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Naphthoquinones' biological activities and toxicological effects

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ABSTRACT

Naphthoquinones are secondary metabolites widespread in nature, comprising a wide variety of chemical structures based on the naphthalene skeleton. They exhibit several substituents and may group together forming dimers, trimers and, more seldomly, tetramers. Naphtoquinones serve as important links in electron transport chains, participate in multiple oxidative processes, and may act as defensive compounds in interspecies chemical warfare. Thus, the biological and toxicological activities of naphthoquinones have been explored by the scientific community in an attempt to discover and develop new drugs. The known naphthoquinones' spectrum of activity includes antibiotic, antiviral, anti-inflammatory, and antiproliferative effects. Their toxicity and potential therapeutic activity depend on their ability to bind nucleophilic molecules or act as oxidizing agents. These abilities allow naphthoquinones to influence gene transcription as well as the activity of multiple enzymes and cellular signalling pathways.

This chapter encompasses a review of the different biological activities and toxicological effects reported for naphthoquinones, including an analysis of their structure-activity relationships.

Keywords: Naphthoquinone; flavoenzymes; vitamin k; antimicrobial; antioxidant; inflammation; antitumor.

CONTENTS

INTRODUCTION

Secondary metabolites are low molecular weight compounds derived from primary metabolites: carbohydrates, amino acids and lipids (Ting 1982). Secondary metabolites contrast with primary metabolites by having limited taxonomic distribution and no apparent direct role in the normal growth, development or reproduction of the plants in which they are found (Bernards 2010). However, secondary metabolites have important roles in defence strategies, in adaptation to new environments and for species survival, including antimicrobial, photoprotective, structure stabilizing and signalling properties. Thus, secondary metabolites are increasingly considered for medicinal, nutritional and cosmetic purposes, being a source of innovation in drug discovery (Edreva *et al*. 2008).

Secondary metabolites in plant species are mainly grouped by biosynthetic origin and structural features (Bernards 2010), with flavonoids, alkaloids, terpenes, coumarins, phenolic acids and quinones being relevant examples.

The quinoid structure is widespread in nature and the quinones encompass over 1200 naturally occurring compounds (Thomson 1987). Quinones have many roles in organisms, being functional constituents of biochemical systems (Babula *et al*. 2009). They play essential roles in mitochondrial respiration (e.g. ubiquinone) and in photosynthesis (e.g. plastoquinone), while other quinones have a defence role, inhibiting bacterial, fungal or parasite growth (Errante *et*

al. 2006; Kapadia *et al*. 2001; Park *et al*. 2005). Part of their properties result from conjugation of two carbonyl functions with the benzenic ring (benzoquinones) or of condensed polycyclic aromatic system: anthracene (anthraquinones) and naphthalene (naphthoquinones) (Bruneton 1999).

Naphthoquinones exist in different organisms, including plants (e.g. Ebenaceae), fungi (e.g. *Fusarium* spp.), lichens (e.g. *Cetraria* spp.), algae (*Landsburgia quercifolia*) and in actinomycetes (*Streptomyces* spp.) (Babula *et al*. 2009). At least three biosynthetic pathways have been reported to generate naphthoquinones in vascular plants. These include the acetate-malonate, the mevalonic and chorismic acids, and the *p*-hydroxybenzoic acid pathways (Bruneton 1999). The chemical structure of monomeric naphthoquinones is based on the naphthalene skeleton with carbonyl groups in positions C1 and C4 (1,4-naphthoquinones) or in C1 and C2 (1,2-naphthoquinones) (**Fig. 1**). In addition to a great variety of possible substituents groups, monomeric naphthoquinones may be joined together forming dimers, trimers and, more seldomly, tetramers (**Fig. 2**) (Babula *et al*. 2009).

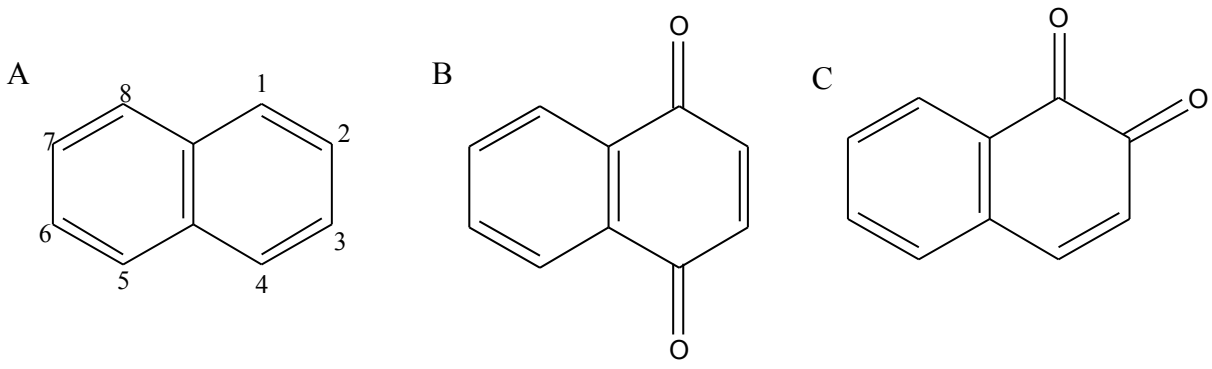

Fig. 1. Naphthalene structure (A) and basic structures of naphthoquinones: 1,4-naphthoquinone (B) and 1,2-naphthoquinone (C).

Naturally occurring napthoquinones play a key environmental role in the context of negative allelopathy (the inhibition of neighbouring organisms via secretion of germination or growth inhibitors). Allelopathy of naphthoquinones was first reported when Davis found that juglone (5-hydroxy-1,4-naphthoquinone) was responsible for growth inhibition of other vegetal species surrounding *Juglans nigra* (Davis 1928). Mechanisms underlying this negative allelopathy are the inhibition of *p*-hydroxyphenylpyruvate dioxygenase (the crucial enzyme in plastoquinone synthesis) and reduction of photosynthesis in leaf tissues (Babula *et al*. 2009; Hejl *et al*. 1993), reduction of H-ATPase activity, impairment of water reuptake by roots and inhibition of transpiration and stomatal conductance (Hejl and Koster 2004). Naphthoquinones are usually coloured and so they also play important roles in attracting pollinators (Babula *et al*. 2009). Their colours, which exist due to double bond conjugation, vary between yellow, orange and brown (Babula *et al*. 2009).

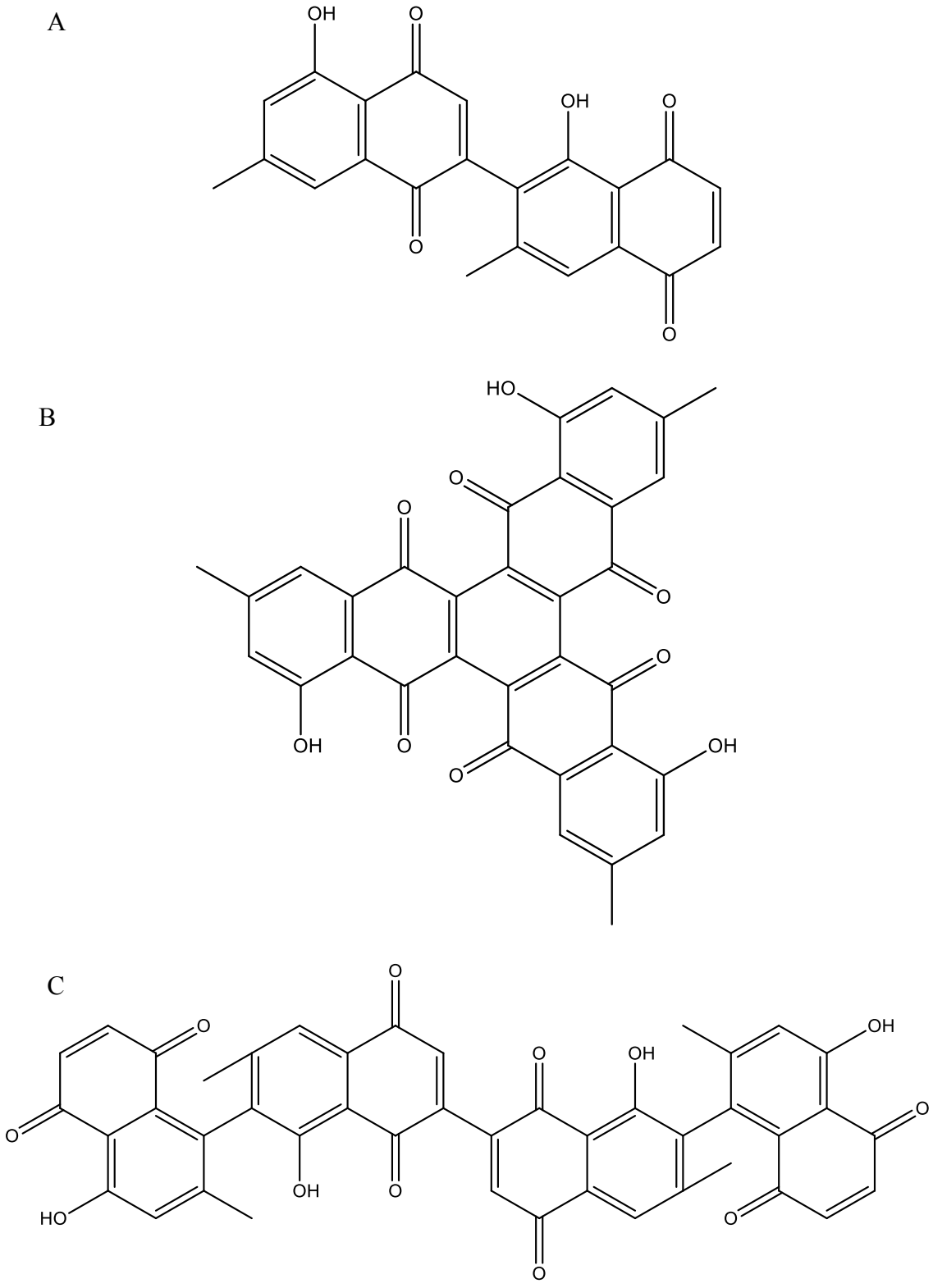

Fig. 2. Examples of non-monomeric naphthoquinones: diospyrin (dimer) (A), xilospyrin (trimer) (B) and bisisodiospyrin (tetramer) (C).

The vitamin K group, which includes menaquinones and phylloquinone, is fundamentally important for biological processes. Menaquinones are isoprenoid naphthoquinones with 2-methyl-1,4-naphthoquinone skeleton substituted at C3 by repeating unsaturated prenyl units (MK-*n*) side chain, where *n* represents the number of 5-carbon units (**Fig. 3A**). Menaquinones are synthesized by a variety of live organisms, comprising plants, bacteria and algae (Babula *et al*. 2009). Phylloquinone (**Fig. 3B**), also named vitamin K1, is produced by plants, while bacteria synthesize a spectrum of molecular forms of menaquinones (Shearer 1995). Since humans and other mammals are unable to synthesize essential isoprenoid naphthoquinones, these compounds must be supplied via food or obtained from symbiotic bacteria living in intestine (Shearer 1995). In mammals, vitamin K is an important cofactor for γ-glutamyl carboxilase, which catalyses the transformation of glutamate residues in γ-carboxyglutamate residues. This is required for coagulation since four procoagulants depend on vitamin K as cofactor (**Fig. 4**) (Babula *et al*. 2009). Although phylloquinone is a major dietary form of vitamin K, the MK-4 menaquinone is found in humans and rodents in concentrations exceeding that of phylloquinone in most of their tissues (Shearer 1995). Some authors defend that MK-4 is the most important vitamin K in humans, and that there may be a human metabolic pathway to derive MK-4 from phylloquinone (Booth and Suttie 1998). Vitamin K has also been reported to modulate cytokines (Nowicka and Kruk 2010), inhibit microsomal lipid peroxidation (Vervoort *et al*. 1997) and prevent neuronal cell death (Li *et al*. 2009).

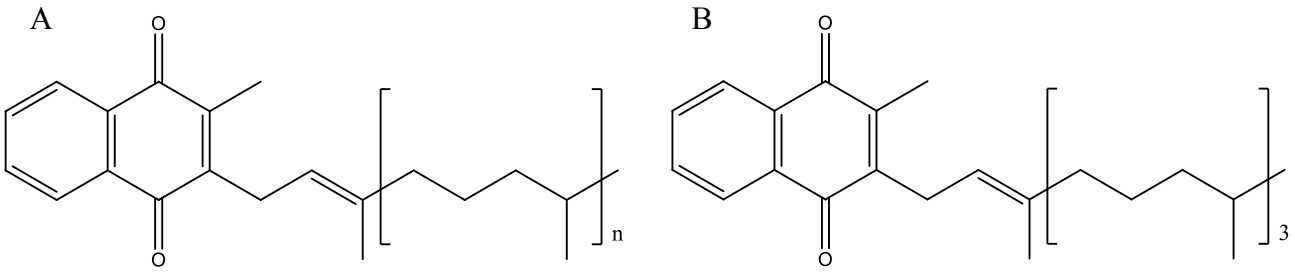

Fig. 3. Chemical structures of vitamin K: MK-*n* where *n* represents the number of 5-carbon units in the molecule (A) and phylloquinone (B).

BIOCHEMICAL IMPORTANCE OF NAPHTHOQUINONES

Most studies addressing the biological potential of naphthoquinones explain their activities via mechanisms of action based on naphthoquinones' pro-oxidant character. This character explains the interactions with nucleophilic biomolecules and formation of reactive oxygen species (ROS) due to naphthoquinones redox cycling (Klaus *et al*. 2010; Murakami *et al*. 2010). The main consequences of these actions are alterations in cell signalling, inhibition of several enzymes (**Table 1**), DNA alterations and reduced glutathione (GSH) depletion.

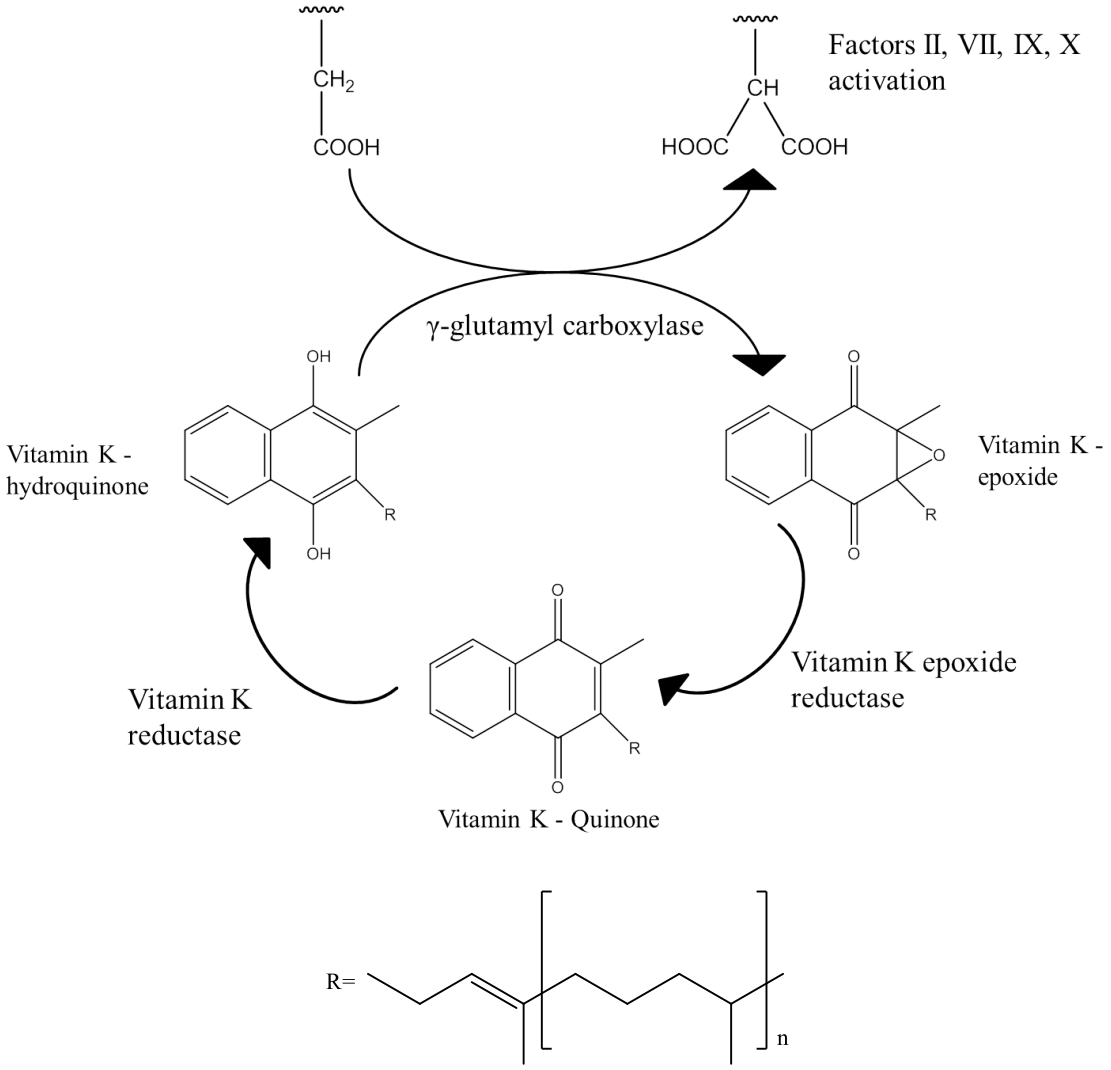

Fig. 4. Vitamin K-dependent carboxylation of coagulation factor precursors. *n* represents the number of 5-carbon units.

Naphthoquinones' pro-oxidant character

In cells, some naphthoquinones undergo redox cycling catalysed by flavoenzymes. Flavoenzymes are involved in a wide range of biological processes, having a central role in aerobic metabolism (Joostenn and van Berkel 2007). Flavoenzymes in the endoplasmic reticulum (NADH cytochrome b5 reductase and NADPH cytochrome c reductase) and mitochondria (NADH dehydrogenase and lipoyl dehydrogenase) catalyse a single electron quinone reduction, thus forming cytotoxic semiquinone radicals (O' Brien 1991). In the cytosol, xanthine oxidase and xanthine dehydrogenase catalyse a one or two-electron reduction of quinone (Nakamura and Yamazaki 1969) and NAD(P)H quinone-oxidoreductase (also called DT-diaphorase; a FAD-dependent flavoenzyme) catalyses only a two-electron reduction of quinones, directly forming hydroquinone (**Fig. 5**). Most semiquinones

formed by one-electron quinone reduction are readily reoxidized in aerobic conditions and can enter a redox cycle with molecular oxygen (O_2), forming superoxide anion radicals. Naphthohydroquinone may also undergo autoxidation, transferring one electron to O_2 with formation of semiquinone and superoxide (Buffinton *et al*. 1989). Once formed, superoxide anion radicals can originate other ROS, as hydrogen peroxide (H_2O_2) and hydroxyl radical. These ROS are powerful oxidising species and they are responsible for the damage caused by naphthoquinones to essential macromolecules in cells (O' Brien 1991). In most cases the reduction of naphthoquinones to hydroquinones is less deleterious to cells than the reduction to semiquinones, because hydroquinones are in general more stable than semiquinones. Therefore, DT-diaphorase has been regarded as a cellular protector against the oxidative stress induced by one-electron reductases (O'Brien 1991; Öllinger and Brunmark 1991). As example, dicoumarol, an inhibitor of DT-diaphorase, increased the loss of cell viability induced by menadione (2-methyl-1,4-naphthoquinone), nearly by 2-fold (Thor *et al*. 1982). However, other studies revealed that DT-diaphorase is an activator agent in some situations. DT-diaphorase substantially enhances the toxicity of *β*-lapachone in human cancer cell lineages overexpressing this enzyme because the hydroquinone resulting from the reduction of *β*-lapachone is unstable and prone to auto-oxidation back to *β*-lapachone, perpetuating a futile redox cycle. This phenomena leads to increased ROS generation, decreased cellular NAD(P)H, loss of Ca^{2+} homeostasis , and ATP depletion (Pink *et al*. 2000; Planchon *et al*. 2001; Tagliarino *et al*. 2001). Naphthoquinones' activities are regulated by detoxification processes, with different naphthoquinones being substrates with different affinities for each flavoenzyme. Also, the capacity of a naphthoquinone to produce ROS depends on several parameters, e.g. reduction potential of quinone, pKa (acid dissociation value) value of hydroquinone and stability constant of the semiquinone (Öllinger and Brunmark 1991). The reduction potential must be high enough to allow efficient reduction of the quinone by cellular reductases but not so high that it reduces the rate of electron transfer from the hydro- and semiquinone to O_2 (Ölinger and Brunmark 1991). Thus, naphthoquinones with a higher redox potential are, in general, considerably more toxic than others with lower redox potential (Bellomo *et al*. 1987). The pKa value of a hydroquinone influences the production of ROS because electron transfer is orders of magnitude higher for the anionic form of the reductant (Öllinger and Brunmark 1991), and thus a high pKa value prevents the autoxidation of the hydroquinone.

The introduction of electron-donating or electron-attracting groups in the benzene or quinone moieties determines the pro-oxidant properties of naphthoquinones (Murakami *et al*. 2010). The following order of autoxidation rates for naphthohydroquinones was reported by Buffinton and collaborators (1989): 5-hydroxy- > 5,8-dihydroxy- >2-methyl-5-hydroxy- > 2,3-dimethoxy- > unsubstituted > 2-methyl- > 2-hydroxy-. Although the introduction of electron-donating hydroxyl group decreases electrophilicity of the quinone moiety, hydroxy-1,4-naphthoquinone has a reduction potential similar to that of 1,4-naphthoquinone (Murakami *et al*. 2010). However, the semiquinone resulting from hydroxy-1,4-naphthoquinone reduction is more stable than the one arising from l,4-naphthoquinone, which may lead to a higher semiquinone concentration and consequently to higher rate of autoxidation (Öllinger and Brunmark 1991). Furthermore, the high autoxidation rate of naphthohydroquinones with a 5-hydroxy substituent has been attributed to an internal hydrogen bond between the 4- and 5-hydroxy groups or to a low pKa of the

naphthohydroquinone (Land *et al*. 1983). This high autoxidation rate explains why naphthoquinones with 5-hydroxyl group in the benzene moiety, such as juglone, shikonin and plumbagin, markedly stimulated lipid peroxidation (Murakami *et al*. 2010) and were highly toxic for hepatocytes (Öllinger and Brunmark 1991) and keratinocytes (Inbaraj and Chignell 2004; Klaus *et al*. 2010). In addition to naphthazarin having a high redox potential, its semiquinone radical anion is very liable, co-existing in tautomeric equilibrium with several isoforms (**Fig. 6**), which increases the steady state level of semiquinone and possibly increasing the efficiency of free radical production after enzymatic reduction (Moore and Scheuer 1966).

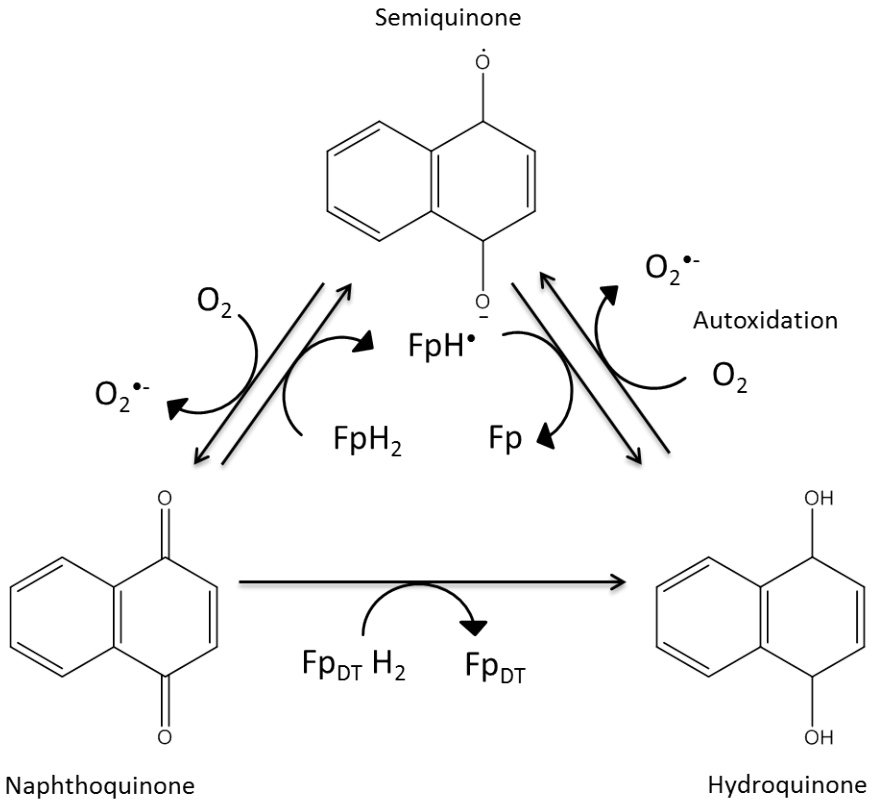

Fig. 5. Redox cycle of naphthoquinones. Flavoenzyme (Fp); DT-diaphorase (Fp_{DT}).

Naphthoquinones with 2-hydroxyl or 2-methyl groups in the quinone moiety, such as lawsone and lapachol, showed no stimulation of lipid peroxidation (Murakami *et al*. 2010). 2-Hydroxylation renders 1,4-naphthoquinones less active, because there is tautomerization of the C2/C3 enol structure, resulting in a saturated C3, no longer allowing for nucleophilic addition reactions in that position. Moreover, this tautomerization stabilizes the quinoid structure, resulting in a very low one-electron reduction potential, rendering one-electron reduction of the naphthoquinones less efficient (Öllinger and Brunmark 1991; Uchimyia and Stone 2009). Methylation at C2 similarly lowers reduction potentials, implying that redox cycling would become less efficient, as well as electrophilic addition (Öllinger and Brunmark 1991).

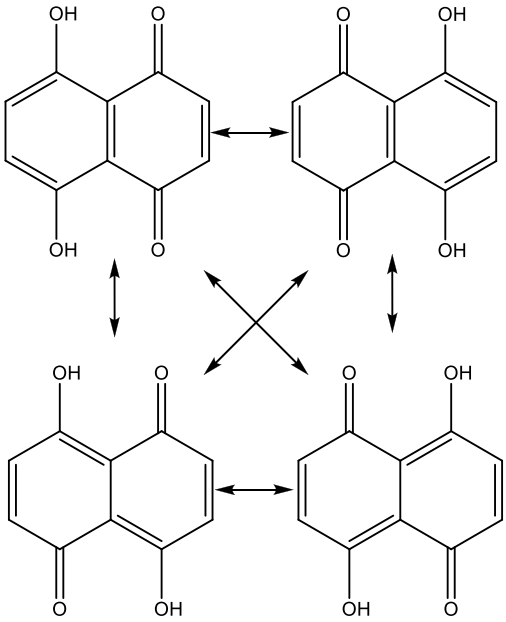

Fig. 6. Tautomeric equilibrium of naphthazarin.

Naphthoquinones with electron-donating groups have been used as a tool to generate oxidative stress in exposed cells and tissues, generating ROS by redox cycling and depleting GSH. The classical example is the use of menadione (Ross *et al*. 1985). Menadione toxicity may result from oxidation of intramitochondrial pyridine nucleotides by activity of NADPH and NADH oxidoreductases, which catalyse the reduction of menadione (Frei *et al*. 1986). More recently, Gerasimenko and collaborators (2002) showed that menadione induces partial mitochondrial depolarisation through induction of the permeability transition pore, which allows release of cytochrome c, leading to caspases activation and apoptosis.

Structural differences correlate with different cytotoxic action of naphthoquinones, e.g. juglone (5-hydroxy-1,4-naphthoquinone) causes cell death of some types of cells, whereas lawsone (2-hydroxy-1,4-napthoquinone) shows little or no cytotoxicity (Kumbhar *et al*. 1996). Although Klaus and collaborators defend that cytotoxicity is largely paralleled by several ROS formation (Klaus *et al*. 2010), diverse toxicological and pharmacological effects of naphthoquinones do not result from direct ROS formation, but are due to their electrophilic character which confer to naphthoquinones the ability to form covalent bonds with cellular nucleophiles, such as protein thiols and basic parts of DNA (Di Monte *et al.* 1984). These covalent bonds occur mainly with sulfhydryl groups, thus all proteins with high cysteine content are vulnerable to naphthoquinones (Fila *et al*. 2008). Therefore, naphthoquinones may directly inhibit proteins and constitute the cause of cell death (Paulsen and Ljungman 2005).

Naphthoquinones and cell signalling

The covalent bond established with macromolecules and the oxidant properties of naphthoquinones may induce alterations in cell signalling. The nuclear factor-κB (NF-κB) and p53 pathways alteration are the most documented. Paulsen and Ljungman discovered that juglone induces loss of p53 (Paulsen and Ljungman 2005), which is a tumour suppressor protein with a central role in multiple response pathways activated by DNA damage. Loss of p53 may result from the inhibition of p53 transcription by juglone (Paulsen and Ljungman 2005). However, Paulsen and Ljungman argue that the main mechanism of action is the oxidation of p53 thiols by juglone, which leads to crosslinking of p53 into aggregates and subsequent proteolytic degradation, since p53 is a redox-sensitive protein that is folded into an active conformation in a reducing environment (Paulsen and Ljungman 2005).

The suppression of constitutive and inducible NF-κB activation and consequent silencing of NF-κB regulated genes by naphthoquinones may explain several of their biological activities. NF-κB regulates several genes involved in cell proliferation (e.g. cyclin

D1 and cyclooxygenase-2 (COX-2)), antiapoptosis (e.g. survivin, inhibitors of apoptosis protein 1 and 2 (IAP1 and IAP2) and B-cell lymphoma-2 (Bcl-2) protein family), angiogenesis (e.g. vascular endothelial growth factor (VEGF)), and invasion (e.g. matrix metallopeptidase 9 (MMP-9)) (Sandur *et al*. 2006). Under resting conditions NF-κB is present in the cytoplasm of all cells as an inactive heterotrimer (the most common is p50, p65 and inhibitor of kB (IkBα)). IkBα is phosphorylated by the IκB kinase (IKK) complex and undergoes proteasommal degradation, allowing binding of NF-κB to specific DNA sequences (Brasier 2006). Plumbagin suppresses IKK activation, resulting in inhibition of IkBα phosphorylation and its degradation. Additionally, Sandur and collaborators argued that naphthoquinones inhibit the connection of p65 subunit of NF-κB to DNA (Sandur *et al*. 2006). These mechanisms of action may explain why some naphthoquinones are, in general, more cytotoxic to cancer vs. non-cancer cells. Various carcinogens and tumour promoters activate NF-κB and constitutive expression of NF-κB is frequently found in tumour cells. This transcription factor regulates several genes involved in tumour initiation, promotion, and metastasis and may induce resistance to chemotherapeutic agents and to radiation (Aggarwal 2004). The NF-κB suppression suggests that naphthoquinones may be effective not only in suppressing invasion, but also in inhibiting angiogenesis and inflammation.

DNA alteration by naphthoquinones

Naphthoquinones may induce DNA alteration by two processes: direct interaction with DNA and inhibition of regulatory enzymes, which have an essential role in DNA maintenance, as the DNA topoisomerases. All these actions are important for the mutagenic and genotoxic activities of naphthoquinones (Medina *et al*. 2008). Naphthoquinones may intercalate into the DNA base pairs by establishing electrostatic bonds (Huang *et al*. 2010). Furthermore, the pro-oxidant character of naphthoquinones allows the induction of direct oxidative damage in DNA (Medina *et al*. 2008).

DNA topoisomerases are enzymes that alter DNA conformation through a concerted breaking and re-joining of the DNA molecule, being involved in many critical functions of DNA (Wang 1985). The inhibition of topoisomerase enzymes induces DNA damage and initiates signalling cascades, including p53-dependent processes that control cell cycle progression and the decision on either DNA repair or apoptosis (Klaus *et al*. 2010). Topoisomerases I (Topo I) and II (Topo II) may be inhibited by naphthoquinones due to alkylation of thiol groups (Ahn *et al.* 1995; Wang *et al*. 2001). The potency of this inhibition depends on several factors, including electrophilicity, with juglone, 1,4-naphthoquinone and plumbagin being 10–100 times more potent than menadione and 1000–10,000 times more potent than lawsone or lapachol (Fujii *et al*. 1992; Wang 1985). Although the electrophilic attack appears to govern the inhibitory activity of topoisomerases, hydrogen bonding might also be involved, because naphthazarin, which is less electrophilic than 5,8-dimethoxy-1,4-naphthoquinone, is more active in inhibiting Topo-I than the last (You *et al*. 1998). Furthermore, the capacity for metal ions chelation by naphthohydroquinones seems to be important for their activity, inhibiting Topo I by binding to its zinc finger domain (Plyta *et al.* 1998).

Topo I is considered an essential target for the family of bisnaphthoquinoids, since isodiospyrin and diospyrin have a great Topo I inhibitory activity (Ray *et al*. 1998; Tazi *et al*. 2005; Ting *et al*. 2003). *β*-Lapachone was shown to inhibit Topo I (Li *et al*. 1993) by blocking the

formation of the Topo I–DNA cleavable complex, a mechanism of action different from that of camptothecin. Other works were developed in order to find a structure with a greater capacity of inhibiting Topo-I, and Plyta and collaborators defend that naphthoquinones bearing at least one phenolic hydroxyl group, as juglone, are potent inhibitors of Topo I (Plyta *et al*. 1998). Shikonin is another antineoplasic compound, whose activity can be related to Topo I inhibition. Structure–activity relationship studies indicated that shikonin analogues with acyl side chain lengths of C2–C6 exerted a strong inhibitory action in Topo I and this activity was potentiated by the presence of double bonds at C5–C7 (Ahn *et al.* 1995).

Salvicine (**Fig. 7**), a diterpenoid naphthoquinone obtained by structural modification of natural compounds, as ferruginol, isolated from *Salvia prionitis* Hance (Cheng *et al*. 2001) is a promising Topo II inhibitor (Meng and Ding 2007). Salvicine acts by multiple mechanisms: binding to the ATPase domain of Topo II, promoting DNA-Topo II binding, inhibiting Topo II mediated DNA relegation and ATP hydrolysis (Meng and Ding 2007). Menadione was also reported to inhibit Topo II (Wang *et al*. 2001).

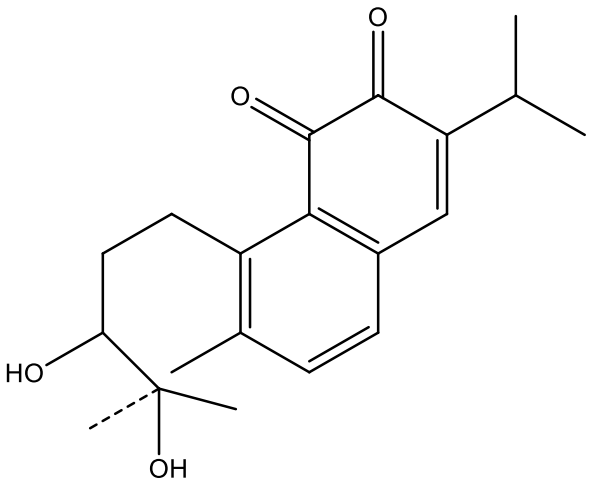

Fig. 7. Chemical structure of salvicine, a diterpenoid naphthoquinone with Topo II inhibitory activity.

Glutathione depletion by naphthoquinones

Naphthoquinones may induce GSH depletion by two ways: by GSH oxidation and by formation of naphthoquinone-GSH adducts (Pritsos *et al*. 1982; Ross *et al*. 1985). In the presence of naphthoquinones and excess GSH, extensive GSH oxidation to oxidized glutathione (GSSG) occurs due to H_2O_2 formed by dismutation of the superoxide radicals (**Fig. 8**) (Ross *et al*. 1985). Once GSH is depleted, cellular macromolecules are alkylated (Hoffmann *et al*. 1985). Gluthatione peroxidase, which metabolises H_2O_2 to H_2O in the presence of gluthatione (Brigelius-Flohé and Kipp 2009), and glutathione reductase, which regenerates GSH from GSSG (Bellomo *et al*. 1987), are important enzymes in avoiding naphthoquinone induced damage. In the work by Öllinger and Brunmark (1991), naphthazarin, among several hydroxyl substituted 1,4-naphthoquinones, was the quinone that most efficiently caused GSH oxidation and loss of total glutathione, suggesting that this compound is efficient in promoting redox cycling.

The chemistry of GSH addition to 1,4-naphthoquinones is simple, because the adjacent benzene ring limits the nucleophilic addition to one side of the quinoid ring. Most 1,4-naphthoquinones form GSH conjugates at the C3 position. One exception is juglone, which forms adduct at C2. The latter probably occurs because a hydrogen bond between the 5-hydroxyl group and the carbonyl oxygen at C4 makes 5-hydroxyl group an electron acceptor, so that the C2 position becomes the preferential site for nucleophilic attack (O'Brien 1991). 2,3-Dichloronaphthoquinone, like 2-hydroxy-1,4-naphthoquinone, is an example of a compound which rapidly forms GSH conjugates, being involved in protein alkylation and not in oxidative stress (Pritsos *et al*. 1982). It is also

known that menadione reacts rapidly with GSH, depleting cellular GSH and elevating intracellular Ca^{2+}. This increase of intracellular Ca^{2+} induces activation of Ca^{2+} and Mg^{2+}-dependent endonuclease, resulting in DNA damage and cell death (Chiou *et al*. 1997).

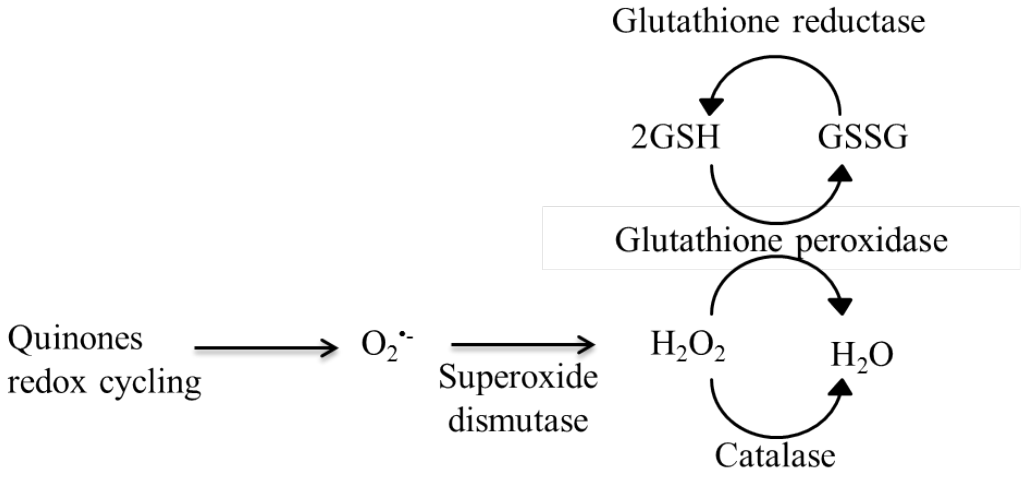

Fig. 8. Involvement of glutathione reductase and glutathione peroxidase in detoxification of ROS.

BIOLOGICAL PROPERTIES AND HEALTH EFFECTS

Naphthoquinones, as other secondary metabolites, are widely recognised by the pharmaceutical industry for their remarkable structural diversity and wide range of pharmacological activities. Some of these structures represent interesting pharmacophores, which make them excellent candidates for building blocks for biologically relevant chemical libraries (Horton *et al*. 2003). Several biological properties of naphthoquinones are known and many plants containing these compounds have been employed in folk medicine for treatment of diverse diseases (Blumenthal 1998; Hegnauer 1966): extracts derived from black walnut trees are used in the treatment of acne, inflammatory diseases and for hair dying (Blumenthal 1998); decoction of *Diospyros tricolor* has been used for leprosy, dysentery and diarrhoea (Hegnauer 1966); roots of *Plumbago zeylanica*, a major source of plumbagin, have been used in the Indian medicine, as an antiatherogenic, cardiotonic, hepatoprotective, and neuroprotective agent (Sandur *et al*. 2006).

Antimicrobial activity

Over time, microorganisms have developed resistance to several antimicrobial agents currently used in therapeutics, prompting the the search of new effective antimicrobials. There are several works documenting the activity of a variety of naphthoquinones against an array of microorganisms, including bacteria, fungi, parasites and viruses.

Antibacterial Activity

Naphthoquinones are compounds with activity against pathogenic bacteria afflicting humans and for which there is a need of new effective

compounds. Examples include *Helicobacter pylori, Clostridium paraputrificum, Clostridium perfringes, Streptococcus pyogenes*, *Bacillus subtilis*, *Mycobacterium chelonae, Mycobacterium tuberculosis* and *Neisseria gonorrhoeae.* Several extracts containing naphthoquinones exert antibacterial activity, which was attributed to the presence of these compounds. As an example, the ethyl acetate extract of *P. zeylanica* L. and the methanol extract of *Tabebuia impetiginosa* Martius exhibited activity against *H. pylori*, a bacterium strongly associated with gastric cancer and peptic ulceration (Park *et al*. 2006; Wang and Huang 2005). The activity of naphthoquinones was compared with that of tetracycline, metronidazole and amoxicillin and it was found that the methyl group in 2 position, as in menadione and in plumbagin, is important for activity, contrarily to the hydroxyl in the same position, as in lawsone, which was less active against this bacteria (Park *et al*. 2006).

T. impetiginosa extract, whose main napthoquinone is lapachol, also showed selective inhibitory activity against several human intestinal bacteria: strong activity for the harmful bacteria *C. paraputrificum* and *C. perfringes*, moderate effect against *Escherchia coli* and weak to moderate activity against the intestinal beneficial bacteria *Bifidobacterium longum*, *Lactobacillus acidophilus* and *Lactobacillus casei.* Thus, lapachol, by its activity toward harmful bacteria combined with almost no growth effects on lactic acid-producing bacteria, could be useful as a new preventive agent against various diseases caused by harmful intestinal bacteria (Park *et al*. 2005).

Furthermore, naphthoquinones have activity against other important human pathogens, like *M. tuberculosis*, as observed for the methanol and dichloromethane extracts of *Diospyros* spp. and its constituintes diospyrone, crassiflorone, plumbagin and diospyrin (Kuete *et al*. 2009; Theerachayanan *et al*. 2007). Lapachol, which can be obtained from *Tabebuia avellanedae* is also active against mycobacteria (Oliveira *et al*. 2010). Diospyrone also exhibited activity against *Neisseria gonorrhoeae* (Kuete *et al*. 2009).

Naphthoquinones may thus prove useful for the development of new antibiotics, since some of them are active at concentrations similar to those of reference drugs. Isodiospyrin is effective against *S. pyogenes*, *B. subtilis* and *M. chelonae* at a range of concentrations identical to those of antibiotics like gentamicin, ampicillin, chloramphenicol, penicillin, streptomycin or erythromycin (Adeniyi *et al*. 2000).

Antifungal activity

Antifungal chemotherapy is in constant need of new and effective compounds, due to the variable efficacy and adverse effects of the drugs currently used, plus the rapid evolution of pathogen resistance and even cross resistance (Eilenberg *et al*. 2010). Systemic fungal infections are one of the serious causes of mortality in Human Immunodeficiency Virus (HIV) infections and the emergence of multiresistance strains is a significant problem (Errante *et al*. 2006).

Candida spp., mainly *Candida albicans*, are among the most significant causes of nosocomial infections (Breger *et al*. 2007). Shikonin and its derivatives revealed to be active against this pathogen, being shikonin more effective than fluconazol (Sasaki *et al*. 2002). Plumbagin is also interesting as antifungic, as it has a spectrum of activity as large as that of ketoconazole, a reference antifungal, showing activity against *Candida* spp. and several filamentous fungi (*Aspergillus* spp. *Cladosporium* spp, *Fusarium* spp, and *Penicillium* sp.) (Dzoyem *et al*. 2007).

In the search of new antifungal agents, Errante and collaborators (2006) introduced arylthiols in naphthoquinones and arylthiols or

arylamines in sulfoxide-naphthoquinones and these compounds derivatives were more active than amphotericin B against *Candida* spp., *Aspergillus* spp., *Fusarium oxysporum* and *Trychophyton* spp.. Other authors defend that 3-arylamino-5-methoxynaphthalene-1,4-diones are the most promising antifungal naphthoquinones (Ryu and Chae 2005).

Interestingly, naphthoquinones may also be synthesized by fungi, having an important role in their phytopathogenicity (Medentsev and Akimenko 1998). In addition, when growing conditons are not favourable fungi also synthesize naphthoquinones to slow or even arrest their growth (Medentsev and Akimenko 1998).

Antiparasitic activity

Naphthoquinones have activity against several parasites, including *Leishmania* spp., *Schistosoma* spp. *Tripanosoma cruzi*, apicomplexans parasites, as *Plasmodium falciparum*, *Babesia equi*, *Toxoplasma gondii* and *Theileria* spp. The mechanism of action of naphthoquinones is common to all apicomplexans parasites: they inhibit cytochrome-containing electron transport chain, by interference with cytochrome c reductase component (complex III), blocking mitochondrial oxidation of ubiquinol. The inhibition of the dihydroorotate dehydrogenase step of *de novo* pyrimidine biosynthesis also occurs. These actions lead to mitochondrial membrane potential collapse and nucleic acid synthesis disruption (Combs 1999; Fry and Pudney 1992).

Malaria is a devastating disease, which affects many people in the world, mainly in poor tropical countries. Although several antimalarial drugs are available, their efficacy has been limited by rapid development of resistant strains of parasites, especially *P. falciparum*, during the last 30 years (White 1999). Atovaquone (**Fig. 9A**), which was first developed for the treatment of *Pneumocystis carinii* pneumonia and toxoplasmosis (McKeage and Scott 2003), is a naphthoquinone with important antimalarial activity. As atovaquone acts by a mechanism different from that of other antimalarials, resistance to atovaquone was considered unlikely (Basco *et al*. 1995). Nevertheless, single point mutations in cytochrome *b* gene of *P. falciparum* have already been identified, which confer a marked reduction in susceptibility to atovaquone. Nowadays, atovaquone is used in association with proguanil as antimalarial, reducing the chance of mutant parasite to survive (White 1999). Atovaquone and proguanil, an inhibitor of dihydrofolate reductase, have synergistic effects (Canfield *et al*. 1995), acting in pre-erythrocytic (hepatic) stages of *P. falciparum* and thereby providing causal prophylaxis (McKeage and Scott 2003).

After atovaquone discovery, several naphthoquinones had been studied in order to assess their antimalarial activity, namely aminonaphthoquinones (Kapadia *et al*. 2001) and bisnaphthoquinones (Theerachayanan *et al*. 2007). Aminonaphthoquinones showed promising antimalarial activity, with primary amino group revealing to be of great importance. Kapadia and collaborators (2001) showed that introduction of a primary amino group increased antimalarial activity of 1,2-naphthoquinone three-fold. Interestingly, introduction of a hydroxyl group into the quinone ring or aryl ring seems to be detrimental to antimalarial activity. However, the introduction of another hydroxyl group in *para* position relatively to the existing hydroxyl in the aryl ring, as in naphthazarin, increases the activity by five-fold as compared to juglone (Kapadia *et al*. 2001). 2-Amino-3-chloro-1,4-naphthoquinone, a relatively nontoxic compound used as herbicide, is a potential lead for the design and development of a novel class of antimalarials, because it has a strong activity toward the chloroquine- resistant strain of *P.*

falciparum and is more active than quinine against cloroquine, pyrimethamine and sulfadoxine-resistant parasite (Kapadia *et al*. 2001).

Menoctone (**Fig. 9B**), a hydroxynaphthoquinone with structural similarities with atovaquone, has great activity against *Theileria parva* and *Theileria annulata*, being more active than methotrexate. However, menoctone manufacture is prohibitively difficult (McHardy 1978). Several hydroxynaphthoquinones were developed after anti-theilerial success with menoctone. Buparvaquone (**Fig. 9C**) and parvaquone (**Fig. 9D**) are compounds with activity against theileriose, being the first 20 times more active than the last (McHardy *et al*. 1985). Buparvaquone also has antileishmanial activity against *Leishmania donovani* (Garnier *et al*. 2007), and a phosphate prodrug was developed to counteract its low aqueous solubility (Mäntyla *et al*. 2004). Buparvaquone and atorvaquone are also active against many other parasites, as *B. equi* (Kuttler *et al*. 1987; Zaugg and Lane 1989).

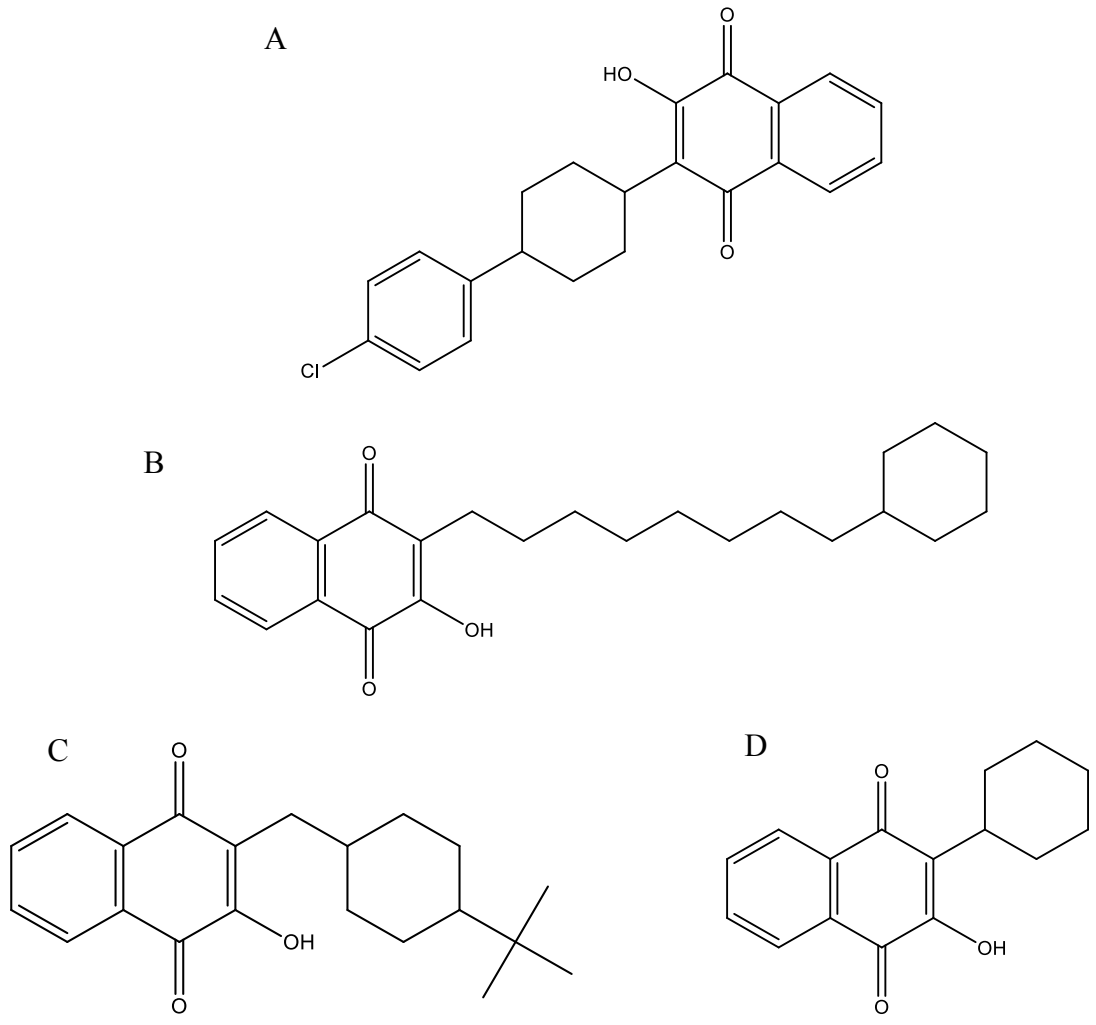

Fig. 9. Some naphthoquinones with antimalarial activity: atovaquone (A), menoctone (B), buparvaquone (C) and parvaquone (D).

Naphthoquinones may be important prophylactic agents in preventing *Schistosoma mansoni* cercarial skin penetration after topical application. Lapachol has great activity against

this parasite, probably due to the 5-carbon atom side chain, which turns the molecule more liposoluble (Pinto *et al*. 1977). Naphthoquinones have activity against *Tripanosoma cruzi*, by inhibition of a parasite-specific flavoenzyme trypanothione reductase (TR) (Krauth-Siegel and Comini 2008). This enzyme has 40% homology with the analogous human glutathione reductase, although their active sites are sufficiently different to allow the development of selective TR inhibitors. The first reported group of TR inhibitors were the naphthoquinones and nitrofuranes, named "subversive substrates" due to the induced futile-cycling of the enzyme. Thus, such substrates prevent the enzyme from reducing trypanothione disulfide, which is its physiological substrate (Henderson *et al*. 1988). Other naphthoquinones displayed TR selectivity, namely 3,3'-[polyaminobis(carbonylalkyl)]-bis(1,4-naphthoquinones), which showed potent *in vitro* activity against *T. cruzi* epimastigotes, without human glutathione reductase toxicity (Pinto and de Castro 2009). The increased lipophilicity by addition of a furan moiety, methoxyl group or an aliphatic side chain, led to an increase in the trypanocidal activity. Conceivably, increased lipophilicity allows for better penetration of the compound through the plasma membrane of the parasite (Pinto and de Castro 2009).

β-Lapachone is another naphthoquinone with activity against *T. cruzi*. *β*-lapachone induces the rearrangement of the chromatin into patches, alterations of the nuclear and cytoplasmic membranes, mitochondrial swelling and inhibition of DNA, RNA and protein synthesis, by release of superoxide anion radical and H_2O_2 (Goijman and Stoppani 1985; Pinto and de Castro 2009). Unfortunately, *β*-lapachone is inactivated either by reduction in the presence of oxyhaemoglobin or by interaction with serum proteins (due to interaction of the quinoidal moiety with free basic NH_2 residues of proteins) (Lopes *et al*. 1978). In order to solve this problem, a *β*-lapachone derivative was synthesized, the allyl-β-lapachone, being considered a potential chemoprophylactic agent for use in blood banks (Pinto *et al*. 1987). Plumbagin is another potential chemoprophylatic agent, as it leads to the total lysis of bloodstream trypomastigotes at a concentration similar to that of crystal violet, the standard drug recommended for the chemoprophylaxis of banked blood (Arias *et al*. 1994).

Antiviral activity

Naphthoquinones were reported to possess antiviral activity, mainly by inhibition of essential proteins for virus survival. Schuerch and Wehrli (1978) reported that *β*-lapachone inhibited reverse transcriptase from myeloblastus virus and Rauscher murine leukaemia virus, although without selectivity, since *β*-lapachone also inhibited eukaryotic DNA polymerase. The effect in both enzymes was related with similar exposed thiol groups in their actives sites.

Naphthoquinones significantly inhibit RNase H activity, an enzyme essential to HIV replication. This activity is higher for non-substituted 1,4-naphthoquinone, while hydroxylation decreases the activity (Min *et al*. 2002). Conocurvone (**Fig. 10**), a natural compound with several naphthoquinones moieties, inhibits HIV integrase and HIV mediated cell fusion. However, due to its high lipophilicity, conocurvone presents low solubility in water. Thus, Crosby and collaborators synthesised trimeric naphthoquinones, which maintained the activity of conocurvone. However, these compounds may have their therapeutic use limited due to their low stomach permeability (Crosby *et al*. 2010).

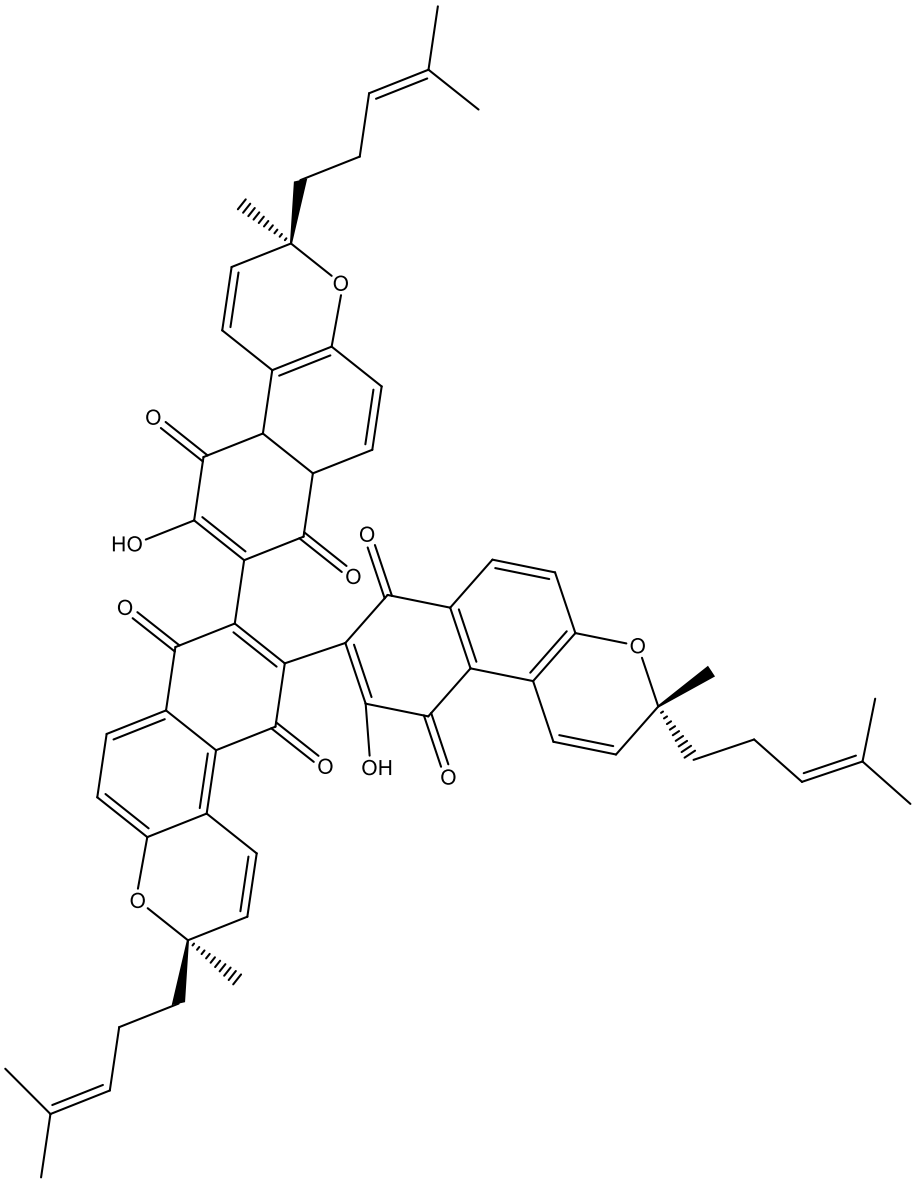

Fig. 10. Chemical structure of conocurvone, a lead compound for development of anti-HIV compounds.

Interaction with immune system and inflammatory process

Naphthoquinones have an active role in the modulation of immune system, mainly by interference with lymphocyte activity, and in the inflammatory process, for which several mechanism of action are known. These biological activities could be beneficial for the treatment of inflammatory, autoimmune and vascular diseases.

For instance, plumbagin revealed immunosuppressive effects in mice, protecting them from lethal Graft-versus-host disease. This results from the inhibition of lymphocytes entry into the S phase of the cell cycle, having an antiproliferative effect. Furthermore, the suppression of T cell activation, proliferation and cytokine production by plumbagin was ascribed to its ability to inhibit the NF-κB activation pathway (Checker *et al*. 2009). Modulation of NF-κB activation pathway has

been shown to have therapeutic potential in the prevention of graft rejection (Vodanovic-Jankovic *et al*. 2006).

Juglone influences cell growth and T cell proliferation by its inhibitory activity on *n*-type voltage-gated K^+ channels in the plasma membrane of human lymphocytes (Gallin 1986; Varga *et al*. 1996) and by its inhibitory action on Pin-1. Pin-1 is a protein that influences type I immune response by modulating the production of T cell cytokines, including INF-γ and IL-2. Furthermore, Pin-1 inhibition has synergistic effects with calcineurin inhibitors (Esnault *et al*. 2007).

In addition to immunosuppressive action, naphthoquinones may also directly interfere with the inflammatory process (Checker *et al*. 2009). Shikonin and its derivatives have been extensively studied as anti-inflammatory drugs. They inhibit cyclooxygenase-2 expression (Subbaramaiah *et al.* 2001), mast cell degranulation *in vitro,* phospholipase C activity (Wang and Kuo 1997) and superoxide generating activity of polymorphonuclear leukocytes by inhibition of formation of the NADPH oxidase complex needed for the activation of the respiratory burst in these cells. Thus, shikonin and its derivatives protect the tissues from injury caused by ROS produced by polymorphonuclear leukocytes (Kawakami *et al.* 1996). The use of shikonin on established collagen-induced arthritis, as anti-inflammatory and immunomodulatory, has been described (Dai *et al*. 2009).

Several studies have been performed in order to evaluate the effect of naphthoquinones on nitric oxide (NO) production (Cheng *et al*. 2008). Naphthoquinones with a pyran ring ((-)-isoeleutherin and (+)-eleutherin) and a lactone ring ((-)-hongconin, (+)-dihydroeleutherinol and eleutherinol) reduce NO production in LPS-activated mouse macrophage RAW 264.7 cells (Han *et al*. 2008). Liu *et al.* reported that *β*-lapachone, another naphthoquinone with a pyran ring, showed anti-inflammatory activity, by inhibition of expression and function of inducible nitric oxide synthase (iNOS) in rat alveolar macrophages and in aortic rings (Liu *et al*. 1999). Similar to *β*-lapachone, α-benzoylamino-1,4-naphthoquinone (PPM-18), a chemically synthesized naphthoquinone, inhibits the expression of iNOS *via* inhibition of the activation of NF-κB (Yu *et al*. 1997). This mechanism of action has been also proposed for other naphthoquinone, as 1,2-naphthoquinones, which reduce iNOS-catalyzed NO production by disruption of IKKβ/NF-κB signalling (Sumi *et al*. 2010). However, naphthoquinones can also inhibit constitutive forms of NOS, like endothelial nitric oxide synthase (eNOS), as will be discussed in the toxicological effects section.

Another aspect of anti-inflammatory activity of naphthoquinones is related with their effects on platelet aggregation (Ko *et al*. 1990). In the work by Kuke and collaborators (1998) isodiospyrin was the most effective compound in preventing tetradecanoyl phorbol acetate (TPA)-induced platelet aggregation, among other binaphthoquinones. This action resulted form the involvement of naphthoquinones with protein kinase C. Kuke and collaborators defend that at least one unsubstituted quinoid ring is required for maximum activity, as in diospyrin or isodiospyrin (Kuke *et al*.1998). Another mechanism proposed for anti-platelet activity of naphthoquinones is the suppression of arachidonic acid and collagen release, by suppression of phosphorylated extracellular signal regulated kinase (ERK1/2) and mitogen activated protein kinase (MAPK) activation (Son *et al*. 2006). This mechanism was proposed by Son and collaborators after verifying that chloroform fractions of methanol extract of *T. impetiginosa* inhibited platelet aggregation and vascular smooth muscle cells (VSMC) proliferation *in vitro* (Son *et al*. 2006). Thus, naphthoquinones may have cardiovascular benefits, which were confirmed by Anufriev and

collaborators. These authors reported that naphthazarin and their hydroxyl derivatives have cardioactive effects under *in vivo* ischemic-reperfusion, reducing the infarction zone by 50% without detectable adverse effects (Anufriev *et al*. 1998).

Several *in vivo* studies confirmed the anti-inflammatory activity of some naphthoquinones described *in vitro*. Shikonin and alkannin inhibited the increase of capillary permeability and thermal oedema in rats (Tanaka *et al*. 1986). This anti-oedematous response is mainly due to the suppression of mast cell degranulation, leading to protection of the vasculature (Wang *et al.* 1995).

Naphthoquinones as antipsoriatic agents

Several quinones are included in cosmetics or colorants applied in skin, as henna dyes and walnut extracts (Klaus *et al*. 2010). The effects of naphthoquinones in skin cells opened another new application for these compounds, as antipsoriatic agents. The toxicity of plumbagin, juglone and *β*-lapachone against HaCat keratinocytes, a transformed epidermal human cell line, suggests that topical preparations containing these naphthoquinones should be used with care, because they may damage the skin (Inbaraj and Chignell 2004; Müller *et al*. 1999). However, the activity of these compounds in inhibiting human keratinocytes growth makes them good candidates for further development in psoriasis treatment, with the activity of *β*-lapachone being similar to the antipsoriatic drug anthralin (Müller *et al*. 1999).

Antioxidant activity

Although redox cycling of naphthoquinones confer them a pro-oxidant behaviour, some authors defend that naphthoquinones may also have antioxidant activity. Gao and collaborators showed that *β*-alkannin exerts strong antioxidant activity against various types of ROS, having a high anti-lipid peroxidative ability. *β*-alkanin reacts directly with ROS, as superoxide radical and *tert*-butyl peroxyl radical, and it has higher reactivity towards singlet oxygen and superoxide radical than either ascorbic acid or α-tocopherol (Gao *et al*. 2000). Similar conclusions were taken concerning the extract of *P. zeylanica* and plumbagin (Tilak *et al*. 2004). Shikonin, a compound related to *β*-alkanin, has been proposed as antioxidant for cigarette filters, due to its effectiveness against a broad range of ROS, as peroxyradicals, heat resistance and nonvolatility (Nishizawa *et al*. 2005).

Elingold and collaborators defend that antioxidant activity of naphthofuranquinone results from the quinone reduction by NADPH-cytochrome P450 reductase and consequent semiquinone oxidation by molecular oxygen. These reactions divert electrons, preventing oxidative damage, as microsomal lipid peroxidation (Elingold *et al*. 2009). Beyond this, naphthoquinones have the ability of iron chelating, thus reducing liposome peroxidation induced by metals (Lebedev *et al*. 2008).

Anti-amyloidogenic activity

Naphthoquinones have potential as lead compounds for prevention of amyloid aggregation and neurotoxicity in Alzheimer's disease. Currently there are still few studies with naphthoquinones in this area. However, a study of Bermejo-Bescós and collaborators showed that naphthoquinones inhibit *β*-secretase. This enzyme plays a crucial role in the rate-limiting step of the amyloid cascade, so its inhibition reduces *β*-amyloid peptide aggregation (Bermejo-Bescós *et al*. 2010).

Antitumoral activity

Antitumoral activity is exhibited predominantly by three main groups of naturally occurring

quinones: benzoquinones, naphthoquinones and anthraquinones (Nohl *et al*. 1986). Particularly, naphthoquinones have been studied as antitumoral against several cell lines (**Table 2**). In the majority of the cases, the great antitumoral activity of naphthoquinones is due to higher levels of cytochrome P450 reductase found in some tumour cells than in the normal ones. This enzyme induces extensive reduction of the quinoid drugs, which explains the higher toxicity and consequently selectivity of naphthoquinones to tumour cells (Stout and Becker 1986).

T. impetiginosa, a species largely sold in markets as Red lapacho has shown activity against several cancer cell lines (Castellanos *et al*. 2009). From the 18 most relevant quinones isolated from Red lapacho tea, lapachol and *β*-lapachone demonstrated clinical importance, and they have been considered as responsible for the pharmacological activity of Red lapacho tea (Oswald 1993).

The great interest in lapachol activity has leaded this compound into clinical trials. However, in phase I studies in which lapachol was administered orally no therapeutic response was observed, mainly because no satisfactory blood levels of the compound were obtained (Suffnes and Douros 1980). *β*-Lapachone, a lapachol isomer, also revealed activity against a wide range of tumour cell lines, including breast, leukaemia and prostate, as well as several multridrug resistance cell lines (Ravelo *et al*. 2004). This naphthoquinone has been under investigation for the treatment of specific cancers associated with elevated DT-diaphorase levels, because this enzyme induces bioactivation of *β*-lapachone, as we described before (Choi *et al*. 2007; Ough *et al*. 2005; Planchon *et al*. 2001). Also, Oswald defends that purification of extract of Red lapacho declines its activity, arguing that Red lapacho action results from the synergism and potentiation of the individual effects of each compound in the extract, being the antitumoral activity attributed to the extract as a whole (Oswald 1993). Despite the antitumoral potential of Red lapacho, it has a similar toxicity to lapachol. Their toxicity is associated with interference in the biological cycle of vitamin K in the body, with perturbation in the clotting formation (Castellanos *et al*. 2009).

Despite the inhibition of NF-κB by naphthoquinones, which may provide selective cytotoxicity for cancer cells, juglone was more cytotoxic for normal human fibroblasts, where a rapid apoptotic but mostly necrotic response occured, than for colonic (HCT116 and HT29) or breast cancer cell lines (MCF-7) (Paulsen and Ljungman 2005). The induction of cell death by juglone is related with the p53 oxidation, DNA damage by phosphorylation of the histone variant H2AX, and consequent inhibition of transcription and of mRNA synthesis (Paulsen and Ljungman 2005). In order to overcome the lack of selectivity, several juglone derivatives were synthetized and evaluated against several tumour and normal cells lines (Montenegro *et al*. 2010). It was found that the introduction of a methoxy group at C5, strongly increases the cytotoxicity of juglone against tumour cells, as well as the introduction of bromo atom in position 2 or 3 of juglone. Moreover, it was demonstrated that 5-methoxy-1,4-naphthoquinone induces apoptosis by an extrinsic pathway, being independent of mitochondrial depolarization (Montenegro *et al*. 2010).

This lack of selectivity of juglone to cancer cells may be due to the existence of several mechanisms of action and of nonspecific consequences of oxidative stress. However, Salustiano and collaborators defend that oxidative stress should be less important for some naphthoquinones, because pentacyclic naphthoquinones were equally active in cells resistant to oxidative stress, as K562 (high content of glutathione) or Lucena-1 cells (K562

resistant to vinca alkaloids) (Salustiano *et al*. 2010).

Contrary to juglone, plumbagin has shown selectivity between normal and cancer cells, becoming an excellent lead compound in the discovery process of anticancer agents with higher therapeutic index (Nazeem *et al*. 2009). Nazeem and collaborators showed that human skin carcinoma cells (A-431 cell line) are highly sensitive to plumbagin by ROS generation *via* cooper redox cycle mechanism. This is an advantage because cooper is often elevated in numerous cancers. The authors reported no significant toxicity to normal cells (Nazeem *et al*. 2009).

Thus, plumbagin has been extensively studied as antitumoral showing activity both in *in vitro* and *in vivo* studies (Nazeem *et al*. 2009). Plumbagin has demonstrated potential as antitumoral in melanoma, prostate, cervical and ovarian cancers (Kumar *et al*. 2009; Powolny and Singh 2008; Srinivas *et al*. 2004a; Srinivas *et al*. 2004b; Wang *et al*. 2008).

Diosquinone revealed a good cytotoxic potential against the multiple drug resistant or vinblastine resistant human nasopharyngeal carcinoma (KB-V V-VLB) (Adeniyi *et al*. 2003). Thus, naphthoquinones may be a future alternative to some resistant cancers, alone or as adjuvants to other treatments. This was observed with the use of menadione as a radiosensitizer in combination with X-ray radiation (Deeley 1962). Plumbagin also induced radiosensitization in experimental mouse tumours, as well as, in tumour cells *in vitro* (Ganasoundari *et al*. 1997; Sandur *et al*. 2006). Furthermore, *in vitro* synergy has been demonstrated between menadione and methotrexate or 5-fluorouracil in L1210 cells, murine leukemia cells (Chlebowski *et al*. 1981). *β*-Lapachone exhibited a synergistic lethality with taxol (Li *et al*.1999) and genistein (Kumi-Diaka *et al*. 2004) on several tumour cell lines implanted into mice.

Beyond naphthoquinones use in cancer treatments, they may have a chemopreventive activity. Sugie and collaborators showed that plumbagin and juglone significantly inhibited azoxymethane-induced intestinal carcinogenesis in rats, and so, they can be promising chemopreventive agents for human intestinal neoplasia (Sugie *et al*. 1998).

TOXICOLOGICAL EFFECTS

In spite of all these possible applications in disease treatment or prevention, naphthoquinones show high degree of toxicity both to eukaryotes and prokaryotes. Naphthalene, the basic nucleus of naphthoquinones, is a volatile aromatic hydrocarbon found in cigarette smoke and in diesel fuel extracts (O' Brien 1991). Numerous toxic effects of naphthalene are known and some of them are similar to toxic effects associated to naphthoquinones (Buonarati *et al*. 1989). Furthermore, in isolated hepatocyte, naphthoquinones were the most toxic metabolites formed from the naphthalene derivative 1-naphthol (Doherty *et al*. 1984).

Tissues from menadione-treated rats revealed lesions in kidney, heart, liver and lungs, being the kidney the most sensitive organ. In what concerns to other targets of menadione toxicity, cardiomyocytes were more susceptible than skeletal muscle cells, smooth muscle cells and hepatocytes (Chiou *et al*. 1997). Besides menadione, renal damage was verified with 2-hydroxy-1,4-naphthoquinones and 2-amino-1,4-naphthoquinone, but not with alkyl or dialkyl derivatives, which led to the suggestion that nephrotoxicity could involve tautomerism of the hydroxyl or amino 1,4-quinones to highly reactive 1,2-naphthoquinones or 1,2-naphthoquinoneimine (**Fig. 11**) (Munday *et al.* 2005). Therefore, nephrotoxicity is a common feature of 1,2-naphthoquinones and prevention of tautomerism in 1,4-naphthoquinones

abolishes nephrotoxicity (Munday *et al.* 2005). As said before, DT-diaphorase can either detoxify or activate quinones depending on the target organ (see above naphthoquinones' pro-oxidant character) (O'Brien 1991; Öllinger and Brunmark 1991). In the case of rats exposed to 2-amino-1,4-naphthoquinones and 2-hydroxy-1,4-naphthoquinones, pre-treatment with DT-diaphorase inducers reduced naphthoquinone nephrotoxicity but aggravated haemolysis (Munday *et al.*, 2005). Man is particularly vulnerable to renal damage by naphthoquinones, due to low level of DT-diaphorase in human liver (Munday *et al*. 2005).

The haemolytic effect of naphthoquinones is other important toxicological effect. Naphthoquinones cause haemolytic anaemia *in vivo* (Munday *et al*. 1991; Munday *et al*. 1995), by occurrence of methamoglobinemia, GSH oxidation and sulfhydryl modification in erythrocytes, which alters cation permeability and induces colloidal osmotic haemolysis (Cohen and Hochstein 1964; Mezick *et al*. 1970). Furthermore, humans with glucose-6-phosphate dehydrogenase deficiency are particularly vulnerable, suffering life-threatening haemolysis after exposure to these substances, because their red cells are more sensitive to oxidative damage (Raupp *et al*. 2001).

The ability of naphthoquinones to inhibit nitric oxide synthase (NOS) has harmful effects, because naphthoquinones also inhibit endothelial NO, inhibiting NO-dependent vasorelaxation. Thus, disruption of NO-dependent vascular tone occurs (Sun *et al*. 2006), causing acute lethal effects due to heart failure (Sumi *et al*. 2010). This vascular dysfunction was observed after treatment of vascular ring and human umbilical vein endothelial cells with naphthazarin and methylnaphthazarin. Due to NOS inhibition and superoxide generation, naphthazarin and its derivative potentiated phenylephrine-induced contraction and inhibited acetylcholine-induced relaxation (Kang *et al*. 2006). Furthermore, evidence suggests that elevated oxidative stress contributes to the endothelial dysfunction associated with atherosclerosis, hypertension, and heart failure (Cai and Harrison 2000).

Chronic exposure to various quinoid compounds may lead to carcinogenic and mutagenic insults. Chesis and collaborators showed that one-electron reduction, catalyzed by NADH-cytochrome P-450 reductase originates mutagenic metabolites, while DT-diaphorase does not lead to mutagenic compounds. The mutagenicity of naphthoquinones is also attributed to ROS generation (Chesis *et al*. 1984). In general, naphthoquinones with a prenyl side chain and bis-naphthoquinones derivatives are non-mutagenic, while naphthoquinones with one or two hydroxyl and/or methyl substituents are mutagenic (Tikkanen *et al*. 1983).

In vitro cytotoxicity assays cannot accurately predict *in vivo* toxicity. For instance, 2-hydroxy-1,4-naphthoquinone has been shown to be much less toxic than 2-methyl-1,4-naphthoquinone in a variety of cell types (Dičkancaitė *et al.* 1997), yet the reverse is true *in vivo.* Similarly, dialkyl naphthoquinones show little effect *in vitro*, but are powerful haemolytic agents in rats (Munday *et al.* 1995). Differences in pharmacokinetics of these compounds can explain the observed differences. For instance, conjugation of some quinones or their reduction products with glucuronide or sulfate in the liver and elimination into the bile constitutes an important route of detoxification of naphthoquinones (O'Brien 1991).

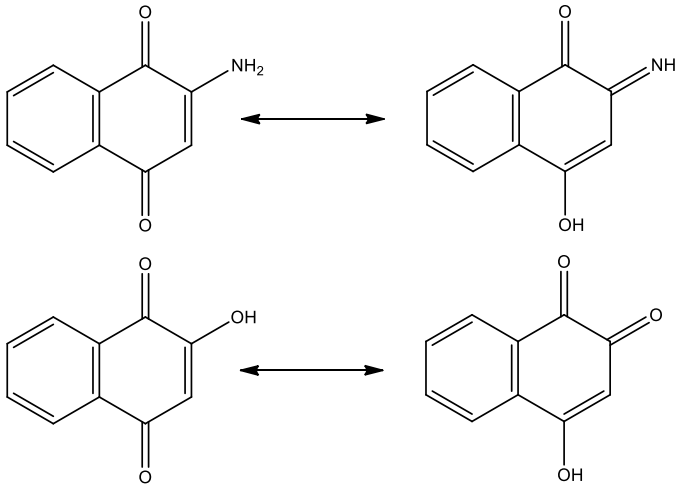

Fig. 11. Tautomerism of the amino and hydroxyl 1,4-naphthoquinones to 1,2-naphthoquinoneimine and 1,2-naphthoquinones.

CONCLUSIONS

Naphthoquinones present multiple biological activities, being interesting lead compounds for the development of new drugs. The pro-oxidant character and electrophilicity of naphthoquinones explain most of the biological and toxicological activities documented for these compounds. Due to nonspecific mechanisms of action, naphthoquinones display significant toxicity. However, adequate modifications in naphthoquinones structures may originate valuable drugs, as shown with the anti-malarial atovaquone.

ACKNOWLEDGEMENTS

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Table 1 Enzymes inhibited by naphthoquinones

Inhibited Enzymes	Naphthoquinones	References
Cytochrome P450 linked monooxygenase systems	Juglone	Muto *et al*. 1987
Integrase HIV	Naphthazarin	Fesen *et al*. 1993
Phosphatidylinositol-3-kinase	Juglone; Methyljuglone	Frew *et al*. 1995
Reverse transcriptase (oncornavirus)	*β*-Lapachone	Schuerch and Wehrli 1978
RNase (HIV virus)	1,4-Naphthoquinone; Juglone; Menadione; Plumbagin	Min *et al*. 2002
RNA polymerase II	Juglone	Chao *et al*. 2001
Topoisomerase I	Naphthazarin derivatives	You *et al*. 1998
	β-Lapachone	Li *et al*. 1993; Planchon *et al*. 2001
	Acetyl shikonin analogues	Ahn *et al*. 1995
	Isodiospyrin	Ting *et al*. 2003
	Diospyrin	Ray *et al*. 1998; Tazi *et al*. 2005
	Alkanin and shikonin derivatives	Plyta *et al*. 1998
Topoisomerase II	Plumbagin Shikonin	Fujii *et al*. 1992
	Eleutherin derivatives	Sperry *et al*. 2009

Table 2 Different cell lines used for naphthoquinones study.

Cancer	Cell Lines	Compounds	References
Prostate	DU-145; PC-3; LNCaP	*β*-Lapachone	Kumi-Diaka *et al*. 2004 ; Li *et al*. 1995 ; Li *et al*. 1999; Planchon *et al*. 1995; Planchon *et al*. 2001;
	LNCaP	Diosquinone	Adeniyi *et al*. 2003
	PC-3; LNCaP; C4-2	Plumbagin	Powoly and Singh 2008
Leukemia	HL-60	*β*-Lapachone	Li *et al*. 1995; Planchon *et al*. 1995
	HL-60	Juglone	Montenegro *et al*. 2009
	HL-60; K-562	Diospyrim	Chakrabarty *et al*. 2002
	Daudi; Lucena-1; K562	Lapachol; α-lapachone	Salustiano *et al*. 2010
Breast	MCF-7; 21 MT; 21PT; 21NT	*β*-lapachone	Li *et al*. 1995; Li *et al*. 1999; Planchon *et al*. 1995 ; Wuerzberger *et al*. 1998
	BC-1	Diosquinone	Adeniyi *et al*. 2003
	MCF-7	Juglone	Paulsen and Ljungman 2005
	MCF-7	Diospyrin	Chakrabarty *et al*. 2002
	MCF-7; MDA-MD-231	Plumbagin	Kuo *et al*. 2006
Ovary	AD 2780 s	*β*-Lapachone	Li *et al*. 1995
	BG1	Plumbagin	Srinivas *et al*. 2004b
Renal	293	*β*-lapachone	Li *et al*. 1995;
Colon	SW1116; HT-29; DLD	*β*-lapachone	Li *et al*. 1995; Li *et al*. 1999
	COL-2	Diosquinone	Adeniyi *et al*. 2003
	HCT-8; HCT116; HT29	Juglone	Montenegro *et al.* 2009; Paulsen and Ljungman 2005
Pulmonary	H596; H520; A549; IMR-90; G480	*β*-Lapachone	Bey *et al.* 2007; Choi *et al*. 2007; Li *et al*. 1995; Li *et al*. 1999
	LU-1	Diosquinone	Adeniyi *et al*. 2003
	A549	Plumbagin	Hsu *et al.* 2006
Laryngeal	HEp-2	*β*-Lapachone	Boothman *et al*. 1987 ; Boothman *et al*. 1989
Pancreatic	MIA PaCa-2; ASPC-1	*β*-Lapachone	Li *et al*. 1999; Ough *et al*. 2005
Skin	U1-Mel; HaCat; SKmel-28	*β*-Lapachone	Boothman *et al*. 1989; Li *et al*. 1999; Müller *et al*. 1999
	HaCat	Plumbagin and juglone	Inbaraj and Chignell 2004
	MDA-MB 435	Juglone	Montenegro *et al.* 2009
	B16F1; A375.S2; A-431	Plumbagin	Kumar *et al*. 2009; Nazeem *et al* 2009; Wang *et al*. 2008
Cervical	HeLa; HeLa-bcl-2	*β*-Lapachone	Li *et al*. 1995
	HeLa	Diospyrin	Chakrabarty *et al*. 2002
	ME-180	Plumbagin	Srinivas *et al*. 2004a
Other	HT-1080; KB; U373; KB-V; SKNSH	Diosquinone	Adeniyi *et al*. 2003
	SF-295	Juglone	Montenegro *et al.* 2009

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