Structure–Activity Relationships of the Antimalarial Agent Artemisinin 10. Synthesis and Antimalarial Activity of Enantiomers of rac-5β-Hydroxy-D-Secoartemisinin and Analogs: Implications Regarding the Mechanism of Action

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Abstract: An efficient synthesis of rac-6-desmethyl-5β-hydroxy-D-secoartemisinin 2, a tricyclic analog of R-(+)-artemisinin 1, was accomplished and the racemate was resolved into the (+)-2b and (−)-2a enantiomers via their Mosher Ester diastereomers. Antimalarial activity resided with only the artemisinin-like enantiomer R-(−)-2a. Several new compounds 9–16, 19a, 19b, 22 and 29 were synthesized from rac-2 but the C-5 secondary hydroxyl group was surprisingly unreactive. For example, the formation of carbamates and Mitsunobu reactions were unsuccessful. In order to assess the unusual reactivity of 2, a single crystal X-ray crystallographic analysis revealed a close intramolecular hydrogen bond from the C-5 alcohol to the oxepane ether oxygen (O-11). All products were tested in vitro against the W-2 and D-6 strains of Plasmodium falciparum. Several of the analogs had moderate activity in comparison to the natural product 1. Iron (II) bromide-promoted rearrangement of 2 gave, in 50% yield, the ring-contracted tetrahydrofuran 22, while the 5-ketone 15 provided a monocyclic methyl ketone 29 (50%). Neither 22 nor 29 possessed in vitro antimalarial activity. These results have implications in regard to the antimalarial mechanism of action of artemisinin.

Keywords: artemisinin; racemate; enantiomers; Mosher ester; diastereomers; Plasmodium falciparum; iron

1. Introduction

Malaria continues to be one of the most lethal parasitic diseases due to its prevalence in remote, economically challenged regions about the tropical belt. Historically, natural products have played a crucial role in the pharmacotherapy of this disease. The emergence and spread of malarial resistance to conventional antimalarials has been a cause for great concern, but the discovery by Chinese researchers of qinghaosu, the active ingredient of the medicinal herb qinghao (Artemisia annua) over four decades ago, was both outstanding and timely. Now referred to as Artemisinin (1), this tetracyclic endoperoxylactone (Figure 1) has nanomolar potency against drug-resistant strains of P. falciparum such as the W2-Indochina (chloroquine resistant) and D6-Sierra Leone (mefloquine resistant) clones.
The unusual tetracyclic structure of 1 containing a peroxidic 1,2,4-trioxane moiety is known to play an essential role in the antimalarial mechanism of action of artemisinin [1]. Interaction of the endoperoxide moiety with heme, or free Fe (II) leads to the formation of free radical intermediates [2–11]. The importance of these free radicals is still not fully understood but is widely believed to result in parasitic death [12,13]. The mechanism of action of peroxides towards Plasmodium falciparum is a matter of debate with general acceptance that ferrous iron is required to generate carbon free radicals, but a metal-free mechanism has been suggested [14]. The fate of the carbon radicals formed by Fe(II) promoted fragmentation of the artemisinin peroxide is complicated by intermolecular vs. intramolecular processes where clearly not every peroxide-Fe(II) reaction leads to parasite death. Heme-artemisinin adducts have been detected in malaria infected mice and a covalent adduct of manganoporphyrin with a fragmented carbon radical from artemisinin has been reported [15]. The interactions of artemisinin or peroxides with achiral radical promoters (i.e., Fe(II), heme) should be independent of peroxide chirality. A sample of simplified peroxide enantiomers was shown to have equal potency in vitro [16,17]. Interestingly, artemisinin has been shown to form a thioether adduct with cysteine in the presence of Fe(II), ex vivo [3,18–20]. More recently, protein targets for the antimalarial effects of artemisinin have been reported [21] and the calcium channel SERCA, pfATP6, has been shown to be a target of artemisinin [22] as has been the translationally controlled tumor protein pfTCTP [23–25]. Both protein targets have been the subject of protein–drug computational modeling, neither have bound Fe(II), and pfTCTP has been shown to form a covalent adduct with artemisinin.

Due to the undesired physiochemical properties of artemisinin, scientists around the globe over the past four decades have established different methodologies to synthesize artemisinin derivatives at different positions (C-3, 6, 7, 9, 10, O-11, O-13 and 16) of the artemisinin skeleton [26–39]. A number of partial analogs substituted at C-4 have been subjected to SAR [40] but the C-5 position has remained synthetically elusive due to the absence of accessible functional groups at or nearby these positions. In regard to total synthetic attempts in our hands, substitution of the side-chain carbon ultimately becoming C5 was unsuccessful except with a cyano moiety [41]. Avery et al., have synthesized numerous modified analogs of artemisinin which include C-13 carbon, N-11 derivatives, C-3 (alkyl, arylalkyl and carboxyalkyl) analogs, C-7 (–OR), C-9 and corresponding 10-deoxo derivatives such as 9β-16-(arylalkyl)-10-deoxoartemisinins. Many of these compounds were more active than artemisinin with improved physiochemical properties, and they present a valuable contribution towards structure–activity relationship (SAR) and quantitative structure–activity relationships (QSAR) [36,38,42–49]. The synthesis of rac-6-desmethyl-5β-hydroxy-d-secoartemisinin 2 (a 1,2,4-trioxane) afforded a starting point upon which to conduct [50] heretofore unexplored SAR about the C-5 position. Additionally, as the C-4 position plays a quintessential mechanistic role in supporting a C-4 radical, C-5 derivatives were hypothesized and synthesized in order to test the effect of stabilization of the C-4
radical. Finally, the racemic C-5 alcohol was derivatized as a mixture of two diastereomers to be separated and by bioassay, test whether chirality is important to biological activity.

2. Results

2.1. Chemistry

Synthesis of the racemic 5-alcohol proved more difficult than reported [50] and required further synthetic studies to produce gram quantities for derivatization and subsequent bioassay. The reported route to 2 was attempted but modified as follows (Scheme 1).

The reported synthesis was modified specifically as follows: ethyl acetoacetate was ketalized under Dean-Stark conditions with ethylene glycol and p-toluenesulfonic acid monohydrate (p-TsOH·H2O) in benzene with azeotropic removal of water to produce 2-carbethoxyethyl-2-methyl-dioxolane 3 [51] which was then reduced to the aldehyde 4 using diisobutylaluminum hydride (DIBAL-H). Temperature control, solvent and mixing rates were crucial to the success of this reduction.

![Scheme 1](image)

Scheme 1. Reagents and conditions: (a) DIBAL-H, −78 °C, DCM; (b) cyclohexanone, LDA, THF, −78 °C; (c) TBS-Cl, 2,6-Lutidine, DMF, at 0 °C to r.t., for 15 h; (d) NaH, Me2S+I−, DMSO, THF, (e) H2O2/ether, Na2MoO4/glycine; (f) p-TsOH, DCM, r.t.

The aldehyde underwent anti-selective aldol condensation with the lithium enolate of cyclohexanone to form the expected alcohol 5. The labile β-hydroxy group of 5 was protected as the tert-butyldimethylsilyl ether 6 which then underwent direct conversion to the spiro-epoxide 7 via the Corey–Chaykovsky reaction [52]. This labile, unexpectedly volatile epoxide was then ring opened using ethereal hydrogen peroxide and the metal catalyst [53] sodium molybdate dihydrate (Na2MoO4·2H2O)/glycine affording the unstable hydroperoxide 8. Finally, simultaneous deprotection, dehydration, desilylation and ring closure were affected readily in a one-pot reaction with p-TsOH·H2O in undried dichloromethane (DCM) to give racemic 2 as a stable, crystalline solid [50] in 32% yield (Scheme 1).

2.1.1. Ester Derivatives

In our efforts to find a suitable method for synthesis of ester derivatives of 2, 1.2 molar equivalents of N-(3-dimethylaminopropyl)-N′-ethylcarbodiimide hydrochloride (EDC) [54] were added to a stirring mixture of rac-2, 1.2 molar equivalents of valeric acid and 19 mol-% of 4-(dimethylaminopyridine) (DMAP) in dry DCM. The progress of the reaction was monitored by TLC analysis and showed slow product formation. After 4 days,
additional equivalents of all reagents were added, and the reaction mixture was stirred for another 24 h but unreacted starting material remained. The mixture was allowed to stir for 1 h at 40 °C and worked up. Compound 9 was obtained only in 11% yield as illustrated in Table 1 and unreacted starting material rac-2 was recovered. In continuation of our efforts to find a suitable method for synthesis of an ester derivative from 2, 2 molar equivalents of benzoyl chloride were added to a suspension of rac-2 and a 2 molar equivalents of both N-methylimidazole (NMI) and N,N,N′,N′-tetramethylethlenediamine (TMEDA) in dry acetonitrile (CH₃CN) at 0 °C to r.t., for 24 h [55]. It was hoped that the highly basic conditions would result in a competition of the intramolecular H-bond for the diamine. However, reaction progress as monitored by TLC analysis was minimal. When one molar equivalent of all reagents were added and the reaction mixture left to stir for 24 h, 10 was obtained in 42% yield as illustrated in Table 1. When the above condition was modified by adding 4 molar equivalents benzoyl chloride to a suspension of 2 and 4 molar equivalents of both trimethylamine (Et₃N) and DMAP in dry CH₃CN at 0 °C to r.t., for 24 h, 10 was afforded in 70% yield. When the same reaction was repeated but using 4 molar equivalents of TMEDA instead of Et₃N, the yield was improved slightly to afford 10 in 74% yield. Thus, we decided to use the protocol of 4 molar equivalents DMAP/TMEDA, which was used for the rest of the esters in Table 1.

Table 1. Various esters derived from rac-2.

| Compound | R                   | % Yield | Compound | R                   | % Yield |
|----------|---------------------|---------|----------|---------------------|---------|
| 9        | CH₃(CH₂)₂CO         | a 11    | 12       | p-MeOC₆H₄CO        | d 68    |
| 10       | C₆H₄CO             | b 42,c 70,d 74 | 13       | C₆H₄(CH₂)₂CO       | d 96    |
| 11       | p-FC₆H₄CO          | d 70    | 14       | p-ClC₆H₄CO         | d 49    |

Reagents and conditions: a 2.4 molar equivalents valeric acid, 1.7 molar equivalents EDC, 0.6 molar equivalents DMAP, dry DCM, r.t., for 96 h; b 3 molar equivalents benzoyl chloride, 3 molar equivalents N-methylimidazole, 3 molar equivalents TMEDA, dry CH₃CN at 0 °C to r.t., for 24 h; c 4 molar equivalents benzoyl chloride, 4 molar equivalents Et₃N, 4 molar equivalents DMAP, dry CH₃CN at 0 °C to r.t., for 24 h; d 4 molar equivalents benzoyl chloride, 4-fluorobenzoyl chloride, 4-methoxybenzoyl chloride, cinnamoyl chloride, 4-chlorobenzoyl chloride, 4 molar equivalents TMEDA, 4 molar equivalents DMAP, dry CH₃CN, 0 °C to r.t., for 24–72 h.

2.1.2. Ketone Derivative

Different methods were applied to oxidize rac-2 to ketone 15. A mild condition including use a combination of Oxone (potassium peroxymonosulfate) and TEMPO (2,2,6,6-tetramethylpiperidin-1-yl)oxidanyl offered an efficient catalyst system to oxidize secondary alcohol 2 to ketone 15. Two different solvents (toluene and DCM) were used to find the optimal condition for the oxidation process. Additionally, tetrabutylammonium bromide (Bu₄NBr) was added as a bromide ion source. The bromide ion was oxidized to hypobromous acid (HOBr) by Oxone and the HOBr promptly oxidizes the nitroxyl radical to the ultimate oxidant, an N-oxo ammonium ion [56]. Upon addition of 1 mol% of TEMPO and 2.2 molar equivalents of Oxone to a solution of the rac-2 and 4 mol% of Bu₄NBr in toluene, the mixture stirred at r.t., for 5 days to afford 15 in 40% yield with some unreacted alcohol 2. The yield was enhanced to 50% by changing the solvent to DCM and increasing the mol% of both TEMPO and Bu₄NBr to 10 mol% and 3 molar equivalents of Oxone. However, the reaction time was the same as before i.e., 5 days as illustrated in Table 2. To increase the yield and decrease the reaction time, and keeping in mind to retain the crucial trioxane system, we investigated the use of the well-known Cr(VI) based oxidant, pyridinium chlorochromate (PCC). When rac-2 was dissolved in DCM and added to a
suspension of 1.5 molar equivalents of PCC in DCM at r.t., after 48 h, 15 was obtained in 55% yield [57].

**Table 2.** Methods used for oxidation of alcohol 2.

| Compound | Method | % Yield |
|----------|--------|---------|
| 15       | a      | 40      |
|          | b      | 50      |
|          | c      | 55      |

Reagents and conditions: a: 1 mol% TEMPO, 2.2 molar equivalents Oxone, 4 mol% Bu4NBr, toluene, r.t., for 5 days; b: 10 mol% TEMPO, 2.2 molar equivalents Oxone, 10 mol% Bu4NBr, DCM, r.t., for 5 days; c: 1.5 molar equivalents PCC, DCM, r.t., for 48 h.

2.1.3. Exomethylene Derivative

Using an excess of both base and phosphonium salt was successful in converting 15 to a methylene olefin but was not the desired 16 [58]. Upon the addition of a solution of 15 to a mixture of 9.5 molar equivalents of both potassium bis(trimethylsilyl)amide (KHMD) and methyltriphenylphosphonium bromide CH3P(C6H5)3Br in dry THF at 0 °C to r.t., for 12 h, an olefin hoped to be 16 was obtained in 24% (Scheme 2).

Scheme 2. Reagents and conditions: a: 9.5 molar equivalents KHMD, 9.5 molar equivalents CH3P(C6H5)3Br, dry THF, 0 °C to r.t., for 12 h.

Tebbe olefination [59] was unsuccessful in converting 15 to **exo**-olefin 16; instead, decomposed fractions were obtained. In retrospect, it was hoped that using a transition metal based reagent would occur more rapidly than radical decomposition of 15/18 but this was not the case.

Attempted Wittig reaction of ketone 15 led to enolization and beta-elimination. The hydroperoxy group could fragment to the alcohol 18x which added back to the enone (Scheme 3) and the nonperoxy ketone 15x could then undergo eventual olefination leading to 16x. Partial structures having a methylene moiety were also seen along with the product 16x, but none were peroxidic.

Scheme 3. Wittig reaction of ketone 15 with excess base.
2.1.4. Separation of the Two Enantiomers 2a and 2b

Previous studies from the literature of separate enantiomeric peroxides suggested that chirality was unimportant for bioactivity [16,17]. It was felt that this could not be true in all cases because clear bioorganic and biological evidence for the intermediacy of protein receptors exists [21–25]. Separating the two enantiomers of the artemisinin-like rac-5-alcohol into (3R, 5R, 5aR, 9aS) and (3S, 5S, 5aS, 9aR) diastereomers led to the discovery that most if not all of the antimalarial activity was associated with the (3R, 5R, 5aR, 9aS) diastereomer. This diastereomer overlays with the X-ray structure of the natural product, R(+)-artemisinin (the 3R trioxane).

Mosher Ester Analysis

Enantiopure (R)-(−)-α-methoxy-α-(trifluoromethyl)phenylacetyl chloride (R)-(−)-MTPA-Cl or Mosher’s acid chloride was used as a chiral derivatizing agent in order to create diastereomeric, alpha-methoxy-alpha-trifluoromethylphenylacetic acid (MTPA) esters 19a and 19b upon reaction with rac-2. These diastereomers were separated by silica gel column chromatography whereupon basic hydrolysis was applied to 19a and 19b to obtain the enantiopure 2a and 2b, respectively. In addition, optical activity of 2a and 2b were compared. Addition of 2 molar equivalents of (R)-(−)-MTPA-Cl to a stirring suspension of rac-2 and 3 molar equivalents of both DMAP and TMEDA in dry CH$_3$CN [54] at 0 °C to r.t., for 72 h was examined by TLC (silica gel with 20% EtOAc/hexane solvent and p-anisaldehyde stain), and showed reaction completion. Two separable spots of high $R_f$ (0.63) and low $R_f$ (0.58) of 19b and 19a was observed in 19% and 22%, respectively.

Hydrolysis of the two Mosher’s esters 19a and 19b occurred smoothly without destruction of the essential trioxane system. They were treated with a solution of 1N sodium hydroxide (NaOH) in methanol (MeOH) [59] at ambient temperature for 36 h, furnishing 2a and 2b in 27 and 24% yield, respectively (Scheme 4). Furthermore, the specific rotations, [α]D$^20$ were calculated for 2a and 2b and found to be -100.5° and +100.5°, respectively.

![Scheme 4](image_url)

**Scheme 4.** Reagents and conditions: (a) 2 molar equivalents (R)-(−)-MTPA-Cl, 3 molar equivalents DMAP, CH$_3$CN 0 °C to r.t., for 72 h; (b) 1N NaOH/MeOH for 36 h.

2.1.5. Rearrangement Chemistry

Within the malaria parasite, heme Fe (II) (reduced hemin) or other sources of ferrous iron can induce chemical decomposition of artemisinin and related tricyclic trioxanes (e.g., rac-2, 15) to generate an oxy radical that subsequently rearranges into distinctive carbon-centered radical species (e.g., 20 and 21; Schemes 5 and 6). Using FeBr$_2$ as a mimic of heme iron(II), we performed ferrous-mediated degradation of racemic 2 and 15. When Fe(II) associated with oxygen 1, an oxy radical 20 was formed and rearrangement occurs to provide a primary carbon-centered radical 21 via a C$_3$-C$_4$ scission process. This interme-
mediate radical species then undergoes ring-contraction to the tetrahydrofuran product 22 (Scheme 5). As mentioned earlier, this reactive intermediate (e.g., 21) could be responsible for alkylation of biomacromolecules such as heme, specific proteins, and other targets that cause the death of malaria parasites.

Scheme 5. Reagents and conditions: a) 2 molar equivalents FeBr₂, THF, r.t., for 2 h.

Upon treatment rac-2 at ambient temperature in THF with 2 equivalents of FeBr₂ for 2 h [45], 22 was isolated by flash chromatograph in 47% yield as the only tractable product from rearrangement of rac-2. Assignment of a structure to 22 was based on spectral evidence; it showed in its IR spectra strong absorption bands at 3394 and 1740 cm⁻¹ corresponding to hydroxyl and acetyl groups, respectively. In the NMR, the presence of a methyl of the acetyl group was clearly indicated as a singlet at δ 2.11. In addition, HMBC and HSQC experiments confirmed the connectivity presented for compound 22. For example, the C-8 protons at δ 4.62 (dd, J = 1.5, 12.3 Hz, 1H, CH₂₃-8), and 4.26 (d, J = 12.3 Hz, 1H, CH₂₅-8), and the methyl at δ 2.11 (s, 3H, CH₃11), showed strong HMBC correlations to C-10, a carbonyl assigned to δ 171.8. Furthermore, the quaternary carbon at δ 81.8 showed strong HMBC correlations with the protons at CH-3, CH₂-8 and CH₂-2, confirming the THF ring in the molecule 22. The nuclear Overhauser effect spectroscopy (NOESY) experiment showed a correlation between the protons at CH-3 and CH-3a indicating a cis configuration between these two protons.

When ketone 15 was treated with 2 molar equivalents of FeBr₂ in THF, for 2 h, 29 was isolated by flash chromatograph in 50% yield as the only tractable product from the rearrangement of 15. Plausible mechanisms for generation of 29 are illustrated in paths (a) and
(b). Association of Fe (II) with oxygen-1 leads to an oxy radical that scissions to the primary carbon-centered radical 23. The stabilized enol radical 23 abstracts a hydrogen atom from THF and generates 24 and 25. Involvement of a trace of water (path a) leads to protonated (tetrahydrofuran-2-yl)oxonium ion 26 that then quenches the Fe(II)oxo intermediate 27 and generates 2-hydroxytetrahydrofuran 28 and the (2-acetyl-1-hydroxycyclohexyl)methyl acetate 29, respectively. In case of truly anhydrous THF, 24 could lose a proton (path b) to protonate the Fe(II)oxo intermediate 27 thus generating 29 and 2,3-dihydrofuran 30 (Scheme 6).

Product 29 showed strong hydroxyl and carbonyl absorption in the IR at 3452, 1740 and 1705 cm\(^{-1}\) and was confirmed in the proton NMR spectra which indicated the presence of an acetyl group at \(\delta 2.08\) and methyl ketone at \(\delta 2.23\). In addition, HMBC and HSQC experiments confirmed the connectivity shown for compound 29. For instance, the C-7 protons at \(\delta 4.24\) (d, \(J = 12.0\) Hz, 1H, CH\(_{2}\)-7), and 4.18 (d, \(J = 12.0\) Hz, 1H, CH\(_{2}\)-7), and the methyl at \(\delta 2.08\) (s, 3H, CH\(_{3}\)10), showed a strong HMBC correlations to C-9, a carbonyl assigned to \(\delta 171.8\). The C-2 carbon at \(\delta 56.4\) showed strong correlations with the protons at CH\(_{2}\)-7 and the methyl signal at \(\delta 2.23\) (s, 3H, CH\(_{3}\)12) confirming the presence of the methyl ketone. Unfortunately, the 5-exomethylene derivative 16 could not be prepared for this study but may have given a similar result to 15.

2.2. Antimalarial Activity

Artemisinin 1 and the analogs rac-2, 2a, 2b, 9-16, diastereomers 19a, 19b, 22 and 29 were tested in vitro in parasitized whole blood against drug-resistant strains of \(P. falciparum\) parasite clones D-6 and W-2 at The National Center for Natural Products Research (NCNPR) at The University of Mississippi using the parasite lactate dehydrogenase (pLDH) assay developed by Makler (Table 3) [60–62]. This assay is based on the ability of the pLDH enzyme of \(P. falciparum\) to reduce APAD to APADH. This reaction is carried out at a slow rate by human red blood cell LDH.

Table 3. In vitro antimalarial activity of the synthesized compounds against \(P. falciparum\).

| Structure | R       | IC\(_{50}\) (ng/mL) | Relative Activity \(\times 100\) |
|-----------|---------|---------------------|---------------------------------|
|           | D-6     | W-2     | D-6     | W-2     | anal. (C, H) |
| 1         | (Artemisinin) | 7.05    | 5.08    | 100     | 100         |
| rac-2     | H       | 1716.22 | 1778.35 | 0.6     | 0.43        |
| 2a        | H       | 1255.56 | 884.89  | 0.43    | 0.44        |
| 2b        | H       | NA      | NA      | NA      | NA          |
| 9         | CH\(_3\)(CH\(_2\))CO | 149.19 | 208.86  | 5.00    | 2.60        |
| 10        | C\(_6\)H\(_4\)CO | 152.81 | 120.97  | 10.40   | 9.50        |
| 11        | p-FC\(_6\)H\(_4\)CO | 80.72  | 97.54   | 20.81   | 12.41       |
| 12        | p-MeOC\(_6\)H\(_4\)CO | 114.96 | 167.22  | 15.13   | 7.50        |
| 13        | C\(_6\)H\(_2\)(CH\(_2\))CO | 130.87 | 148.09  | 13.14   | 8.37        |
| 14        | p-CIC\(_6\)H\(_4\)CO | 144.65 | 119.95  | 12.18   | 10.58       |
| 15        | Ketone  | 31.83   | 23.34   | 33.30   | 32.72       |
| 16        | Exomethylene | 344.84 | 346.31  | 3.05    | 2.18        |
| 19a       | C\(_6\)H\(_2\)(OCH\(_2\))(CF\(_3\))CO | 150.64 | 357.24  | 7.13    | 2.17        |
| 19b       | C\(_6\)H\(_2\)(OCH\(_2\))(CF\(_3\))CO | 2169.31 | 2255.40 | 0.50    | 0.34        |
| 22        | Scheme 5 | NA      | NA      | NA      | NA          |
| 29        | Scheme 6 | NA      | NA      | NA      | NA          |

\(\text{a Relative activity} = 100 \times \frac{\text{IC}_{50} \text{ (artemisinin, control value)} \text{/ IC}_{50} \text{ (analog)}}{\text{MW (analog)/MW (artemisinin)}}\). \(\text{b Not active. None of the synthesized compounds were cytotoxic against VERO cells up to} \text{15.80 \mu M.}\)
The formation of APADH was monitored colorimetrically by the addition of nitroblue tetrazolium which was reduced to a blue formazan product. Two P. falciparum malaria parasite clones, designated as Indochina (W-2) and Sierra Leone (D-6), were used in susceptibility testing. The W-2 clone is chloroquine-resistant and mefloquine-sensitive, while the D-6 clone is chloroquine-sensitive but mefloquine resistant. The relative potency values for these analogs were derived from the IC\textsubscript{50} value for artemisinin (1) and adjusted for molecular weight differences by multiplication of the ratio of the molecular weight of the analog divided by the molecular weight of artemisinin. This approach to reporting activity was based on the fact that the analogs were tested on different occasions in which the IC\textsubscript{50} for the control, artemisinin, had varied anywhere from 5 to 7 ng/mL based on parasitemia levels and clone.

2.3. X-ray Crystallography

A single crystal X-ray diffraction analysis was conducted to verify the structure of rac-2. The crystal used was not in a racemic space group and was a pure enantiomer. The racemate likely crystallized as a conglomerate, that is the (+) and (−) enantiomers formed separate crystals, from which 2b was chosen randomly. The X-ray confirms that the crystal structure of 2b is an orthorhombic (P2\textsubscript{1} 2\textsubscript{1} 2\textsubscript{1}) with cell dimensions of a = 5.7823 Å, b = 7.6135 Å, and c = 23.795 Å. The total volume of the unit cell is 1047.5 Å\textsuperscript{3}. It also confirmed that a hydrogen bond was present between H4O and O1 with a total length of 2.3 Å. In addition, a crystal structure was also generated for better visualization utilizing commercially available molecular software (Sciencomics, MAPS 3.4) and was drawn as 2b. The *cif coordinates file (generated from the X-ray analysis) was imported to molecular dynamics software and gave the atomic representation (Figure 2a). The atomic structure is represented by the following color code: hydrogen white balls, carbon is grey balls and oxygen is blue balls. The hydrogen bond is also evident as represented by the dashed line (Figure 2b).

![Figure 2. (a) (left) X-ray crystallographic Analysis of 2b; (b) (right) intramolecular hydrogen-bond in X-ray of 2b.](image_url)

3. Discussion

Antimalarial activities of the new analogues synthesized are presented in Table 3. The relative activities range from 0.62% (D-6) and 0.43% (W-2) in case of rac-2 to 33.30% (D-6) and 32.72% (W-2) in case of the ketone 15. We were not surprised to find that enantiopure (−)-2a was responsible for the antimalarial activity with relative activities of 0.43% (D-6) and 0.44% (W-2), values almost the same as rac-2; while enantiopure (+)-2b was devoid of antimalarial activity. Of the ester derivatives, 4-fluoro ester 11 was the most active with relative activities of 20.81% (D-6) and 12.41% (W-2) while the enantiomer 19b was the least active with relative activities of 0.50% (D-6) and 0.34% (W-2). The Fe (II)-induced rearrangement products 22 and 29 had no antimalarial activity due to loss of the natural product-like 1,2,4-trioxane ring.
The ketone 15 and exomethylene 16 were designed taking into consideration likely rearrangement behavior relative to alcohols or esters derived from 2. We were thus pleased to find that the ketone 15 was 55–77 times more potent than the alcohol rac-2 or 2a (i.e., 2). Structures 15 and 2 have very similar overall shape due to their highly constrained tricyclic systems. The only major difference between the alcohol 2 and ketone 15 is the intramolecular H-bond in 2. While there may be an effect on the initial O-O homolytic cleavage of 2 due to the IMH-bond, relative to 15, a more important difference is likely related to the intermediate alkylating species. C-radical 21 should be less stable than C-radical 23 (Schemes 5 and 6) and furthermore, we know that radical 21 undergoes intramolecular ring closure quenching the carbon radical while carbon radical 23 is forced to react in an intermolecular sense, avoiding self-immolation. In the active site of a protein target, 23 produced from 15 should be longer-lived than 21 from 20. It might be argued that 23 is more active than 21 because 23 would have a better chance to react in an intermolecular sense than 21 and, thus, would be more likely to alkylate protein. While this is only one example of this theory, synthesis of 16 could be a laudable goal.

Analysis of 2a by DFT calculations (Jaguar, Schrödinger 21-1 release; see Figures 3 and 4) revealed the importance of the intramolecular H-bond in stabilizing the structural energy. Two structures, local minimum 2a no-IMHB, and local minimum 2a-IMHB 2-up were apparent with a ΔE of 5.1 kcal/mol. However, a global minimum revealed 2a-IMHB 2-down, identical to the X-ray structure 2b (Figures 2 and 3), was 10.4 kcal/mol more stable than 2a without an IMHB. The 2a-IMHB 2-down boat overlaps with the unstable trioxane (+)-artemisinin1down (Figure 4). The large difference between H-bonded and the non–H-bonded conformers corresponds to an exclusive intramolecular H-bond (IMHB) in 2a-IMHB-2-down. The significance of this H-bond in dictating the reactivity of rac-2 was unforeseen during its synthesis. Once its abnormal properties became apparent with rac-2 in hand, we hoped reaction solvent changes and/or additives could have effected the extent of IMHB, but we were disconcerted by the unreactive nature of rac-2 as became apparent vide supra.

Figure 3. Quantum mechanics calculations regarding the intramolecular H-bond in rac-2.
Continuing the analysis of conformational effects, we noticed that two ring-flipped variants existed for artemisinin, both trioxane rings adopting a boat conformation. One of these, the lower E variant, we referred to as O1-UP because O1 appears higher than O2. As shown in Figure 4, this is illustrated as (+)-artemisinin1UP and the local minimum (+)-artemisinin1DOWN was also obtained and it had a ∆E of 12.56 kcal/mol relative to Artemisinin1UP, the later representing the global minimum and corresponding to the conformation found in the X-ray structure of the natural product. One can see that the effect of the IMHB in 2a is to ring-flip the trioxane to 2-down with a ∆E of 10.4 kcal/mol. No other artemisinin derivatives have the “1DOWN” conformation as seen with the benzoate 10. The ketone 15, having increased rigidity, transmits conformational effects to the peroxide to effect a ring flip to 15 2-down. How this preference for a natural trioxane conformation in 2a-IMHB 2-down and ketone 15 2-down compare to unnatural 10 2-up in regards to rearrangement/radical chemistry and thence, antimalarial activity awaits further study.

Overall, these comparisons show that 2a exists in an exclusive IMH-bonded conformation by X-ray analysis and DFT calculations. Thus, the poor or absent reactivity of alcohol 2 towards many acylation partners must be related to a reticent H-bond, and ultimately inhibited a more detailed SAR/QSAR. With more 2a analogs in hand, application of QM parameterization to QSAR could reveal the effects of peroxide geometry on antimalarial activity. Several reports [63–68] described the application of different quantum mechanical approaches to studying the geometrical parameters and chemical reactivity descriptors of artemisinin. As the valence-separate basis set 6-31G** of DFT/B3LYP method showed the best results as suggested by Santos et al. [69], we implemented this technique in our calculations.

4. Materials and Methods
4.1. Chemistry
4.1.1. General Information

Optical rotations were recorded using Rudolph Research Analytical Autopol V Polarimeter. Melting points were measured on an OptiMelt® V.1061 (Stanford Research systems) instrument and were uncorrected. 1H and 13C NMR spectra were obtained on Bruker NMR spectrometers model DRX 600, DRX 500 and DRX 400 NMR spectrometers with standard pulse sequences, operating at 600, 500 and 400 MHz in 1H and 150, 125 and 100 MHz in 13C, respectively. The chemical shifts values were reported in parts per million units (ppm) from trimethylsilane (TMS) using known solvent CDC3, C6D6 chemical shifts. Coupling constants were recorded in Hertz (Hz), standard pulses were used for COSY, HSQC, HMBC, and NOESY experiments. Infrared (IR) spectra were recorded on a PerkinElmer Spectrum 100FT-IR Spectrometer. High resolution mass spectra (HRMS) were measured with a Waters Q-TOF Micromass spectrometer using the electrospray ionization (ESI) source in negative or positive mode. Flash chromatography was performed using silica gel (Whatman 60Å, 230–400 mesh). Analytical thin layer chromatography (TLC) was performed on EMD Chemical INC 25 TLC aluminum sheets, silica gel 60 F254 or GP Analtech TLC plates. All reaction solvents were purchased as HPLC grade and, where
appropriate, distilled from CaH₂ and then stored over 3 or 4 Å molecular sieves. Most commercial reagents were used without further purification unless otherwise noted in the procedure. All reagents and dry solvents were purchased from Sigma-Aldrich, Fluka, or Thermo Fisher Scientific. All round bottom flasks were dried properly in a vacuum oven prior to reactions. Solvents and reagent transfers were accomplished via dried syringes or cannulas. All reactions were performed under argon atmosphere, unless otherwise specified.

General information this: The NMR spectra and crystalographic data are available in the Supplementary Materials.

4.1.2. Preparation and Characterization of Synthesized Compounds

Ethyl 2-(2-methyl-1,3-dioxolan-2-yl) acetate (3)

A solution of ethyl acetoacetate (150.0 g, 1.15 mol), ethylene glycol (214 g, 3.44 mol), and p-TsOH·H₂O (9.70 g, 51 mmol) in 1100 mL of benzene in a 2 L round bottom flask was refluxed for 5 h with continuous azeotropic separation of H₂O via a Dean-Stark trap. The mixture was cooled to ambient temperature, concentrated to 500 mL in vacuo and then washed sequentially with sat. aq. NaHCO₃ (2 × 100 mL) and brine (100 mL), filtered over anhydrous Na₂SO₄ and evaporated in vacuo. The crude material was distilled under vacuum (20–25 mbar) at 109–110 °C to afford 3 (167.3 g, 83%) as a colorless oil; ¹H-NMR (400 MHz, CDCl₃): δ: 4.09 (q, J = 7.1 Hz, 2H), 3.91 (s, 4H), 2.59 (s, 2H), 1.43 (s, 3H), 1.20 (t, J = 7.1 Hz, 3H) ppm; ¹³C-NMR (100 MHz, CDCl₃): δ: 169.3, 107.5, 64.7, 60.4, 44.1, 24.4, 14.1 ppm; IR (neat): 2986, 2889, 1721, 1380, 1293, 1148, 1115, 1050, 948, 863 cm⁻¹; ESI-HRMS: calcd for C₆H₁₂O₄ [M + H]+: 175.0900, found: 175.0973.

2-(2-Methyl-1,3-dioxolan-2-yl)acetaldehyde (4)

To a 1000 mL 2-necked round-bottomed flask equipped with argon line and septa dry DCM (250 mL) and the ester 3 (55.09 g, 0.32 mol) were added. The mixture was vigorously stirred at −78 °C for 15 min, whereupon DIBAL-H (57.92 g, 0.41 mol, 1.0 M solution in toluene) was added dropwise via cannula and the reaction mixture was then stirred at −78 °C for 5 h. The reaction was checked by TLC for completion, and then quenched with MeOH (40 mL). The mixture was allowed to warm to ambient temperature and stirred for 40 min. A saturated aqueous solution of Rochelle salt (200 mL) was added and the reaction mixture was stirred overnight. The aqueous layer was separated and extracted with DCM (3 × 120 mL). The combined organic layers were washed with brine (2 × 150 mL) and dried over anhydrous MgSO₄ and then the crude product was filtered through a pad of silica gel atop a pad of celite and the clear solution was concentrated by rotary evaporation. The crude product was distilled under vacuum (41 mbar) at 98–100 °C to afford 4 (31.6 g, 76%) as a pale yellow oil; ¹H-NMR (400 MHz, CDCl₃): δ: 9.73 (t, J = 2.9 Hz, 1H), 3.99 (m, 4H), 2.70 (d, J = 2.9 Hz, 2H), 1.41 (s, 3H) ppm; ¹³C-NMR (100 MHz, CDCl₃): δ: 200.2, 107.6, 64.8, 52.2, 24.9 ppm; IR (neat): 2986, 2889, 1721, 1380, 1293, 1148, 1115, 1050, 948, 863 cm⁻¹; ESI-HRMS calcd for C₆H₁₆NaO₃ [M + Na]⁺: 153.0528, found: 153.0689.

2-(1-Hydroxy-2-(2-methyl-1,3-dioxolan-2-yl)ethyl)cyclohexan-1-one (5)

To a 300 mL round-bottomed flask equipped with argon line and septum n-BuLi (3.63 g, 56 mmol, 2.5 M solution in hexanes) was added to a −78 °C solution of i-Pr₂NH (5.73 g, 56 mmol) in dry THF (150 mL). The mixture was warmed to 0 °C and stirred for 30 min. and then the bath temperature was lowered to −78 °C. A solution of cyclohexanone (5.05 g, 51 mmol) in dry THF (40 mL) was added dropwise via cannula. The mixture was stirred at −78 °C for 2 h at which time the aldehyde 4 (6.71 g, 51 mmol) was added via syringe and the mixture stirred for 1 h at −78 °C. The reaction was quenched with glacial acetic acid (6.19 g, 103 mmol) and the bath was allowed to warm to ambient temperature naturally and the reaction mixture was stirred overnight. Saturated aqueous NH₄Cl (100 mL) was added, followed by Et₂O (120 mL). Phases were separated and the aqueous layer back-extracted with Et₂O (3 × 100 mL). The combined organic layers were washed with water and brine (2
× 100 mL) and dried over anhydrous Na$_2$SO$_4$. After filtration, the solvent was removed by rotary evaporation. The reaction product was then chromatographed over silica gel with 30% EtOAc/hexane to afford 5 as a colorless oil (7.8 g, 66%); $^1$H-NMR (400 MHz, CDCl$_3$) δ: 4.17 (m, 1H), 3.97 (br s, 4H), 3.54 (d, $J$ = 2.8 Hz, 1H), 2.49 (dt, $J$ = 11.3, 5.4 Hz, 1H), 2.41–2.25 (m, 2H), 2.13 (m, 1H), 2.03 (m, 1H), 1.86 (m, 2H), 1.81–1.62 (m, 3H), 1.51 (m, 1H), 1.39 (s, 3H), 1.34 (s, 3H), 1.30–1.17 (m, 4H), 0.88 (s, 9H), 0.78 (s, 6H) ppm; IR (neat): 2931, 2856, 1708, 1472, 1378, 1251, 1165, 1113, 1046, 1030, 923, 830, 773 cm$^{-1}$; ESI-HRMS calcd for C$_{12}$H$_{20}$NaO$_4$ [M + Na]$^+$: 251.1259, found: 251.1264.

2-((Tert-butyldimethylsilyl)oxy)-2-(2-methyl-1,3-dioxolan-2-yl)ethyl)cyclohexan-1-one (6)

To a 100 mL round-bottomed flask equipped with argon line and septum, a solution of alcohol 5 (2.6 g, 11.3 mmol) in dry DMF (30 mL) was added and stirred at 0 °C for 5 min. Then, 2,6-Lutidine (5.9 g, 55 mmol) was added via syringe and the reaction mixture was stirred for 20 min. TBSCI (2.51 g, 16.7 mmol) was added to the above mixture followed by 22 mol% of DMAP and stirred at 0 °C for 2 h at ambient temperature. Saturated aqueous NH$_4$Cl (20 mL) was added followed by Et$_2$O (80 mL). Phases were separated and the aqueous layer back-extracted with Et$_2$O (3 × 50 mL). The combined organic layers were washed with water (100 mL) and brine (2 × 100 mL) and dried over anhydrous Na$_2$SO$_4$. Solvent was removed by rotary evaporation. The crude product was purified by silica gel flash chromatography (30% EtOAc/hexane) to give 6 as a colorless oil (2.82, 72%); $^1$H-NMR (400 MHz, CDCl$_3$) δ: 4.02–3.87 (m, 4H), 2.46 (m, 1H), 2.33 (m, 1H), 2.25–2.15 (m, 2H), 2.03–1.84 (m, 3H), 1.70–1.52 (m, 4H), 1.41 (s, 3H), 0.88 (s, 9H), 0.07 (d, $J$ = 17.0 Hz, 6H) ppm; $^{13}$C-NMR (100 MHz, CDCl$_3$) δ: 211.3, 110.3, 67.6, 64.3, 64.0, 56.7, 43.5, 41.8, 28.9, 25.9, 24.7, 18.0, −4.5 ppm; IR (neat): 2931, 2856, 1702, 1449, 1405, 1378, 1313, 1250, 1201, 773 cm$^{-1}$; ESI-HRMS calcd for C$_{18}$H$_{34}$NaO$_4$Si [M + Na]$^+$: 365.2124, found: 365.2111.

Tert-butyldimethyl(2-(2-methyl-1,3-dioxolan-2-yl)-1-(1-oxaspiro[2.5]octan-4-yl)ethoxy)silane (7)

To a 100 mL round-bottomed flask equipped with argon line and septum, a suspension of NaH (0.83 g, 34 mmol, in 60% (w/w) mineral oil) in dry DMSO (30 mL) was added and stirred at ambient temperature for 5 h. A solution of ketone 2 (2.6 g, 11.3 mmol) in dry DMF (30 mL) was added and stirred at 0 °C for 40 min. A solution of ketone 2 (2.02 g, 5.9 mmol) in dry THF (12 mL) was added to the above mixture and stirred for 20 min at 0 °C. The mixture was then allowed to warm to ambient temperature and stirred for 15 h before being partitioned between Et$_2$O (100 mL) and brine (60 mL). Phases were separated and the aqueous layer back-extracted with Et$_2$O (3 × 50 mL). The combined organic layers were washed with water (100 mL) and brine (2 × 100 mL) and dried over anhydrous Na$_2$SO$_4$. Solvent was removed by rotary evaporation. The crude product was purified by silica gel flash chromatography (30% EtOAc/hexane) and the relevant fractions were combined. Normal rotary evaporative solvent removal at 20 mm Hg led to lower yields with some product in the condensate. More careful evaporation afforded the epoxide 7 as a light, volatile yellow oil (1.14 g, 54%). Again, significant loss of epoxide occurred to evaporation and could provide a means of improvement in yield. $^1$H-NMR (400 MHz, CDCl$_3$) δ: 3.91 (s br, 4H), 3.03 (d, $J$ = 4.3 Hz, 1H), 2.41 (d, $J$ = 4.3 Hz, 1H), 2.06 (d, $J$ = 14.6 Hz, 1H), 1.94 (m, 1H), 1.92–1.80 (m, 2H), 1.79–1.60 (m, 2H), 1.54–1.38 (m, 2H), 1.34 (s, 3H), 1.30–1.17 (m, 4H), 0.89 (s, 9H), 0.08 (s, 6H) ppm; $^{13}$C-NMR (100 MHz, CDCl$_3$) δ: 109.8, 68.6, 64.2, 64.0, 61.0, 50.4, 45.5, 41.5, 36.2, 25.9, 25.7, 24.8, 24.3, 23.6, 18.1, −3.9, −4.3 ppm; IR (neat): 2930, 2856, 1472, 1375, 1252, 1170, 1100, 1037, 944, 771 cm$^{-1}$; ESI-HRMS calcd for C$_{19}$H$_{36}$NaO$_4$Si [M + Na]$^+$: 379.2281, found: 379.2276.
(2-((tert-butylimidethanol)oxy)-2-(2-methyl-1,3-dioxolan-2-yl)ethyl)-1-hydroperoxycyclohexyl)methanol (8)

To a 100 mL round-bottomed flask equipped with argon line and septum, a solution of epoxide 7 (1.80 g, 5.1 mmol) in ethereal H2O2 (Et2O washed with saturated NaCl 90% H2O2, 50 mL) was added and stirred at ambient temperature for 5 min before the catalyst (Na2MoO4 2H2O and glycine, 0.21 g, 0.5 mmol) was added. The mixture was stirred for 24 h, whereupon water (60 mL) was added to the reaction mixture which was then extracted with EtOAc (3 × 80 mL). The combined organic layers were washed with water and brine (100 mL) and dried over anhydrous Na2SO4. Solvent was removed by rotary evaporation to afford the unstable hydroperoxide 8 as a light-yellow oil (1.20 g, 61%).

3-Methyloctahydro-1H-3,9a-epidioxybenzo[c]oxepin-5-ol, (rac-2)

To a 150 mL round-bottomed flask 8 (1.15 g, 2.9 mmol), DCM (80 mL) and p-TsOH·H2O (0.28 g, 1.47 mmol) were added. The mixture was stirred at ambient temperature for 24 h. Saturated aqueous NaHCO3 (100 mL) was added, then the reaction mixture was extracted with Et2O (3 × 125 mL). The combined organic layers were washed with water (100 mL) and brine (100 mL) and dried over anhydrous Na2SO4. Solvent was removed by rotary evaporation and the crude product was chromatographed on silica gel (20% EtOAc/hexane) to afford the rac-2 as a crystalline solid (0.20 g, 32%); m.p. 109–111 °C; 1H-NMR (400 MHz, C6D6) δ: 4.02 (d, J = 11.3 Hz, 1H, CH2-10), 3.86 (d, J = 11.3 Hz, 1H, CH2-10), 3.75 (m, 1H, CH-5), 3.64 (d, J = 11.9 Hz, 1H, OH), 2.70 (dd, J = 3.2, 15.0 Hz, 1H, CH2a-4), 2.35 (dd, J = 3.1, 15.0 Hz, 1H, CH2b-4), 1.75 (m, 1H, CH2-7), 1.67–1.62 (m, 3H, CH-5a, CH2b-7, CH2a-9), 1.52 (dd br, J = 12.8 Hz, 1H, CH2a-6), 1.32 (m, 1H, CH2b-8), 1.31 (s, 3H, CH3-12), 1.21 (d, J = 4.5, 13.7 Hz, 1H, CH2b-9), 0.98 (m, 1H, CH2b-6), 0.81 (tt, J = 3.6, 13.4 Hz, 1H, CH2a-8) ppm; 13C-NMR (100 MHz, C6D6): δ: 105.1 (C-3), 82.8 (C-9a), 68.3 (C-5), 63.5 (C-10), 51.9 (C-5a), 44.9 (C-4), 35.2 (C-9), 27.1 (C-7), 26.2 (C-12), 25.5 (C-5), 23.6 (C-8) ppm; IR (neat): 3413, 2928, 2856, 1463, 1361, 1252, 1069, 1006, 938, 850, 771 cm−1; ESI-HRMS calcd for C11H18NaO4 [M + Na]+: 237.1103, found: 237.0937; C11H19O4 [M + H]+: 215.1283, found: 215.1059.

3-Methyloctahydro-1H-3,9a-epidioxybenzo[c]oxepin-5-yl pentanoate (9)

To a 10 mL round-bottom flask equipped with argon line and septum a solution of rac-2 (16 mg, 0.075 mmol) in dry DCM (2mL), valeric acid (0.09 mmol, 9.83 µL), EDC (0.09 mmol, 17.17 mg) and DMAP (1.82 mg, 0.015 mmol) was added. After stirring at ambient temperature for 4 days, additional valeric acid (9.83 µL), EDC (8 mg) and DMAP (4 mg) were added and stirred continuously for another 24 h. The reaction mixture was then stirred at 40 °C for 1 h. The mixture was filtered, and the filtrate was collected and diluted by DCM (15 mL). Subsequently, the filtrate was washed by 0.1 N HCl (10 mL), saturated aqueous NaHCO3 (10 mL) and brine (10 mL), dried over anhydrous Na2SO4, concentrated by rotary evaporation and the crude product was chromatographed on silica gel (20% EtOAc/hexane) to afford 9 (2.5 mg 11%); 1H-NMR (400 MHz, CDCl3) δ: 5.26 (s br, CH-5), 4.24 (d, J = 10.4 Hz, 1H, CH2a-10), 4.05 (d, J = 0.9, 10.4 Hz, 1H, CH2b-10), 2.70 (dd, J = 3.2, 15.8Hz, 1H, CH2b-4), 2.38–2.30 (m, 3H, CH2-2', CH2b-4), 2.06 (m, 1H, CH-5a), 1.87 (d br, J = 12.5 Hz, 1H, CH2a-9), 1.78–1.60 (m, 5H, CH2a-6, CH2b-7, CH2-8, CH2-3'), 1.49 (m, 1H, CH2b-6), 1.30–1.20 (m, 6H, CH2b-9, CH3-12, CH2-7, CH2b-8, CH2-4'), 0.93 (m, 3H, CH3-5') ppm; 13C-NMR (100 MHz, CDCl3) δ: 173.6 (C-1'), 103.0 (C-3), 82.5 (C-9a), 69.4 (C-5), 63.4 (C-10), 49.9 (C-5a), 43.1 (C-4), 35.8 (C-9), 34.5 (C-2'), 27.1 (C-7), 26.2 (C-3'), 26.1 (C-12), 25.3 (C-6), 23.6 (C-8), 22.3 (C-4'), 13.7 (C-5') ppm; ESI-HRMS calcd for C16H26CsO5 [M + Cs]+: 431.0835, found: 431.0945.

3-Methyloctahydro-1H-3,9a-epidioxybenzo[c]oxepin-5-yl benzoate (10)

To a 5 mL round-bottom flask equipped with argon line and septum rac-2 (20 mg, 0.093 mmol), CH3CN (1 mL), DMAP (45 mg, 0.37 mmol) and TMEDA (56 µL, 0.37 mmol) were added. The mixture was stirred at 0 °C for 10 min. Then benzoyl chloride (43 µL, 0.37
mmol) was added and the reaction mixture stirred for 1 h. The mixture was allowed to warm to ambient temperature and stirred for 24 h. Water (2 mL) was added to the stirred mixture, which was extracted with EtOAc (15 mL). The organic phase was washed with brine (10 mL), dried over anhydrous Na₂SO₄, and concentrated by rotary evaporation. The crude product was purified by flash chromatography over silica gel (30% EtOAc/hexane) to give 10 as a white solid (22 mg, 74%); m.p. 100–102 °C; ¹H-NMR (400 MHz, CDCl₃) δ: 8.07 (d br, J = 7.4 Hz, 2H, CH-2' and CH-6'), 7.57 (t br, 7.8 Hz, 1H, C-4'), 7.46 (t, J = 7.8 Hz, 2H, CH-3' and CH-5'), 5.55 (ddd, J = 3.1, 6.4, 6.3 Hz, C-5), 4.37 (dd, J = 10.9 Hz, 1H, CH₂-9), 4.14 (dd, J = 1.7, 10.8 Hz, 1H, CH₂-10), 2.81 (dd, J = 3.5, 15.8 Hz, 1H, CH₂-4), 2.45 (dd, J = 2.9, 15.8 Hz, 1H, CH₂-4), 2.15 (m, 1H, CH-5a), 1.91 (d br, J = 12.2 Hz, 1H, CH₂-9), 1.78–1.66 (m, 3H, CH₂-6, CH₂-7, CH₂-8), 1.52 (td, J = 3.4, 12.9 Hz, 1H, CH₂-6), 1.37 (m, 1H, CH₂-9), 1.35 (s, 3H, CH₃-12), 1.21 (m, 2H, CH₂-7, CH₂-8), ppm; ¹³C-NMR (100 MHz, CDCl₃) δ: 166.2 (C=O), 132.9 (C-4'), 130.6 (C-1'), 129.7 (C-2' and C-6'), 128.5 (C-3' and C-5'), 103.2 (C-3), 82.6 (C-9a), 70.0 (C-5), 63.6 (C-10), 50.3 (C-5a), 43.3 (C-4), 35.8 (C-9), 26.4 (C-6), 26.1 (C-12), 25.2 (C-7), 23.6 (C-8) ppm; IR (neat): 2934, 2864, 1710, 1601, 1499, 1374, 1314, 1274, 1220, 1205, 1183, 1160, 1136, 1111, 1070, 1055, 1024, 966, 91, 887, 838, 821, 783 cm⁻¹; ESI-HRMS calcd for C₁₉H₂₂O₃S [M + Cs]⁺: 451.0522, found: 451.0526.

3-Methyloctahydro-1H-3,9a-epidioxybenzo[c]oxepin-5-yl 4-fluorobenzoate (11)

To a 15 mL round-bottom flask equipped with argon line and septum rac-2 (20 mg, 0.093 mmol), CH₃CN (5 mL), DMAP (45 mg, 0.37 mmol) and TMEDA (56 µL, 0.37 mmol) were added. The mixture was stirred at 0 °C for 10 min. Then, 4-fluorobenzoyl chloride (44 µL, 0.37 mmol) was added and the reaction mixture stirred for 1 h. The mixture was allowed to warm to ambient temperature and stirred for 48 h. Water (5 mL) was added to the stirred mixture, which was extracted with EtOAc (20 mL). The organic phase was washed with brine (20 mL), dried over anhydrous Na₂SO₄, and concentrated by rotary evaporation. The crude product was purified by flash chromatography over silica gel (20% EtOAc/hexane) to give 11 as a yellow solid (22 mg, 70%); m.p. 88–90 °C; ¹H-NMR (400 MHz, CDCl₃) δ: 8.10 (m, 2H, CH-2' and CH-6'), 7.13 (t br, J = 8.6 Hz, 2H, CH-3' and CH-5'), 5.55 (m, C-5), 4.36 (d, J = 11.0 Hz, 1H, CH₂-10), 4.15 (dd, J = 1.8, 11.0 Hz, 1H, CH₂-9), 2.82 (dd, J = 3.6, 15.8 Hz, 1H, CH₂-4), 2.44 (dd, J = 2.9, 15.8 Hz, 1H, CH₂-4), 2.14 (m, 1H, CH₂-9), 1.91 (d br, J = 13.1 Hz, 1H, CH₂-9), 1.79–1.69 (m, 3H, CH₂-6, CH₂-7, CH₂-8), 1.46 (m, 1H, CH₂-6), 1.38 (m, 1H, CH₂-9), 1.36 (s, 3H, CH₃-12), 1.20 (m, 2H, CH₂-7, CH₂-8), ppm; ¹³C-NMR (100 MHz, CDCl₃) δ: 165.8 (C-4'; J₇C = 252.4 Hz, 162.5 (C=O), 132.2 (C-2' and C-6'; J₇C = 9.2 Hz), 126.9 (C-1'), 115.5 (C-3' and C-5'), J₇C = 21.8Hz), 103.2 (C-3), 82.6 (C-9a), 70.2 (C-5), 63.6 (C-10), 50.3 (C-5a), 43.3 (C-4), 35.8 (C-9), 26.4 (C-6), 26.1 (C-12), 25.3 (C-7), 23.6 (C-8) ppm; IR (neat): 2931, 2862, 1704, 1603, 1507, 1453, 1410, 1374, 1279, 1223, 1161, 1111, 1070, 1055, 1024, 966, 91, 887, 838, 821, 783 cm⁻¹; ESI-HRMS calcd for C₁₉H₂₁FNaO₅ [M + Na]⁺: 359.1271, found: 359.2250.

3-Methyloctahydro-1H-3,9a-epidioxybenzo[c]oxepin-5-yl 4-methoxybenzoate (12)

To a 15 mL round-bottom flask equipped with argon line and septum rac-2 (20 mg, 0.093 mmol), CH₃CN (5 mL), DMAP (45 mg, 0.37 mmol) and TMEDA (56 µL, 0.37 mmol) were added. The mixture was stirred at 0 °C for 10 min. Then, 4-methoxybenzoyl chloride (50 µL, 0.37 mmol) was added and the reaction mixture stirred for 1 h. The mixture was allowed to warm and stir at ambient temperature for 72 h. Water (5 mL) was added to the stirred mixture, which was extracted with EtOAc (20 mL). The organic phase was washed with brine (20 mL), dried over anhydrous Na₂SO₄, and concentrated by rotary evaporation. The crude product was purified by flash chromatography over silica gel (20% EtOAc/hexane) to give 12 as a white solid (22 mg, 68%); m.p. 155–157 °C; ¹H-NMR (400 MHz, CDCl₃) δ: 8.04 (d br, J = 8.8 Hz, 2H, CH-2' and CH-6'), 6.94 (d br, J = 8.8 Hz, 2H, CH-3' and CH-5'), 5.53 (ddd, J = 3.2, 6.4, 6.4 Hz, C-5), 4.37 (dd, J = 10.9 Hz, 1H, CH₂-10), 4.14 (dd, J = 1.8, 10.9 Hz, 1H, CH₂-10), 3.87 (s, 3H, OCH₃), 2.82 (dd, J = 3.6, 15.8 Hz, 1H, CH₂-4), 2.43 (dd, J = 2.8, 15.8 Hz, 1H, CH₂-4), 2.13 (m, 1H, CH-5a), 1.90 (dd br, J = 1.9, 11.8
Hz, 1H, CH₂a-9), 1.77–1.69 (m, 3H, CH₂a-6, CH₂a-7, CH₂a-8), 1.49 (ddd, δ = 3.5, 12.8, 16.4 Hz, 1H, CH₂b-6), 1.36 (m, 1H, CH₃-2g), 1.35 (s, 3H, CH₃-12), 1.21 (m, 2H, CH₂b-7, CH₂b-8) ppm; ²³C-NMR (100 MHz, CDCl₃) δ: 165.9 (C=O), 163.4 (C-4’), 131.7 (C-2’ and C-6’), 123.0 (C-1’), 113.7 (C-3’ and C-5’), 103.2 (C-3), 82.6 (C-9a), 69.6 (C-5), 63.6 (C-10), 50.3 (C-5a), 43.4 (C-4), 35.9 (C-9), 26.4 (C-6), 26.1 (C-12), 25.3 (C-7), 23.6 (C-8) ppm; IR (neat): 2923, 2859, 1710, 1604, 1512, 1578, 1604, 1710, 1512, 1350, 1248, 1276, 1160, 1117, 1101, 1045, 1018, 967, 853, 820, 771 cm⁻¹; ESI-HRMS calcd for C₁₉H₂₄NaO₆ [M + Na]⁺: 371.1471, found: 371.1747; C₁₉H₂₄CsO₆ [M + Cs]⁺: 481.0627, found: 481.0659.

3-Methyloctahydro-1H-3,9a-epidioxybenzo[c]oxepin-5-yl cinnamate (13)

To a 15 mL round-bottom flask equipped with argon line and septum rac-2 (50 mg, 0.23 mmol), CH₃CN (7 mL), DMAP (114 mg, 0.93 mmol) and TMEDA (139 µL, 0.93 mmol) were added. The mixture was stirred at 0 °C for 10 min. Then, cinnamoyl chloride (155 mg, 0.95 mmol) was added and the reaction mixture stirred for 1 h. The mixture was warmed and allowed to stir at ambient temperature for 72 h. Water (10 mL) was added to the stirred mixture, which was extracted with EtOAc (20 mL). The organic phase was washed with brine (20 mL), dried over anhydrous Na₂SO₄, and concentrated by rotary evaporation. The crude product was purified by flash chromatography over silica gel (20% EtOAc/hexane) to give 13 as yellow oil which solidified upon storage in the refrigerator (29 mg, 36%); ¹H-NMR (400 MHz, CDCl₃) δ: 7.71 (d, δ = 16.0 Hz, 1H, CH-3’), 7.55 (s br, 2H, CH-2’ and CH-6’), 7.40 (s br, 3H, CH-2”, CH-6”), 7.37 (d, δ = 16.0 Hz, 1H, CH-2’), 5.45 (m, 1H, C-5), 4.32 (d, δ = 10.8 Hz, 1H, CH₂b-10), 4.11 (d, δ = 10.8 Hz, 1H, CH₂b-10), 2.78 (dd, δ = 3.7, 15.9 Hz, 1H, CH₂a-4), 2.43 (dd, δ = 2.8, 15.9 Hz, 1H, CH₂b-4), 2.10 (m, 1H, CH₃a-9), 1.90 (d br, δ = 14.0 Hz, 1H, CH₂a-9), 1.74–1.65 (m, 3H, CH₂a-6, CH₂a-7, CH₂a-8), 1.54 (m, 1H, CH₂b-6), 1.47 (m, 1H, CH₂b-9), 1.36 (s, 3H, CH₃-12), 1.22 (m, 2H, CH₂b-7, CH₂b-8) ppm; ¹³C-NMR (100 MHz, CDCl₃) δ: 166.8 (C=O), 144.9 (C-3’), 134.5 (C-1’), 130.3 (C-4’), 128.8 (C-3’” and C-5’”), 128.1 (C-2’” and C-6”’), 118.5 (C-2’”), 103.1 (C-3”), 82.6 (C-9a), 69.6 (C-5), 63.6 (C-10), 50.3 (C-5a), 43.3 (C-4”), 35.9 (C-9’), 26.3 (C-6”), 26.1 (C-12”), 25.3 (C-7”), 23.6 (C-8) ppm; IR (neat): 2931, 2863, 1702, 1636, 1449, 1374, 1310, 1275, 1202, 1180, 1156, 1130, 1072, 1054, 1024, 979, 965, 916, 887, 836, 820, 767, 711 cm⁻¹; ESI-HRMS calcd for C₂₀H₂₄ClCsO₅ [M + Cs]⁺: 477.0678, found: 477.0681.

3-Methyloctahydro-1H-3,9a-epidioxybenzo[c]oxepin-5-yl 4-chlorobenzoate (14)

To a 15 mL round-bottom flask equipped with argon line and septum rac-2 (30 mg, 0.14 mmol), CH₃CN (5 mL), DMAP (68 mg, 0.56 mmol) and TMEDA (83 µL, 0.56 mmol) were added. The mixture was stirred at 0 °C for 10 min. Then, 4-chlorobenzoyle chloride (72 µL, 0.56 mmol) was added and the reaction mixture stirred for 1 h. The mixture was allowed to warm and stir at ambient temperature for 24 h. Water (5 mL) was added to the stirred mixture, which was extracted with EtOAc (20 mL). The organic phase was washed with brine (20 mL), dried over anhydrous Na₂SO₄, and concentrated by rotary evaporation. The crude product was purified by flash chromatography over silica gel (20% EtOAc/hexane) to give 14 as white solid (24 mg, 49%); m.p. 110–112 °C; ¹H-NMR (400 MHz, CDCl₃) δ: 8.02 (d br, δ = 8.6 Hz, 2H, CH-2’ and CH-6’), 7.44 (d, δ = 8.6 Hz, 2H, CH-3’ and CH-5’), 5.55 (ddd, δ = 2.4, 6.0, 6.1 Hz, C-5), 4.35 (d, δ = 11.0 Hz, 1H, CH₂b-10), 4.15 (ddd, δ = 1.8, 10.9 Hz, 1H, CH₂a-10), 2.83 (dd, δ = 3.5, 15.8 Hz, 1H, CH₂a-4), 2.45 (dd, δ = 2.8, 15.8 Hz, 1H, CH₂b-4), 2.16 (m, 1H, CH₃a-9), 1.92 (dd br, δ = 1.0, 13.1 Hz, 1H, CH₂a-9), 1.78–1.65 (m, 3H, CH₂a-6, CH₂a-7, CH₂a-8), 1.46 (ddd, δ = 3.6, 13.2, 16.4 Hz, 1H, CH₂b-6), 1.37 (m, 1H, CH₂b-9), 1.36 (s, 3H, CH₃-12), 1.22 (m, 2H, CH₂b-7, CH₂b-8) ppm; ¹³C-NMR (100 MHz, CDCl₃) δ: 165.3 (C=O), 139.4 (C-4’), 131.1 (C-2’ and C-6’), 129.0 (C-1’), 128.8 (C-3’ and C-5’), 103.2 (C-3), 82.6 (C-9a), 70.4 (C-5), 63.6 (C-10), 50.2 (C-5a), 43.3 (C-4’), 35.8 (C-9’), 26.4 (C-6), 26.1 (C-12), 25.3 (C-7), 23.6 (C-8) ppm; IR (neat): 2929, 2860, 1701, 1591, 1487, 1450, 1400, 1368, 1271, 1222, 1161, 1136, 1088, 1014, 965, 854, 819, 750 cm⁻¹; ESI-HRMS calcd for C₁₉H₂₂ClC₁₇O₅ [M + Cs]⁺: 485.0132, found: 485.0169.
3-Methylhexahydro-1H-3,9a-epidioxybenzo[c]oxepin-5(5aH)-one (15)

To a 25 mL round-bottom flask equipped with argon line and septum PCC (80 mg, 0.37 mmol) and dry DCM (10 mL) were added. The mixture was stirred at ambient temperature for 5 min before a solution of rac-2 (53 mg, 0.25 mmol) in dry DCM (5 mL) was added via cannula to the above mixture and stirred for 48 h. The mixture was diluted with EtO (10 mL), decanted and concentrated by rotary evaporation. The crude material was purified by flash chromatography over silica gel (20% EtOAc/hexane) to give 15 as a yellow oil which solidified upon storage in the refrigerator (29 mg, 55%); $^1$H-NMR (400 MHz, CDCl$_3$) δ: 4.14 (d, $J = 11.4$ Hz, 1H, CH$_2$-10), 4.11 (d, $J = 11.4$ Hz, 1H, CH$_2$-10), 3.56 (d, $J = 15.6$ Hz, 1H, CH$_2$-4), 2.93 (d, $J = 15.6$ Hz, 1H, CH$_2$-4), 2.36 (m, 3H, CH-5a), 2.32 (m, 1H, CH-2a), 1.92–1.87 (d br, $J = 14.1$ Hz, 1H, CH$_2$-9), 1.80–1.70 (m, 2H, CH$_2$-7, CH$_2$-8), 1.44 (ddd, $J = 4.4$, 13.5, 18.0 Hz, 1H, CH$_2$-9), 1.37 (s, 3H, CH$_3$-12), 1.30–1.12 (m, 3H, CH$_2$-6, CH$_2$-7, CH$_2$-8), ppm; $^{13}$C-NMR (100 MHz, CDCl$_3$) δ: 204.9 (C-5), 103.0 (C-3), 83.4 (C-9a), 63.6 (C-10), 57.6 (C-5a), 54.9 (C-4), 34.3 (C-9), 25.4 (C-7), 24.6 (C-6), 24.6 (C-7), 55.1 (OCH$_3$) ppm; IR (neat): 2940, 2852, 1746, 1460, 1376, 1271, 1161, 1097, 1055, 909, 881, 863, 827, 778 cm$^{-1}$; ESI-HRMS calcd for C$_{11}$H$_{17}$O$_4$ [M + H]$^+$: 213.1127, found: 213.1140.

(3R,5R,5aR,9aS)-3-methyloctahydro-1H-3,9a-epidioxybenzo[c]oxepin-5-yl(R)-3,3,3-trifluoro-2-methoxy-2-phenylpropanoate (19a) and (3S,5S,5aR,9aR)-3-methyloctahydro-1H-3,9a-epidioxybenzo[c]oxepin-5-yl(R)-3,3,3-trifluoro-2-methoxy-2-phenylpropanoate (19b)

To a 50 mL round-bottom flask equipped with argon line and septum rac-2 (195 mg, 0.91 mmol), CH$_3$CN (25 mL), DMAP (334 mg, 2.73 mmol) and TMEDA (0.41 mL, 2.73 mmol) were added. The mixture was stirred at 0 °C for 10 min. Then, (R)-(−)-MTPA-Cl (0.34 mL, 1.82 mmol) was added and the reaction mixture stirred for 1 h. The mixture was allowed to warm and stirred at ambient temperature for 72 h. Water (20 mL) was added to the stirred mixture, which was extracted with EtOAc (2 × 25 mL). The organic phases were washed with brine (30 mL), dried over anhydrous Na$_2$SO$_4$, and concentrated by rotary evaporation. The crude product was purified by flash chromatography over silica gel (15% EtOAc/hexane) to give two diastereomers. The higher $R'$ material 19b (76 mg, 19%) was a viscous oil; $^1$H-NMR (600 MHz, CDCl$_3$) δ: 7.71 (m, 2H, CH-2" and CH-6"), 7.42 (m, 3H, CH-3", CH-5", CH-4"), 5.40 (dt, $J = 3.3$, 9.6 Hz, 1H, C-5), 4.19 (d, $J = 11.0$ Hz, 1H, CH$_2$-10), 4.05 (dd, $J = 1.7$, 11.1 Hz, 1H, CH$_2$-10), 3.65 (s, 3H, OCH$_3$), 2.79 (dd, $J = 3.0$, 16.2 Hz, 1H, CH$_2$-4), 2.50 (dd, $J = 2.8$, 16.2 Hz, 1H, CH$_2$-4), 2.10 (m, 1H, CH-5a), 1.88 (d br, $J = 11.5$ Hz, 1H, CH$_2$-9), 1.69–1.63 (m, 2H, CH$_2$-7, CH$_2$-8), 1.54 (m, 1H, CH$_2$-6), 1.34 (s, 3H, CH$_3$-12), 1.32 (m, 1H, CH$_2$-9), 1.19–1.12 (m, 3H, CH$_3$-6, CH$_3$-7, CH$_3$-8), ppm; $^{13}$C-NMR (150 MHz, CDCl$_3$) δ: 166.1 (C-1", 131.7 (C-1"), 129.3 (C-3" and C-5"), 127.2 (C-2" and C-6"), 125.5 (C-3"), 204.9 (C-5), 103.0 (C-3), 83.4 (C-9a), 63.6 (C-10), 57.6 (C-5a), 54.9 (C-4), 34.3 (C-9), 25.4 (C-7), 24.6 (C-6), 24.6 (C-7), 55.1 (OCH$_3$) ppm; IR (neat): 2935, 2865, 1715, 1450, 1409, 1376, 1335, 1320, 1268, 1247, 1213, 1190, 1167, 1118, 1097, 1055, 909, 881, 863, 827, 778 cm$^{-1}$; ESI-HRMS calcd for C$_{11}$H$_{17}$O$_4$ [M + Cs]$^+$: 563.0668, found: 563.0663. The lower $R'$ material 19a (82 mg, 22%) was a viscous oil; $^1$H-NMR (400 MHz, CDCl$_3$) δ: 7.56 (m, 2H, CH-2" and CH-6"), 7.43 (m, 3H, CH-3", CH-5", CH-4"), 5.40 (dt, $J = 3.1$, 9.4 Hz, 1H, CH-5), 4.06 (d, $J = 11.0$ Hz, 1H, CH$_2$-10), 3.97 (dd, $J = 1.7$, 11.0 Hz, 1H, CH$_2$-10), 3.50 (s, 3H, OCH$_3$), 2.76 (dd, $J = 3.5$, 16.1 Hz, 1H, CH$_2$-4"), 2.45 (dd, $J = 2.8$, 16.1 Hz, 1H, CH$_2$-4"), 2.08 (m, 1H, CH-5a), 1.84 (d br, $J = 13.6$ Hz, 1H, CH$_2$-9), 1.68–1.56 (m, 3H, CH$_2$-6, CH$_2$-7, CH$_2$-8), 1.35–1.26 (m, 2H, CH$_2$-6, CH$_2$-7, CH$_2$-8), 1.24 (s, 3H, CH$_3$-12), 1.19–1.10 (m, 2H, CH$_2$-7, CH$_2$-8) ppm; $^{13}$C-NMR (100 MHz, CDCl$_3$) δ: 166.4 (C-1"), 131.7 (C-1"), 129.6 (C-4"), 128.4 (C-3" and C-5"), 127.8 (C-2" and C-6"), 127.5 (C-3"), 102.6 (C-3), 82.5 (C-9a), 73.2 (C-5), 50.2 (C-5a), 42.7 (C-4), 35.7 (C-9), 26.0 (C-6), 26.0 (C-12), 25.4 (C-7), 23.5 (C-8) ppm; IR (neat): 2940, 2852, 1746, 1450, 1376, 1271, 1161, 1127, 1023, 837, 718 cm$^{-1}$; ESI-HRMS calcd for C$_{21}$H$_{25}$F$_3$O$_5$ [M + Cs]$^+$: 563.0658, found: 563.0651.
To a 25 mL round-bottom flask containing 19a (20 mg, 0.05 mmol), a warm solution of 1N NaOH in MeOH (10 mL) was added. The mixture was allowed to stir at ambient temperature for 36 h. The mixture was then neutralized with a few drops of dilute HCl. After removal of the MeOH by rotary evaporation, the residue was dissolved in CHCl₃ (10 mL) and washed with H₂O (10 mL). The aqueous layer was extracted with CHCl₃ (2 × 10 mL). The combined organic layers were filtered over anhydrous Na₂SO₄ and concentrated by rotary evaporation. The crude product was purified by flash chromatography over silica gel (20% EtOAc/hexane) to afford 2a as a crystalline solid (2.6 mg, 27%); m.p. 108–110 °C; ¹H-NMR (400 MHz, CDCl₃) δ: 4.19 (d, J = 11.3 Hz, 1H, CH₂-10), 4.11 (dd, J = 1.4, 11.3 Hz, 1H, CH₂-10), 3.84 (m, 1H, CH-5), 3.64 (d, J = 11.7 Hz, 1H, OH), 2.74 (dd, J = 3.3, 15.2 Hz, 1H, CH₂-4), 2.44 (dd, J = 3.1, 15.2 Hz, 1H, CH₂-4), 1.90–1.58 (m, 6H, CH₂-7, CH₂-8, CH₅a, CH₂-7, CH₂-9, CH₂-6), 1.40 (s, 3H, CH₃-12), 1.33–1.20 (m, 3H, CH₂-8, CH₂-9, CH₂-6) ppm; ¹³C-NMR (100 MHz, CDCl₃) δ: 105.2 (C-3), 83.8 (C-9a), 68.6 (C-5), 63.6 (C-10), 51.7 (C-5a), 44.8 (C-4), 35.1 (C-9), 27.0 (C-7), 26.3 (C-12), 25.4 (C-6), 23.8 (C-8) ppm; [α]D ⁰ = -100.5 ° (c = 0.016, CHCl₃); IR (neat): 3426, 2928, 2856, 1462, 1449, 1376, 1361, 1252, 1161, 1048, 1030, 830, 771 cm⁻¹; ESI-MS calcd for C₁₁H₁₇O₄ [M + H]⁺: 213.1127, found: 213.0793.

To a 25 mL round-bottom flask containing 19b (24 mg, 0.06 mmol), a warm solution of 1N NaOH in MeOH (10 mL) was added. The mixture was allowed to stir at ambient temperature for 36 h. The mixture was then neutralized with few drops of dilute HCl. After removal of the MeOH by rotary evaporation, the residue was dissolved in CHCl₃ (10 mL) and washed with H₂O (10 mL). The aqueous layer was extracted with CHCl₃ (2 × 10 mL). The combined organic layers were filtered over anhydrous Na₂SO₄ and concentrated by rotary evaporation. The crude product was purified by flash chromatography over silica gel (20% EtOAc/hexane) to afford 2b as a crystalline solid (2.9 mg, 24%); m.p. 107–109 °C; ¹H-NMR (400 MHz, CDCl₃) δ: 4.19 (d, J = 11.3 Hz, 1H, CH₂-10), 4.11 (dd, J = 1.4, 11.3 Hz, 1H, CH₂-10), 3.84 (m, 1H, CH-5), 3.64 (d, J = 11.7 Hz, 1H, OH), 2.74 (dd, J = 3.3, 15.2 Hz, 1H, CH₂-4), 2.44 (dd, J = 3.1, 15.2 Hz, 1H, CH₂-4), 1.90–1.58 (m, 6H, CH₂-7, CH₂-8, CH₅a, CH₂-7, CH₂-9, CH₂-6), 1.40 (s, 3H, CH₃-12), 1.33–1.20 (m, 3H, CH₂-8, CH₂-9, CH₂-6) ppm; ¹³C-NMR (100 MHz, CDCl₃) δ: 105.2 (C-3), 83.8 (C-9a), 68.6 (C-5), 63.6 (C-10), 51.7 (C-5a), 44.8 (C-4), 35.1 (C-9), 27.0 (C-7), 26.3 (C-12), 25.4 (C-6), 23.8 (C-8) ppm; [α]D ⁰ = +100.5 ° (c = 0.020, CHCl₃); IR (neat): 3417, 2931, 2862, 1449, 1387, 1357, 1240, 1221, 1160, 1111, 1092, 1030, 984, 903, 882, 853, 832, 816 cm⁻¹; ESI-MS calcd for C₁₁H₁₇O₄ [M + H]⁺: 213.1127, found: 213.0741.

To a 5 mL round-bottom flask rac-2 (19 mg, 0.09 mmol), THF (1.2 mL), and FeBr₂ (0.038 g, 0.177 mmol) were added. The mixture was stirred at ambient temperature under argon atmosphere for 2 h and then directly chromatographed over silica gel (60% EtOAc/hexane). The product 22 was isolated in 47% yield (9 mg); m.p. 123–125 °C; ¹H-NMR (400 MHz, CDCl₃) δ: 4.62 (dd, J = 1.5, 12.3 Hz, 1H, CH₂-8), 4.42 (td, J = 1.5, 5.5 Hz, 1H, CH-3), 4.26 (d, J = 12.3 Hz, 1H, CH₂-8), 4.23 (dd, J = 5.2, 10.5 Hz, 1H, CH₂-2), 3.85 (dd, J = 1.6, 10.5 Hz, 1H, CH₂-2), 2.30 (dt, J = 3.3, 6.7 Hz, 1H, CH₂-7), 2.11 (s, 3H, CH₃-11), 1.91 (m, 1H, CH₂-5), 1.83 (m, 1H, CH₂-4), 1.76 (m, 1H, CH₂-6), 1.54 (m, 1H, CH₃a), 1.46 (m, 1H, CH₂-4), 1.35–1.26 (m, 3H, CH₂-7, CH₂-5, CH₂-6) ppm; ¹³C-NMR (150 MHz, CDCl₃) δ: 171.8 (C-10), 81.8 (C-7a), 75.2 (C-2), 72.2 (C-3), 61.8 (C-8), 53.4 (C-3a), 33.6 (C-7), 25.6 (C-5), 22.6 (C-6), 21.7 (C-4), 21.0 (C-11) ppm; IR (neat): 3394, 2944, 2881, 1740, 1481, 1451, 1387, 1368, 1353, 1311, 1242, 1125, 1148, 1124, 1081, 1036, 1018, 1008, 973, 926, 870, 845, 755 cm⁻¹; ESI-HRMS calcd for C₁₁H₁₈NaO₄ [M + Na]⁺: 237.1103, found: 237.1145.
(2-Acetyl-1-hydroxycyclohexyl)methyl acetate (29)

To a 5 mL round-bottom flask 15 (20 mg, 0.09 mmol), THF (1.2 mL), and FeBr$_2$ (0.041 g, 0.177 mmol) were added. The mixture was stirred at ambient temperature under argon atmosphere for 2 h and then directly chromatographed over silica gel (60% EtOAc/hexane). The product 29 was isolated in 50% yield (10 mg); $^1$H-NMR (400 MHz, CDCl$_3$) $\delta$: 4.24 (d, $J = 12.0$ Hz, 1H, CH$_2$-7), 4.18 (d, $J = 12.0$ Hz, 1H, CH$_2$-7), 2.68 (dd, $J = 4.4$, 9.8 Hz, 1H, CH-2), 2.23 (s, 3H, CH$_3$-1), 2.08 (s, 3H, CH$_3$-10), 2.01–1.92 (m, 2H, CH$_2$-6, CH$_2$-3), 1.68–1.59 (m, 3H, CH$_2$-6, CH$_2$-4, CH$_2$-5), 1.43–1.34 (m, 3H, CH$_2$-3, CH$_2$-4, CH$_2$-5) ppm; $^{13}$C-NMR (100 MHz, CDCl$_3$) $\delta$: 211.6 (C-11), 171.8 (C-9), 72.8 (C-1), 67.3 (C-7), 56.4 (C-2), 34.5 (C-6), 30.9 (C-12), 25.2 (C-3), 23.8 (C-4), 22.1 (C-5), 20.8 (C-10) ppm; IR (neat): 3452, 2924, 2854, 1740, 1705, 1366, 1259, 1236, 1186, 1090, 1014, 924, 800, 737 cm$^{-1}$; ESI-HRMS calcd for C$_{11}$H$_{18}$O$_4$ [M + Na]$^+$: 237.1103, found: 237.1132.

4.2. Single Crystal X-ray Diffraction Experiment

Compound rac-2

Racemic 2 crystallized as a conglomerate, from which 2b was randomly selected. Crystal data for C$_{11}$H$_{18}$O$_4$ (M = 214.25 g/mol): clear colorless prism (0.38 $\times$ 0.30 $\times$ 0.16 mm), orthorhombic, space group P2$_1$2$_1$2$_1$, $a = 5.7823$ (5) Å, $b = 7.6135$ (10) Å, $c = 23.795$ Å, $v = 1047.5$ Å$^3$ and $z = 4$. $d_{calc} = 1.359$ mg x m$^{-3}$, 4872 independent reflections measured out to $\theta_{max} = 36.6^\circ$ with a Nonius KappaCCD diffractometer using Mo K$_\alpha$ radiation ($\lambda = 0.71073$ Å) with a graphite monochromator in the incident beam. The data were collected at room temperature by using the $\omega$ scan technique. Multi-scan absorption correction was applied using Denzo and Scalepack [70]. The structure was solved by direct methods as implemented by the SHELXTL97 system of programs [70]. Full-matrix least-squares refinement was performed on 141 parameters using the 4872 reflections. The C-H distances were fixed at 0.98–1.00 Å and placed in idealized positions.

4.3. Antimalarial Activity Assay

Synthesized compounds were tested in vitro against two P. falciparum malaria parasite clones, designated as Indochina (W-2) and Sierra Leone (D-6). The W-2 clone is chloroquine resistant whereas the D-6 clone is a chloroquine-sensitive strain.

4.3.1. Reagents and Materials

The two P. falciparum clones, W-2 and D-6 were obtained from Walter Reed Army Institute. Human blood and human serum were obtained from Interstate Blood Bank. Roswell Park Memorial Institute medium (RPMI 1640 medium), acetic acid and 96-well microplate were purchased from Thermo Fisher Scientific. APAD, NBT, PES, artemisinin, chloroquine, DMSO, amikacin, doxorubicin, phosphate-buffered saline (PBS) and neutral red were purchased from Sigma-Aldrich. Vero cells (African green monkey kidney fibroblast) purchased from American Type Culture Collection (ATCC).

4.3.2. In Vitro Antimalarial Assay

A suspension of red blood cells infected with W-2 or D-6 strain of P. falciparum that contains 2% parasitemia and 2% hematocrit in RPMI 1640 medium supplemented with 10% human serum and 60 µg/mL amikacin was dispensed into the wells of a 96-well flat-bottomed microtiter plate containing 10 µL of serially diluted test samples. The plates were incubated at 37 °C in an environment of 90% N$_2$, 5% O$_2$, and 5% CO$_2$ for 72 h. Next, 100 µL aliquots of the Malstat reagent were added to each well of a new 96-well microtiter plate. The cultures were resuspended from the assay plate by mixing each well up and down several times. A total of 20 µL from each well of the resuspended culture was removed and added to the plate containing the Malstat reagent and the plate was incubated at r.t., for 30 min. Further, to each well, 20 µL of a NBT/PES (1:1) solution (2 and 0.1 mg/mL, respectively) were added. The plate was incubated in the dark for 1 h. The reaction was terminated by the addition of 100 µL of a 5% acetic acid solution. The 139
plate was then read at 650 nm. Artemisinin and chloroquine were included in each assay as antimalarial drug controls. The IC$_{50}$ values were computed from the dose–response curves using XLfit 4.2.

4.3.3. In Vitro Cytotoxicity Assay

The assay was performed in 96-well tissue culture-treated plates as described earlier. Vero cells were seeded to the wells of 96-well plate at a density of 25,000 cells/well and incubated for 24 h. Tested compounds at different concentrations were added and plates were again incubated for 48 h. The number of viable cells was determined by Neutral Red assay [71,72]. IC$_{50}$ values were obtained from dose–response curves. Doxorubicin was used as a positive control for cytotoxicity.

Neutral Red Assay

Briefly, after incubating with the tested compounds, the cells were washed with PBS and incubated for 90 min with the medium containing Neutral Red (166 µg/mL). The cells were washed to eliminate extracellular dye. A solution of acidified isopropanol (0.33% HCl) was then added to lyse the cells. As a result, the absorbed dye was released from the viable cells. The absorbance was read at 540 nm. IC$_{50}$ (the concentration of the test compounds that caused a growth inhibition of 50% after 48 h of exposure of the cells) was calculated from the dose curves created by plotting percent growth vs. the test concentration on a logarithmic scale using Microsoft Excel. All assays were performed in triplicate.

5. Conclusions

The 5β-hydroxy-secoartemisinin rac-2 proved to be resistant to esterification except with highly reactive acylation partners such as acid chlorides/DMAP; softer electrophiles such as formyl chlorides/DMAP failed to acylate, with the outcome being only a limited qualitative SAR was possible. Since many of the dozen compounds would succumb to esterase hydrolysis at different rates, QSAR was not expected to provide useful results. Fortunately, the Mosher esters of rac-2 could be formed and separated leading to chiral alcohols $R(-)$-2a and $S(+)$-2b. Of these, the alcohol $R(-)$-2a mimics the natural product and is the only alcohol of the pair with antimalarial activity, an important finding in light of previous generalizations regarding the unimportance of chirality for antimalarial activity [16,17]. Clearly, at least in this case, chirality is important to biological activity. Given the probability of protein targets such as pf-ATP6 [21–25] and, in the antimalarial effects of peroxides, the issue of chirality in antimalarial drug design is important.

The corresponding 5-ketone, 15, holds potential for derivation of both C-4 and C-5 although we were unable to methylene 15 to give 16. Perhaps this or some other idea can be accomplished in the future to access analogs of artemisinin at the C4/C5 positions via ketone 15. The antimalarial activity of ketone 15 was substantially improved over the corresponding alcohol 2 and interestingly showed a different ultimate rearrangement pathway under the influence of ferrous ion. The greater antimalarial effect of the ketone 15 relative to the alcohol 2 may be related to the more stable $\alpha$-keto radical intermediate 23 compared to the more reactive $\beta$-hydroxy radical intermediate 21 which undergoes self-immolation rather than reside long enough for intermolecular processes to occur.

Supplementary Materials: The following are available online, Figures S1–S87. The crystal structure has been deposited to the CCDC with the number 2006194.

Author Contributions: M.J. and M.A.A. designed chemical synthesis, performed chemical synthesis experiments, analyzed results, and wrote the manuscript; F.L. analyzed results; F.R.F. performed single-crystal X-ray diffraction analysis; K.M.E. carried out the DFT calculations, J.R. supervision of M.J., and project administration; S.I.K. performed malaria testing. All authors have read and agreed to the published version of the manuscript.
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