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# Understanding cancer and the anticancer activities of naphthoquinones – a review

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The non-communicable disease, cancer, is one of the major causes of death across the world and is forecast to increase by 75% to reach close to 25 million cases over the next two decades. Radiotherapy and surgical approaches have been unsuccessful in controlling the incidence of most cancers. The development of chemotherapeutic strategies involving novel small molecule antitumour agents has therefore been the focus area of cancer chemotherapy for several decades as another strategy to combat and control the incidence of cancer. Many natural products as well as several synthetic drugs have a naphthoquinone chromophore. The anticancer activities of naphthoquinones have been the focus of much research to discover novel anticancer agents. The naturally occurring 1,2-naphthoquinone-based compound,  $\beta$ -lapachone (ARQ 761), is currently being assessed for its anti-tumour activity against advanced solid tumours. This review describes the most recent applications of naphthoquinones and their derivatives in cancer drug discovery. The biology relevant to the design of novel naphthoquinone anticancer agents is also discussed. Furthermore, the discussion of the biology will contribute to understanding cancer as well as the applications of naphthoquinones as anticancer agents.

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## Introduction

Cancer, as a single entity, is the major cause of death globally with an estimated 8.2 million deaths being attributed to it in 2012.<sup>1</sup> There was a 11% increase in cancer cases to reach an estimated 14.1 million cases in 2012.<sup>2</sup> Lung (1.8 million cases, 13.0% of the total), breast (1.7 million, 11.9%) and large bowel (1.4 million, 9.7%) cancers were the most prevalent diagnosed cancers. Globally cancer cases are forecast to increase by 75% to reach close to 25 million cases over the next two decades. The most common causes of cancer death were due to lung (1.6 million, 19.4% of the total), liver (0.8 million, 9.1%), and stomach (0.7 million, 8.8%) cancers.

In developing countries the growing and aging populations are inexplicably affected by the increasing numbers of cancer cases. Africa, Asia, Central and South America account for more than 60% of the world's total cases and about 70% of the world's cancer deaths. It is anticipated that 80% of the increase in the number of all cancer deaths will occur in less developed regions by 2025.<sup>3</sup>

The cancer burden is destructive to the economies of wealthy nations and is placing unmanageable pressure on health-care systems. Premature deaths (aged 30 to 69 years) across the world are currently estimated at 4.2 million and are anticipated to increase well beyond 5 million per annum by 2025 unless practical strategies are applied to address cancer.<sup>4</sup>

Cancer is a class of non-communicable diseases that is characterised by uncontrolled cell growth. To date, there are more than 100 different types of cancers of which each have been classified according to the cell type that was initially affected.

Uncontrolled growth of damaged cells results in the formation of lumps or masses of tissue called tumours. The latter can interfere with the nervous, digestive and circulatory systems.<sup>5</sup> Tumours can also release hormones that alter body function. Tumours are considered benign when they are localised and have limited growth. Two things must happen for a tumour to be considered malignant. The first is that a cancer cell has to spread throughout the body and destroy healthy tissue by a process called invasion.<sup>5</sup> Second is that it must divide, grow and form new blood vessels by angiogenesis.<sup>5</sup> A tumour has metastasised when it has successfully spread to other areas of the body. This is a critical condition that is very hard to treat and is responsible for about 90% of human cancer deaths.<sup>6–8</sup>

Different treatments are available to patients who have been diagnosed with cancer. The conventional cancer treatments are surgery, radiation and chemotherapy.

Surgery is the oldest and the primary treatment modality. It is most effective in the treatment of localized primary tumours and associated regional lymphatics.<sup>9</sup> Disadvantages are that healthy tissues or organs (lymph nodes) can be damaged, metastasised cancer and cancer cells or tumours that are not visible cannot be removed and that “latent” small tumours can be activated thus causing further proliferation.<sup>10,11</sup>

Radiation therapy (by X-rays and gamma rays) is an important treatment modality which shrinks tumours.<sup>12</sup> A disadvantage is that healthy cells may be damaged.<sup>12</sup> The most prevalent side effect is fatigue which may last through treatment and for many months afterwards. Other side effects include hair loss, skin irritation, possible hearing problems, nausea, vomiting, loss of appetite, and neurologic effects (permanent memory and speech problems).<sup>12</sup>

Chemotherapy is a systemic treatment which means that the anticancer drugs are circulated throughout the body by the blood circulatory system to reach cancer cells wherever they are. The goal of chemotherapy is to annihilate both cancerous colonies and metastasised cancer cells within a patient's body. The side effects of chemotherapy are anaemia, diarrhoea, nausea, vomiting, hair loss and weakening of the immune system. Resistance to chemotherapeutic drugs may also be developed by cancer cells.<sup>11,13,14</sup> Most cancers cannot be cured with only chemotherapy.

Quinones are widely distributed in nature and occur in animals, plants and microorganisms. They often perform vital roles in the biochemistry of energy production by providing essential links in the respiratory chain of living cells.<sup>15</sup> Quinones display various biological activities and have therefore been the subject of much research.<sup>16</sup> Seminal studies on several synthetic and natural quinones conducted by the National Cancer Institute (NCI-USA), nearly four decades ago, showed that these compounds possess anticancer activity.<sup>17</sup> Both natural quinones and their analogues are vital sources of cytotoxic compounds.<sup>18–21</sup> The anthracycline antibiotics (daunorubicin, doxorubicin, idarubicin and mitoxantrone), bleomycins, dactinomycin and mitomycin-C are examples of quinones that have been used clinically for cancer chemotherapy.<sup>22–26</sup>

The most important and widely distributed chemical class in the quinone family is the 1,4-naphthoquinones. Their derivatives have exhibited a variety of biological responses which include antiallergic,<sup>27–29</sup> antibacterial,<sup>30,31</sup> antifungal,<sup>30–33</sup> anti-inflammatory,<sup>27–33</sup> antithrombotic,<sup>34,35</sup> antiplatelet,<sup>27–29,36–39</sup> antiviral,<sup>30,35,40,41</sup> apoptosis,<sup>42–44</sup> lipoxigenase,<sup>45,46</sup> radical scavenging<sup>47</sup> and anti-ringworm<sup>30</sup> activities. Anticancer activity has also been reported for the 1,4-naphthoquinones.<sup>31,32,35–37</sup> The biological activities and structural properties of these compounds have led to them being regarded as privileged structures in Medicinal Chemistry.<sup>48</sup> This is because of biological activities particularly against pathogenic protozoa and cancer cells.<sup>49,50</sup> The clinical importance of 1,4-naphthoquinones has stimulated enormous research interest in this class of compounds.<sup>51</sup> A pertinent research area in cancer chemotherapy has been the development of novel antitumour agents.<sup>52,53</sup>

In this article we review the synthesis of naphthoquinone derivatives that have been synthesised over the last five years (2009–2013) to discover novel antitumour agents for cancer chemotherapy. We will also discuss other topics in this article that are of relevance to cancer drug discovery such as oxidative stress, reactive oxygen species (ROS), multidrug resistance, mechanisms of action of quinone anti-tumour agents, reductive activation of quinones, and quinone-based drugs in cancer therapy.

## Oxidative stress and cancer

ROS are due to metabolic reactions in the mitochondria of eukaryotic cells. Specific subcellular processes require low concentrations of ROS for example disulfide bond formation, enzyme activation, signal transduction and gene expression. Low concentrations of ROS are also required when new proteins fold in the endoplasmic reticulum and for controlling caspase activity that is initiated in the apoptotic mechanism. ROS are the most abundantly produced compounds from oxidative metabolism with half-lives ranging from a few nanoseconds to hours depending on the stability of the molecule. ROS include the *hydroxyl radical* ( $\cdot\text{OH}$ ), *hydrogen peroxide* ( $\text{H}_2\text{O}_2$ ), *superoxide radical anion* ( $\text{O}_2^{\cdot-}$ ), *superoxide anion* ( $\text{O}_2^-$ ), *singlet oxygen* ( $^1\text{O}_2$ ) and *ozone* ( $\text{O}_3$ ).<sup>54</sup> After ROS has been used for the subcellular events they are eliminated from normal cells. Cancer cells, however, require *high ROS concentrations* to maintain their *high proliferation rate*.

### Internal sources of oxidative stress

These encompass peroxisomes and enzymes such as xanthine oxidase, the detoxifying enzymes from the P450 complex, and the nicotinamide adenine dinucleotide (NADPH) oxidase complexes which include the Nox family. The mitochondria, where the majority of these enzymes function, are the primary origin of oxidative stress.<sup>55</sup>

### External sources of oxidative stress

These encompass UV radiation and chemical compounds from smoking, alcohol, exercise and environmental pollutants. Reactive species have been classified into four groups based on the main atom involved: (i) reactive chloride species (RCS); (ii) reactive nitrogen species (RNS); (iii) ROS and; (iv) reactive sulfur species (RSS).<sup>55</sup>

### Damage by ROS in cells

ROS damage in cells depends on their *intracellular concentration* and on the *equilibrium* between the ROS and the *endogenous antioxidant species*. Oxidative stress is generated as a result of the loss of the pro-oxidant/anti-oxidant equilibrium. This oxidative stress *alters* and *damages* many intracellular molecules such as *proteins, lipids, DNA and RNA*.<sup>56</sup>

ROS causes several types of *DNA damage* such as *base modification, strand breakage* and *DNA-protein cross-linkage*.<sup>57</sup> 8-Hydroxy-2'-deoxyguanosine (8-OH-dG) is one of the major oxidatively modified DNA base products *in vivo* which causes mutations in DNA resulting in *enhanced aging* and *carcinogenesis* (Fig. 1).<sup>57–60</sup> The hydroxyl radical ( $\cdot\text{OH}$ ), singlet oxygen ( $^1\text{O}_2$ ) or photodynamic action is responsible for the formation of 8-OH-dG.<sup>57,61</sup>

The cell membrane, which is rich in polyunsaturated lipids, is susceptible to oxidation by ROS. ROS induces lipid peroxidation reactions which *increases the permeability of the cell membrane* that could lead to *cell death*.<sup>62</sup>

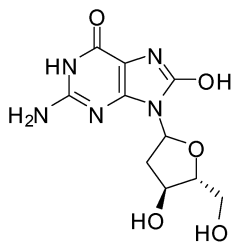


Fig. 1 8-Hydroxy-2'-deoxyguanosine responsible for mutations in DNA.

The *high concentration of ROS* affects proteins the most and leads to a generation and accumulation of *carbonyl* (i.e., aldehydes and ketones) and *thiol groups* (–SH) within the proteins. The thiol groups may be converted into *sulfur reactive radicals*.<sup>63</sup> Oxidation induced modification leads to an *alteration* in the protein structure resulting in *changes or loss of protein function*.

### Tumour development and progression

Several aspects can be promoted by ROS which have been classified into the following biological processes: (i) *angiogenesis* [e.g., the release of vascular endothelial growth factor (VEGF) and angiopoietin]; (ii) *cellular proliferation* [e.g., ligand independent RTK activation and extracellular-regulated kinase 1/2 (ERK1/2) activation]; (iii) *tissue invasion and metastasis* [e.g., metalloproteinase (MMP) secretion into the extracellular matrix (ECM), Rho–Rac interaction, and Met overexpression], and; (iv) *evasion of apoptosis or anoikis* [e.g., Src, NF-κB and phosphatidylinositol-3 kinase (PI3K)/Akt activation].<sup>64–66</sup>

### Biochemical pathways affected by oxidative stress

Several biochemical pathways pertaining to cellular proliferation are affected. These include the epidermal growth factor receptor (EGFR) that encompasses vital signaling proteins such as Ras, Raf, PKC, nuclear factor erythroid 2-related factor 2 (Nrf2) and kelch-like protein 19 (Keap1). In addition, the mitogen activated protein kinases (MAPK) e.g. p38α, c-myc, ERK1/2, c-Jun N-terminal kinase (JNK), MEK and p53 are also involved.<sup>64–66</sup> The master regulator of the antioxidant response is Nrf2 while p38α acts as a key sensor of oxidative stress. The redox sensing function of p38α is essential in the control of tumour development.<sup>67</sup> Unlike other MAPKs, p38α suppresses tumorigenesis by either promoting apoptosis or blocking proliferation.

## Reactive oxygen species (ROS)

### Formation in the cell

The production of ROS is a natural process of normal cells and these species are *by-products* of the *oxidative phosphorylation* process in the mitochondria. *Electrons* can escape from the mitochondria and *react with molecular oxygen* during electron transfer through the electron transport chain. *Superoxide radical anions* ( $O_2^{\cdot-}$ ) anions are formed from up to 2% of the oxygen that is consumed during ATP synthesis in the

mitochondria.<sup>68</sup> ROS can also be produced from the detoxifying enzymes such as cytochrome P450s.

A *1-electron reduction* produces a *superoxide radical anion* ( $O_2^{\cdot-}$ ). This radical has a *short lifetime* in the cell because it reacts quickly with antioxidants or is transformed to another ROS, such as *hydrogen peroxide* ( $H_2O_2$ ).<sup>69</sup> Amongst the common ROS species, *peroxides* are the *least reactive* and can therefore *persist longer in the cell* than the *superoxide radical anion* and the *hydroxyl radical* ( $\cdot OH$ ).<sup>69</sup> The uncharged *hydroxide radical*, despite having a short lifetime, can be *very damaging* since it can react readily with a variety of cellular macromolecules.<sup>69</sup> Because ROS can cause damage to the cell, endogenous cellular systems for scavenging ROS have evolved.

### Defence against ROS in cells

The redox balance can be maintained and the amount of ROS limited by endogenous cellular systems within the cell. The *enzymatic* and *small molecule antioxidant defences* involve *superoxide dismutase* (SOD), *glutathione peroxidase* (GPX), *catalase* (CAT), *ascorbic acid* (vitamin C), *α-tocopherol* (vitamin E), *β-carotene*, *glutathione* (GSH), and *vitamin A*.<sup>70–74</sup> In this way mammalian cells can defend themselves against ROS damage.

*ROS neutralizing enzymes* such as *catalase* and *superoxide dismutases* (SOD) can detoxify radicals and maintain the cellular redox balance (Fig. 2).<sup>75</sup>

SOD catalyses the formation of hydrogen peroxide from superoxide while catalase transforms hydrogen peroxide into molecular oxygen and water.<sup>75</sup> Hydrogen peroxide, even though it is not as reactive as superoxide, can damage the cell by oxidising DNA.<sup>76</sup> NADPH provides the reducing power for many of the enzymes involved in ROS neutralization.

*ROS scavengers*, thioredoxin (a protein) and glutathione (a small molecule) neutralize ROS by the oxidation of dithiols (Fig. 3).<sup>75</sup> Different enzymes, by reduction of the disulfide bond, recycle the thioredoxin and glutathione for re-use.

In more highly oxygenated locations where there is a greater probability of ROS damage Vitamin E, the enzymes SOD, CAT and GPX, as well as substrates (GSH) tend to be in a higher concentration.<sup>77</sup>

### ROS in cancer cells

*Cancer cells* have a *greater concentration of endogenous ROS* compared to normal cells.<sup>77,78</sup> Several theories have been proposed to explain this phenomenon. One theory proposes that because cancer cells are more metabolically active than normal cells, they require more ATP. As a result of the additional metabolic burden (stresses and respiration) on the electron transport chain, more superoxide radical anions ( $O_2^{\cdot-}$ ) are formed.<sup>73</sup>

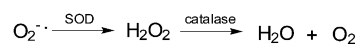


Fig. 2 ROS neutralizing enzymes: (a) superoxide dismutase reduces superoxide radical anions to peroxide and; (b) catalase transforms peroxide into molecular oxygen and water.

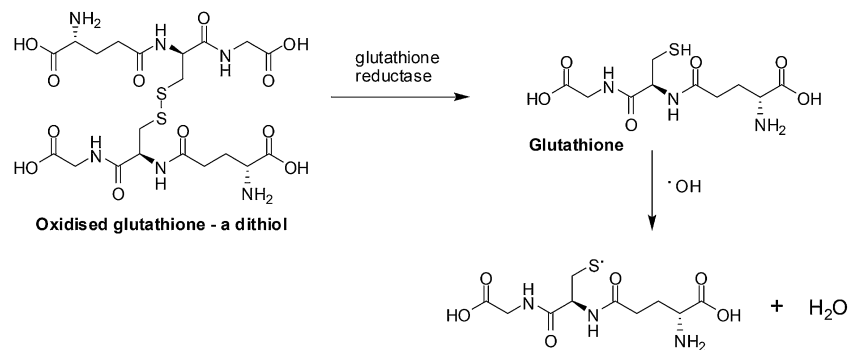


Fig. 3 ROS scavengers. The neutralization of ROS is achieved by glutathione forming dithiols and the enzyme, glutathione reductase, reduces the oxidized glutathione.

ROS can damage mitochondrial DNA which leads to mutations in members of the oxidative phosphorylation process resulting in more ROS being produced. ROS is believed to *increase cancer cell proliferation which leads to uncontrolled tumour growth*. One mechanism that has been proposed is that ROS interferes with the MAPK signaling pathway thus disrupting normal metabolic regulation and allows uncontrolled metabolism and growth.<sup>69,79</sup>

A second mechanism by which ROS promotes cancer cell survival is through DNA damage (Fig. 4).

When the DNA is damaged beyond repair, mutations may occur during replication that may be advantageous to cancer

cell growth. Faulty respiration proteins are coded for in the cycle of mitochondrial DNA damage which then allows for even greater ROS leakage.<sup>69</sup> Proteins can also be *oxidized* by ROS thus *damaging the mitochondrial membrane* and the *proteins involved in ATP synthesis* thus *promoting further ROS production*.<sup>80</sup>

It is believed that the sustained imbalance in oxidative stress in cancer cells can be exploited for chemotherapeutic selectivity. By increasing the ROS in a cancer cell the already strained redox balance could be pushed to a critical level that could overpower the ROS buffering capacity of the cancer cell and cause cell death.<sup>69,75,80</sup>

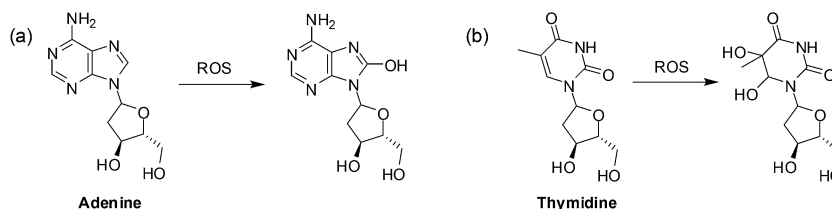


Fig. 4 Oxidation of DNA bases by ROS: (a) purines can be monohydroxylated and; (b) pyrimidines can be dihydroxylated at the double bond.

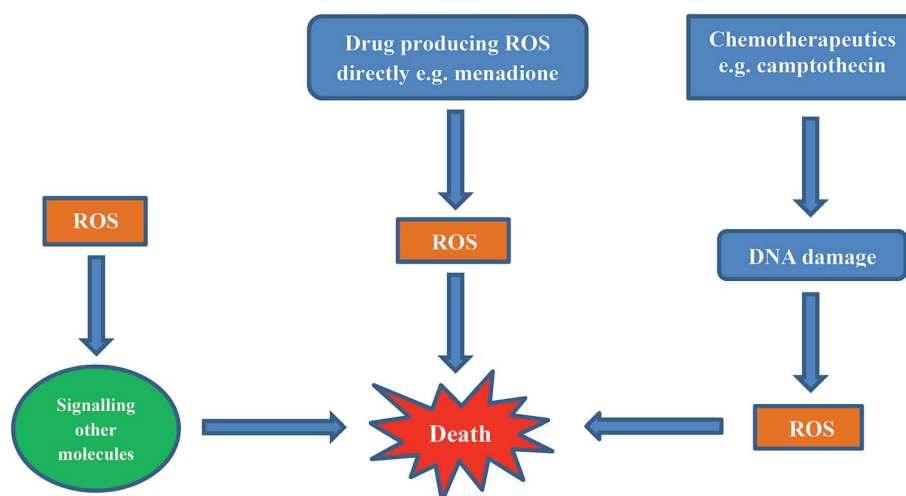


Fig. 5 Death induced by cellular ROS by at least three mechanisms.

## Mechanisms of cell death involving ROS

Inducing cancer cell death by ROS is a direct method which is being employed as an anti-cancer strategy.<sup>69,75,80–82</sup> There are three mechanisms by which ROS can affect a cell (Fig. 5).<sup>69,80,81,83</sup>

The first mechanism entails cells exhibiting ROS damage as a by-product from cytotoxins in combination with another primary mechanism involving an anticancer agent (*e.g.* cisplatin, camptothecin). The ROS produced during cisplatin treatment as well as *after* DNA crosslinking by cisplatin, probably contribute to cell death, but these are not the primary cause for the induction of cell death.

The second mechanism by which cells die is from ROS exposure to compounds that induce a small amount of ROS. These ROS then cause downstream *signaling for the actual molecules* that are responsible for *inducing cell death*.

The third, and most direct mechanism, is when macromolecules in the cell are damaged by ROS as a result of *reduction and oxidation* of a compound *e.g.* menadione. When a sufficient amount of ROS is generated and intracellular stores of antioxidant molecules are exhausted, the cells cannot recover from ROS-induced damage.

## Reductive activation of quinones

Enzymes and the oxygen environment modulate the biological activity of quinones and other molecules. Quinones undergo bio-reductive activation by two major enzymes such as the cytochrome P450's and the cytochrome P450 reductases. In addition, a hypoxic (oxygen deficient) environment also has an effect on some anticancer agents.

Quinones can undergo bio-reductive activation by two pathways *i.e.* a 1 electron or a 2 electron reduction (Fig. 6). A 1 electron reduction affords a semiquinone while a 2 electron reduction affords the corresponding hydroquinone. The

hydroquinone and quinone are in equilibrium. The semiquinone can be oxidized to the quinone under *normal oxygen levels*. In this process O<sub>2</sub> is reduced resulting in the production of *superoxide radical anions* (O<sub>2</sub><sup>•−</sup>).

### Quinone activation by a 1-electron reduction

The *cytochrome P450 reductases* are the main enzymes responsible for the 1-electron reduction of anticancer drugs.<sup>84–86</sup> The cofactors, FADH and FMNH, are utilised by P450 reductase to transfer electrons from NADP for a 1 electron quinone reduction.<sup>87</sup> Since Cytochrome P450 reductase does not have a metal center only a 1 electron reduction of a molecule can be done.<sup>87</sup>

Cytochrome P450 enzymes are found in nearly all tissues in the body and have many different functions. They accept various substrates that can be part of biosynthesis, degradation as well as activation of both xenobiotics and endogenous compounds.<sup>88</sup> It is the Cytochrome P450s that are responsible for the activation of some investigational drugs to active molecules. The enzymatic heme core (a mononuclear Fe center) of cytochrome P450s uses NADPH to reduce molecules by 2 electrons resulting in the formation of the active species of some drugs.<sup>89,90</sup>

### Quinone activation by a 2-electron reduction

The enzyme, NAD(P)H: quinone oxidoreductase 1 (NQO1, DT-diaphorase), reduces a quinone by 2 electrons to afford a hydroquinone (Fig. 7).<sup>91</sup> The catalytic action is accomplished through the cofactors FAD, NAD(P)H, and the enzyme pocket since it does also not contain a metal center.<sup>91</sup> The detoxification of many quinones may be due to NQO1 which results in the excretion of the *less reactive hydroquinone* (Fig. 8).

The 2-electron reduction process has made some compounds *biologically active* (Fig. 7).<sup>92</sup> Since quinones exert their activities after reduction many are regarded as pro-drugs. For a particular target the corresponding hydroquinone may have greater pharmacological activity than the parent quinone. Semiquinones or hydroquinones may be formed after quinone reduction followed by subsequent generation of ROS and oxidative stress.

### Activation in hypoxic environments

Hypoxia (also known as hypoxiation or anoxemia) is a condition in which the body or a region of the body is deprived of

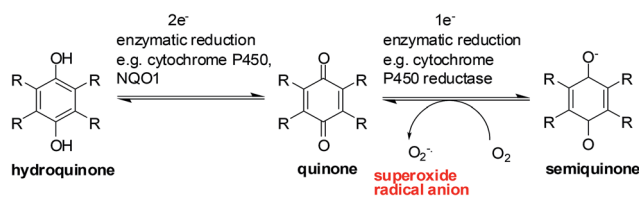


Fig. 6 A general scheme for quinone reduction.

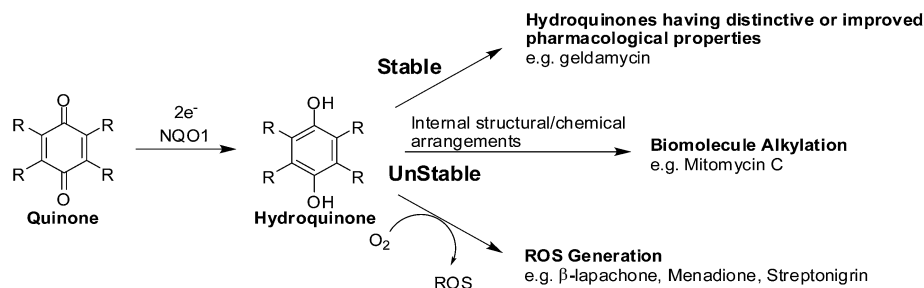


Fig. 7 The bio-reductive activation of antitumour quinones by NQO1.



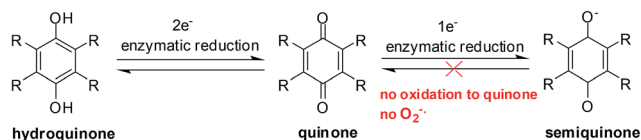


Fig. 8 Semiquinones cannot re-oxidize to quinones without oxygen and can thus accumulate in cells.

adequate oxygen supply. It is commonly defined at 0.1–2% oxygen in the local environment, compared to ~20% oxygen in normoxic tissues.<sup>93</sup> Hypoxia has been identified as a major contributor to cancer progression and to treatment failure. By decreasing the availability of oxygen patient treatment resistance increases and tumour progression is favoured.

Hypoxia, a major feature of solid tumours, offers several treatment challenges. Hypoxia, in rapidly growing tumours, is a result of either a reduced number or incorrectly formed blood vessels.<sup>93</sup> Availability of a drug is reduced when the blood vessels are reduced in number and in integrity. The resistance to drugs also increases due to slower cellular metabolism. Furthermore, solid tumours also become resistant to radiation treatments as oxygen is not present to repair DNA damage.<sup>93</sup>

In the absence of oxygen, semiquinones, created by a 1-electron reduction, cannot be oxidized to quinones and results in the accumulation of semiquinone and less hydroquinone (Fig. 4). Differences between normoxic and hypoxic cells offer a method of selective cancer treatment.<sup>93</sup>

## Mechanisms of action of quinone anti-tumour agents

The mechanisms of action of these compounds have been the subject of intense investigation.<sup>18,94</sup> In the structure of most of the anti-tumour agents it is the *para*-quinone moiety that participates in the cell redox cycle and acts as a precursor of ROS which leads to oxidative stress.<sup>18</sup>

Under aerobic conditions in organs with a sufficient blood supply (normoxia) the highly redox active quinones undergo a one-electron reduction resulting in free-radical intermediates that undergo back oxidation in the presence of oxygen, releasing ROS.<sup>95,96</sup> The ROS are generated *via* their intermediate semiquinone or hydroxy radicals. It is the hydroxyl radicals that are responsible for DNA strand breaks.<sup>97</sup>

Under anaerobic conditions (hypoxia), a two-electron reduction of the quinone to the hydroquinone, is followed by its inactivation through subsequent glucuronidation and/or sulfation. The *in situ* reduction of the quinone to the hydroquinone is an alternative pathway.<sup>96</sup> This alternative pathway leads to conjugated intermediates which are powerful alkylating agents.<sup>97–101</sup> This is believed to be the dominant mechanism under anaerobic conditions.<sup>102</sup>

When severe oxidative stress occurs within cells as a result of the formation of ROS, oxidized cellular macromolecules such as proteins, lipids and DNA can be formed and a number of signaling pathways can also be activated.<sup>103–106</sup>

## Quinone-based drugs in cancer therapy

The goal of anticancer drug design is to develop drugs which are selectively toxic to tumour cells with minimal toxicity to normal cells. A requirement for a drug to be used in therapy is that it must replace the natural chelators that bind to natural cellular receptors.<sup>107</sup> Natural regulative agents (agonists) may be imitated by the drug or the activity of natural ligands may be inhibited by blocking the receptor spot.

Some drugs can be administered in an inactive form into the tissue and become activated upon interaction with the cytoplasmic reticulum *e.g.* doxorubicin (adriamycin) and mitomycin. These drugs only become active when they have been reduced in the cytoplasmic reticulum. Drug entry into the cell occurs by passive diffusion.<sup>108</sup> Quinone-based drugs used in chemotherapy such as the anthracyclines, anthraquinones, mitomycin C and streptonigrin will be discussed in this section.

### 1. Anthracyclines

The anthracyclines (doxorubicin, daunorubicin and idarubicin) are quinone-containing antitumour agents used in treatment of cancers such as leukemias, lymphomas, breast, uterine, ovarian, and lung cancers (Fig. 9). Doxorubicin has been used clinically to treat solid tumours,<sup>109</sup> daunorubicin is used to treat acute lymphoblastic and myeloblastic leukaemias,<sup>110</sup> and idarubicin is a first line treatment for acute myeloid leukemia.<sup>111</sup>

**Mechanisms of action.** Multiple mechanisms have been cited for the action of the anthracyclines.<sup>25</sup> One mechanism involves interference with DNA synthesis which may be an indication of initial events involving intercalation, enzyme inhibition, and oxidative stress. The likely primary mode of action of topoisomerase II inhibition at a concentration *in vivo* appears to entail *interaction with the enzyme–DNA complex* which is then followed by *DNA strand breaks*. It was found that resistant tumour cells possess reduced levels of topoisomerase II which was accompanied by a decrease in the degree of DNA cleavage.<sup>25</sup>

Another mechanism proposes that anthracyclines can also undergo *redox cycling to generate ROS* by a process that involves *enzyme catalyzed one-electron reduction* to the *semiquinone radical* which, on interaction with oxygen, generates the *super-oxide anion radical* ( $O_2^{\cdot-}$ ) and *hydrogen peroxide* ( $H_2O_2$ ). Damage to tumour cells is due to the ability to undergo enzymatic reduction to the *semiquinone radical*.<sup>112,113</sup>

The action of doxorubicin (adriamycin) was shown to be more complicated.<sup>114</sup> *Formaldehyde* was formed from the oxidation of the keto side chain by hydrogen peroxide which subsequently affected covalent bonding of the drug *via* its amino group with the 2-amino moiety of a DNA guanine. The anthracycline thus also functioned as an alkylating agent. It was shown that the synthetic anthracycline formaldehyde conjugates, precursors for formaldehyde generation, could circumvent multi-drug resistance and could potentially be used in the treatment of resistant cancers.<sup>114</sup>

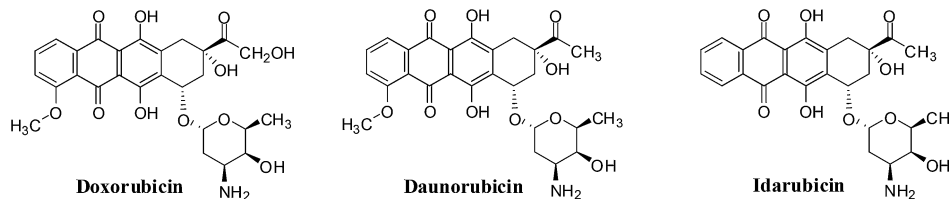


Fig. 9 The anthracycline drugs.

A major disadvantage of all the anthracyclines is the cardiac damage that is produced which can result in serious and even life-threatening complications.<sup>115</sup>

## 2. Anthracenediones

They were developed in a program to find a cytotoxic agent with less cardiotoxicity than that of the anthracyclines.<sup>116</sup> The anthracenediones differ in structure from the anthracyclines since they do not have a glycoside substituent.

The synthetic anthracenedione, mitoxantrone, is used to treat mostly metastatic breast cancer, acute myeloid leukemia, and non-Hodgkin's lymphoma (Fig. 10).<sup>117–119</sup> The survival rate of children suffering from first relapse of acute lymphoblastic leukaemia was also improved by mitoxantrone treatment.<sup>120</sup>

The antitumour activity spectrum of mitoxantrone is less than that of doxorubicin. It also has less cardiac toxicity as a result of a decreased ability to participate in ROS generation. The main disadvantage of mitoxantrone is its cardiac toxicity.<sup>121</sup> It has a similar activity to that of doxorubicin but produces significantly less cardiac toxicity in patients with previously treated breast cancer.<sup>122</sup> Mitoxantrone has also been used clinically to treat solid tumours.<sup>109</sup>

**Mechanisms of action.** Mitoxantrone intercalates into DNA and instigates DNA crosslinking and DNA strand breaks,<sup>123</sup> and also meddles with ribonucleic acid (RNA). Mitoxantrone is also a potent inhibitor of the enzyme, topoisomerase II, which is responsible for the uncoiling and repairing of damaged DNA.<sup>124</sup>

## 3. Mitomycin C

The mitomycins from *Streptomyces* sp. are quinone-containing alkylating agents. Mitomycin C (MMC) is a quinone containing antibiotic that was isolated from *Streptomyces caespitosus* (Fig. 11). MMC is considered a prototype bio-reductive alkylating drug.<sup>125</sup>

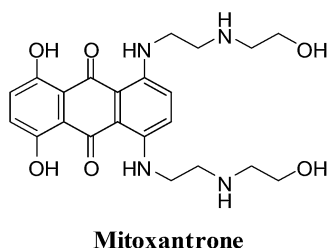


Fig. 10 A potent topoisomerase II inhibitor and DNA intercalator.

The term “bio-reductive alkylating agent” refers to drugs which generate electrophilic species upon reduction which then bind covalently to cellular macromolecules<sup>126</sup> and was first used by Sartorelli and co-workers.<sup>127</sup> Enzymatic reduction, by both one- and two-electron reductases, is required to achieve intracellular activation of bio-reductive alkylating agents. Bioactivation affords mono- and bifunctional alkylating species (bifunctional alkylating agents can cross-link DNA) and/or generates ROS.

Bio-reductive chemotherapy exploits the differences in oxygen content and cellular pH between normal tissue and tumour tissue and can therefore be utilised to selectively attack carcinomas.<sup>128</sup> This treatment is most successful in certain cancers such as breast, colon, head and lung cancer that are rich in reductive activation proteins.

Hypoxic cells of solid tumours, though resistant to most chemotherapeutic agents, create an environment which favours activation of MMC through reductive processes. MMC exhibits a greater cytotoxicity to oxygen-deficient cells than to their oxygenated counterparts and are thus ideal for attacking hypoxic regions of solid tumours.

**Mechanisms of action.** The mechanism of action of MMC involves intracellular bio-reductive activation which leads to DNA interstrand crosslinking.<sup>92</sup> Cytochrome P450 reductase is believed to be responsible for catalysing the formation to the semiquinone which then rearranges and reacts further to give the active alkylating species.<sup>129</sup> Under normoxia, NQO1 converts MMC to the hydroquinone and then to the alkylating species after internal structural/chemical rearrangements.<sup>92</sup>

This FDA approved drug, which has been used clinically for more than 30 years, is used for the treatment of solid tumours including stomach, pancreas, breast and lung. Studies showed that there was an excellent correlation between tumour cell kill and formation of ROS and that the drug, covalently bound to DNA, remained redox active resulting in oxy radicals.<sup>130</sup>

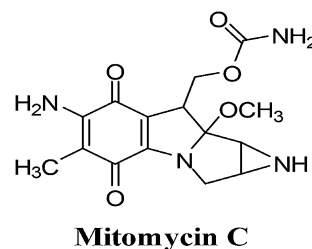
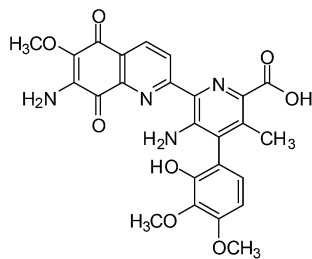


Fig. 11 A bio-reductively activated anticancer drug.



Streptonigrin

Fig. 12 An aminoquinone containing anticancer agent.

#### 4. Streptonigrin

This quinoline analogues of naphthoquinone shows a broad range of activity against breast, lung, lymphoma, melanoma cancer as well as head and neck cancer (Fig. 12).<sup>131–136</sup>

A side-effect of streptonigrin (SN), which has limited its use in cancer therapy, is prolonged bone marrow depression.<sup>137–142</sup> Positive results were, however, reported for SN in the treatment of leukemias, lymphomas and melanomas.<sup>143–147</sup> DT-diaphorase, an enzyme showing increased activity in certain tumours such as human non-small cell lung cancer, efficiently bio-activates SN.<sup>148,149</sup> It was therefore proposed SN could be used against tumours with increased DT-diaphorase function.

**Mechanisms of action.** There are several different cellular mechanisms responsible for anticancer activity such as *interference with cell respiration, impairment of DNA synthesis and direct damage to DNA*.<sup>150–154</sup> When the aminoquinone moiety is blocked (as in azastreptonigrin) the anticancer activity is lost. It therefore appears that the *aminoquinone moiety is essential for cytotoxicity*.<sup>155</sup>

For SN to produce its *DNA-damaging effects*, autoxidation of the quinone moiety to *semiquinone* in the presence of NADH is required which leads to the production of *free radical species*.<sup>150,153,156,157</sup>

### Multidrug resistance (MDR) in cancer

Cancer multidrug resistance is defined as the cross-resistance or insensitivity of cancer cells to the cytostatic or cytotoxic actions of various anticancer drugs which are structurally or functionally unrelated and have different molecular targets.<sup>158</sup> This phenomenon is prevalent in various types of cancers and is a major impediment to the effective chemotherapeutic treatment. The resistance of cancer cells to multiple cancer drugs is also the main reason for the failure of cancer treatments.<sup>159</sup> Resistance entails cellular and non-cellular mechanisms which are utilised by cancer cells to overcome the cytotoxic actions of various drugs. These mechanisms form part of the strategy that confers resistance to cancer cells.

#### Mechanisms of multidrug resistance in cancer

The numerous mechanisms that have been proposed to mediate cancer multidrug resistance can be categorized as cellular or non-cellular depending on the factors contributing to

MDR development.<sup>160</sup> Cellular mechanisms involve *enzymes and transport systems* while non-cellular mechanisms involve factors that are extracellular such as the *cell growth environment* or *limited vascular accessibility*.

#### Cellular MDR mechanisms

There are two cellular MDR mechanisms which are utilised by the cell for the removal of a drug. The first has been classified as *classical/transport* based and the second, *non-classical/non-transport* based.

The first mechanism involves the efflux of a drug from the cell by various energy dependant membrane transport proteins and occurs in the classical/transport based cellular mechanism of MDR. This *prevents* the drug from reaching its *therapeutic concentration* inside the cell.<sup>161</sup>

The superfamily of proteins that mediate this MDR *via* the ATP-dependent drug efflux pumps are the *ATP-binding cassette (ABC) transporters*.<sup>162</sup> ABC transporters are *proteins that occur throughout the cell membrane and can actively transport both endogenous compounds and xenobiotics*. MDR is a result of overexpression of the ABC transporters.<sup>163</sup>

P-glycoprotein (Pgp), multidrug resistance-associated protein-1 (MRP1), its homologs MRP2-6 and the breast cancer resistance protein (BCRP) are all transport proteins of the ABC superfamily.<sup>164–166</sup> These proteins are *overexpressed* in cancer cells and *pump anticancer drugs out* of the cell. Among the ABC transporters *Pgp confers the strongest resistance to a wide variety of compounds* and has been expressed in cancers of the gastrointestinal (GI) tract (small and large intestine, liver cancer, and pancreatic cancer), cancers of the genitourinary system (kidney, ovary, testicle), cancers of the hematopoietic system (myeloma, lymphoma, leukemia), as well as childhood cancers (neuroblastoma, fibrosarcoma).<sup>167</sup>

Many cellular processes require transport of metabolites or substrates across the cell membrane and utilise the ABC transporters. The ABC transporters are thus key elements to consider in the discovery and development of drugs targeting MDR in cancer cells.

The second mechanism, the non-classical/non-transport based one, employs enzymatic systems that restrict the required drug activity without changing its effective concentration within the cell. The enzyme, glutathione-S-transferase (GST), is an important enzyme responsible for xenobiotic metabolism. The excretion of organic molecules can be expedited through biotransformation by GST which catalyses their conjugation with polar molecules. Drugs are modified into end products with reduced activity and an enhanced rate of excretion. Elevated levels of GST have been reported in various resistant cancer cell-lines like MCF-7.<sup>168,169</sup>

#### Non-cellular MDR mechanisms

*In vivo* tumour growth leads to *non-cellular drug resistance* which is typically associated with *solid tumours*. For cells to be considered cancerous, growth beyond their natural boundaries is required. This growth is usually accompanied by a vasculature that is well developed. In certain solid tumours, where



angiogenesis has been compromised, the vasculature is poor.<sup>170</sup> Poor vasculature can lead to limited drug access to regions within these solid tumours and thus protect them from cytotoxicity thus conferring resistance. Lactic acid generation by hypoxic tumour cells generates an acidic environment in tumours which can confer a resistance mechanism against weak bases where cellular uptake across membranes is dependent on the pH gradient. The physiological properties of solid tumours can result in tumour regions that lack oxygen (hypoxia) and nutrients which can confer resistance to cancer cells particularly against drugs that act on actively dividing cells.<sup>171</sup>

### The three main mechanisms responsible for cancer MDR

The three mechanisms identified are as follows: the membrane mechanism determining the low drug level in resistant cells associated with the existence of specific membrane proteins (P-gp and MRP);<sup>172–174</sup> the mechanism associated with change in the effectiveness of the glutathione cycle;<sup>168,169</sup> the mechanism associated with the decrease in DNA topoisomerases expression.<sup>161,175</sup>

Knowledge about mechanisms of cancer drug resistance may aid in the design and development of strategies to circumvent resistance. This knowledge may also contribute to the development of new drugs that are less prone to the known resistance mechanisms. Several reviews have been written on cancer MDR and these can be consulted for further information.<sup>161,176–182</sup>

## Studies on synthetic naphthoquinones and derivatives of natural naphthoquinones

In this section we will discuss the anticancer activity of the synthesised synthetic naphthoquinones as well as synthesised derivatives of natural naphthoquinones. The reaction schemes are only shown for reports where the synthetic route to the target molecule is short.

### 1. Aminonaphthoquinones

A subject of study for many years have been 1,4-naphthoquinones possessing an amino or a substituted amino group in the 2-position. This was inspired by various medical and biological applications such as antituberculars, antimalarials, antibacterials, antitumour agents, larvicides and molluscicides, herbicides, and fungicides.<sup>183–186</sup> The nitrogen atom in this position enables geometric modification of the neutral molecules and of their reduction intermediates and modulation of the substituent's effects on the electronic properties of the quinone system.

The commercial anti-neoplastic agents actinomycin<sup>187</sup> and streptonigrin<sup>187</sup> as well as the antibiotics, mitomycin<sup>188</sup> and rifamycin,<sup>189</sup> are also based on an aminoquinone. The amino group is in the 2-position in mitomycin C, streptonigrin,<sup>187</sup> actinomycin<sup>189</sup> (and the structurally related aurantins<sup>190</sup>) as well as in the ansa-antibiotics, rifamycin<sup>189</sup> and geldanamycin.<sup>191</sup> The biological activity exhibited by these drugs have inspired research into new routes to the synthesis of aminoquinones.

A series of side chain homologated derivatives of 2-chloro-3-(*n*-alkylamino)-1,4-naphthoquinone {*n*-alkyl: pentyl; hexyl; heptyl; and octyl} were synthesised by Pal *et al.* (Scheme 1).<sup>192</sup>

Antiproliferative activities of **1** to **8** {*n*-alkyl: methyl; **1**, ethyl; **2**, propyl; **3** and butyl; **4**} were studied in a panel of cancer cells consisting of colon (COLO205), brain (U87MG) and pancreas (MIAPaCa2) cell-lines using the XTT assay.

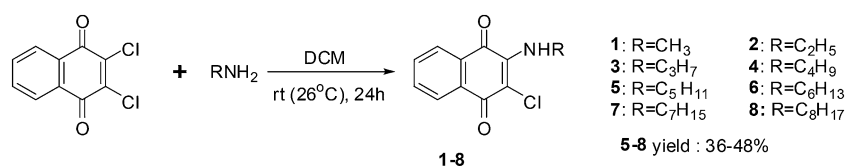
Compounds **1**, **2** and **3** were active against MIAPaCa2 (**1** = **2** < **3**) and COLO205 (**2** = **3** > **1**) and inactive against U87MG. Compound **3** was the most active compound (IC<sub>50</sub> = 1.3 ± 2 mM). It was established that homologation of 2-chloro-3-(*n*-alkylamino)-1,4-naphthoquinone with saturated methyl groups afford tissue specific compounds such as **1** (for MIAPaCa2) and **3** (for COLO205) with ideal activity.

Bhasin *et al.* used 1,4-naphthoquinone, a 1,4-naphthoquinone derivative, Juglone, Menadione and Plumbagin as lead molecules in structure–activity relationship studies of a series of substituted 1,4-naphthoquinones.<sup>193</sup> The synthetic routes are shown in Scheme 2 below.

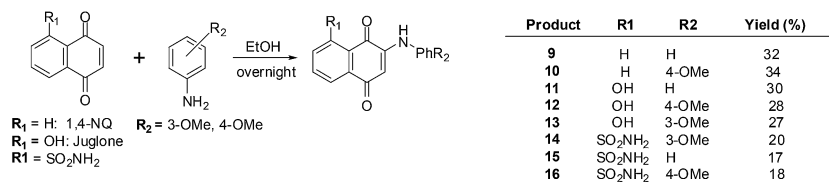
The compounds were screened against a panel of cell-lines using the Promega CellTiter 96 Aqueous Non-Radioactive Cell Proliferation assay. Enhanced antiproliferative activity was observed in prostate (DU-145), breast (MDA-MB-231) and colon (HT-29) cancer cell-lines in contrast with juglone, the lead molecule. Compounds **9** and **10** only exhibited potent activity against prostate cancer, but compounds **11–13** exhibited potent activity against all three cancer cell-lines. The most potent compounds were **12–16** which had IC<sub>50</sub> values in the 1–7 μM range in all three cancer cell-lines.

Wellington *et al.* reported on the synthesis of a series of aminonaphthoquinones obtained by laccase catalysis (Scheme 3).<sup>194</sup>

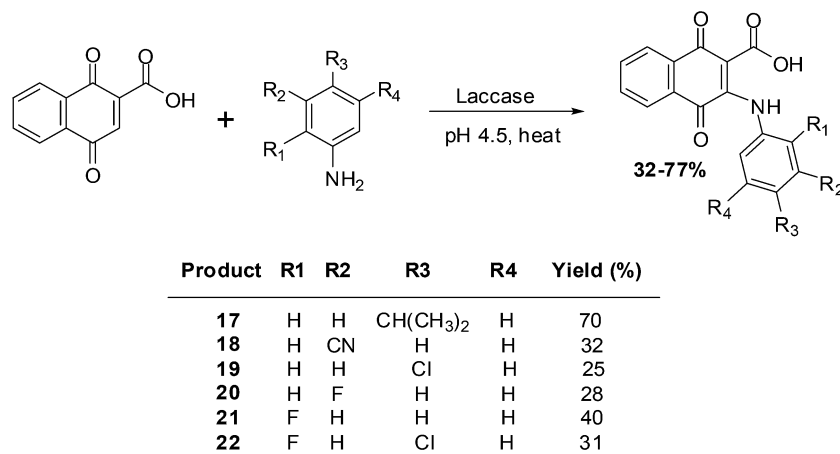
The compounds were screened against renal (TK10), melanoma (UACC62), breast (MCF7) and cervical (HeLa) cancer cell-lines using the sulforhodamine assay to determine their growth inhibitory activity. From the screening results it was apparent that the aminonaphthoquinones exhibited potent cytostatic effects mainly against the melanoma cancer cell-line (GI<sub>50</sub> = 3.98–7.54 μM).



Scheme 1 Synthesis of aminonaphthoquinones **1–8**.



Scheme 2 Synthesis of aminonaphthoquinones 9–16.



Scheme 3 Laccase-catalysed synthesis of aminonaphthoquinones.

Potent cytostatic effects against both the renal and the melanoma cancer cell-lines were exhibited by compound **17**. Against the renal cell-line the activity ( $GI_{50} = 8.38 \mu\text{M}$ ) was nearly as good as that of the anticancer agent, etoposide ( $GI_{50} = 7.19 \mu\text{M}$ ). Potent cytostatic effects against both the melanoma and the breast cancer cell-lines were also exhibited by compounds **20** and **21** ( $GI_{50} = 5.28\text{--}9.84 \mu\text{M}$ ).

Most compounds exhibited a total growth inhibition (TGI) which was better than that of etoposide against the melanoma cell-line. The potent cytostatic effects of compounds **18**, **19** and **22** ( $TGI = 7.17\text{--}7.94 \mu\text{M}$ ) against the melanoma cell-line was 7- to 8-fold better than that of etoposide ( $TGI = 52.71 \mu\text{M}$ ).

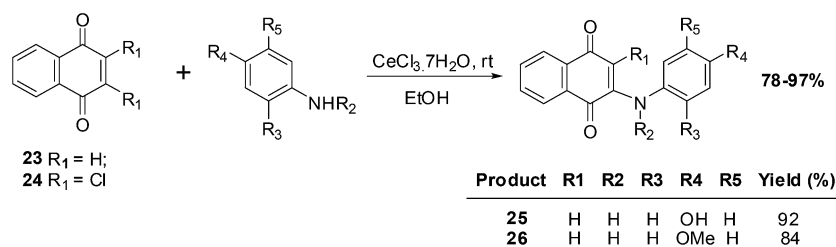
The melanoma cancer cell-line was the most susceptible to the aminonaphthoquinones.

Benites *et al.* synthesised a series of phenylamino-1,4-naphthoquinones to investigate structure-activity relationships (SARs) and the anticancer activity of this scaffold (Scheme 4).<sup>195</sup>

The cytotoxic effects of the aminoquinones were evaluated *in vitro* using the MTT reduction assay against breast (MCF7), prostate (DU145) and bladder (T24) cell-lines as well as healthy fibroblasts (BALB/3T3). The half maximal effective concentration ( $EC_{50}$ ) was determined for each of the compounds.

From a SAR analysis of the aminonaphthoquinone series it was shown that insertion of a methyl group at the nitrogen atom of the donor phenylamino group and/or insertion of a chlorine atom in the acceptor quinone nucleus induced significant changes in cytotoxic activity.

The aminoquinones, **25** and **26**, exhibited a high safety index (5.73 and 6.29, respectively), low hydrophobicity ( $\log P$  of 1.16 and 1.43, respectively), and low redox potential ( $-775$  and  $-856$  mV, respectively). Since they had a high safety index they were further investigated in various assays: cell proliferation (clonogenic assay), caspase-3 activation (DEVDase activity), and the intracellular content of ATP. Aminoquinone **25** impaired the



Scheme 4 Synthesis of phenylaminonaphthoquinones.

proliferative capacity of T24 cells without activating caspase-3 and also strongly influenced ATP levels.

PI-083, an aminonaphthoquinone from the NCI chemical library (Fig. 13), was discovered in a search for inhibitors against the chymotrypsin-like (CT-L) activity of the proteasome.

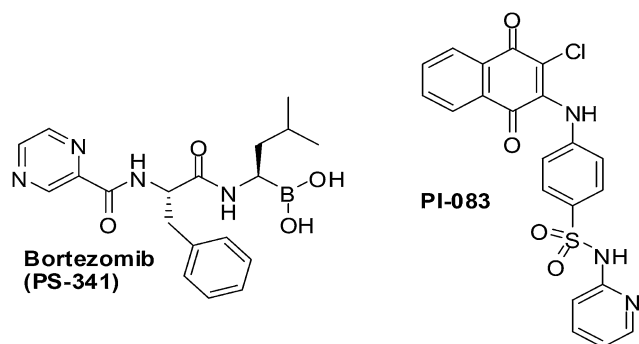


Fig. 13 The chemical structures of PI-083 and Bortezomib.

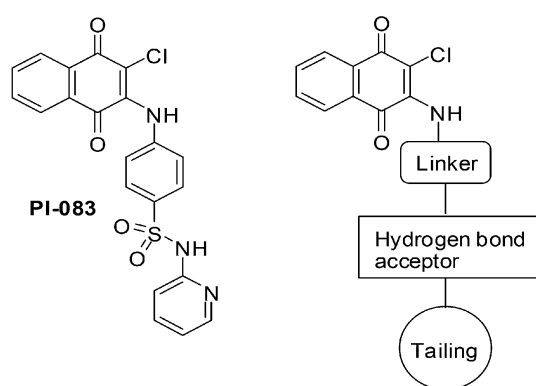


Fig. 14 Structure of PI-083 and the general formula of the target compounds.

It is a non-peptidic proteasome inhibitor that has exhibited broader antitumour activity than bortezomib, the first 20S proteasome inhibitor approved by the US FDA (Fig. 13).

CT-L activity and cell proliferation is inhibited by PI-083 which also induces apoptosis selectively in cancer cells (ovarian T80-Hras, pancreatic C7-Kras and breast MCF-7) as compared to their normal/immortalized counterparts (T80, C7 and MCF-10A, respectively). PI-083 was found to induce its antitumour effects quicker than Bortezomib in a range of cancer cells such as Multiple Myeloma (MM), breast, pancreatic, ovarian, lung, prostate cancer cell-lines as well as fresh MM cells from patients.<sup>196,197</sup>

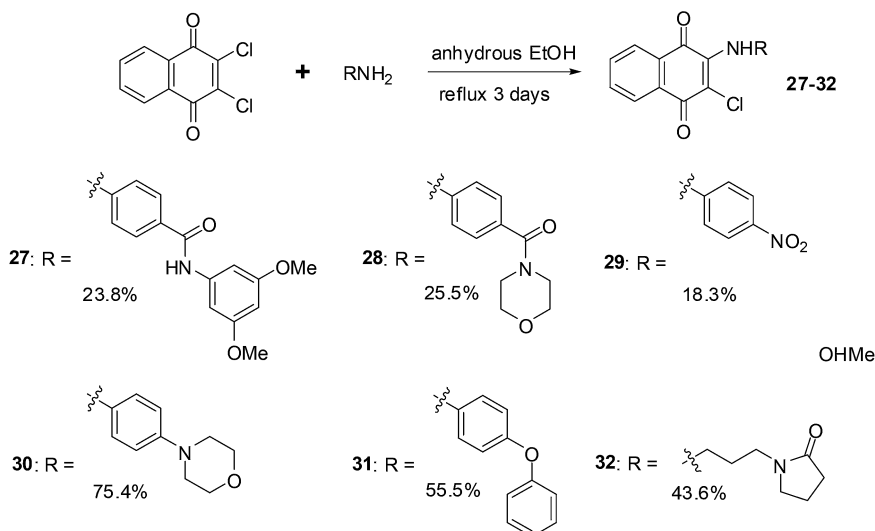
Xu *et al.* synthesised a series of naphthoquinone derivatives that were designed as analogues of the reputed proteasome inhibitor, PI-083 (Fig. 14).<sup>198</sup>

The synthesis route for the aminonaphthoquinones is shown in Scheme 5 below.

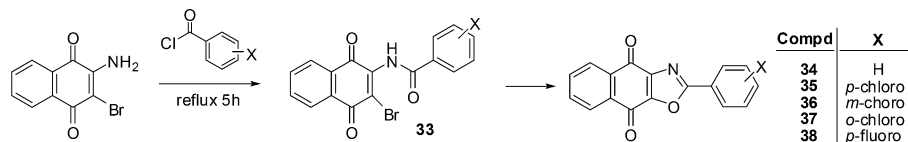
The compounds were evaluated for antiproliferative activity against lung (A549), prostate (DU145), nasopharyngeal (KB), and vincristine-resistant nasopharyngeal (KBVIN) cancer cell-lines. Antiproliferative activities comparable to that of PI-083 were obtained for six compounds (27, 28, 29, 30, 31, and 32). Compound 31 exhibited potent activity ( $IC_{50} = 1.3\text{--}8.12\text{ }\mu\text{M}$ ) against all four cancer cell-lines. Compound 29 was confirmed as a 20S proteasome inhibitor in both *in vitro* and in cell-based assays (Scheme 5).

## 2. 2-Arylnaphtho[2,3-*d*]oxazole-4,9-dione derivatives

It has been shown that naphthoquinones with fused five-membered rings reduce multidrug resistance and also potentially increases cytotoxicity.<sup>199</sup> This prompted the synthesis of compounds 34–38 which were synthesised by refluxing 2-amino-3-bromo-1,4-naphthoquinone with appropriate benzoyl chloride analogues at elevated temperatures (Scheme 6).<sup>200</sup> The reaction is thought to progress by first forming the 2-amido-3-bromo-1,4-naphthoquinone derivative 33 (Scheme 6).



Scheme 5 Synthesis of the aminonaphthoquinones – analogues of the proteasome inhibitor PI-083.



Scheme 6 Synthesis of aryl-substituted oxazonaphthoquinones.

These compounds were evaluated *in vitro* for their cytotoxic effects by screening against androgen-dependent, LNCaP, and androgen-independent, PC3, human prostate cancer cell-lines using the MTT assay. The aryl-substituted oxazonaphthoquinones displayed potent cytotoxicity against both LNCaP ( $IC_{50} = 0.01\text{--}0.40\ \mu\text{M}$ ) and PC3 ( $IC_{50} = 0.08\text{--}0.36\ \mu\text{M}$ ) prostate cancer cell-lines after 5 days of drug exposure. These compounds exhibited slightly stronger cytotoxicity against the LNCaP cell-line than on the PC3 cell-line.

Analogue 37, having the *ortho*-chloro substituent, had the strongest cytotoxicity ( $IC_{50} = 0.01 \pm 0.002\ \mu\text{M}$ ) followed by the *meta*-chloro-analogue 36 ( $IC_{50} = 0.03 \pm 0.002\ \mu\text{M}$ ) against the LNCaP cell-line. For the PC3 cell-line, the most potent compounds were the *meta*-chloro-analogue 36 ( $IC_{50} = 0.08 \pm 0.007\ \mu\text{M}$ ) and the *para*-chloro-analogue 35 ( $IC_{50} = 0.08 \pm 0.01\ \mu\text{M}$ ). The *meta*-substituted product 36 had the best cytotoxicity against both cell-lines ( $IC_{50} = 0.03\ \mu\text{M}$  for LNCaP and  $0.08\ \mu\text{M}$  for PC3). When the cytotoxicities of the two *para*-substituted analogues on the androgen-independent PC3 cell-line were compared it was found that the *p*-chloro-analogue 35 ( $IC_{50} = 0.08 \pm 0.01\ \mu\text{M}$ ) showed better activity than the *p*-fluoro-analogue 38 ( $IC_{50} = 0.20 \pm 0.012\ \mu\text{M}$ ). It appears that both the position and the electronegativity of the substituent on the aryloxazole group are important in modulating the cytotoxicity of this class of compounds.

### 3. 2-Aryl-1,4-naphthoquinone-1-oxime methyl ethers

The cytotoxicity of a variety of quinone monooxime derivatives was evaluated against a HeLa S3 cell-line in an initial screen to determine SARs.<sup>201</sup> The active compounds were then further screened against a panel of eight cancer cell-lines comprised of ovarian cancer (HeLa), renal cancer (Caki-1 and 786-O), lung cancer (A549), breast cancer (MCF-7) and mesothelioma cancer (H28, H2052, and MSTO-211H). The cytotoxic activity was evaluated against HeLa S3 cells by a methylene blue staining method and the  $IC_{50}$  values were determined using the Litchfield and Wilcoxon method.

These naphthoquinone oximes effectively inhibited HeLa ( $IC_{50} = 0.10 \pm 0.02\ \mu\text{M}$  for 39 and  $0.23 \pm 0.04\ \mu\text{M}$  for 40) and MCF-7 ( $IC_{50} = 0.20 \pm 0.07\ \mu\text{M}$  for 39 and  $0.51 \pm 0.09\ \mu\text{M}$  for 40) cancer cell-lines (Fig. 15).

The 2-aryl-6,7-methylenedioxy-1,4-naphthoquinone-1-oxime methyl ethers showed cytotoxic activity against a wide range of cancer cell-lines of which the HeLa and MCF-7 cell-lines were most susceptible. The quinone monooxime core is therefore a promising scaffold for the discovery of bioactive small molecules against cancer.

### 4. Azanaphthoquinone pyrrolo-annulated derivatives

Shanab *et al.* reported on the synthesis of a series of azanaphthoquinone pyrrolo-annulated derivatives with basic side chains (Fig. 16).<sup>202</sup>

Amongst this series compound 42a exhibited significant anti-proliferative activity which was due to the induction of caspase 3/7 activity and not due to intercalation. The rationale for the reported synthesis was to determine whether different annulation patterns of the pyridine ring to the quinone nucleus and substitution by equally electron-withdrawing heteroaromatic ring systems such as pyrazine or pyrimidine would enhance cytotoxicity.

The compounds were evaluated against five different cancer cell-lines such as cervical carcinoma (KB/HeLa), ovarian carcinoma (SKOV-3), CNS glioma (SF-268), non-small cell lung carcinoma (NCI-H460), and colon adenocarcinoma (RKO27, RKO27IND) using two independent XTT cytotoxicity assays.

The quinazolines 43 exhibited the highest inhibition across all the cell-lines with 43a, having the dimethylaminoethyl chain, displaying the highest inhibition ( $EC_{50} = 0.082 \pm 0.006\ \mu\text{g mL}^{-1}$  against NCI-H460 and  $0.107 \pm 0.020\ \mu\text{g mL}^{-1}$  against SF-268). The quinazoline derivative 43b having a dimethylamino-propyl chain was slightly less active ( $EC_{50} = 0.177 \pm 0.007\ \mu\text{g mL}^{-1}$  against NCI-H460 and  $0.198 \pm 0.025\ \mu\text{g mL}^{-1}$  against SF-268). The quinoxaline, 46a, was the next most active compound within the series and was nearly 10-fold less active. The isoquinolines 41a,b and 42a,b were at least two-fold less potent than 46a. Compound 45b, having a dimethylaminopropyl chain was inactive as well as the quinolines 44a,b and 45a,b.

From the results it was apparent that activity clearly falls into groups of positional isomers. The best cytotoxicity was exhibited by ring systems containing two nitrogens (quinazolines and quinoxalines), while ring systems containing one nitrogen (with the exception of 42) exhibited lower cytotoxicity.

The effect of the most active compound 43a, on cell cycle and intercalation was also investigated. Standard DNA intercalating UV experiments showed that 43a does not intercalate into DNA.

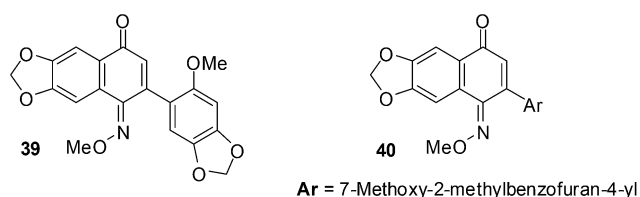


Fig. 15 Compounds exhibiting activity against cancer.



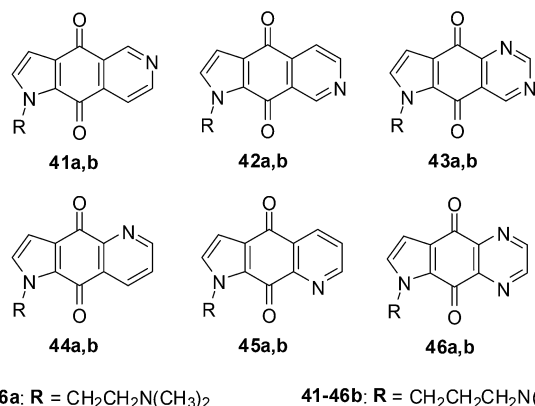


Fig. 16 Compounds 41a,b–46a,b exhibiting significant antiproliferative activity.

These nitrogen heterocyclic compounds have exhibited interesting anticancer activity that can be further investigated for potential application as anticancer agents.

## 5. Naphthoquinone amides and esters

The synthesis of fourteen new naphthoquinone aliphatic amides and seventeen naphthoquinone aliphatic esters were reported by Kongkathip *et al.*<sup>203</sup> The synthesis was inspired by the anticancer activity reported for the long chain rhinacanthins (Fig. 17). The nine to ten steps synthesis from 1-hydroxy-2-naphthoic acid afforded a 9–25% overall yield for the amides, and a 16–21% overall yield for the esters.

For the amide synthesis the crucial step was the coupling reaction between amine and various aliphatic acids using the coupling agent, 4-(4,6-dimethoxy-1,3,5-triazin-2-yl)-4-methylmorpholinium chloride (DMTMM). DCC/DMAP or CDI was used as the coupling reagent between aliphatic acids and naphthoquinone alcohol for the ester synthesis.

The compounds were evaluated for their anticancer activity against oral cavity cancer (KB), small cell lung cancer (NCI-H187), using the resazurin microplate assay (REMA) and normal Vero cell-lines by a green fluorescent protein (GFP)-based assay.

The ester series having chain lengths between 3- to 7-carbon atoms ( $IC_{50} = 1.76\text{--}41.01\text{ }\mu\text{M}$ ) inhibited KB cells stronger than the amide series ( $IC_{50} = 28.21\text{--}101.86\text{ }\mu\text{M}$ ) having the same chain length. Compounds **49** and **50** exhibited potent activity

against the KB cancer cell-line *i.e.* 1.76 and 4.65  $\mu\text{M}$ , respectively.

The naphthoquinone aliphatic amides exhibited better anticancer activity than the esters when the chain length exceeds 7-carbon atoms. The amides **47** (14-carbon atoms) and **48** (16-carbon atoms) exhibited very strong activity against the KB cancer cell-line with  $IC_{50}$  values of 5.12 and 6.35  $\mu$ M, respectively. The optimum chain length of amides is expected to be 16-carbon atoms. Much stronger anticancer activity was observed for naphthoquinone aliphatic esters having an  $\alpha$ -methyl group on the ester moiety compared to the straight chains.

Compounds **47** and **48** have almost the same potency against Vero cells ( $IC_{50} = 5.60$  and  $5.76 \mu M$ , respectively) as against KB cells. The naphthoquinone esters **49** and **50** were more potent against Vero cells ( $IC_{50} = 0.87$  and  $0.12 \mu M$  respectively) than against the KB cancer cell-line.

The decatenation assay was used to determine whether the compounds can inhibit human topoisomerase II alpha (hTopoII $\alpha$ ). The enzyme, hTopoII $\alpha$ , catalyzes the ATP-dependent decatenation of long-chain, catenated DNA molecules into free, relaxed and supercoiled forms. The assay results showed that a naphthoquinone amide **47** with a 16-carbon atom chain can completely inhibit hTopoII $\alpha$  activity at 15 and 20 mM. Only moderate inhibition was obtained at 10 mM.

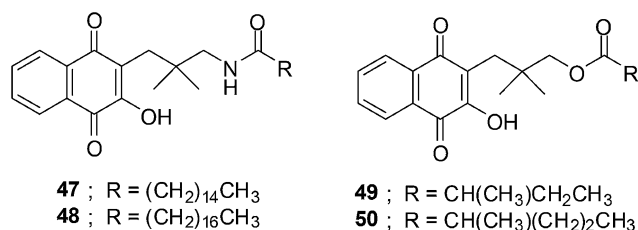
The same trend was observed in molecular docking studies as that in the cytotoxicity and decatenation assays.

Overall, the potent compounds are not selective for cancer cells over Vero cells. Further structural modification is required to make these compounds more selective for KB cancer cells.

Pradidphol *et al.* reported the synthesis of sixteen novel naphthoquinone aromatic amide analogues of rhinacanthin (naphthoquinone ester, Fig. 18).<sup>204</sup> The synthesis entailed a new synthetic route which started from 1-hydroxy-2-naphthoic acid. The products were obtained in nine or ten steps in good to excellent yield (54–98%). The amide formation reaction was carried out by using the coupling reagent, 4-(4,6-dimethoxy-1,3,5-triazin-2-yl)-4-methylmorpholinium chloride (DMTMM). DMTMM is a useful condensing agent that affords carboxamides in high yield. Oxidation with Fremy's salt followed by hydroxylation with *tert*-butyl hydroperoxide and triton B was the key step for converting naphthol to 3-hydroxynaphthoquinone.

The anticancer activity of the naphthoquinone amides were evaluated against human cancer cell-lines such as oral cavity cancer (KB), small cell lung cancer (NCI-H187), and breast cancer (MCF-7) using the resazurin microplate assay (REMA) and normal Vero cell-lines by a green fluorescent protein (GFP)-based assay. Benzamide **51** ( $IC_{50} = 8.83 \pm 1.79 \mu M$ ) showed potent inhibition against small cell lung cancer (NCI-H187) cell-line while naphthamides **52** ( $IC_{50} = 9.00 \pm 2.17 \mu M$ ) and **54** ( $IC_{50} = 8.62 \pm 2.26 \mu M$ ) were the most potent against the oral cavity cancer (KB) cell-line. The activity of compound **55** ( $IC_{50} = 41.23 \pm 15.93 \mu M$ ) was at least 4.5-fold less than that of **54**. Most of the naphthoquinone amides showed weak toxicity against normal Vero cell-lines.

The decatenation assay was used to determine the inhibitory activity of novel naphthoquinone aromatic amides against



**Fig. 17** The structures of the naphthoquinone amides and aliphatic esters.

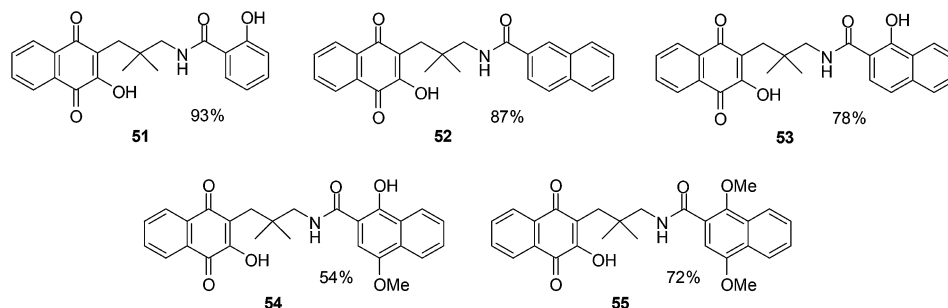


Fig. 18 Naphthoquinone amides, analogues of the rhinacanthin ester.

human Topoisomerase II $\alpha$  (hTopoII $\alpha$ ). The results showed that compound 53 and 54 inhibit hTopoII $\alpha$  activity at a concentration of 20 mM while compounds 51, 52 and 55 exhibited hTopoII $\alpha$  inhibitory activity at 50 mM. The same trend was observed in the docking experiment as the cytotoxicity and decatenation assay.

From this study it was concluded that the naphthoquinone amides 53 and 54 are promising target molecules for anticancer drug development.

## 6. Dimeric naphthoquinones

The anticancer activity of a novel series of twelve dimeric naphthoquinones against prostate cancer was reported by Ross *et al.*<sup>205–209</sup>

The compounds were screened against androgen-independent (PC3, DU145) and androgen-responsive (LNCaP, 22RV1) prostate cancer cell-lines as well as prostate epithelial cells (PrECs). Cytotoxicity at 15  $\mu$ M against all the prostate cancer cell-lines were observed for 56–60 (Fig. 19).<sup>209</sup>

A study in clonogenic assays showed that the ability of prostate cancer cells to form colonies was limited after treatment with the compounds. It was found that cellular ROS formation increased after exposure of prostate cancer to these compounds. ATP production decreased and apoptosis was promoted.<sup>209</sup> Cytotoxicity was dependent on NADP(H):quinone oxidoreductase activity in DU-145 cells but not in PrECs, PC3 and 22RV1 cells.

The accumulation of ROS and mitochondrial dysfunction probably contributed to the cytotoxic effect of the dimeric naphthoquinones.

## 7. Dioncoquinones and dioncoquinone derivatives

A family of natural naphthoquinones that have exhibited promising anti-tumoural and anti-infective activities are the dioncoquinones. Amongst these the dioncoquinones A 61 and B 62 are highly active against *Leishmania major* and multiple myeloma cells with low toxicity toward normal blood cells (Fig. 20).<sup>210</sup> Their activities against multiple myeloma cell-lines were comparable to melphalan. The latter is a documented DNA-alkylating agent effective against both multiple myeloma and B cell lymphoma.

The first total synthesis of the highly oxygenated anticancer agent dioncoquinone B 62 and the isolation of its new, even higher-oxygenated analogues, dioncoquinones C 63, D 64 and E 65, from cell cultures of *Triphyophyllum peltatum* was reported (Fig. 20).<sup>210</sup> Several derivatives of these compounds were synthesised, including dioncoquinone C 63.

The dioncoquinones B 62 and C 63 and related naphthoquinones were screened against a malignant human multiple myeloma cell-line (INA-6) and non-malignant peripheral blood mononuclear cells (PBMC). Apoptotic and viable cell fractions were determined by staining with annexin V-FITC and propidium iodide (PI). Dioncoquinones A 61 and B 62 showed promising results with B 62 exhibiting especially high ( $EC_{50}$  = 11  $\mu$ M) and selective activity against multiple myeloma (MM)

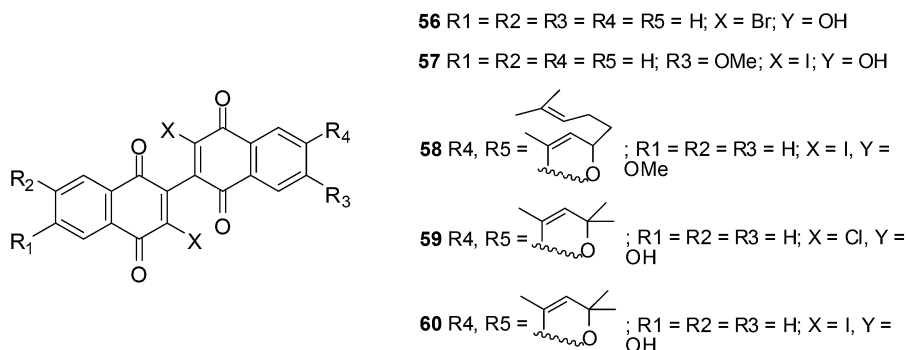


Fig. 19 Dimeric naphthoquinones.

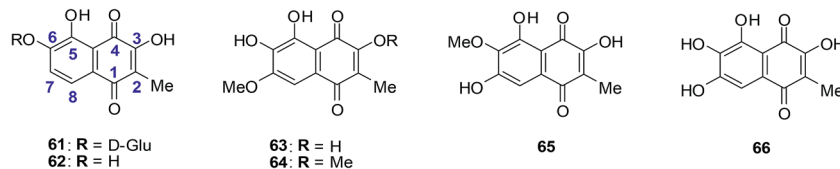


Fig. 20 Higher-oxygenated naphthoquinones **61**–**65** isolated from *T. peltatum* cell cultures and compound **66** exhibiting anticancer activity.

cells. Dioncoquinone C **63** ( $EC_{50} = 14 \mu\text{M}$ ) showed a similar activity against the MM cells as that of dioncoquinone B **62** which was inactive against PBMcs. The 7-hydroxy-dioncoquinone B **66** ( $EC_{50} = 7 \mu\text{M}$ ) was 10-times more selective for MM INA-6 than for PBMcs ( $EC_{50} = 70 \mu\text{M}$ ) (Fig. 20).

From a SAR study it was determined that all three hydroxyl groups (at C-3, C-5, and C-6) are necessary for enhanced anti-cancer activities and reduced cytotoxicities. This can be seen from the similar activities of dioncoquinone B **62**, dioncoquinone C **63**, and 7-hydroxyl-dioncoquinone B **66**. Dioncoquinone C **63** was the only new compound that strongly induced apoptosis in the MM cell-line (INA-6) without any noteworthy toxicity against PBMcs. Dioncoquinones B **62** and C **63** are two encouraging elementary structures for developing anti-MM candidates.

## 8. Glycosylated derivatives of juglone and related 1,4-naphthoquinones

Antifungal, immunomodulatory, and antitumour properties have been reported for the glycosylated derivatives of the physiologically active natural compound juglone as well as related 1,4-naphthoquinones. There were, however, no reports on their antileukemic properties and their SARs have been insufficiently studied. In light of this, a series of fifty 1,4-naphthoquinone derivatives were therefore evaluated for their antileukemic effects and subjected to a structure–activity study (SAR).<sup>211</sup>

The compounds were screened against human promyelocytic leukemia (HL-60) cells using the MTS method for determining cell viability. The compounds inhibited the HL-60 cells at a wide range of concentrations.

From the SAR study it was apparent that the juglone derivatives were among the most active compounds and that the hydroxyl group at position 5 of the quinone moiety was essential for the increase of cytotoxicity. The most active compounds were the glycosides of juglone **67**–**77** ( $IC_{50} = 0.6$ – $4.8 \mu\text{M}$ ). The structures of compounds with  $IC_{50}$  value less than  $1 \mu\text{M}$  are shown in Fig. 21.

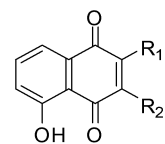
The products of intramolecular cyclisation of the *S*-glycosides of 1,4-naphthoquinones were also very active ( $IC_{50} = 1$ – $5 \mu\text{M}$ ). It was found that electronegative groups like  $-\text{OH}$ ,  $-\text{Cl}$ ,  $-\text{NH}_2$ ,  $-\text{OMe}$ ,  $-\text{OAc}$  at the  $\alpha$ -position to the sugar moiety dramatically reduced antileukemic activity of the compounds. The structures of compounds with an  $IC_{50}$  value less than or equal to  $1.5 \mu\text{M}$  are shown in Fig. 22.

There is potential for the development of *O*- or *S*-glycosylated derivatives of juglone and related 1,4-naphthoquinones as new antileukemic agents.

## 9. Lapachol derivatives

Lapachol **83**, a naturally occurring 1,4-naphthoquinone, has its origins in several tree species from the Bignoniaceae family found in the Western Hemisphere including Brazil and has a history of cancer cell cytotoxicity.<sup>212,213</sup>  $\beta$ -Lapachone **84**, a secondary metabolite, has shown anticancer activity against various cancer molecular targets.<sup>214–216</sup> It is in phase II clinical trials in the USA for the treatment of advanced solid tumours.<sup>217</sup>

A series of synthetic 1,4-naphthoquinones related to the secondary metabolites lapachol,  $\alpha$ - and  $\beta$ -lapachone were synthesised (Fig. 23).<sup>218</sup> The secondary metabolites lapachol,  $\alpha$ - and  $\beta$ -lapachone and the synthetic 1,4-naphthoquinones were screened against the oesophageal cancer cell-line (WHCO1) using the MTT assay. Most of the compounds exhibited potent activity ( $IC_{50} = 1.6$ – $7.3 \mu\text{M}$ ).



Juglone:  $R_1 = R_2 = \text{H}$

- 67:**  $R_1 = \text{Ac}_4\beta\text{D-GlcO}$ ;  $R_2 = \text{H}$   
**68:**  $R_1 = \text{H}$ ;  $R_2 = \text{Ac}_4\beta\text{D-GlcO}$   
**69:**  $R_1 = \text{Ac}_7\beta\text{D-MalO}$ ;  $R_2 = \text{H}$   
**70:**  $R_1 = \text{H}$ ;  $R_2 = \text{Ac}_7\beta\text{D-MalO}$   
**71:**  $R_1 = \text{H}$ ;  $R_2 = \text{Ac}_4\beta\text{D-GlcS}$   
**72:**  $R_1 = \text{Ac}_4\beta\text{D-GlcS}$ ;  $R_2 = \text{Me}$   
**73:**  $R_1 = \text{H}$ ;  $R_2 = \text{Ac}_4\beta\text{D-GalS}$   
**74:**  $R_1 = \text{H}$ ;  $R_2 = \text{Ac}_3\beta\text{D-XylS}$   
**75:**  $R_1 = \text{H}$ ;  $R_2 = \text{Ac}_3\text{-}\alpha\text{-L-AraS}$   
**76:**  $R_1 = \text{H}$ ;  $R_2 = \text{Ac}_7\beta\text{D-MalS}$   
**77:**  $R_1 = \text{H}$ ;  $R_2 = \text{Ac}_4\beta\text{D-ManS}$

Fig. 21 Juglone glycosides **67**–**77** highly active against leukemia (HL-60) cells.

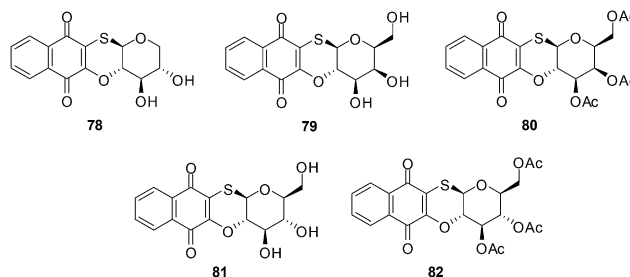


Fig. 22 *S*-glycosides of 1,4-naphthoquinones highly active against leukemia (HL-60) cells.

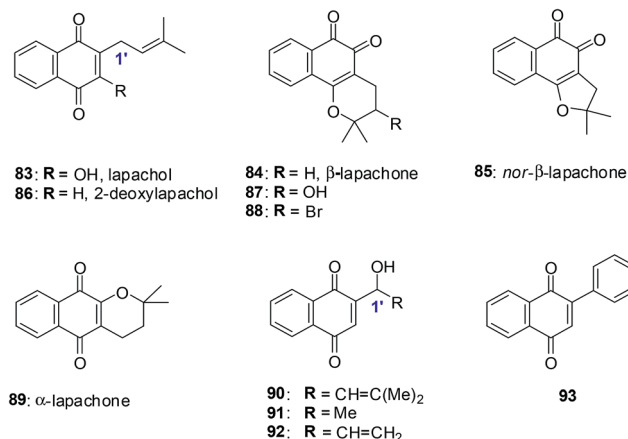


Fig. 23 *Para*- and *ortho*-naphthoquinones evaluated against the oesophageal cancer cell-line.

The synthetic compounds **94**, **95** and **96** were screened against a normal fibroblast cell-line (NIH3T3). The two new synthetic halogenated compounds **95** and **96** (IC<sub>50</sub> = 3.0 and 7.3  $\mu$ M, respectively) and the previously reported compound **94** (IC<sub>50</sub> = 3.9  $\mu$ M), were found to be non-toxic (Fig. 24). Compounds **95** and **96** are also more cytotoxic than cisplatin the current drug of choice against oesophageal cancer.

Compound **94** was used to determine the mechanism of action of cell death in oesophageal cancer cells. Cell death entailed processes involving PARP cleavage (indicative of apoptosis) caused by **94** which was shown to be associated with elevated c-Jun levels.

This investigation identified compounds that are useful as a starting point for new and improved pharmacologically improved chemotherapeutic agents against oesophageal cancer.

The natural naphthoquinone, Lapachol **83**, has been investigated mainly due to its antibacterial,<sup>219–221</sup> antifungal,<sup>222</sup> trypanocidal<sup>223</sup> and anticancer activities (Fig. 25).<sup>224</sup> The hydroxyl derivative, 5-hydroxylapachol **97**, was isolated from the root

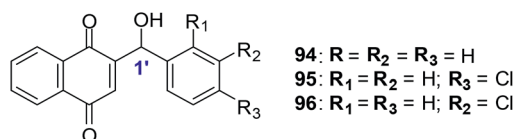


Fig. 24 Synthetic 1,4-naphthoquinones related to lapachol, and  $\alpha$ - and  $\beta$ -lapachone.

heart wood of *Tectona grandis*. This compound, like lapachol **83**, was also found to be cytotoxic to *Artemia salina* (brineshrimp) with an LC<sub>50</sub> of 5 ppm.<sup>225</sup> The cyclisation product of lapachol **83** is  $\beta$ -Lapachone **84** which has been under intense investigation for clinical use in cancer chemotherapy (Fig. 25).<sup>214,226,227</sup>

Bonifazi *et al.* reported on a series of 5-hydroxy-1,4-naphthoquinone analogues which were synthesised from juglone.<sup>228</sup> The antiproliferative activity of these compounds was evaluated against a panel of six human cancer cell-lines such as ovarian (A2780), breast (HBL-100), cervical (HeLa), non-small cell lung (SW1573), breast (T-47D), and colon (WiDr) using the sulforhodamine B (SRB) assay.

The most potent compounds, 5-hydroxylapachol **97**,  $\beta$ -Lapachone **84** and **98** (Fig. 25), had GI<sub>50</sub> values of 0.42–8.1  $\mu$ M, 0.69–2.3  $\mu$ M and 0.80–2.2  $\mu$ M, respectively. The comparison between lapachol **83** and the hydroxyl derivative **97** showed that the occurrence and location of a hydroxyl group attached to the aromatic ring does affect the capacity to inhibit the progression of cancer cell-lines. Thus, it is concluded that the presence of a phenolic hydroxyl group at C-5 plays an important role in increasing the antiproliferative activity.

## 10. $\beta$ -Lapachone-based 1,2,3-triazoles

A variety of  $\beta$ -lapachone derivatives were synthesised by different synthetic routes and were obtained in moderate to high yields (Fig. 26).<sup>229</sup> The compounds were screened against leukemia (HL-60), melanoma (MDA-MB435), colon (HCT-8) and central nervous system (SF295) cancer cell-lines using the MTT assay. Some of the compounds were highly active exhibiting IC<sub>50</sub> values below 2  $\mu$ M. The  $\beta$ -lapachone-based 1,2,3-triazoles had the best activity.

The most significant activity was observed toward HL-60 and the compounds **100**, **101** and **103** were highly active with IC<sub>50</sub> values of 1.43, 1.01 and 1.20  $\mu$ M, respectively. These compounds were as active as the prototype,  $\beta$ -lapachone **84** (IC<sub>50</sub> = 1.65  $\mu$ M). Compounds **101**–**104** were highly active against MDA-MB435 with IC<sub>50</sub> values below 2  $\mu$ M. Only substance **101** was considered highly active against HCT-8 cell-lines with an IC<sub>50</sub> value of 1.37  $\mu$ M. The compounds **101**–**104** exhibited an activity between 1.55–3.01  $\mu$ M against SF295.

The addition of 1-bromoheptane that deplete reduced glutathione (GSH) content and the use of *N*-acetylcysteine that protects cells against apoptotic cellular death gave an understanding of the ROS mechanism of anticancer action. An analysis for the formation of thiobarbituric acid reactive substances (TBARS) was also done. Furthermore the comet assay which utilises the bacterial enzymes, formamidopyrimidine DNA-

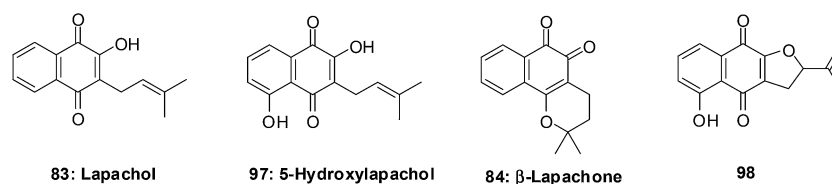


Fig. 25 The chemical structures of natural quinones.



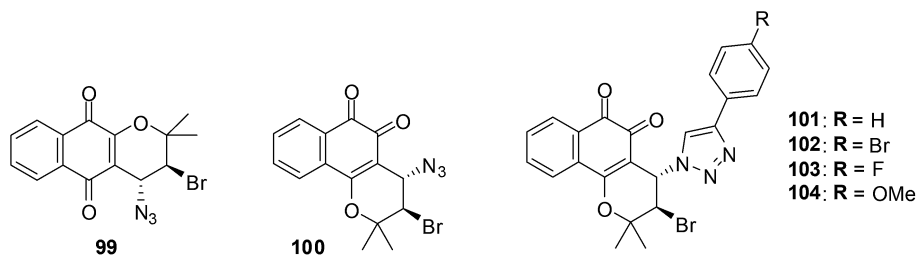


Fig. 26 Lapachone derivatives.

glycosylase (FPG) and endonuclease III (ENDIII), was used to detect for DNA damage after treatment. It was found that compounds **101** and **103** promoted cell death by apoptosis and genotoxicity and suggests that quinone-induced apoptosis is associated with ROS production.

The  $\beta$ -lapachone-based 1,2,3-triazoles are promising prototypes for application in cancer chemotherapy.

### 11. *Nor*- $\beta$ -lapachone derivatives

Several 3-arylamino and 3-alkoxy-*nor*- $\beta$ -lapachone derivatives were synthesised in moderate to high yields (Fig. 27).<sup>230</sup> These compounds were evaluated for their anticancer activity against central nervous system (SF295), colon (HCT8), melanoma (MDA-MB435), and leukemia (HL60) cancer cell-lines using the MTT assay. Most of the arylamino-*nor*- $\beta$ -lapachone derivatives exhibited potent activity against all cancer cell-lines. Several compounds were found to be highly active with  $IC_{50}$  values below 2  $\mu$ M and were also more active than  $\beta$ -lapachone **84** an anti-prostate cancer prototype in phase II clinical studies.

Compounds **105** and **114** were the two most active compounds against all cancer cells with  $IC_{50}$  values below 1  $\mu$ M which was even better than that of the standard, doxorubicin (toward MDA-MB435).

MDA-MB435 was the most sensitive cell-line with compounds **105–109**, **110**, **111–118** exhibiting activity more potent than doxorubicin. Toward the HL60 cancer cell compounds **105**, **106**, **109–112**, **114** and **118** were superior in activity when compared to  $\beta$ -lapachone **84** ( $IC_{50}$  = 1.65  $\mu$ M) and *nor*- $\beta$ -lapachone **85** ( $IC_{50}$  = 1.75  $\mu$ M) with compounds **111** and **114** having similar activity ( $IC_{50}$  = 0.28  $\mu$ M).

Toward the HCT8 and SF295 cancer cell-lines, two compounds **114** ( $IC_{50}$  = 0.15  $\mu$ M and 0.39  $\mu$ M, respectively) and **105** ( $IC_{50}$  = 0.76  $\mu$ M and 0.82  $\mu$ M, respectively) were more potent than *nor*- $\beta$ -lapachone **85** ( $IC_{50}$  = 1.36 and 1.58  $\mu$ M) and  $\beta$ -lapachone **84** ( $IC_{50}$  = 0.83 and 0.91  $\mu$ M).

The arylamino *para*-nitro **105** and the 2,4-dimethoxy substituted naphthoquinones **114** exhibited the best activity while the *ortho*-nitro **112** and the 2,4-dimethoxy substituted compound **114** were more selective than doxorubicin and similar to the precursor lapachones. Compound **114** exhibited the best anticancer activity and selectivity index which was even better than doxorubicin, the positive control.

These compounds are promising new lead compounds for anticancer drug development.

### 12. *Nor*- $\beta$ -lapachone arylamino derivatives

A variety of naphthoquinones such as *nor*- $\beta$ -lapachone and its arylamino derivatives, iodinated and methylated naphthoquinones and *nor*- $\beta$ -lapachone-based 1,2,3-triazoles were synthesised (Fig. 28).<sup>231</sup> These compounds were evaluated for their anticancer activities against four human leukemia cell-lines (HL-60, K562, Molt-4 and Jurkat) using the MTT assay. It was found that *nor*- $\beta$ -lapachones with arylamino substituents exhibited potent activity.

Naphthoquinones **119–122** ( $IC_{50}$  = 0.33–2.30  $\mu$ M) were the most active. Compounds **123–125**, the iodinated and methylated derivatives, were less active against the evaluated cancer cell-lines ( $IC_{50}$  = 4.05–3.44  $\mu$ M) except for **125** for HL-60 ( $IC_{50}$  = 1.11  $\mu$ M) and compound **124** for Jurkat ( $IC_{50}$  = 1.38  $\mu$ M). Compounds **119–122** (for HL-60), **122** (for K562), **120** and **122** (for Molt-4) and **119**, **120** and **122** (for Jurkat) were found to be more active than *nor*- $\beta$ -lapachone, the naphthoquinoidal precursor.

The standard comet assay was used to determine how these compounds caused DNA damage. Compounds **119–122** were found to induce oxidative DNA damage by ROS generation and treatments with these compounds impair DNA repair activity resulting in the triggering of apoptosis. The mechanism of action of these compounds does not involve topoisomerase inhibition.

A study was conducted in Chinese hamster lung fibroblasts (V79 cells) to determine the capacity of the compounds to

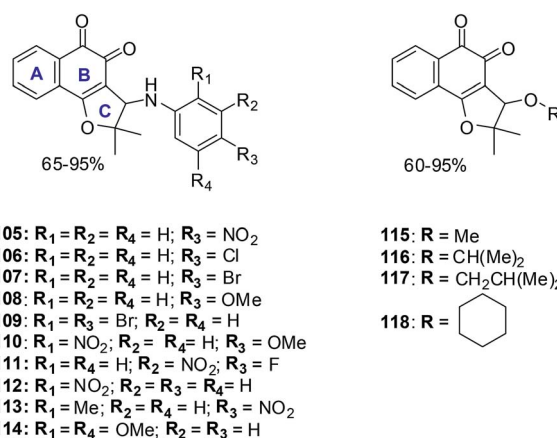


Fig. 27 Compounds exhibiting potent anticancer activity.

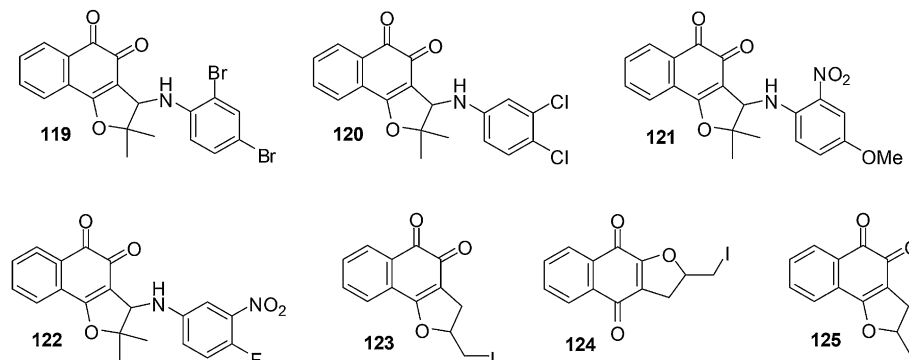


Fig. 28 Compounds exhibiting anticancer activity.

stimulate apoptosis and chromosomal aberrations as micronuclei. After drug treatment morphological apoptotic nuclei and micronuclei stimulation were detected signifying a link between DNA damage and apoptosis.

Since compounds **119–122** lack selectivity between cancer and non-cancer cells, their therapeutic potential may be limited as anticancer agents.

### 13. $\alpha$ - and $\beta$ -lapachone derivatives

The synthesis of a series of compounds having  $\alpha$ - and  $\beta$ -lapachone containing hydroxyl or methoxyl groups on the benzene ring was achieved through a selective acid catalysed cyclization of suitable lapachol analogues (Fig. 29).<sup>232</sup> Compounds were evaluated for their anticancer activity against breast (HBL-100), cervical (HeLa), lung (SW1573), and colon (WiDr) cancer cell-lines using the SRB assay.

From the data it was apparent that there was a clear difference in activity between the three series of compounds. The activity order was as follows:  $\beta$ -lapachone analogues >  $\alpha$ -lapachone analogues  $\approx$  lapachol analogues. Compounds **126**, **127**, **128** and **129** exhibited activity less than 2.2  $\mu\text{M}$  against all the cell-lines. The sensitivity of the cell-lines was in the order HBL-100 > SW1573 > HeLa > WiDr. From this evaluation the  $\beta$ -lapachone analogue **127** was found to be the most active of the series ( $\text{GI}_{50} = 0.029\text{--}2.0\ \mu\text{M}$ ). This compound exhibited better activity than the parent drug,  $\beta$ -lapachone ( $\text{GI}_{50} = 0.38\text{--}2.0\ \mu\text{M}$ ).

From cell cycle studies, protein expression experiments, and ROS analysis it was determined that, likewise to  $\beta$ -lapachone, ROS formation and DNA damage are vital aspects in the cellular toxicity of **127**. This may be the leading mechanism of action of this compound since human DNA topoisomerase II $\alpha$  is not inhibited in the decatenation assay.

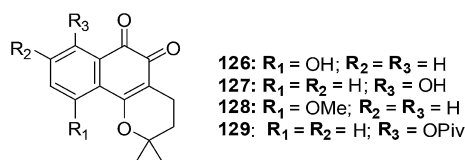


Fig. 29  $\beta$ -Lapachone analogues exhibiting potent anticancer activity.

Compound **127** is promising for lead development in cancer chemotherapy.

### 14. *Nor*- $\beta$ -lapachone-based 1,2,3-triazoles

da Silva Júnior *et al.* reported on the anticancer activity of five *nor*- $\beta$ -lapachone-based 1,2,3-triazoles and a precursor azido-naphthoquinone against central nervous system (SF-295), colon (HCT-8), melanoma (MDAMB-435), leukaemia (HL-60), prostate (PC-3), murine melanoma (B-16) and a normal murine fibroblast L-929 using the MTT assay (Fig. 30).<sup>233</sup> Most of the compounds are strongly or moderately active ( $\text{IC}_{50} = 0.43\text{--}9.48\ \mu\text{M}$ ) against all cancer cell-lines.

Compound **130** ( $\text{IC}_{50} = 1.19\ \mu\text{M}$ ) was selectively active against MDAMB-435. The triazolic naphthoquinone **134** exhibited the best activity against cancer cells ( $\text{IC}_{50} = 0.43\text{--}1.83\ \mu\text{M}$ ) with the strongest activity being against MDAMB-435. In addition, it also exhibited high selectivity (2–4 fold more selective) for cancer cells over normal cells (L-929,  $\text{IC}_{50} = 2.85\ \mu\text{M}$ ). The activity of compound **134** against melanoma cells was comparable to that of the initial quinones, *nor*- $\beta$ -lapachone and  $\beta$ -lapachone, and better than that of doxorubicin. Compound **134** was also more active ( $\text{IC}_{50} = 1.17\ \mu\text{M}$ ) than *nor*- $\beta$ -lapachone **85** ( $\text{IC}_{50} = 1.75\ \mu\text{M}$ ) and  $\beta$ -lapachone **84** ( $\text{IC}_{50} = 1.65\ \mu\text{M}$ ) towards HL-60.

An attempt was made to correlate physicochemical parameters (reduction potentials and calculated  $\log P$ ) with cytotoxic activity. There was no correlation found between reduction potentials and cytotoxicity. There was a correlation between cytotoxicity and the calculated  $\log P$ : the higher the lipophilicity values ( $c\ \log P$ ) the better the cytotoxicity. Compounds **133–135** could be exploited for the preparation of analogues for the development of new anticancer drugs.

### 15. Mansonone F derivatives

Naturally occurring Mansonone F (MF) is an *o*-quinone sesquiterpene that exists in plants such as *Ulmus pumila* and *Mansonia altissima*.<sup>234,235</sup> It has antibacterial<sup>236,237</sup> and anti-proliferative activities<sup>238,239</sup> and also inhibits the activity of thioredoxin reductase, a potential target for anticancer drugs.<sup>240,241</sup>

A series of MF derivatives were designed and synthesised by different synthetic routes and were obtained in moderate to

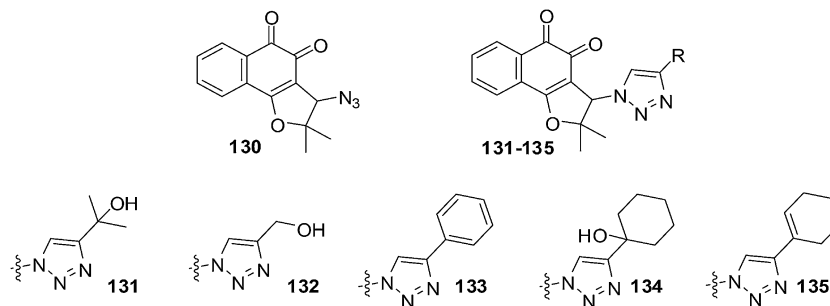


Fig. 30 Compounds exhibiting anticancer activities.

high yields (Fig. 31).<sup>242</sup> The compounds were screened against topoisomerase I and II and found to be potent inhibitors of topoisomerase II. The inhibitory activity exhibited by the best inhibitor against topoisomerase II was 20 times stronger than that of the positive control, Etoposide.

The MTT assay was used to determine the growth inhibitory concentration ( $IC_{50}$ ) of the MF derivatives against the human lung adenocarcinoma cell-line (Glc-82) and the human nasopharyngeal carcinoma cell-line (CNE-2). Potent anticancer activity was exhibited by compounds **136–139** ( $IC_{50}$  = 2.75–7.93  $\mu$ M), **140–142** ( $IC_{50}$  = 3.18–9.76  $\mu$ M), and **146** and **147** ( $IC_{50}$  = 2.88–9.19  $\mu$ M) against both CNE-2 and Glc-82 cell-lines. From a SAR study it was determined that the *o*-quinone group and the pyran ring are important for cytotoxic activity.

## 16. Naphthoquinone-based STAT3 inhibitors

The signal transducer and activator of transcription 3 (STAT3) is active in a large variety of cancers.<sup>243</sup> It is activated by a number of cytokines and hormones.<sup>244–247</sup> The STATs undergo homo- and/or hetero-dimerization after the phosphorylation of a specific tyrosine residue.<sup>248,249</sup> STAT3 is activated upon phosphorylation at Tyr705 (ref. 249) and it signals as a homodimer or as a STAT1–STAT3 heterodimer.<sup>250</sup>

Strategies to target STAT3 have been directed toward its aminoterminal, DNA-binding and SH2 domains. The SH2 domain is involved in both receptor binding and dimerization and has therefore received much attention.

The first small molecule inhibitor of the STAT3 SH2 domain was STA-21 **148** (Fig. 32).<sup>251</sup> The structural complexity of STA-21 has made the synthesis of analogues for structure–activity relationship (SAR) studies difficult. This led to the simplification of the structure and the design and synthesis of the initial compounds LLL3 **149** and LLL12 **150** which are substituted anthraquinones.<sup>252</sup> LLL12 **150** is an exceptionally effective compound that shows elevated levels of antiproliferative activity.

The synthesis and assessment of 1,4-naphthoquinone analogues targeting STAT3 was attempted to design more potent analogues with the 1,4-naphthoquinone scaffold (Fig. 33).<sup>253</sup>

These analogues were evaluated against breast (MDA-MB-231), prostate (DU-145 and PC-3) and colon (HT-29) cancer cell-lines. The 1,4-naphthoquinone methyl ketone derivative **151** (DU145:  $IC_{50}$  =  $6.4 \pm 0.5$   $\mu$ M; HT29:  $IC_{50}$  =  $4.2 \pm 0.8$   $\mu$ M) and methyl ester derivative **152** ( $IC_{50}$  =  $5.8 \pm 1.4$   $\mu$ M, HT29) were found to be the most potent.

The STAT3 fluorescence polarization (FP) assay was used to evaluate selected 1,4-naphthoquinones for their abilities to bind to the STAT3 SH2 domain and inhibit STAT3. The compounds inhibited the FP signal and therefore STAT3 is one of their molecular targets.

The results from this study are promising for further development of these compounds for potential application in cancer chemotherapy.

Cpd	R <sub>1</sub>	R <sub>2</sub>	Yield (%)	Cpd	R <sub>1</sub>	R <sub>2</sub>	Yield (%)
<b>136</b>	-Cl	-Ph	40	<b>143</b>	-	-	40
<b>137</b>	-Cl	-CH <sub>2</sub> Ph	53	<b>144</b>	-Cl	-CH <sub>2</sub> CH <sub>2</sub> CH <sub>3</sub>	91
<b>138</b>	-Cl	-CH <sub>2</sub> CH <sub>2</sub> CH <sub>3</sub>	51	<b>145</b>	-Cl	-CH <sub>2</sub> CH=CH <sub>2</sub>	50
<b>139</b>	-Cl	-CH <sub>2</sub> CH=CH <sub>2</sub>	65	<b>146</b>	-H	-Ph	90
<b>140</b>	-H	-Ph	48	<b>147</b>	-H	-CH <sub>2</sub> Ph	85
<b>141</b>	-H	-CH <sub>2</sub> Ph	50				
<b>142</b>	-H	-4-F-Ph	45				

Fig. 31 Compounds exhibiting potent activity against both CNE-2 and Glc-82 cell-lines.

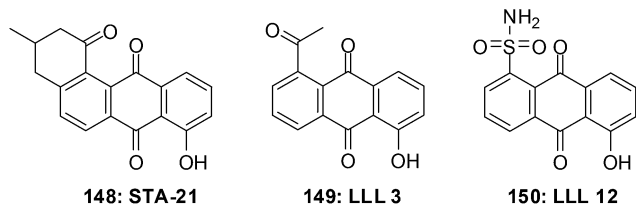


Fig. 32 Small molecule inhibitors of the STAT3 SH2 domain.

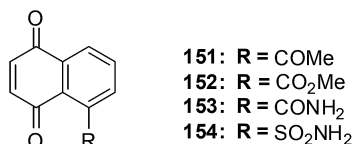


Fig. 33 Compounds 151–154 were synthesised to bind to the STAT3 SH2 domain and inhibit STAT3.

### 17. Pentacyclic 1,4-naphthoquinones

Salustiano *et al.* conducted a study which entailed the comparison of the cytotoxic effects of pentacyclic 1,4-naphthoquinones, lapachol and  $\alpha$ -lapachone on human leukemic cells.<sup>254</sup> The pentacyclic 1,4-naphthoquinones of type 1<sup>36</sup> were designed as molecular hybrids of the cytotoxic naphthoquinones, lapachol and  $\alpha$ -lapachone and natural pterocarpan<sup>255–257</sup> (Fig. 34).

The compounds were screened against two human leukemic cell-lines K562 (oxidative stress-resistant), Lucena-1 (MDR phenotype), Daudi, MCF-7 and PBMC using the MTT colorimetric assay. The pentacyclic 1,4-naphthoquinones 155–158 were found to be cytotoxic ( $IC_{50} = 2–7 \mu M$ ) to the leukemic cell-lines including fresh leukemic cells obtained from patients, some with the MDR phenotype. Lapachol **83** and  $\alpha$ -lapachone **89** were ineffective against these cell-lines while MMC inhibited cell proliferation at concentrations as low as  $0.45 \pm 0.04 \mu M$ . It was shown in previous work that *in situ* bioactivation activates these compounds and after rearrangement enones are formed that are powerful alkylating agents.

The little activity exhibited by lapachol **83** and  $\beta$ -lapachone **84** is because they cannot be activated by reduction. These compounds exhibited low toxicity against lymphocytes activated

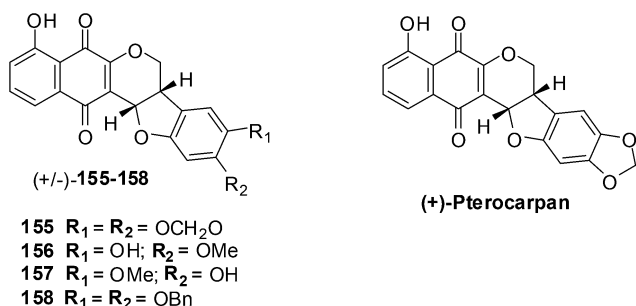


Fig. 34 The pentacyclic naphthoquinones 155–158 and natural pterocarpan.

by phytohemagglutinin thus indicating that they are selective for the leukemic cancer cells.

Compounds 155–158 were active against oxidative stress-resistant K562 and Lucena-1 cell-lines which indicated that bioreductive activation of pentacyclic naphthoquinones type 1 could be responsible for the cytotoxic effect on these cells. By flow cytometry it was determined that the pentacyclic 1,4-naphthoquinones 155 and 157 induced cancer cell death by apoptosis.

### 18. Peptidomimetic compounds containing redox active quinones

Healthy cells are different from their cancer counterparts in that they do not exhibit a disturbed intracellular redox balance. Some cancers are naturally under oxidative stress (OS) such as prostate and breast cancer cells while others are hypoxic, *i.e.* their cells are more reducing than normal ones such as solid lung carcinoma. The ROS levels of these cells, when compared to healthy cells, are much closer to the critical redox threshold at which cell death is induced.<sup>258</sup> Selective and effective redox drugs can be designed to exploit the biochemical differences between healthy and cancerous cells.<sup>259,260</sup>

Shabaan *et al.* used the Passerini multicomponent reaction for the synthesis of di-, tri- and tetra-functional redox agents containing multiple chalcogen and quinone redox sites (Fig. 35).<sup>261</sup>

Compounds **162**, **163**, **165** and **167** were submitted for a one-dose screen at the National Institute of Health (NIH) which included 58 cell-lines. These cell-lines were grouped into breast cancer, renal cancer, colon cancer, prostate cancer, ovarian cancer, CNS cancer, non-small-cell lung cancer, leukemia, and melanoma cell-lines. All the evaluated compounds displayed a noteworthy cytotoxicity against the cancer cell-lines that were then selected for 5-dose testing. The compounds exhibited  $GI_{50}$  values that were in the low to sub-micromolar range.

From a COMPARE analyses it was determined that the compounds' pattern activities link with cisplatin which is used to treat numerous forms of cancers, including sarcomas, lymphomas, and germ cell tumours. The compounds' pattern activities also correlated with methyl MMC which has anti-tumour antibiotic activity against breast cancer, and with the anthracycline-based redox agents representing menogaril, deoxydoxorubicin, and MX2 HCl, which are used in the treatment of prostate cancer and leukemia.

The cytotoxic activity of the test compounds was further evaluated against human breast adenocarcinoma (MCF-7), human kidney carcinoma (A-498), and human epidermoid carcinoma (A-431). The MTT assay was used and after 24 h the  $IC_{50}$  values are estimated from the dose-response curves.

Compounds with four redox centers, **161** and **162**, were more cytotoxic than their homologs with two and three redox centers. The most toxic compounds, **161**, **162**, **163**, **165** and **167**, possess a direct attachment of the selenium to the quinone. The exceptions were compounds **163** and **165** which have only two redox centers but were also among the most active compounds.



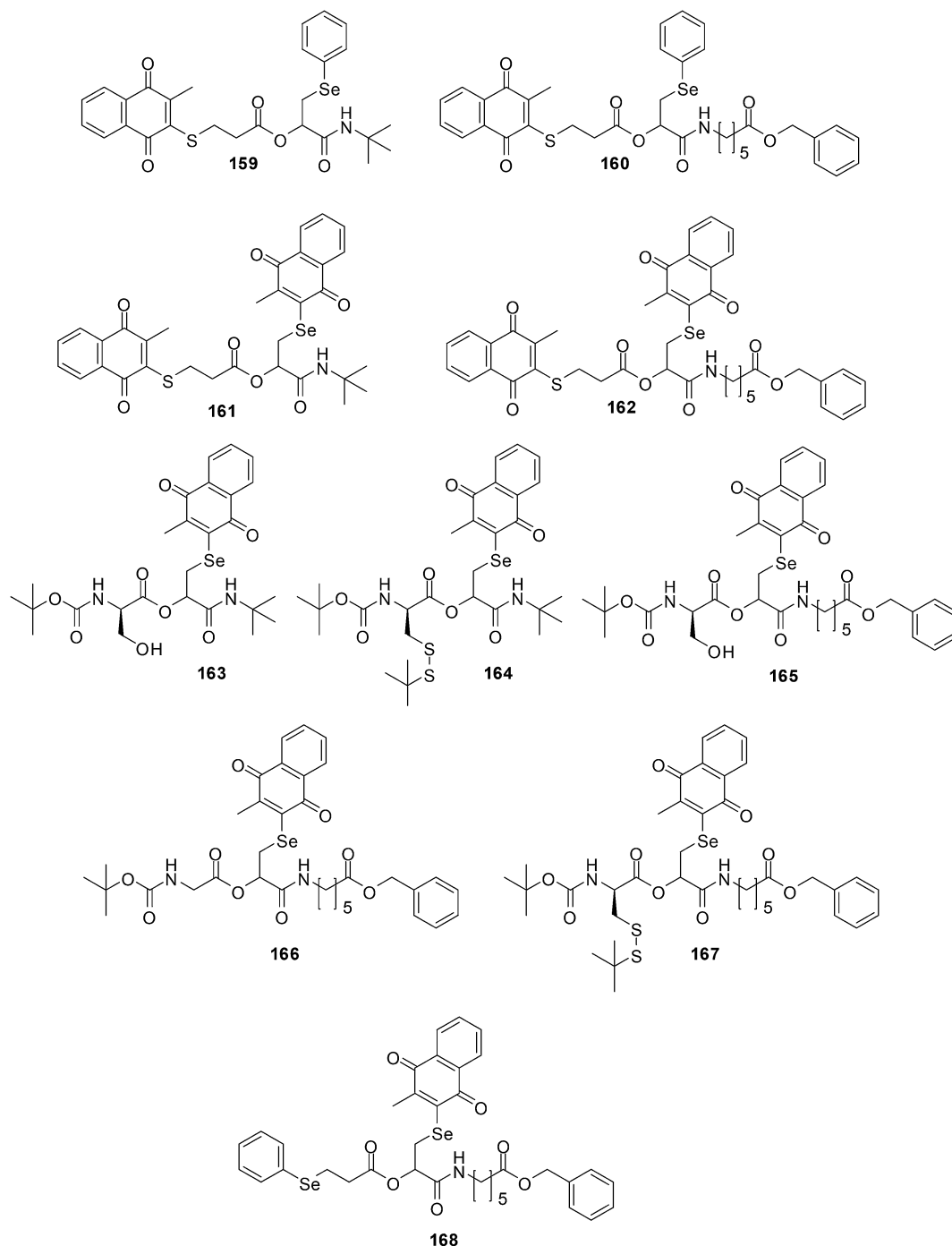


Fig. 35 The Passerini multicomponent reaction was used for the synthesis of di-, tri- and tetra-functional redox agents **159–168** containing multiple chalcogen and quinone redox sites.

The compounds were also evaluated for their toxicity against primary human fibroblasts (HF) and human umbilical vein endothelial cells (HUVEC). Only compounds **159**, **160**, **165** and **166** were non-toxic, most were toxic. Amongst the non-toxic compounds **165** clearly showed a lower cytotoxicity against both primary cells than against the three cancer cell-lines.

The mediation of oxidative stress by the production of the superoxide radical ( $O_2^{\cdot-}$ ) and hydrogen peroxide ( $H_2O_2$ ) were

also investigated. The DCF and DHE assays were used to determine ROS. It was found that the compounds were able to significantly increase the intracellular ROS levels in A-431 cells.

During cell cycle arrest there is an increase in ROS-induced DNA damage. A sub-lethal dose of a cytotoxic compound can prevent the progression of the cell cycle by causing a delay in G0/1. A significant delay of cell cycle progression was observed when MCF-7 cells were treated with **163**, **164** and **168** at their

respective  $IC_{50}$  concentrations for 24 h. In the G2/M phase there was a decrease in cells while in the G0/1 phase an increase in comparison to the negative control.

The ER stress-mediated apoptotic pathway or the mitochondrial-mediated apoptotic pathway or both pathways can be activated when a high level of ROS is present. These apoptotic pathways depend on the activation of caspases, particularly caspase-3 and -7, for the final execution of apoptosis. It was found that compound **168** (concentration = 2.8  $\mu$ M) strongly activated caspase-3/7 activity in A-431 cells after 24 h of treatment.

Mitochondrial dysfunction and induction of apoptosis are linked to low levels of glutathione (GSH), thus decreasing chemoresistance. The intracellular levels of reduced GSH were estimated using the DTNB assay. Compounds **163**, **164**, **166** and **168**, at a concentration of 5  $\mu$ M, caused a decrease of the intracellular GSH level in breast cancer (MCF-7) cells by 46%, 59%, 62%, and 76%, respectively.

This study has afforded interesting results and thus opens the door to a range of follow-up studies in the areas of synthetic chemistry (organoselenium and organotellurium compounds), redox chemistry, biochemistry and, ultimately, drug development. Future studies will have to focus on improving the selectivity of these compounds for cancer cells.

## 19. Pyranonaphthoquinones

AKT,<sup>262</sup> a serine/threonine kinase, is a key player in the signaling pathway that starts with phosphoinositide 3'-kinase (PI3K $\alpha$ ) activation. The latter generates phosphatidylinositol triphosphate (PIP3) which then binds and activates AKT. The phosphatase and tensin (PTEN) homologue, deleted on chromosome 10, dephosphorylates PIP3 and antagonizes AKT activation. Activated AKT has been found in a wide variety of human cancers and is often due to a mutational loss of PTEN activity. Its presence has been correlated with poor prognoses.<sup>263</sup>

AKT has been the focus of intense research. It has been the target of small molecule inhibitors such as allosteric site binders, ATP-competitive active site binders, peptide or peptidomimetic pseudosubstrates, and analogues or mimics of PIP3.<sup>264</sup>

The known natural product antibiotic, lactoquinomycin **169**, an amino-C-glycoside-pyranonaphthoquinone (PNQ) lactone (medermycin<sup>265</sup>) was found to inhibit AKT1 ( $IC_{50}$  = 149 nM) (Fig. 36). Kalafungin<sup>266</sup> **170**, frenolicin B **171**, and related PNQ lactones, were found to have similar inhibitory activity (Fig. 36).

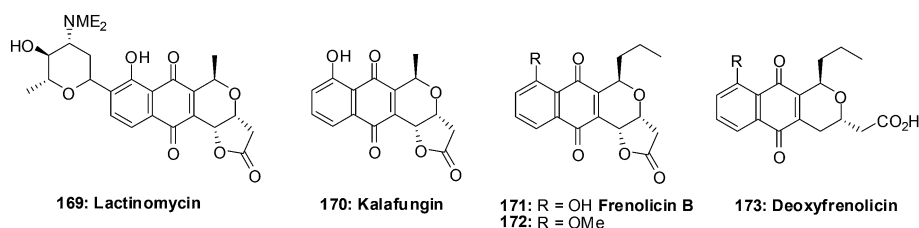


Fig. 36 Natural and semisynthetic PNQs **169**–**173** that inhibit AKT.

A series of synthetic PNQs were synthesised for a SAR study (Fig. 37).<sup>267</sup> Compounds were screened against the AKT and breast cancer (MDA468) cell-lines. The inhibition of AKT activity in cells was determined by the immunoblot assay of phosphorylation of downstream targets of AKT.

It was found that PNQ lactones inhibit the proliferation of breast cancer containing activated AKT and show expected effects on cellular biomarkers. Compounds **174**–**186** exhibited potent activity and were highly active against breast cancer ( $IC_{50}$  = 0.23–1.60  $\mu$ M). They also exhibited potent inhibitory activity against AKT ( $IC_{50}$  = 0.044–1.440  $\mu$ M).

From the results of the screening assays it was apparent that there was no clear correlation between the level of enzyme inhibition and the cell antiproliferative activity in the series of analogues. Compounds **185** and **186**, the least potent AKT inhibitors, had antiproliferative activities equal to or greater than some of the more active compounds.

A possible mechanism of action for AKT inhibition by the PNQs is the bioreductive alkylation mechanism. PNQ lactones, upon reduction *in vivo*, form a hydroquinone that can subsequently form a quinone methide which can then alkylate a nucleophilic site on a protein. Biochemical data supported the proposed bioreductive alkylation mechanism of action of a regulatory loop cysteine.

These pyranonaphthoquinone lactones are a highly selective new class of AKT kinase inhibitors.

## 20. Shikonin derivatives

The traditional oriental herb, *Lithospermium erythrorhizon*, is the source of the natural product, Shikonin, which is isolated from its roots (Fig. 38).<sup>268</sup> Significant cytotoxic activity has been

Compd	R	Stereo
<b>174</b>	H	(+)
<b>175</b>	2 thienyl	(+)- <i>anti</i>
<b>176</b>	CH <sub>2</sub> OBn	(+/-)- <i>anti</i>
<b>177</b>	4-hydroxyphenyl	(+/-)- <i>syn</i>
<b>178</b>	3 thienyl	(-)- <i>anti</i>
<b>179</b>	3 thienyl	(+)- <i>anti</i>
<b>180</b>	methyl	(+)- <i>anti</i>
<b>181</b>	<i>n</i> -propyl	(+/-)- <i>anti</i>
<b>182</b>	(CH <sub>2</sub> ) <sub>4</sub>	(+/-)
<b>183</b>	3-hydroxyphenyl	(+/-)- <i>syn</i>
<b>184</b>	<i>n</i> -propyl	(+/-)- <i>syn</i>
<b>185</b>	4-aminophenyl	(+/-)- <i>syn</i>
<b>186</b>	<i>gem</i> -dimethyl	(+/-)

Fig. 37 Pyranonaphthoquinone lactones **174**–**186** that inhibit AKT and exhibit anticancer activity.

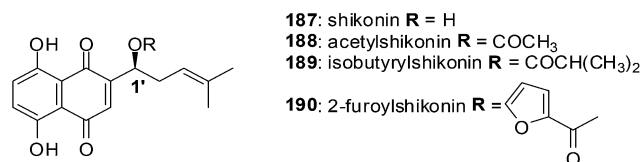
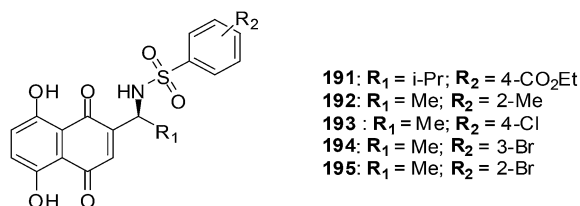
Fig. 38 Shikonin **187** and its derivatives **188–190**.

Fig. 39 Shikonin analogues.

exhibited against various cancers both *in vitro* and *in vivo* by Shikonin and its derivatives.<sup>269,270</sup>

For the mechanism of action the primary cause of cytotoxicity is the generation of ROS in which a semiquinone radical is believed to cause cell damage. Another plausible mechanism is bio-reductive alkylation which may also be the cause of their cytotoxic behavior.<sup>271</sup> Shikonin and its derivatives could also strongly inhibit both topoisomerase I and II, and induce DNA cleavage and cell apoptosis.<sup>272–274</sup> The acylated shikonin derivatives, acetylshikonin (**188**, Fig. 38) and isobutyrylshikonin (**189**, Fig. 38), have been reported to be more potent inhibitors of topoisomerases than shikonin.<sup>272</sup>

A novel series of shikonin analogues with various side chains have been synthesised in several synthesis steps (Fig. 39).<sup>275</sup> These compounds were evaluated for their anticancer activity against cervical carcinoma cells (HeLa) and promyelocytic leukemia cells (HL60) using the SRB and MTT assays. Most of the compounds exhibited better inhibitory effects ( $IC_{50} = 0.02\text{--}0.35\text{ }\mu\text{M}$ ) against HL60 than those of the positive controls (VP16:  $IC_{50} = 0.82\text{ }\mu\text{M}$ ; shikonin **187**:  $IC_{50} = 0.44\text{ }\mu\text{M}$ ).

Two compounds, **192** and **193**, were selected for further evaluation for inhibitory activity against breast cancer (MDA-MB-231), colon cancer (HCT116), lung cancer (A549), rhabdomyosarcoma (RH30), oral epidermoid cancer (KB-3-1 and KB/VCR), and leukemia (K562). Both compounds **191** and **192** exhibited potent inhibitory activity against these cancer cell-lines with the average  $IC_{50}$  value of  $8.6\text{ }\mu\text{M}$  and  $7.3\text{ }\mu\text{M}$ , respectively. In addition, **191** and **193** also showed significant activity against KB/VCR (the vincristine-selected multidrug-resistant subline) with  $IC_{50}$  of  $13.2\text{ }\mu\text{M}$  and  $12.4\text{ }\mu\text{M}$ , respectively.

Hypoxia is a common characteristic of cancer cells and the hypoxia-inducible factor  $1\alpha$  (HIF- $1\alpha$ ) is the most important transcript factor in response to intracellular oxygen pressure. It is enhanced to accumulate significantly in a hypoxic environment but under normoxia it is almost undetectable.

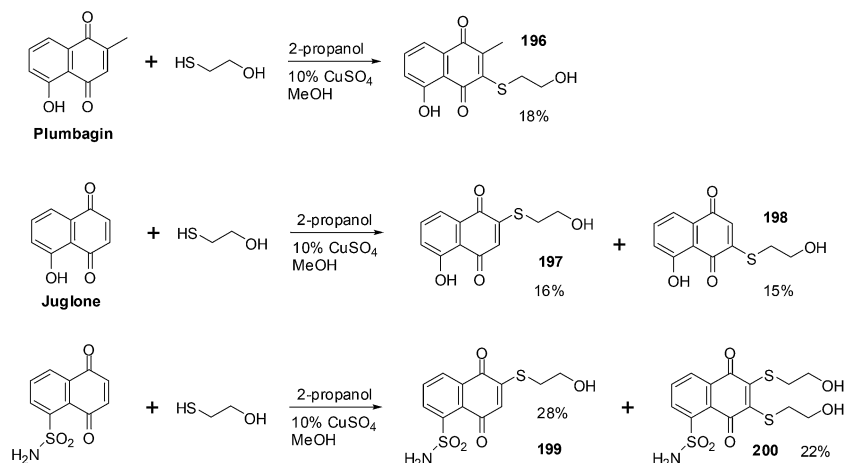
There is a correlation of HIF- $1\alpha$  with increased patient mortality in many different cancers such as brain, breast, cervix, colon, ovary and lung cancer. The overexpression of HIF- $1\alpha$  in human cancer cells increases tumour growth, angiogenesis and metastasis. HIF- $1\alpha$  is a validated therapeutic target.

Under hypoxic conditions compounds **192**, **193**, **194**, **195** and shikonin **187** reduced the expression level of HIF- $1\alpha$  in breast cancer cells MDA-MB-231. These results demonstrate the potential of these compounds for further development as cancer chemotherapeutics.

## 21. Thiolated naphthoquinones

Thiolated derivatives of menadione, juglone and 1,4-naphthoquinone were synthesised for a structure–activity study of their anticancer properties (Scheme 7).<sup>193</sup>

These compounds were screened against prostate (DU-145), breast (MDA-MB-231) and colon (HT-29) cancer cell-lines using the Promega CellTiter 96 Aqueous Non-Radioactive Cell Proliferation assay. Compounds **196**, **197**, **198** and **199** ( $IC_{50} = 1.6\text{--}9.7\text{ }\mu\text{M}$ ) exhibited potent activity against all three cancer cell-lines. Their antiproliferative activity was also better than that of the lead molecules. Inhibition of STAT3 dimerization

Scheme 7 Synthesis of mono- and dithiolated naphthoquinone derivatives **196–200**.

was studied using the Fluorescent polarization assay and it was found that **196** and **197** were potent STAT3 dimerization inhibitors.

## 22. Promising candidates

The tumour-selective cytotoxic properties of the natural product,  $\beta$ -Lapachone **84** (ARQ 761), resulted in its advancement into clinical trials. It is currently being assessed for its anti-tumour activity against advanced solid tumours.<sup>276</sup>

## Conclusions

The synthesised synthetic naphthoquinones and derivatives of natural naphthoquinones have a broad range of structural diversity and anticancer activities. These compounds have also exhibited inhibitory activity against MDR cancer cell-lines. Some of the compounds were also shown to inhibit enzymes such as topoisomerase I and II, STAT3 as well as AKT kinases.

The potent inhibitory activities coupled with low toxicities of some of these compounds have qualified them for further development as potential cancer chemotherapeutics. The results of these studies warrants continued investigation of the naphthoquinone class of compounds for the discovery of novel anticancer agents.

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