

TITLE: SPIRO AND DISPIRO 1,2,4-TRIOXOLANE ANTIMALARIALS

FIELD OF THE INVENTION

This invention relates to compositions and methods for treating malaria.

5 Specifically, this invention relates to pharmaceutical compositions including spiro and dispiro trioxolanes, and methods of their use and manufacture.

BACKGROUND OF THE INVENTION

10 Malaria is an acute and often chronic infectious disease resulting from the presence of protozoan parasites within red blood cells. Caused by single-celled parasites of the genus *Plasmodium*, malaria is transmitted from person to person by the bite of female mosquitos.

15 Although once prevalent in North America and other temperate regions of the world, today malaria occurs mostly in tropical and subtropic countries. Each year, between 400 million and 600 million people contract the disease, and 1.5 million to 2.7 million die of the disease.

Four species of *Plasmodium* protozoan parasites are generally responsible for malaria, including *Plasmodium vivax*, *Plasmodium falciparum*, *Plasmodium malariae*, and *Plasmodium ovale*. Of the four, *Plasmodium falciparum* is the most dangerous, accounting for half of all clinical cases of malaria and 90% of deaths from the disease.

20 The transmission of malaria begins when a female mosquito bites a human already infected with the malaria parasite. When the infected mosquito bites another human, sporozoites in the mosquito's saliva are transferred into the blood, which then travel to the liver. In the liver, the sporozoites divide rapidly, then enter the bloodstream where they invade red blood cells. Inside these blood cells, the merozoites multiply rapidly until they
25 cause the red blood cells to burst, releasing into the blood stream a new generation of merozoites that then infect other red blood cells.

The symptoms associated with malaria are generally associated with the bursting of the red blood cells. The destruction of the red blood cells spills wastes, toxin, and other debris into the blood. This in turn causes an intense fever that can leave the infected
30 individual exhausted and bedridden. More severe symptoms associated with repeat infections and/or infection by *Plasmodium falciparum* include anemia, severe headaches, convulsions, delirium and, in some instances, death.

The treatment of malaria has been especially difficult due to the ability of malaria parasites to develop resistance to drugs. Quinine, an antimalarial compound that is extracted from the bark of the South American cinchona tree, is one of the oldest and most effective pharmaceuticals in existence. The downside to quinine is that it is short-acting, and fails to prevent disease relapses. Further, quinine is associated with side effects ranging from dizziness to deafness.

Chloroquine is a synthetic chemical similar to quinine. It became the drug of choice for malaria when it was developed in the 1940s due to its effectiveness, ease of manufacture, and general lack of side effects. However, in the last few decades, malaria parasites in many areas of the world have become resistant to chloroquine.

Mefloquine is another synthetic analog of quinine that has been used in the treatment of malaria. Malaria parasites have also developed resistance to mefloquine, however. Mefloquine is also associated with undesirable central nervous side effects in some patients, including hallucinations and vivid nightmares.

Antifolate drugs are effective against malaria parasites by inhibiting their reproduction. Although the parasites have also developed a resistance to antifolate drugs, the drugs can still be used effectively in combination with other types of antimalarials. The use of combination therapies in treating malaria has the drawbacks of being inconvenient and expensive, however.

More recent developments in the treatment of malaria have involved the use of the peroxide functional group, as exemplified by the drug artemisinin, which contains a unique 1,2,4-trioxane heterocyclic pharmacophore. The antimalarial action of artemisinin is due to its reaction with the iron in free heme molecules in the malaria parasite with the generation of free radicals leading to cellular destruction.

The discovery of artemisinin (qinghaosu), a naturally occurring endoperoxide sesquiterpene lactone (Meshnick et al., 1996; Vroman et al. 1999; Dhingra et al., 2000) initiated a substantial effort to elucidate its molecular mechanism of action (Jefford, 1997; Cumming et al., 1997) and to identify novel antimalarial peroxides (Dong and Vennerstrom, 2001). Many synthetic 1,2,4-trioxanes, 1,2,4,5-tetraoxanes, and other endoperoxides have been prepared.

Although the clinically useful semisynthetic artemisinin derivatives are rapid acting and potent antimalarial drugs, they have several disadvantages including recrudescence, neurotoxicity, (Wesche et al., 1994) and metabolic instability. (White, 1994). A fair number of these compounds are quite active *in vitro*, but most suffer from low oral activity.

5 (White, 1994; van Agtmael et al., 1999). Although many synthetic antimalarial 1,2,4-trioxanes have since been prepared (Cumming et al., 1996; Jefford, 1997), there exists a need in the art to identify new peroxide antimalarial agents, especially those which are easily synthesized, are devoid of neurotoxicity, and which possess improved pharmacokinetic properties, e.g. improved stability, oral absorption, etc.

10 Accordingly, it is a primary objective of the present invention to provide compositions and methods for prophylaxis and treatment of malaria using spiro and dispiro 1,2,4-trioxolanes.

It is a further objective of the present invention to provide a composition and method for prophylaxis and treatment of malaria using spiro and dispiro 1,2,4-trioxolanes
15 that is nontoxic.

It is a further objective of the present invention to provide a composition and method for prophylaxis and treatment of malaria using spiro and dispiro 1,2,4-trioxolanes that is metabolically stable and orally active.

It is yet a further objective of the present invention to provide a composition and
20 method for prophylaxis and cost-effective treatment of malaria using spiro and dispiro 1,2,4-trioxolanes that do not involve a treatment regimen of more than three days.

It is a further objective of the present invention to provide compositions and methods for prophylaxis and treatment of malaria using spiro and dispiro 1,2,4-trioxolanes that can be used either as stand-alone medicaments or in combination with other agents.

25 It is still a further objective of the present invention to provide novel intermediates for synthesizing compositions for prophylaxis and treatment of malaria.

The method and means of accomplishing each of the above objectives as well as others will become apparent from the detailed description of the invention which follows hereafter.

30 **SUMMARY OF THE INVENTION**

The invention describes a method and composition for treating malaria with spiro and dispiro 1,2,4-trioxolanes, their prodrugs and analogues. The trioxolanes of this invention are sterically hindered on one side of the trioxolane heterocycle in order to provide chemical and metabolic stability to the trioxolane ring for better *in vivo* activity. In one embodiment, the spiro and dispiro trioxolanes are sterically hindered with an unsubstituted, mono-, di-, or poly-substituted C₅-C₁₂ spiro cycloalkyl group, which may be spiroadamantane. In this embodiment, the spiro and dispiro trioxolanes may include a spirocyclohexyl that is functionalized or substituted at the 4-position or a spiropiperidyl ring that is functionalized or substituted at the nitrogen atom. In another embodiment, the trioxolanes of this invention include an alkyl bridge from the 4-position of the spirocyclohexyl ring connecting a substituent that is most preferably a weak base. The invention embraces achiral, achiral diastereomers, racemic mixtures, as well as enantiomeric forms of the compounds.

The trioxolanes of this invention possess excellent potency and efficacy against *Plasmodium* parasites, and a low degree of neurotoxicity. In addition, several of the trioxolanes are suitable for both oral and non-oral administration. Moreover, in comparison to artemisinin semisynthetic derivatives, the compounds of this invention are structurally simple, easy and inexpensive to synthesize, and can be used effectively alone or in conjunction with other antimalarials.

DETAILED DESCRIPTION OF THE PREFERRED EMBODIMENT

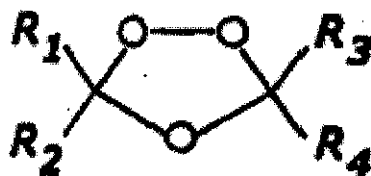
The present invention relates to the development of spiro and dispiro 1,2,4-trioxolanes for use in the prophylaxis and treatment of malaria. The present invention is predicated upon the unexpected discovery that trioxolanes that are relatively sterically hindered on at least one side of the trioxolane heterocycle provide metabolic and chemical stability to the trioxolane ring, thereby providing better *in vivo* activity, especially with respect to oral administration.

As used herein the term "prophylaxis-effective amount" refers to a concentration of compound of this invention that is effective in inhibiting or preventing infection and subsequent disease by malarial parasites. Likewise, the term "treatment-effective amount" refers to a concentration of compound that is effective in treating malaria in terms of preventing an increase in the concentration of malarial parasites, decreasing the

concentration of malarial parasites, and/or "curing" a malaria infection, i.e. survival for 30 days post-infection.

Tetrasubstituted trioxolanes are relatively stable peroxidic compounds based on literature precedent (Griesbaum et al., 1997a; 1997b). This may be due, in part, to the lack of α -hydrogen atoms. The present inventors have synthesized new compounds in the trioxolane class having both superior antimalarial potency and oral efficacy. Furthermore, the compounds of this invention have low toxicity, and half-lives conducive to treatment of malaria which are believed will permit short-term treatment regimens comparing favorably to other artemisinin-like drugs. These compounds may also be used in malaria prophylaxis.

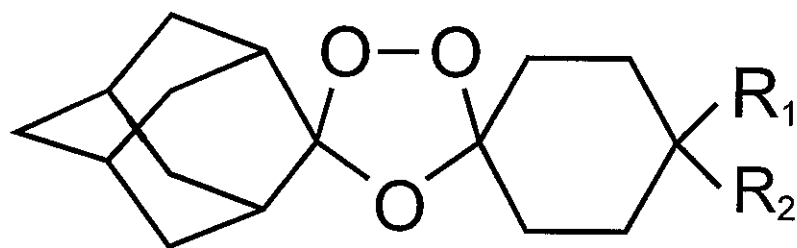
In previous application, the present inventors disclosed certain novel tetrasubstituted trioxolanes having the following structural formula:



wherein R_1 , R_2 , R_3 , and R_4 represent combinations of ring systems, acyclic systems, and functional groups that provide sufficient steric hindrance about the trioxolane ring in order to give the ring chemical and metabolic stability. R_1 , R_2 , R_3 and R_4 may be the same or different, and may be a linear or branched alkyl, aryl, or alkaryl group which is optionally substituted. In the alternative, R_1 and R_2 taken together and/or R_3 and R_4 taken together may form an alicyclic group which is optionally interrupted by one or more oxygen, sulfur or nitrogen atoms and which group is optionally substituted. In no event may any of R_1 , R_2 , R_3 or R_4 be hydrogen.

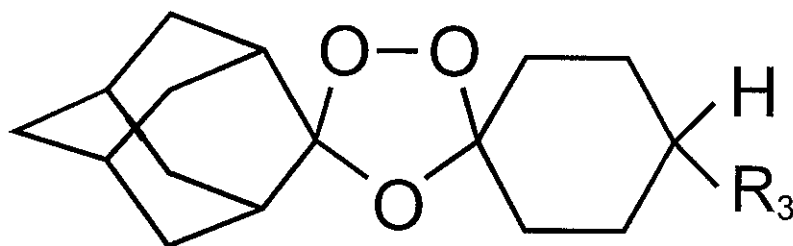
In one embodiment, the compounds include those whereby R_1 and R_2 taken together and/or R_3 and R_4 taken together is a mono- or di-substituted C_5 - C_{12} spirocycloalkyl group which is optionally interrupted by one or more oxygen, sulfur, or nitrogen atoms, and which group is optionally substituted. In another embodiment, R_1 and R_2 taken together or R_3 and R_4 is spiroadamantane.

The present invention discloses a new embodiment of trioxolane compounds having the following structure:



The spirocyclohexyl ring may be optionally interrupted by one or more oxygen, sulfur or nitrogen atoms. In this regard, R_1 and R_2 may be the same or different, and may be hydrogen, substituted or unsubstituted linear or branched alkyl, aryl, and alkaryl groups and substituted or unsubstituted alicyclic groups that may be interrupted by one or more oxygen, sulfur or nitrogen atoms, substituted or unsubstituted aromatic or heterocyclic groups that may be interrupted by one or more oxygen, sulfur or nitrogen atoms, a hydroxy group, or a halogen. In one embodiment, R_1 or R_2 is an amide. It has been unexpectedly found that amide-containing substituents at the 4-position provide antimalarial compounds with good oral absorption, good antimalarial activity, and good pharmacokinetics, i.e. rates of absorption, metabolism, and elimination that are suitable and advantageous for the prophylaxis and treatment of malaria.

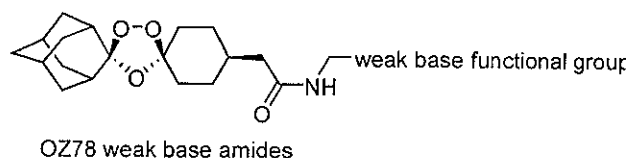
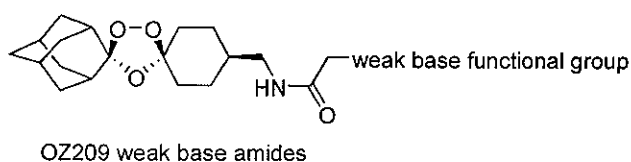
In another embodiment, the compounds of this invention have the following structural formula:



whereby R_3 is $(CH_2)_n-Y$. In this formula, Y represents a functional group that, in one embodiment, is non-acidic, and in another embodiment is a weak base. The Y functional group may be an alkyl, ketone, acid, alcohol, amine, amide, sulfonamide, guanidine, ether,

ester, oxime, urea, oxime ether, sulfone, lactone, carbamate, semicarbazone, phenyl, or heterocycle. In one embodiment, $n = 1$. The alkyl "bridge" group has been found to improve the metabolic stability (i.e. oral activity and pharmacokinetics) of the antimalarial compounds of this invention.

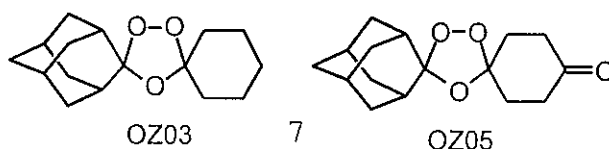
5 In another embodiment of the invention, the trioxolanes are weak bases, which provide an ideal combination of high intrinsic potency and good oral activity. Two promising trioxolane structural subtypes are weak base amides of trioxolane amine OZ209 and trioxolane acid OZ78. These compounds have one of the following two structural formulas:



15 Other substituents at the 4-position of the spirocyclohexyl ring are also possible that fall within the scope of this invention. The spirocyclohexyl ring may also be substituted at other positions besides the 4-position. For instance, the inventors have synthesized several compounds substituted at the 2-position of the spirocyclohexyl ring that provide excellent antimalarial potency.

20 In another embodiment of this invention, the compounds include an alkyl group connecting the substituent at the 4-position to the spirocyclohexyl ring. In one embodiment, the alkyl group is methyl or ethyl. In another embodiment, the alkyl group is methyl. The substituent may also be directly attached to the 4-position of the spirocyclohexyl ring.

25 The present inventors have identified two orally active lead dispiro-1,2,4-trioxolanes, OZ03 and OZ05:

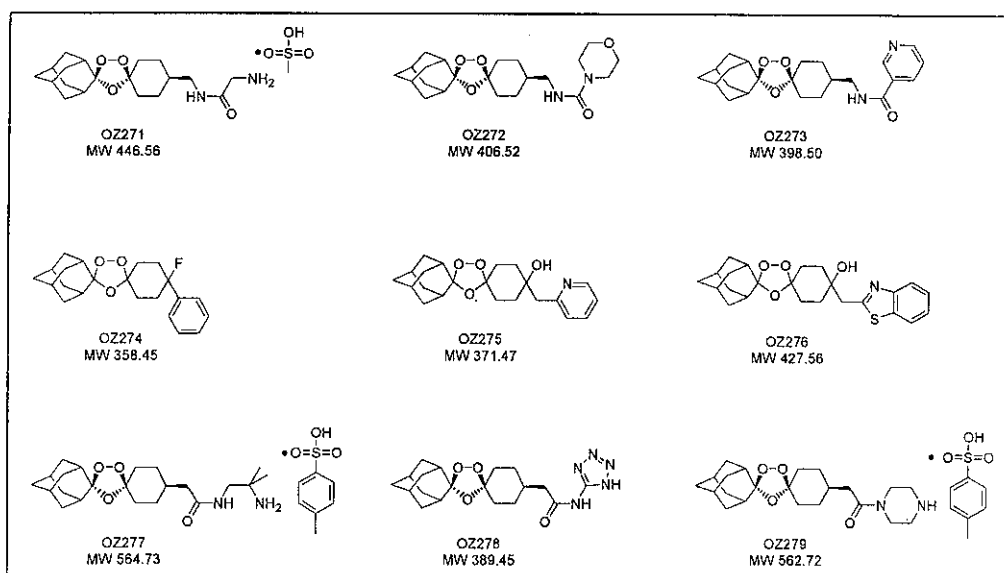


These trioxolanes have IC_{50} s between 1 and 5 ng/ml against *P. falciparum* *in vitro*, and presumably possess good therapeutic indices as no toxicity is evidence for either compound in a neuroblastoma cell line or at single 640 mg/kg doses in mice in the Rane test. These results contrast with published data (de Almeida Barbosa et al., 1992; 1996) disclosing the weak *in vitro* antimalarial potency of several tricyclic trioxolanes, the best of which has an IC_{50} of 2000 ng/ml against *P. falciparum* *in vitro*.

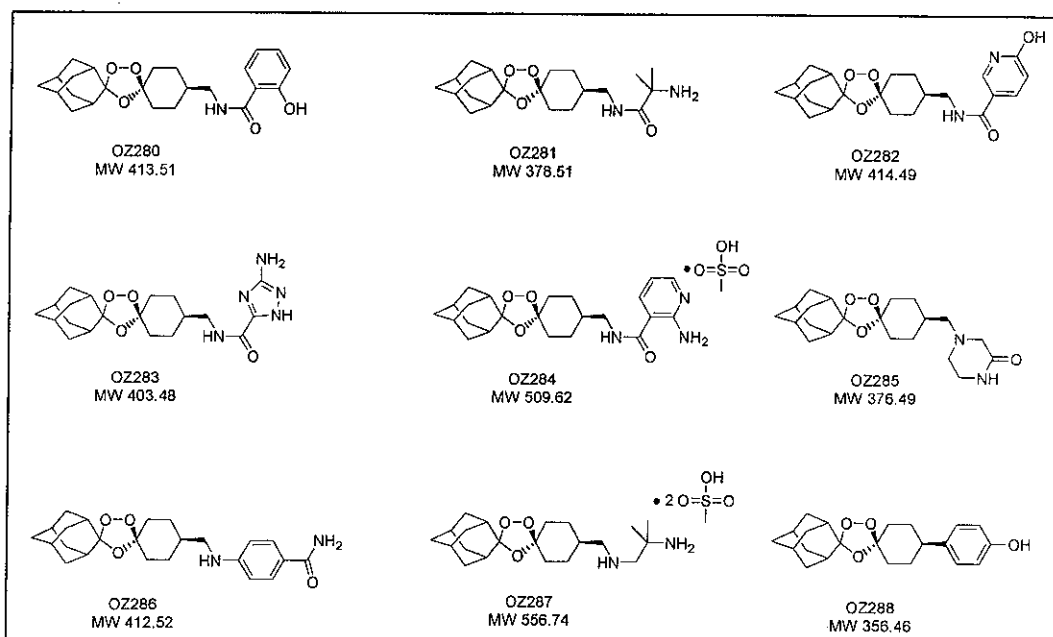
A notable feature of these trioxolanes in comparison to the artemisinin semisynthetic derivatives is their structural simplicity. A potential advantage of trioxolanes over both trioxanes (Jefford, 1997; Cumming et al., 1997) and tetraoxanes (Vennerstrom et al., 2000) is a more convenient access to structurally diverse, non-symmetrical, and in many cases, achiral compounds.

Below are several dispiro 1,2,4-trioxolanes synthesized in accordance with the teachings of this invention. "OZ" is an internal designation for these compounds that will be used throughout the remainder of the application for convenience. The structures of OZ01-OZ270 have been previously disclosed in prior applications U.S. Serial No. 09/886,666 (U.S. Patent No. 6,486,199) and PCT/02/19767, and are therefore not repeated here.

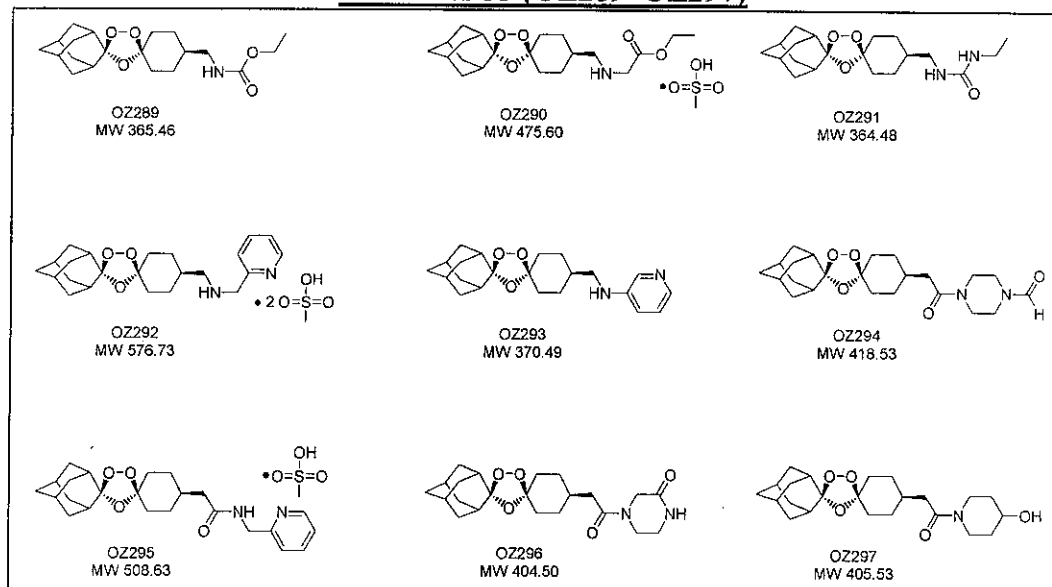
OZ Series 31 (OZ271–OZ279)



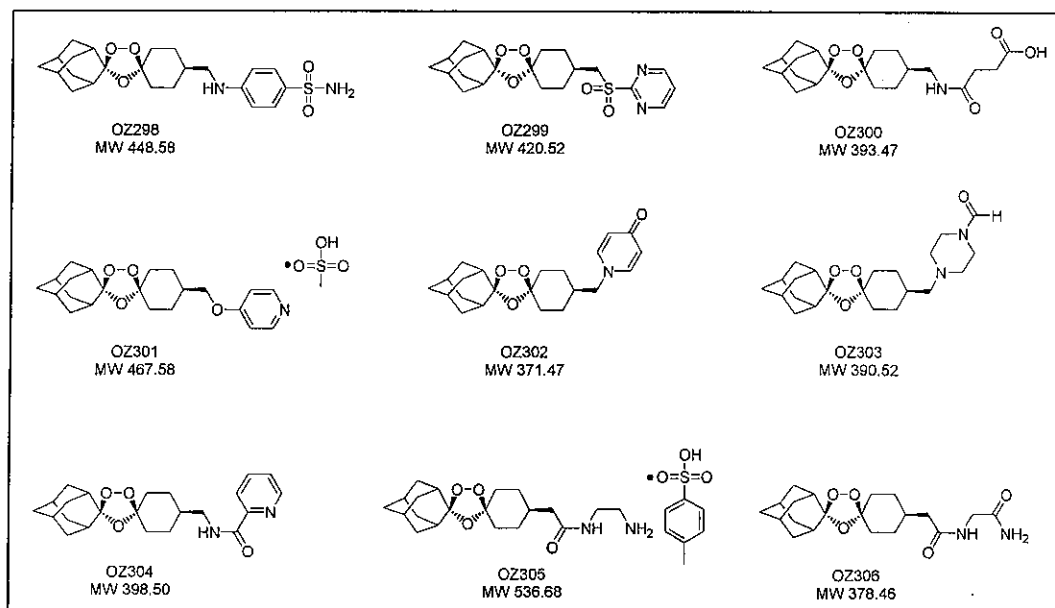
OZ Series 32 (OZ280–OZ288)



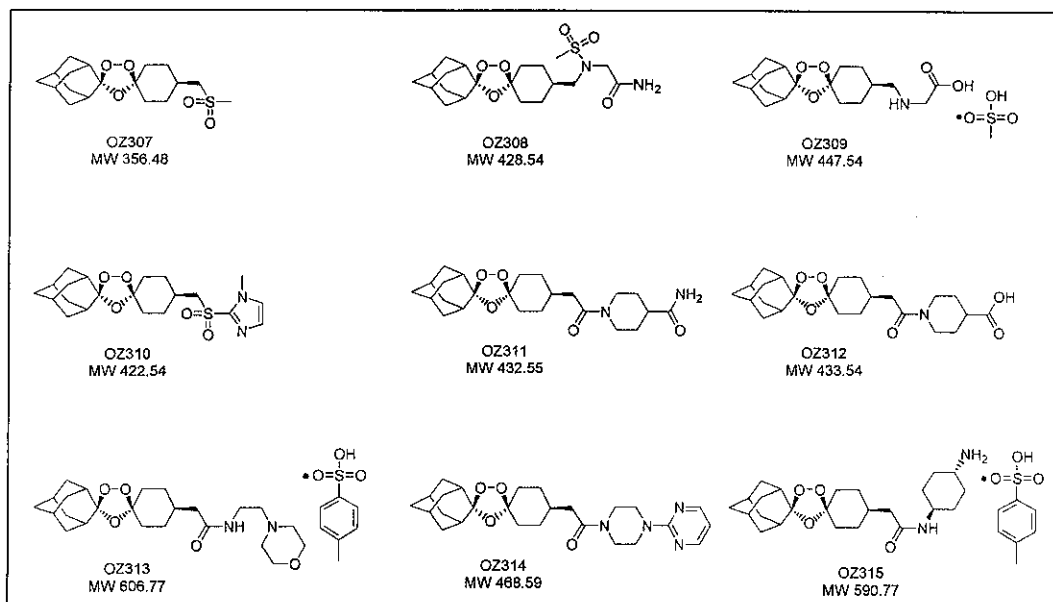
OZ Series 33 (OZ289–OZ297)



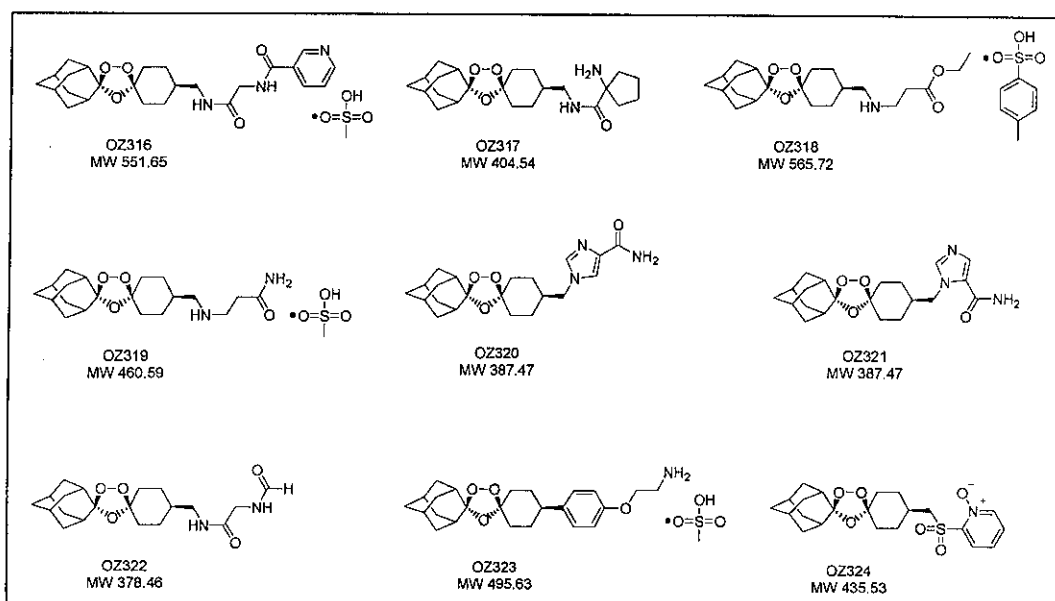
OZ Series 34 (OZ298–OZ306)



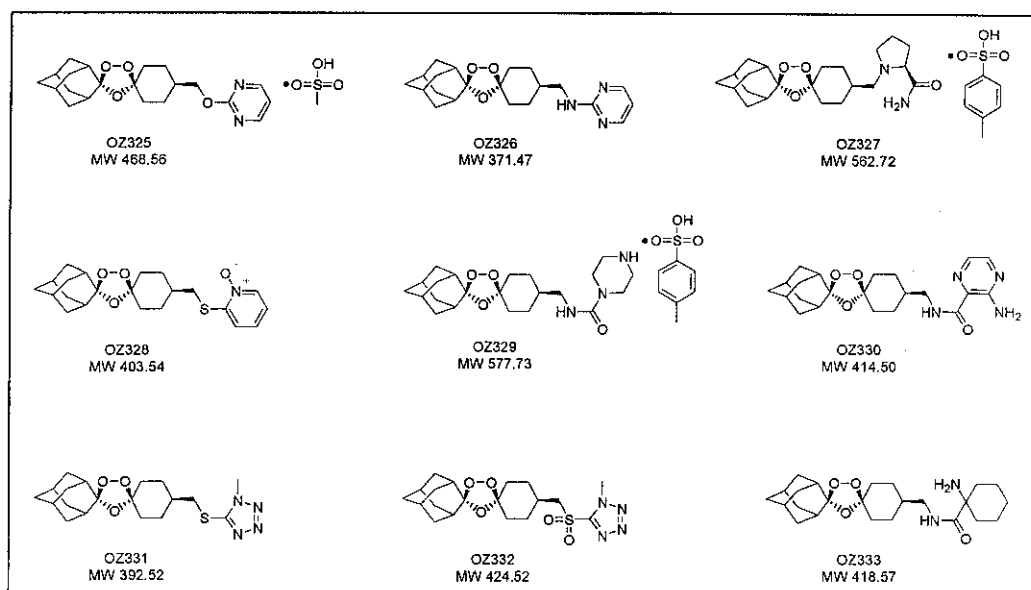
OZ Series 35 (OZ307–OZ315)



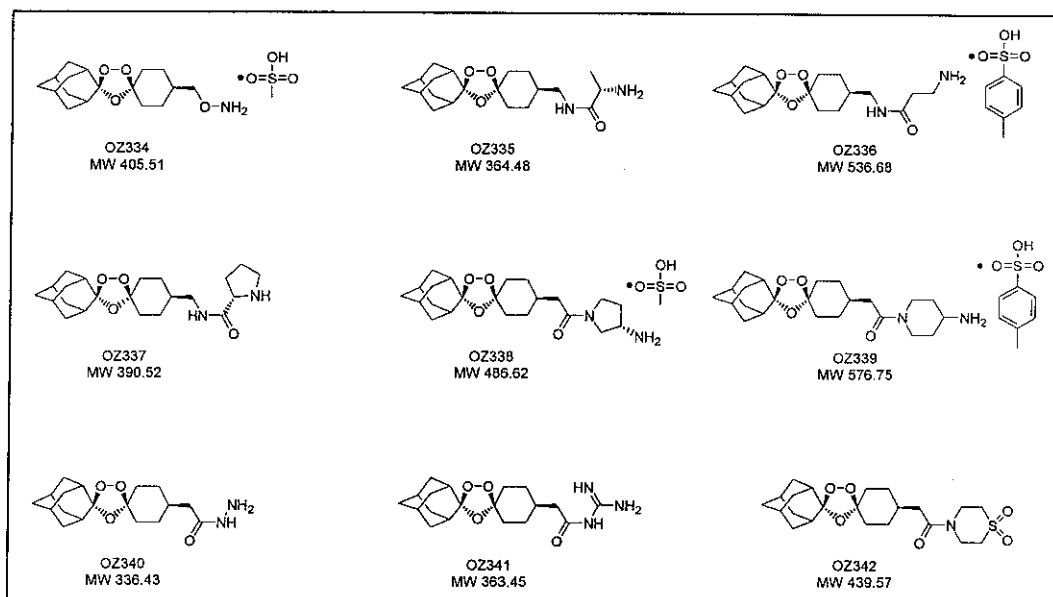
OZ Series 36 (OZ316–OZ324)



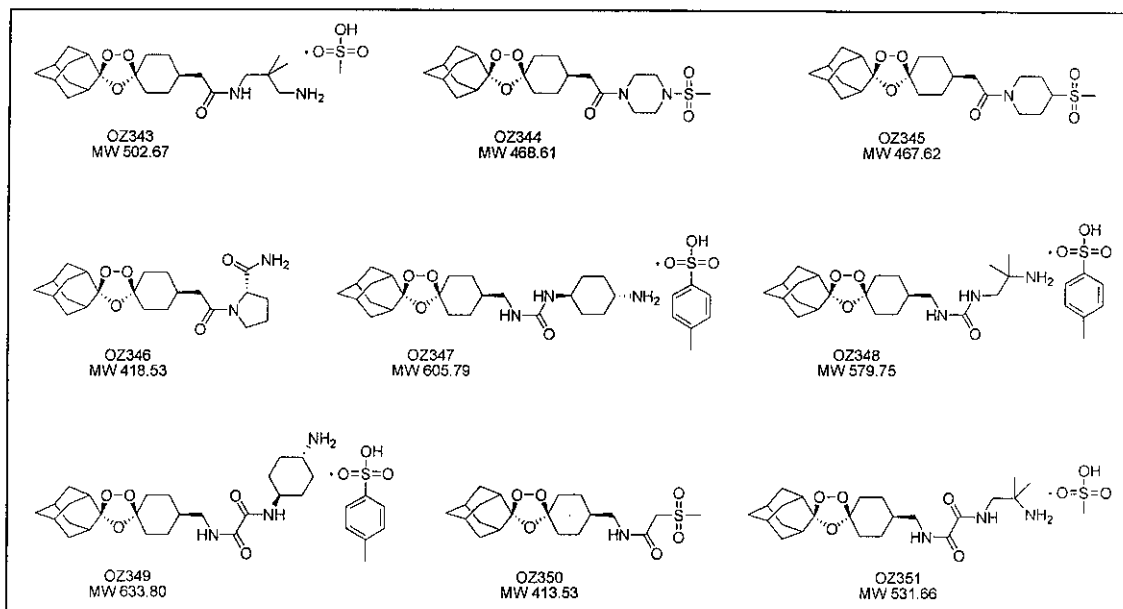
OZ Series 37 (OZ325–OZ333)



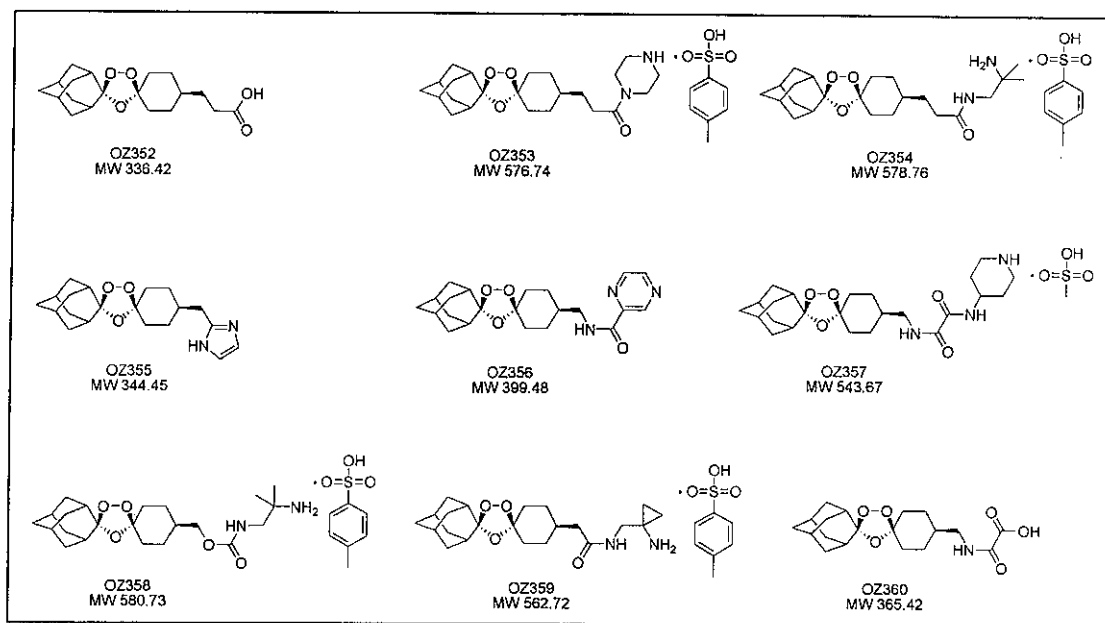
OZ Series 38 (OZ334-OZ342)



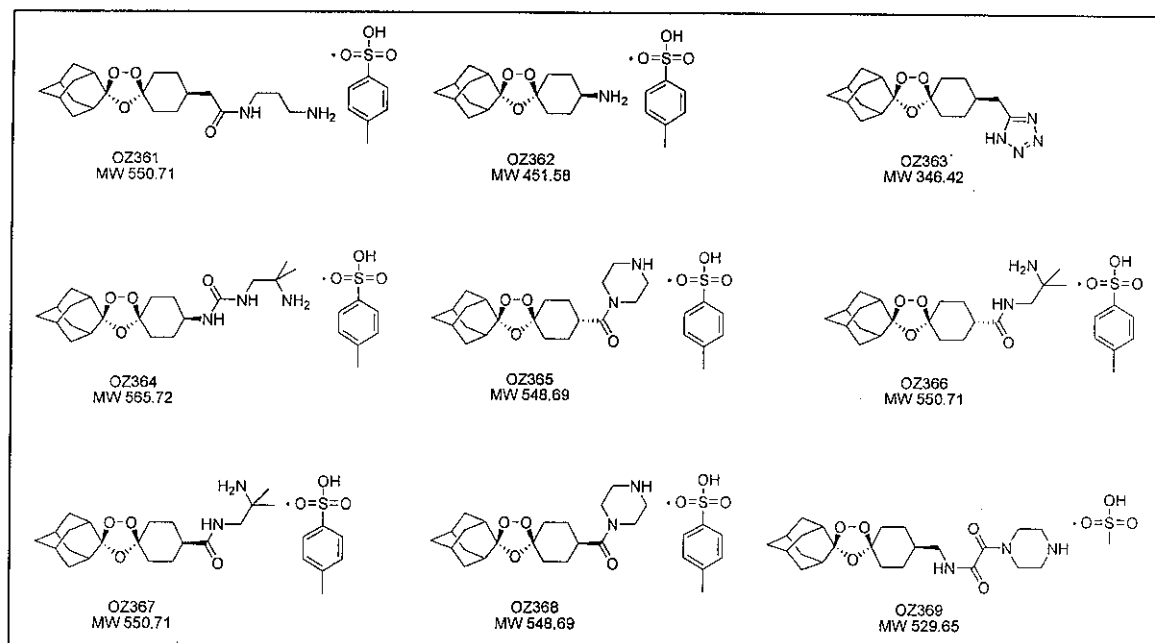
OZ Series 39 (OZ343-OZ351)



OZ Series 40 (OZ352-OZ360)



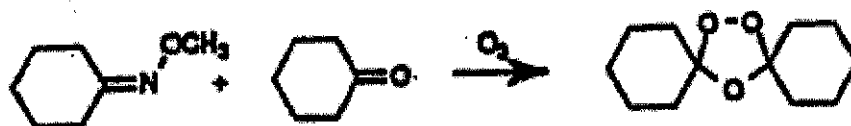
OZ Series 41 (OZ361-OZ369)



The prototype trioxolanes of this invention are OZ03 and OZ05. Preferred compounds identified thus far include OZ03, OZ05, OZ11, OZ25, OZ27, OZ61, OZ71, OZ78, OZ127, OZ145, OZ156, OZ163, OZ175, OZ177, OZ179, OZ181, OZ189, OZ205, OZ207, OZ209, OZ210, OZ219, OZ227, OZ229, OZ235, OZ255, OZ256, OZ257, OZ263,

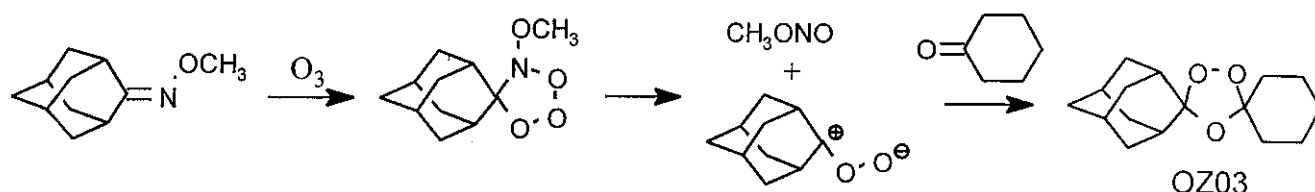
OZ264, OZ265, OZ266, OZ267, OZ268, OZ269, OZ270, OZ271, OZ277, OZ281, OZ279, OZ288, OZ289, OZ290, OZ296, OZ297, OZ298, OZ301, OZ305, OZ309, OZ315, OZ317, OZ319, OZ320, OZ323, OZ329, OZ333, OZ335, OZ336, OZ337, OZ338, OZ339, OZ343, OZ349, OZ351, OZ353, OZ354, OZ357, OZ358, OZ359, OZ365, and OZ368. The most preferred compounds are OZ78, OZ163, OZ181, OZ207, OZ209, OZ255, OZ256, OZ257, OZ263, OZ264, OZ267, OZ271, OZ277, OZ279, OZ301, OZ305, OZ315, OZ317, OZ319, OZ323, OZ329, OZ338, OZ339, OZ349, OZ351, OZ354, OZ357, OZ359, and OZ368, with OZ277 and OZ279 being the best of those compounds identified thus far. In general, the highest *in vitro* potency against malarial parasites is obtained for trioxolanes functionalized or substituted at the 4-position of the spirocyclohexyl ring. As a general rule, non-symmetrical, achiral trioxolanes are also preferred.

Notable features of these spiro and dispiro 1,2,4-trioxolanes in comparison to the artemisinin semisynthetic derivatives are their structural simplicity and ease of synthesis. For example, dispiro trioxolanes may be easily synthesized by the coozonolysis of the *O*-methyl oximes of cycloalkanones in the presence of the requisite cycloalkanone derivatives according to the method of Griesbaum et al. (1997a; 1997b) as illustrated below for the symmetrical dispiro cyclohexyl trioxolane:



If yields are low in this coozonolysis reaction, yields can improve dramatically when the *O*-methyloxime and ketone are "reversed." This novel procedure provides a uniquely convenient method to synthesize spiro and dispiro trioxolanes. Advantages of the oxime ether route over the alkene approach include convenient synthesis of starting materials (oxime ethers vs. tetrasubstituted alkenes), higher yield and selectivity of formation of desired trioxolanes by the judicious selection of paired reaction substrates. The trioxolanes may be purified by crystallization or by flash column chromatography. Their structures and purity may be confirmed by analytical HPLC, ¹H and ¹³C NMR, IR, melting point and elemental analysis.

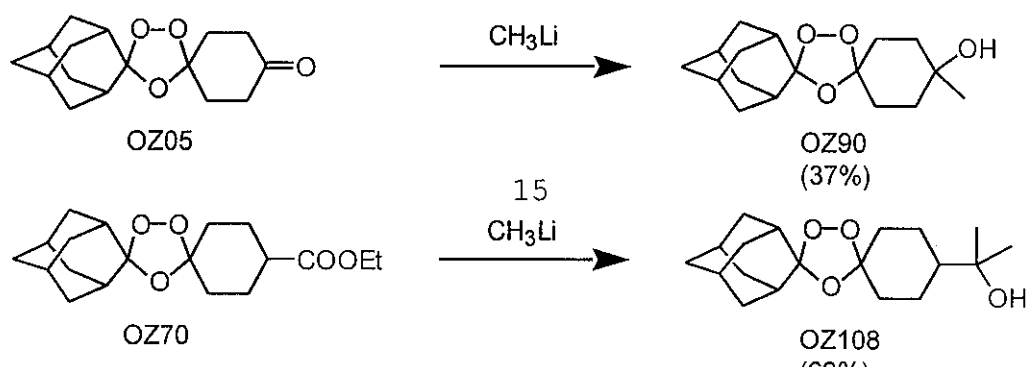
Formation of a trioxolane from an oxime ether and a ketone is presumed to be a three-step process. The sequence begins by the electrophilic addition of ozone to the oxime double bond to form a primary ozonide. Second, the very unstable primary adduct fragments to a reactive carbonyl oxide driven in part by the concomitant expulsion of the relatively stable methyl nitrite. Third, the carbonyl oxide undergoes a [3 + 2] cycloaddition with a ketone to give the secondary ozonide or 1,2,4-trioxolane. It remains to be



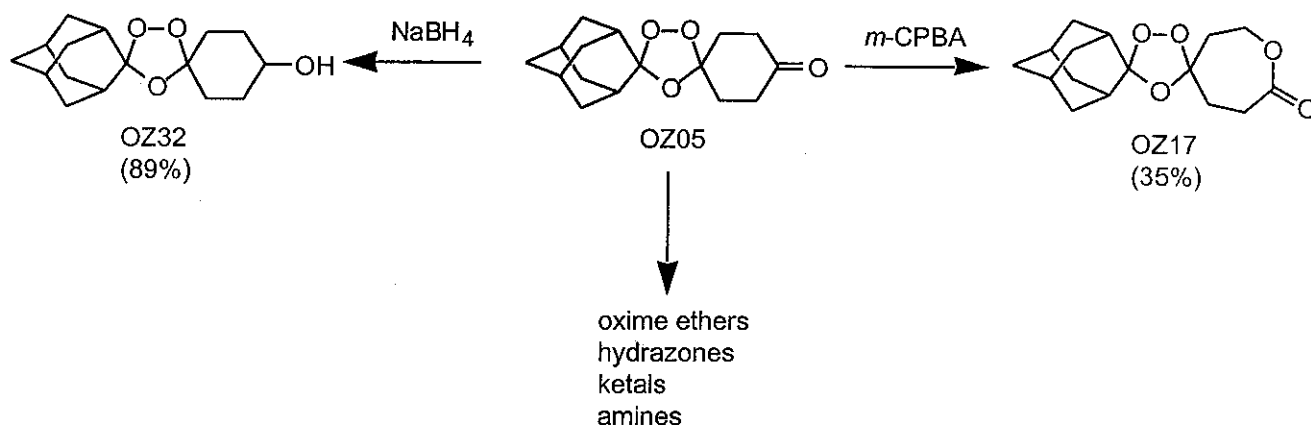
determined whether this is a stepwise or a concerted recombination process.

As illustrated above by the synthesis of OZ03, most of the new dispiro trioxolanes contain a spiroadamantane and can be synthesized by the coozonolysis of adamantanone *O*-methyl oxime in the presence of the requisite cycloalkanone derivative. The preferred reaction solvents for the coozonolysis reactions are hydrocarbon solvents such as pentane or cyclohexane; more polar solvents tend to decrease the yield of the reaction. When ketones are not readily soluble in pentane or cyclohexane, a mixed solvent (pentane/methylene chloride) or methylene chloride alone may be used. Several factors govern the ratio of oxime ether to ketone. In some reactions, in order to avoid diperoxide (1,2,4,5-tetraoxane) formation, to preclude diozonide formation from diketones, and to promote the reaction with readily pentane soluble ketones, excess ketone (2:1) is used. Most commonly in the discovery synthesis stage, and especially in cases where ketones are not readily soluble in pentane, expensive, or difficult to remove in the reaction workup, a 1:1 ratio of ketone to oxime ether may be used. In large scale trioxolane syntheses, a 1.5-fold excess of oxime ether can be used to achieve higher conversions of ketones into the desired product trioxolanes without causing purification problems.

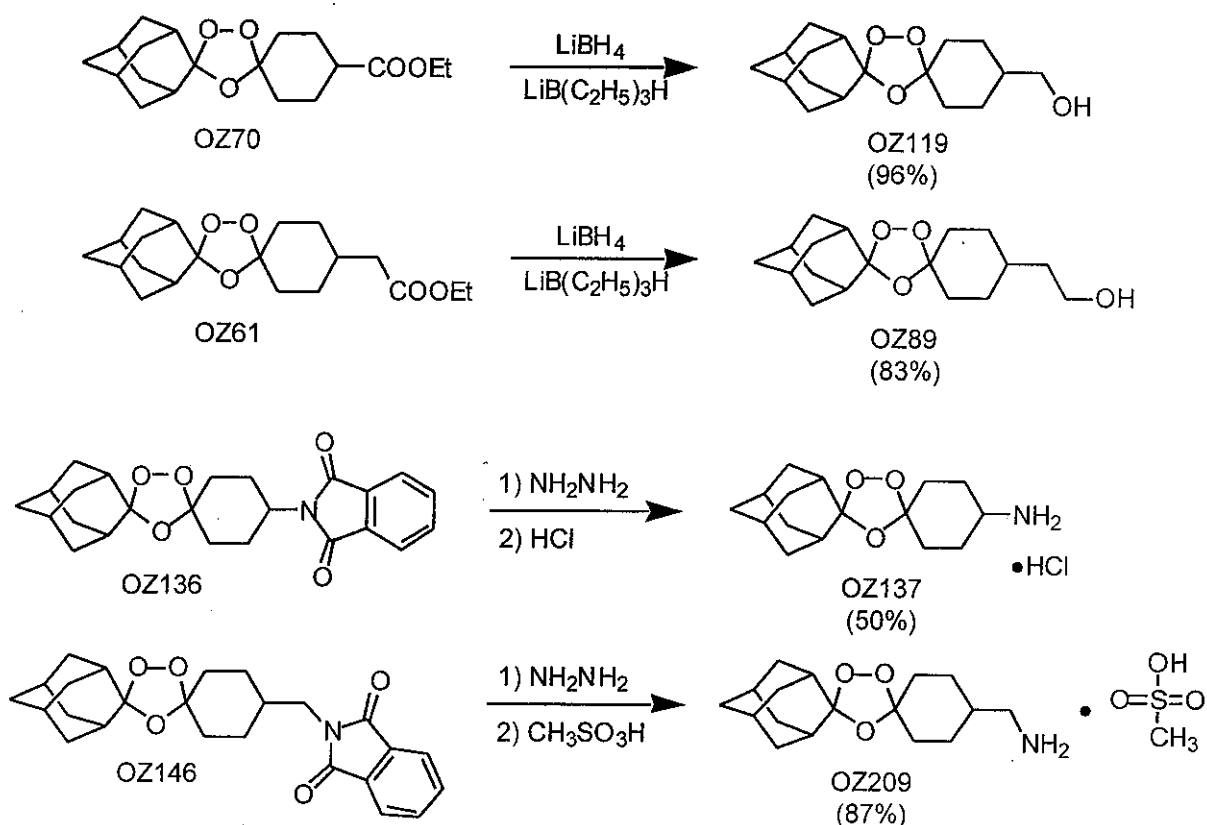
There are several examples of where post-ozonolysis transformations were used to obtain trioxolane target compounds difficult, or in some cases, impossible to obtain directly (Kashima et al., 1987) by the coozonolysis method. Trioxolane tertiary alcohols OZ90 and



OZ108 can be obtained by methyllithium treatment of trioxolane ketone OZ05 and trioxolane ester OZ70, respectively. In other reactions, trioxolane lactone OZ17 and trioxolane alcohol OZ32 were obtained by treatment of OZ05 with *m*-CPBA and sodium borohydride, respectively. In addition, various oxime ethers, hydrazones, ketals, and amines (reductive amination with sodium triacetoxyborohydride) were also obtained from trioxolane ketone OZ05 in good to excellent yields. In the examples noted above, it is evident that trioxolane ketone OZ05 is a key intermediate as its ketone functional group provides a convenient means for functional group transformation.

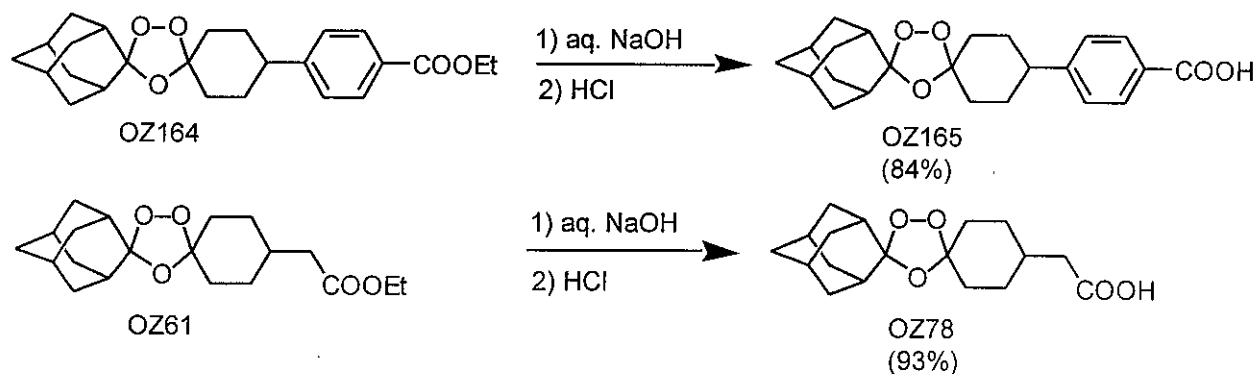


Further evidence of the stability of these trioxolanes to reducing agents is shown by the reduction of trioxolane esters OZ70 and OZ61 into their corresponding trioxolane alcohols OZ119 and OZ89 with a mixture of lithium borohydride and lithium triethylborohydride, and the hydrazinolysis of the trioxolane phthalimides OZ136 and



OZ146 into their corresponding trioxolane amines OZ137 and OZ209.

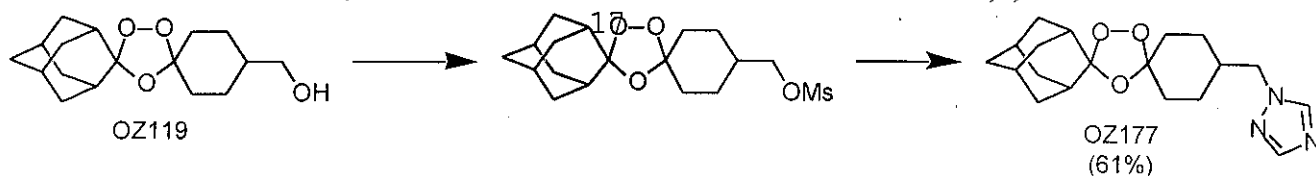
As shown in the examples below, trioxolane esters can be conveniently converted into their corresponding trioxolane acids.



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In addition to trioxolane ketone OZ05, trioxolane amine mesylate OZ209, trioxolane ester OZ61 and trioxolane acid OZ78, trioxolane alcohols OZ119 and OZ89 have and will continue to be key intermediates for post-ozonolysis synthetic transformations. A recent example is the synthesis of trioxolane triazole OZ177 in a

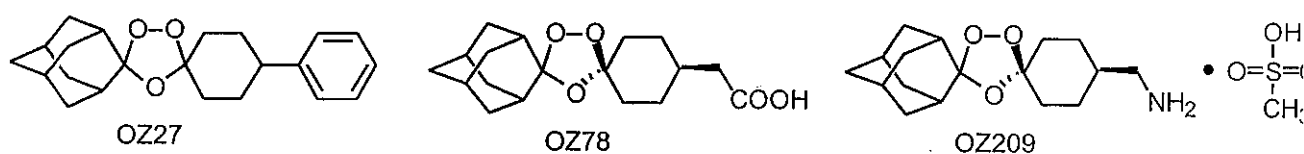
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It has been found that the coozonolysis method using oxime methyl ethers offers a rapid, flexible, and predictable access to structurally diverse trioxolanes. In fact, several key trioxolanes that have served as important building blocks have been prepared in large scale including OZ05 (100 mmol), OZ61 (100 mmol), and OZ146 (60 mmol), with no decrease in reaction yields over the usual 5-10 mmol scale. Furthermore, both OZ61 and OZ146 can be conveniently isolated as white solids by addition of ethanol to the crude reaction mixtures.

Differential scanning calorimetry (DSC) experiments (Cammenga, and Epple, 1995) reveal that these compounds have good thermal stability, comparable to artemisinin. The average $T_{m, dec}$ was $160 \pm 15^\circ\text{C}$ compared to a $T_{m, dec}$ of 181°C for artemisinin. It is presumed that thermal decomposition of these trioxolanes was initiated by formation of a 1,5 diradical produced by homolytic cleavage of the peroxide bond of the trioxolane ring.

Since most of the target trioxolanes contain the symmetrical spiroadamantane structural framework, their stereochemistry is largely a function of the starting material ketone structure or reagents used in post-ozonolysis reactions. For OZ27 and other similarly 1,4-substituted trioxolanes, two achiral diastereomers are possible. However, as exemplified by OZ27, the majority of these trioxolanes were isolated as single achiral diastereomers rather than as mixtures of two achiral diastereomers. For example, in OZ27, no chirality is present since the trioxolane ring and phenyl substituent are in a 1,4 relationship in a six membered ring. Such compounds possess a plane of symmetry.



As determined by X-ray crystallography, the assignment of stereochemistry for OZ78, OZ209 and their derivatives was determined to be *cis* where the peroxide oxygens are in an axial position.

The starting material 2-adamantanone may be obtained from Aldrich Chemical Co. or from TCI American Organic Chemicals or may also be synthesized. Persons skilled in the art can readily ascertain other appropriate means of synthesizing the starting materials and compounds in accordance with this invention.

The spiro and dispiro trioxolane compositions of the present invention may be generally used for the prophylaxis and treatment of malaria. The trioxolane compositions of the present invention are administered along with a pharmaceutically acceptable carrier. Any pharmaceutically acceptable carrier may be generally used for this purpose, provided
5 that the carrier does not significantly interfere with the stability or bioavailability of the trioxolane compounds of this invention.

The trioxolanes of this invention can be administered in any effectively pharmaceutically acceptable form to warm blooded animals, including human and other animal subjects, e.g. in topical, lavage, oral, suppository, parenteral, or infusible dosage
10 forms, as a topical, buccal, sublingual, or nasal spray or in any other manner effective to deliver the agents. The route of administration will preferably be designed to optimize delivery and/or localization of the agents to target cells.

In addition to the active compounds i.e. the trioxolanes, the pharmaceutical compositions of this invention may contain suitable excipients and auxiliaries which
15 facilitate processing of the active compounds into preparations which can be used pharmaceutically. Oral dosage forms encompass tablets, capsules, and granules. Preparations which can be administered rectally include suppositories. Other dosage forms include suitable solutions for administration parenterally or orally, and compositions which can be administered buccally or sublingually.

The pharmaceutical preparations of the present invention are manufactured in a
20 manner which is itself well known in the art. For example the pharmaceutical preparations may be made by means of conventional mixing, granulating, dragee-making, dissolving, lyophilizing processes. The processes to be used will depend ultimately on the physical properties of the active ingredient used.

Suitable excipients are, in particular, fillers such as sugars for example, lactose or sucrose mannitol or sorbitol, cellulose preparations and/or calcium phosphates, for
25 example, tricalcium phosphate or calcium hydrogen phosphate, as well as binders such as starch, paste, using, for example, maize starch, wheat starch, rice starch, potato starch, gelatin, gum tragacanth, methyl cellulose, hydroxypropylmethylcellulose, sodium
30 carboxymethylcellulose, and/or polyvinyl pyrrolidone. If desired, disintegrating agents may be added, such as the above-mentioned starches as well as carboxymethyl starch,

cross-linked polyvinyl pyrrolidone, agar, or alginic acid or a salt thereof, such as sodium alginate. Auxiliaries are flow-regulating agents and lubricants, for example, such as silica, talc, stearic acid or salts thereof, such as magnesium stearate or calcium stearate and/or polyethylene glycol. Oral dosage forms may be provided with suitable coatings which, if
5 desired, may be resistant to gastric juices.

For this purpose concentrated sugar solutions may be used, which may optionally contain gum arabic, talc, polyvinylpyrrolidone, polyethylene glycol and/or titanium dioxide, lacquer solutions and suitable organic solvents or solvent mixtures. In order to produce coatings resistant to gastric juices, solutions of suitable cellulose preparations such
10 as acetylcellulose phthalate or hydroxypropylmethylcellulose phthalate, dyestuffs and pigments may be added to the tablet coatings, for example, for identification or in order to characterize different combination of compound doses.

Other pharmaceutical preparations which can be used orally include push-fit capsules made of gelatin, as well as soft, sealed capsules made of gelatin and a plasticizer
15 such as glycerol or sorbitol. The push-fit capsules can contain the active compounds in the form of granules which may be mixed with fillers such as lactose, binders such as starches, and/or lubricants such as talc or magnesium stearate and, optionally, stabilizers. In soft capsules, the active compounds are preferably dissolved or suspended in suitable liquids, such as fatty oils, liquid paraffin, or liquid polyethylene glycols. In addition stabilizers may
20 be added. Possible pharmaceutical preparations which can be used rectally include, for example, suppositories, which consist of a combination of the active compounds with the suppository base. Suitable suppository bases are, for example, natural or synthetic triglycerides, paraffin hydrocarbons, polyethylene glycols, or higher alkanols. In addition, it is also possible to use gelatin rectal capsules which consist of a combination of the active
25 compounds with a base. Possible base material include for example liquid triglycerides, polyethylene glycols, or paraffin hydrocarbons.

Suitable formulations for parenteral administration include aqueous solutions of active compounds in water-soluble or water-dispersible form. In addition, suspensions of the active compounds as appropriate oily injection suspensions may be administered.
30 Suitable lipophilic solvents or vehicles include fatty oils for example, sesame oil, or synthetic fatty acid esters, for example, ethyl oleate or triglycerides. Aqueous injection

suspensions may contain substances which increase the viscosity of the suspension, including for example, sodium carboxymethyl cellulose, sorbitol and/or dextran. Such compositions may also comprise adjuvants such as preserving, wetting, emulsifying, and dispensing agents. They may also be sterilized, for example, by filtration through a bacteria-retaining filter, or by incorporating sterilizing agents into the compositions. They can also be manufactured in the form of sterile solid compositions which can be dissolved or suspended in sterile water, saline, or other injectable medium prior to administration.

In addition to administration with conventional carriers, active ingredients may be administered by a variety of specialized delivery drug techniques which are known to those of skill in the art, such as portable infusion pumps.

The trioxolane compositions of the present invention are administered along with a pharmaceutically acceptable carrier in an amount sufficient to prevent malarial infection and/or treat an active infection. The trioxolane compounds of this invention have extremely low toxicity and a low degree of side effects even at high doses. The dosing range of the trioxolane compositions will vary depending on a number of factors, such as whether it is used for prophylaxis or treatment of an active infection, route of administration, dosing schedule, etc. In general, the therapeutic dose of trioxolane may range between about 0.1-1000 mg/kg/day, with between about 1-100 mg/kg/day being preferred. The foregoing doses may be administered as a single dose or may be divided into multiple doses for administration. The trioxolane compositions may be administered once to several times daily. For malaria prevention, a typical dosing schedule could be, for example, 2.0-1000 mg/kg weekly beginning 1-2 weeks prior to malaria exposure taken up until 1-2 weeks post-exposure.

The spiro and dispiro trioxolanes of this invention may be administered as any pharmaceutically effective salt form. Such salts are well known in the art and include, but are not limited to acetate, adipate, alginate, citrate, aspartate, benzoate, benzene sulfonate, bisulfate, butyrate, camphorate, camphor sulfonate, digluconate, glycerophosphate, hemisulfate, heptanoate, hexanoate, fumarate, hydrochloride, hydrobromide, hydroiodide, 2-hydroxyethansulfonate (isothionate), lactate, maleate, methane sulfonate, nicotinate, 2-naphthalene sulfonate, oxalate, palmitoate, pectinate, persulfate, 3-phenylpropionate, picrate, pivalate, propionate, succinate, tartrate, thiocyanate, phosphate, glutamate,

bicarbonate, p-toluene sulfonate and undecanoate salts. Preferred salts are those that increase the bioavailability of the trioxolane compounds. This will depend upon a number of factors, including the chemical structure of the trioxolane, the carrier to which it is incorporated, the route of administration, etc.

5 The spiro and dispiro trioxolanes of this invention have been found to be effective in the treatment of schistosomiasis. Schistosomiasis ranks second behind malaria in terms of socioeconomic and public health importance in tropical and subtropical areas. The disease is endemic in 74 developing countries, infecting more than 200 million people in rural agricultural and peri-urban areas. An estimated 500-600 million people worldwide
10 are at risk from the disease.

 The major forms of human schistosomiasis are caused by five species of water-borne flatworm, or blood flukes, called schistosomes. One of these species is *Schistosoma mansoni*, which has been reported in 53 countries in Africa, the Eastern Mediterranean, the Caribbean, and South America. The parasites enter the body through contact with infested
15 surface water, primarily among people engaged in agriculture and fishing. The parasites normally infect the host during the cercaria, or larval stage. Once inside the host, the cercaria develop into adults or schistosomes.

 Current treatments for schistosomiasis have focused primarily on prophylaxis, i.e. prevention of host infection by cercaria. Currently, praziquantel is the most widely used
20 drug for treatment of schistosomiasis. While artemether has demonstrated activity in the prophylaxis of schistosomiasis, it has not shown any activity against adult *S. mansoni*.

 It has now been unexpectedly discovered that the spiro and dispiro trioxolanes of this invention are active against both cercaria and adult *S. mansoni*, *S. japonicum* when administered in the dosages and manner outlined above with respect to treatment of
25 malarial parasites. It is also believed the trioxolanes of this invention will be active against *S. haematobium*. Preferred compounds identified for use in the treatment of schistosomiasis include OZ05, OZ11, OZ23, OZ25, OZ28, OZ32, OZ71, OZ78, OZ89, OZ90, OZ119, OZ145, OZ179, OZ205, OZ207, and OZ209. Most preferred compounds are OZ78, OZ207, and OZ209. Preferred dosing levels of the spiro and dispiro trioxolanes
30 are about 100-200 mg/kg/day orally. The prototype trioxolanes of this invention are OZ03 and OZ05.

The spiro and dispiro trioxolanes of this invention may also have effectiveness in the treatment of cancer. Compounds having an endoperoxide moiety that is reactive with heme and iron have shown an ability to kill cancer cells. (See e.g. U.S. Pat. No. 5,578,637, the disclosure of which is hereby incorporated by reference). As noted with respect to artemisinin, trioxolanes' mechanism of action against malarial parasites is based on the ability of trioxolane compounds to react with the iron in free heme molecules in malaria parasites, with the generation of free radicals leading to cellular destruction. Similarly, trioxolanes are selective against cancer cells due to the higher concentration of transferrin receptors on cancer cell membranes that pick up iron at a higher rate than normal cells. In the presence of the trioxolanes of this invention, the cancer cells will accumulate high concentrations of free radicals, leading to cell death. For cancer treatment, the trioxolanes of this invention may be administered in the doses and manner outlined above.

Other drugs besides trioxolanes which are compatible with the carrier ingredients may also be incorporated into the carrier. Such drugs may be readily ascertained by those of ordinary skill in the art and may include, for instance, antibiotics, other antimalarials, antiinflammatory agents, etc.

It is understood that the present invention contemplates the use of not only the above-stated trioxolane compounds themselves, but their prodrugs which metabolize to the compound and the analogues and biologically active salt forms thereof, as well as optical isomers which provide the same pharmaceutical results.

The following examples are offered to illustrate but not limit the invention. Thus, they are presented with the understanding that various formulation modifications as well as method of delivery modifications may be made and still be within the spirit of the invention.

EXAMPLE 1

Antimalarial Activity of OZ271-OZ369

Antimalarial Assays

Various OZ compounds were tested by the semiautomated microdilution assay against intraerythrocytic forms of *Plasmodium falciparum* derived from asynchronous stock cultures. The culture medium used was RPMI 1640 supplemented with 10% human

type A⁺ serum, 25 mM HEPES, 25 mM NaHCO₃ (pH 7.3). Human type A⁺ erythrocytes served as host cells. The culture was kept at 37°C in an atmosphere of 3% O₂, 4% CO₂, and 93% N₂ in humidified modular chambers.

Compounds were dissolved in DMSO (10 mg/ml), pre-diluted in complete culture medium, and titrated in duplicate in serial twofold dilutions over a 64-fold range in 96-well microtiter plates. After addition of the parasite cultures with an initial parasitemia (expressed as the percentage of erythrocytes infected) of 0.75% in a 2.5% erythrocytes suspension, the test plates were incubated under the conditions described above for 72 h. Growth of the parasites cultures was measured by the incorporation of radiolabelled [³H]-hypoxanthine added 16 h prior to termination of the test. Fifty percent inhibitory concentration (IC₅₀) were estimated by Logit regression analysis. Compounds were tested against reference *P. falciparum* strains, K1 strain (Thailand resistant to chloroquine) and NF54 strain (an airport strain of unknown origin that is sensitive to standard antimalarials).

In the single dose STI *in vivo* screen, Moro SPF or NMRI mice infected with the ANKA strain of *P. berghei* (groups of 3-5 mice) were used. Trioxolanes were administered as single oral (po) 3 mg/kg and 10 mg/kg doses in standard suspending vehicle (SSV). SSV consists of 0.5% w/v CMC, 0.5% v/v benzyl alcohol, 0.4% v/v Tween 80, and 0.9% w/v sodium chloride in water. Antimalarial activity was measured by percent reduction in parasitemia on day three post-infection and survival times compared to an untreated control group. Survival to day 30 post-infection is considered to be a cure. In PCT/02/19767, Table 1 presented data for trioxolanes OZ01-OZ270 along with the controls, fenozan, artemisinin, arteether, artemether, and artesunate. The data showed that antimalarial activity falls off both when the trioxolane peroxide bond is too exposed or is sterically inaccessible to iron(II) species. Other factors influencing antimalarial activity include the stability of carbon radicals formed by β-scission subsequent to the initial electron transfer to the peroxide bond and the influence of steric effects remote from the peroxide bond on the interactions between carbon radicals and potential drug targets. The data also demonstrated that trioxolane carboxylic acids are usually less active than their hydrocarbon, ester, and hydroxamic acid counterparts.

Below is the activity data for OZ271-OZ369.

Table 1

Compd	IC ₅₀ (ng/ml) K1/NF54	Activity (%) 10/3 mg/kg	Survival (days) 10/3 mg/kg
NONE	---	0	5.6 ± 0.3
OZ271	0.36/0.29	99.93/99.52	8.7/8.6
OZ272	1.1/1.3	98/49	10.3/6.6
OZ273	0.97/1.0	99.58/64	11.7/7.2
OZ274	5.1/5.7	11/ND	7.0/ND
OZ275	0.66/0.74	68/ND	7.3/ND
OZ276	0.86/0.94	17/ND	7.3/ND
OZ277	0.57/0.58	99.98/99.28	9.3/9.6
OZ278	23/39	56/ND	7.3/ND
OZ279	0.21/0.24	99.98/99.42	9.3/8.4
OZ280	1.2/1.4	0/ND	5.7ND
OZ281	0.50/0.30	99.87/96	8.7/10.2
OZ282	2.0/2.4	98.5/53	8.3/7.2
OZ283	1.7/2.0	99.81/56	8.3/8.2
OZ284	1.0/1.4	97/61	9.0/7.8
OZ285	1.3/1.8	99.81/72	8.7/7.6
OZ286	0.94/1.6	99.73/60	8.7/7.0
OZ287	0.49/0.83	95/68	8.3/7.2
OZ288	1.7/2.7	99.96/82	11.0/8.0
OZ289	0.40/0.56	99.94/92	13.4/7.4
OZ290	0.42/0.45	99.64/95	9.0/7.0

OZ291	0.52/0.72	98/34	8.0/6.8
OZ292	0.19/0.26	74/46	7.0/6.6
OZ293	0.25/0.34	99.58/77	8.8/7.4
OZ294	0.39/0.63	99.79/70	8.6/7.4
OZ295	0.44/0.75	47/35	7.0/6.4
OZ296	0.60/0.89	99.22/90	10.0/9.2
OZ297	0.49/0.76	99.69/83	9.8/7.6
OZ298	2.0/3.0	99.95/98	9.4/9.0
OZ299	6.8/6.3	36/0	6.6/6.4
OZ300	71/97	6/0	6.8/7.0
OZ301	2.1/2.8	100/97	11.8/8.4
OZ302	1.9/2.9	99.60/88	9.0/9.0
OZ303	1.6/2.4	99.01/69	8.6/7.6
OZ304	1.6/2.3	98/61	8.2/8.8
OZ305	1.8/1.9	99.93/96	9.2/9.6
OZ306	3.9/3.6	99.58/87	9.2/8.2
OZ307	0.62/0.88	98/74	9.4/7.8
OZ308	1.2/1.7	86/30	7.8/6.6
OZ309	11/17	99.80/86	8.8/7.8
OZ310	0.82/1.1	81/38	7.8/8.2
OZ311	1.2/1.8	80/40	8.6/7.0
OZ312	19/27	43/13	7.2/6.2
OZ313	0.55/0.76	98.9/58	10.4/7.4
OZ314	11/17	13/11	6.6/6.6

OZ315	2.8/3.0	99.97/92	11.2/9.6
OZ316	1.0/1.7	69/7	10.0/8.0
OZ317	0.33/0.36	99.92/99.20	9.8/10.0
OZ318	0.56/0.82	88/29	12.4/7.6
OZ319	0.41/0.91	99.92/99.14	10.6/10.8
OZ320	0.68/1.30	99.76/92	10.4/11.8
OZ321	0.58/0.97	99.77/64	14.0/9.8
OZ322	0.90/1.5	98/61	10.0/9.4
OZ323	0.85/1.1	99.98/99.92	15.6/9.2
OZ324	2.4/3.4	60/26	9.4/7.4
OZ325	0.62/1.4	99.43/30	8.8/6.2
OZ326	0.65/1.2	80/7	7.4/6.0
OZ327	0.85/1.1	97/72	9.8/7.0
OZ328	1.4/3.0	99.77/75	8.6/7.6
OZ329	0.43/0.68	99.97/99.50	11.2/9.2
OZ330	0.55/1.2	99.96/82	12.2/7.2
OZ331	0.50/1.2	99.47/66	9.0/7.0
OZ332	1.7/2.5	8/0	6.0/6.0
OZ333	0.24/0.44	99.86/96	9.2/8.0
OZ334	28/20	47/13	6.8/6.6
OZ335	0.29/0.28	99.95/97	9.2/10.0
OZ336	1.4/0.91	99.93/98	9.2/9.6
OZ337	0.29/0.25	99.92/97	8.6/11.6

OZ338	0.38/0.45	99.93/99.80	9.8/8.0
OZ339	0.35/0.39	99.95/99.65	10.2/9.2
OZ340	4.9/2.9	93/36	9.2/6.4
OZ341	2.4/2.0	97/32	8.0/7.0
OZ343	0.46/0.49	99.94/99.21	9.4/10.0
OZ344	1.2/2.1	17/0	7.8/7.4
OZ345	1.9/2.4	75/0	9.2/6.6
OZ346	3.8/4.7	85/51	8.8/7.6
OZ347	11/11	2/0	6.6/7.6
OZ348	3.2/3.5	97/24	9.2/6.6
OZ349	0.70/1.3	99.92/99.88	11.2/9.6
OZ350	1.1/2.2	99.17/69	10.4/9.6
OZ351	0.40/0.54	99.90/99.69	9.4/9.0
OZ352	19/20	99.66/77	8.2/7.2
OZ353	0.35/0.56	99.96/99.91	9.2/9.6
OZ354	0.55/0.82	99.97/99.63	9.6/10.0
OZ355	0.29/0.42	71/0	7.0/6.2
OZ356	1.4/1.1	99.64/87	8.6/8.0
OZ357	0.56/0.79	99.99/99.81	10.2/8.8
OZ358	0.22/0.36	99.96/99.02	10.4/9.6
OZ359	0.40/0.52	99.94/96	9.4/8.8
OZ360	58/59	38/0	6.8/6.2
OZ361	3.5/2.3	99.90/85	8.4/7.8
OZ362	0.81/0.31	98/58	9.8/7.0

OZ363	46/55	40/10	6.8/6.0
OZ364	8.4/6.2	63/80	7.0/7.3
OZ365	0.79/0.25	99.57/92	8.0/8.0
OZ366	1.4/0.74	84/62	8.2/7.0
OZ367	0.66/0.25	96/59	8.6/6.8
OZ368	0.63/0.43	99.94/99	9.0/9.2
OZ369	0.69/0.54	99.90/0	8.4/5.8
AM	0.45/0.36	99.75/79	9.4/8.7
AS	1.4/1.5	87/66	7.0/8.0
CQ	76/4.4	99.92/82	9.0/8.0
MFQ	2.2/5.0	99.11/9	17/6.3

For comparative analysis, data is also presented for the control antimalarial drugs artemether (AM), artesunate (AS), chloroquine (CQ), and mefloquine (MFQ).

5

The new activity data demonstrates that trioxolane carboxylic acids are usually less active than their hydrocarbon, ester, amide, and hydroxamic acid counterparts. The position of ionizable functional groups such as carboxylic acids and amines is also critical to activity.

10 The best combination of high intrinsic potency and good oral activity is found when a weak base functional group is present.

EXAMPLE 2

Onset of Action and Recrudescence of OZ11, OZ27, OZ78, OZ156, OZ175, OZ177, OZ207, OZ209, OZ277, and OZ279

15

Onset of Action and Recrudescence Experiments

The onset of drug action was determined after a single fixed dose of 100 mg/kg (SSV vehicle) po to groups of five animals on day +3 post- infection (day 0). Parasitemias

at this point are usually between 25–40%. The infected controls do not survive beyond day +6 post-infection. The reduction of parasitemia is monitored 12, 24, and 48 h after treatment, and the time of recrudescence (> 5% parasitemia) is assessed by daily blood smears for 14 days, followed by intermittent assessment for up to 60 days.

The onset part of this experiment reveals how rapidly a compound reduces parasite load; the recrudescence part of the experiment provides information about the efficacy of the compound against the parasite. A long delay in recrudescence can be due to a very good antiparasitic effect of the compound or to a compound with a long half-life.

Both the trioxolanes and the artemisinins produced a rapid decline in parasitemia, confirming that they are rapidly acting antimalarial agents. In contrast to both chloroquine and these peroxidic antimalarials, mefloquine has a slow onset of action. Recrudescence (> 5% parasitemia) occurs quite rapidly for artemisinin and artesunate. The time of recrudescence increased for the more lipophilic artemisinin derivatives artemether and arteether.

In contrast to artemether, recrudescence occurred much more slowly for the lipophilic trioxolanes OZ11 and OZ27; the recrudescence time for OZ27 was especially marked, superior to that of mefloquine. However, recrudescence times for the relatively polar trioxolanes OZ78, OZ175, and OZ277 were very similar to that of artemether. The more lipophilic trioxolane (OZ156) of the OZ156/OZ177 pair produced the longest delay in recrudescence, longer than chloroquine, but less than mefloquine. The recrudescence times for OZ177 and OZ279 were roughly equivalent to that of chloroquine.

Strikingly, there was no recrudescence observed for OZ207 and OZ209, two different salt forms (OZ207 – tosylate, OZ209 – mesylate) of aminomethyl trioxolane OZ163 (hydrochloride). The recrudescence data for these two trioxolanes suggests that they are either more powerful antimalarial agents or have longer half-lives than any of the semisynthetic artemisinins.

Table 2

Compd	Time of Recrudescence (days)
OZ11	22.2
OZ27	22.0 (3/5), > 60 (2/5)
OZ78	11.2

OZ156	19.0 (4/5), > 60 (1/5)
OZ175	13.0
OZ177	18.5
OZ207	> 60
OZ209	> 60
OZ277	13.0
OZ279	15.0
Artemisinin	8.4
Artesunate	8.6
Artemether	12.0
Arteether	11.4
Chloroquine	17.8
Mefloquine	28.0

EXAMPLE 3

Effect of Trioxolanes on *Schistosoma* Species

Effect of Trioxolane OZ207 on *Schistosoma japonicum*

Table 3

Comparative effect of OZ207 and artemether in mice infected with *Schistosoma japonicum*

Drug	Age of worm	Dose (mg/kg × 1)	Mice without ♀ worm	MTWB/ x ± SD	WRR/ %	MFWB/ x ± SD	FWR R/ %
Control	-	-	0/8	26.6 ± 4.2	-	11.6 ± 2.4	-
OZ207	35 days	200	4/7	9.1 ± 3.9	66	0.6 ± 0.7	95
OZ207	35 days	400	4/6	4.3 ± 1.2	84	0.7 ± 1.2	94
Artemether	35 days	400	0/7	10.1 ± 4.4	62	3.4 ± 1.6	71
OZ207	7days	200	0/8	5.4 ± 2.4	81	2.1 ± 1.0	82

MTWB, mean total worm burden; WRR, worm reduction rate MFWB, mean female worm burden; FWRR, female worm reduction rate.

Table 3 illustrates that the mean total worm burden and mean female worm burden in OZ207 400 mg/kg group was significantly lower than those in artemether 400 mg/kg group ($P < 0.01$). The mean female worm burden in OZ207 200 mg/kg group was also significantly lower than that in artemether group ($P < 0.01$).

Effect of Trioxolanes on 21-day-old schistosomules

Mice were infected with 100 *Schistosoma mansoni* cercariae on day 21 post-treatment. Each group was treated per os with trioxolanes at a single dose of 200 mg/kg. Untreated mice served as the control. All groups were killed 4 weeks after treatment and the liver and intestine were removed and separated. The liver and intestine were compressed and alive male and female worms could be seen and counted. The effect of the compounds was evaluated by mean total and female worm burden. The results are shown in Table 4.

Effect of Trioxolanes on adult schistosomes (49-day-old)

Mice were infected with 100 *Schistosoma mansoni* cercariae on day 49 post-treatment. Each group was treated per os with OZ compounds at single doses of 200, 400, and 600 mg/kg. Untreated mice served as the control. All groups were killed 4 weeks after treatment and the liver and intestine were removed and separated. The liver and intestine were compressed and alive male and female worms could be seen and counted. The effect of the compounds was evaluated by mean total and female worm burden, and the results are set forth in Table 4.

Table 4

IN VIVO ACTIVITY AGAINST SCHISTOSOMA MANSONI (MICE female INFECTED)					
OZ COMPOUNDS TESTED	% reduction of schistosomule growth at day 21 after per os application of 200 mg/kg		% reduction of adult worms growth at day 49 after per os application of mg/kg		
			200	400	600
	TWR (%)	FWR (%)	TWR (%) / DEAD WORM (%)		
OZ 271	88	86		0 / 6	
OZ 277	91	92		0 / 0	17 / 14
OZ 279	86	88		0 / 12	33 / 20
OZ 281	91	90		12 / 15	
OZ 285 liquid	86	85		17 / 22	
OZ 288	95	96		52 / 45	
OZ 296	85	85		1 / 13	
OZ 309	97	100		19 / 17	

1 x 600 sc.
39 / 17

1 x 600 sc.
21 / 27

OZ 312	94	93		16 / 9	
OZ 323	95	97		20 / 23	
OZ 329	90	90		15 / 6	
OZ 349	ND	ND		36 / 16	
OZ 352	89	91		16 / 26	
OZ 360	17	13		0 / 0	
ARTEMETHER	(n4) 85	(n4) 85	(n2)	53 / 29	
PRAZIQUANTEL	0	0	(n2)	96 / 96	100 / 100

EXAMPLE 4

Activity of Trioxolanes Against *P. berghei*

- 5 In the single dose ED₅₀/ED₉₀/ED₉₉ determinations, Moro SPF or NMRI mice (group of three) infected with the ANKA strain of *Plasmodium berghei* were treated on day one post-infection. Trioxolanes were dissolved or suspended in the standard suspending vehicle (SSV) and administered as single 10, 6, 3, 1, 0.3, and 0.1 mg/kg doses po and sc.
- 10 The SSV consists of 0.5% w/v CMC, 0.5% v/v benzyl alcohol, 0.4% v/v Tween 80, and 0.9% w/v sodium chloride in water. Antimalarial activity was measured by percent reduction in parasitemia on day three post-infection. The ED₅₀/ED₉₀ values were calculated by nonlinear fitting.

Table 5

Compd	ED ₅₀ (mg/kg)	ED ₉₀ (mg/kg)	ED ₉₉ (mg/kg)
OZ277	0.78	2.0	4.4
OZ279	0.63	1.8	3.9
Artesunate	4.7	19	60
Artelinate	4.8	10	18
Artemether	2.2	4.2	7.1
Chloroquine	1.8	3.5	5.9

Mefloquine	4.0	5.4	6.8
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Table 5 shows ED50/ED90/ED99 data obtained by po administration of trioxolanes in the SSV formulation. The relatively lipophilic artemether is substantially more active than the more polar artesunate and artelinate. In contrast, the most active trioxolanes (OZ181, OZ207, OZ209) - different salt forms of the same amino trioxolane, and amino and amide trioxolanes OZ277 and OZ279, are relatively polar compounds.

EXAMPLE 5

Dosing of OZ279, OZ277, OZ256, and OZ209

Based on results of dosing OZ279, OZ277, OZ256, and OZ209 in rats and dogs, the inventors determined projected optimal dosing of the same compounds in humans. Artesunate is listed as a reference compound.

Table 6

Parameter	Ideal	Accept	Artes	OZ 279	OZ 277	OZ 256	OZ 209
Rat Data							
IV t1/2 (10 mg/kg)	180 min	60 min	40 (DHA)	100.5	77.2	94.0	150.0
Oral Bioavailability							
10 mg/kg	>30%	>20%	not done	37.2	36.9	18.6	12.4
25 mg/kg	>30%	>20%	21 (DHA)	71.1	44.1	51.9	22.4
Oral t1/2 (25 mg/kg)	180 min	60 min	not done	166.8	90.5	73.3	101.5
Dog Data							
IV t1/2 (10 mg/kg)	180 min	60 min	not done	177.5	95.0	85.4	182.8

Oral							
Bioavailability	>30%	>20%	not done	32.8 (V)	87.9	42.0 (V)	24.5 (V)
10 mg/kg	>30%	>20%	not done	55.7 (V)	96.1	38.3 (V)	15.9 (V)
25 mg/kg							
Oral t _{1/2} (10 mg/kg)	180 min	60 min	not done	195.3	148.1	82.8	127.3
Human Data							
Projected daily dose mg/day (% BA)	150 mg	300 mg	150-300 (actual)	105-154 (30%)	28-56 (30%)	91-133 (20%)	35-70 (20%)

EXAMPLE 6

Effectiveness of Selected OZ Compounds in the Treatment and Prophylaxis of Malarial Infections

Moro NMRI male mice (Fü Albino specific pathogen free) weighing 18 ± 2 g were infected intravenously (i.v.) with 2×10^7 *P. berghei* ANKA strain-infected erythrocytes from donor mice on day 0 of the experiment. From donor mice with circa 30% parasitemia, heparinized blood was taken and diluted in physiological saline to 10^8 parasitized erythrocytes per ml. An aliquot (0.2 ml) of this suspension was injected i.v. into experimental and control groups of mice. In untreated control mice, parasitemia rose regularly to 40 to 50% by day 3 post-infection and 70 to 80% by day 4 post-infection. The mice died between days 5 and 7 post-infection. Throughout the experiments, mice were kept in groups of three or five animals in Makrolon type II cages in an air-conditioned animal room at 22 to 23°C. A diet with *p*-aminobenzoic acid (PABA) of 45 mg (NAFAG FUTTER® food N° 9009 PAB-45) per kg of body weight, and tap water is available *ad libitum*.

OZ compounds were prepared at an appropriate concentration, either as a solution or a suspension containing SSV (0.5% w/v CMC, 0.5% v/v benzyl alcohol, 0.4% v/v Tween 80, and 0.9% w/v sodium chloride in water). They were administered per os (p.o.) in a total volume of 0.01 ml per gram of mouse. The activity of the compound was

determined by a variety of methods outlined in subsequent sections. Survival time was also recorded, and survival to day 30 post-infection was considered to be a cure.

The first experiment conducted consisted of administration of a divided 3 x 10 mg/kg p.o. dose administered on days 1, 2, and 3 post-infection vs. a single 1 x 30 mg/kg po dose administered on day 1 post-infection. On day 4 post-infection, blood smears of all animals were prepared and stained with Giemsa. Parasitemia was determined microscopically, and the difference between the mean value of the control group (taken as 100%) and those of the experimental groups was calculated and expressed as percent reduction. Compounds were administered orally in the SSV vehicle. The results are shown in Table 7 below:

Table 7

	1 x 30 mg/kg			3 x 10 mg/kg		
	Activity %	Survival (days)	Cures	Activity (%)	Survival (days)	Cures
OZ	p.o. SSV			p.o. SSV		
209	100	>30	0/5	100	>30	3/3
271	99.97	14	0/5	100	27.8	4/5
277	99.92	10.4	0/5	100	27.6	4/5
279	99.95	14.8	0/5	100	25.4	3/5
301	NA	NA	NA	100	>30	5/5
315	NA	NA	NA	100	>30	5/5
CQ	99.94	9.5	0/5	99.99	14.3	0/5
MFQ	99.94	20.3	0/5	99.92	23.3	0/5
AS	83.83	9	0/5	98.62	11	0/5

As shown by Table 7, a 3 x 10 mg/kg dose of these trioxolanes cured between 3/5 and 5/5 of the infected mice. At this same dose, none of the standard antimalarial drugs cured any of the infected mice. At the 1 x 30 mg/kg dose, all tested trioxolanes showed activities > 99.9% on day 3 post-treatment.

The second experiment consists of administration of divided 3 x 3 mg/kg and 3 x 1 mg/kg po doses administered on days 1, 2, and 3 post-infection. On day 4 post-infection, blood smears of all animals were prepared and stained with Giemsa. Parasitemia was determined microscopically, and the difference between the mean value of the control

group (taken as 100%) and those of the experimental groups was calculated and expressed as percent reduction. Compounds were administered orally in the SSV vehicle. The results are shown in Table 8.

Table 8

	3x3 mg/kg			3x1 mg/kg	
	Activity (%)	Survival (days)	Cures	Activity (%)	Survival (days)
OZ	p.o. SSV			p.o. SSV	
209	100	16.4	0\5	99.51	9.4
271	99.99	16.2	0\5	87	8.8
277	100	14	0\5	83	9.4
279	100	14.8	0\5	83	8.8
281	100	12.4	0\5	92	13
288	99	10.2	0\5	49	8.4
289	100	17.2	0\5	41	7.4
290	93	10.6	0\5	14	6.8
296	94	9.4	0\5	49	7.8
297	89	9.4	0\5	22	6.4
298	99.99	16.4	0\5	93	11
301	100	23	1\5	58	8.8
302	99.51	13.4	0\5	87	13.4
305	99.91	12.2	0\5	87	9.6
306	99.75	7.6	0\5	85	11
309	99	9.2	0\5	66	9.4
315	99.99	22	0\5	81	12.2
317	100	16.8	0\5	73	11.4
319	99.97	11.2	0\5	92	13
320	96	9.6	0\5	50	8.6
323	99.95	14.4	0\5	66	14.4
329	100	27	2\5	99.86	11
330	99	12.6	0\5	45	9.2
333	99	10.2	0\5	64	9.4
335	99.99	15.4	0\5	98	10
336	100	20.8	0\5	99.14	10.4
337	99.98	14.4	0\5	96	9.4
338	100	25.6	0\5	98	9.4
339	100	27	3\5	97	9.2
343	100	22.2		87	9.4
349	99.98	25.2	2\5	98	9.4
351	100	22.8		99	9.6
343	100	22.2		87	9.4
349	99.98	25.2	2\5	98	9.4
351	100	22.8		99	9.6
353	99.99	16.4		91	10
354	99.99	24.4		95	8
357	100	22.4	1\5	98	9.2
358	99.98	9.8		79	9.8

359	99.65	8.6		79	8
365	99.96	12		79	8.4
368	99.99	22.2		91	8.6
CQ	99.54	10		25	7.2
MFQ	98	12		2	6.2
AM	86	9.4		51	7.2
AS	78	9.4		39	6.8

As shown by Table 8, at the 3 x 3 mg/kg dose, fourteen trioxolanes had activities of 100% and produced high survival numbers. Of these, OZ301, OZ329, OZ339, OZ349, and OZ357 cured 1/5, 2/5, 3/5, 2/5, and 1/5 of the infected mice, respectively. At the 3 x 1 mg/kg dose, most of the trioxolanes were more potent than the reference antimalarial drugs; sixteen of these had activities $\geq 90\%$. OZ209, OZ329, and OZ336 were the only trioxolanes with activities greater than 99% at the 3 x 1 mg/kg dose. All of OZ343-OZ368 that were tested were more active than the reference antimalarial drugs.

Prophylactic activities of the compounds were compared after administering po single dose of 100 mg/kg to different groups of five animals at various times before infection. All groups including an untreated control group, were then infected at the same time. Parasitemia was determined for each animal on day 3 post-infection, and percent of reduction of the level of parasitemia compared to levels for animals given no drug is determined. The results are shown in Table 9.

Table 9

				Prophylactic Activity (%)						
	AM	AS	CQ	MFQ	209	256	271	277	279	281
72 h -				99.97	99.92	13	99.89	9	14	8
48 h -			57.49	99.92	99.9	29	99.98	7	27	45

24 h -	0	6.28	99.92	100	100	82	100	25	97	99.23
0 h	100	92.44	100	100	100	100	100	100	100	100

The unique prophylactic property of OZ209 (3-day protection, same as MFQ) was found also for OZ271.

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EXAMPLE 7

Chemical Properties of OZ277 Salts

The following tosylate, maleate, hydrochloride, tartarate, and succinate salts of the base form of OZ277 were synthesized:

cis-Adamantane-2-spiro-3'-8'-[[[(2'-amino-2'-methylpropyl)amino]carbonyl]methyl]-1',2',4'-trioxaspiro[4.5]decane *p*-tosylate

cis-Adamantane-2-spiro-3'-8'-[[[(2'-amino-2'-methylpropyl)amino]carbonyl]methyl]-1',2',4'-trioxaspiro[4.5]decane hydrogen maleate

cis-Adamantane-2-spiro-3'-8'-[[[(2'-amino-2'-methylpropyl)amino]carbonyl]methyl]-1',2',4'-trioxaspiro[4.5]decane hydrochloride

cis-Adamantane-2-spiro-3'-8'-[[[(2'-amino-2'-methylpropyl)amino]carbonyl]methyl]-1',2',4'-trioxaspiro[4.5]decane hydrogen tartarate

cis-Adamantane-2-spiro-3'-8'-[[[(2'-amino-2'-methylpropyl)amino]carbonyl]methyl]-1',2',4'-trioxaspiro[4.5]decane hydrogen succinate

Their properties are shown in Table 10:

30

Table 10

<u>Selection criteria</u>	<u>Maleate</u>	<u>Tosylate</u>	<u>HCl</u>	<u>Tartarate</u>	<u>Succinate</u>
Water solubility	5 mg/mL	0.4 mg/mL	2.5 mg/mL	2 mg/mL	1.4 mg/mL
Crystallinity	Crystalline	Crystalline	Crystalline	More towards amorphous	Crystalline
Hygroscopicity	Non-hygroscopic	Non-hygroscopic	Non-hygroscopic	Hygroscopic	Non-hygroscopic
Chemical	Stable	Stable	Stable	Comparatively	Stable

Stability				less stable	
Physical stability	Stable	Stable	Stable	Stable	Stable
Particle size (90% of particles less than)	~100 μm	~50 μm	~230 μm	~200 μm	~150 μm
Stability in analytical solution (up to 24 hrs)	Stable	Stable	Stable	Stable	Stable
Taste	Not bitter	Not bitter	Not bitter	Not bitter	Not bitter

EXAMPLE 8

Preparation of OZ277 Maleate Salt

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Raw Materials:

<u>Description</u>	<u>Quantity</u>	<u>Mole Equivalent</u>
OZ277 (base form)	60.00 g	1.00
Maleic acid	17.40 g	0.98
Ethanol	240 ml	
Heptane	780 ml	

Process:

- 10 -Charge ethanol (150 ml) at 20-25°C;
 -Charge OZ277 (60 gm) at 20-25°C under stirring;
 -Stir at 20-25°C for 10 min to get clear solution;
 -Add the solution of maleic acid (17.4 g in 90 ml ethanol) drop wise at 20-25°C
 under stirring in 1 hr.;
- 15 -Charge Heptane (720 ml) at 20-25°C under stirring;
 -Stir for 4 hr at 20-25°C;
 -Filter the solid and wash with heptane (60 ml);
 -Suck dry the product;
 -Dry the product under vacuum at 20-25°C.

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The resulting product has a weight of 65-70 grams.

It should be appreciated that the spiro and dispiro 1,2,4-trioxolane compositions of this invention may contain trioxolanes within the scope of the formulas described above, or
5 prodrugs or analogues of these compounds or a racemic mixture of either the D or the L form. The invention is also intended to include all biologically active salt forms of the compounds. Also, minor dosage and formulation modifications of the composition and the ranges expressed herein may be made and still come within the scope and spirit of the present invention.

10 Having described the invention with reference to particular compositions, theories of effectiveness, and the like, it will be apparent to those of skill in the art that it is not intended that the invention be limited by such illustrative embodiments or mechanisms, and that modifications can be made without departing from the scope or spirit of the invention, as defined by the appended claims. It is intended that all such obvious modifications and
15 variations be included within the scope of the present invention as defined in the appended claims. The claims are meant to cover the claimed components and steps in any sequence which is effective to meet the objectives there intended, unless the context specifically indicates to the contrary.

What is claimed is:

1. A spiro or dispiro 1,2,4-trioxolane, wherein the spiro or dispiro 1,2,4-trioxolane is selected from the group consisting of: OZ271, OZ272, OZ273, OZ274, OZ275, OZ276,
5 OZ277, OZ278, OZ279, OZ280, OZ281, OZ282, OZ283, OZ284, OZ285, OZ286, OZ287, OZ288, OZ289, OZ290, OZ291, OZ292, OZ293, OZ294, OZ295, OZ296, OZ297, OZ298, OZ299, OZ300, OZ301, OZ302, OZ303, OZ304, OZ305, OZ306, OZ307, OZ308, OZ309, OZ310, OZ311, OZ312, OZ313, OZ314, OZ315, OZ316, OZ317, OZ318, OZ319, OZ320, OZ321, OZ322, OZ323, OZ324, OZ325, OZ326, OZ327, OZ328, OZ329, OZ330, OZ331,
10 OZ332, OZ333, OZ334, OZ335, OZ336, OZ337, OZ338, OZ339, OZ340, OZ341, OZ342, OZ343, OZ344, OZ345, OZ346, OZ347, OZ348, OZ349, OZ350, OZ351, OZ352, OZ353, OZ354, OZ355, OZ356, OZ357, OZ358, OZ359, OZ360, OZ361, OZ362, OZ363, OZ364, OZ365, OZ366, OZ367, OZ368, and OZ369.
- 15 2. The spiro or dispiro 1,2,4-trioxolane of claim 1 that is selected from the group consisting of OZ277, OZ349, OZ351, OZ354, OZ357, OZ359, and OZ368.
3. A pharmaceutical composition for prophylaxis and treatment of malaria comprising: a malaria prophylaxis or malaria treatment-effective amount of a spiro or
20 dispiro 1,2,4-trioxolane, its prodrugs and optical isomers thereof, selected from the group consisting of OZ271, OZ272, OZ273, OZ274, OZ275, OZ276, OZ277, OZ278, OZ279, OZ280, OZ281, OZ282, OZ283, OZ284, OZ285, OZ286, OZ287, OZ288, OZ289, OZ290, OZ291, OZ292, OZ293, OZ294, OZ295, OZ296, OZ297, OZ298, OZ299, OZ300, OZ301, OZ302, OZ303, OZ304, OZ305, OZ306, OZ307, OZ308, OZ309, OZ310, OZ311, OZ312,
25 OZ313, OZ314, OZ315, OZ316, OZ317, OZ318, OZ319, OZ320, OZ321, OZ322, OZ323, OZ324, OZ325, OZ326, OZ327, OZ328, OZ329, OZ330, OZ331, OZ332, OZ333, OZ334, OZ335, OZ336, OZ337, OZ338, OZ339, OZ340, OZ341, OZ342, OZ343, OZ344, OZ345, OZ346, OZ347, OZ348, OZ349, OZ350, OZ351, OZ352, OZ353, OZ354, OZ355, OZ356, OZ357, OZ358, OZ359, OZ360, OZ361, OZ362, OZ363, OZ364, OZ365, OZ366, OZ367,
30 OZ368, and OZ369, and a pharmaceutically acceptable carrier.

4. The composition of claim 3 wherein the trioxolane comprises a salt selected from the group consisting of acetate, adipate, alginate, citrate, aspartate, benzoate, benzene sulfonate, bisulfate, butyrate, camphorate, camphor sulfonate, digluconate, glycerophosphate, hemisulfate, heptanoate, hexanoate, fumarate, hydrochloride, hydrobromide, hydroiodide, isothionate, lactate, maleate, methane sulfonate, nicotinate, 2-naphthalene sulfonate, oxalate, palmitoate, pectinate, persulfate, 3-phenylpropionate, picrate, pivalate, propionate, succinate, tartrate, thiocyanate, phosphate, glutamate, bicarbonate, p-toluene sulfonate and undecanoate.

5. The composition of claim 4 wherein the trioxolane is selected from the group consisting of *cis*-adamantane-2-spiro-3'-8'-[[[(2'-amino-2'-methylpropyl)amino]carbonyl]methyl]-1',2',4'-trioxaspiro[4.5]decane *p*-tosylate; *cis*-adamantane-2-spiro-3'-8'-[[[(2'-amino-2'-methylpropyl)amino]carbonyl]methyl]-1',2',4'-trioxaspiro[4.5]decane hydrogen maleate; *cis*-adamantane-2-spiro-3'-8'-[[[(2'-amino-2'-methylpropyl)amino]carbonyl]methyl]-1',2',4'-trioxaspiro[4.5]decane hydrochloride; *cis*-adamantane-2-spiro-3'-8'-[[[(2'-amino-2'-methylpropyl)amino]carbonyl]methyl]-1',2',4'-trioxaspiro[4.5]decane hydrogen tartarate; and *cis*-adamantane-2-spiro-3'-8'-[[[(2'-amino-2'-methylpropyl)amino]carbonyl]methyl]-1',2',4'-trioxaspiro[4.5]decane hydrogen succinate.

6. *Cis*-adamantane-2-spiro-3'-8'-[[[(2'-amino-2'-methylpropyl)amino]carbonyl]methyl]-1',2',4'-trioxaspiro[4.5]decane hydrogen maleate.

7. A method of preventing or treating malaria comprising:

administering a malaria prevention or malaria treatment effective amount of a spiro or dispiro 1,2,4-trioxolane in a pharmaceutically acceptable carrier, said trioxolane being selected from the group consisting of OZ271, OZ272, OZ273, OZ274, OZ275, OZ276, OZ277, OZ278, OZ279, OZ280, OZ281, OZ282, OZ283, OZ284, OZ285, OZ286, OZ287, OZ288, OZ289, OZ290, OZ291, OZ292, OZ293, OZ294, OZ295, OZ296, OZ297, OZ298, OZ299, OZ300, OZ301, OZ302, OZ303, OZ304, OZ305, OZ306, OZ307, OZ308, OZ309, OZ310, OZ311, OZ312, OZ313, OZ314, OZ315, OZ316, OZ317, OZ318, OZ319, OZ320, OZ321, OZ322, OZ323, OZ324, OZ325, OZ326, OZ327, OZ328, OZ329, OZ330, OZ331,

OZ332, OZ333, OZ334, OZ335, OZ336, OZ337, OZ338, OZ339, OZ340, OZ341, OZ342, OZ343, OZ344, OZ345, OZ346, OZ347, OZ348, OZ349, OZ350, OZ351, OZ352, OZ353, OZ354, OZ355, OZ356, OZ357, OZ358, OZ359, OZ360, OZ361, OZ362, OZ363, OZ364, OZ365, OZ366, OZ367, OZ368, and OZ369.

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8. A method of manufacturing a composition for prophylaxis and treatment of malaria comprising: mixing a malaria prophylaxis or malaria treatment-effective amount of a spiro or dispiro 1,2,4-trioxolane, its prodrugs and optical isomers thereof, with a pharmaceutically acceptable carrier, said trioxolane being selected from the group

10 consisting of: OZ271, OZ272, OZ273, OZ274, OZ275, OZ276, OZ277, OZ278, OZ279, OZ280, OZ281, OZ282, OZ283, OZ284, OZ285, OZ286, OZ287, OZ288, OZ289, OZ290, OZ291, OZ292, OZ293, OZ294, OZ295, OZ296, OZ297, OZ298, OZ299, OZ300, OZ301, OZ302, OZ303, OZ304, OZ305, OZ306, OZ307, OZ308, OZ309, OZ310, OZ311, OZ312, OZ313, OZ314, OZ315, OZ316, OZ317, OZ318, OZ319, OZ320, OZ321, OZ322, OZ323, 15 OZ324, OZ325, OZ326, OZ327, OZ328, OZ329, OZ330, OZ331, OZ332, OZ333, OZ334, OZ335, OZ336, OZ337, OZ338, OZ339, OZ340, OZ341, OZ342, OZ343, OZ344, OZ345, OZ346, OZ347, OZ348, OZ349, OZ350, OZ351, OZ352, OZ353, OZ354, OZ355, OZ356, OZ357, OZ358, OZ359, OZ360, OZ361, OZ362, OZ363, OZ364, OZ365, OZ366, OZ367, OZ368, and OZ369.

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9. A method of treating cancer comprising: administering a cancer treatment effective amount of a spiro or dispiro 1,2,4-trioxolane in a pharmaceutically acceptable carrier, said trioxolane being selected from the group

consisting of: OZ271, OZ272, OZ273, OZ274, OZ275, OZ276, OZ277, OZ278, OZ279, 25 OZ280, OZ281, OZ282, OZ283, OZ284, OZ285, OZ286, OZ287, OZ288, OZ289, OZ290, OZ291, OZ292, OZ293, OZ294, OZ295, OZ296, OZ297, OZ298, OZ299, OZ300, OZ301, OZ302, OZ303, OZ304, OZ305, OZ306, OZ307, OZ308, OZ309, OZ310, OZ311, OZ312, OZ313, OZ314, OZ315, OZ316, OZ317, OZ318, OZ319, OZ320, OZ321, OZ322, OZ323, OZ324, OZ325, OZ326, OZ327, OZ328, OZ329, OZ330, OZ331, OZ332, OZ333, OZ334, 30 OZ335, OZ336, OZ337, OZ338, OZ339, OZ340, OZ341, OZ342, OZ343, OZ344, OZ345, OZ346, OZ347, OZ348, OZ349, OZ350, OZ351, OZ352, OZ353, OZ354, OZ355, OZ356,

OZ357, OZ358, OZ359, OZ360, OZ361, OZ362, OZ363, OZ364, OZ365, OZ366, OZ367, OZ368, and OZ369.

10. A method of prophylaxis or treatment of schistosomiasis comprising:

- 5 administering a schistosomiasis prophylaxis or treatment effective amount of a spiro or dispiro 1,2,4-trioxolane in a pharmaceutically acceptable carrier, said trioxolane being selected from the group consisting of: OZ271, OZ272, OZ273, OZ274, OZ275, OZ276, OZ277, OZ278, OZ279, OZ280, OZ281, OZ282, OZ283, OZ284, OZ285, OZ286, OZ287, OZ288, OZ289, OZ290, OZ291, OZ292, OZ293, OZ294, OZ295, OZ296, OZ297, OZ298, 10 OZ299, OZ300, OZ301, OZ302, OZ303, OZ304, OZ305, OZ306, OZ307, OZ308, OZ309, OZ310, OZ311, OZ312, OZ313, OZ314, OZ315, OZ316, OZ317, OZ318, OZ319, OZ320, OZ321, OZ322, OZ323, OZ324, OZ325, OZ326, OZ327, OZ328, OZ329, OZ330, OZ331, OZ332, OZ333, OZ334, OZ335, OZ336, OZ337, OZ338, OZ339, OZ340, OZ341, OZ342, OZ343, OZ344, OZ345, OZ346, OZ347, OZ348, OZ349, OZ350, OZ351, OZ352, OZ353, 15 OZ354, OZ355, OZ356, OZ357, OZ358, OZ359, OZ360, OZ361, OZ362, OZ363, OZ364, OZ365, OZ366, OZ367, OZ368, and OZ369.