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Dihydroartemisinin and dihydroartemisitene dimers as anti-cancer and anti-infective agents

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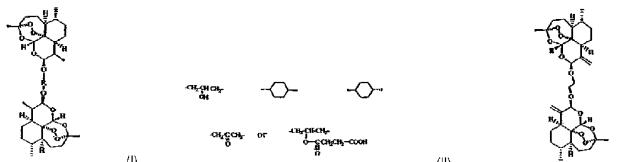
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(54) Title: DIHYDROARTEMISININ AND DIHYDROARTEMISITENE DIMERS AS ANTI-CANCER AND ANTI-INFECTIVE AGENTS



(57) Abstract: This invention relates to a compound of the formula where R is or of the formula: The invention also relates to the use of these compounds in pharmaceutical compositions as anticancer and anti-infective agents.

DIHYDROARTEMISININ AND DIHYDROARTEMISITENE DIMERS AS
ANTI-CANCER AND ANTI-INFECTIVE AGENTSFIELD OF INVENTION

The present invention relates to dihydroartemisinin and dihydroartemisitene dimers and their use in the treatment of cancer and as antiprotozoal agents.

Background of the Invention

Cancer deaths in the U.S. alone were over 500,000 in 2001, and in spite of many advances, cancer remains one of the leading killers (1). There is a critical need for the development of new anti-cancer agents, especially those with novel and selective mechanisms of action. Although some of the promise of non-cytotoxic therapies is beginning to be realized (e.g. immunostimulants, growth factor antagonists, anti-sense therapy), the mainstay of the treatment of most cancers remains with cytotoxic drugs. In view of the limited success rates, incidence of toxicities, and development of resistance to such agents, there is a dire need for new classes of these drugs, especially those that may act by new mechanisms or exhibit exploitable selectivity. There is also a need for a better understanding of dosing, scheduling, and concomitant therapies in order to optimize treatment protocols.

Natural products have historically been a rich source of new, successful prototype classes of lead compounds from which analogs have been developed. According to a recent review, 60% of the anti-infective and anti-cancer drugs that have successfully advanced to the clinic are derived from natural products (2). Examples of these among currently used anti-cancer agents include the anthracycline class (e.g., doxorubicin), the Catharanthus (Vinca) alkaloids, paclitaxel, and derivatives of podophyllotoxin and camptothecin. A recently published tabulation of natural product-based anti-tumor drugs shows more than 25 agents currently in Phase I or II (3). This and other recent reviews are important reminders of the critical role of natural products as a resource for the discovery of new anti-tumor agents (4,5).

The natural product artemisinin (**1**) is a sesquiterpene endoperoxide first isolated in 1971 from the Chinese plant *Artemisia annua* (6). The compounds as numbered

hercin are depicted in Figure 1. The compound was shown to have anti-malarial activity against both chloroquine-sensitive and chloroquine-resistant strains of *Plasmodium falciparum*.

Because of the importance of the clinical effects of artemisinin in treating malaria, many derivatives were prepared in order to develop the most effective and least toxic anti-malarial agent. Initially, simple derivatives were prepared – e.g., dihydroartemisinin (DHA, in which the lactone carbonyl is reduced resulting in a hemiacetal), artemether (the methyl ether of DHA) and several other ether and ester analogs. The sodium salt of the hemisuccinate ester (sodium artesunate) was one of these derivatives that showed more activity and less toxicity than artemether, the latter being more active than artemisinin itself. Continued interest in the activity of artemisinin and DHA analogs later resulted in the preparation of artemisinin acetal dimers through reaction of dihydroartemisinin with borontrifluoride-etherate.

In addition to its anti-malarial activity, artemisinin had been reported to have cytotoxic effects against EN-2 tumor cells (7), P-388, A549, HT-29, MCF-7, and KB-tumor cells (8). As more analogs were evaluated for anti-tumor activity, it was reported that the unsymmetrical dimer (2) showed strong cytotoxic activity and was more potent than cisplatin (9). The symmetrical dimer (3) also showed pronounced cytotoxic activity (10).

This finding stimulated interest in other types of DHA dimers. Posner *et al.* (11) prepared dimers linked with a polyethylene glycol spacer (3 units of ethylene glycol), an eight carbon glycol, and a dithio- derivative. The authors also prepared simpler trioxane dimers. Posner *et al.* also prepared several dimers of DHA where the linking units between the two molecules of dihydroartemisinin were dicarboxylic acids of different types (12). Zhang and Darbie (13,14) also proposed several dihydroartemisinin dimers to be linked via different coupling agents. Some of these artemisinin dimers and some of the simpler trioxanes had anti-malarial effects, anti-cancer activity, and anti-proliferative effects with some compounds being as active as calcitriol in an anti-proliferative assay in murine keratinocytes.

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The discussion of documents, acts, materials, devices, articles and the like is included in this specification solely for the purpose of providing a context for the present invention. It is not suggested or represented that any or all of these matters formed part of the prior art base or were common general knowledge in the field relevant to the present invention as it existed before the priority date of each claim of this application.

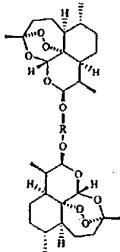
5 Where the terms "comprise", "comprises", "comprised" or "comprising" are used in this specification (including the claims) they are to be interpreted as specifying the presence of the stated features, integers, steps or components, but not precluding the presence of one or more other features, integers, steps or components, or group thereof.

0 **Summary of the invention.**

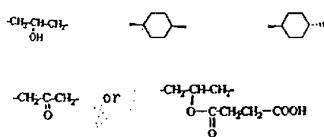
This invention comprises compositions containing dihydroartemisinin and dihydroartemisinene dimers with activity as anticancer agents and anti-protozoal, including anti-malarial and anti-leishmanial properties. This invention also describes methods of preparation of these compositions and methods of 5 use of such compositions for the treatment of cancer, and protozoal infections, including malaria, or leishmaniasis. The compositions of this invention have not been previously described.

The compounds of this invention represent a potential new class of anti-tumor agents, one that has shown promising activity against solid tumors, and with a pattern of selectivity that suggests a possible new mechanism of action.

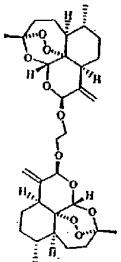
10 In one aspect the present invention provides a method of treating cancer comprising administering to a subject suffering from cancer an effective amount of at least one compound of the formula:



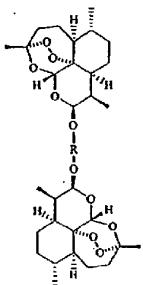
where R is



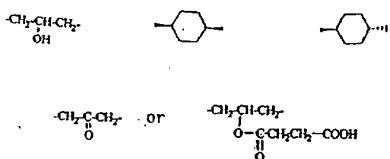
25 or a compound of the formula;



In another aspect the present invention provides a method of treating a protozoal infection comprising administering to a subject suffering from such an infection an effective amount of at least 5 one compound of the formula:

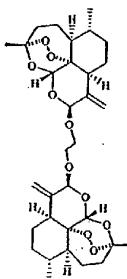


where R is

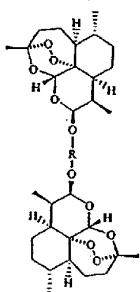


or a compound of the formula:

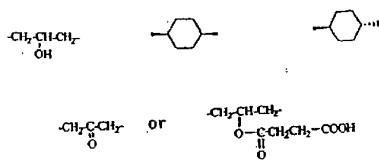
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In a further aspect the present invention provides a compound of the formula:

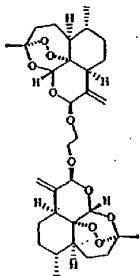


5 where R is

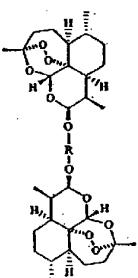


or of the formula:

3b

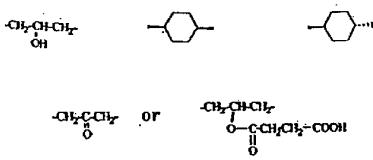


In still a further aspect the present invention provides use of at least one compound of the formula:



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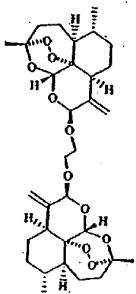
where R is



or a compound of the formula:

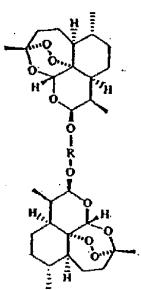
3c

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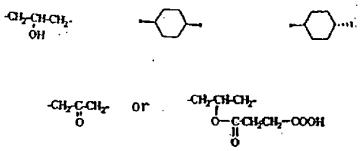


in the manufacture of a medicament for the treatment of cancer.

In still another aspect the present invention provides use of at least one compound of the
5 formula:

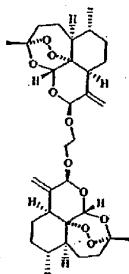


where R is



or a compound of the formula:

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in the manufacture of a medicament for the treatment of a protozoal infection.

5 **Description of the invention.**

In the interest of development of new chemotherapeutic agents, artemisinin dimers were prepared in this invention by condensation of DHA with a variety of vicinal and non-vicinal glycols. These dimers have been evaluated in the NCI anti-tumor screening program, and all passed to the 60-cell line screen (4-9). Several of these compounds have been advanced into further testing by the NCI 0 into the Hollow Fiber Assay (HFA) protocol (**Compounds 4, 5, and 6**). Additional supplies of **Compounds 5 and 6** have been recently prepared and provided to the NCI for testing in xenograft tumor models.

Although the mechanism of action of these DHA dimers remains to be determined, some clues regarding possible molecular targets are suggested. Use of the NCI COMPARE analysis revealed that 5 the cell sensitivity profile of these compounds in the 60-cell line assay was similar to platinum compounds. These compounds inhibit cell replication by forming DNA intrastrand cross-links. Correlations on micro-array data for the 60 cell lines also indicate that cells most sensitive to these dimers contain lower levels of the mRNAs encoding proteins involved in integrin and hypoxia signaling. Lower levels of expression of these proteins may result in enhanced sensitivity either 20 because these proteins are direct

targets, or because their reduced expression reflects a condition within the cell (e.g., redox potential) that augments sensitivity.

Administration of the instant dimers may be by any of the conventional routes of administration, for example, oral, subcutaneous, intraperitoneal, intramuscular, intravenous or rectally. In the preferred embodiment, the compound is administered in combination with a pharmaceutically-acceptable carrier which may be solid or liquid, dependent upon choice and route of administration. Examples of acceptable carriers include, but are not limited to, starch, dextrose, sucrose, lactose, gelatin, agar, stearic acid, magnesium stearate, acacia, and similar carriers. Examples of liquids include saline, water, edible oils, e.g. peanut and corn.

When administered in solid form, the compound and diluent carrier may be in the form of tablets, capsules, powders, lozenges, suppositories prepared by any of the well known methods. When given as a liquid preparation, the mixture of active compound and liquid diluent carrier may be in the form of a suspension administered as such. The compound is administered in a non-toxic dosage concentration sufficient to inhibit the growth and/or destroy cancer or to destroy protozoal organisms such as malaria and leishmania. The actual dosage unit will be determined by the well recognized factors as body weight of the patient and/or severity and type of pathological condition the patient might be suffering with. With these considerations in mind, the dosage unit for a particular patient can be readily determined by the medical practitioner in accordance with the techniques known in the medical arts.

The compounds of this invention have been prepared by reaction of dihydroartemisinin or dihydroartemistene with a variety of optionally substituted 1,2-, 1-3- or 1,4 glycols under acidic conditions (borontrifluoride etherate) in dry ether followed by chromatography of the reaction mixture to isolate the desired product. Optional substituents include, for example, alkoxy or acyloxy groups. The dimers of the present invention can also be prepared by the reaction of dihydroxy ketones such as, for example, dihydroxyacetone, with DHA or dihydroartemistene followed by reduction of the keto-group and reaction of the

hydroxy group formed in the reduction of the ketone with hydroxy reactive compounds such as mono or dicarboxylic acids as their acid halides and acid anhydrides. The starting material (dihydroartemisinin) is prepared by sodium borohydrite reduction of the natural product artemisinin (1). The latter compound is isolated from the leaves of *Artemisia annua* following the procedures previously described (15, 16). The compounds of the invention were tested in the NCI anti-tumor screen and in the anti-malarial and anti-Leishmanial screens. The activities are shown in Tables 1-8 as shown in Figures 2A, 2B, 3A, 3B, 4A, 4B and Figures 5 to 9.

Examples

Reactions were run in oven dried round-bottomed flasks. Diethyl ether (ether) was distilled from sodium benzophenone ketyl prior to use under an atmosphere of argon. All chemicals were purchased from Sigma-Aldrich and used without further purification, except the diols, which were dried over grade I alumina prior to use. Artemisinin (1) was isolated from locally grown *Artemisia annua* L. plants, using a literature procedure (15,16), and was reduced to dihydroartemisinin as previously reported (17).

Column chromatography was performed using flash chromatography, using silica gel purchased from Merck (particle size 230 – 400 mesh). Analytical thin-layer chromatography (TLC) was performed with silica gel 60 F₂₅₄ plates (250 μ m thickness; Merck), using *n*-hexane-EtOAc mixtures as solvent systems. Visualization was accomplished by spraying with *p*-anisaldehyde spray reagent followed by heating using a hot-air gun (18).

Mp's were recorded on an Electrothermal 9100 instrument. IR spectra were obtained using AATI Mattson Genesis Series FTIR. Optical rotations were recorded at ambient temperature using JASCO, DIP-370, digital polarimeter. 1D and 2D NMR spectra were obtained on Bruker Avance DRX 500 spectrometers at 500 MHz (¹H) and 125 MHz (¹³C) or Bruker DRX 400 spectrometer using the solvent peak as the internal standard. HRESIFTMS were

obtained using a Bruker Bioapex FT-MS in ESI mode. Low resolution MS were measured on a ThermoQuest aQa LC/MS.

Preparation of the 1,3- β , β -Dihydroartemisinin Dimer with Glycerol (Compound 4)

Example 1

To a stirred solution of dihydroartemisinin (160 mg, 0.56 mmol) in a round bottomed flask (50 mL) in dry ether (10 mL), was added dry glycerol (26 mg) and BF_3OEt_2 (267 μL) using a hypodermic syringe. The mixture was stirred under argon for 70 min. then quenched and worked up as usual to leave a gummy residue (199 mg). Upon crystallization from ether, it yielded 4 (52 mg) as cubic crystals, 28.7 %; $[\alpha]_D^{25} +173^\circ$ (c 0.022, CHCl_3); IR (film) ν_{max} : 3525 (OH), 2953, 2933, 2881, 1449, 1376, 1194, 1176, 1134, 1107, 1027, 991 cm^{-1} ; ^1H NMR (CDCl_3 , 500 MHz, for one of the two identical monomeric units): δ 5.40 (1H, s, H-5), 4.79 (1H, d, J $=$ 3.9, H-12), 4.78 (1H, d, J $=$ 3.5 Hz, H-12'), 3.87 (1H, m, H-16, H-16', H-17); 3.49 (1H, dd, J $=$ 5.9, 4.3 Hz, H-18), 3.42 (1H, q, J $=$ 5.4 Hz, H-18'), 2.63 (1H, m, H-11), 2.34 (1H, ddd, J $=$ 14.0, 4.0, 3.9 Hz, H-3), 2.00 (1H, m, H-3'), 1.85 (1H, m, H-2), 1.68 (3H, m, H-2', H-8, H-9), 1.46 (2H, m, H-7, H-8'), 1.39 (3H, s, Me-15), 1.34 (1H, m, H-10), 1.21 (1H, m, H-1), 0.92 (3H, d, J $=$ 6.4 Hz, Me-14), 0.90 (3H, d, J $=$ 7.4 Hz, Me-13), 0.89 (3H, d, J $=$ 7.3 Hz, H-13'), 0.87 (1H, m, H-9'); ^{13}C NMR (CDCl_3 , 125 MHz): δ 104.5 (s, C-4), 103.13 (d, C-12), 103.06 (s, C-12'), 88.3 (d, C-5), 81.4 (s, C-6), 70.3 (t, C-16), 70.1 (t, C-18), 70.0 (d, C-17), 52.9 (d, C-1), 44.7 (d, C-7, C-7'), 37.7 (d, C-10), 36.8 (t, C-3), 35.0 (t, C-9), 31.27 (d, C-11), 31.25 (d, C-11'), 26.5 (q, C-15), 25.0 (t, C-2), 25.0 (t, C-8), 20.7 (q, C-14), 13.4 (q, C-13); HRESIFTMS [m/z] 625.3512 [M+H] $^+$ (calcd for $\text{C}_{33}\text{H}_{53}\text{O}_{11}$, 625.3582).

Preferred Procedure for Preparation of Compound 4.

Example 2

The preferred method of preparing **Compound 4** was to first prepare the ketone precursor through condensation of dihydroxyacetone with dihydroartemisinin

in the presence of boron trifluoride-etherate followed by sodium borohydride reduction of the resulting ketone to give **Compound 4**. This is detailed in the following examples.

Preparation of the β,β -Dihydroartemisinin Dimer with Dihydroxyacetone (Compound 7)

Example 3

Dihydroartemisinin (284 mg, 1 mmol) and 1,3-dihydroxyacetone dimer (45.05 mg, 0.25 mmol) were suspended in diethylether (10 mL). To the mixture (cooled to 5°C under argon) was then added 35.5 mg $\text{BF}_3\text{-Et}_2\text{O}$ (0.25 mmol, 31 μL) and the mixture stirred at 5°C for 20 minutes then at room temperature for 1 hr. Workup as usual gave 319 mg of residue.

The residue was chromatographed on silica gel column (30 g) and eluted with hexane:EtOAc (95:5) with polarity increasing to 80:20. Fractions were collected and pooled according to TLC similarities to give four major fractions. The most polar fraction (140.2 mg) was identified as **Compound 7** (converts to **Compound 4** upon NaBH_4 reduction):

$^1\text{H-NMR}$ in CDCl_3 at 500 MHz: δ 5.44 (2H, s, H-5 & H-5'), 4.805 (2H, d, J = 3.39 Hz, H-12 & H-12'), two broad doublets (2H each J = 17.59) centered at 4.46 & 4.285 (H-16 & H-18), 2.665 (2H, m, H-11 & H-11'), 2.355 (2H, ddd, H-3), 2.025 (2H, m, H-3'), 1.88 (2H, m, H-2), 1.81 (4H, m, H-9 & H-9'), 1.675 (2H, m, H-8), 1.475 (4H, H-7, H-7', H-10 & H-10'), 1.41 (6H, s, Me-15 & Me-15'), 1.255 (2H, m, H-1 & H-1'), 0.99-0.95 (12H, Me-14, Me-14', Me-13 & Me-13').

$^{13}\text{C-NMR}$ in CDCl_3 at 124 MHz: δ 204.8 (s, C=O), 104.53 (s, C-4), 102.87 (d, C-12), 88.51 (d, C-5), 81.36 (s, C-6), 72.06 (t, C-16 & C-17), 52.89 (d, C-1), 44.67 (d, C-7), 37.75 (d, C-10), 36.77 (t, C-3), 34.99 (s, C-9), 31.13 (d, C-11), 26.47 (q, C-15), 25.03 (t, C-2), 24.74 (t, C-8), 20.69 (q, C-14), 13.43 (q, C-13).

Example 4

To a suspension of 2.84 g dihydroartemisinin (10 mmol) and 450 mg (2.4 mmol) of 1,3-dihydroxyacetone dimer in ether (100 mL) was added 127 μ L of $\text{BF}_3\text{Et}_2\text{O}$ (142 mg, 1 mmol) at 5°C. The mixture was stirred at room temperature for 30 minutes, then a second portion (127 μ L) of $\text{BF}_3\text{Et}_2\text{O}$ was added. A third portion and a fourth portion (254 μ L) of $\text{BF}_3\text{Et}_2\text{O}$ were added at 15-minute intervals making up a total of 4 mmols. Stirring was continued for 1.5 hr. Workup as usual provided an oily residue which was chromatographed in a manner similar to that described under **Example 3** and fractions were combined based on their TLC similarities.

The fractions with R_f values corresponding to the dimer prepared in **Example 3** were combined and the solvent evaporated to produce 2.05 g of an oily residue which foamed in vacuum. This material was identical to that prepared under **Example 3 (Compound 7)**.

Example 5

To a suspension of 3.3 g dihydroartemisinin (11.6 mmols) and 522 mg, 2.9 mmols) of 1,3-dihydroxyacetone dimer (0.25 equivalent) in ether (100 mL) was added 0.88 mL of $\text{BF}_3\text{Et}_2\text{O}$ (0.986 g, 6.9 mmol, 0.6 equivalent) at 5°C. The mixture was then stirred at room temperature for 3 hr, then worked up as usual to provide an oily residue. The residue was chromatographed over silica gel column (130 g) and eluted with mixtures of hexane-EtOAC ranging from 95:5 to 85:15 to give several fractions which were combined according to TLC similarities. Fractions containing the desired product (identical to that prepared under **Example 3**) were combined to give 1.628 g of **Compound 7**.

Preparation of the 1,3- β , β -Dihydroartemisinin Dimer of Glycerol (Compound 4) Starting from Compound 7.

Example 6

The ketone intermediate (**Compound 7**), (1.94 g, 312 mmols) was dissolved in 225 mL of a mixture of THF and water (2:1). The solution was stirred and NBH_4

(474 mg, 4 molar equivalent) was then added in portions at room temperature over a 15 minute period. The mixture was then neutralized with 2N HCl. The THF was then evaporated under vacuum. The precipitate was filtered and washed with water and air dried to give 1.8 g (92.5% yield) of **Compound 4**, identical to that prepared under **Example 1**.

Example 7

The same procedure described under **Example 6** was repeated using 0.778 g of **Compound 7** to yield 0.73 g of **Compound 4**.

Preparation of the β,β -Dihydroartemisinin Dimer with Cyclohexanediol (Compounds 5 and 6).

Example 8

In a round-bottomed flask (100 mL) was introduced dihydroartemisinin (850 mg, 3.0 mmol) and dry ether (25 mL) then the mixture was stirred at room temperature with cyclohexane-1,4-diol (mixture of *cis* and *trans*) (170 mg). To the stirred solution, $\text{BF}_3 \cdot \text{OEt}_2$ (570 μL) was then added using a hypodermic syringe. The stirring was continued for 80 min., then the reaction was quenched and worked up as usual to leave a gummy residue (1.13 g). The residue was loaded on Si gel column (170 g) and eluted with increasing amounts of EtOAc in *n*-hexane (15 \rightarrow 50%). Fractions of 5 mL were collected and similar fractions were pooled by guidance of TLC to afford **Compound 5** (238 mg, oil). Earlier fractions were pooled and re-chromatographed on a silica gel column to yield **6** (70 mg, white amorphous solid).

Compound 5; $[\alpha]_D +142^\circ$ (c 0.036, MeOH); IR (film) ν_{max} : 2938, 2872, 1448, 1375, 1227, 1194, 1122, 1099, 1029 cm^{-1} ; ^1H NMR (CDCl_3 , 500 MHz, for one of the identical monomereric units): δ 5.42 (1H, s, H-5), 4.90 (1H, d, J = 3.3, H-12), 3.78 (1H, brs, H-16), 2.62 (1H, m, H-11), 2.36 (1H, ddd, J = 14.0, 12.4, 3.8 Hz, H-3), 2.04 (1H, m, H-3'), 1.88 (2H, m, H-2, H-8), 1.77 (2H, m, H-2', H-8'), 1.71 (2H, m, H-17a, H-17b), 1.65-1.56 (4H, m, H-9, H-9', H-17'a, H-17'b), 1.48 (1H, m, H-7), 1.43 (3H, s, Me-15), 1.32 (1H, m, H-10), 1.25 (1H, m, H-1), 0.95 (3H, d, J = 6.2 Hz, Me-14), 0.90 (3H, d, J = 7.2 Hz, Me-13); ^{13}C NMR (CDCl_3 , 125 MHz): δ 104.4 (s,

C-4), 100.0 (d, C-12), 88.4 (d, C-5), 81.5 (s, C-6), 72.7 (d, C-16), 53.0 (d, C-1), 44.9 (d, C-7), 37.9 (d, C-10), 36.9 (t, C-3), 35.2 (t, C-9), 31.2 (d, C-11), 26.6 (q, C-15), 24.9 (t, C-2), 25.1 (t, C-8), 20.7 (q, C-14), 13.5 (q, C-13); HRESIFTMS [m/z] 647.3445[M-1]⁺ (calcd for C₃₆H₅₅O₁₀, 647.3510).

Compound 6; *R*_f 0.42 (*n*-hexane:EtOAc, 8:2), [α]_D +114° (c. 0.042, CHCl₃); IR (film) no OH absorption; ¹H NMR (CDCl₃, 400 MHz): δ 5.41 (1H, s, H-5), 4.88 (1H, d, *J* = 3.3 Hz, H-12), 3.75 (1H, brs, H-16), 1.47 (1H, m, H-7), 1.43 (3H, s, Me-15), 1.26 (2H, m, H-1, H-10), 0.95 (3H, d, *J* = 6.0 Hz, Me-14), 0.87 (3H, d, *J* = 7.3 Hz, Me-13); ¹³C NMR (CDCl₃, 100 MHz): δ 104.4 (s, C-4), 100.4 (d, C-12), 88.4 (d, C-5), 81.6 (s, C-6), 73.9 (t, C-16), 53.0 (d, C-1), 44.9 (m, C-7), 37.9 (d, C-10), 36.9 (t, C-3), 35.1 (t, C-9), 31.2 (d, C-11), 30.4 (t, C-17), 27.5 (t, C-17), 26.6 (q, C-15), 24.9 (t, C-2), 25.1 (t, C-8), 20.8 (q, C-14), 13.5 (q, C-13); HRESIFTMS [m/z] 671.3772 [M+Na]⁺ (calcd for C₃₆H₅₆O₁₀Na, 671.3765).

Example 9

A mixture of dihydroartemisinin (372 mg 1.31 mmol) and 1,4-cyclohexanediol (*cis* and *trans* mixture) (74.4 mg, 0.64 mmol) were suspended in 10 mL dry ether and 0.25 mL of BF₃·Et₂O (280 mg, 1.97 mmol) was added at 0°C. The mixture was then stirred at room temperature for 80 minutes followed by workup by shaking with a sodium bicarbonate solution and separation of the ether layer. The aqueous layer was washed with ether (2 x 10 mL). The ether layers were combined and washed with water and brine and the ether layer was dried over anhydrous sodium sulfate. Evaporation of the ether resulted in an oily residue (429 mg) which was chromatographed on silica gel column (30 g) and eluted with hexane:ether mixtures ranging from 97:3 to 80:20 and fractions were pooled together according to their TLC similarities. **Compound 6** (56 mg) was isolated as white solid and **Compound 5** (24 mg) was isolated as an oil which foamed under vacuum. These were found to be identical to **Compounds 5** and **6** prepared under **Example 8**.

Example 10

A mixture of dihydroartemisinin (744 mg, 2.62 mmol) and 1,4-cyclohexanediol (149 mg, 1.28 mmol) of the *cis* and *trans* mixture were stirred in 20

mL dry ether at 5°C. To the mixture was added $\text{BF}_3\text{Et}_2\text{O}$ (83 μL , 0.655 mmol) and stirring was continued at room temperature for 1 hr. A second portion of $\text{BF}_3\text{Et}_2\text{O}$ (83 μL) was added, and the mixture continued to stir for 1 hr. The reaction mixture was then worked up as usual to give 890 mg of an oily residue. Column chromatography of the residue using silica gel (32 g) and elution with hexane:ether 92:2, 96:4, 94:6, and then 90:10 (200 mL each) yielded several fractions pooled according to TLC similarities. **Compound 6** was isolated as cubic crystals (238 mg, melting point 146-148 °C). **Compound 5** was isolated from later fractions as an amorphous foam (184 mg, melting point 93-97 °C). These were found to be identical to those previously prepared under **Examples 8 and 9**.

Example 11

A mixture of dihydroartemisinin (10 g, 35.2 mmol) and 1,4-cyclohexanediol (*cis* and *trans* mixture) (2 g, 17.2 mmol) were suspended in 260 mL dry ether and 1.11 mL of $\text{BF}_3\text{Et}_2\text{O}$ was added at 0°C under argon. Two additional portions of $\text{BF}_3\text{Et}_2\text{O}$ (1.11 mL each) were added after 1 hr intervals. The mixture was then stirred at room temperature for 1 hr after the last addition of $\text{BF}_3\text{Et}_2\text{O}$ followed by workup as usual to give 12 g of an amorphous residue which was chromatographed on silica gel column to produce 2.69 g of **Compound 5** and 2.78 g of **Compound 6**. These were found to be identical to **Compounds 5 and 6** prepared under **Examples 8-10**.

It must be mentioned that should only **Compound 5** or only **Compound 6** be desired that only the *cis* or *trans* 1,4-Cyclohexanediol be used as the starting material to produce the desired product.

Preparation of the Hemisuccinate Ester of Compound 4 (Compound 8)

Example 12

To a stirred solution of **Compound 4** (200 mg, 0.32 mmol) in dry methylene chloride (4 mL) were added triethylamine (0.14 mL, 1.3 equiv), dimethylaminopyridine (16 mg, 0.4 equiv) and succinic anhydride (92 mg, 3 equiv). The resulting solution was slowly stirred at room temperature for 16 hr. Following

evaporation of the solvent under reduced pressure, the residue was purified over a silica gel column using hexane:acetone (6:4) as the eluent. The product of the reaction was isolated as white amorphous solid (156 mg) with R_f value of 0.68 (hexane:acetone, 1:1) identified as the hemisuccinate ester of **Compound 4** (**Compound 8**), based on spectral data (see details under **Example 13**).

Example 13

The reaction of **Example 12** was repeated on a larger scale (starting with 550 mg of **Compound 4**) where all reactants were scaled up proportionally. Purification of the reaction product in the same manner produced 355 mg of **Compound 8** as amorphous white powder with the following spectral characteristics:

$^1\text{H-NMR}$ (acetone-d₆, 500 MHz): δ 5.442 (1H, s, H-5), 5.396 (1H, s, H-5'), 5.181 (1H, t, J = 4.9 Hz, H-17), 4.755 (1H, d, J = 3.4 Hz, H-12), 4.740 (1H, d, J = 3.4 Hz, H-12'), 3.990 (2H, m, H-16), 3.608 (1H, dd, J = 4.5 Hz & 4.5 Hz, H-18), 3.560 (1H, q, J = 5.19 Hz, H-18), 2.652 (4H, m, CO - CH₂-CH₂-CO-), 2.548 (2H, m, H-11 & H-11'), 2.304 (2H, ddd, J = 3.0, 3.89 & 3.0 Hz, H-3), 2.065 (2H, m, H-3'), 1.886 (2H, m, H-2), 1.786 (2H, m, H-2), 1.786 (2H, m, H-9), 1.686 (2H, m, H-8), 1.534 (2H, m, H-2'), 1.489 (2H, m, H-7 & H-10), 1.332 (6H, s, Me-15 & Me-15'), 1.213 (2H, m, H-1 & H-1'), 0.980-0.937 (12H, Me-14, Me-13, Me-14' & Me-13').

$^{13}\text{CNMR}$ (acetone-d₆, 125 MHz): δ 172.99 (s, CO-OH), 171.7 (s, -CO-0-), 103.9 (s, C-4), 102.46 (d, C-12), 102.32 (d, C-12'), 88.02 (d, C-5), 87.99 (d, C-5'), 80.97 (s, C-6), 71.92 (d, C-17), 66.74 (t, C-16), 66.59 (t, C-18), 53.09 (d, C-1), 44.89 (d, C-7), 37.63 (d, C-10), 36.70 (t, C-3), 34.98 (t, C-8), 31.33 (d, C-11), 31.29 (d, C-11'), 29.48 & 28.78 (t, methylenes of CO - CH₂-CH₂-CO), 25.72 (q, C-15), 24.98 (t, C-2), 24.7 (t, C-9), 20.23 (q, C-14), 12.81 (q, C-13). HRESIFTMS (m/z) 723.362 [M-H]⁺, (Calcd. for C₃₇H₅₅O₁₄)

Preparation of the β,β -Dihydroartemisitene Dimer with Ethylene Glycol**(Compound 9)****Example 14**

To a stirred solution of dihydroartemisitene (prepared from artemisinin as previously described (19) (75 mg) in dry ether (15 mL) and ethylene glycol (52 mg), was added BF_3OEt_2 (18 μL) and the reaction mixture was allowed to stand for 24 hr, then quenched and worked up as usual. Column chromatography of the crude reaction mixture using a gradient of EtOAc in *n*-hexane (20 %→50 %) afforded **Compound 9** as a gum (7 mg), $[\alpha]_D +181^\circ$ (*c* 0.022, MeOH); IR (film) ν_{max} : 2937, 2875, 1681, 1449, 1376, 1191, 1102, 987 cm^{-1} ; ^1H NMR (CDCl_3 , 400 MHz): δ 5.89 (1H, s, H-5), 5.38 (1H, s, H-12), 5.08 (1H, s, H-13a), 4.94 (1H, s, H-13b), 3.89 (1H, d, J = 7.5 Hz, H-16a), 3.57 (1H, d, J = 7.6 Hz, H-16b), 2.31 (1H, m, H-7), 1.44 (3H, s, Me-15), 1.25 (2H, m, H-1, H-10), 0.7 (3H, d, J = 6.2 Hz, Me-14); ^{13}C NMR (CDCl_3 , 100 MHz): δ 143.1 (s, C-11), 114.5 (t, C-13), 103.5 (s, C-4), 101.2 (d, C-12), 88.1 (d, C-5), 80.8 (s, C-6), 66.6 (t, C-16), 51.9 (d, C-1), 48.3 (d, C-7), 37.0 (d, C-10), 36.5 (t, C-3), 34.4 (t, C-9), 31.4 (t, C-8), 24.6 (t, C-2), 25.8 (q, C-15), 20.0 (q, C-13). HRESIFTMS [*m/z*] 613.2943 [M+Na]⁺ (calcd for $\text{C}_{32}\text{H}_{46}\text{O}_{10}\text{Na}$, 613.2983).

Example 15

Compounds of this invention were subjected to anti-cancer activity screen carried out by the National Cancer Institute (NCI) following their standard protocol against 60 different cancer cell lines. The activity of the compounds of this invention against selected cell lines fro leukemia, non-small cell lung cancer, colon cancer, CNS cancer, melanoma, ovarian cancer, renal cancer, prostate cancer, and breast cancer were determined in terms of GI50 (Table 1), TGI (Table 2), and LC50 (Table 3) concentrations. GI50 is the concentration which inhibits 50% of the growth of the cells, TGI is the concentration causing total growth inhibition, and LC50 is the concentration which kills 50% of the cells.

Example 16

Compounds of this invention were further tested under the NCI's Hollow Fiber Assay Standard Protocol which assesses the *in vivo* activity. Compounds are considered to have enough activity to progress into further testing if the combined IP and SC scores were ≥ 20 or if the SC score was ≥ 8 or if there was a net cell kill of one or more cell lines. Table 4 (Figure 5) shows the results of the testing of compounds of this invention in this assay.

Example 17

Compounds of this invention were subjected to *in vitro* assays to assess their anti-angiogenic activity. These assays are carried out by the NCI according to their standard protocol for HUVEC assays for initial *in vitro* testing. The three assays are the Growth Inhibition Assay, the Cord Formation Assay, and the Cell Migration Assay. Compounds are considered for further testing if activity is shown in at least one of the above assays. Table 5 (Figure 6) shows the activity of compounds of this invention in these assays.

Example 18

Compounds of this invention were subjected to anti-protozoal screens at the National Center for Natural Products Research (NCNPR) at the University of Mississippi following standard protocols for assessing anti-malarial and anti-

Leishmanial activity. Compounds' activities against these two organisms were compared to the activity of standard medications for each organism.

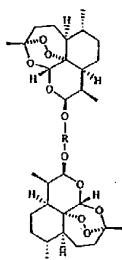
Table 6 (Figure 7) shows the activity of compounds of this invention against two strains of the malaria parasite (*Plasmodium falciparum*), one is chloroquine sensitive (D6 clone) and one is chloroquine resistant (W2 clone). The cytotoxicity of the compounds was also assessed using Vero cells. The data show that compounds of this invention are more active than chloroquine or artemisinin as anti-malarial drugs.

Table 7 (Figure 8) shows the activity of a selected group of compounds of this invention against the malaria parasite. These are from different synthetic lots than those tested in Table 6 (Figure 7). This confirms the activity of compounds of this invention as anti-malarial agents.

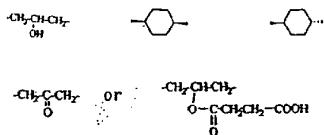
Table 8 (Figure 9) shows the activity of compounds of this invention against the leishmania parasite with activity comparable to that of pentamidine.

The Claims Defining The Invention Are As Follows

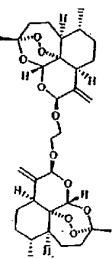
1. A method of treating cancer comprising administering to a subject suffering from cancer an effective amount of at least one compound of the formula:



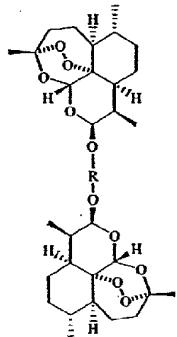
5 where R is



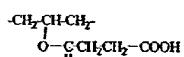
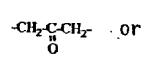
or a compound of the formula;



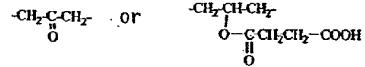
2. A method of treating a protozoal infection comprising administering to a subject suffering from such an infection an effective amount of at least one compound of the formula:



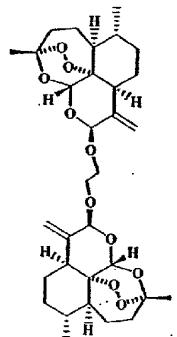
where R is



or

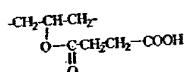
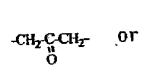
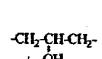
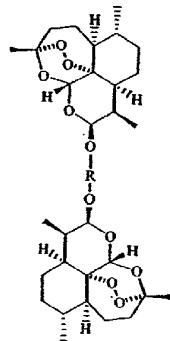


or a compound of the formula:

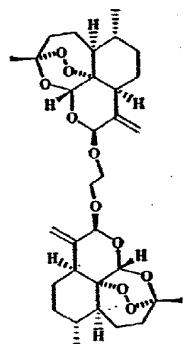


3. A compound of the formula:

where R is



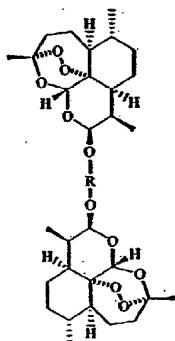
or of the formula:



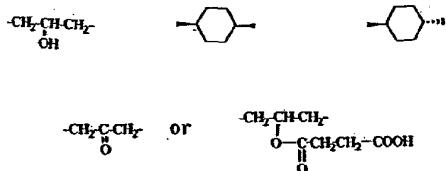
4. A pharmaceutical composition comprising at least one of the compounds according to claim 3 and pharmaceutically acceptable carrier and/or excipient.

5. A method of making compounds of the formulas of claim 3, comprising reacting dihydroartemisin or dihydroartemisitene with an appropriate optionally substituted 1, 2 or 1,3 glycol or dihydroxyacetone under acidic conditions in the presence of boron trifluoride etherate.

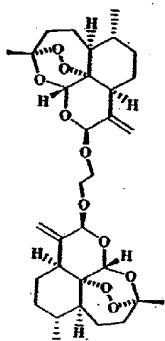
6. Use of at least one compound of the formula:



where R is

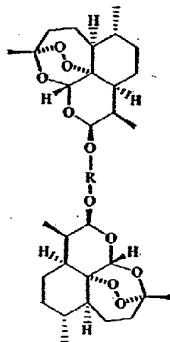


or a compound of the formula:



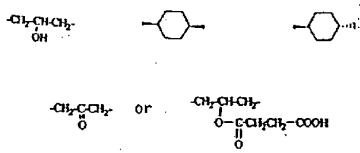
in the manufacture of a medicament for the treatment of cancer.

7. Use of at least one compound of the formula:

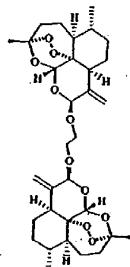


2003282543 12 Feb 2009

where R is



or a compound of the formula:



5

in the manufacture of a medicament for the treatment of a protozoal infection.

8. A method according to claim 1, substantially as hereinbefore described with reference to any one of the Examples 1-17 and Figures 1-6.

0

9. A method according to claim 2, substantially as hereinbefore described with reference to any one of the Examples 1-14 and 18 and Figures 7-9.

10. A compound according to claim 3, substantially as hereinbefore described with reference to any one of the Examples or the Figures.

15 11. A use according to claim 6, substantially as hereinbefore described with reference to any one of the Examples 1-17 and Figures 1-6.

20 12. A use according to claim 7, substantially as hereinbefore described with reference to any one of the Examples 1-14 and 18 and Figures 7-9.

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Fig. 1

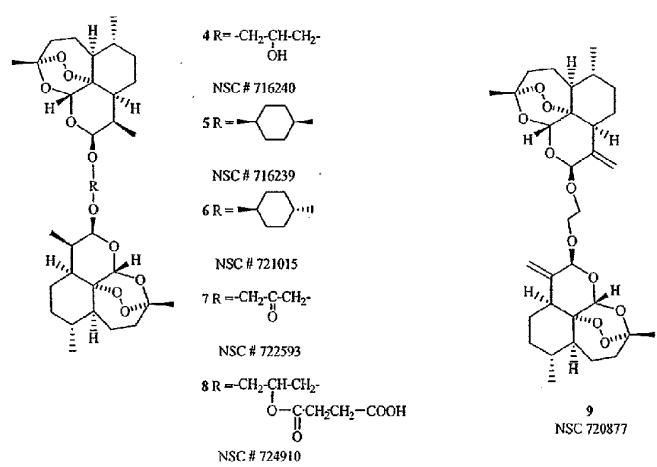
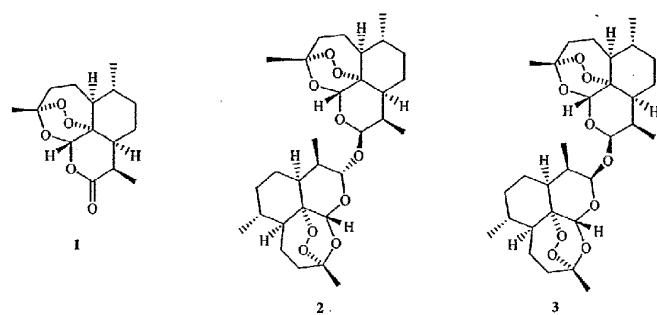


Table 1. Results of *in vitro* anti-cancer testing of compounds 4-9

Compound	4	5	6	7	8	9
NSC #	716240	716239	721015	722593	724910	720877
Panel / Cell Line						
Leukemia						
CCRF-CEM	0.104	<0.005	<0.01		<0.01	0.098
HL-60 (TB)	0.062	0.017	0.032	0.01	<0.01	0.098
K-562	0.06	0.014	0.041	0.01	<.01	0.050
MOLT-4	0.298	0.02	0.1	0.01	0.98	0.120
RPMI-8226	0.024	<0.005	<0.01	0.01		0.013
SR	0.178	0.014	0.306	0.01		0.028
Non-Small Cell Lung Cancer						
A549/ATCC	0.505	0.02	0.0188	<0.01	13.6	5.370
EK-VX	0.307	0.024	0.0277		19.5	0.479
HOP-62	13.3	12.3		11.5	11.3	6.918
HOP-92	0.384	0.017		0.18	0.08	0.741
NCI-H226	61.7	0.347	0.0247	<0.01	18.1	
NCI-H23	0.135	0.022	0.0247	0.01	<0.01	1.072
NCI-H322M	62.5	16.6	0.67	8.42	10.3	11.220
NCI-H460	----	0.017	0.028	<0.01	<0.01	5.495
NCI-H522	0.52	0.021	0.036	0.01		0.398
Colon Cancer						
COLO-205	0.04	0.007	<0.01	0.01		0.123
HCC-2998	7.02	0.018	0.018			6.026
HCT-116	0.049	0.016	<0.01	0.01	<0.01	0.027
HCT-15	0.027	0.014	<0.01	0.01	<0.01	0.012
HT29	0.268	0.026	0.011	0.01	<0.01	0.043
KM12	0.166	0.012	0.011	0.01	<0.01	0.093
SW-620	0.099	0.012	0.024	0.01		0.062
CNS Cancer						
SF-268	13.7	0.034	0.11	11.1	<0.01	6.310
SF-295	9.52	0.028	0.061	2.93		7.586
SF-539	8.64	0.02	0.092		8.3	5.370
SNB-19	27.6	0.099	0.37	0.0213		10.965
SNB-75	8.25	0.03	0.2	6.01	11.7	
U251	----	0.019	0.016	<0.01	<0.01	0.288

Fig. 2A

Table 1, Contd

Compound	4	5	6	7	8	9
NSC #	716240	716239	721015	722593	724910	720877
Panel / Cell Line						
Melanoma						
LOXIMVI	0.11	0.017	0.013	0.01	<0.01	0.204
MALME-3M	14.9	0.027	0.06	0.01	4.05	7.244
M14	8.82	0.044	0.07	0.01	3.42	3.162
SK-MEL-2	14.2	0.024	0.3	0.031	15.5	6.026
SK-MEL-28	9.39	0.085	98.8	11.7	0.501	
SK-MEL-5			0.49	<0.010		
UACC-257	0.257	0.027	0.074	<0.010	11.0	0.479
UACC-62	0.296	0.022	0.045	<0.010		7.244
Ovarian Cancer						
IGROVI	13.1	6.75	0.055	1.74	14.3	0.617
OVCAR-3	0.171	0.015	0.067	<0.010		0.107
OVCAR-4	0.209	0.024	0.053	0.069		0.072
OVCAR-5	17.7	0.152	0.042	<0.010	0.01	5.012
OVCAR-8	0.252	0.019	0.057	<0.010	17.7	0.372
SK-OV-3	21.4	0.034	0.166			10.965
Renal Cancer						
786-0	----	0.031	0.027	<0.010	<0.01	0.347
A498	15.8	0.04	0.37	14.3		50.119
ACHN	----	0.027	0.042	<0.010		6.310
CAKI-1	0.458	0.037	0.33	0.025	0.01	1.778
RXF 393	0.213	0.018	0.29	15.4	<0.01	6.457
SN12C	0.303	0.025	0.097	<0.010	8.82	7.244
TK-10	14.1	0.029	0.12	<0.010	<0.01	0.219
UO-31	0.127	0.018	0.12	<0.010	6.95	0.182
Prostate Cancer						
PC-3	0.0354	0.0105	<0.01	<0.010	<0.01	0.063
DU-145	13.8	0.028	0.34	11.2		7.586
Breast Cancer						
MCF7	0.095	0.0158	0.042	<0.010	<0.01	0.148
NCI/ADR-RES	0.34	0.077	0.13	<0.010	0.29	0.537
MDA-MB-231/ATCC	45.8	0.092	0.52	<0.010	0.01	13.183
HS 578T	12.5	0.049	0.52		17.5	0.074
MDA-MB-435	----	0.028	0.07	<0.010	<0.01	0.447
MDA-N	0.368	0.024	0.077			0.513
BT-549	0.408	0.042		0.05	19.4	7.586
T-47D	0.027	<0.005	<0.01	<0.010		0.014

Fig. 2B

Table 2. Results of *in vitro* anti-cancer testing of compounds 4-9
TGI (uM)

Compound NSC #	4 716240	5 716239	6 715015	7 722593	8 724910	9 720877
Panel / Cell Line						
Leukemia						
CCRF-CEM	18.3	8.32	>100	<0.01	>100	8.318
HL-60 (TB)	0.18	0.03	0.44	12.4		12.832
K-562	>62.5	7.67	>100	12.1	>100	11.222
MOLT-4	>62.5	9.24	>100	<0.01	>100	11.220
RPMI-8226	11.2	0.276	0.024	<0.01		0.234
SR	6.96	0.762	58.6	4.4		8.318
Non-Small Cell Lung Cancer						
A549/ATCC	19.3	>50	>100	17.4	57.6	16.218
EKVVX	>62.5	15.4			72.4	9.772
HOP-62	>62.5	>50		24.0	26.0	14.454
HOP-92	42.2	---		22.9	21.2	13.490
NCI-H226	>62.5	27	38.9	14.8	35.0	
NCI-H23	>62.5	>50	>100	21.2	12.1	25.704
NCI-H322M	>62.5	>50	>100	24.7	37.9	32.359
NCI-H460	27.1	26.1	>100	17.0	16.5	13.490
NCI-H522	>62.5	18.5	0.41	16.1	37.5	10.715
Colon cancer						
COLO 205	0.21	0.016	0.012	<0.01		0.501
HCC-2998	19.3	8.86				13.183
HCT-116	>62.5	8.3	0.042	14.2	15.6	8.710
HCT-15	>62.5	>50	0.054	<0.01	10.3	7.413
HT29	>62.5	>50	>100	20.3	>100	8.913
KM12	>62.5	18.6	>100	12.5	<0.01	12.303
SW-620	13.4	>50	>100	16.3		21.878
CNS Cancer						
SF-268	>62.5	>50	>100	36.6	20.7	17.378
SF-295	>62.5	>50	>100	20.9		21.878
SF-539	19.1	13.8	>100	25.3	38.6	11.482
SNB-19	>62.5	>50	>100	18		50.119
SNB-75	>62.5	42	>100	39.2	35.3	
U251	49.2	>50	>100	17.7	20.2	11.749

Fig. 3A

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Table 2, cont

Compound	4	5	6	7	8	9
NSC #	716240	716239	721015	722593	724910	720877
Panel / Cell Line						
Melanoma						
LOXIMVI	2.77	2.25	0.099	<0.01		
MALME-3M	>62.5	>50	>100	12.8	10.4	14.791
M14	>62.5	>50	>70.6	15.3	26.9	16.218
SK-MEL-2	>62.5	26.2	2.51	18.6	>100	17.783
SK-MEL-28	>62.5	>50	>100	28.7	>100	13.490
SK-MEL-5	>62.5	0.56	12.7	15.6		21.380
UACC-257	>62.5	21.7	>100	13.6	53.2	22.909
UACC-62	>62.5	11.9	>100	14.8		14.791
Ovarian Cancer						
IGROVI	>62.5	>50	27.4	7.59	>100	2.951
OVCAR-3	20.1	6.15	3.3	17.4		10.233
OVCAR-4	>62.5	>50	>100	27.4		1.288
OVCAR-5	>62.5	>50	>100	24.2	13.4	14.454
OVCAR-8	>62.5	>50	0.242	0.128	63.5	11.220
SK-OV-3	>62.5	>50	>100	23.5		37.154
Renal Cancer						
786-0	16.5	>50	0.452	15	18.5	9.333
A498	>62.5	>50	>100	30.1		50.119
ACHN	>62.5	15.8	>100	11.6		12.589
CAKI-1	48.8	7.7	97.3	13.2	0.66	14.791
RXF393	24.8	0.44	>100	34.1	0.21	16.982
SN12C	16	9.37	>100	<0.01	25.7	1.445
TK-10	>62.5	>50	>100		21.2	21.878
UO-31	1.31	0.75	25.8	1.94	>100	0.912
Prostate Cancer						
PC-3	>62.5	>50	>100	13.2	15.2	31.623
DU-145	>62.5	23.6	>100	23.3		15.488
MCF7	36.8	10.8	>100	22.6	20.6	42.658
NCI/ADR-RES	>62.5	>50	>100	16.6	16.8	8.913
MDA-MB-231/ATCC	>62.5	>50	>100	3.27		24.547
HS 578T	>62.5	>50	>100	19	48.5	9.333
MDA-MB-435	>62.5	23.9	>100	12.3	14.4	12.023
MDA-N	>62.5	>50	>100			13.490
BT-549	>62.5	>50	>100	22.3	40.7	26.303
T-47D	>62.5	0.038	0.091	<0.01		21.878

Fig. 3B

Table 3. Results of *in vitro* anti-cancer testing of compounds 4-9
LC50 (μM)

Compound NSC #	4 716240	5 716239	6 721015	7 722593	8 724910	9 720877
Panel / Cell Line						
Leukemia						
CCRF-CEM	>62.5	>50.0	>100	19.1	>100	41.687
HL-60 (TB)	0.35 ¹	0.17	>100	96.2	>100	50.119
K-562	>62.5	>50.0	>100	90.9	>100	32.359
MOLT-4	>62.5	>50.0	>100	5.2	>100	45.709
RPMMI-8226	>62.5	>50.0	58.8	20.7		26.915
SR	>62.5	45.4	>100	33.7		38.019
Non-Small Cell Lung Cancer						
A549/ATCC	>62.5	>50.0	>100	54.6	>100	48.978
EKVVX	>62.5	>50.0	>100		>100	25.704
HOP-62	>62.5	>50.0	>100	50.0	59.5	29.512
HOP-92	>62.5	>50.0	>100	67.7	71.0	50.119
NCI-H226	>62.5	>50.0	>100	51.4	67.9	
NCI-H23	>62.5	>50.0	>100	66.8	37.1	50.119
NCI-H322M	>62.5	>50.0	>100	65.0	>100	50.119
NCI-H460	>62.5	>50.0	>100	65.7	50.1	33.113
NCI-H522	>62.5	>50.0	>100	62.1	>100	26.915
Colon cancer						
COLO 205	----	0.035	>100	<0.01		7.244
HTCC-2998	53.2	>50.0	72.1			28.840
HCT-116	>62.5	44.2	>100	45.8	40.4	21.380
HCT-15	>62.5	>50.0	>100	23.1	40.2	19.953
HT29	>62.5	>50.0	>100	>100.0	>100	21.380
KM12	>62.5	>50.0	>100	43.7	13.2	36.308
SW-620	>62.5	>50.0	>100	44.0		50.119
CNS Cancer						
SF-268	>62.5	>50.0	>100	>100.0	69.3	47.863
SF-295	>62.5	>50.0	>100	48.2		50.119
SF-539	42.3	>50.0	>100	55.9	>100	24.547
SNB-19	>62.5	>50.0	>100	42.4		50.119
SNB-75	>62.5	>50.0	>100	>100.0	>100	
U251	>62.5	>50.0	>100	42.1	48.1	27.542

Fig. 4A

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Table 3, Cont
LC50 (uM)

Compound	4	5	6	7	8	9
NSC #	716240	716239	721015	722593	724910	720877
Panel / Cell Line						
Melanoma						
LOXIMVI	>62.5	27.1	>100			71.1
MALME-3M	>62.5	>50	>100	57.0	79.5	35.481
M14	>62.5	>50	>100	73.2	>100	50.119
SK-MEL-2	>62.5	>50	>100	70.1	>100	29.512
SK-MEL-28	>62.5	>50	>100	39.5		50.119
SK-MEL-5	>62.5	15.6	>100	40.7	>100	50.119
UACC-257	>62.5	>50	>100	60.9		50.119
UACC-62	>62.5	>50	>100	60.9		30.903
Ovarian Cancer						
IGROV1	>62.5	>50	>100	33.7	>100	13.803
OVCAR-3	>62.5	32.5	>100	43.8		36.308
OVCAR-4	>62.5	>50	>100	>100.0		26.303
OVCAR-5	>62.5	>50	>100	74.8	48.0	40.738
OVCAR-8	>62.5	>50	>100	38.8	>100	35.481
SK-OV-3	>62.5	>50	>100	55.4		50.119
Renal Cancer						
786-0	43.2	>50	>100	39.3	55.8	22.387
A498	>62.5	>50	>100	63.4		50.119
ACHN	>62.5	>50	>100	34.1		25.119
CAKI-1	>62.5	32	>100	37.4	23.2	50.119
RXF 393	>62.5	33.6	>100	75.7	21.8	44.668
SN12C	47	>50	>100	28.2	68.4	28.840
TK-10	>62.5	>50	>100	34.4	>100	50.119
UO-31	11.3	13.8	>100	8.08	>100	2.692
Prostate Cancer						
PC-3	>62.5	>50	>100	63.5	79.3	50.119
DU-145	>62.5	>50	>100	48.5		31.623
Breast Cancer						
MCF7	>62.5	>50	>100	>100.0	>100	50.119
NCI/ADR-RES	>62.5	>50	>100	68.1	60.5	26.915
MDA-MB-231/ATCC	>62.5	>50	>100	27.6	39.8	46.774
HS578T	>62.5	>50	>100	78.7	>100	50.119
MDA-MB-435	>62.5	>50	>100	37.7	42.3	34.674
MDA-N	>62.5	>50	>100			43.652
BT-549	>62.5	>50		57.0	85.6	50.119
T-47D	>62.5	>50	>100	36.1		50.119

Fig. 4B

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Table 4. *In vivo* Hollow Fiber Assay Scores for Compounds 4, 5, and 6

Compound	NSC Number	IP Score	SC Score	Total Score	Cell Kill
4	716240	12	2	14	Y
5	716239	26	10	36	N
6	721015	18	6	24	N

Fig. 5

Table 5. *In Vitro* Anti-Angiogenesis Activity of Compounds 4, 5, 7 and 8

Compound	NSC No.	IC ₅₀ (UM)		
		Growth Inhibition Assay	Cord Formation	Chemotaxis
4	716240	0.077	30.79	1.778
5	716239	0.042	38.75	3.33
7	722593	0.057	>50	28.5
8	724910	0.056	45.8	45.3
Control	Taxol	0.00165	0.05	0.1
Control	TNP 470	0.00316	1.00	0.5

Fig. 6

Table 6. Anti-malarial activity of Compounds 2-6 and 9.

Compound	<i>P. falciparum</i> (D6 Clone)		<i>P. falciparum</i> (W2 Clone)		Cytotoxicity (Vero) TC 50 (ng/mL)
	IC 50 (ng/mL)	S.i.	IC 50 (ng/mL)	S.i.	
Artemisinin (1)*	6-10	>24	6-10	>24	NC
2*	13	>37	14	>34	NC
3*	6.5	>73	3.8	>125	NC
4	1.0	>47.6	0.74	>64.3	NC
5	19	>25.0	13.0	>37.0	NC
6	24.0	19.9	30.0	>15.9	NC
9	11	>43	3.0	>159	NC

*Previously published compounds, included for the purpose of comparison

IC50 = Concentration causing death to 50% of the cells

S.I. = Selectivity index (IC50 vero cells/IC50 *Plasmodium falciparum*)

TC50 = Concentration toxic to 50% of the cells

NC = Not cytotoxic at highest concentration tested

Highest concentration tested for compounds of this invention = 476 ng/mL.

Fig. 7

Table 7. Anti-malarial Activity of Compounds 4-7

Compound	<i>Plasmodium falciparum</i> (D6 Clone)		<i>Plasmodium falciparum</i> (W2 Clone)		Cytotoxicity (Vero)
	IC50 (ng/mL)	S.I.	IC50 (ng/mL)	S.I.	
4	0.2	>23,800	0.74	>6,430	>4,760
5	3.0	>1,590	5.2	>920	>4,760
6	3.5	>1,360	8.6	>553	>4,760
7	0.7	>7,000	0.2	>23,800	>4,760
8	1.0	ND	1.5	ND	ND
Chloroquine	9.5		>238		
Artemisinin	2.8		4.5		

IC50 = Concentration causing death to 50% of the cells.

S.I. = Selectivity index (IC50 vero cells/IC50 *Plasmodium falciparum*)

TC50 = Concentration toxic to 50% of the cells

Highest concentration tested for compounds of this invention = 4,760 ng/mL.

ND = Not Determined

Fig. 8

Table 8. Anti-Leishmanial Activity of Compounds 4-7

Compound Number	IC50 (ug/L)	IC90 (ug/mL)
4	3.1	10
5	6.5	>50
6	5.0	>50
7	4.8	12
Pentamidine	1.18	5
Amphotericin B	0.08	0.14

IC50 and IC90 are the compound concentrations that kill 50% and 90% of the cells, respectively, compared to solvent controls.

Highest concentration tested of compounds of this invention = 50 ug/mL.

Fig. 9