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Synthesis of antitrypanosomal 1,2-dioxane derivatives based on a natural product scaffold

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ABSTRACT

A short practical synthesis of a new natural product based scaffold (**6**), based on antitrypanosomal and antimalarial compounds isolated from different *Plakortis* species is described. The scaffold contains a peroxide unit that is surprisingly stable to chemical manipulation elsewhere in the molecule, enabling it to be elaborated into a small library of derivatives. It is stable to ozonolysis, reductive work-up with dimethylsulfide and the Wittig reaction with stabilized phosphorus ylides. The scaffold along with its Wittig analogues has displayed low to sub-micro molar (0.2–3.3 μM) antitrypanosomal activity.

Human African Trypanosomiasis (HAT) and Malaria are two of the world's deadliest diseases, with more than one million deaths worldwide in 2008, most of them in sub-Saharan Africa and Asia.[1] Owing to the complexity of these diseases and to the emergence of parasites that are resistant to currently available anti-HAT and antimalarial drugs, there is an urgent need for new, effective and affordable drugs.[2]

During the course of our on-going drug discovery program aimed at new antitrypanosomal compounds from natural sources, we found that 11,12-didehydro-13-oxo-plakortide Q (**1**), isolated from the Australian marine sponge *Plakortis* species, exhibited antitrypanosomal activity at an IC_{50} of 49 nM against *Trypanosoma brucei brucei* (one of the parasites responsible for HAT).[3] Interestingly, the methyl ester of peroxyplakortic acid B_3 (**2**) from the Okinawan marine sponge *Plakortis* sp.[4] and plakortin[5] (**3**), along with a number of analogues from *Plakortis simplex,* have shown sub-micromolar to low micromolar in vitro antimalarial activity.[6] A comparison of **1**–**3** with the known antimalarial artemisnin[7] (**4**) and yingzhaosu A[8] (**5**) suggested that the substituted 1,2-dioxane (endoperoxide) moiety might be the core skeleton responsible for the antimalarial and antitrypanosomal activity (Fig. 1).

Although a number of cyclic peroxides with antimalarial, anti-HAT and cytotoxic profiles have been reported, effective methods for their synthesis or for generating analogues are limited.[9a,b] This is not only because of the limited number of methods available for generating cyclic peroxides, but importantly, the 1,2-dioxane system is not stable to many of the reaction conditions required for further functional group manipulations. For example, the peroxide moiety is not stable to metal-mediated reactions, most reducing agents and strong bases.[10a,b] Thus the synthesis of compounds such as **1**–**3**, and analogues required for structure–activity relationship (SAR) studies, presents a significant challenge, often involving

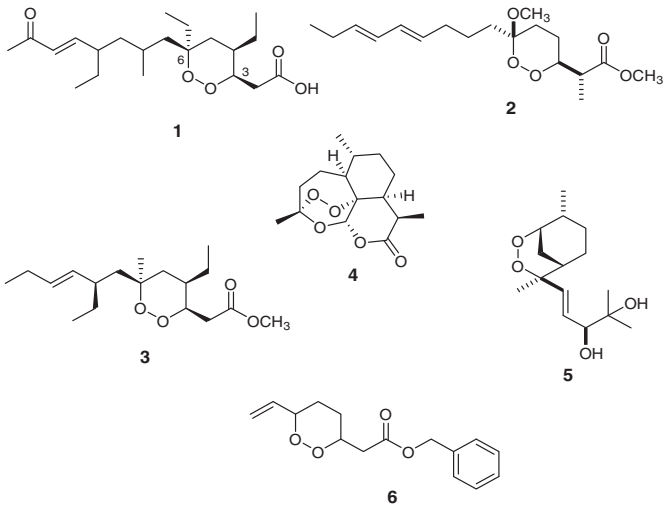

Figure 1.

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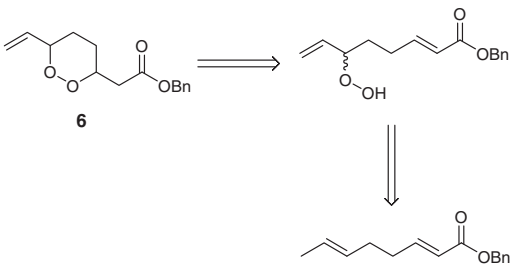
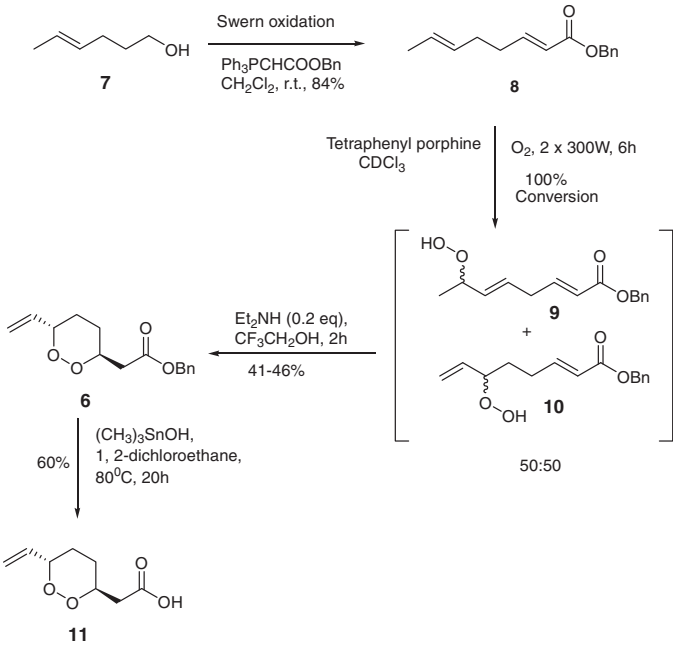


Scheme 1. Retrosynthesis of scaffold **6**.



Scheme 2. Synthesis of scaffold **6**.

rather lengthy syntheses with introduction of the peroxide functionality late in the synthesis.[11–14] For example, the most recent synthesis of the dihydro analogue of **3** requires 18 steps.[11a] Such a lengthy synthesis would generally preclude the development of an affordable drug to treat HAT. An alternative approach to drug discovery, and one that we are exploring, is to prepare a natural product-based scaffold or building block[15] (<6-step synthesis) and to convert this into a small focused library of analogues. An example of this approach is outlined below.

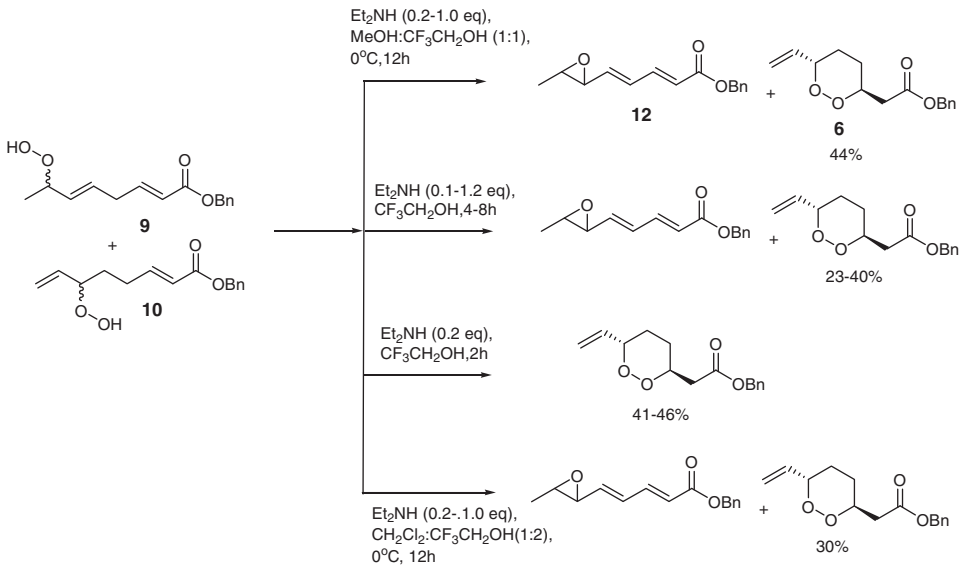
Our initial target was the scaffold **6**, which contains both the 1,2-dioxane ring and carboxylate moieties present in **1**–**3**, as well as a vinyl 'handle' with potential for further synthetic elaboration. The benzyl ester was chosen for convenience (UV chromophore), and we used established chemistry to develop a short synthesis of **6**.

A retrosynthetic analysis (Scheme 1), suggested that the 1,2–dioxane could be generated by intramolecular Michael addition of a hydroperoxy enoate, which in turn could be obtained by a singlet oxygen ene-reaction (Schenck reaction) of the corresponding 2,6-dienoate.[16]

Swern oxidation of (*E*)-hex-4-en-1-ol (**7**) followed by Wittig reaction with benzyl triphenylphosphoranylidene acetate in a one-pot procedure gave the desired (2*E*,6*E*)-benzyl octa-2,6-dienoate (**8**) in 84% isolated yield (Scheme 2).[17] The singlet oxygen ($^1O_2$) ene-reaction on **8** was achieved by photo oxidation using two 300 W flood lamps in $CDCl_3$ at 5–10 °C for 6 h with a catalytic amount of tetraphenylporphine as sensitizer. This resulted in the formation of the allylic hydroperoxides **9** and **10** as a regioisomeric mixture (50:50). Changing the solvent (CD_2Cl_2, CD_3COCD_3), temperature (−78 °C to room temperature), or sensitizer (methylene blue, rose bengal) did not improve the yield of the desired regioisomer, (*E*)-benzyl 6-hydroperoxyocta-2,7-dienoate (**10**). Attempted separation of **9** and **10** by flash chromatography was unsuccessful, however it was found that the presence of the undesired regioisomer **9** in the reaction mixture did not affect the subsequent intramolecular Michael addition of hydroperoxide to the α,β-unsaturated benzyl ester in **10** (Scheme 2).

The Michael addition to give the scaffold **6** was carried out using the protocol employed by Kobayashi and co-workers.[13a] Small changes in the reaction conditions resulted in significant changes in the yield of **6** (Scheme 3).

A side reaction observed when more than 0.5 equiv of Et_2NH were employed, was the formation of the epoxy dialkenoate **12**, presumably formed by nucleophilic attack by the 5,6-double bond



Scheme 3. Standardization for intramolecular Michael addition of allylic hydroperoxide to scaffold **6**.

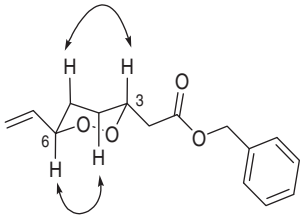


Figure 2. Key NOESY correlations of (**6**).

Table 1
Reaction conditions for one pot ozonolysis and Wittig reaction on (**6**)

S. No.	Condition	Yield (%)
1	Heat for 12 h	30–50
2	Et_3N (2.0 equiv)	Nil
3	4-Methoxymorpholine *N*-oxide (3.0 equiv)	Nil
4	K_2CO_3 (2.0 equiv)	Nil
5	Me_2S (6.0 equiv) for 2 h	30
6	Me_2S (10.0 equiv) for 12 h	60–70

on the hydroperoxide **9** with loss of hydroxide and (possibly concerted) removal of a proton from the 4-position to give the conjugated diene and the 6,7-epoxide ring. Use of 0.2 equiv of Et_2NH in CF_3CH_2OH at room temperature for 2 h gave a reasonable yield (41–46%) of scaffold **6**, with negligible amounts of the epoxy dialkenoate. Interestingly, only one diastereomer of the scaffold **6** was obtained. By employing 2D NOESY analysis in deuterated pyridine, the relative configuration of the two substituents on the scaffold was found to be *trans*, with the benzyl ester and alkene side chains at the 3 and 6 positions preferring equatorial orientations as expected (Fig. 2).

The hydrolysis of the benzyl ester group using LiOH led to cleavage of the peroxide bond. However, Nicolaou's mild method of hydrolysis employing trimethyltin hydroxide at 80 °C in 1,2-dichloroethane was found to be effective in removal of the benzyl group to give peroxy acid **11** (the free acid form of scaffold **6**).[18]

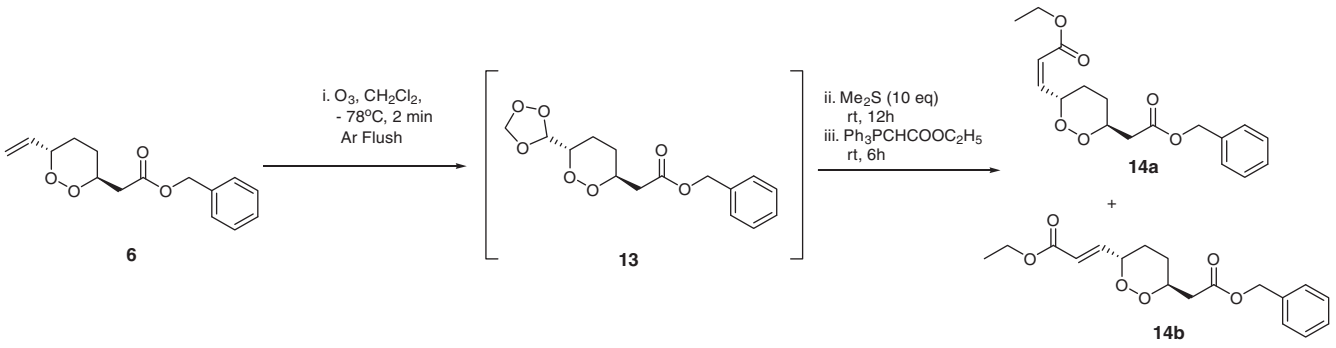
With the synthesis of the scaffold accomplished, generating different analogues using the vinyl side chain at C6 as a handle was the next task. Initially we attempted to extend the side chain via a cross-metathesis reaction employing 4-methylstyrene and either Grubbs I or II as catalyst. Unfortunately, the peroxide linkage was incompatible with the Grubbs catalyst and although we observed some cross metathesis products, none contained the peroxide moiety. We then turned our attention to a protocol involving oxidative cleavage of the vinyl group to an aldehyde followed by elaboration with a Wittig reagent (Scheme 4). The scaffold **6** was treated with ozone in anhydrous dichloromethane at −78 °C until the color of the reaction mixture turned to blue (an indication of excess ozone and complete conversion of the scaffold to its ozonide **13**; the reaction was also followed by TLC and NMR). Excess dimethylsulfide was then added and the mixture stirred at room temperature for 12 h to ensure decomposition of the ozonide. This was followed by addition of a range of stabilized phosphorus ylides (Table 2) to obtain the corresponding chain-extended products as mixtures of *E*- and *Z*- isomers that could be separated in all cases except the nitrile **19**.[21]

Initially the one-pot reaction was carried out by adding the phosphorus ylide directly to the reaction mixture immediately following ozonolysis according to the procedure of Hon et al.[19] The reaction was continued at room temperature for 12 h to obtain a 30–50% combined yield of *E* and *Z* products. In order to improve the yield, we also tried various bases, as shown in Table 1.[20] However, use of either triethylamine or 4-methoxymorpholine-*N*-oxide, led to cleavage of the O–O bond. Best results were obtained using dimethylsulfide for reductive work-up of the ozonide. Interestingly, dimethylsulfide appears to selectively react with the ozonide, and not the peroxide under these conditions (room temperature for 12 h).

The synthesized peroxy ester derivatives along with the scaffold **6** were screened for antitrypanosomal activities against *T. brucei brucei* BF427 (Table 3).[22] Interestingly, the scaffold **6** itself displayed significant (IC_{50} 3.03 μM) activity, providing support for our hypothesis that the substituted 1,2-dioxane moiety is the core skeleton responsible for the antimalarial and antitrypanosomal activity. Further, elaboration of the scaffold to give the analogues **14**–**19** generally resulted in improved potency, with analogues **15b** and **16a**, for example, displaying a 10-fold increase in potency (IC_{50} 0.2 and 0.24 μM, respectively). This provides some support for our strategy of natural product scaffold-based drug discovery.

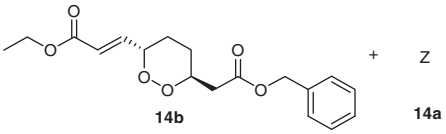
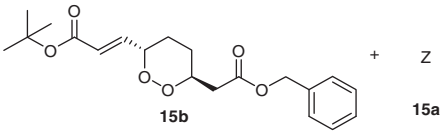
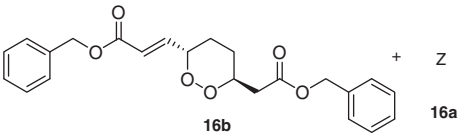
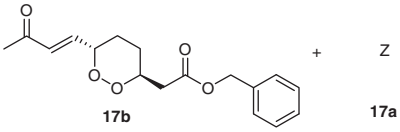
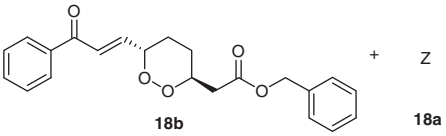
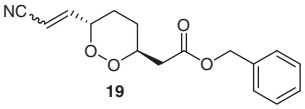
The fact that neither the size of the substituents nor the stereochemistry of the analogues **14**–**18** had a major effect on the activity suggests that the binding pocket at this site might be fairly large or open. The in silico physicochemical profiling of the compounds carried out with Instant *J*-Chem 2.5.2 software (Table 3), showed that all of the synthesized compounds obey Lipinski's rule of five for drug likeness.[23] All compounds have molecular weight (MW) less than 500 Da and partition coefficient (*c*Log*P*) along with hydrogen bond accepter count (HBA) less than 5. The percent polar surface area (%PSA) was also found to be less than 140 $Å^2$ which satisfies the parameter for oral bioavailability of these compounds.[24]

In conclusion, we have developed a short and practical route for synthesizing a new 1,2-dioxane scaffold based on the structure of several bioactive natural products. The scaffold contains a vinyl group at C6 that can be readily chain-extended while keeping intact the peroxide bond. The scaffold along with a



Scheme 4. One-pot ozonolysis followed by Wittig reaction on (**6**).

Table 2
Wittig derivatives of scaffold **6**

S. No.	Phosphorus Ylide	Product	Yield (*E* + *Z*) (%)
1	$Ph_3P{=}COOC_2H_5$	 **14b** + Z **14a**	(47 + 27)
2	$Ph_3P{=}COOC(CH_3)_3$	 **15b** + Z **15a**	(40 + 23)
3	$Ph_3P{=}COOCH_2C_6H_5$	 **16b** + Z **16a**	(45 + 22)
4	$Ph_3P{=}COCH_3$	 **17b** + Z **17a**	(16 + 5)
5	$Ph_3P{=}COC_6H_5$	 **18b** + Z **18a**	(45 + 14)
6	$Ph_3P{=}CHCN$	 **19**	(61)

E-trans isomer; *Z-cis* isomer.

Table 3
Antitrypanosomal activities of scaffold (**6**) and its Wittig analogues

Compound	Physicochemical parameter[a]					IC_{50} ± SD (μM) *T. b. brucei* BF427
	MW	*c* log *P*	HBA	HBD	% PSA	
6	262.30	3.15	3	0	11.3	3.03 ± 0.78
14a	334.36	3.31	4	0	14.1	1.35 ± 0.05
14b	334.36	3.31	4	0	14.1	0.62 ± 0.12
15a	362.42	4.01	4	0	12.5	0.37 ± 0.09
15b	363.42	4.01	4	0	12.5	0.2 ± 0.025
16a	396.43	4.68	4	0	12.3	0.24 ± 0.017
16b	396.43	4.68	4	0	12.3	0.64 ± 0.09
17a	304.34	2.91	4	0	13.6	1.88 ± 0.44
17b	304.34	2.91	4	0	13.6	0.9 ± 0.032
18a	366.15	4.33	4	0	11.7	0.58 ± 0.11
18b	366.15	4.33	4	0	11.7	0.51 ± 0.07
19	287.31	2.80	4	0	16.6	3.14 ± 0.42
Pentamidine isethionate salt						0.00086 ± 0.007

HBA-hydrogen bond donor; HBD-hydrogen bond acceptor.
[a] In silico calculations by Instant *J*-Chem 2.5.2 software.

small library of analogues displayed low to sub-micro molar (0.2–3.3 μM) antitrypanosomal activity. Synthesis of a range of analogues of this and related scaffolds is being pursued for comprehensive SAR studies.

Acknowledgements

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Supplementary data

Supplementary data (detailed procedure and NMR data) associated with this article can be found, in the online version, at doi:10.1016/j.bmcl.2011.06.059.

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21. *General procedure for one pot ozonolysis followed by Wittig reaction on scaffold* ***6***: A solution of scaffold (**6**) (1 equiv) in 5 mL dry CH_2Cl_2 at −78 °C was treated with stream of O_3/O_2 for approximately 2 min until the solution turned steady blue in color. Excess ozone was removed from the solution by flushing with Argon. Dimethyl sulfide (10 equiv) was added to this solution. The solution was allowed to stir for 12 h at room temperature followed by addition of respective stabilized triphenylphosphorane (2 equiv) and further stirred for 4 h. Then, the reaction mixture was quenched with water and extracted with excess CH_2Cl_2 and brine. The organic phase was separated, dried over $MgSO_4$ and evaporated under reduced pressure. The crude mass was subjected to column chromatography. The *E* and *Z* products were separated using hexane/ethyl acetate as eluent. The spectral data for selected products. *Compound* ***14a***: (*Z*)-ethyl 3-[(3*S*,6*S*)-6-(2-(benzyloxy)-2-oxoethyl)-1,2-dioxan-3-yl]acrylate; pale yellow oil; IR (KBr): ν_{max} 2954, 1720, 1193, 1024, 820 cm^{-1}; ^1H NMR (500 MHz, $CDCl_3$): δ 7.35 (m, 5H), 5.98 (dd, J = 11.8, 7.2 Hz, 1H), 5.83 (dd, J = 11.8, 1.1 Hz, 1H), 5.61 (br t, 1H), 5.14 (s, 2H), 4.59 (m, 1H), 4.17 (q, J = 7.0 Hz, 1H), 2.58 (dd, J = 15.8, 7.4 Hz, 1H), 2.44 (dd, J = 15.8, 5.6 Hz, 1H), 2.06 (m, 1H), 1.91 (m, 1H), 1.67 (m, 2H), 1.29 (t, J = 7.1 Hz, 3H); ^{13}C NMR (125 MHz, $CDCl_3$): δ 169.7, 165.2, 144.1, 135.6, 128.5, 128.2, 128.1, 120.8, 78.8, 77.5, 66.6, 60.4, 38.4, 28.4, 27.9, 14.1. HRMS (ESI): calcd for $C_{18}H_{22}O_6Na$ $[M+Na]^+$ 357.1308, found 357.1302.
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