSTRACT

We have synthesised a focused library of derivatives of natural products containing the pyranonaphthoquinone moiety including the first report of such a scaffold with an appended tetrazole functionality. Examples include kalafungin derivatives as well as analogues of nanaomycin and eleutherin. These compounds were assessed for cytotoxic activation by breast cancer cell lines engineered to express the prototypic human one- and two-electron quinone bioreductive enzymes, NADPH: cytochrome P450 oxidoreductase (POR) and NAD(P)H: quinoneoxidoreductase 1 (NQO1; DT-diaphorase), respectively. Several compounds were observed to be cytotoxic at sub-micromolar level and a pattern of increased aerobic potency was observed in cells over expressing POR. A subset of analogues was assessed under anoxic conditions, where cytotoxicity was reduced, implicating redox cycling as a major mechanism of toxicity. The substrate specificity for reductive enzymes is relevant to the future design of bioreductive prodrugs to treat cancer.

1. Introduction

Natural products and derivatives thereof, are a useful source of novel, chemically complex compounds and have provided approximately half of all drugs which are currently used to treat cancer.[1–3] A broad range of natural products containing the quinone moiety, notably the anthracyclines and the mitomycins, are currently used to treat a range of human cancers.[4] These compounds exert their cytotoxicity through a variety of mechanisms[4,5] but of particular interest to the current research is the potential for quinone containing compounds to be activated by reductive mechanisms to selectively kill cancer cells,[6] a concept first proposed by Sartorelli et al. in 1972.[7]

Quinones can be activated under reductive conditions to form reactive electrophilic quinone-methides via either a one or two electron transfer pathway (Fig. 1). The reactive quinone–methides can then form covalent bonds with essential cellular nucleophilic macromolecules including the purines of DNA and thiols of proteins, ultimately leading to death of the exposed cell. In addition to causing cellular damage via alkylation mechanisms, quinones reduced via a one electron pathway to form semiquinone radicals, can cause cell death by general oxidative stress either due to the direct toxic effects of the semiquinone radical itself, or under aerobic conditions, due to the redox cycling of this reactive intermediate.

Enzymes which have been identified to catalyse the one electron and two electron reductive pathways are of increasing interest as they are found to be differentially expressed in normal and cancerous tissues.[8] NADPH-cytochrome P450 oxidoreductase (POR) operates via a one electron mechanism, reducing quinones to the corresponding reactive radical semiquinone methides. Anti-cancer efficacy of quinone drugs has been directly correlated to levels of POR at the tumour site, indicating that this enzyme does indeed activate this family of prodrugs.[9–11] The ability to activate bioreductive agents has led to POR being the focus of emerging site specific gene-delivery cancer therapy and as such, the discovery of substrates specific for this enzyme is of increasing importance.[12,13] Additionally, the potential for hypoxia-selective activation following single-electron reduction by flavoenzymes such as POR may enable quinone-based drug discovery to be used for therapeutic advantage.[14] Two electron bioreductive enzymes normally serve a detoxification purpose, essentially acting as antioxidants by metabolising and thus reducing toxic metabolites. DT-diaphorase (DTD; also known as NAD(P)H quinoneoxidoreductase 1, NQO1), found to be elevated in many tumours,[15] catalyses the reduction of quinones via a two electron mechanism, thus forming reactive hydroquinone methides. Identifying compounds with specificity for this enzyme has the potential to enable selective enzyme-direc-

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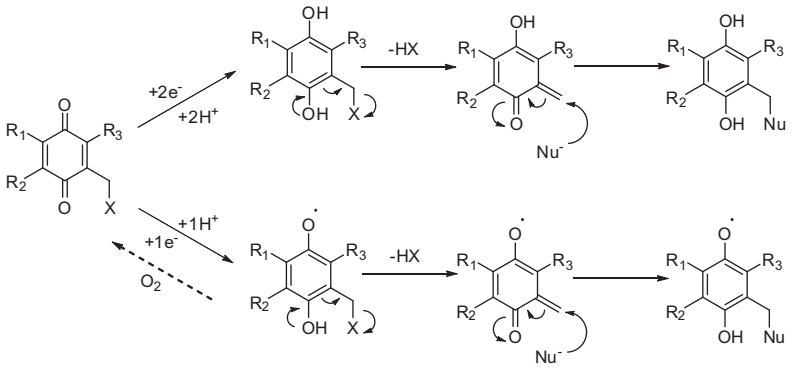

Figure 1. Proposed mechanism of action of quinone bioreductive alkylating agents.

ted targeting of chemotherapies to tumour cells found to be over-expressing DTD.[16,17] Although possibly offering selectivity towards some cancerous cells, compounds which are substrates for DTD need to be treated with caution as this reductase has been found to be ubiquitously expressed at relatively high levels in critical normal tissues.[18–21] Indeed cytotoxic compounds which are not further activated by such enzymes may themselves offer prospective therapeutic value. Thus, information surrounding substrate selectivity of cytotoxic compounds can provide valuable information for future drug development endeavours.

It is difficult to predict the effect of a quinone substituent on substrate specificity for the reducing enzymes although several studies have surprisingly found steric factors to be more relevant than electronic effects when assessing the bioreductive activation of simple quinones by DTD.[22,23] In addition, a QSAR study on the anti-cancer activities of 1,4-naphthoquinones determined that cytotoxic activity was largely dependent on overall hydrophobicity.[24] By synthesizing a series of analogues related to known bioreductive natural products, it is envisaged that knowledge surrounding the substrate specificity of reducing enzymes could be used to develop new therapeutic agents. In the present work, several structural analogues of the pyranonaphthoquinone family of natural products were therefore synthesized for assessment of cytotoxicity in the breast cancer cells line MDA-MB-231 that expressed differing levels of either one and two electron oxidoreductases.

The pyranonaphthoquinone family of natural products including kalafungin **1**, dihydrokalafungin **2**, nanaomycin A **3**, and eleutherin **4** (Fig. 2) display a range of biological activities with medicinal potential,[25,26] and have been postulated to act as bioreductive alkylating agents.[27–29] Our ongoing interest in the synthesis of members of this family,[30–35] have resulted in the procurement of a focused library of 23 analogues closely related to kalafungin, nanomycin and eleutherin (Fig. 2). Principally kalafungin and dihydrokalafungin derivatives together with analogues of eleutherin were synthesized alongside *O*-methylated nanaomycin analogues in which the carboxylic acid functionality was replaced with the isosteric triazole moiety. In addition to this 'click' triazole analogue series of compounds we herein report the first synthesis of a tetrazole appended pyranonaphthoquinone.

2. Results and discussion

2.1. Synthesis

Given that the triazole analogues of the natural products **2**, **3** and **4** were readily available in our group,[36–42] attention turned to the synthesis of tetrazole **5**. As tetrazoles are known to possess superior bioavailability and metabolic stability than carboxylic acids,[43] one aim of this project was to probe whether the potent bioactivity exhibited by the acid-appended pyranonaphthoquinones was still observed for the 1H-tetrazole analogue **5**.

Our planned route to tetrazole **5** relied on the commonly employed cycloaddition between a nitrile and an appropriate azide source[44] and as such, the synthesis of a pyranonaphthoquinone nitrile was considered (Scheme 1). As we had synthetically useful quantities of tosylate **7** in hand,[42] its conversion to nitrile **6** was facile. The use of sodium azide in the cycloaddition reaction failed to effect formation of tetrazole **8**, despite screening several catalysts including AcOH/Et_3N,[45] $Et_3N{\cdot}HCl$,[46,47] $ZnBr_2$,[48] morpholine-HCl,[49] and ammonium chloride.[50] $TMSN_3$[51] and CuN_3[52] also failed to provide any of the desired tetrazole **8**. Oxidative demethylation of naphthopyran **6** gave pyranonaphthoquinone **9**, which also resisted all attempts at its conversion to tetrazole **5**.[19–26] At this stage it was considered that due to the *cis*-configuration of the pyran ring

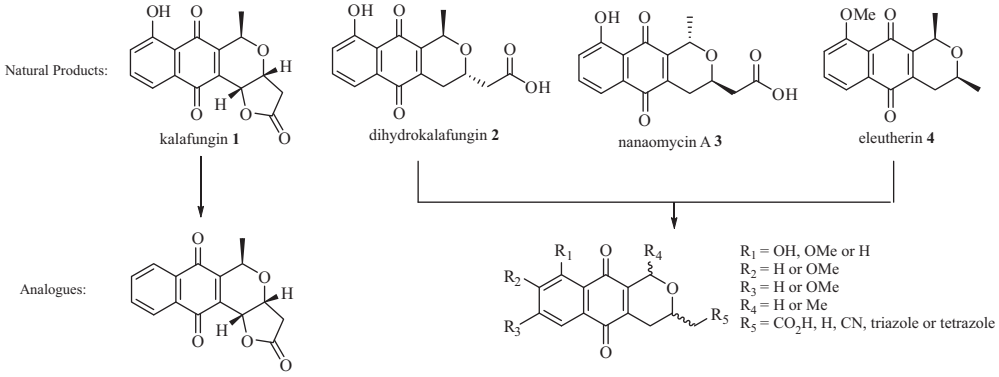

Figure 2. Pyranonaphthoquinone natural products and synthetic analogues.