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THE SYNTHESIS OF 8,8-DISUBSTITUTED TRICYCLIC ANALOGS OF ARTEMISININ

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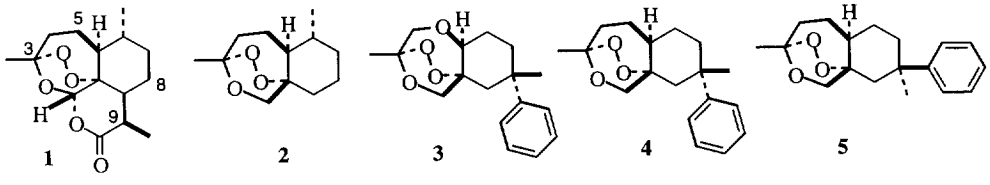
Abstract: Two tricyclic analogs of artemisinin were designed based on a Comparative Molecular Field Analysis (CoMFA) model, and synthesized for antimalarial testing as part of a program to construct and validate modeling tools for drug design of novel antimalarial agents based on the natural product lead, (+)-artemisinin.

Each year the WHO estimates that 300-500 million cases of malaria occur with mortality in children approaching one million deaths. Alarmingly, multi-drug resistance of malaria (*Plasmodium falciparum*) to conventional agents such as chloroquine and sulfadoxine/pyrimethamine has risen to overwhelming proportions. Thus, a significant impetus exists to develop new antimalarials functioning by novel mechanisms of action.[1] Among mechanistically unique antimalarials is (+)-artemisinin **1**, a natural product from *Artemisia annua* L.[2] Clinically fielded first generation antimalarials derived from artemisinin such as artemether have recently shown neurotoxicity in animal models,[3] fueling interest in next-generation antimalarials. Well over 200 analogs of artemisinin have appeared in the literature over the past decade, but very few quantitative structure-activity relationship (QSAR) studies have been reported. A classical QSAR study on derivatives of dihydroartemisinin was reported a number of years ago,[4] and was recently followed by 3D-QSAR studies of limited datasets.[5] We have recently expanded our QSAR efforts in this area[6,7] by completing a 3D-QSAR study of 202 diverse analogs of artemisinin.[8] With this larger dataset, we have obtained crossvalidated correlation coefficients (q^2 or cvr^2) in the range of 0.8 ($F = 327$, $s = 0.475$, conventional $r^2 = 0.91$), a value suggesting that the model should have excellent predictive capability. In order to validate this model we have undertaken the synthesis of several analogs designed to gather test data for comparison against their predicted activities.

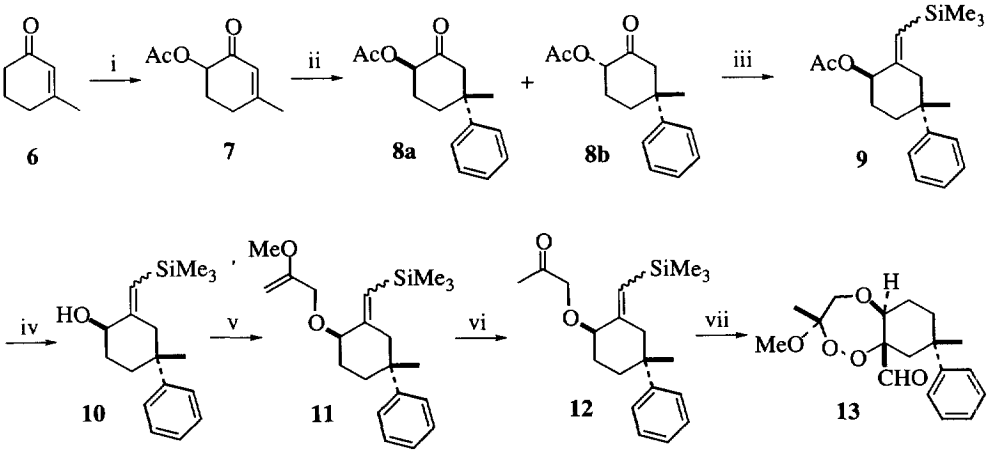


Our original plan was to combine heteroatom substitution in the B-ring (oxepane ring) with alkyl substitution at C-8. It was hoped that potency could be enhanced both by circumventing metabolic hydroxylation at C-5, a major pathway for arteether in mammalian systems,[9] and by incorporating substituents suggested to be beneficial by CoMFA. The analogs to be presented here, **3-5**, are structural modifications of **2**, a tricyclic analog of artemisinin lacking the D-ring reported by our group in 1990.[10]

Utilizing visual information gleaned from our CoMFA fields, we determined that substitution of the C-8 position might allow for greater activity than that of the base structure **2** by partially filling the volume of **2** in the area formerly occupied by the δ-lactone of artemisinin. In particular, an α-phenyl moiety was suggested by the *de novo* design tool, Leapfrog (Tripos Assoicates, Inc.). Predictions using the CoMFA model have shown that a β-butyl group placed at C-8 should substantially increase the activity of the compounds, as should alkyl substitution of the 3'-position of the phenyl ring. Analogs **3-5** were built and their activities predicted in the model.

The synthesis of analog **3** was planned utilizing ozonolysis chemistry of vinylsilanes as described previously in our laboratory for numerous artemisinin analogs.[6] While a peroxyaldehyde normally results from the ozonolysis of isolated vinyl silanes, when an adjacent pendent carbonyl is available, the intermediate 1,2-dioxetane can collapse in the presence of acid to a 1,2-dioxepane ring charactaristic of the A and B-rings of artemisinin.

Scheme I

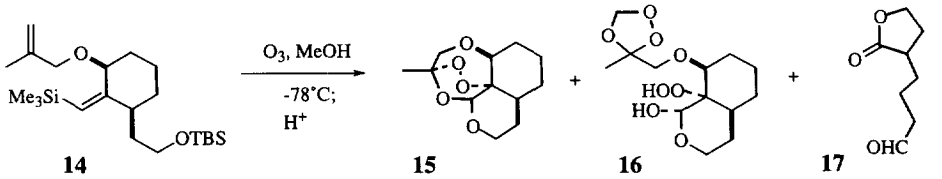


Reagents: (i) $Mn(OAc)_3$, PhH, reflux; 72% yield; (ii) CuBr-Me_2S, PhMgCl, THF, 0 °C; 68%; (iii) $Me_2(MeO)SiCH_2SiMe_3$, t-BuLi, pentane; then **8a**, THF; 15%; (iv) NaOH, MeOH; 42%; (v) NaH, DMF, $BrCH_2CH(OMe)=CH_2$, 0 °C; (vi) oxalic acid, SiO_2, CH_2Cl_2; 62% (overall yield for v and vi); (vii) O_3/O_2, MeOH; then BF_3-Et_2O; less than 5%.

In order to synthesize the vinylsilane precursor **12**, we used the modified Peterson olefination methodology reported by Thomas and coworkers in 1989.[11] In their approach, methoxy substitution for one of the methyl groups of bis(trimethylsilyl)methyl lithium gives the reagent, [(methoxydimethylsilyl)

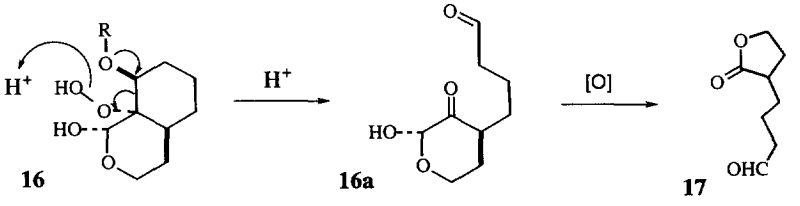
(trimethylsilyl)methyl]lithium, which reacts with enolizable ketones and aldehydes to form vinylsilanes rather than the exclusive deprotonation seen for the lithium anion of bis(trimethylsilyl)methane.

Scheme I shows the route we chose for forming the vinylsilane to be utilized in the ozonolysis. Conversion of 3-methylcyclohexenone to the α-acetoxyketone **7** was accomplished using $Mn(OAc)_3$ in refluxing benzene.[12] The resultant enone was then treated with phenylmagnesium chloride and 10 mol% of $CuBr\text{-}Me_2S$ to form the ketone **8**. Due to an obvious visual difference in crystal shapes, the two diastereomers of the ketone were easily isolated after recrystallization by mechanical separation. Compound **8a** was transformed into the vinylsilane using the modified Peterson olefination described above. The acetate group was then cleaved and the free alcohol was alkylated using methoxyallyl bromide to form compound **11**. Deprotection with oxalic acid resulted in the ketone, **12**. Frustratingly, ozonolysis of this vinylsilane proved to give only low yields of the peroxyaldehyde, **13**. We feel that the oxygen atom plays an important mechanistic role in disrupting the ability of ketone **12** to cleanly undergo post-ozonolytic rearrangement to desired products. Several by-products were observed and not identified in this system; however, by-products were determined in a related system. For example, a major side-reaction in the ozonolysis of **14** is the formation of the lactone **17**.



14 **15** **16** **17**

While product **16** could be circumvented by utilizing the side-chain ketone corresponding to **14**, the preponderance of ring cleaved structures such as **17** are indicative of an acid catalyzed fragmentation of peroxy-ethers like **16**, perhaps via **16a**. Further oxidation of the glycal intermediate could then account for the production of the butyrolactone **17**.



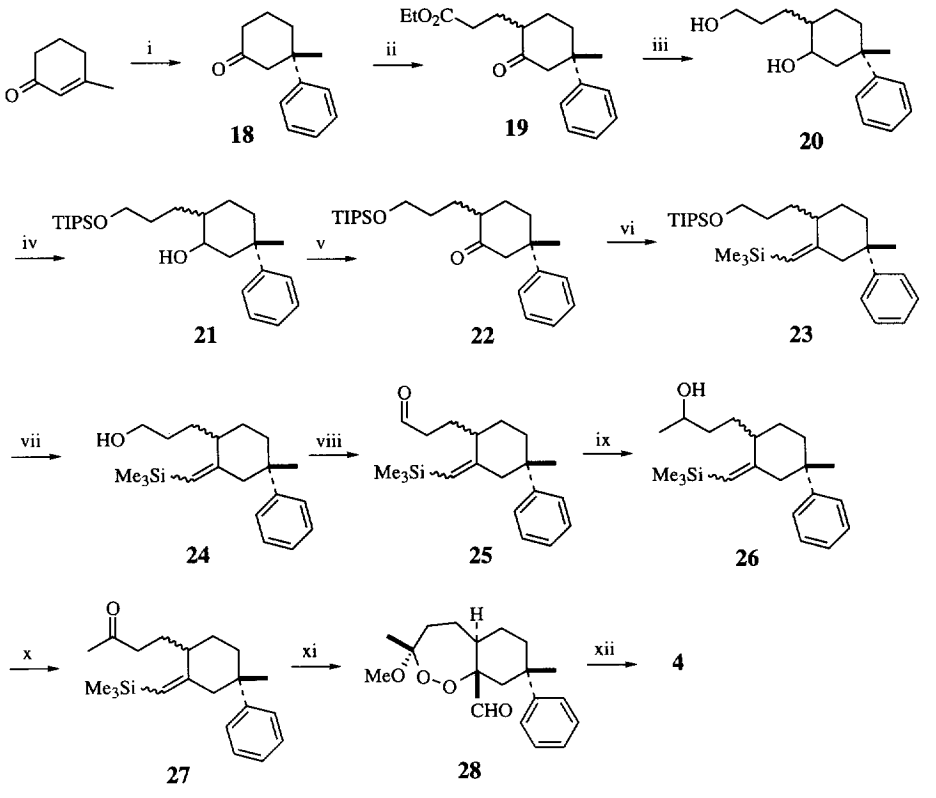
16 **16a** **17**

We abandoned the ozonolytic route to 5-oxa analogs and pursued a different, perhaps more pedestrian, avenue to a structurally related analog. This new compound, **4**, is structurally similar to compound **3** with a carbon replacing the oxygen at the 5-position. While any potential benefit in regards to reduced metabolism is thus lost for **4** relative to **3**, it was anticipated that the synthesis of **4** would at least be achievable by the vinylsilane-ozonolysis approach.

The synthesis of compound **4** is shown in Scheme II. Starting with 3-methylcyclohexenone, we utilized a Normant reaction to form ketone **18**. The pyrrolidine enamine of this ketone was then generated, followed by 1,4-addition of ethyl acrylate to afford keto-ester **19**. The resulting diastereomeric mixure was reduced to diol **20**

using lithium aluminum hydride and the primary alcohol was then protected using triisopropylsilyl chloride to furnish **21**. Swern oxidation of **21** produced the desired cyclohexanone **22**. Peterson olefination using the Bates reagent described above resulted in production of the vinylsilane **23** along with recovered starting material. While **23** existed as a predominant isomer corresponding ultimately to the desired stereoisomer, purification at this stage proved tedious and thus the mixture was carried forward without separation. However, at each stage the isomers were separated on an analytical scale and gave satisfactory analytical and spectral data.

Scheme II



Reagents: (i) PhMgCl, CuBr-Me_2S, THF, 0 °C; 45% yield; (ii) (a) pyrrolidine, PhH, reflux; (b) ethyl acrylate, 1,4-dioxane, reflux; 46%; (iii) LAH, 0 °C; 92%; (iv) TIPSCl, Et_3N, CH_2Cl_2; 91%; (v) Swern oxidation; 93%; (vi) $Me_2(MeO)SiCH_2SiMe_3$, t-BuLi, pentane; then **22**, THF/pentane; 39%; (vii) TBAF; 91%; (viii) Swern oxidation; 91%; (ix) MeMgBr; THF; 78%; (x) Swern oxidation; 83%; (xi) O_3, MeOH; then BF_3-Et_2O; 40%; (xii) (a) $NaBH_4$, (b) pTsA, CH_2Cl_2; 51%.

The triisopropylsilyl protecting group was then removed with fluoride ion to give **24**, which was then oxidized to aldehyde **25**. Finally, treatment of the aldehyde with methylmagnesium bromide, (to give **26**) followed by Swern oxidation yielded the penultimate ketone **27** required for the final reactions. This vinylsilane

ketone was a mixture of isomers and purification resulted in two fractions. The fraction with the highest R_f contained only one isomer, while the lower fraction was a mixture containing two other compounds. One of these compounds was the regioisomer of the vinylsilane, while the other compound was a diastereomer of the higher R_f compound. Ozonolysis of the higher fraction resulted in the formation of the peroxyaldehyde **28**, which by analogy to earlier work was reduced and cyclized to form the target compound **4**. The relative stereochemistry of the methyl group at C-8 was determined by DNOE experiments and was subsequently proven for compound **4** by single crystal X-ray diffraction analysis.[13]

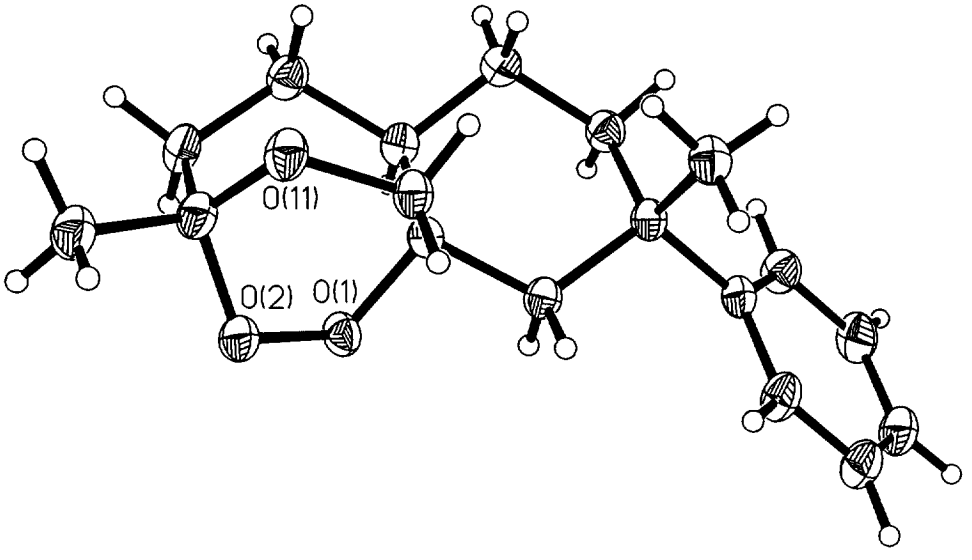


Figure 1. Perspective drawing of the X-ray structure for **4**. Non-hydrogen atoms are represented by thermal vibration ellipsoids drawn to encompass 50% of their electron density; hydrogen atoms are represented by arbitrary spheres not representative of thermal motion. The oxygen atoms are labeled.

Ozonolysis of the lower fraction followed by reduction and cyclization as before resulted in a mixture of compounds **4** and **5**. These isomers were separated by chromatography and pure **5** was obtained as a crystalline solid. Unlike **4**, **5** displayed no substantial DNOE between the trioxane methylene hydrogens and the ring methyl at C-8; otherwise, compounds **4** and **5** had similar NMR spectra.[14] Compounds **4** and **5** have undergone antimalarial testing *in vitro* and displayed activity at 13 and 132 ng/ml, respectively, in the W-2 clone of *P. falciparum* while the IC_{50} value for artemisinin was 4.2 ng/ml. These results will be discussed in relation to the QSAR model in the near future.[8]

Acknowledgment:

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13. Spectral data for compound **4**, (±)-Hexahydro-(1a,5aα,6β,7,8,8aα)-3,8β-dimethyl-3β,1aβ-[epoxymethano]-8α-phenylbenzo-1,2-dioxepine. mp = 112.5-113.5 °C. ^{1}H NMR ($CDCl_3$) δ 7.32-7.13 (m, 5H), 4.44 (dd, 1H, *J* = 0, 11.2 Hz), 4.15 (dd, 1H, *J* = 0, 11.2 Hz), 2.42 (ddd, 1H, *J* = 4.0, 12.4, 14.6 Hz), 2.05 (dd, 1H, *J* = 1.9, 14.1 Hz), 2.03 (ddd, 1H, *J* = 3.2, 3.3, 13.45 Hz), 1.87-1.82 (m, 2H), 1.77-1.65 (m, 5H), 1.55 (dd, 1H, *J* = 0, 14.2 Hz), 1.33 (s, 3H), 1.22 (s, 3H). ^{13}C NMR δ 151.3, 128.2, 125.8, 124.5, 103.8, 83.9, 65.6, 46.8, 46.7, 38.7, 37.5, 35.9, 27.7, 27.6, 26.7, 25.8. CIMS for $C_{18}H_{24}O_3$: calcd 288.1725, found 288.1743. Analysis calculated for $C_{18}H_{24}O_3$: C 74.95, H 8.39; found C 75.23, H 8.48%. Coordinates are available from the Cambridge Structural Database.
14. Spectral data for compound **5**, (±)-Hexahydro-(1a,5aα,6β,7,8,8aα)-3,8α-dimethyl-3β,1aβ-[epoxymethano]-8β-phenylbenzo-1,2-dioxepine. mp = 103.5-105 °C. ^{1}H NMR ($CDCl_3$) δ 7.38-7.14 (m, 5H), 4.10 (dd, 1H, *J* = 0, 10.6 Hz), 3.70 (dd, 1H, *J* = 0, 10.6), 2.59 (dd, 1H, *J* = 2.7, 13.9 Hz), 2.50 (ddd, 1H, *J* = 3.0, 5.9, 13.9 Hz), 2.25-2.15 (m, 2H), 1.95-1.85 (m, 1H), 1.8-1.7 (m, 2H), 1.42-1.21 (m, 6H), 1.22 (s, 3H), 1.12 (s, 3H). ^{13}C NMR δ 146.8, 127.8, 125.8, 125.4, 103.5, 81.7, 68.7, 46.2, 44.0, 37.5, 36.6, 36.5, 35.9, 26.7, 26.1, 25.8. CIMS for $C_{18}H_{24}O_3$: calcd (M+H) 289.1808, found (M+H) 289.1803. Analysis calculated for $C_{18}H_{24}O_3$: C 74.95, H 8.39; found C 74.85, H 8.65%.

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