

Anti-Infective Quinone Derivatives of Recent Patents

Junko Koyama*

Faculty of Pharmaceutical Sciences, Kobe Pharmaceutical University, Higashinada-ku, Kobe 658-8558, Japan

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Abstract Quinones are important naturally occurring pigments widely distributed in nature and are well known to demonstrate various physiological activities as antimicrobial and anticancer compounds. This review will focus on the preparation, therapeutic application, and administration of several benzoquinones, naphthoquinones, and anthraquinones having anti-infective, e.g. antiviral and antibacterial activities, in recent patents.

Keywords: Benzoquinone, naphthoquinone, anthraquinone, anti-infective, antiviral activity, antibacterial activity, antimalarial activity, patent.

INTRODUCTION

Infectious diseases have not yet been overcome. The worldwide death toll is 52 million people a year according to the estimate by WHO, and a third of them, 17 million people, die from infectious diseases. It is reported that most infections around the world are the diarrhea syndrome and 4 billion patients occur every year, those who are infected with malaria amount to 500 million, and pneumonia attacks 400 million. Considering that the earth's population is 6 billion, such infections are a threat to human. The viruses related to hepatitis, such as HEV and HCV, and the new infectious diseases, such as the Ebola virus, *Legionella pneumophila*, and human immunodeficiency virus (HIV), have been discovered in the past 20 years. Furthermore, the appearance of a multidrug-resistant strain by antibacterial medicine use is a big factor.

One of the more significant achievements of the 20th century has been the discovery and commercial development of numerous therapeutic agents that now provide reliably effective treatments for many infectious diseases that had previously caused extensive mortality, morbidity, and fear. The search for suitable drugs effective for the cure of human diseases is a continuing process. A number of methods can be used to perform susceptibility tests with antibacterial agents in a clinical laboratory setting or for research purpose, such as assessing the activities of new antimicrobial agents. These methods include the broth microdilution, disk diffusion, antibiotic gradient (epsilometer-test), and automated-instrument methods.

For the antibacterial agents often used, quinone compounds are tetracyclines, new quinolones, anthracinones, and mitomycins. More than 2000 naturally occurring quinones, for example - anthraquinones, naphthoquinones, and benzoquinones (Fig. 1), are now known and widely distributed in nature as pigments and as intermediates in cellular respiration and photosynthesis [1, 2]. Some quinones have important roles in the biochemistry of energy

production and serve as vital links during electron transport. Most quinones, which are aromatic compounds present in bacteria and eukaryotes [3], are often involved in electron transport and include, ubiquinones and menaquinones [4]. They provide a defense role as a result of their effectiveness at inhibiting the growth of bacteria, fungi, or parasites [5-8]. Therefore, a number of them have various physiological activities as antimicrobial and anticancer compounds [9]. The mechanism of toxicity is still under investigation, but two theories dominate the literature [10], with some quinones proposed to exhibit one or both mechanisms. Redox cycling is the concept in which compounds catalytically cycle and generate oxidative radicals, such as hydrogen peroxide and superoxide, which then damage the cell. Alkylation is when quinones are activated inside cells and become covalently attached to proteins, DNA, or other targets. The most important reaction of quinones as far as biology is concerned is their reversible reduction to the corresponding hydroquinone (Fig. 2).

This study covers the preparation, the therapeutic application, and the administration of several benzoquinones, naphthoquinones, and anthraquinones having anti-infective, e.g., antiviral and antibacterial effects, in recent patents (2000-2005). The quinone compounds in 11 recent patents are classified into four categories based on targets; antibacterial, antiviral, antifungal, and antiprotozoal application, and reviewed.

ANTIBACTERIAL APPLICATION

Bacteria are very small, relatively simple, single-celled organisms whose genetic material is not enclosed in a special nuclear membrane. Two types of chemotherapeutic agents are synthetic drugs and antibiotics. In this section, 5 patents are classified into 1) antimycobacterial, 2) antichlamydial and/or anti-methicillin-resistant *Staphylococcus aureus* (MRSA), and 3) antibiotic.

1) Antimycobacterial

Naphthoquinone Derivatives and their Use in the Treatment and Control of Tuberculosis [11]

This invention relates to the use of the naphthoquinone derivative for the treatment and control of tuberculosis caused by *Mycobacterium tuberculosis*.

*Address correspondence to this author at the Faculty of Pharmaceutical Sciences, Kobe Pharmaceutical University, Higashinada-ku, Kobe 658-8558, Japan; Tel: +81-441-7549; Fax: +81-441-7550; E-mail: j-koyama@kobepharmaceutical-u.ac.jp

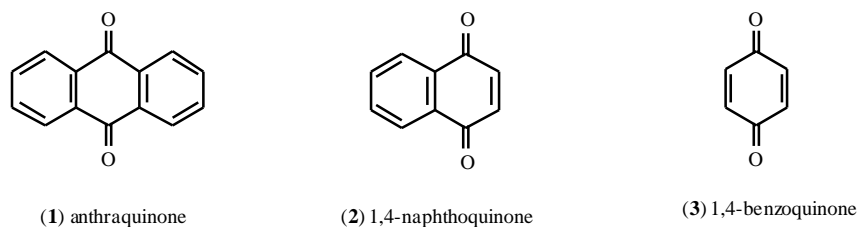


Fig. (1). Structures of quinones.

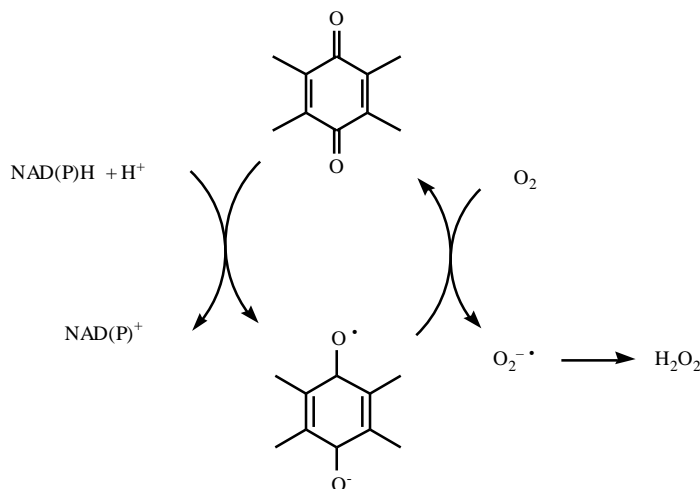


Fig. (2). Redox cycling of NADH or NAD(P)H and quinone.

Tuberculosis (TB) remains a major global public health problem. Nearly 2 million people died of TB, with a global case fatality rate of 23% but reaching > 50% in some African countries due to high rates of coexisting HIV infection. Man infected with HIV are very susceptible to TB [12]. If control of tuberculosis is not further strengthened, WHO estimates that between 2000 and 2020, nearly one billion people will be newly infected, 200 million people will become sick, another 35 million people will die from TB.

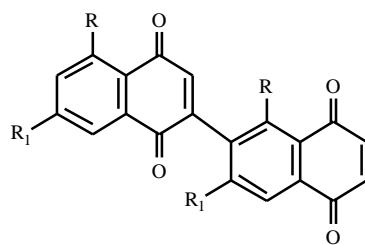
Mycobacteria are aerobic, non-endospore-forming, nonmotile, slightly curved or straight rod. These bacteria are relatively resistant to normal staining procedures. Once stained, however, mycobacteria are not easily decolorized, even with acid-alcohol and are therefore classified as acid-fast. This characteristic reflects the unusual composition of the cell wall which contains mycolic acids together with free lipids.

Treatment options of tuberculosis are limited, the drugs have significant side effect, and no new antibiotics have been developed against any mycobacteria since the 1970s. The present treatment regimes for TB are based on multidrug therapy with usually 3 or 4 antituberculosis drugs. However, the problem of multidrug resistant *tubercle bacilli* is emerging for various drugs. The need for new antituberculosis agents is urgent due to the increasing resistance of mycobacteria to the classic antituberculosis drugs. It is essential to have new antituberculosis agents,

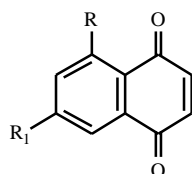
preferably those that can readily and simply be produced from some local sources.

Twenty South Africa medicinal plants used to treat pulmonary diseases were screened for activity against the drug-resistant and sensitive strains of *M. tuberculosis*. A preliminary screening of acetone and a water plant extract, against a drug-sensitive strain of *M. tuberculosis*, H37Rv, was carried out using the agar plate method. The minimal inhibitory concentration (MIC) for the extract of *Croton pseudopulchellus*, *Ekebergia capensis*, *Euclea natalensis*, *Nidorella anomala* and *Polygala myrtifolia* was 0.1 mg/ml against the H37Rv using the radiometric method. The extracts of *Chenopodium ambrosioides*, *Ekebergia capensis*, *Euclea natalensis*, *Helichrysum melanacme*, *Nidorella anomala* and *Polygala myrtifolia* were active against the resistant strain at 0.1 mg/ml.

In this invention, the naphthoquinone derivatives of Formulas **A** and **B** have been found to be particularly effective against *Mycobacterium tuberculosis* [13]. (Fig. 3) In particular diospyrin (**4**) and methyljuglone (**5**) have been found to inhibit several antibiotic resistant as well as antibiotic susceptible strains of *M. tuberculosis*. Diospyrin and methyljuglone [14-16] were isolated from *E. natalensis* and other species in this genus. A combination treatment of diospyrin and methyljuglone, which may be more effective than a single drug treatment of the two naphthoquinones, is also being considered. The oral administration of diospyrin or methyljuglone in an appropriate pharmaceutical



[Formula A]

(4) diospyrin: R= OH, R₁= CH₃

[Formula B]

(5) methyljuglone: R= OH, R₁= CH₃**Fig. (3).** Structures of naphthoquinones.

composition with suitable diluents and carriers will typically be used to treat or control tuberculosis.

There are some papers about the mechanism such as extrusion pumps, cell penetration, and redox cycling, of naphthoquinone [17-20]. However, the mechanism is still under investigation.

2) Antichlamydia and/or Anti-MRSA

Antichlamydia Agent [21]:

This invention relates to obtaining an antichlamydia agent which shows an antimicrobial effect, e.g., detergency, disinfection, infection prophylactic effect or the like on chlamydia using bamboo extract.

Many problems exist during the treatment of chlamydia, including widely spread urogenital infections [22]. Chlamydia, which involves intracellular parasites, is hardly accessible to the majority of existing compounds. The chlamydia genus that is a pathogenic microorganism is neither a bacillus nor a virus from 1974, and is independently classified. As for chlamydia, three kinds (*C. trachomatis*, *C. pneumoniae*, and *C. psittaci*) are known.

Chlamydia is bimorphic which is a fancy way of saying that they come in 2 stages: the elementary body (EB) and the reticular body (RB). The EB is the only infectious stage of the chlamydial developmental cycle. It functions as a tough "spore-like" body whose purpose is to permit chlamydial survival in the non-supportive (to chlamydiae) environment outside the host cell. They have either no or a very small amounts of peptidoglycan. Chlamydia is treated with antibiotics as doxycycline and not penicillin. It is not an antichlamydia agent that improves the infection prevention to EB, though the antibiotic such as the tetracycline derivatives used for a treatment after infecting chlamydia.

In this invention, the antichlamydia agent is obtained from the extract of bamboo, e.g., *Phyllostachys pubescens*

[23] or the like, and an alcohol or ether as an extraction raw material and an extracting solvent, respectively, as the active ingredient. The infection preventive effect is due to inhibit the protein synthesis catalyzed ATP. This antichlamydia agent contains benzoquinone in the extract, improves the infection prevention of somatic cell to EB, and provide the certain antibacterial activity by kill out RB in the cell. The more specific antimicrobial effect is exhibited when the extract contains liquid benzoquinone in a formation amount of 0.2 - 0.5%.

Anti-Drug Resistant Strain Agents and Antichlamydia Agents [24]:

The invention relates to novel antibacterial agents for drug-resistant bacteria and antichlamydia agents which comprise highly active furanonaphthoquinone derivatives as effective compounds.

In recent years, MRSA has been seriously considered as the casual bacterium of hospital infection. Since this MRSA is a multiple drug resistant bacterium for a variety of antibiotics, there is a limitation to the drugs that may be effectively used as therapeutic agents and not antibacterial agents which show a stronger antibiotic activity against MRSA than against MSSA (sensitive bacterium). Accordingly, the antibacterial agents for drug-resistant bacteria with a high antibacterial activity have been desired.

The *Chlamydiae* differ from the other main order of intracellular bacteria, the *Rickettsiales*, in their characteristic dimorphic growth cycle. Chlamydia, which is known to be the casual bacterium of parrot fever, comes from infected pet birds and infections caused by sexual intercourse, urethritis, cervicitis, lymphogranuloma, etc. Antibiotics such as macrolide derivatives and tetracycline derivatives have been used for its treatments. Since recent new drugs have a broad spectra, the acquisition of resistance to other bacteria against drugs has become a problem. Therefore, the development of novel highly specific antichlamydia agents of which the

action mechanism is different from that of the drugs known so far, is anticipated.

This invention provides antibacterial agents for drug-resistant bacteria and antichlamydia agents comprising, as the effective component, the furanonaphthoquinone derivative (Fig. 4 wherein each R may be the same or different representing any one of the following a) to e)). These various compounds may be produced by a variety of known methods of chemical synthesis [25] or by methods such as the extraction of naphthoquinones as natural products. For example, 8-hydroxy-2-methylnaphtho[2,3-*b*]furan-4,9-dione (**6**, FNQ13) was tested for 14 strains of MRSA and 12 strains of MSSA as the control, and six fluconazole-resistant *Candida albicans* (FRCA) and fluconazole-sensitive *Candida albicans* (FSCA) as the control. It was found that the MIC of MRSA was 5.36 µg/ml, and MSSA 11.98 µg/ml, indicating that FNQ13 exhibits a stronger antimicrobial activity against MRSA. FNQ13 exhibits the same degree of antibacterial activity against the FRCA of *Candida albicans* as that against FSCA. Furthermore, antibiotics by concomitant use with a low concentration of FNQ13 decreases the resistance of MRSA against antibiotics such as ampicillin, cefaclor, vancomycin, etc., from 1/2 to 1/4.

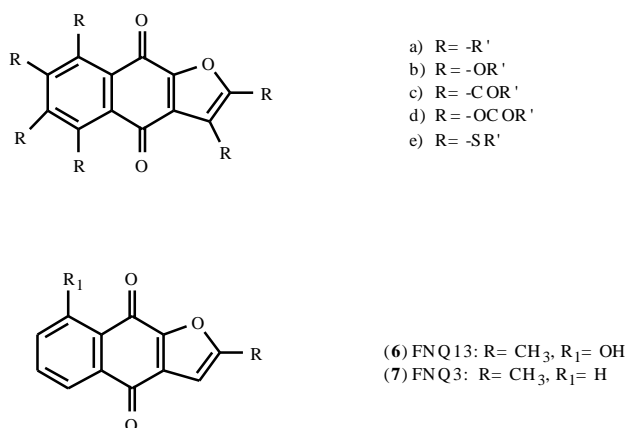


Fig. (4). Structures of furanonaphthoquinones.

2-Methylnaphtho[2,3-*b*]furan-4,9-dione (**7**, FNQ3) and 2-hydroxymethylnaphtho[2,3-*b*]furan-4,9-dione inhibited the growth of all the tested strains of chlamydia at a low concentration of 0.25 to 1.0 µg/ml. In addition, as for the toxicity to human cells, the concentration that caused 100% necrosis in human cancer cells is about 5 µg/ml, while that for normal human cells is about 20 µg/ml [26]. Each compound of the present invention, which may be used as the effective component in antibacterial agents against drug-resistant strains, are advantageous in that it exhibits a drug efficacy as an anti-cancer agent, but shows no toxicity to normal cells and causes no side effects, at effective concentrations as antibacterial agents. When A549 cells were treated with **7**, reactive oxygen species was produced in mitochondria and carcinoma cells compared to normal cells damaged as 10-fold [27]. The same reaction may be occurred in the bacteria.

Antibacterial Compound Against VRE and/or MRSA [28, 29]

This invention relates to the specific quinone methide compounds having a strong antibacterial activity against vancomycin-resistant *Enterococcus* (VRE) and MRSA, and the synthetic methods of these new compounds (Fig. 5).

In recent years, infections caused by bacteria resistant to multiple antibiotics have been significant problems. Especially, MRSA and VRE are spreading to medical institutions all over the world. It was found that tolarol, abietane diterpene, had a strong antibacterial activity for MRSA by searching for a new antibacterial compound [30, 31]. Abietane diterpenes are widely distributed in nature with various biological activities, e.g., antiviral [32, 33], antibacterial [33-35], antimalarial [36], antioxidant [37], and antitumor activities [38, 39]. The inventors reported the synthesis of oxidized abietane diterpene derivatives and their antibacterial activities against MRSA and VRE, and that the quinone methide compound showed a very potent activity against both MRSA and VRE [40, 41]. In this invention, the compounds having a more potent antibacterial activity against MRSA and VRE were synthesized using a previously reported synthetic route [40, 41]. From the previously reported results, it was suggested that the C ring structure of this compound has a greater influence than the structure of the A or B ring against the antibacterial activity. Therefore, even if the structure of the A or B ring of the compound shown in the figure is somewhat different, it is thought that the compound which has the same partial structure as the C ring has the same antibacterial activity. For example, the synthetic route of compounds, **11-15** were reported. The antibacterial activities against MRSA and VRE of the synthesized abietane diterpenes and their derivatives were evaluated. The test organisms, three strains of MRSA (MRSA-664, MRSA-730 and MRSA-996) and three strains of VRE (VanA, VanB and VanC) were obtained from the Department of Arterial and Blood Products, National Institute of Infection Disease of Japan. MIC of the test compounds are listed in (Table 1). In Table, compounds, **12**, **13**, and **15** showed potent antibacterial activity.

At present, antibacterial mechanism is not clear. Many strains of MRSA possess efflux pumps such as the specific TetK and MsrA transporters which export certain tetracyclines and macrolide, and the multidrug resistance proteins NorA and QacA which confer resistance to a wide range of structurally unrelated antibiotics. The activity is likely to be related to the inhibition of pumps [42].

3) Antibiotic

New Anthraquinone Derivatives [43]

This invention relate to the new bianthraquinone derivatives, for example, 2,2',4,4',5,5',8-heptahydroxy-7,7'-dimethoxy[1,1'-bianthracene]-9,9',10,10'-tetrone (**16**, YM 187781) and 2,2',4,4',5,5'-hexahydroxy-7,7'-dimethoxy[1,1'-bianthracene]-9,9',10,10'-tetrone (**17**, YM187787) were obtained by culturing a strain belonging to the genus *Verticillium* (A Hyphomycetes *Verticillium lecanii* Q57371) that have an antibacterial activity toward various bacteria (Fig. 6).

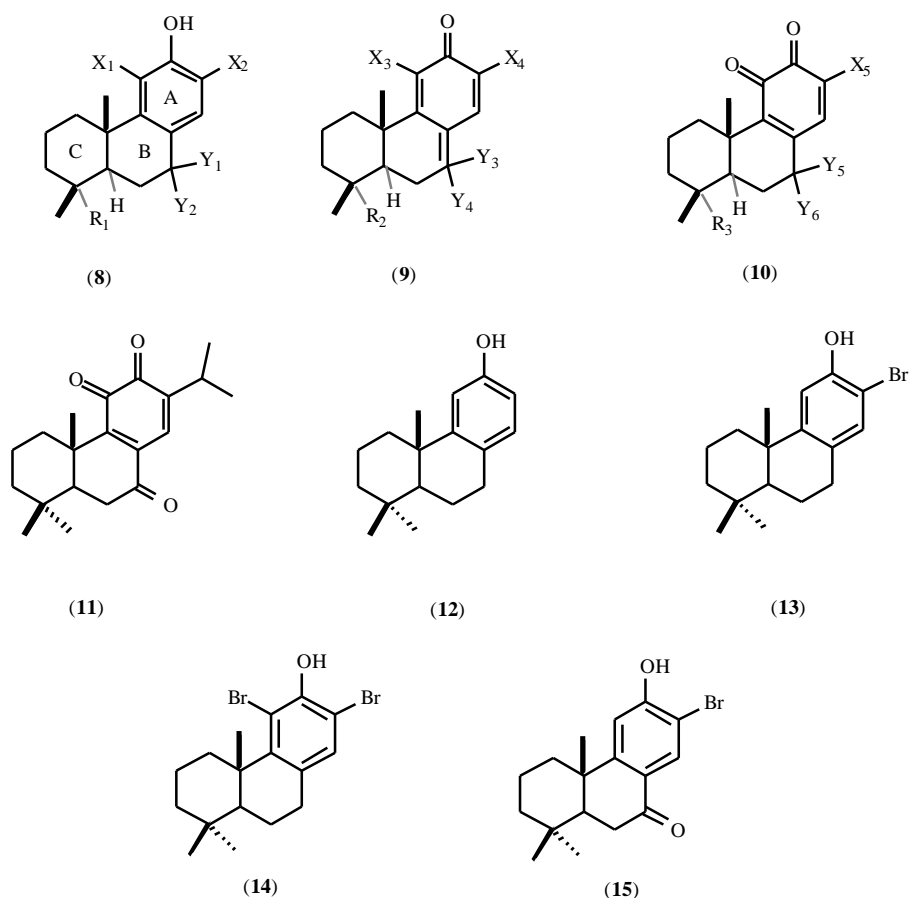


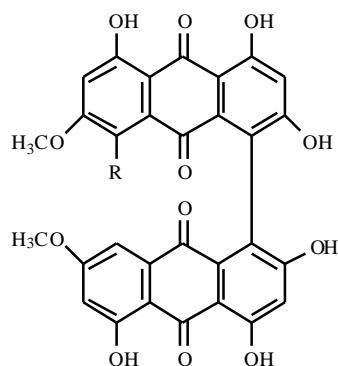
Fig. (5). Structures of abietane diterpene compounds.

Table 1. Minimum Inhibitory Concentrations (MIC) of Abietane Derivatives

compound	strain			MIC ($\mu\text{g/mL}$)		
	VRE			MRSA		
	VanA	VanB	VanC	996	730	664
11	64	64	128	64	64	64
12	-	-	-	2	2	4
13	4	4	4	4	4	4
14	16	32	16	32	64	64
15	8	8	16	8	8	8
vancomycin	256	128	16	2	2	2

Conventionally, there are the β -lactam antibiotics, such as penicillin and cephalosporin, macrolide antibiotics, such as erythromycin, and aminoglycoside antibiotics, such as kanamycin, which the microbes produce. These inventors describe new antibacterial compounds, a manufacturing process with a microbe having the ability to produce a new compound, and a new microbe. As a result of a detailed study, they discovered a microbe which has the ability to

produce a compound having an antibacterial activity, and they identified it with the Hyphomycetes *Verticillium lecanii* Q57371 strain which belonged to the genus *Verticillium* that was separated from soil collected on Yaku island in Kagoshima, Japan [44]. In order to manufacture new antibacterial compounds, a cultivation is started by inoculating the *Verticillium lecanii* Q57371 strain into the culture medium containing the source of nutrition of the



(16) YM187781: R = OH

(17) YM187787: R = H

Fig. (6). Structures of bianthraquinones.

Verticillium lecanii Q57371 strain and growing it aerobically. The cultivation method performed by the same production method used for general antibiotics.

These antibacterial compounds react with acids and form inorganic salts or organic salts. The active compounds described here may be formulated for addition to a pharmaceutical carrier in accordance with known techniques [45].

ANTIVIRAL APPLICATION

Viruses contain a single type of nucleic acid, either DNA or RNA surrounding by a protein coat and are obligatory intracellular parasites. They multiply by using the host cell's synthesizing machinery to cause the synthesis of specialized elements that can transfer the viral nucleic acid to other cells. In this section, 4 patents are classified into two categories; 1) HIV and 2) others.

1) HIV

Anti-Viral Multi-Quinone Compounds and Regiospecific Synthesis Thereof [46]

The present invention relates to a method of the regiospecific synthesis of multi-quinone compounds and to novel biquinones and trimeric quinones, those that have antiviral activity and can be used to treat viral infections, particularly HIV infections.

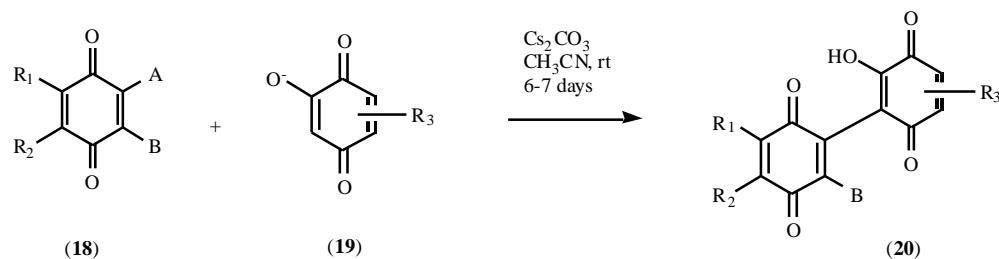
Acquired immune deficiency syndrome (AIDS) is a fatal disease caused by HIV that afflicts millions of people worldwide. Many current commercially available drugs used to treat HIV act by inhibiting either the enzymes reverse transcriptase or protease. The use of combinations or cocktails of these two classes of drugs has enabled a great number of HIV-infected individuals to keep the virus in check and remain alive. However, there are some patients who cannot respond to multi-drug therapy, and the side effects of several drugs can be also serious. Since HIV came to gain resistance to existing drugs, there is a pressing need to discover new anti-HIV medicines.

Conocurvone, being a trimeric naphthoquinone, was isolated from a plant of the genus *Conospermum*, commonly known as the western Australian smoke bush by Boyd *et al.* [47-49]. The Boyd patents disclose that conocurvone had been found to inhibit the growth and replication of viruses, and particularly retroviruses such as HIV, and synthesized trimeric naphthoquinones through the acid-coupling or base-coupling of 2,3-deoxy-1,4-naphthoquinone with two other naphthoquinone monomers. The monomeric and dimeric naphthoquinones were both found to be devoid of antiviral activity in the Boyd patents. Conocurvone and other trimeric quinones may possess a completely novel mechanism of HIV-inhibition by acting against integrase and the fusion of HIV to CD4 T-lymphocytes [47]. The synthetic method that is regiospecific and produces a good yield is thus needed [50, 51].

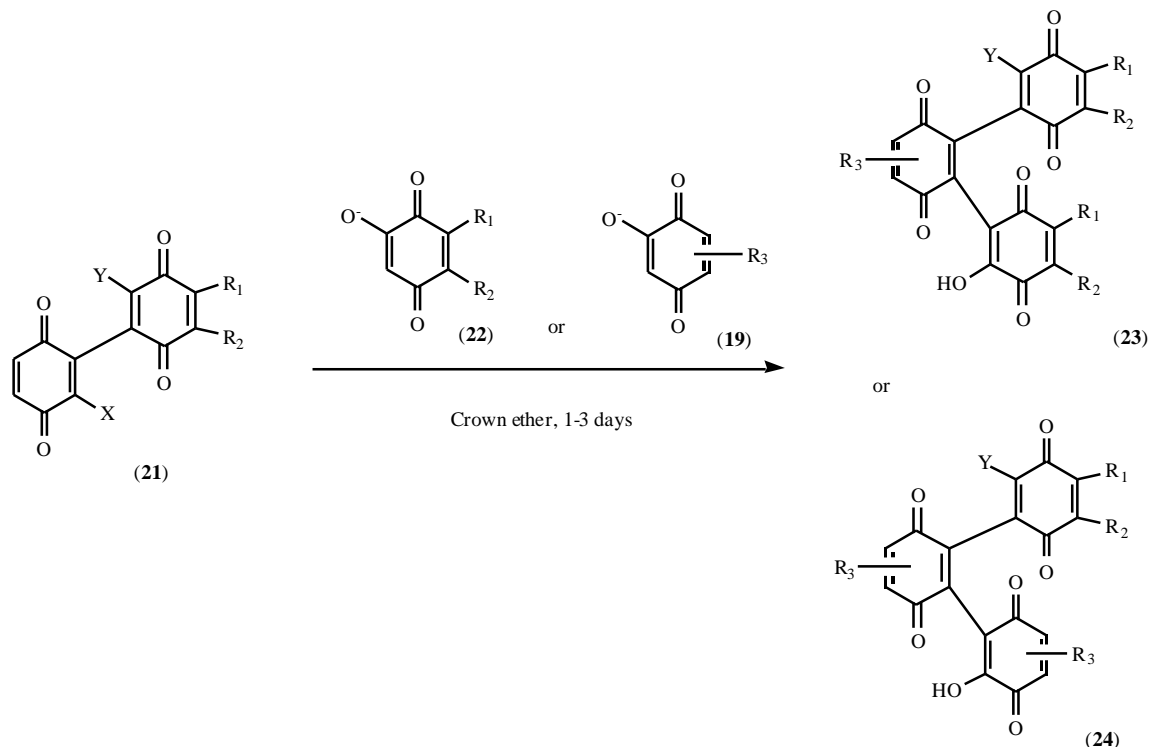
The quinone includes various quinone derivatives including benzoquinones and naphthoquinones. The multi-quinone compounds can include identical quinone monomers or two or more different quinone monomers, such as a biquinone having a benzoquinone monomer bonded to a naphthoquinone monomer. The first quinone includes at least two directing groups at the C-2 position of the first quinone and a second directing group at C-3. The first directing group is selected from a group consisting of a halogen, and a non-halogen, and the second directing group is selected from a group consisting of a halogen and non-halogen. Using the first directing group that is different from the second directing group allows for the efficient regiospecific bonding of the hydroxyquinone anion obtained by reacting a hydroxyquinone in the presence of a base, such as potassium hydroxide or cesium carbonate to the first quinone. The reaction can occur between any hydroxyquinone anion and any first quinone in a solution containing cesium carbonate and acetonitrile in an inert atmosphere at room temperature in about six to seven days (Scheme 1).

In one embodiment of this invention, the representative biquinone can be further reacted in the presence of a base and or a chemical reagent to substitute the hydroxyl group for any chemical group. The biquinone can also be further reacted with a nucleophile. The nucleophile can substitute for the other in the first and second directing group. The nucleophile, for example, can be an amine analog or a second hydroxyquinone anion. Reacting the biquinone with the second hydroxyquinone anion results in a trimeric quinone in a polar aprotic solvent at about 60°C in about 1-3 days (Scheme 2).

One aspect of this invention relates to a method for treating a viral infection demonstrating through *in vitro* antiviral assays [52]. Multi-quinone compounds of the present invention have been shown to inhibit retroviruses, particularly the human immunodeficiency virus, including different strains of HIV-1. The multi-quinone compounds may be formulated into various compositions for use in therapeutic antiviral treatment compositions. Antiviral compositions of this invention include one or more antiviral multi-quinones of this invention, as well as a pharmaceutically acceptable carrier. Pharmaceutically acceptable carriers and methods of administration are well-known to those skilled in the art.



Scheme (1).



Scheme (2).

Inhibition of Carbohydrates Metabolism by Quinone Compounds [53]

The present invention relates to an optically pure enantiomer of a synthetically prepared avarol. The enantiomers of avarol and derivatives are demonstrated to be potent and selective inhibitors of α -glucosidase and α -mannosidase. The inhibition of these two enzymes is useful for a variety of assays and probes, and offers particular utility in the treatment of retroviral infection-associated syndromes, such as AIDS.

Studies conducted with simple achiral quinone have suggested that their toxic activity can be attributed not only to their ability to undergo redox cycling but also to their potential binding and alkylation of nucleic acids and proteins [54]. Given the facile conversion of hydroquinones to quinones under aerobic conditions [55, 56], it stands to reason that chiral substituents on a hydroquinone nucleus might impart a degree of selectivity to the interaction between the respective quinone and asymmetric cellular components such as nucleic acids and highly organized

proteins. Glycosyl hydrolases [57] are important enzymes that catalyze the hydrolysis of glycosidic bonds in polysaccharides and glycoproteins. The ability to inhibit the biosynthetic pathways to carbohydrate and carbohydrate-protein conjugates is significant in the study of cellular and extracellular events and in the development of antiviral [58], antidiabetic [59], and antitumor [60] chemotherapeutic strategies. All the currently approved drugs target one of two key retroviral enzymes, reverse transcriptase or protease, which are essential for replication and survival of the virus. Another promising strategy indirectly targets the initial association and recognition event between the HIV virus and the host cell. The CD4 surface protein has been shown to be a specific cellular receptor for HIV [61, 62]. The inhibitors of certain glycosidases having a profound effect on both the cell surface expression and function and topology of glycoproteins [63], are potential candidates for the therapeutic treatment of HIV infection.

In 1974, avarol and avarone, having various biological effects [64, 65], were isolated from the marine sponge *Dysidea avara* by Minale *et al.* [66] (Fig. 7).

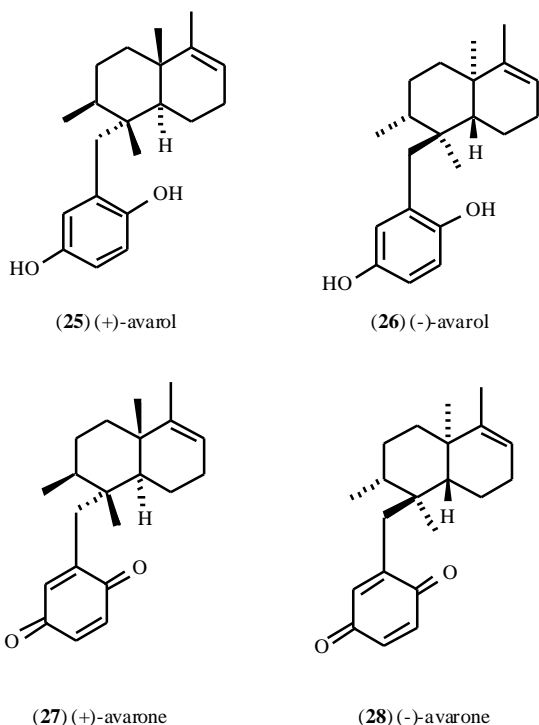


Fig. (7). Structures of avarol and avarone.

In this invention, optically pure enantiomers of avarol were synthetically prepared. A survey of the potential inhibitory effect by avarol against twelve glycosidases was performed according to general method [67], and the avarols proved to be selective, potent inhibitors of α -glucosidase and α -mannosidase. The selective inhibition of α -glucosidase (Type IV, brewer's yeast, EC 3.2.1.20) and α -mannosidase (hack bean, EC 3.2.1.24) was observed with virtually no inhibitory activity against the other assayed enzymes. The value of K_i (9.5 and 25 μM) of (-)-avarol and natural (+)-avarol were obtained from Lineweaver-Burk analyses [68]. Interestingly, the unnatural isomer (-)-avarol was significantly more active than the naturally occurring enantiomer in both cases. The IC_{50} for the unnatural (-)-avarol was 7.6 μM and the natural (+)-isomer was greater than 20 μM . The magnitude of inhibition of α -glucosidase (yeast) by avarol is comparable to that exhibited by deoxynojirimycin ($K_i=23 \mu\text{M}$, yeast α -glucosidase) and the castanospermine derivative ($K_i=1.27 \mu\text{M}$, cellular α -glucosidase) which are currently under investigation as potential anti-HIV drugs. Avarol and its derivatives [69, 70] and avarone [71] had their pharmaceutical compositions described and used as AIDS agents. New potent anti-HIV agents may be prepared by incorporating into avarol some of the salient chemical functionality inherent to several known glycosidase inhibitors while ideally retaining the documented low toxicity of both avarol and avarone.

2) Others

Heterocyclic Quinones as Pharmaceutical Agents [72]

This invention relates to synthetic methods for the preparation of pyrrolylquinones and indolylquinones, the compounds so prepared, and uses thereof in the treatment of

disease, viral infections, neurodegenerative disease, and proliferative disease.

A large class of natural products derived from *Aspergillus* fungi is based upon the dihydroxy-bis-indolylquinone unit that is prenylated in various ways and sometimes O-methylated, and are called asterriquinones. They have the ability to activate the insulin receptor, the TrkA nerve growth factor (neurotrophin) receptor [73, 74], and antitumor activity. They have further developed a synthetic version identified as "compound 29" [73-78] (Fig. 8).

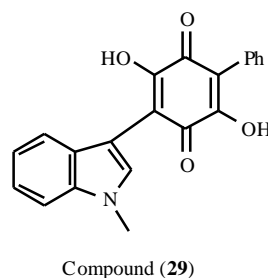


Fig. (8). Structure of compound (29).

A first aspect of the present invention is the compound of Formula I and an acid-catalyzed method of producing a compound of Formula I (Fig. 9) by reacting a substituted or unsubstituted 2,5-dichloro-1,4-benzoquinone with at least one pyrrole in a polar organic solvent, for example, tetrahydrofuran, and in the presence of an acid, such as HCl, H_2SO_4 , AcOH or a mixture to produce a first intermediate, and then reacting the first intermediate with an oxidation agent, such as dichlorodicyanobenzoquinone, Ag_2CO_3 , or a mixture to produce the said compound of Formula I. The method may further include reacting Formula I with an alkali metal hydroxide to produce a compound of the compound 30 ($\text{R}_1=\text{R}_3=\text{OH}$ in Fig. 9).

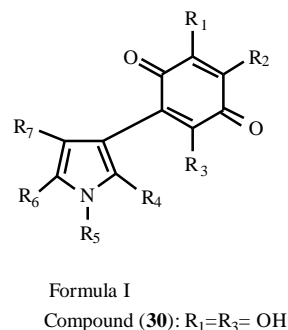
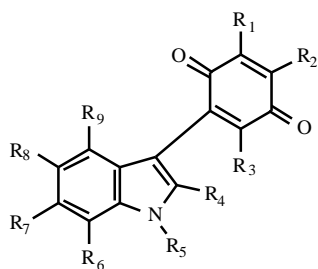


Fig. (9). Structure of Formula I.

A second aspect of the invention is the compound of the Formula II and an acid-catalyzed method of producing these compounds (Fig. 10).

A further aspect of this invention is a method of treating a viral infection, a proliferative disease, and neurodegenerative disease. The method includes the administration



Formura II

Fig. (10). Structure of Formura II.

of compounds of Formulas I and II. The active compounds described here may be formulated for administration in a pharmaceutical carrier in accordance with known techniques [79]. The administration routes of these active compounds are in pharmaceutically acceptable carriers for oral, rectal, topical, buccal, parenteral, intramuscular, intradermal, intravenous, and transdermal administration. The preferred routes of parenteral administration include intrathecal injection, including directly into the tumor, and intraventricular injection into a ventricle of the brain. There are comparatively fewer antivirals than there are antibiotics. Since viruses engage in much of their infective activity by hijacking a cell's machinery and essentially directing the cell to manufacture virus particles, agents with an antiviral effect may additionally inhibit cellular functions in non-infected cells. For example, iododeoxyuridine, one of the first antiviral agents, depresses the DNA synthesis by inhibiting the incorporation of thymidine. The present invention describes use against several families of viruses, both in traditional antiviral targets and in families that currently have no antiviral medication. In one embodiment of the invention, compounds of the invention are effective in treating an infection by the viruses of the family *Poxviridae*, such as variola and vaccinia. Compounds included in this invention may have utility against *Filoviridae*, such as Ebola and Marburg, *Hepadnaviridae*, such as Hepatitis B, *Herpesviridae*, such as HSV-1, and *Retroviridae*. Tested compounds of this invention inhibited phosphatase Cdc25B.

Cysteine Protease Inhibitors [80]

The present invention relates to the compounds having one of the structures represented by 117 formulas, quinone and quinone analogs (examples in Fig. 11) useful for pharmaceutical preparations which inhibit cysteine proteases, in particular, the caspases and 3C-cysteine proteases. The cysteine protease inhibitor is used for treatment of viral diseases, neurodegenerative diseases, and inflammatory diseases.

Cysteine proteases are a major family of peptide-bond-cleaving hydrolases isolated from viruses, bacterial protozoa, plants, mammals and fungi, wherein the thiol group of the cysteine residue serves as a nucleophile in the catalytic process. A variety of physiological disorders or diseases have been attributed to the presence of excessive or insufficient levels of cysteine proteases. The caspases (one family of cysteine proteases) are involved in the biochemical

pathway that mediates apoptosis. Apoptosis is one method by which multicellular organisms eliminate unwanted cells. Normally, apoptosis is a means for regulating the cell number, facilitating morphogenesis, and eliminating harmful, abnormal or nonessential cells. Inappropriate apoptosis has been implicated in a number of diseases. Modulators of apoptosis are a potential target for therapeutics for these diseases. Inhibitors of caspases have been shown to be useful for the treatment of diseases in which excessive apoptosis occurs, such as Alzheimer, Parkinson, etc., and enhancers of caspases in which insufficient apoptosis occurs, such as cancer, viral infections and certain autoimmune diseases [81-84]. Recognized as important proteins in the maturation of the picornaviral life cycle, the 3C and 2A proteases have been a prime target for extensive structural and mechanistic investigations during the past few years [85]. While a variety of compounds have been identified to treat viral diseases by reacting with certain 3C protease or 3C protease-like proteins, which are essential for viral replication and the activity of various proteins [86-88]. Several chemical compounds useful as inhibitors of cysteine proteases, in particular, caspases and 3C cysteine proteases have been found. These inhibitors can be used in *in vitro* applications as well as pharmaceutical preparations.

Antifungal Application

Fungi includes yeasts, molds, and freshly fungi (mushrooms). Yeasts are unicellular and molds are multicellular filamentous organisms. Cell type of fungi is eucaryotic with well-defined nuclear membrane. Cell membranes contain sterols and cell walls contain glucans, mannans, and chitin. Of the more than 100,000 species of fungi, only about 100 are pathogenic for humans and other animals.

Benzonaphthacenequinone Derivative [89]

This invention relates to a novel compound that is prepared by covalently bonding a benzonaphthacenequinone having an antimicrobial activity to a specific organic compound, thus providing the antimicrobial activity of the benzonaphthacenequinone, and increasing the solubility in water to attain the efficient and selective transportation of medicines with high safety and reduced side-effects.

During the screening for microbial products as lead compounds for the treatment of mycoses, benanomycin was isolated from *Actinomadura* sp. by Gomi [90] and pradimicin was from *Actinomadura hibisca* by Oki [91]. Benanomycin and pradimicin, termed Mannose-Binding Quinone glycosides (MBQ) [92], are composed of a polyketide-derived benzo[*a*]naphthacenequinone aglycon, a D-amino acid and monosaccharide residue. MBQ recognizes D-mannosides and binds to the yeast cell surface [93, 94] in the presence of calcium ions. This binding is essential for MBQ to exert its fungicidal action. MBQ has an ideal profile for an antifungal agent, with high selectivity, fungicidal activity, low toxicity, and broad spectrum. Although this development has been withdrawn due to side-effects, the MBQ derivative is believed to be one of the most promising candidates.

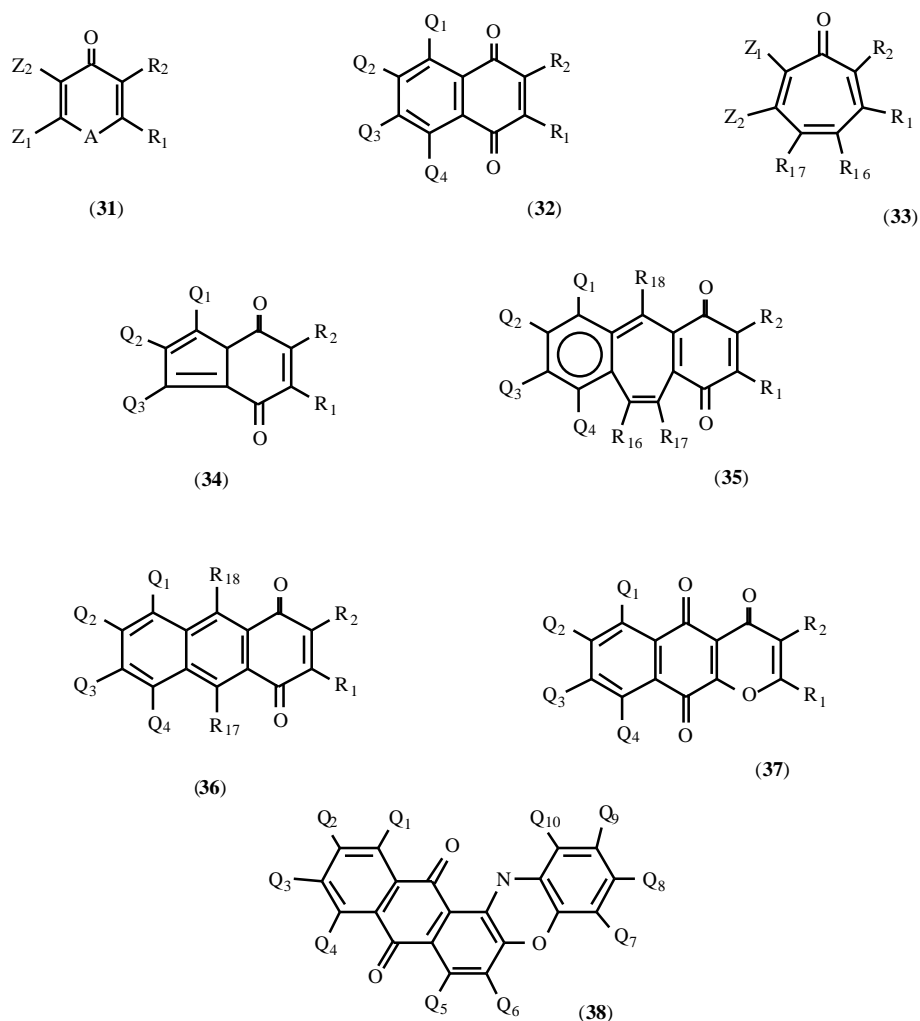


Fig. (11). Structures of quinone analogs.

In this invention, a new compound is a benzonaphthacenequinone covalently bonded to a polyether or glycosaminoglycan represented by the formula (R₁ is H, lower alkyl, lower hydroxyalkyl; R₂ is hydroxy group, amino group, mono- or di(lower alkyl)amino; R₃ is H, nonsulfated or sulfated D-xylosyl, D-glucosyl; R₄ is H, lower alkyl, D-xylosyl), typically monodecyloxy-tetraethylene glycol-benanomicin A. The compound is prepared, for example, by protecting the hydroxyl groups, then allowing the carboxyl group to react with the amino groups or hydroxyl groups to form the acid amide bonds or ester bonds. Also claimed are pharmaceutical compounds containing the derivatives or their salts (Fig. 12).

For example, 8.7 mg of benanomicin in DMF was treated with 29.0 mg of ω -amino-hexaethylene glycol methyl ether in the presence of dicyclohexylcarbodiimide to produce 8.5 mg of the amide product. The benanomicin A polyethylene glycol derivative showed a dose-dependant antifungal activity against *Candida albicans* 3143, and the activity was enhanced in the presence of calcium.

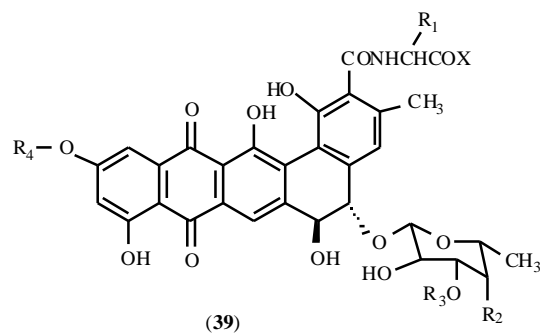


Fig. (12). Structures of benzonaphthacenequinone derivative.

The derivative of this invention can be used in the medical treatment of various diseases (infective disease, HIV, cancer, etc.) of mammals including humans.

ANTIPROTOZOAL APPLICATION

Protozoans are one-celled, eucaryotic organisms that belong to the Protista. All protozoans live in areas with a

large supply of water. Protozoans are mostly aerobic heterotrophs and classified by their means of locomotion: the Sarcidina move by amoeboid motion; the Mastigophora use flagella for motility; the Sporozoa lack a means of locomotion and are obligate parasites; the Ciliata possess cilia.

Pharmaceutical combination of 2-[4-(4-chlorophenyl)cyclohexyl]-3-hydroxy-1,4-naphthoquinone and proguanil and pharmaceutical preparation [95]: The present invention relates to the synergistic combination of 2-[4-(4-chlorophenyl)cyclohexyl]-3-hydroxy-1,4-naphthoquinone (atovaquone) and proguanil which have anti-parasitic activity.

The compound 2-[4-(4-chlorophenyl)cyclohexyl]-3-hydroxy-1,4-naphthoquinone (atovaquone) has previously been disclosed, for example in EP123238 [96] which relates to the 2-substituted 3-hydroxy-1,4-naphthoquinones of Formula III having antiprotozoal activity. Specifically, compounds of Formula III wherein n is zero are said to be active against the human malaria parasite *Plasmodium falciparum* and also against *Eimeria* species. Among the compounds specifically named is atovaquone having the formula in which n is zero, R₁ is hydrogen and R₂ is the 4-chlorophenyl group (Fig. 13).

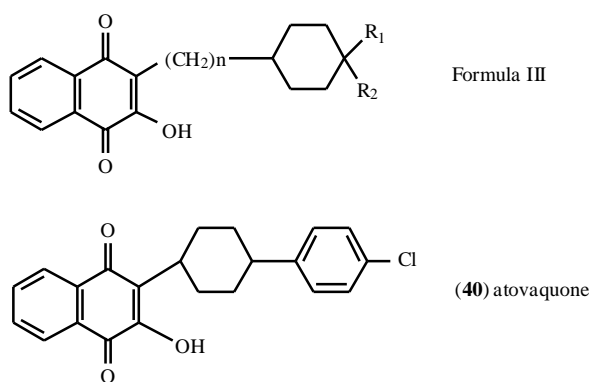


Fig. (13). Structures of atovaquone derivatives.

Proguanil is a well-known drug for prophylaxis, but not for the treatment of malaria. It is one of the safest antimalarial drugs. However, a resistance of *P. falciparum* to proguanil has appeared, particularly in southeast Asia.

In order to combat drug resistance, it is becoming standard practice to use combinations of more than one antimalarial, either simultaneously or sequentially. However, many such combinations are antagonistic, resulting in a reduced effectiveness.

In this invention, it has been found that potentiation of the antiparasitic and antimalarial activities is achieved by combining, either concomitantly or sequentially, atovaquone and proguanil. Atovaquone inhibits the electron transport system in the mitochondria of parasites, thereby blocking nucleic acid synthesis and inhibiting replication [97]. Proguanil also acts against the asexual erythrocytic stage of the parasite by selectively inhibiting plasmodial dihydrofolate reductase. But it significantly enhanced the

ability of atovaquone to cause collapse of the mitochondrial membrane potential when used in combination [98]. The present invention provides a method for the treatment and/or prophylaxis of a protozoal parasitic infection such as malaria and toxoplasmosis, and an infection caused by *P. carinii* in mammals including humans, which comprises administering an effective amount of atovaquone or a physiologically acceptable salt thereof and concomitantly or sequentially administering an effective amount of proguanil. The pharmaceutical combination of proguanil and atovaquone is present in a weight ratio ranging from 1:1 to 1:3. Preferred are the pharmaceutical preparations containing 50 mg to 3 g of each of the agents, more preferably 500 mg of atovaquone and 200 mg of proguanil.

CURRENT & FUTURE DEVELOPMENTS

Quinone compounds are intermediates in many pathways of gene regulation, enzyme protein induction, feedback control, and waste product elimination in addition to the role as substrates and products in metabolism. Quinones play a pivotal role in energy metabolism, many other key processes, and even in chemotherapy where redox cycling drugs are utilized. However, the molecular mechanisms involved in quinone cytotoxicity and pharmaceutical activity are still mostly unknown. Their widespread use as antibiotics, antiparasitic agents, antitumor agents, and a variety of other agents makes it imperative to understand their effects on cellular function. Until this is clarified, it is not possible to use a rational approach to search for or design more effective quinone agents with less side-effects, and the current approach of random screening and analog development will continue.

Malarone (atovaquone and proguanil) as antimalarial agent and the bamboo extract containing benzoquinone as antichlamidial detergent in this review will be approved for use because of their commercial clinical use and low toxicity. The fate of new other compounds will be decided in clinical trials. It is unclear that research will yield the next breakthrough discovery, but it is certain that therapeutic advances will continue to happen.

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