Perspectives on Medicinal Properties of Benzoquinone Compounds

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Abstract: Benzoquinones are class of natural quinones found chiefly in higher plants, fungi, bacteria and animal kingdom. They are involved in important biological functions such as bioenergetic transport, oxidative phosphorylation and electron transport processes. In recent years it has become increasingly clear that some of them possess potent antioxidant, anti-inflammatory and anticancer activities. There is clearly a common thread running through these activities and there have been a large number of studies carried out to unravel the mechanisms of these activities. In the present review we have provided a brief account of these studies especially covering these aspects. Although antioxidant potentials of these compounds constitute the basis of their biological activities its nature and scope is dictated by many microscopic biological environments. One of the important advantages offered by these compounds is the ease with which they can be synthesized and chemically manipulated. This can easily provide impetus for further research in developing some potentially useful drug molecules.

Keywords: Anticancer, anti-inflammatory, antioxidant, benzoquinone, benzoquinone analogs, quinone.

1. INTRODUCTION

Benzoquinones constitute a class of compounds with important biological functions in bioenergetic transport [1, 2], oxidative phosphorylation and electron transfer processes [3]. They possess diverse pharmacological properties such as anticancer [4-7], anti-inflammatory [8], antimicrobial [9, 10] and antiviral activities [11]. The compounds are used as potential synthetic building blocks in the design and synthesis of various heterocyclic drug molecules. The impetus for research on these compounds in recent years is perhaps provided by the discovery of chemopreventive and therapeutic properties observed for a naturally occurring benzoquinone compound, viz. Thymoquinone, which is found in the Black Cumin (*Nigella sativa*) seed oil [12]. The black cumin seeds have long been used as a spice in the Mediterranean region and in Western Asian countries including India, Pakistan and Afghanistan. This herb goes by many different names such as 'Panacea' in Latin, 'Habbah Sawda' in Arabic, 'Hak Jung Chou' in Chinese or 'Kalonji' in India. Investigations on the extracts of this plant have indicated potent antitumor activities without serious toxic effects suggesting that compounds having benzoquinone nucleus may be developed into useful therapeutic agents [12, 13].

In the present review we provide a brief account of some important benzoquinone compounds having potent antioxidant, anti-inflammatory and anticancer properties reported until December 2009.We also provide a short review of the chemistry of these compounds in order to help understand structural relevance to their medicinal properties.

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2. CHEMISTRY OF BENZOQUINONE DERIVATIVES

Benzoquinones are the simplest structural quinones which are widely distributed in plants, animals and bacteria. Most of the investigations on the biogenesis of benzoquinones have been carried out with moulds such as *Aspergillus fumigatus* and *Pencillium patulum* [14]. Majority of the fungal benzoquinones appear to be formed by the acetate-malonate pathway yielding ubiquinones having polyprenyl side chains [15]. These compounds are found in most aerobic organisms from bacteria to higher plants and animals and are involved in mitochondrial electron transport chains. The *Streptomyces* bacteria also produce large number of nitrogen containing heterocyclic quinones [16]. Studies on insects using radio-labelled precursors have indicated two biosynthetic routes for the formation of benzoquinone compounds such as methylbenzoquinone, 2, 3-dimethyl and 2, 5-dimethylbenzoquinone which serve as important toxic defence for many insects and arthropods. Comparatively less is known about the biogenesis of benzoquinones in higher plants [17].

The methods employed to isolate naturally occurring benzoquinones are common to natural products chemistry and involve sequential extraction with solvents of increasing polarity while the appropriate fractions are purified by column or thin layer/preparative thin layer chromatography. Ultrasonic or critical fluid extractions carried out in recent years offer advantages in terms of avoiding residual solvent levels [18].

Chemically 1, 4-benzoquinone is a simple six-membered, non-aromatic compound which is the oxidised derivative of 1, 4-dihydroquinone.It is a multi-functional moiety exhibiting properties of a ketone, oxidant and an alkene undergoing addition reactions. The compound can be easily reduced to 1,

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4-benzenediol. An equimolar quantity of 1, 4-benzoquionone and 1, 4-benzenediol is known as quinhydrone which is used as a standard electrode material [19]. Several features associated with the quinonoidal structure make it an attractive motif for building supramolecular assemblies. Similarly, the quinonoidal carbonyl groups are capable of evolving hydrogen bonded assemblies yielding inclusion compounds [20, 21]. The electron deficient benzoquinone system also yields charge transfer complexes very easily [22-27]. The facile redox cycling associated with benzoquinones make them excellent oxidizing agents [28].They are known to react with acetic anhydride and sulphuric acid to give triacetate of 1, 3, 4-trihydroxybenzene and the reaction is known as Thiele reaction [29].

The benzoquinones easily undergo nucleophilic addition reactions and serve as an excellent dienophiles in the Diels-Alder reaction. They react with large number of primary and secondary aliphatic or aromatic amines yielding mostly disubstituted compounds. However, single substitution is observed with secondary amines under selected conditions. During such substitutions one or two moles of reduced benzoquinone molecules are also formed as shown in Fig. (**1**) [30]. Harley-Mason and Laird [31] have reported on the formation of 1, 2, 3, 4-tetrahydro-1, 4, 5, 8-tetra-aza-anthracene after reacting 2, 5-dihydroxy-p-benzoquinone with ethylenediamine in presence of air in aqueous media. A similar reaction with 2,3-dimethoxy-p-benzoquinone and ethylenediamine yields 2, 5-bis(2-hydroxyethylamino)-3, 6-dichloro-1, 4-benzoquinone which on further heating for several hours gives tetra-aza-anthracene (Fig. **2**) [32]. Aromatic amines also undergo nucleophilic substitution at 2 and 5 position of p-benzoquinone. For example, Aseed and Omer have synthesised 2, 5-Diamino-3, 6-dibromo-1, 4-benzoquinone by reacting various amines with 2, 3, 5, 6- tetrabromo-1, 4-benzoquinone (Fig. **3**) which exhibit antibacterial activity against *Aspergillus niger* and *Candida albicans* respectively [33]

The Diels-Alder reaction (also known as the Diene Synthesis) is one of the most commonly used reactions for cycloadditions. The original reaction involved interaction of 1, 3-butadiene with an alkene to form a cyclohexene. The method was extended to the reaction of cyclopentadiene with p-benzoquinone by Diels and Alder who found out that in this reaction the electron deficient ring of quinones act as dienophile and undergoes Diels-Alder 4+2 cyclo-addition reactions or photochemical 2+2 cyclo-addition reactions leading to interesting organic compounds [34, 35]. These quinone cyclo-addition reactions are important since they can be extended to synthesis of various natural products. For example, the cyclopentadiene-benzoquinone adduct has been employed in the synthesis of dendrobine (Fig. **4**) [36].

Lora-Tamayo has summarized the characteristic features of addition reactions of substituted benzoquinones. Accordingly addition occurs only at non-substituted ethylene linkage of benzoquinone and no addition occurs if substituents are present on both ethylene linkages. Secondly, the reaction generally accompanies tetra-dehydrogenation to yield a phenanthrenequinone derivative [37]. However, the orientation of the quinone is not clarified by these authors. After studying the reaction between styrene and methoxy-benzoquinone other workers have concluded that the main product of the reaction is 3-methoxybenzoquinone (Fig. **5**) [38]. The compound 2-methyl-1, 4 benzoquinone has been used in synthesis of vitamin K_3 using a multi-component Diels-Alder reaction and microwave irradiation under the conditions shown in Fig. (**6**) [39]. Buckle *et al.* have evaluated the relative rates of the Diels-Alder reactions of the unsymmetrical diene, viz. 2-(trimethylsilyloxy)-1, 3-cyclohexadiene, and its 6, 6- and 5, 5-dimethyl derivatives. They have found almost synchronous Diels-Alder reaction with symmetrical, ethylenic dienophiles like para-benzoquinone, maleic anhydride and N-phenylmaleimide, while the reaction was sufficiently asynchronous with tetracyanoethylene and diethyl acetylenedicarboxylate. The addition also leads to different rates of reaction with dimethyl-dienes [40].

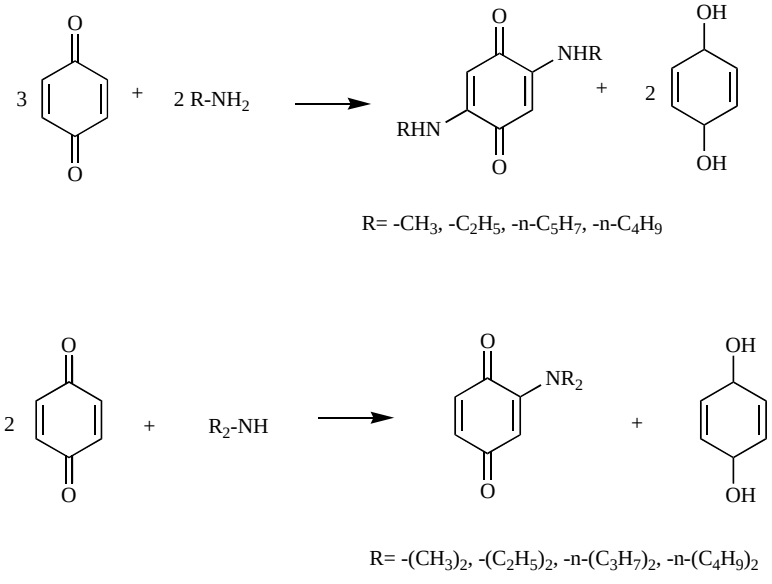


Fig. (1). Reaction of 1, 4-benzoquinone with amines.

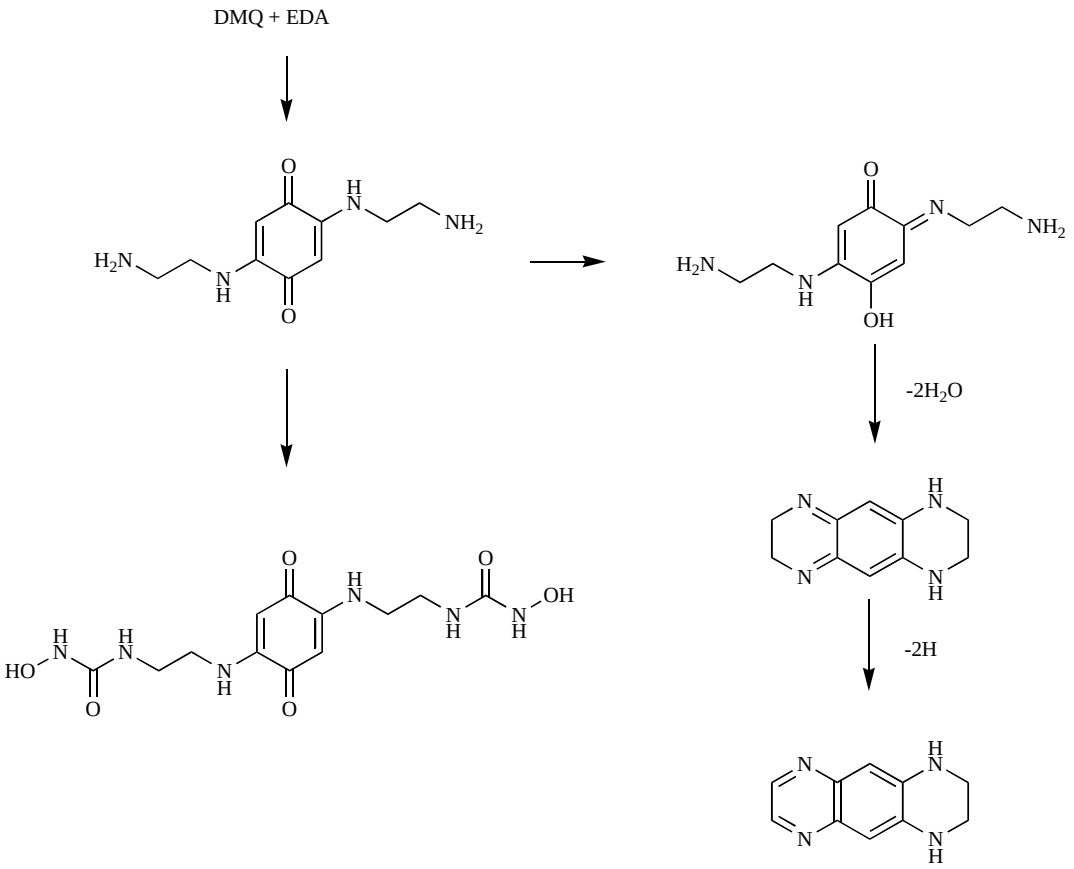


Fig (2). Preparation of tetra-aza-anthracene from reaction of 2,5-dihydroxy-1,4-benzoquinone with ethylene diamine (EDA).

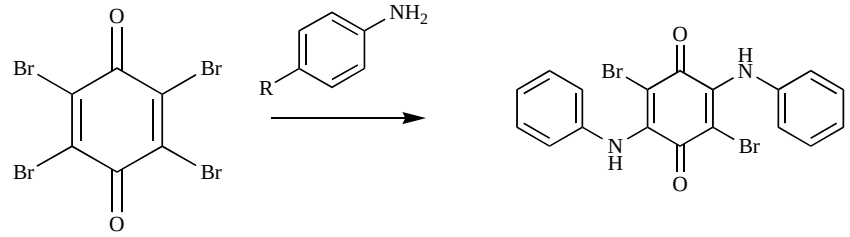


Fig (3). Reaction of tetrabromo-substituted 1, 4-benzoquinone with amines.

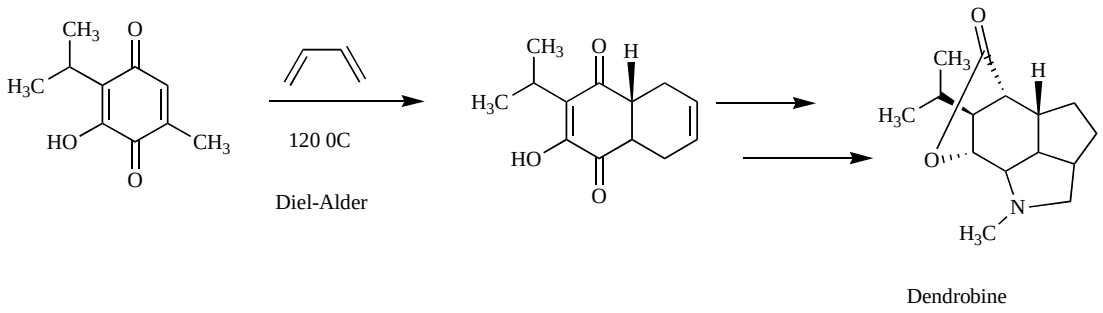


Fig (4). Synthesis of dendrobine from benzoquinone via Diels-Alder's reaction.

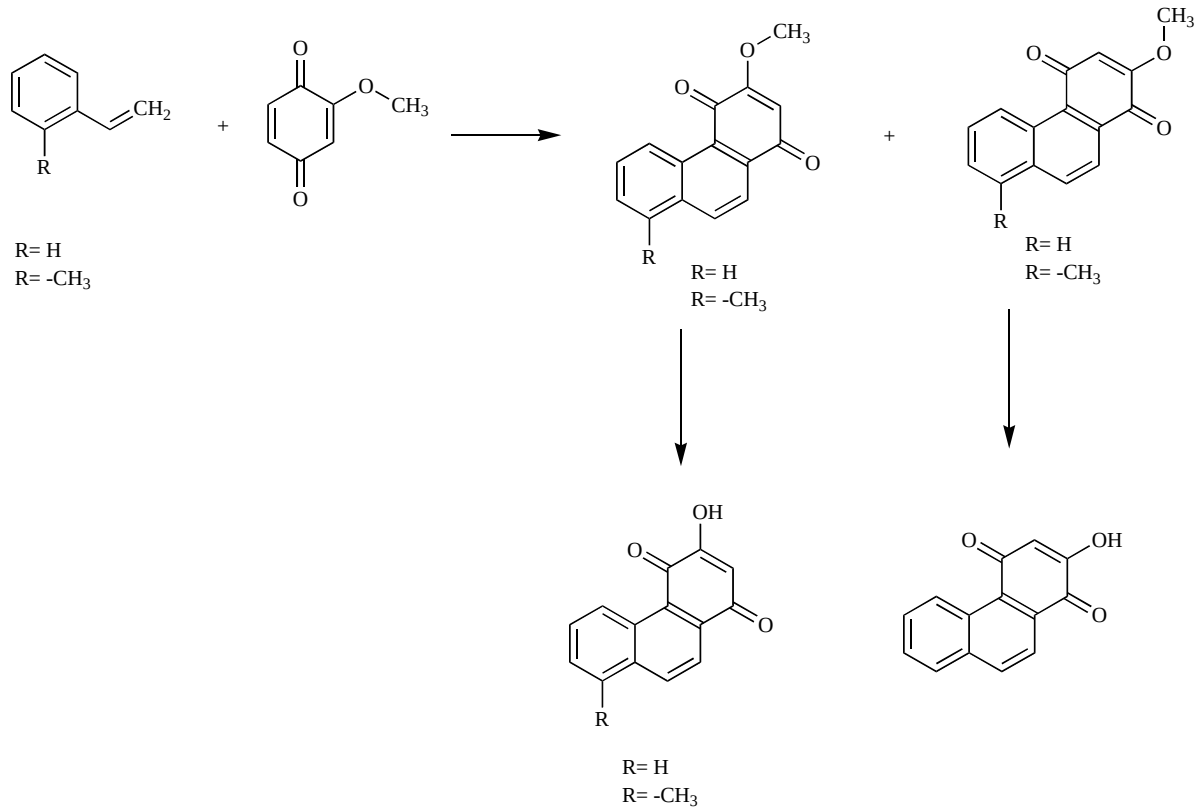


Fig (5). Reaction between styrene and methoxy-benzoquinone yielding 2- and 3-methoxyphenanthrene-1, 4-quinone.

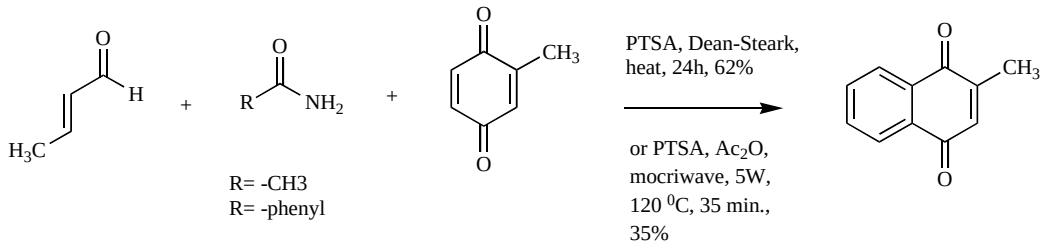


Fig (6). Synthesis of vitamin K3 by using 2-methyl-1, 4 benzoquinone.

Finally, the redox properties of quinones have found applications in pH determination and catalytic redox reactions [41] as well as in electrochemical redox switches [42]. The redox active quinone polymers have also been employed in light-emitting devices [43]. The biological activities of quinonoidal compounds have been explained in terms of "Bioreductive alkylation" which is the term used to explain a series of mechanisms by which a drug can be reduced to its reactive intermediate which can undergo nucleophilic addition [44]. Benzoquinones have been known to undergo bioreductive alkylation under biological conditions to generate methides and reactive oxygen species (ROS) [45]. Although no specific enzyme has been identified as quinone reductase as yet, the enzymes like microsomal NADPH-cytochrome P-450 reductase, mitochondrial NADH dehydrogenase and cytosolic xanthine oxidase have been shown to function as quinone reductases (Fig. **7**) [46]. The anticancer activities of some quinonoidal antitumor compounds like mitomycin C, adriamycin, daunomycin, mitozantrone and 3,6-diaziridinyl-2,5-bis (carboethoxyamino)-1,4-benzoquinone (Fig. **8**) have been explained on the basis of bioreductive alkylation and oxidative stress induced by the ROS [47].

3. BIOLOGICAL ACTIVITIES

3.1. Antioxidant Activity

Several studies have reported on the antioxidant potential of natural and synthetic benzoquinone analogs. One of the important natural benzoquinone compound is Coenzyme Q10 (ubiquinone, **1**), which is 2, 3-dimethoxy-5-methylbenzoquinone with a side chain comprising of ten carbon isoprene units in *trans* configuration with one double bond. It plays a major role in mitochondrial bioenergetics and is present in plasma and subcellular fractions [48]. Recent reports suggest that ubiquinone affects gene expression involved in human cell signalling, metabolism and transport [49]. It exerts its neuroprotective effect by enhancing antioxidant enzyme levels in hippocampus of rats in pilocarpine

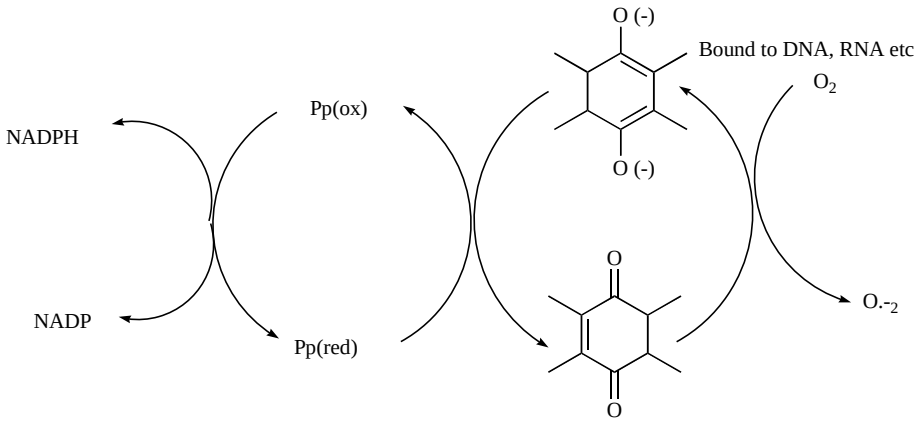


Fig (7). Bioreductive alkylation of benzoquinones.

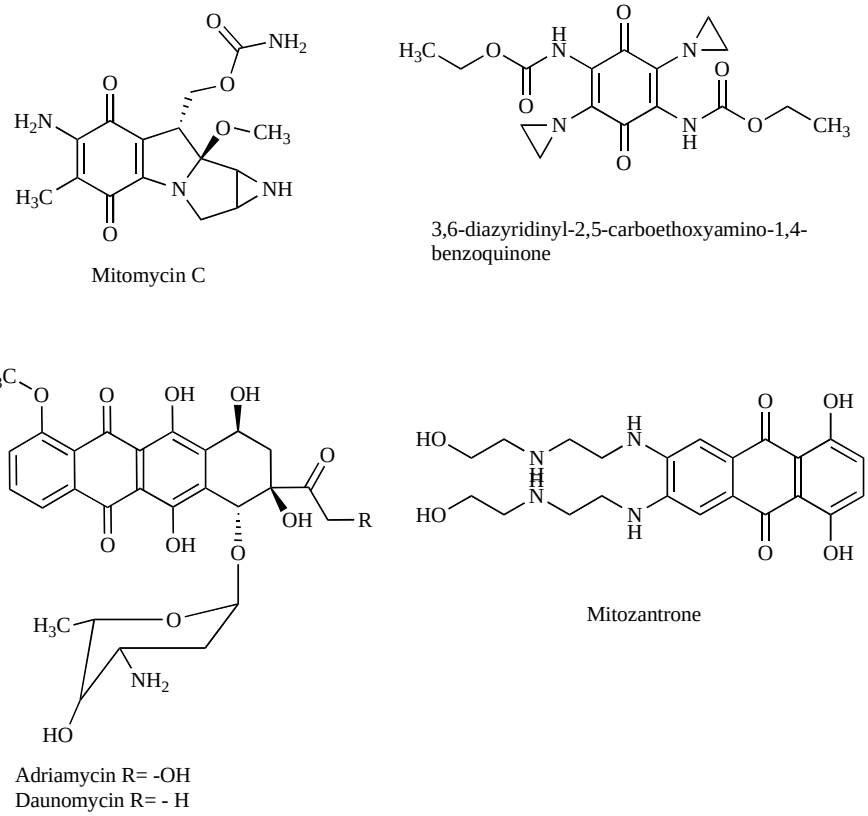
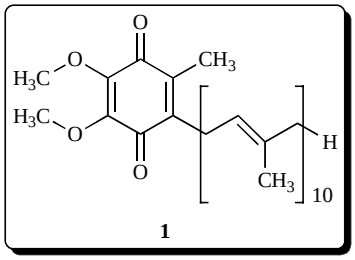


Fig (8). Structures of some quinonoidal antitumor compounds.

induced seizures [50]. Littarru and Tiano have summarized the antioxidant effects of ubiquinone [51]. The compound is endogenously synthesized as a lipid soluble antioxidant which when reduced protects the system from protein degradation, DNA oxidation and lipid peroxidation. It prevents peroxidation of lipids of cell membrane and lipoprotein lipids in the circulation [52]. Anti-atherogenic effect of ubiquinone has been evaluated in apo-lipoprotein-E-deficient mice fed with a high-fat diet. In this model, supplementation with ubiquinone resulted in lowering of the absolute concentration of lipid hydroperoxides and size of atherosclerotic lesions in the whole aorta [53]. Recent data has shown that ubiquinone can have a direct effect on endothelial function. In patients with stable moderate congestive heart failure, supplementation with ubiquinone has been shown to ameliorate cardiac contractility and endothelial dysfunction as well as increase in Co-enzyme-Q9 and ubiquinone content in homogenates of liver, heart, kidney, skeletal muscle and brain [54-57]. The compound shows a strong correlation between endothelium bound extra cellular SOD (ecSOD) and flow-dependent endothelial-mediated dilation which is commonly used as a biomarker of vascular function [58].



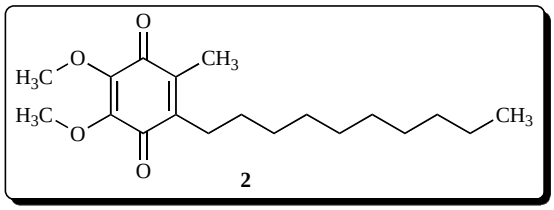
1

Recently, Mancuso *et al.* have suggested involvement of ubiquinone in oxidative phosphorylation and cellular antioxidant protection for the treatment of mitochondrial disorders, like Parkinson's disease, Huntington's disease and Friedreich's ataxia [59]. Ratnam *et al.* have shown that encapsulated nanoparticles of ellagic acid in combination with ubiquinone enhanced glucose and triglyceride lowering activity in high fat diet induced hyperlipidemic rats [60]. Sohet and co-workers have studied the effect of ubiquinone on hepatic metabolism and inflammatory disorders associated with diet-induced obesity and glucose intolerance in C57bl6/j mice and have concluded that the compound decreased the global hepatic mRNA expression of inflammatory and metabolic stress markers without changing obesity and tissue lipid peroxides [61]. Kumar and co-workers have summarised the rationale behind the use of this compound in cardiac disease, hypertension and Meniere-like syndrome. Besides the antioxidant and free radical scavenging activity the compound also shows a vasodilatory effect and LDL oxidation inhibitory effects which lead to improvement in atherosclerosis. The compound decreases the release of pro-inflammatory cytokines and blood viscosity which is useful for patients with heart failure and coronary artery disease. It was found to improve ischemic and reperfusion injury to coronary revascularisation [62]. Stawiarska-Pieta *et al.* have shown that at a dose of 200 μg/rat/24h the compound exerts protective effect against sodium fluoride-induced oxidative degeneration of rat pancreas and heart through histopathological examination [63]. Okello and co-workers have proposed that statins and ubiquinone show synergistic improvement in cardiac function by enhancing expression of superoxide dismutase [64].

In animal models of Parkinson's and Huntington's diseases, the antioxidant effect of ubiquinone shows additive neuroprotective effect in combination with creatine in the transgenic R6/2 HD mice [65]. Cordero *et al.* have found that oxidative stress due to defect in distribution and metabolism of ubiquinone in cells and tissues in the patients, may be one of the contributing factors to fibromyalgia and protection offered by ubiquinone in mononuclear cells indicate beneficial effects of its supplementation in fibromyalgia patients [66]. A study involving the supplementation of ubiquinone and α-tocopherol on metabolic control and pancreatic mitochondria of GK rats have shown that the compounds lower the glycated hemoglobin and pancreatic lipid peroxidation but do not prevent the pancreatic lesions in type-2 diabetes [67]. Ayaz *et al.* have concluded that ubiquinone prevents diabetes-induced shift of actively contributing nerve fibers towards slower conduction velocity and restores velocities toward those of aged-matched control group. They have also suggested that protective effects of ubiquinone on mitochondrial alterations in diabetic neuropathy were mediated by its antioxidant activity [68]. Nakajima *et al.* have studied the neuroprotective action of ubiquinone against retinal damage induced by hydrogen peroxide *in vitro* on cultured retinal ganglion cells and intra-vitreal N-methyl-d-aspartate (NMDA) injection (at 10 mg/kg dose) in mice *in vivo* [69].

Kim and co-workers have investigated effect of nanoparticles of ubiquinone on photoaging in the hairless mouse skin induced by ultraviolet B (UVB) irradiation where the treatment was found to reduce oxidative stress significantly by enhancing manganese superoxide dismutase (SOD2) and glutathione peroxidase activity in the skin [70]. Another study involving administration of ubiquinone for 6-24 months has shown decrease in age associated peroxidizability index, enhanced catalase activity and modulation of aging related changes in mitochondrial electron transport in skeletal muscle of rats fed on polyunsaturated fatty acid (PUFA)-rich diet inducing age related oxidative stress [71]. In yet another clinical study the effect of combined modality of ubiquinone (100 mg), riboflavin (10 mg) and niacin (50 mg) with tamoxifen (10 mg twice a day) for 90 days on serum lipids and lipoprotein levels in postmenopausal women with breast cancer was undertaken. Various lipid parameters were calculated on 45^{th} and 90^{th} day in 78 un-treated, tamoxifen-treated and combination-treated groups with age and sex controls. Results suggest that all lipid parameters are altered in un-treated group when compared to normal counterparts, while serum triglyceride and VLDL-C are found to be elevated and reduced LDL-C in tamoxifen-treated patients. All altered lipid parameters were found to be near normalized after 90 days treatment of combination [72]. Upaganlawar *et al.* have investigated antioxidant effects of ubiquinone (10 mg/kg/day, i.p.) alone and in combination with green tea (25 mg/kg/day, p.o.) against gentamicin-induced nephrotoxicity in rats. Treatment with ubiquinone or green tea alone showed decrease in serum urea, creatinine and tissue lipid peroxidation content and increase in antioxidant enzymes, while combined treatment showed better activity [73].

Decylubiquinone (2, 3-Dimethoxy-5-methyl-6-decyl-1,4-benzoquinone) (**2**) which is an analog of ubiquinone, has been shown to block ROS production induced by glutathione depletion and prevent activation of the MPT (mitochondrial permeability transition) thereby leading to apoptosis of HL60 cells [74]. Leber's hereditary optic neuropathy is a mitochondrial disease characterized by visual loss resulting from retinal ganglion cell degeneration and associated with free radical induced defect in glutamate uptake. Administration of decylubiquinone partially restores glutamate transport impairment occurring in trans-mitochondrial cybrid cell lines constructed using enucleated carrying the most severe 3460/ND1 mutation and the osteosarcoma-derived mtDNA-less cells [75].



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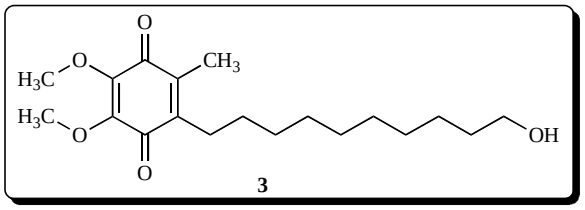
Idebenone (2, 3-dimethoxy-5-methyl-6-(10-hydroxydecyl)-1, 4-benzoquinone) (**3**) is another synthetic analogue of ubiquinone which is currently used to improve cognitive status in patients with clinical history of stroke, Alzheimer's disease, and multiinfarct dementia [76-77]. Idebenone was initially developed by Takeda Pharmaceuticals Company Ltd. for the treatment of patients with cognitive disorders and Alzheimer's disease and was approved in Japan in 1986 for the treatment of those with decreased violition and emotional disturbances associated with cerebrovascular disease. Its neuroprotective action was mediated by compound's antioxidant properties through inhibition of lipid peroxidation in brain homogenates and mitochondrial membranes [78] as well as in neural cell lines subjected to oxidative stress [79]. Voronkova and Meleshkov have conducted a clinical study by using idebenone at dose of 120 mg/day for six months in the treatment of patients aged 60-86 years with Alzheimer's-type dementia, mixed dementia, and memory impairments not reaching the stage of dementia. They have shown that 37% of the patients showed improvements in short-term and long-term memory and attention, with improvements in speech functions, performance of kinesthetic, spatial, and dynamic praxis tests, and in visuo-spatial gnosis, thought, and writing [80]. Idebenone was also found to inhibit glycerophosphate and succinate-dependent, ferricyanide-activated ROS production. It was found to be more efficient in scavenging ROS with IC_{50} value of 0.052 μM.



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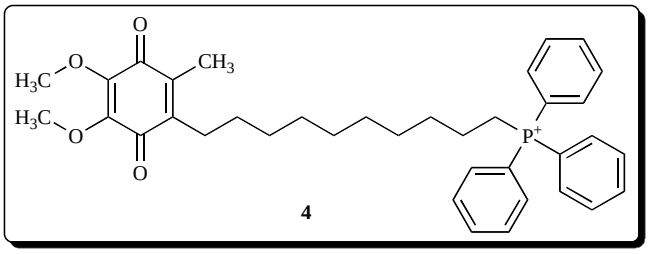
Liposomally-entrapped idebenone prevented the ethanol induced injury on astroglial cells and hence was implicated in the treatment of brain disorders [81]. Idebenone-loaded polyethyl-2-cyanoacrylate (PECA) nanocapsules were tested against oxidative stress induced by diethylmaleate on Human non-immortalized fibroblasts *in vitro*. The results suggest that the compound entrapped within Tween 80-coated PECA nanocapsules at concentration 0.5 μM are capable of reducing oxidative damage to fibroblasts than free drug [82]. Idebenone alone reduced oxidative brain stress following transient circulatory arrest in the rat by scavenging free radicals at a dose of 100 mg/kg [83]. Mordente *et al.* have proposed that the compound functions by virtue of the electron-donating properties of its hydroquinone form. Thus, neuroprotective effects of idebenone can be attributed to its antioxidant ability, involving redox cycling [84].

Mitoquinone (**4**) is another analog of ubiquinone wherein a triphenyphosphonium cation is linked to ubiquinone moiety by a linear alkyl chain, which is a potential mitochondrial targeting antioxidant. It is taken up quickly by isolated mitochondria driven by the mitochondrial membrane potential. The antioxidant effect of mitoquinone is due to its ubiquinol form and redox cycling associated with it [85]. Some recent reports have summarized potential antioxidant effects of mitoquinone and its benefits in diseases originating from oxidative stress [86-88]. The compound is an orally active antioxidant that is under development by Antipodean Pharmaceuticals Inc and is being currently studied in phase II clinical study for Parkinson's disease and liver damage associated with HCV infection [86].



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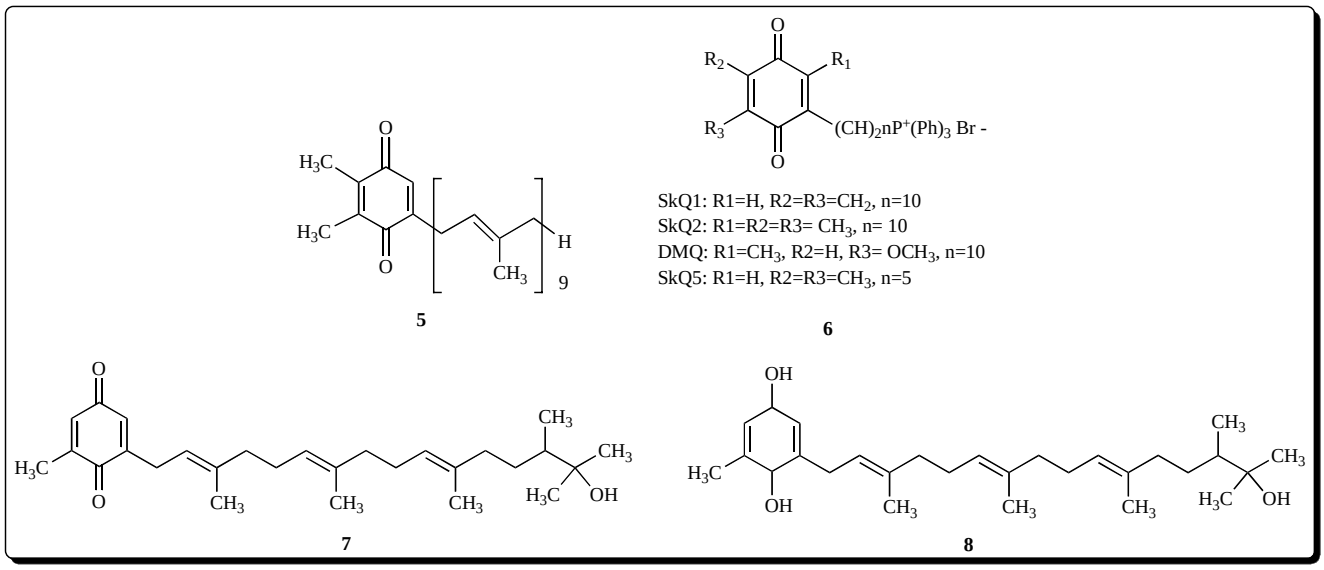
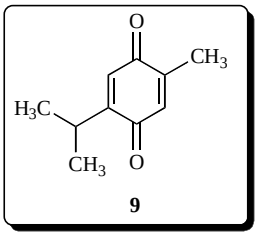
Plastoquinone (**5**) is another benzoquinone derivative which is involved in chloroplast electron transfer chain in plants. Some reports have suggested that plastoquinone is a better antioxidant than ubiquinone [89-91]. In the photosynthetic process oxygen producing chloroplasts get more exposed to oxidative stress than oxygen consuming mitochondria and hence it is likely that plants may be using plastoquinone to scavenge these radicals in chloroplasts. Antonenko *et al.* have recently synthesized cationic plastoquinone derivatives (**SkQs**) (**6**) where positively charged phosphonium or rhodamine moieties connected to plastoquinone by decane or pentane linkers. They have found out that these compounds can easily penetrate through planar mitochondrial and outer cell membranes and exhibit potent antioxidant activity at nanomolar concentration in lipid micelles, liposomes and isolated mitochondria as well as intact cells [92, 93]. **SkQ1** has also been studied in various oxidative stress-induced ocular diseases. Small amount of food supplemented with **SkQ1** (50 nmol/kg per day) showed prevention of age-induced cataract and retinopathies of the eye, lipid peroxidation and protein carbonylation in skeletal muscles, as well as decrease in bone mineralization in ROS induced progeria. The instillation of drops of 250 nM of **SkQ1** showed reversal of uveitis, cataract and retinopathies in 3-12-month-old (but not in 24-month-old) OXYS rats [94]. Iwashima *et al.* have isolated two plastoquinone derivatives from methanolic extract of the brown alga *Sargassum micracanthum* which were identified as 2-geranylgeranyl-6-methylbenzoquinone (**7**) and its hydroquinone (**8**), which showed inhibition of DPPH free radials and lipid peroxidation respectively [95].

Thymoquinone (**9**) is an active constituent of volatile oil of black *Nigella sativa* seed whose chemistry and biological activities have recently been summarized by us [13, 96]. The compound has a good safety profile with LD_{50} value of 104.7 mg/kg after i.p. injection and 870.3 mg/kg after oral treatment in mice [97]. Ragheb *et al.* have studied the effect of thymoquinone on cyclosporine A or hyperlipidemia induced atherosclerosis alone or in combination in a rabbit model. They have found that thymoquinone attenuates the oxidative stress and atherogenesis as well as hyperlipidemia by decreasing aortic MDA [98]. Rats treated with thymoquinone-rich fraction (and thymoquinone at various doses) showed significant inhibitory activity on liver RNA expression and up-regulation of the antioxidant superoxide dismutase 1 (SOD1), catalase, and glutathione peroxidase 2 (GPX) genes respectively in the liver of hypercholesterolemic rats [99].

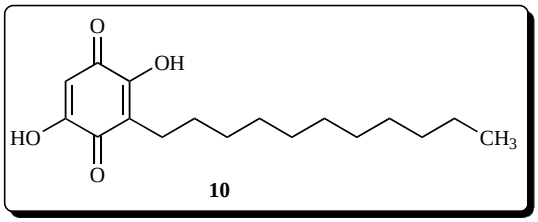


Thymoquinone was found to restore streptozotocin-diabetes-induced changes in cardiac creatine kinase levels in muscle and brain as well as brain monoamines due to its antioxidant properties [100]. Chandra *et al.* have shown that thymoquinone had protective effects against highly active antiretroviral therapy inducing a metabolic syndrome manifesting cardiovascular dysfunction and lipodystrophy as well as insulin resistance [101]. The compound can ameliorate oxidative damage and proliferative response induced by mercuric chloride in rat kidney under nephropathic condition [102].



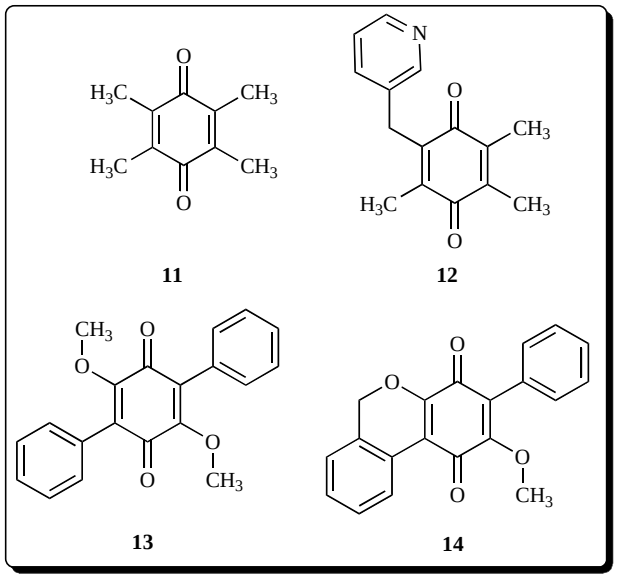
Various reports have shown that the antioxidant effects of thymoquinone are mediated by scavenging of various reactive oxygen species including superoxide radical anions and hydroxyl radicals respectively [103-105]. Thymoquinone has a potent chemopreventive potential of inhibiting the process of carcinogenesis by modulating lipid peroxidation and cellular antioxidant milieu [106,107]. The compound significantly restores hepatic antioxidant enzymes such as superoxide dismutase, catalase and glutathione peroxidase respectively thereby inhibiting iron dependent microsomal lipid peroxidation in rats with doxorubicin-induced hyperlipidemic nephropathy [108]. It also shows protection against gentamicin induced nephrotoxicity [109]. El-Saleh and coworkers [110] have shown that active antioxidant components of black seeds of *Nigella sativa* plants are capable of rendering protection against the development of methionine-induced hyperhomocysteinemia (HHcy) and its associated state of oxidative stress in rats. Thymoquinone was also found to be effective in acetic acid-induced colitis in rats [111]. The treatment with thymoquinone has been found to suppress DOX-induced proteinuria, albuminuria, and urinary excretion of N-Acetyl Glucosamine (NAG) in doxorubicin (DOX)-induced hyperlipidemic nephropathy and oxidative stress in rats [112]. The neuroprotective effect of thymoquinone is mediated by down-regulation of the elevated levels of MDA upregulation of antioxidant enzymes in transient forebrain ischemia-induced neuronal damage in the hippocampus of rats. Thymoquinone inhibits *in vitro* non-enzymatic lipid peroxidation in hippocampal homogenate induced by iron-ascorbate with the IC_{50} value of 3 μM respectively which makes thymoquinone a promising agent in pathologies implicating neurodegeneration such as cerebral ischemia [113].

Embelin (2, 5-dihydroxy-3-undecyl-1, 4-benzoquinone) (**10**) has been isolated from the seeds of the plant *Embelia ribes* (Myrsinaceae), which is commonly known as 'Vidanga' in Indian sub-continent [114]. The compound shows potential antioxidant activity in diabetic animals [115, 116]. It can be synthesized by peroxide alkylation of 2, 5-dihydroxy 1, 4-benzoquinone [117]. Embelin is chemically an interesting molecule due to presence of quinone and phenolic group on the same ring with a long alkyl chain at C-3 position conforming lipid solubility and cell permeability. The adjacent quinone and phenolic groups are involved in intramolecular hydrogen bonding which seems to facilitate metal complexation with Mn (II), Ni (II), Cu (II) and Zn (II) [118, 119]. It is found to enhance antioxidant enzymes and decrease lipid peroxidation in the carbon tetrachloride-induced free radical generation and hepatotoxicity [114]. The compound was found to scavenge DPPH radicals and inhibit deoxyribose degradation induced by hydroxyl radicals. It



also showed inhibition of lipid peroxidation and up-regulation of Mn-superoxide dismutase level. Joshi and co-workers have suggested that the compound can act as a competitive antioxidant under physiological conditions [120].

Duroquinone (**11**) is a tetramethyl-*p*-benzoquinone which has also been studied for its antioxidant activity [121]. Various other synthetic benzoquinone analogs have also been screened for antioxidant activity. For example, Terao *et al.* have synthesized 2, 3, 5-trimethyl-6-(3-pyridylmethyl)-1, 4-benzoquinone (**12**) which is found to be a scavenger of reactive oxygen species [122]. Betulinans A (**13**) and B (**14**) isolated from the methanolic extract of *Lenzites betulina* have been found to inhibit lipid peroxidation with IC_{50} values of 0.46 and 2.88 μg/ml respectively [123]. A series of arylthiolated 2, 3-dimethoxy-1, 4-benzoquinones have been found to be efficient exogenous radical scavengers for the inhibition of lipid peroxidation [124].



3.2. Anti-Inflammatory Activity

Choi *et al*. have studied the effect of ubiquinone on cutaneous healing in skin-incised mice and found that the treatment increased the level of collagen and collagen-like polymer and decreased the level myeloperoxidase and phospholipase A_2. These workers have also shown that ubiquinone did not act as antioxidant as judged by an *in vitro* DPPH assay but did possess potent antioxidant activity by reducing malondialdehyde and enhancing superoxide dismutase levels in cell cultures of Raw 264.7 cells [125]. Other workers have shown that the diet supplemented with fat-soluble antioxidants can suppress phosphorylation of NF-kB, I-kB kinase and SAPK/JNK proteins, thereby preventing the activation of NF-kB kinase and SAPK/JNK signalling pathways in LPS-treated mice suggesting the potential of such antioxidants in developing novel therapeutic combinations [126]. Recently, influence of ubiquinone in affecting expression of several genes involved in activation of inflammatory pathways has been elucidated. For example, Novoselova *et al*. have identified signalling pathways of G-protein coupled receptors, JAK/STAT, and integrin which have several ubiquinone sensitive genes. Consequently treatment with ubiquinone has been found to reduce the expression of LPS-induced TNF-α response in apoE-3 or apoE-4 cells respectively [127]. Lee and co-workers have shown that ubiquinone partially attenuated the effect of TNF-α on PPAR-γ but did not alter its effect on PPAR-α [128]. Chew and Watts suggested that ubiquinone improves endothelial dysfunction by re-coupling eNOS and mitochondrial oxidative phosphorylation and acting synergistically with anti-atherogenic agents such as fibrates and statins, to improve endotheliopathy in diabetes [129].

The effects of mitoquinone have been studied *in vitro* in an endothelial cell model of sepsis and *in vivo* in rat model of sepsis. Mitoquinone lowered rate of ROS formation, while maintaining mitochondrial membrane potential thereby lowering the oxidative stress and protecting mitochondria from damages. It also suppressed pro-inflammatory cytokine release from cells *in vitro*. The compound decreased the levels of biochemical markers of acute liver and renal dysfunction *in vivo*, while improving mitochondrial membrane potential in most of the organs as studied by lipopolysaccharide-peptidoglycan rat model of the organ dysfunction [130].

Idebenone was found to inhibit enzymatic metabolism of arachidonic acid in astroglial homogenates although it was less effective in platelets. Oxidised form of idebenone showed preferential cyclooxygenase inhibition, while the reduced form did not distinguish between cyclooxygenase and lipooxygenase forms [131].

Thymoquinone has been reported to be a potent inhibitor of leukotriene formation in human blood cells. Mansour and his group have studied effects of thymoquinone on leukotriene formation in human blood cells and have found concentration-dependent inhibition of both LTC4 and LTB4 formation from endogenous substrate with IC50 values of 1.8 and 2.3 μM respectively [132]. It also shows dose as well as time-dependent inhibition of both 5-lipooxgenase and leukotriene-C4 synthase (LT-4 synthase) activity. Since treatment with thymoquinone suppresses the production of NO by macrophages, it is helpful in ameliorating the inflammatory and autoimmune responses [133]. Treatment with thymoquinone showed decrease in the levels of myeloperoxidase and platelet activating factor, while glutathione level was found to be increased leading to complete protection against acetic acid-induced colitis in rats [111]. However, Juhas *et al.* were not able to confirm anti-inflammatory effects of thymoquinone in TNBS colitis [134].

The anti-inflammatory activity of thymoquinone was demonstrated in lipopolysaccharide (LPS)-induced pro-inflammatory cytokine production in RBL-2H3 cells which was mediated by inhibition of IL-5 and IL-13 mRNA expression as well as protein production but not the production of IL-10 [135]. The intraperitoneal administration of Black cumin seed oil (at doses between 100- 400 μl/kg) showed anti-inflammatory effect against carrageenan-induced paw edema in rats and croton oil-induced ear edema in mice. Thymoquinone also showed significant analgesic activity in acetic acid-induced writhing, formalin and light tail flick tests. Since analgesic effect of the oil could not be reversed by an opioid antagonist naloxone, it was concluded that opioid receptors were not involved in these analgesic effects

[136]. Thymoquinone has significant inhibitory effects on lipopolysaccharide-induced IL-5 and IL-13 mRNA expression, transcription of GATA-1 and -2 genes and protein production, but did not affect IL-10 production, expression of AP-1 protein subunits, c-Jun and c-Fos in rat mast cells [137]. It showed inhibitory effects on activation of the redox-sensitive transcription factor NF-kB and interleukin-6 (IL-6) *in vitro* [138].

Recently, Ragheb *et al.* have summarised the antioxidant and anti-inflammatory effects of thymoquinone in acute renal injury which indicate the therapeutic value of the compound in delaying end stage renal diseases in diabetics [139]. Kanter has shown that thymoquinone exerted protective effects by reducing morphological alterations and myelin breakdown in streptozotocin-induced diabetic rats indicating potential of thymoquinone treatment on peripheral neuropathy [140]. Collectively, these results suggest that NF-kB is a molecular target of thymoquinone among many other legitimate targets. El-Gazzar and colleagues have investigated the effect of thymoquinone on LPS-induced TNF-α production in the rat basophile cell line, RBL-2H3 [135]. They found that thymoquinone did not alter NF-kB cytosolic activation or nuclear expression in LPS-stimulated cells but it significantly increased the amount of the repressive NF-kB p50 homodimer and decreased the amount of trans-activating NF-kB p65:p50 heterodimer bound to the TNF-α promoter as revealed by electrophoretic mobility shift and chromatin immunoprecipitation assays. These results suggest that thymoquinone attenuates the pro-inflammatory response in LPS-stimulated mast cells by modulating nuclear transactivation of NF-kB and TNF-α production.

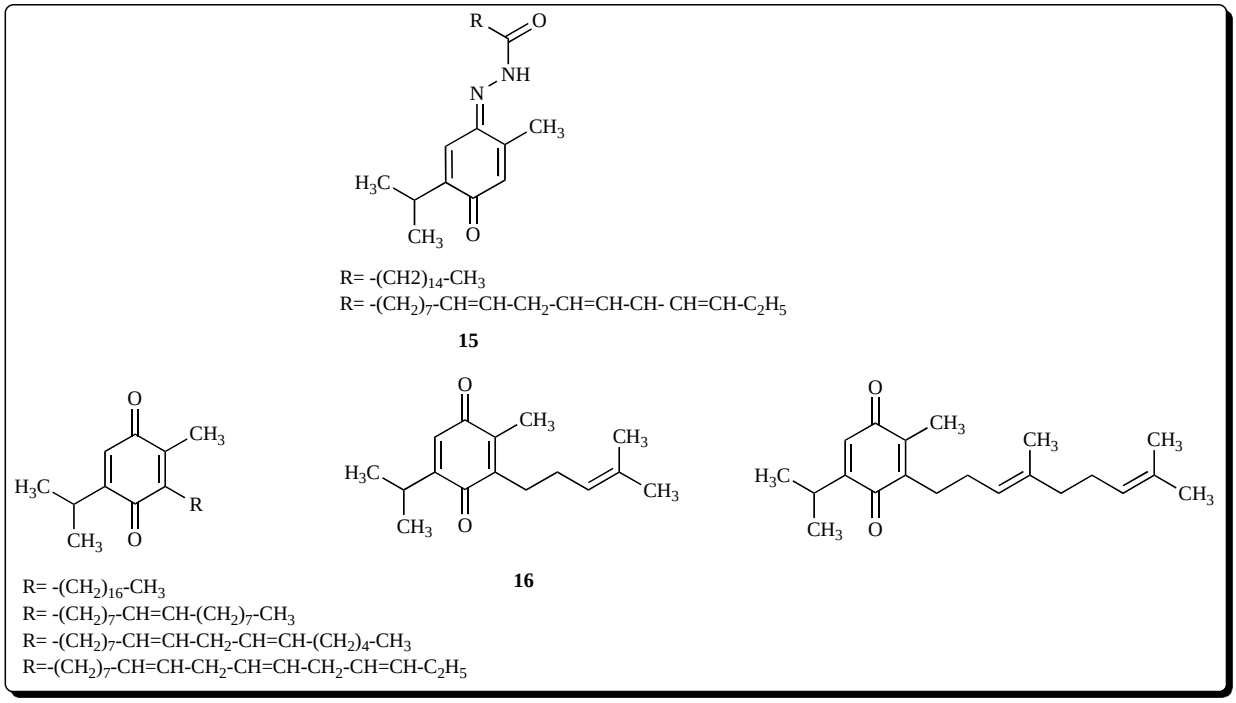
Embelin is known to produce analgesic and anti-inflammatory effects in animals [141]. The compound was found to inhibit both the inducible as well as constitutive forms of NF-kB activated by TNF-α, interleukin-1β, lipopolysaccharide, phorbol myristate acetate, okadaic acid, hydrogen peroxide, and cigarette smoke condensate. It showed suppression of NF-κB-dependent reporter gene transcription induced by TNF-α, TNF receptor-1 (TNFR1), TNFR1-associated death domain protein, TNFR-associated factor-2, NF-kB-inducing kinase, and I-kBα kinase respectively [142]. Gupta *et al.* have synthesised some semi-synthetic derivatives of embelin and have found that its di-salt also exhibit analgesic, antipyretic and anti-inflammatory activities [143].

The 17-allylamino-17-demethoxy derivatives of Geldanamycin (**20**) have been found to significantly suppress the LPS-induced increase in retinal leukocyte adhesion; vascular leakage; NF-kB and PI-3K activity as well as VEGF and IL-1β levels [144]. The potent anti-inflammatory effects of geldanamycin on brain glial cells have been suggested to be therapeutically beneficial in neuro-inflammatory diseases [145]. Malhotra and co-workers demonstrated that geldanamycin inhibited TNF-α-mediated IL-8 gene and NF-kB expression in A549 human respiratory epithelial cells by inhibiting activation of the IL-8 promoter. Geldanamycin showed inhibition of TNF-α-mediated luciferase activity in the cells transiently transfected with an IL-8 promoter-luciferase reporter plasmid. These workers concluded that geldanamycin acted directly by reducing the formation of the NF-kB/DNA complex [146]. The compound has also shown dose-dependent inhibition of carrageenan-induced mouse paw edema [147].

Alhosin *et al.* [148] have analyzed the effects of thymoquinone on p53-deficient acute lymphoblastic leukemia (ALL) Jurkat cell line. The results showed that thymoquinone inhibits the proliferation of Jurkat cells and induces G1 cell cycle arrest in a dose-dependent manner. Thymoquinone -induced apoptosis was confirmed by the presence of hypodiploid G0/G1 cells which was associated with a rapid and sharp re-expression of p73 and dose-dependent changes in the levels of caspase-3 cleaved subunits. Knockdown of p73 expression restores anti-apoptotic and epigenetic integrator (UHRF1) expression and reactivates cell cycle progression leading to inhibition of thymoquinone-induced apoptosis. El-Najjar and co-workers [149] have shown that thymoquinone inhibited proliferation of a panel of human colon cancer cells (Caco-2, HCT-116, LoVo, DLD-1 and HT-29), without exhibiting cytotoxicity to normal human intestinal FHs74Int cells. Further investigations on DLD-1 cells revealed that apoptotic cell death is the mechanism for thymoquinone-induced growth inhibition as confirmed by flow cytometry, M30 cytodeath and caspase-3 activation. Apoptosis was probably induced *via* generation of reactive oxygen species (ROS) as evidenced by the abrogation of thymoquinone apoptotic effect in cells pre-incubated with the strong anti-oxidant such as N-acetyl cysteine (NAC). Thus, pro-oxidant effects of thymoquinone are linked with its apoptotic effects in colon cancer and proving a protective role of MAPK.

In a recent study by Sarkar *et al,* [150] have reported for the first time, the chemosensitizing effect of thymoquinone to conventional chemotherapeutic agents both *in vitro* and *in vivo* using an orthotropic model of pancreatic cancer. *In vitro* studies revealed that pre-exposure of cells with thymoquinone (25 μM/L) for 48 h followed by gemcitabine or oxaliplatin resulted in 60-80% growth inhibition compared with 15-25%when gemcitabine or oxaliplatin was used alone. Moreover, it was observed that thymoquinone could potentiate the killing of pancreatic cancer cells induced by chemotherapeutic agents by down-regulation of NF-kB, Bcl-2 family, and NF-kB-dependent antiapoptotic genes (X-linked inhibitors of apoptosis, survivin, and cyclooxygenase-2). Interestingly, NF-kB, was inactivated in animal tumors pre-treated with thymoquinone. These results provide strong *in vivo* molecular evidence in support of the hypothesis that thymoquinone could abrogate gemcitabine- or oxaliplatin-induced activation of NF-kB, resulting in the chemosensitization of pancreatic tumors to conventional therapeutics.

Breyer and co-workers [151] have studied the 4-Acylhydrazones (**15**) and 6-alkyl derivatives of thymoquinone (**16**) and tested them for growth inhibition of human HL-60 leukemia, 518A2 melanoma, KB-V1/Vbl cervix, and MCF-7/Topo breast carcinoma cells. Unsaturated side chains conferred greater activities than equally long saturated chains. The 6-hencosahexaenyl conjugate was most active in all resistant tumor cells, with IC_{50} (72 h) values as low as 30 nM in MCF-7/Topo cells. The conjugates seem to operate by mechanisms different from those of thymoquinone, For example, the compounds induced distinct caspase-independent



apoptosis in HL-60 and 518A2 cells concomitant with a loss of mitochondrial membrane potential and subsequent rise in the levels of reactive oxygen species. It has been shown that thymoquinone triggers apoptosis in HCT-116 cells in a dose and time-dependent manner, which is associated with a 2.5 to 4.5 fold increase in p53 and $p21^{WAF1}$ mRNA expression and a significant decrease in Bcl-2 protein levels [152]. These results suggest role of thymoquinone in influencing cell cycle regulators involved in apoptosis as well as in down-regulating the anti-apoptotic proteins. Similar effects were observed on primary mouse keratinocytes, papilloma (SP-1) and spindle carcinoma cells respectively. At longer incubation times (48 h) the compound induced apoptosis in both cell lines by increasing the ratio of Bax/Bcl-2 protein expression and down-regulating the Bcl-xL protein.

Thymoquinone has been shown to initiate apoptosis *via* p53-independent pathways through activation of caspase-3, 8 and 9 in p53-null myeloblastic leukemia HL-60 cells [153]. It was observed that caspase-8 activity was highest after 1 h following the treatment of thymoquinone, while caspase-3 activity was highest after 6 h respectively. These observations are based upon the up-regulation of pro-apoptotic Bax protein along with down-regulation of anti-apoptotic Bcl-2 proteins resulting in enhanced Bax/Bcl-2 ratio. It is thus evident that thymoquinone induces apoptosis through multiple protein targets and can act as a potent phytochemical for treatment of various cancers. These results are also supported by reports in prostate and other cancer cells [12, 154, 155] A recent report has identified checkpoint kinase-1 homolog, CHEK1 which is a serine/threonine kinase, as the target of thymoquinone, leading to apoptosis in $p53^{+/+}$ colon cancer cells [156].

The principle activity of thymoquinone was found to be due to its effects on the expression of cell cycle regulatory proteins. The treatment of cells with 30 μM concentration of thymoquinone for 48 h induced G1 cell-cycle arrest in papilloma cells, which correlated with a sharp increase in the expression of the cyclin-dependent kinase inhibitor p16 and down-regulation of cyclin D1 protein expression [157]. In Flow cytometric studies of DNA content by propidium iodide staining it has been revealed that thymoquinone induces G1 cell-cycle arrest of osteosarcoma cancer cells (COS31) as well as human colon cancer cells (HCT-116), at 100μM concentration treated for 48 h [158]. Roepke and colleagues [159] have evaluated the anti-proliferative and pro-apoptotic effects of thymoquinone in two human osteosarcoma cell lines with different p53 mutation status. Cell viability was reduced more selectively in MG63 tumor cells than in normal human osteoblasts. Reindl and colleagues have reported that thymoquinone and its synthetic C-1 Schiff base analog, Poloxin, are good inhibitors of Plk-1 PBD *in vitro* and cause deregulation of its cellular localization, chromosomal defects, mitotic arrest and apoptosis in HeLa cells [160]. Ivankovic and colleagues [161] have investigated the anti-tumor activity of thymoquinone and its hydroquinone analog in L929 mouse fibroblasts and two other tumor cell lines, viz. squamous cell carcinoma (SCC VII) and fibrosarcoma (FsaR), respectively. Both compounds showed dose-dependent potent cytotoxicities, which was more pronounced in tumor cells compared to L929 normal fibroblasts. Badary and colleagues have shown that thymoquinone protects mice against benzopyrene-induced fore-stomach carcinogenesis and chromosomal aberrations in mouse bone marrow cells when supplemented in the drinking water [162].

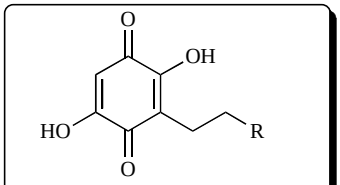
Tan and colleagues have examined the effects of thymoquinone on the proliferation and viability of PANC-1 cell line. The compound was found to be the potent inhibitor of human pancreatic carcinoma, reducing their propagation activities [163]. It significantly decreased proliferation and metastasis of human breast (MCF-7), colon (Caco-2) and

prostate (DU-145) cancer cells at concentration of 100 μM [164]. Thymoquinone has been explored further for combination with other known chemopreventive agents like selenomethione and lycopene on SiHa cells in the presence or absence of estrogen. Their results indicate that selenomethione alone appeared to be chemo-protective [165].

Dai *et al.* [166] have evaluated the effects of embelin, on colon cancer, with a particular focus on whether PPAR-γ is required for this compound to exert its effect. The compound inhibited proliferation and induced apoptosis in HCT116 cells with marked up-regulation of PPAR-γ. In addition, it significantly inhibited the expressions of survivin, cyclin D1, and c-Myc. It is known that PPAR-γ, PPAR-γ (+/-) mice are more susceptible to DMH-induced colon carcinogenesis than PPAR-γ (+/+) mice and embelin significantly reduced the incidence of colon cancer in PPAR-γ (+/+) mice but not in PPAR-γ (+/-) mice. It also inhibited NF-kB activity in PPAR-γ (+/+) mice but marginally so in PPAR-γ (+/-) mice.

Recently Xu and co-workers [167] have evaluated biological activities of the 5- O-ethylembelin and 5-O-methylembelin derivatives. The compounds exhibit antiproliferative activity against a panel of human tumor cell lines. They arrested HL-60 cells in the G(0)/G(1) phase of the cell cycle in a dose- and time-dependent manner. In HeLa cells, exposure to these compounds at 100 μM concentration for 6 h induced a complete disassembly of the microtubule network increasing number of cells blocked in mitotic stages. Treatment with 10 μM for 24 h induced apoptosis in HL-60 cells. This evidence suggests that both compounds are promising novel anti-mitotic and anticancermolecules targeting microtubular proteins. Mori *et al.* [168] have observed that TRAIL-induced apoptosis was restored by the combination of FLIP antisense and embelin. Since pancreatic cancer cells gain resistance to TRAIL-induced apoptosis *via* expression of the anti-apoptotic proteins XIAP and FLIP. The enhanced effect of TRAIL in the presence of FLIP antisense and embelin suggests that a low molecular weight XIAP inhibitor like embelin could be a lead compound for the development of effective XIAP inhibitors.

Chen and co-workers [169] have reported a series of new embelin analogs (**17**) as inhibitors of XIAP. The compounds were tested for the inhibitory activity of cell growth in MDA-MB-231 (2LMP) human breast cancer line and PC-3 human prostate cancer cell line. The most effective compound shows inhibition of cell growth with IC_{50} values of 5.0 and 5.5 μM in the MDA-MB-231 and PC-3 cell lines respectively. Podolak and co-workers [170] have evaluated cytotoxicity of embelin in murine melanoma (B16) and sarcoma (XC) cells, where it was found to be most active against XC cells, but slightly less active against B16 cells.

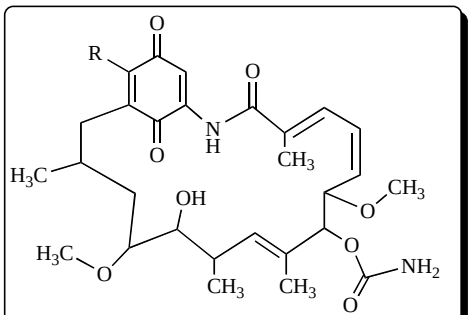


R= $-C_9H_{19}$, -H, $-C_2H_4-C_6H_5$,
$-C6H_5-C_4H_9$,$-C_6H_5-C2H_4-C_6H_5$

17

In a recent study Ahn and co-workers [133] found that embelin inhibited TNF-α- induced NF-kB activation. Both inducible and constitutive NF-kB activation were also abrogated by embelin. It also suppressed NF-kB-dependent reporter gene transcription as well as down-regulated gene products involved in cell survival, proliferation, invasion, and metastasis of the tumor. Thus it was concluded that embelin is a novel NF-kappaB blocker and potential suppressor of tumorigenesis.

In a study by Miyata [171] it was observed that geldanamycin (**18**) had anti-proliferative activity on tumor cells transformed by oncogene kinases such as v-Src. Geldanamycin neither binds nor inhibits oncogene kinases directly, but specifically binds and inhibits a major molecular chaperone, viz. Heat shock protein Hsp90. Geldanamycin specifically inhibits the essential ATPase activity of Hsp90. Thus, treatment of cells with Geldanamycin results in inactivation, destabilization, and degradation of Hsp90 client proteins. Since Hsp90 client proteins play important roles in regulation of cell cycle, cell growth, cell survival, apoptosis, and oncogenesis, Geldanamycin obstructs the proliferation of cancer cells and shows anti-cancer activity in experimental animals. Thecompounds 17-Dimethylaminoethylamino-17-demethoxygeldanamycin (DMAG, **19**) and 17-allylamino-17-demethoxygeldanamycin (17-AAG, **20**) are two derivatives of geldanamycin that are currently undergoing clinical evaluation as anticancer agents. These agents bind to heat shock protein 90 (Hsp90), resulting in destabilization of the client proteins and inhibition of tumor growth. Cysyk and coworkers [172] have found that geldanamycin and above derivatives react chemically (i.e., non-enzymatically) with glutathione. The reaction occurs at pH 7.0 and physiological concentration of glutathione, indicating that cellular glutathione could play a role in modulating the cellular toxicity of these agents and therefore be a factor in their mechanism of differential toxicity. Moreover, reactions with thiol groups of critical cellular proteins could be important to the mechanism of toxicity with this class of anticancer agents.

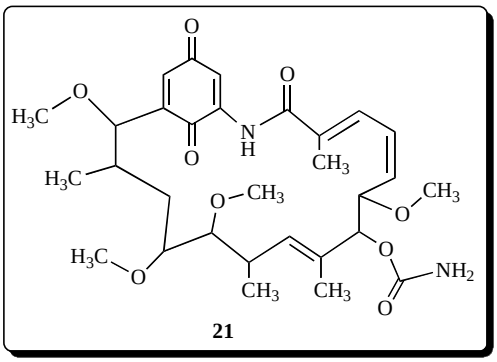


18 R= $-OCH_3$,
19 R= $-NH-CH_2-CH_2-N(CH_3)_2$,
20 R= $-NH-CH_2-CH=CH$

Using the glucocorticoid receptor as a model system Rosenhagen and co-workers [173] analyzed the effects of radicicol and various benzoquinone ansamycins. All com-

pounds efficiently abolished glucocorticoid receptor - dependent transactivation. Surprisingly, whenever one of the ansamycin was applied in combination with Radicilol, synergistic inhibition of glucocorticoid receptor-dependent transcription and hormone binding of glucocorticoid receptor was observed. In contrast, combination of two ansamycins showed no synergy. These observations may lead to the exploration of different ways to target the HSP proteins as potential anticancer target. Kaur *et al.* [174] have investigated the antiangiogenic properties of 17-(dimethylamino-ethylamino)-17-demethoxygeldanamycin, which is a water-soluble derivative, on HUVEC (human umbilical vein endothelial cells). The protein level of heat shock protein (Hsp) 90 and client proteins were examined by Western blot in FGF-2 and VEGF-stimulated HUVEC cells. *In vitro*, the compound inhibited the migration and the extracellular matrix-invasiveness of HUVEC and their capacity to form capillary like structures in Matrigel in dose-dependent manner.

The benzoquinonoid ansamycin antibiotics, geldanamycin and herbimycin A (**21**), are potent cytotoxins against tumor cells *in vitro*. Benchekroun and co-workers [175] have examined the mechanism of their *in vitro* cytotoxicity against human breastadenocarcinoma (MCF-7) cells and have found that multidrug-resistant MCF-7/ADR^R cells that exhibit the MDR phenotype and the overexpression of P-170-glycoprotein were cross-resistant to these two antibiotics. The compounds were reductively activated by the NADPH-cytochrome cytochrome P450-reductase and generated hydroxyl radicals. The formation of these radicals was significantly lower in resistant cells. These observations indicate that lowering of free radical formation and interactions with P170 glycoprotein may both be important contributing factors towards developing resistance against these agents. In an attempt to synthesize water soluble inhibitors of Hsp90, Tian *et al.* [176] have synthesized library of over sixty 17-alkylamino-17-demethoxygeldanamycin analogs and compared their affinity for Hsp90 and ability to inhibit growth of SKBr3 mammalian cells. Over 20 analogs showed cell growth inhibition potencies similar to that of 17-allylamino-17-demethoxygeldanamycin. One of the most potent and water-soluble analogs in the series was 17-(2-dimethylaminoethyl) amino-17-demethoxygeldanamycin. In a recent report by Yun and co-workers [177] the authors have investigated the mechanism of inhibition of TGF-beta signalling by geldanamycin. Western blot analysis revealed that Geldanamycin-induced degradation of TGF-β type I and type II receptors through a proteasome-dependent pathway.



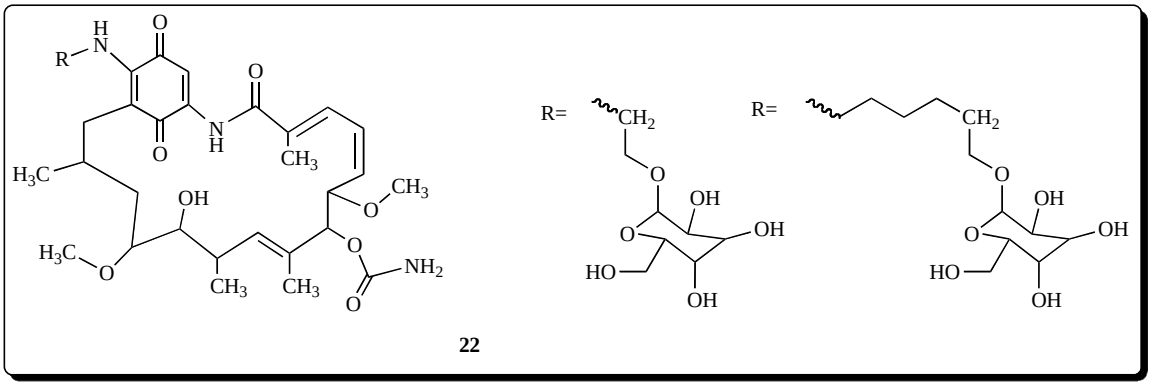
21

Rhabdomyosarcoma is a highly metastatic tumor, mostly observed in children and adolescence. Lukasiewicz *et al.* [178] have shown that geldanamycin and its analogs, can profoundly affect proliferation of rhabdomyosarcoma cell by blocking of HSP90 function and inducing apoptosis of tumor cells. Cells exposed to geldanamycin and its analogs exhibit strong reduction of MET receptor expression and subsequent inhibition of HGF-dependent tumor cell migration and invasion. In another study by Georgakis and co-workers, [179] the authors have determined the role of HSP90 in promoting growth and survival of Hodgkin's lymphoma and to determine the molecular consequences of inhibiting HSP90 function by 17-allylamino-17-demethoxy-geldanamycin. The compound induced cell cycle arrest and apoptosis, which were associated with a decrease in CDK 4, CDK 6, and polo-like kinase 1 (PLK-1) protein inducing apoptosis by caspase-dependent and caspase-independent mechanisms. Lee *et al.* [180] have synthesized a new series of geldanamycin derivatives using a semi-synthetic approach involving genetically engineered biosynthetic intermediates. These analogues were then evaluated for anti-proliferation activity in human cancer cell lines, SK-Br3 and SK-Ov3 respectively. Most of the synthesized compounds exhibited potent *in vitro* anti-proliferative activity toward both cell lines. Onyuksel and co-workers [181] have formulated one of the geldanamycin analog, (viz. 17-AAG) in biocompatible and biodegradable phospholipid nanomicelles (SSM). Cytotoxicity of these nanomicelles to MCF-7 cells is retained implying high affinity for VIP receptors overexpressed on these cells mediate their intracellular uptake thereby improving drug potency.

In an effort to reduce the severe toxicity of geldanamycin, Cheng and co-workers [182] have synthesized a series of carbohydrate-geldanamycin conjugates (**22**) for enzyme-specific activation and improving tumor selectivity. The conjugation was carried out at the C-17-position of geldanamycin. The anticancer activity was tested in a number of cancer cell lines and enzyme-specific activation was evaluated with *â*-galactosidase and *â*-glucosidase. The compounds showed anticancer activity with IC_{50} of 70.2-380.9 nM in various cancer cells. The results suggest that geldanamycin can be inactivated by glycosylation of C-17-position and reactivated for anticancer activity by *â*-galactosidase. Tian *et al.* [183] have synthesized C-11 modified derivatives of geldanamycin and have measured their affinity for Hsp90 as well as ability to inhibit growth of human cancer cells. These analogues showed *in vitro* cytotoxicity against number of human cancer cell lines.

3.4. Miscellaneous Activity

Fararh and co-workers [184] have examined the effect of thymoquinone on hyperglycemia and energy metabolism related enzymes in leukocytes of streptozotosized diabetic rats. Plasma glucose, cholesterol and triglycerides levels were significantly reduced after thymoquinone treatment, whereas immuno-reactive insulin levels showed significant increase. The activities of malate dehydrogenase in cytosolic and mitochondrial fractions of peripheral blood leukocytes were significantly higher in rats treated with thymoquinone and insulin as compared to those in diabetic controls. The results of this study demonstrated that thymoquinone significantly increased insulin level and activities of cytosolic and



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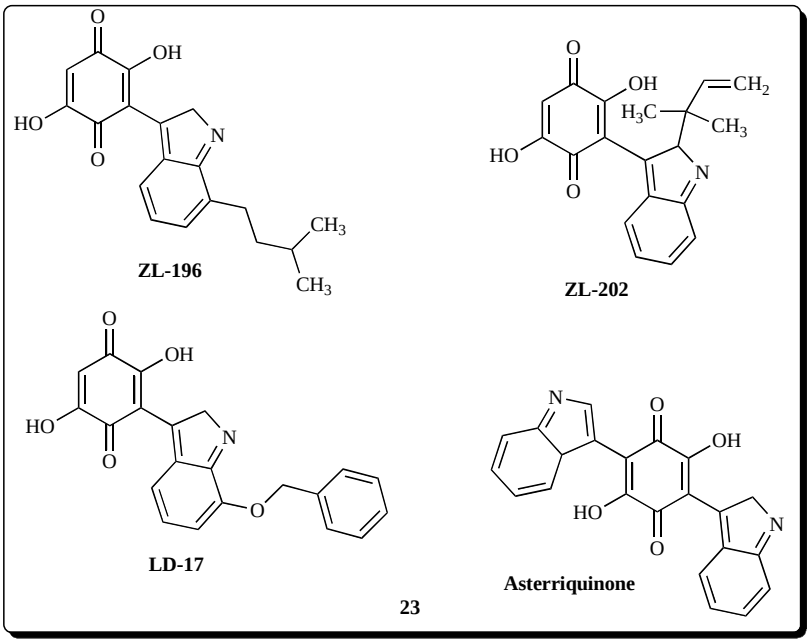
mitochondrial malate dehydrogenase in leukocytes of streptozotosized-diabetic rats. Pari *et al.* [185] have also evaluated antihyperglycemic potential of thymoquinone on the activities of key enzymes of carbohydrate metabolism in streptozotocin-nicotinamide-induced diabetes in rats. Oral administration of thymoquinone for 45 days improved the glycemic status in streptozotocin-nicotinamide induced diabetic rats in a dose-dependent manner. The levels of insulin and hemoglobin showed increase with significant decrease in glucose and glycated hemoglobin (HbA-1C) levels. The altered activities of carbohydrate metabolic enzymes were restored to near normal. No significant changes were noticed in normal rats treated with thymoquinone. Treatment of the compound reduced the glomerular size, thickening of capsular, glomerular and tubular basement membranes, increased amounts of mesangial matrix and tubular dilatation and renal function as compared with diabetics untreated. It was suggested that therapy with this compound offers renal morphological and functional improvement in streptozotocin-induced diabetes in rats.

Oral hypoglycemic agents have great potential for the treatment of both type-1 and type-2 diabetes. In this connection indole substituted benzoquinones (**23**) described by Lin *et al.* [186] are interesting compounds which are capable of activating the insulin receptor under *in vitro* and *in vivo* conditions and mimic the ability of insulin to stimulate glucose uptake, glycogen synthesis and lipid synthesis in 3T3-L1 adipocytes. However, the compounds did not mimic the mitogenic effects of insulin. In animals, these compounds have oral hypoglycemic effects in both normal C57BL6 mice as well as diabetic db/db mice.

Finally, Embelin has been screened for wound healing activity by excision, incision and dead space wound models on Swiss Albino Rats by Kumara Swamy and co-workers [187]. Significant wound healing activity was observed for embelin-treated group where epithelialization of the incision wound was faster with a high rate of wound contraction. The histological examination of the granulation tissue in embelin-treated group showed increased cross-linking of collagen.

4. CONCLUSIONS

Benzoquinone compounds are widely distributed in higher plants, fungi, bacteria and animal kingdom. They are involved in important biological functions such as bioener-



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getic transport, oxidative phosphorylation and electron transfer process. In recent years, it has become increasingly clear that some of them possess a wide range of potent medicinal properties and several possible mechanisms of action have been elucidated. Accumulating evidences suggest that some of these compounds can be developed as cancer chemopreventive agents and be useful in cancer therapy as chemosensitizers for existing anticancer drugs. The possible generation of reactive oxygen species during their use as therapeutic compounds raises some concern about their safety and hence long-term toxicity studies need to be conducted before their clinical use.

ACKNOWLEDGEMENTS

ACV is grateful to Head of Department of Environmental Sciences, University of Pune, Pune for extending the laboratory facilities while SBP would like to acknowledge encouragement and assistance received from the authorities of Dr. D. Y. Patil University, Pune.

REFERENCES

[1] Pink, J.J.; Planchon, S.M.; Tagliarino, C.; Varnes, M.E.; Siegel, D.; Boothman, D.A. NAD(P)H: Quinone oxidoreductase activity is the principal determinant of β-lapachone cytotoxicity. *J. Biol. Chem*., **2000**, *275*, 5416-5424.

[2] Ross, D.; Kepa, J.K.; Winski, S.L.; Beall, H.D.; Anwar, A.; Siegel, D. NAD(P)H: quinone oxidoreductase 1 (NQO1): chemoprotection, bioactivation, gene regulation and genetic polymorphisms. *Chem. Biol. Interact.*, **2000**, *129*, 77-97.

[3] Arntzen, C.J.; Pakrasi, H.B. Photosystem II reaction center: Polypeptide subunits and functional cofactors. In: *Encyclopedia of Plant Physiology*. New series, Staehelin; L.Al; Arntzen, C.J.; Eds.; Springer-Verlag:Berlin; **1986**, Vol *19*, pp. 457-467.

[4] Lindsey, R.H.; Jr, Bromberg, K.D.; Felix, C.A.; Osheroff, N. 1,4-Benzoquinone is a topoisomerase II poison. *Biochemistry*, **2004**, *43*, 7563-7574.

[5] Lee, C.S. Excision repair of 2, 5-diaziridinyl-1,4-benzoquinone (DZQ)-DNA adduct by bacterial and mammalian 3-methyladenine-DNA glycosylases. *Mol. Cell*, **2000**, *10*, 723-727.

[6] Beall, H.D.; Murphy, A.M.; Siegel, D.; Hargreaves, R.H.; Butler, J.; Ross, D. Nicotinamide adenine dinucleotide (phosphate): quinone oxidoreductase (DT-diaphorase) as a target for bioreductive antitumor quinones: quinone cytotoxicity and selectivity in human lung and breast cancer cell lines. *Mol. Pharmacol*., **1995**, *48*, 499-504.

[7] Winski, S.L.; Hargreaves, R.H.; Butler, J.; Ross, D. A new screening system for NAD(P)H:quinone oxidoreductase (NQO1)-directed antitumor quinones: identification of a new aziridinylbenzoquinone, RH1, as a NQO1-directed antitumor agent. *Clin. Cancer Res*., **1998**, *4*, 3083-3088.

[8] Sagnou, M.; Strongilos, A.; Hadjipavlou-Litina, D.; Couladouros, E. A. Synthesis of Novel Benzoquinones with Anti-Inflammatory Activity. *Lett. Drug Des. Discov*., **2009**, *6*, 172-177.

[9] Yezerski, A.; Ciccone, C.; Rozitski, J.; Volingavage, B. The effects of a naturally produced benzoquinone on microbes common to flour. *J. Chem. Ecol.*, **2007**, *33*, 1217-1225.

[10] Awino, O.S.; Kiprono, P.C.; Keronei, K.P.; Kaberia, F.; Obala, A.A. Antimicrobial activity of 2,5-dihydroxy-3-methyl-1,4-benzoquinone from Embelia schimperi. *Z Naturforsch. C*., **2008**, *63*, 47-50.

[11] Bogdanova, N.S.; Pershin, G.N.; Nikolaeva, I.S.; Grinev, A.N.; Shvedov, V.I. Antiviral activity of p-benzoquinone and hydroquinone derivatives. *Farmakol. Toksikol.*, **1970**, *33*, 488-496.

[12] Aggarwal, B.B.; Kunnumakkara, A.B.; Harikumar, K.B.; Tharakan, S.T.; Sung, B.; Anand, P. Potential of Spice-Derived Phytochemicals for Cancer Prevention. *Planta Med*., **2008**, *74*, 1560-1569.

[13] Padhye, S.; Banerjee, S.; Ahmad, A.; Mohammad, R.; Sarkar, F.H. From here to eternity - the secret of Pharaohs: Therapeutic potential of black cumin seeds and beyond. *Cancer Ther*., **2008**, *6*, 495-510.

[14] Packter, N. M. Studies on the biosynthesis of quinones in fungi. Incorporation of 6-methylsalicylic acid into fumigatin and related compounds in *Aspergillus fumigatus* I.M.I. 89353, *Biochem J*., **1965**, *97*, 321-332.

[15] Thomson, R.H. *Naturally Occurring Quinones*, 2nd ed. Academic Press: London, **1971**.

[16] Mikami, Y.; Takahashi, K.; Yazawa, K.; Arai, T.; Namikoshi, M.; Iwasaki, S.; Okuda, S. Biosynthetic studies on Safaramycin A: Quinone antitumor antibiotic produced by *Streptomyces lavendulae*. *J. Biol.Chem.*, **1985**, *260*, 344-348.

[17] Morton, R.A. *Biochemistry of Quinones*, Academic Press: New York, **1965**.

[18] Patel, I.C.; Skauen, D.M. Ultrasonic extraction of *Cassia acutifolia*. *J.Pharm.Sci.*, **1969**, *58*,1135-1139.

[19] Scholz, F.; Düsse, H.; Meyer, B. A new pH-sensor based on quinhydrone. *Fresenius' J. Anal. Chem.*, **1993**, *347*, 458-459.

[20] Hashimoto, M.; Takagia, H.; Yamamuraa, K. Three dimensional supramolecules of triptycene-quinone and its 6, 7-dimethyl derivative formed by weak intermolecular π-π interactions and C-H···O hydrogen bonds. *Tetrahedron. Lett.*, **1999**, *40*, 6037-6040.

[21] Berkovitch-Yellin, Z.; Leiserowitz, L. The role played by C-H···O and C-H···N interactions in determining molecular packing and conformation. *Acta Crystallogr*., **1984**, *B40*, 159-165.

[22] Laskowski, D. E. Influence of structural features on formation of solid charge-transfer complexes of quinones with carcinogenic and related non-carcinogenic electron donors. *Cancer Res.*, **1967**, *27*, 903-911.

[23] Inaba, K.; Takahashi, Y-H.; Ito. K.; Hayashi, S. Critical role of a thiolate-quinone charge transfer complex and its adduct form in de novo disulfide bond generation by DsbB. *Proc. Natl. Acad. Sci. USA*, **2006**, *103*, 287-292.

[24] Bangal, P. R. Hydrogen-bonding and protonation effects on the formation of charge transfer complex between para-benzoquinone and 2,6-dimethoxy phenol. *Chem. Phys. Lett*., **2005**, *401*, 200-204.

[25] Regeimbal, J,; Gleiter, S.; Trumpower, B. L.; Yu, C-A.; Diwakar, M.; Ballou, D. P.;Bardwell, J. C.A. Disulfide bond formation involves a quinhydrone-type charge-transfer complex. *Proc. Natl. Acad. Sci. USA*, **2003**, *100*, 13779-13784.

[26] Fedyanin, I. V.; Lyssenko, K. A.; Vorontsov,a, N. V.; Rozenberg, V.I.; Antipin, M. Y. Transannular interaction in 4,7-[2.2] paracyclophane quinone. *Mendleev Commun*., **2003**, *13*, 15-16.

[27] Sugiyama, H.; Kamogawa, H. Studies on polymers containing functional groups. III. Chargetransfer interaction between quinone and aza polymers. *J. Polym. Sci. Part A-1: Polym. Chem.*, **2003**, *4*, 2281-2288

[28] Uliana, M. P., Vieira, Y. W.; Donatoni, M.C.; Corrêa, A. G.; Brocksom, U.; Brocksom, T.J. Oxidation of Mono-Phenols to *para*-Benzoquinones: a Comparative Study. *J. Braz. Chem. Soc*., **2008**, *19*, 1484-1489.

[29] Thiele, J. Thiele Reaction: Formation of triacetoxy aromatic compounds. *Ber*., **1898**, *31*, 1247-1249.

[30] Hikosaka, A. The reaction of aliphatic amines with p-benzoquinone, The effect of the alkyl group of amines on the reaction. *Bull. Chem. Soc. Jpn*., **1970**, *43*, 3928-3929.

[31] Harley-Mason and Laird, A.H. Isolation and structure of the fluorescent substances formed in the oxidative reaction of adrenaline and noradrenaline with ethylenediamine. *Tetrahedron*, **1959**, *7*, 70-76.

[32] Asahara, T.; Seno, M.; Teshirogi, T. Reaction of p-benzoquinone derivatives with ethylenediamine. *Bull. Chem. Soc. Jpn*., **1971**, *44*, 1687-1689.

[33] Saeed, A. E. M., and Omer, N. M. A. Synthesis of some 2,5- diamino-3,6- dibromo -1,4-Benzoquinones. *Afr. J. Pure. Appl. Chem*., **2009**, *3*, 275-280.

[34] Cameron, D.W.; Feutrill, G.I.; Keep, P.L.C. Dichloro quinones as dienophiles: synthesis of alizarin derivatives. *Tetrahedron Lett.*, **1989**, *30*, 5173-5176.

[35] Brown, J.R.; Imam, S.H. Recent studies on doxorubicines and its analougues. In: *Progress in Medicinal Chemistry*, Ellis, G.P.; West, G.B., Eds. Elsevier Science Publisher B.V.: Amsterdam, **1984**; Vol. *21*, pp. 169-236.

[36] Kende, A.S.; Bentley, T.J.; Mader, R.A.; Ridge, D. Simple total synthesis of (+)-dendrobine. *J. Am. Chem. Soc*., **1974**, *96*, 4332-4334.

[37] Lora-Tamayo, M. Some results obtained in the utilisation of quinones as philodiene. *Tetrahedron*, **1958**, *4*, 17-25.

[38] Inouye, Y.; Kakisawa, H. Diels-Alder reaction of benzoquinone. 1. Reactions of methoxy-benzoquinone with styrene and o-methoxystyrene. *Bull. Chem. Soc. Jpn.*, **1971**, *44*, 563-564.

[39] Vieira, Y. W.; Brocksom T. J. In: *Vitamin K3 synthesis by a multicomponent Diels-Alder reaction, using microwave irradiation*, 13th Brazilian meeting on organic synthesis, São Carlos, SP-Brazil, **2009**, pp. 565-905.

[40] Buckle, R. N.; Liu, P.; Roberts, E. W.D; Burnell, D. J. Differences in rates of diels-alder reactions as experimental indicators of synchronous or asynchronous transition states. *Tetrahedron*, **1999**, *55*, 11455-11464.

[41] Sollner, S.; Deller, S.; Macheroux, P.; Palfey BA. Mechanism of flavin reduction and oxidation in the redox-sensing quinone reductase Lot6p from Saccharomyces cerevisiae. *Biochemistry*, **2009**, *48*, 8636-8643.

[42] Sollner, S., Schober, M., Wagner, A., Prem, A., Lorkova, L., Palfey, B.A., Groll, M., Macheroux, P. Quinone reductase acts as a redox switch of the 20S yeast proteasome. *EMBO Rep.*, **2008**, *10*, 65-70.

[43] Malpica, R.; Franco, B.; Rodriguez, C.; Kwon, O.; Georgellis, D. Identification of a quinonesensitive redox switch in the ArcB sensor kinase. *Proc. Natl. Acad. Sci. USA*, **2004**, *101*, 13318-13323.

[44] Moore, H.W. Bioactivation as a model for drug design. Bioreductive alkylation. *Science*, **1977**, *197*, 527-532.

[45] Kleyer, D.L.; Koch, T.H. Mechanistic investigation of reduction of daunomycin and 7-deoxydaunomycinone with bi-(3, 5, 5-trimethyl-2-oxomorpholin-3-yl). *J. Am. Chem. Soc.*, **1984**, *106*, 2380-2387.

[46] Bachur, N.R.; Gee, M.V.; Friedman, R.D. Nuclear catalyzed antibiotic free radical formation. *Cancer Res.*, **1982**, *42*, 1078-1081.

[47] Bachur, N.R.; Gordon, S.L.; Gee, M.V. A general mechanism for microsomal activation of quinone anticancer agents to free radicals. *Cancer Res.*, **1978**, *38*, 1745-1750.

[48] Dallner, G.; Stocker, R. Coenzyme Q. In: *Encyclopedia of dietary supplements*, Marcel Dekker: New York, **2005**, pp.121-131

[49] Groneberg, D.A.; Kindermann, B.; Althammer, M.; Klapper, M.; Vormann, J.; Littarru, G.P.; Döring, F. Coenzyme Q10 affects expression of genes involved in cell signalling, metabolism and transport in human CaCo-2 cells. *Int. J. Biochem. Cell Biol.*, **2005**, *37*, 1208-1218.

[50] Sales Santos, I.M.; de Freitas, R.L.; da Silva, E.P.; Feitosa, C.M.; Saldanha, G.B.; Souza, G.F.; da RochaTomé, A.; Feng, D.; de Freitas, R.M. Effects of ubiquinone on hydroperoxide concentration and antioxidant enzymatic activities in the rat hippocampus during pilocarpine-induced seizures. *Brain Res.*, **2010**, *1315*, 33-40.

[51] Littarru, G.P.; Tiano, L. Bioenergetic and antioxidant properties of coenzyme Q10: recent developments. *Mol. Biotechnol.*, **2007**, 37, 31-37.

[52] Stocker, R.; Bowry, V.W.; Frei, B. Ubiquinol-10 protects human low density lipoprotein more efficiently against lipid peroxidation than does a-tocopherol. *Proc. Natl. Acad. Sci. USA*, **1991**, *88*, 1646-1650.

[53] Witting, P.K.; Pettersson, K.; Letters, J.; Stocker, R. Anti-atherogenic effect of coenzyme Q10 in apolipoprotein E gene knockout mice. *Free Radic. Biol. Med.*, **2000**, *29*, 295-305.

[54] Digiesi, V.; Cantini, F.; Oradei, A.; Bisi, G.; Guarino, G.C.; Brocchi, A.; Bellandi, F.; Mancini, M.; Littarru, G.P. Coenzyme Q10 in essential hypertension. *Mol. Aspects Med.*, **1994**, *15*, 257-263.

[55] Belardinelli, R.; Muçaj, A.; Lacalaprice, F.; Solenghi, M.; Seddaiu, G.; Principi, F.; Tiano, L.; Littarru, G.P. Coenzyme Q10 and exercise training in chronic heart failure. *Eur. Heart J.*, **2006**, *27*, 2675-2681.

[56] Kwong, L.K.; Kamzalov, S.; Rebrin, I.; Bayne, A.C.; Jana, C.K.; Morris, P.; Forster, M.J.; Sohal, R.S. Effects of coenzyme Q (10) administration on its tissue concentrations, mitochondrial oxidant generation, and oxidative stress in the rat. *Free Radic. Biol. Med.*, **2002**, *33*, 627-638.

[57] Sohal, R.S.; Kamzalov, S.; Sumien, N.; Ferguson, M.; Rebrin, I.; Heinrich, K.R.; Forster, M.J. Effect of coenzyme Q10 intake on endogenous coenzyme Q content, mitochondrial electron transport chain, antioxidative defenses, and life span of mice. *Free Radic. Biol. Med.*, **2006**, *40*, 480-487.

[58] Tiano, T., Belardinelli, R., Carnevali, P., Principi, F., Seddau, G., Littarru, G. P. Effect of Coenzyme Q10 administration on endothelial function and extracellular superoxide dismutase in patients with ischemic heart disease: a double blind randomized controlled study. *Eur. Heart J.*, **2007**, *28*, 2249-2255.

[59] Mancuso, M.; Orsucci, D.; Volpi, L.; Calsolaro, V.; Siciliano, G. Coenzyme Q10 in neuromuscular and neurodegenerative disorders. *Curr. Drug Targets*, **2010**, *11*, 111-121.

[60] Ratnam, D.V.; Chandraiah, G.; Meena, A.K.; Ramarao, P.; Kumar, M.N. The co- encapsulated antioxidant nanoparticles of ellagic acid and coenzyme Q10 ameliorates hyperlipidemia in high fat diet fed rats. *J. Nanosci. Nanotechnol.*, **2009**, *9*, 6741-6746.

[61] Sohet, F.M.; Neyrinck, A.M.; Pachikian, B.D.; de Backer, F.C.; Bindels, L.B.; Niklowitz, P.; Menke, T.; Cani, P.D.; Delzenne, N.M. Coenzyme Q10 supplementation lowers hepatic oxidative stress and inflammation associated with diet-induced obesity in mice. *Biochem. Pharmacol.*, **2009**, *78*, 1391-1400.

[62] Kumar, A.; Kaur, H.; Devi, P.; Mohan, V. Role of coenzyme Q10 (CoQ10) in cardiac disease, hypertension and Meniere-like syndrome. *Pharmacol. Ther.*, **2009**, *124*, 259-268.

[63] Stawiarska-Pieta, B.; Paszczela, A.; Grucka-Mamczar, E.; Szaflarska-Stojko, E.; Birkner, E. The effect of antioxidative vitamins A and E and coenzyme Q on the morphological picture of the lungs and pancreata of rats intoxicated with sodium fluoride. *Food Chem. Toxicol.*, **2009**, *47*, 2544-2550.

[64] Okello, E.; Jiang, X.; Mohamed, S.; Zhao, Q.; Wang, T. Combined statin/coenzyme Q10 as adjunctive treatment of chronic heart failure. *Med. Hypotheses*, **2009**, *73*, 306-308.

[65] Yang, L.; Calingasan, N.Y.; Wille, E.J.; Cormier, K.; Smith, K.; Ferrante, R.J.; Beal, M.F. Combination therapy with coenzyme Q10 and creatine produces additive neuroprotective effects in models of Parkinson's and Huntington's diseases. *J. Neurochem.*, **2009**, *109*, 1427-1439.

[66] Cordero, M.D.; Moreno-Fernández, A.M.; Gomez-Skarmeta, J.L.; de Miguel, M.; Garrido-Maraver, J.; Oropesa-Avila, M.; Rodríguez-Hernández, A.; Navas, P.; Sánchez-Alcázar, J.A. Coenzyme Q10 and alpha-tocopherol protect against amitriptyline toxicity. *Toxicol. Appl. Pharmacol.*, **2009**, *235*, 329-337.

[67] Sena, C.M.; Nunes, E.; Gomes, A.; Santos, M.S.; Proença, T.; Martins, M.I.; Seiça, R. Supplementation of coenzyme Q10 and alpha-tocopherol lowers glycated hemoglobin level and lipid peroxidation in pancreas of diabetic rats. *Nutr. Res.*, **2008**, *28*, 113-121.

[68] Ayaz, M.; Tuncer, S.; Okudan, N.; Gokbel, H. Coenzyme Q (10) and alpha-lipoic acid supplementation in diabetic rats: conduction velocity distributions. *Methods Find Exp. Clin. Pharmacol.*, **2008**, *30*, 367-374.

[69] Nakajima, Y.; Inokuchi, Y.; Nishi, M.; Shimazawa, M.; Otsubo, K.; Hara, H. Coenzyme Q10 protects retinal cells against oxidative stress *in vitro* and *in vivo*. *Brain Res.*, **2008**, *1226*, 226-233.

[70] Kim, D.W.; Hwang, I.K.; Kim, D.W.; Yoo, K.Y.; Won, C.K.; Moon, W.K.; Won, M.H. Coenzyme Q{10} effects on manganese superoxide dismutase and glutathione peroxidase in the hairless mouse skin induced by ultraviolet B irradiation. *Biofactors*, **2007**, *30*, 139-147.

[71] Ochoa, J.J.; Quiles, J.L.; López-Frías, M.; Huertas, J.R.; Mataix, J. Effect of lifelong coenzyme Q10 supplementation on age-related oxidative stress and mitochondrial function in liver and skeletal muscle of rats fed on a polyunsaturated fatty acid (PUFA)-rich diet. *J. Gerontol. A. Biol. Sci. Med. Sci.*, **2007**, *62*, 1211-1218.

[72] Yuvaraj, S.; Premkumar, V.G.; Vijayasarathy, K.; Gangadaran, S.G.; Sachdanandam, P. Ameliorating effect of coenzyme Q10, riboflavin and niacin in tamoxifen-treated postmenopausal breast cancer patients with special reference to lipids and lipoproteins. *Clin. Biochem.*, **2007**, *40*, 623-628.

[73] Upaganlawar, A.; Farswan, M.; Rathod, S.; Balaraman, R. Modification of biochemical parameters of gentamicin nephrotoxicity by coenzyme Q10 and green tea in rats. *Indian J. Exp. Biol.*, **2006**, *44*, 416-418.

[74] Armstrong, J.S.; Whiteman, M.; Rose, P.; Jones, D.P. The Coenzyme Q10 analog decylubiquinone inhibits the redox-activated mitochondrial permeability. *J. Biol. Chem.*, **2003**, *278*, 49079-49084.

[75] Sala, G.; Trombin, F.; Beretta, S.; Tremolizzo, L.; Presutto, P.; Montopoli, M.; Fantin, M.; Martinuzzi, A.; Carelli, V.; Ferrarese, C. Antioxidants partially restore glutamate transport defect in leber hereditary optic neuropathy cybrids. *J. Neurosci. Res.*, **2008**, *86*, 3331-3337.

[76] Bergamasco, B.; Scarzella, L.; La Commare, P. Idebenone, a new drug in the treatment of cognitive impairment in patients with dementia of the Alzheimer type. *Funct. Neurol*., **1994**, *9*, 161-168.

[77] Gillis, J.C.; Benefield, P.; McTavish, D. Idebenone. A review of its pharmacodynamic and pharmacokinetic properties, and therapeutic use in age-related cognitive disorders. *Drugs Aging*, **1994**, *5*, 133-152.

[78] Suno, M.; Nagaoka, A. Inhibition of lipid peroxidation by a novel compound, idebenone (CV-2619). *Jpn. J. Pharmacol.*, **1984**, *35*,196-198

[79] Hirai, K.; Hayako, H.; Kato, K.; Miyamoto, M. Idebenone protects hippocampal neurons against amyloid beta-peptide-induced neurotoxicity in rat primary cultures. *Naunyn Schmiedebergs Arch. Pharmacol.*, **1998**, *358*, 582-585.

[80] Voronkova, K.V.; Meleshkov, M.N. Use of Noben (idebenone) in the treatment of dementia and memory impairments without dementia. *Neurosci. Behav. Physiol.*, **2009**, *39*, 501-506.

[81] Muscoli, C.; Fresta, M.; Cardile, V.; Palumbo, M.; Renis, M.; Puglisi, G.; Paolino, D.; Nisticò, S.; Rotiroti, D.; Mollace, V. Ethanol-induced injury in rat primary cortical astrocytes involves oxidative stress: effect of Idebenone. *Neurosci. Lett.*, **2002**, *329*, 21-24.

[82] Palumbo, M.; Russo, A.; Cardile, V.; Renis, M.; Paolino, D.; Puglisi, G.; Fresta, M. Improved antioxidant effect of Idebenone-loaded polyethyl-2-cyanoacrylate nanocapsules tested on human fibroblasts. *Pharm. Res.*, **2002**, *19*, 71-78.

[83] Grieb, P.; Ryba, M.S.; Debicki, G.S.; Gordon-Krajcer, W.; Januszewski, S.; Chrapusta, S.J. Changes in oxidative stress in the rat brain during post-cardiac arrest reperfusion, and the effect of treatment with the free radical scavenger Idebenone. *Resuscitation*, **1998**, *39*, 107-113.

[84] Mordente, A.; Martorana, G.E.; Minotti, G.; Giardina, B. Antioxidant properties of 2, 3-dimethoxy-5-methyl-6-(10-hydroxydecyl)-1, 4-benzoquinone (idebenone). *Chem. Res. Toxicol.*, **1998**, *11*, 54-63.

[85] Fink, B.D.; O'Malley, Y.; Dake, B.L.; Ross, N.C.; Prisinzano, T.E.; Sivitz, W.I. Mitochondrial targeted coenzyme Q, superoxide, and fuel selectivity in endothelial cells. *PLoS One*, **2009**, *4*, e4250.

[86] Tauskela, J.S. MitoQ-a mitochondria-targeted antioxidant. *IDrugs*, **2007**, *10*, 399-412.

[87] Subramanian, S.; Kalyanaraman, B.; Migrino, R.Q. Mitochondrially targeted antioxidants for the treatment of cardiovascular diseases. *Recent Pat. Cardiovasc. Drug Discov.*, **2009**, *5*, 54-65.

[88] Milagros, R.M.; Victor, V.M. Targeting antioxidants to mitochondria and cardiovascular diseases: the effects of mitoquinone. *Med. Sci. Monit.*, **2007**, *13*, RA132-145.

[89] Kruk, J., Jemiola-Rzeminska, M., Strzalka, K. Plastoquinol and α-tocopherol quinol are more active than ubiquinol and α-tocopherol in inhibition of lipid peroxidation. *Chem. Phys. Lipids*, **1997**, *87*, 73-80.

[90] Loshadkin, D., Roginsky, V., and Pliss, E. Substituted p-hydroquinones as a chain-breaking antioxidant during the oxidation of styrene. *Int. J. Chem. Kinet.*, **2002**, *34*, 162-171.

[91] Roginsky, V., Barsukova, T., Loshadkin, D., and Pliss, E. Substituted *p*-hydroquinones as inhibitors of lipid peroxidation. *Chem. Phys. Lipids*, **2003**, *125*, 49-58.

[92] Antonenko, Y.N.; Avetisyan, A.V.; Bakeeva, L.E.; Chernyak, B.V.; Chertkov, V.A.; Domnina, L.V.; Ivanova, O.Y.; Izyumov, D.S.; Khailova, L.S.; Klishin, S.S.; Korshunova, G.A.; Lyamzaev, K.G.; Muntyan, M.S.; Nepryakhina, O.K.; Pashkovskaya, A.A.; Pletjushkina, O.Y.; Pustovidko, A.V.; Roginsky, V.A.; Rokitskaya, T.I.; Ruuge, E.K.; Saprunova, V.B.; Severina, I.I.; Simonyan, R.A.; Skulachev, I.V.; Skulachev, M.V.; Sumbatyan, N.V.; Sviryaeva, I.V.; Tashlitsky, V.N.; Vassiliev, J.M.; Vyssokikh, M.Y.; Yaguzhinsky, L.S.; Zamyatnin, A.A. Jr; Skulachev, V.P. Mitochondria-targeted plastoquinone derivatives as tools to interrupt execution of the aging program. 1. Cationic plastoquinone derivatives: synthesis and *in vitro* studies. *Biochemistry (Mosc).*, **2008**, *73*, 1273-1287.

[93] Antonenko, Y.N.; Roginsky, V.A.; Pashkovskaya, A.A.; Rokitskaya, T.I.; Kotova, E.A.; Zaspa, A.A.; Chernyak, B.V.; Skulachev, V.P. Protective effects of mitochondria-targeted antioxidant SkQ in aqueous and lipid membrane environments. *J. Membr. Biol.*, **2008**, *222*, 141-149

[94] Bakeeva, L.E.; Barskov, I.V.; Egorov, M.V.; Isaev, N.K.; Kapelko, V.I.; Kazachenko, A.V.; Kirpatovsky, V.I.; Kozlovsky, S.V.; Lakomkin, V.L.; Levina, S.B.; Pisarenko, O.I.; Plotnikov, E.Y.; Saprunova, V.B.; Serebryakova, L.I.; Skulachev, M.V.; Stelmashook, E.V.; Studneva, I.M.; Tskitishvili, O.V.; Vasilyeva, A.K.; Victorov, I.V.; Zorov, D.B.; Skulachev, V.P. Mitochondria-targeted plastoquinone derivatives as tools to interrupt execution of the aging program. 2. Treatment of some ROS- and age-related diseases (heart arrhythmia, heart infarctions, kidney ischemia, and stroke). *Biochemistry (Mosc)*, **2008**, *73*, 1288-1299.

[95] Iwashima, M.; Mori, J.; Ting, X.; Matsunaga, T.; Hayashi, K.; Shinoda, D.; Saito, H.; Sankawa, U.; Hayashi, T. Antioxidant and antiviral activities of plastoquinones from the brown alga *Sargassum micracanthum*, and a new chromene derivative converted from the plastoquinones. *Biol. Pharm. Bull.*, **2005**, *28*, 374-377.

[96] Tageja, N.; Padhye, S.; Dandawate, P.; Al-Katib, A; Mohammad, R.M. New targets for the treatment of follicular lymphoma. *J. Hematol. Oncol.*, **2009**, *23*, 2:50

[97] Al-Ali, A.; Alkhawajah, A.A.; Randhawa, M.A.; Shaikh, N.A. Oral and intraperitoneal LD50 of thymoquinone, an active principle of Nigella sativa, in mice and rats. *J. Ayub. Med. Coll. Abbottabad*, **2008**, *20*, 2025-2027.

[98] Ragheb, A.; Attia, A.; Elbarbry, F.; Prasad, K.; Shoker, A. Attenuated combined action of cyclosporine a and hyperlipidemia on atherogenesis in rabbits by thymoquinone. *Evid Based Complement Alternat. Med*., **2009**, [Epub ahead of print]

[99] Ismail, M.; Al-Naqeep, G.; Chan, K.W. Nigella sativa thymoquinone-rich fraction greatly improves plasma antioxidant capacity and expression of antioxidant genes in hypercholesterolemic rats. *Free Radic. Biol. Med*., **2009**, *48*, 664-672.

[100] Hamdy, N.M.; Taha, R.A. Effects of Nigella sativa oil and thymoquinone on oxidative stress and neuropathy in streptozotocin-induced diabetic rats. *Pharmacology*, **2009**, *84*, 127-134.

[101] Chandra, S.; Mondal, D.; Agrawal, K.C. HIV-1 protease inhibitor induced oxidative stress suppresses glucose stimulated insulin release: protection with thymoquinone. *Exp. Biol. Med. (Maywood).*, **2009**, *234*, 442-453.

[102] Fouda, A.M.; Daba, M.H.; Dahab, G.M.; Sharaf El-Din, O.A. Thymoquinone ameliorates renal oxidative damage and proliferative response induced by mercuric chloride in rats. *Basic Clin. Pharmacol. Toxicol*., **2008**, *103*, 109-118.

[103] Kruk, I.; Michalska, T.; Lichszteld, K.; Kladna, A.; boul-Enein. H.Y. The effect of thymol and its derivatives on reactions generating reactive oxygen species. *Chemosphere*, **2000**, *41*, 1059-1064.

[104] Mansour, M.A.; Nagi, M.N.; El-Khatib, A.S.; Al-Bekairi, A.M.. Effects of thymoquinone on antioxidant enzyme activities, lipid peroxidation and DT- diaphorase in different tissues of mice: a possible mechanism of action. *Cell. Biochem. Funct.*, **2002**, *20*, 143-151.

[105] Al-Enazi, M.M. Effect of thymoquinone on malformations and oxidative stress-induced diabetic mice. *Pak. J. Biol. Sci.*, **2007**, *10*, 3115-9.

[106] Badary, O.A.; al-Shabanah, O.A.; Nagi, M.N.; al-Rikabi, A.C.; Elmazar, M.M. Inhibition of benzo(a)pyreneinduced forestomach carcinogenesis in mice by thymoquinone. *Eur. J. Cancer Prev.*, **1999**, *8*, 435-440.

[107] Badary, O.A.; Abd-Ellah, M.F.; El-Mahdy, M.A.; Salama, S.A.; Hamada, F.M. Anticlastogenic activity of thymoquinone against benzo(a)pyrene in mice. *Food Chem. Toxicol.*, **2007**, *45*, 88-92.

[108] Badary, O.A.; Abdel-Naim, A.B.; Abdel-Wahab, M.H.; Hamada, F.M. The influence of thymoquinone on doxorubicin-induced hyperlipidemic nephropathy in rats. *Toxicology*, **2000**, *143*, 219-226.

[109] Sayed-Ahmed, M.M.; Nagi, M.N. Thymoquinone supplementation prevents the development of Gentamicin induced acute renal toxicity in rats. *Clin. Exp. Pharmacol. Physiol.*, **2007**, *34*, 399-405.

[110] El-Saleh, S.C.; Al-Sagair, O.A.; Al-Khalaf, M.I. Thymoquinone and Nigella sativa oil protection against methionine-induced hyperhomocysteinemia in rats. *Int. J. Cardiol.*, **2004**, *93*, *19*-23.

[111] Mahgoub, A.A. Thymoquinone protects against experimental colitis in rats. *Toxicol. Lett.*, **2003**, *143*, 133-143.

[112] Al-Shabanah, O.A.; Badary, O.A.; Nagi, M.N.; Al-Gharably, N.M.; Al-Rikabi, A.C.; Al-Bekairi, A.M. Thymoquinone protects against doxorubicin-induced cardiotoxicity without compromising its antitumor activity. *J. Exp. Clin. Cancer Res.*, **1998**, *17*, 193- 198.

[113] Al-Majed, A.A.; Al-Omar, F.A.; Nagi, M.N. Neuroprotective effects of thymoquinone against transient forebrain ischemia in the rat hippocampus. *Eur. J. Pharmacol.*, **2006**, *543*, 40-47.

[114] Singh, D.; Singh, R.; Singh, P.; Gupta, R.S. Effects of Embelin on Lipid Peroxidation and Free Radical Scavenging Activity against

Liver Damage in Rats. *Basic Clin. Pharmacol. Toxicol.*, **2009** [Epub ahead of print].

[115] Bhandari, U.; Kanojia, R.; Pillai, K.K. Effect of ethanolic extract of Embelia ribes on dyslipidemia in diabetic rats. *Int. J. Exp. Diabetes Res.*, **2002**, *3*, 159-162.

[116] Bhandari, U.; Jain, N.; Pillai, K.K. Further studies on antioxidant potential and protection of pancreatic beta-cells by Embelia ribes in experimental diabetes. *Exp. Diabetes Res.*, **2007**, *15*, 1-6.

[117] Fieser, L.F.; Chamberlin, E.M. Synthesis of embelin, rapoanne and related quinones by peroxide alkylation. *J. Am. Chem. Soc.*, **1948**, *70*, 71-75.

[118] Rasheed, K.K.A.; Chacko, J.; Nambisan, P.N.K. Thermal, spectral and magnetic studies on some transition metal complexes of Embelin. *Polyhedron.*, **1983**, *2*, 293-299.

[119] Abraham, R.; Yussuf, K.K.M. Copper (II) complexes of embelin and 2- aminobenzimidazole encapsulated in zeolite Y-potential as catalysts for reduction of dioxygen. *J. Mol. Catal. A: Chem.*, **2003**, *198*, 175-183.

[120] Joshi, R.; Kamat, J.P.; Mukherjee, T. Free radical scavenging reactions and antioxidant activity of embelin: biochemical and pulse radiolytic studies. *Chem. Biol. Interact.*, **2007**, *167*, 125-134.

[121] Shatalin, I.V.; Naumov, A.A.; Potselueva, M.M. A comparison of antioxidant properties of hypoxen and duroquinone by the method of chemiluminescence. *Biofizika*, **2008**, *53*, 100-106.

[122] Terao, S.; Ohkawa, S.; Terashita, Z.; Shibouta, Y.; Nishikawa, K. Synthesis and biological evaluation of 2,3,5-trimethyl-6-(3-pyridylmethyl)-1,4-benzoquinone (CV-6504), a dual inhibitor of TXA2 synthase and 5-lipoxygenase with scavenging activity of active oxygen species (AOS). *Adv Prostaglandin Thromboxane. Leukot. Res.*, **1991**, *21A*, 173-176.

[123] Lee, I.K.; Yun, B.S.; Cho, S.M.; Kim, W.G.; Kim, J.P.; Ryoo, I.J.; Koshino, H.; Yoo, I.D. Betulinans A and B, two benzoquinone compounds from Lenzites betulina. *J. Nat .Prod.*, **1996**, *59*, 1090-1092.

[124] Mori, K.; Ushio, T.; Okamoto, T.; Kishi, T.; Sayo, H. Effect of arylthiolated 2, 3- dimethoxy-1,4-benzoquinones on respiratory activity and lipid peroxidation in bovine heart mitochondria. *Biol. Pharm. Bull.*, **1998**, *21*, 293-296.

[125] Choi, B.S.; Song, H.S.; Kim, H.R.; Park, T.W.; Kim, T.D.; Cho, B.J.; Kim, C.J.; Sim, S.S. Effect of coenzyme Q10 on cutaneous healing in skin-incised mice. *Arch. Pharm. Res.*, **2009**, *32*, 907-913.

[126] Novoselova, E.G.; Lunin, S.M.; Novoselova, T.V.; Khrenov, M.O.; Glushkova, O.V.; Avkhacheva, N.V.; Safronova, V.G.; Fesenko, E.E. Naturally occurring antioxidant nutrients reduce inflammatory response in mice. *Eur. J. Pharmacol.*, **2009**, *615*, 234-240.

[127] Schmelzer, C.; Kitano, M.; Rimbach, G.; Niklowitz, P.; Menke, T.; Hosoe, K.; Döring, F. Effects of ubiquinol-10 on microRNA-146a expression *in vitro* and *in vivo*. *Mediators Inflamm.*, **2009**, *2009*, 415437.

[128] Lee, T.I.; Kao, Y.H.; Chen, Y.C.; Chen, Y.J. Proinflammatory cytokine and ligands modulate cardiac peroxisome proliferator-activated receptors. *Eur. J. Clin. Invest.*, **2009**, *39*, 23-30.

[129] Chew, G.T.; Watts, G.F. Coenzyme Q10 and diabetic endotheliopathy: oxidative stress and the 'recoupling hypothesis. *Q J. Med.*, **2004**, *97*, 537-548.

[130] Lowes, D.A.; Thottakam, B.M.; Webster, N.R.; Murphy, M.P.; Galley, H.F. The mitochondria-targeted antioxidant MitoQ protects against organ damage in a lipopolysaccharide-peptidoglycan model of sepsis. *Free Radic. Biol. Med.*, **2008**, *45*, 1559-1565.

[131] Civenni, G.; Bezzi, P.; Trotti, D.; Volterra, A.; Racagni, G. Inhibitory effect of the neuroprotective agent idebenone on arachidonic acid metabolism in astrocytes. *Eur. J. Pharmacol.*, **1999**, *370*, 161-167.

[132] Mansour, M.; Tornhamre, S. Inhibition of 5-lipoxygenase and leukotriene C4 synthase in human blood cells by thymoquinone. *J. Enzyme Inhibit. Med. Chem.*, **2004**, *19*, 431-436.

[133] El-Mahmoudy, A.; Matsuyama, H.; Borgan, M.A.; Shimizu, Y.; El-Sayed, M.G.; Minamoto, N.; Takewaki, T. Thymoquinone suppresses expression of inducible nitric oxide synthase in rat macrophages. *Int. Immunopharmacol.*, **2002**, *2*, 1603- 1611.

[134] Juhás, S.; Cikos, S.; Czikková, S.; Veselá, J.; Il'ková, G.; Hájek, T.; Domaracká, K.; Domarácký, M.; Bujnáková, D.; Rehák, P.; Koppel, J. Effects of borneol and thymoquinone on TNBS-induced colitis in mice. *Folia Biol. (Praha).*, **2008**, *54*, 1-7.

[135] El Gazzar, M.A. Thymoquinone suppressses *in vitro* production of IL-5 and IL-13 by mast cells in response to lipopolysaccharide stimulation. *Inflamm. Res.*, **2007**, *56*, 345- 351.

[136] Hajhashemi, V.; Ghannadi, A.; Jafarabadi, H. Black cumin seed essential oil, as a potent analgesic and antiinflammatory drug. *Phytother. Res.*, **2004**, *18*, 195-199.

[137] El Gazzar, M.A.; El Mezayen, R.; Marecki, J.C.; Nicolls, M.R.; Canastar, A.; Dreskin, S.C. Anti-inflammatory effect of thymoquinone in a mouse model of allergic lung inflammation. *Int. Immunopharmacol.*, **2006**, *6*, 1135-1142.

[138] Sayed, A.A.; Morcos, M. Thymoquinone decreases AGE-induced NF-kappaB activation in proximal tubular epithelial cells. *Phytother. Res.*, **2007**, *21*, 898-899.

[139] Ragheb, A.; Attia, A.; Eldin, W.S.; Elbarbry, F.; Gazarin, S.; Shoker, A. The protective effect of thymoquinone, an anti-oxidant and anti-inflammatory agent, against renal injury: a review. *Saudi J. Kidney Dis. Transpl.*, **2009**, *20*, 741-752.

[140] Kanter, M. Protective effects of thymoquinone on streptozotocin-induced diabetic nephropathy. *J. Mol. Histol.*, **2009**, *40*, 107-115.

[141] Chitra, M.; Sukumar, E.; Suja, V.; Devi, C.S. Antitumor, anti-inflammatory and analgesic property of embelin, a plant product. *Chemotherapy*, **1994**, *40*, 109-113.

[142] Ahn, K.S.; Sethi, G.; Aggarwal, B.B. Embelin, an inhibitor of X chromosome-linked inhibitor-of-apoptosis protein, blocks nuclear factor-kappaB (NF-kappaB) signaling pathway leading to suppression of NF-kappaB-regulated antiapoptotic and metastatic gene products. *Mol. Pharmacol.*, **2007**, *71*, 209-219.

[143] Gupta, O.P.; Ali, M.M.; Ray Ghatak, B.J.; Atal, C.K. Some pharmacological investigations of embelin and its semisynthetic derivatives. *Indian J. Physiol. Pharmacol.*, **1977**, *21*, 31-39.

[144] Poulaki, V.; Iliaki, E.; Mitsiades, N.; Mitsiades, C.S.; Paulus, Y.N.; Bula, D.V.; Gragoudas, E.S.; Miller, J.W. Inhibition of Hsp90 attenuates inflammation in endotoxin-induced uveitis. *FASEB J.*, **2007**, *21*, 2113-2123.

[145] Murphy, P.; Sharp, A.; Shin, J.; Gavrilyuk, V.; Dello Russo, C.; Weinberg, G.; Sharp, F.R.; Lu, A.; Heneka, M.T.; Feinstein, D.L. Suppressive effects of ansamycins on inducible nitric oxide synthase expression and the development of experimental autoimmune encephalomyelitis. *J. Neurosci. Res.*, **2002**, *67*, 461-470.

[146] Malhotra, V.; Shanley, T.P.; Pittet, J.F.; Welch, W.J.; Wong, H.R. Geldanamycin inhibits NF-kappaB activation and interleukin-8 gene expression in cultured human respiratory epithelium. *Am. J. Respir. Cell Mol. Biol.*, **2001**, *25*, 92-97.

[147] Bucci, M.; Roviezzo, F.; Cicala, C.; Sessa, W.C.; Cirino, G. Geldanamycin, an inhibitor of heat shock protein 90 (Hsp90) mediated signal transduction has anti-inflammatory effects and interacts with glucocorticoid receptor *in vivo*. *Br. J. Pharmacol.*, **2000**, *131*, 13-16.

[148] Alhosin, M.; Abusnina, A.; Achour, M.; Sharif, T.; Muller, C.; Peluso, J.; Chataigneau, T.; Lugnier, C.; Schini-Kerth, V.B.; Bronner, C.; Fuhrmann, G. Induction of apoptosis by thymoquinone in lymphoblastic leukemia Jurkat cells is mediated by a p73-dependent pathway which targets the epigenetic integrator UHRF1. *Biochem. Pharmacol.*, **2010**, *79*, 1251-1260.

[149] El-Najjar, N.; Chatila, M.; Moukadem, H.; Vuorela, H.; Ocker, M.; Gandesiri, M.; Schneider-Stock, R.; Gali-Muhtasib, H. Reactive oxygen species mediate thymoquinone-induced apoptosis and activate ERK and JNK signaling. *Apoptosis*, **2010**, *15*, 183-195

[150] Banerjee, S.; Kaseb, A.O.; Wang, Z.; Kong, D.; Mohammad, M.; Padhye, S.; Sarkar, F.H.; Mohammad, R.M. Antitumor activity of gemcitabine and oxaliplatin is augmented by thymoquinone in pancreatic cancer. *Cancer Res.*, **2009**, *69*, 5575-5583

[151] Breyer, S.; Effenberger, K.; Schobert, R. Effects of thymoquinone-fatty acid conjugates on cancer cells. *ChemMedChem.*, **2009**, *4*, 761-768.

[152] Yamasaki, L. Role of the RB tumor suppressor in cancer. *Cancer Treat Res.*, **2003**, *115*, 209-239

[153] El-Mahdy, M.A.; Zhu, Q.; Wang, Q.E.; Wani, G.; Wani, A.A. Thymoquinone induces apoptosis through activation of caspase-8 and mitochondrial events in p53-null myeloblastic leukemia HL-60 cells. *Int. J. Cancer*, **2005**, *117*, 409-417.

[154] Kaseb, A.O.; Chinnakannu, K.; Chen, D.; Sivanandam, A.; Tejwani, S.; Menon, M.; Dou, Q.P.; Reddy, G.P. Androgen receptor and E2F-1 targeted thymoquinone therapy for hormone-refractory prostate cancer. *Cancer Res.*, **2007**, *67*, 7782-7788.

[155] Sethi, G.; Ahn, K.S.; Aggarwal, B.B. Targeting nuclear factor-{kappa}B activation pathway by thymoquinone: role in suppression of antiapoptotic gene products and enhancement of apoptosis. *Mol. Cancer Res.*, **2008**, *6*, 1059-1070.

[156] Gali-Muhtasib, H.; Kuester, D.; Mawrin, C.; Bajbouj, K.; Diestel, A.; Ocker, M.; Habold, C.; Foltzer-Jourdainne, C.; Schoenfeld, P.; Peters, B.; ab-Assaf, M.; Pommrich, U.; Itani, W.; Lippert, H.; Roessner, A.; Schneider-Stock, R. Thymoquinone triggers inactivation of the stress response pathway sensor CHEK1 and contributes to apoptosis in colorectal cancer cells. *Cancer Res.*, **2008**, *68*, 5609-5618.

[157] Gali-Muhtasib, H.; ab-Assaf, M.; Boltze, C.; Al-Hmaira, J.; Hartig, R.; Roessner, A.; Schneider-Stock, R. Thymoquinone extracted from black seed triggers apoptotic cell death in human colorectal cancer cells *via* a p53-dependent mechanism. *Int. J. Oncol.*, **2004**, *25*, 857-866.

[158] Gali-Muhtasib, H.U.; bou Kheir, W.G.; Kheir, L.A.; Darwiche, N.; Crooks, P.A. Molecular pathway for thymoquinone-induced cell-cycle arrest and apoptosis in neoplastic keratinocytes. *Anticancer Drugs*, **2004**, *15*, 389-399.

[159] Roepke, M.; Diestel, A.; Bajbouj, K.; Walluscheck, D.; Schonfeld, P.; Roessner, A., Schneider-Stock, R.; Gali-Muhtasib, H. Lack of p53 augments thymoquinone- induced apoptosis and caspase activation in human osteosarcoma cells. *Cancer Biol. Ther.*, **2007**, *6*, 160-169

[160] Reindl, W.; Yuan, J.; Kramer, A.; Strebhardt, K.; Berg, T. Inhibition of polo-like kinase 1 by blocking polobox domain-dependent protein-protein interactions. *Chem. Biol.*, **2008**, *15*, 459-466.

[161] Ivankovic, S.; Stojkovic, R.; Jukic, M.; Milos, M.; Milos, M.; Jurin, M. The antitumor activity of thymoquinone and thymohydroquinone *in vitro* and *in vivo*. *Exp. Oncol.*, **2006**, *28*, 220-224.

[162] Badary, O.A.; bd-Ellah, M.F.; El-Mahdy, M.A.; Salama, S.A.; Hamada, F.M. Anticlastogenic activity of thymoquinone against benzo(a)pyrene in mice. *Food Chem. Toxicol.*, **2007**, *45*, 88-92.

[163] Tan, M.; Norwood, A.; May, M.; Tucci, M.; Benghuzzi, H. Effects of (-) epigallocatechin gallate and thymoquinone on proliferation of PANC-1 cell line in culture. *Biomed. Sci. Instr.*, **2006**, *42*, 363-371.

[164] Bawadi, H.A.; Bansode, R.R.; Losso, J.N. In: *Thymoquinone in the control of hypoxia-induced angiogenic disease biomarkers: Insight into the mechanism of action in vitro*, IFT Annual Meeting 2004, Las Vegas NV, USA, **2004**, pp 7-12.

[165] Brewer, J.; Benghuzzi, H.; Tucci, M. Effects of thymoquinone, lycopene, and selenomethione in the presence of estrogen on the viability of SiHa cells *in vitro*. *Biomed. Sci. Instr.*, **2006**, *42*, 37-41.

[166] Dai, Y.; Qiao, L.; Chan, K.W.; Yang, M.; Ye, J.; Ma, J.; Zou, B.; Gu, Q.; Wang, J.; Pang, R.; Lan, H.Y.; Wong. B. C. Peroxisome proliferator-activated receptor-gamma contributes to the inhibitory effects of Embelin on colon carcinogenesis: *Cancer Res.*, **2009**, *69*, 4776-4783.

[167] Xu, M.; Cui, J.; Fu. H.; Proksch, P.; Lin, W.; Li, M. Embelin derivatives and their anticancer activity through microtubule disassembly. *Planta Med.*, **2005**, *71*, 944-948.

[168] Mori, T.; Doi, R.; Kida, A.; Nagai, K.; Kami, K.; Ito, D.; Toyoda, E.; Kawaguchi, Y.; Uemoto, S. Effect of the XIAP inhibitor Embelin on TRAIL-induced apoptosis of pancreatic cancer cells. *J. Surg. Res.*, **2007**, *142*, 281-286.

[169] Chen, J.; Nikolovska-Coleska, Z.; Wang, G.; Qiu, S.; Wang. S. Design, synthesis, and characterization of new embelin derivatives as potent inhibitors of X-linked inhibitor of apoptosis protein. *Bioorg. Med. Chem. Lett.*, **2006**, *16*, 5805-5808.

[170] Podolak, I.; Galanty, A.; Janeczko, Z. Cytotoxic activity of embelin from Lysimachia punctata. *Fitoterapia*, **2005**, *76*, 333-335.

[171] Miyata, Y. Hsp90 inhibitor geldanamycin and its derivatives as novel cancer chemotherapeutic agents. *Curr. Pharm. Des.*, **2005**, *11*, 1131-1138

[172] Cysyk, R.L.; Parker, R.J.; Barchi, J.J.; Jr, Steeg, P.S.; Hartman, N.R.; Strong, J.M. Reaction of geldanamycin and C17-substituted analogues with glutathione: product identifications and pharmacological implications. *Chem. Res. Toxicol.*, **2006**, *19*, 376-381.

[173] Rosenhagen, M.C.; Young, J.C.; Wochnik, G.M.; Herr, A.S.; Schmidt, U.; Hartl, F.U.; Holsboer, F.; Rein, T. Synergistic inhibition of the glucocorticoid receptor by radicicol and benzoquinone ansamycins. *Biol. Chem.*, **2001**, *382*, 499-504.

[174] Kaur, G.; Belotti, D.; Burger, A.M.; Fisher-Nielson, K.; Borsotti, P.; Riccardi, E.; Thillainathan, J.; Hollingshead, M.; Sausville, E.A.; Giavazzi, R. Antiangiogenic properties of 17-(dimethylaminoethylamino)-17-demethoxygeldanamycin: an orally bioavailable heat shock protein 90 modulator. *Clin. Cancer Res.*, **2004**, *15*, 4813-4821.

[175] Benchekroun, M.N.; Myers, C.E.; Sinha, B.K. Free radical formation by ansamycin benzoquinone in human breast tumor cells: Implications for cytotoxicity and resistance. *Free Radic. Biol. Med.*, **1994**, *17*, 191-200.

[176] Tian, Z.; Liu, Y.; Zhang, D.; Wang, Z.; Dong, S.D.; Carreras, C.W.; Zhou, Y.; Rastelli, G.; Santi, D.V.; Myles, D.C. Synthesis and biological activities of novel 17-aminogeldanamycin derivatives. *Bioorg. Med. Chem.*, **2004**, *12*, 5317-5329

[177] Yun, C.H.; Yoon, S.Y.; Nguyen, T.T.; Cho, H.Y.; Kim. T.H.; Kim, S.T.; Kim, B.C.; Hong, Y. S.; Kim, S.J.; Lee, H.J. Geldanamycin inhibits TGF-beta signaling through induction of Hsp70. *Arch. Biochem. Biophys.*, **2010**, *495*, 8-13.

[178] Lukasiewicz, E.; Miekus, K.; Kijowski, J.; Gozdzik, J.; Wilusz, M.; Bobis-Wozowicz, S.; Wiecha, O.; Majka, M. High anti tumor activity against rhabdomyosarcoma cells and low normal cells cytotoxicity of heat shock protein 90 inhibitors, with special emphasis on 17-[2-(pyrrolidin-1-yl)ethyl]-aminno-17-demethoxygeldanamycin. *J. Physiol. Pharmacol.*, **2009**, *60*, 161-166.

[179] Georgakis, G.V.; Li, Y.; Rassidakis, G.Z.; Martinez-Valdez, H.; Medeiros, L.J.; Younes, A. Inhibition of heat shock protein 90 function by 17-allylamino-17-demethoxy-geldanamycin in Hodgkin's lymphoma cells down-regulates Akt kinase, dephosphorylates extracellular signal-regulated kinase, and induces cell cycle arrest and cell death. *Clin. Cancer Res.*, **2006**, *12*, 584-590

[180] Lee, K.; Ryu, J.S.; Jin. Y.; Kim, W.; Kaur, N.; Chung, S.J.; Jeon, Y.J.; Park, J.T.; Bang, J.S.; Lee, H.S.; Kim, T.Y.; Lee, J.J.; Hong, Y.S. Synthesis and anticancer activity of geldanamycin derivatives derived from biosynthetically generated metabolites. *Org. Biomol. Chem.*, **2008**, *6*, 340-348.

[181] Onyüksel, H.; Mohanty, P.S.; Rubinstein, I. VIP-grafted sterically stabilized phospholipid nanomicellar 17-allylamino-17-demethoxy geldanamycin: a novel targeted nanomedicine for breast cancer. *Int. J. Pharm.*, **2009**, *365*, 157-161.

[182] Cheng, H.; Cao, Z.; Xian, M.; Fang, L.; Cai, T.B.; Ji, J.J.; Tunac, J.B.; Sun, D.; Wang, P.G. Synthesis and enzyme-specific activation of carbohydrate-geldanamycin conjugates with potent anticancer activity. *J. Med. Chem.*, **2005**, *48*, 645-652.

[183] Tian, Z.Q.; Wang, Z.; MacMillan, K.S.; Zhou, Y.; Carreras, C.W.; Mueller, T.; Myles, D.C.; Liu, Y. Potent cytotoxic C-11 modified geldanamycin analogues. *J. Med. Chem.*, **2009**, *52*, 3265-3273.

[184] Fararh, K.M.; Ibrahim, A.K.; Elsonosy, Y.A. Thymoquinone enhances the activities of enzymes related to energy metabolism in peripheral leukocytes of diabetic rats. *Res Vet Sci.*, **2009** [Epub ahead of print].

[185] Pari, L.; Sankaranarayanan, C. Beneficial effects of thymoquinone on hepatic key enzymes in streptozotocin-nicotinamide induced diabetic rats. *Life Sci.*, **2009**, *85*, 830-834.

[186] Lin, B.; Li, Z.; Park, K.; Deng, L.; Pai, A.; Zhong, L.; Pirrung, M.C.; Webster, M.J.C. Identification of Novel Orally Available Small Molecule Insulin Mimetics. *J. Pharmacol. Exp. Ther.*, **2007**, *323*, 579-585.

[187] Kumara Swamy, H.M.; Krishna, V.; Shankarmurthy, K.; Abdul Rahiman, B.; Mankani, K.L.; Mahadevan, K.M.; Harish, B.G.; Raja Naika, H. Wound healing activity of Embelin isolated from the ethanol extract of leaves of Embelia ribes Burm. *J. Ethnopharmacol.*, **2007**, *109*, 529-534.

Received: December 11, 2009 Revised: March 04, 2010 Accepted: March 10, 2010