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# The synthesis and evaluation of thymoquinone analogues as anti-ovarian cancer and antimalarial agents

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ABSTRACT

Thymoquinone (TQ), 2-isopropyl-5-methyl-1,4-benzoquinone, a natural product isolated from *Nigella sativa* L., has previously been demonstrated to exhibit antiproliferative activity *in vitro* against a range of cancers as well as the human malarial parasite *Plasmodium falciparum*. We describe here the synthesis of a series of analogues of TQ that explore the potential for nitrogen-substitution to this scaffold, or reduction to a hydroquinone scaffold, in increasing the potency of this antiproliferative activity against ovarian cancer cell lines and *P. falciparum*. In addition, alkyl or halogen-substituted analogues were commercially sourced and tested in parallel. Several TQ analogues with improved potency against ovarian cancer cells and *P. falciparum* were found, although this increase is suggested to be moderate. Key aspects of the structure activity relationship that could be further explored are highlighted.



The seeds of *Nigella sativa* L., belonging to the Ranunculaceae family, are commonly known as 'black cumin' or 'black onion'. As well as a common spice, these seeds have been widely used as a traditional medicine in the treatment of a range of diseases such as cancer, diabetes, hypertension, fever, arthritis, inflammation, and gastro-intestinal disturbances.[1] Studies that have explored the anti-inflammatory, antidiabetic, antimicrobial, antioxidant, immunomodulatory and antitumor activities of the essential oil from these seeds have identified thymoquinone (TQ, **1**), 2-isopropyl-5-methyl-1,4-benzoquinone (Fig. 1) as a key bioactive component.[2–5] Further, studies specifically investigating the cytotoxic activity of TQ have shown this compound to inhibit proliferation of several cancer cell lines, including ovarian, prostate, colon, breast, pancreatic cancers, leukaemia and osteosarcoma.[2,4,6] Recently, TQ has been shown to block substrate recognition by the Polo-Box domain of Polo-like-kinase 1 (Plk1), a mitotic regulator that when overexpressed causes cancer.[7] TQ-based inhibitors of Plk1, such as poloxin, have been developed and validate the potential of targeting Plk1 using non-peptide inhibitors of protein-protein interactions at the Polo-Box domain.[8] Further modifications to TQ, including; TQ-fatty acid[9] and -terpene[10] conjugates, 6-alkyl thymoquinone,[11] 2,5-bis (alkyl/aryl-amino) 1,4-benzoquinone,[12] TQ-gallate conjugate,[13] and TQ-artesunic acid hybrid[14] have similarly demonstrated the utility of TQ-analogues as antiproliferatives in cancer cell lines. In addition to the anticancer potential of TQ, it has also been shown to have *in vitro* anti-plasmodial activity with an $IC_{50}$ of 0.2 μg/ml (1.2 μM).[15] *P. falciparum* lacks polo-like kinases, although a number of mitotic kinases have been described.[16,17]

Whilst TQ and TQ-analogues appear relatively safe, efforts have been focussed on improvement of the antiproliferative activity and increased solubility to enhance its bioavailability.[18] For example, only a preliminary structure-activity relationship (SAR) of TQ analogues against pancreatic cancer cell lines explored the potential of amino-substituted 2-methyl-naphthoquinone and 1,4-benzoquinone.[12] Thymoquinone has been demonstrated to be effective against ovarian cancer cells *in vitro* and *in vivo*.[19–22] In particular, treatment of syngeneic mice of ovarian cancer by TQ alone resulted in a 2-fold increase in ascites volume after 60 days compared to vehicle-treated mice. A further combination of TQ and cisplatin caused increased reduction in peritoneal implants and mesenteric tumors compared to either drug alone.[19] TQ has been shown to induce apoptosis by regulation of Bcl-2 and Bax[21] and increase of reactive oxygen species in ovarian cancer cells.[20] However, there is no SAR study on TQ against ovarian cancer and plasmodial parasites. In particular, the effect of the two alkyl substitution groups (methyl and 2-isopropyl groups) on the quinone ring, the additional substitution of the quinone ring of TQ by amine or halogen groups, and the reduction of quinones to quinol forms on the

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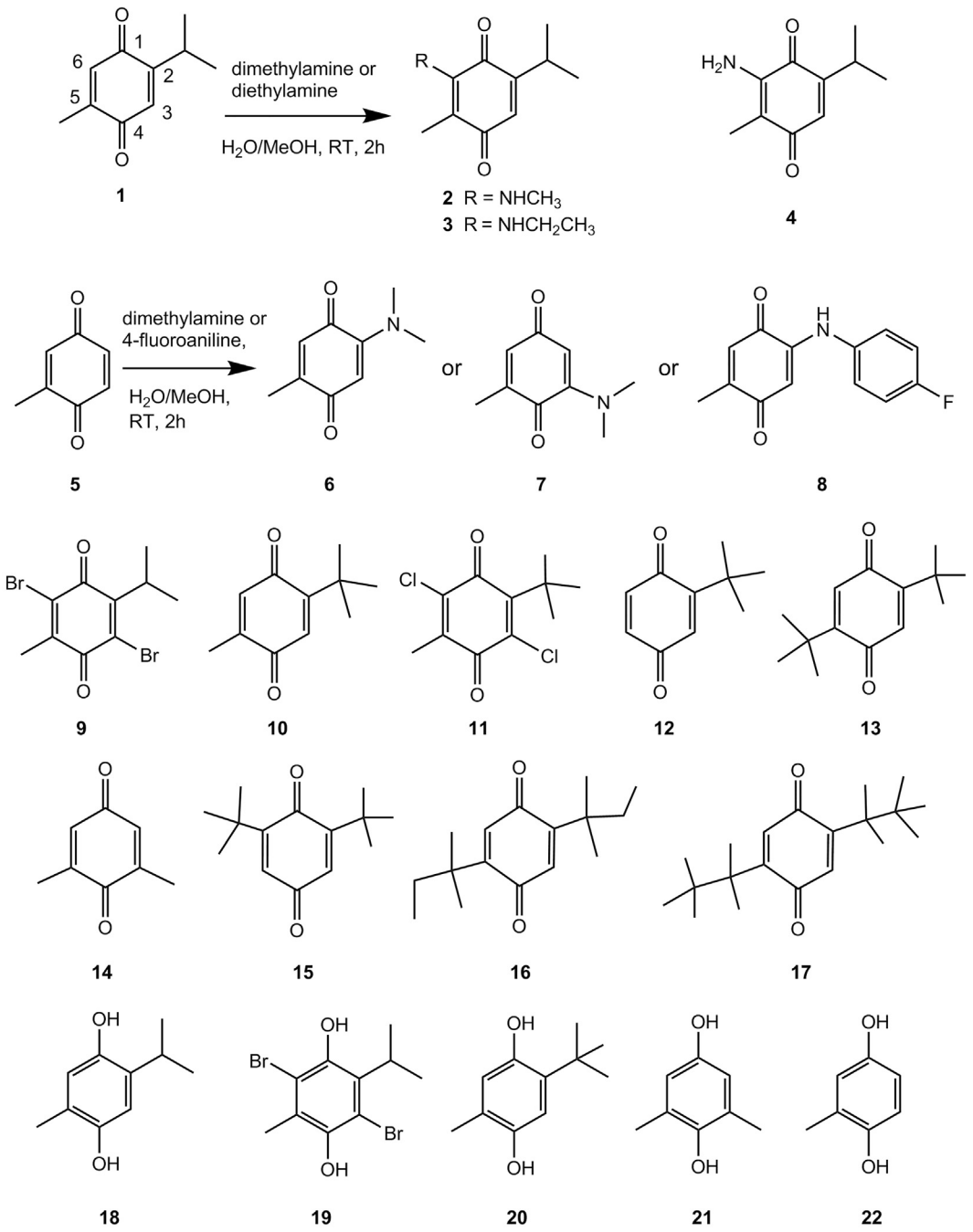


**Fig. 1.** Structure and schemes for the synthesis of TQ analogues.

*in vitro* anti-ovarian cancer and antiplasmodial activities has not been investigated.

This study aims to discover potent cytotoxic/antiplasmodial analogues of TQ. Eleven TQ analogues including six nitrogen-substituted TQ analogues (**2**–**4**, **6**–**8**) and five reduced hydroquinones (**18**–**22**) were synthesized (Supporting information), and ten others (**5**, **9**–**17**) with different substituted groups (e.g. variation of length of alkyl chains and halogen atoms) were procured (Fig. 1). Both the synthetic and procured TQ analogues were investigated for their growth inhibition in three human ovarian cancer cell lines and immortalized human ovarian epithelial cell line (HOE) by determining their $IC_{50}$ values using sulforhodamine B (SRB) cytotoxicity assay[23,24] (Table 1). Eleven TQ analogues (**6**, **9**, **10**–**12**, **14**, **18**–**22**) showed $IC_{50}$ less than 10 μM as TQ in A2780 cell line; they also showed potent cytotoxicity in OVCAR-8 and CIS-A2780 (cisplatin resistant) cancer cell lines with the former one being more resistant. Compound **10** with a *tert*-butyl group (an additional methyl group to the isopropyl group of TQ) and a methyl group on the quinone ring does not increase its cytotoxicity, however its SI increased from 2.3 of TQ to 7.5. Further increase of the bulkiness and hydrophobicity of the side chains either at the 5-methyl or 2-isopropyl group significantly decreases their cytotoxicity as seen for compounds (**8**, **13**, **16**, and **17**). These findings are consistent with loss of cytotoxicity in pancreatic cancer cell lines of the synthetic TQ analogues with bulkier substitution groups via amino addition as previously reported.[12]

Substitution of CH of isopropyl group of TQ by a single nitrogen atom in **6** results in a 2-fold increase in cytotoxicity against the