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Artemisinin-dipeptidyl vinyl sulfone hybrid molecules: design, synthesis and preliminary SAR for antiplasmodial activity and falcipain-2 inhibition

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Abstract

A series of artemisinin-vinyl sulfone hybrid molecules with the potential to act in the parasite food vacuole *via* endoperoxide activation and falcipain inhibition was synthesized and screened for antiplasmodial activity and falcipain-2 inhibition. All conjugates were active against the *Plasmodium falciparum* W2 strain in the low nanomolar range and those containing the Leu-hPhe core inhibited falcipain-2 in low micromolar range.

Keywords

antimalarial; artemisinin; FP-2; Vinylsulfone

Artemisinin, **1**, a sesquiterpene lactone isolated from the *Artemisia annua* Chinese herb, and its analogues (e.g. artemether, **2**, arteether, **3**, and artesunate, **4**) were a major breakthrough in malaria chemotherapy because they produce a very rapid therapeutic response, particularly against multidrug-resistant *Plasmodium falciparum* malaria.[1,2] Despite the rapid clearance of parasites, the short half-lives of these compounds lead to many late recrudescences after monotherapy.[3] Thus, artemisinin-based combination therapy (ACT) has now been recommended by the World Health Organisation as standard therapy for falciparum malaria.[4]

Cysteine proteases from malaria parasites are of particular interest as therapeutic targets due to their role in parasite development.[5] *P. falciparum* expresses four cysteine proteases from the papain family known as falcipains, of which falcipain-2 (FP-2)[6,7] and falcipain-3 (FP-3)[7,8] are the most relevant as therapeutic targets. Peptidyl vinyl sulfones, e.g. **5**, are potent irreversible inhibitors of falcipains, acting as Michael acceptors of the catalytic cysteine residue.[9] Falcipain inhibitors have been shown to inhibit the development of cultured erythrocytic parasites by blocking the hydrolysis of host hemoglobin and to cure mice infected with lethal malaria infections.[10]

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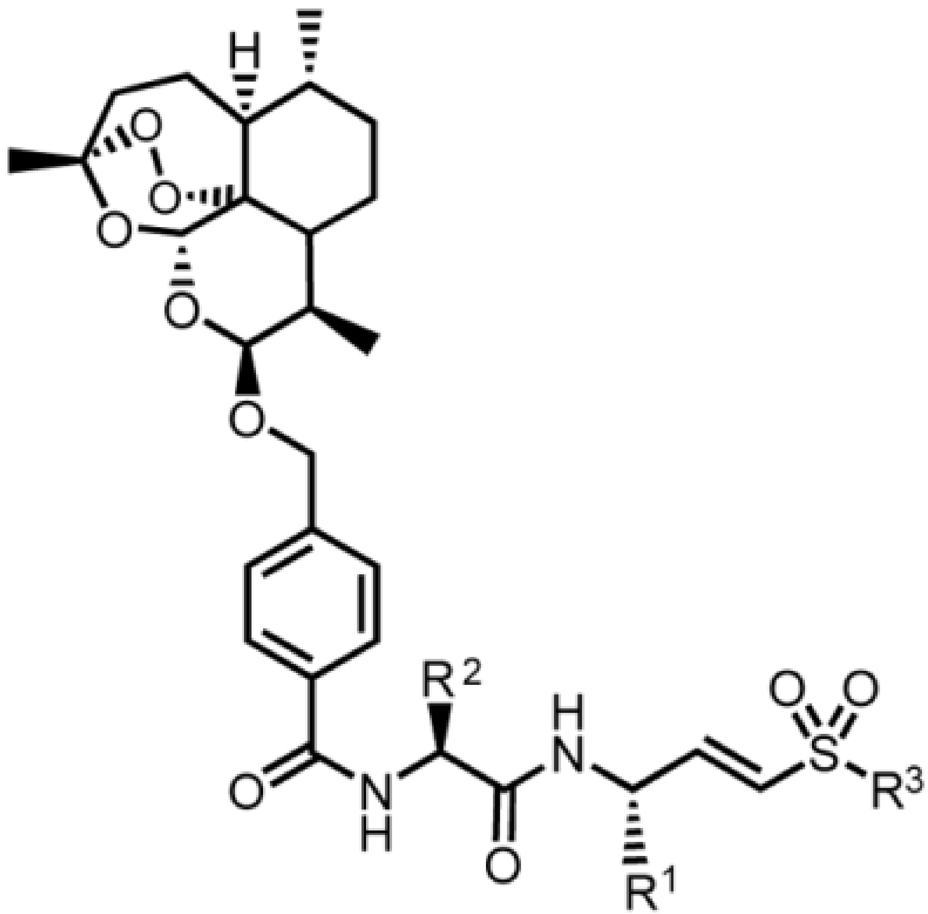
A concern regarding the use of protease inhibitors as antimalarials is that selection of drug-resistant mutants will eventually occur. Indeed, parasites resistant to a dipeptidyl vinyl sulfone have been selected in the laboratory, although this resistance was somewhat unstable.[11] Thus, dipeptidyl vinyl sulfones are obvious candidates for combination antimalarial therapy as a strategy to retard the development of resistance. This information prompted us to design artemisinin-vinyl sulfone hybrid molecules with the potential to help prevent multi-drug resistance in *P. falciparum* malaria. It has recently been shown that hybrid molecules in which the two antimalarials are combined via a linker can offer an effective means of delivering these agents to the parasite site of action.[12–14]



6

R^1 CH_2CH_2Ph, CH_2Ph

R^2 CH_2CHMe_2, CH_2Ph, H

R^3 Ph, Me

Structure-activity relationship (SAR) data for the inhibition of FP-2 reveals that peptidyl vinyl sulfones containing a Leu residue at the P_2 position and a hPhe (homophenylalanine) at the P_1 position, e.g. **5**, are the most active, with IC_{50} values in the low-nM range.[6,8,15,16] With this information in hand we designed hybrid molecules **6**, in which the vinyl sulfone component is linked to the endoperoxide moiety via the *N*-terminus, using a 4-hydroxymethylbenzoic acid linker. In addition to the Leu-hPhe sequence, compounds **6** containing the Phe-hPhe and Phe-Phe moieties were also prepared based on the SAR against falcipains [16] and cruzain, [17] a related cysteine protease from *Trypanosoma cruzi*. The substituent at the P_1' position was a methyl or phenyl group.

The synthesis of compounds **6** involved the preparation of aldehydes **9** containing the P_1 residue, using Weinreb chemistry and the appropriate N^{α}-Boc-protected amino acids **7** (Scheme 1).[18,19]. The Horner-Wadsworth-Emmons reaction of **9** with the appropriate sulfones **10**, prepared by oxidation of the corresponding sulfide with H_2O_2 in AcOH,[18] afforded the amino acyl vinyl sulfones, **11**. The Boc group was removed with trifluoroacetic acid (TFA) and the resulting trifluoroacetates were then reacted with the second N^{α}-Boc-protected amino acid and TBTU, to yield the N^{α}-Boc-protected dipeptidyl vinyl sulfones **12a–f**. These were quantitatively deprotected to **13a–f** with TFA. Finally, compounds **13** were converted in reasonable to good yields into the target compounds, **6a–f**, by reaction with **15** (artelinic acid, synthesized from dihydroartemisinin, **14** 20) and TBTU.[21] The hybrid molecules **6a–f** were isolated as single isomers as shown by the ^{1}H-NMR spectra [21], which presented (i) only one singlet at δ ca. 5.4 ppm, corresponding to the H-12 signal and (ii) a small coupling constant for the H-10 signal at δ 4.9 ppm, *J* ca 4 Hz, indicative of a vicinal equatorial-axial coupling with H-9. This result, which is similar to that for precursor **15** (*J* = 3.7 Hz for the H-10 signal), is consistent with the β-isomer at C-10.

The semi-synthetic artemisinin derivatives **6a–f** were screened for FP-2 inhibition and compared to dipeptidyl vinyl sulfones and E64 (Table 1). Selectivity assays were also carried out by testing compounds **6a–d** against chabaupain-1 (CP-1), a cysteine protease from the murine parasite *P. chabaudi*.[22] Inspection of the data in Table 1, shows that the Leu-hPhe sequence leads to a higher level of FP-2 inhibition than the Phe-hPhe (**6d** *versus* **6c** and **6f** *versus* **6e**) or Phe-Phe counterparts (**6c** *versus* **6b**), in line with the SAR for dipeptidyl vinyl sulfones.[15,16] In contrast, a Phe residue at the P_2 position is preferred for CP-1 inhibition (e.g. **6b** and **6c**), implying that this enzyme presents a different structural requirement for molecular recognition at this position. The presence of the endoperoxide moiety at the amino (P_3) terminus of the dipeptide sequence decreases significantly the inhibitory potency. The IC_{50} ratio for compounds **6d**, **12d** and **5** is 1650:70:1, which suggests that voluminous groups at position P_3 in dipeptidyl vinyl sulfones are deleterious for FP-2 inhibition. Finally, the effect of substituents at the P_1' position seems to be dependent on the dipeptide core. Interestingly, the reduction in activity observed when the phenyl group is exchanged for a methyl at P_1' in compounds with the Phe-hPhe moiety (**6c** *versus* **6e**) is in line with that reported for Mu-Phe-hPhe vinyl sulfones.[16]

The antiplasmodial activity of compounds **6a–f** was screened against the chloroquine-resistant W2 strain of *P. falciparum* (Table 1). All hybrids **6** displayed activity in the nM range, being more active than artemisinin and equipotent to artelinic acid, **15**. This result strongly suggests that the endoperoxide pharmacophore is the major contributor to the antiplasmodial activity exerted by compounds **6**. This hypothesis is further supported by the absence of swollen food vacuoles in trophozoites incubated with **6**. We have previously shown that this specific abnormality, observed when parasites are incubated with dipeptidyl vinyl sulfones and E64, is indicative of a block in hemoglobin hydrolysis.[23] The lack of a food vacuole abnormality for derivatives **6** can be explained by the relatively poor activity of hybrids against FP-2 and/or limited access to the food vacuole. Compounds **6a**, **6e** and **6f** were also screened against 4 additional *P falciparum* strains with different phenotypes: FCR3 (atovaquone resistant), 3D7 (chloroquine-sensitive), V1/S (chloroquine and pyrimethamine resistant) and D6 (chloroquine sensitive, mefloquine resistant) (Table 2). The IC_{50} values show the superior activity of compounds **6a**, **6e** and **6f** when compared to chloroquine and artemisinin against all strains.

In summary, a new class of hybrid molecules, **6**, based on dipeptidyl vinyl sulfone and artemisinin cores has been synthesized and shown to display potent antiplasmodial activity against a panel of *P. falciparum* chloroquine-sensitive and multidrug-resistant strains, with IC_{50} values ranging from 2 to 5 nM. Despite the fact that these hybrids incorporate the structural elements required for falcipain inhibition (e.g. Leu residue at P_2), they inhibited FP-2 only in the μM range. These results indicate that, although the artemisinin core or the linker may not be suitable for optimal enzyme binding, there is space to improve the bi-functional molecules as the SAR for both activities is better understood. The synthesis of novel hybrid molecules incorporating vinyl sulfone and artemisinin cores is underway.

Acknowledgments

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21. Compound 6d. To a solution of 15 (103.7 mg, 0.248 mmol) in DMF (2 ml) stirred at 0°C were added TBTU (86.3 mg, 0.258 mmol), Et_3N (35 μl, 0.249 mmol) and a solution of 13d (129.6 mg, 0.245 mmol) and Et_3N (35 μl, 0.249 mmol) in DMF (2 ml). The reaction was allowed to warm slowly to

room temperature and monitored by TLC. After completion, the reaction mixture was diluted with AcOEt (25 ml) and then poured into saturated $NaHCO_3$ (25 ml). The layers were separated and the aqueous layer was extracted twice with AcOEt (15 ml). The combined organic layers were treated with saturated $NaHCO_3$, HCl 1N and brine and then dried over Na_2SO_4. The solvent was removed and the crude product was purified by column chromatography using AcOEt/hexane (1:1), to give 6d, 53 % (106.7 mg) yield, as a white solid, mp 102–104 °C. ^{1}H NMR (400 MHz, $CDCl_3$) δ_H (ppm) 7.89 (2H, m), 7.74 (2H, d, 8.4 Hz), 7.65 (1H, m), 7.56 (2H, t, 7.6 Hz), 7.40 (2H, d, 8.4 Hz), 7.20 (3H, m), 7.03 (2H, m), 6.93 (1 H, dd, 15.2, 5.2 Hz), 6.70 (1H, d, 8.4 Hz), 6.48 (1H, dd, 15.2, 1.6 Hz), 6.44 (1H, d, 8.0 Hz), 5.47 (1H, s), 4.96 (1H, d, 13.2 Hz), 4.93 (1H, d, 3.6 Hz), 4.74-4.65 (1H, m), 4.64-4.55 (1H, m), 4.58 (1H, d, 13.2 Hz), 2.71 (1H, m), 2.59 (2H, m), 2.40 (1H, m), 2.07 (1H, m), 2.02-1.73 (5H, m), 1.70-1.60 (5H, m), 1.57-1.25 (7H, m), 1.02-0.90 (12H, m). ^{13}C NMR (100 MHz, $CDCl_3$) δ_C (ppm) 171.5, 167.7, 145.4, 142,9, 140.3, 140.1, 133.6, 132.3, 130.9, 129.4, 128.6, 128.4, 127.7, 127.3, 127.1, 126.3, 104.2, 101.6, 88.1, 81.1, 69.1, 52.5, 52.2, 49.2, 44.3, 40.2, 37.4, 36.4, 35.6, 34.6, 31,8, 30.9, 26.2, 25.0, 24.7, 24.5, 22.9, 22.2, 20.3, 13.1. Anal. (C, H, N): Cal. for $C_{46}H_{58}N_2O_9S$: C, 67.73; H, 7.12; N, 3.44; Found: C, 67.58; H, 7.20; N, 3.35. ESI/MS (m/z): Cal. for $C_{46}H_{58}N_2O_9S.Na^+$: 838.04; Found: 838.24.

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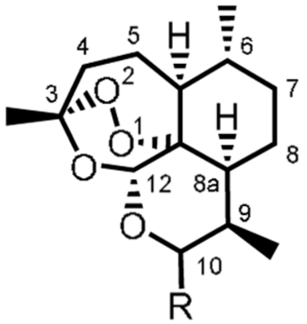
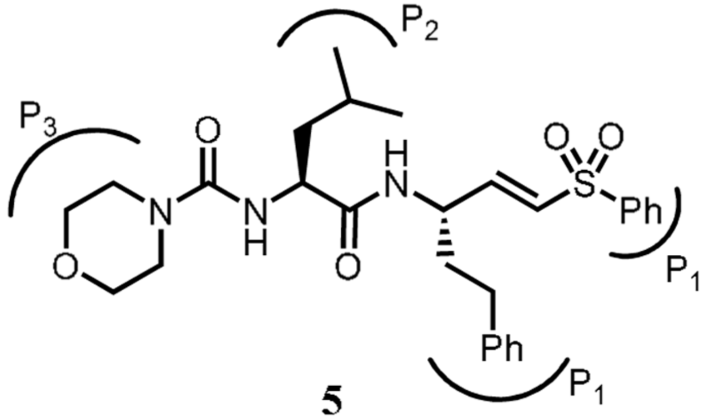


1, R = =O

2, R = β-Me

3, R = β-Et

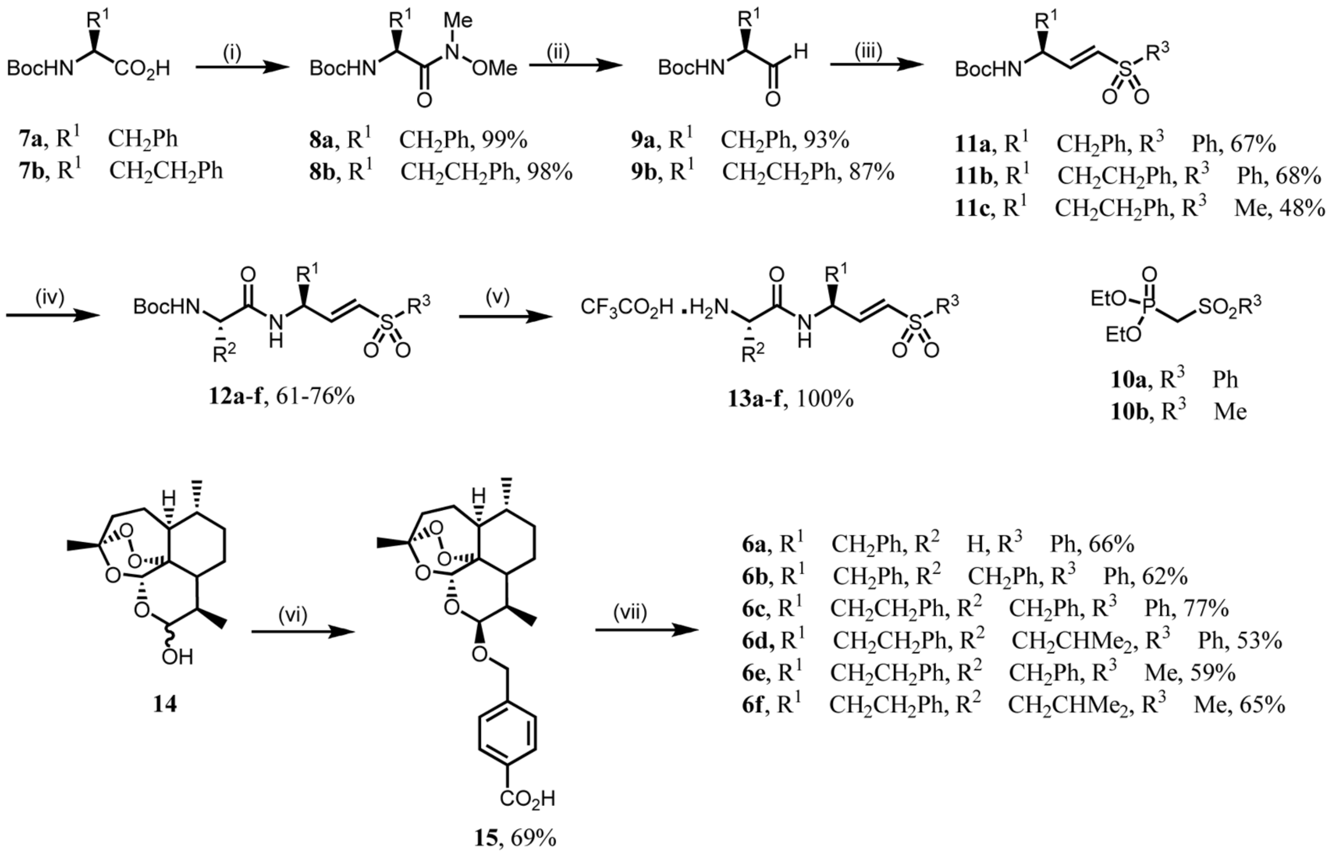
4, R = α-$CO(CH_2)_2CO_2Na$



5

Figure 1.

Structures of artemisinin **1**, artemether, **2**, arteeether, **3**, sodium artesunate, **4**, and a dipeptidyl vinyl sulfone, Mu-Leu-hPhe-VSPh, **5**.



Scheme 1.

Reagents and conditions: (i) TBTU, TEA, HN(Me)OMe, DCM; (ii) $LiAlH_4$, THF; (iii) **10a** or **10b**, NaH, THF; (iv) a) TFA, DCM; b) BocAAOH, TBTU, HOBt, TEA, DMF; (v) TFA, DCM; (vi) $BF_3.OEt_2$, $HOCH_2C_6H_4CO_2H$; (vii) **13a–f**, TBTU, TEA, DMF, rt.

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Table 1

Effect of hybrid compounds **6**, artemisinin (**1**), artelinic acid (**15**), dipeptidyl vinyl sulfones **5** and **12d** and E64 on the inhibition of falcipain-2, chabaupain-1, and growth of *P. falciparum* W2 strain.

Compound	R^1	R^2	R^3	IC_{50}/μM		IC_{50}/nM
				FP-2[a]	CP-1[b]	W2 *P. falciparum*[a]
Artemisinin	--	--	--	ND	ND	12.0±1.97
15	--	--	--	ND	ND	5.66±0.58
6a	CH_2Ph	H	Ph	16.5	56.8	4.09±0.13
6b	CH_2Ph	CH_2Ph	Ph	22.4	0.40	2.27±0.73
6c	CH_2CH_2Ph	CH_2Ph	Ph	9.22	0.538	3.94±0.28
6d	CH_2CH_2Ph	CH_2CHMe_2	Ph	4.95	2.29	2.08±0.89
6e	CH_2CH_2Ph	CH_2Ph	Me	21.6	ND	4.81±0.12
6f	CH_2CH_2Ph	CH_2CHMe_2	Me	0.35	ND	4.21±0.56
12d	CH_2CH_2Ph	CH_2CHMe_2	Ph	0.21	ND	>10000
5	CH_2CH_2Ph	CH_2CHMe_2	Ph	0.003[c]	ND	22.0[d]
E64	--	--	--	0.084	0.009	1955±121

[a]Assays of falcipain inhibition and parasite development were determined as described earlier [15]; assay of chabaupain inhibition was performed as described previously [22];

[c]non-recombinant falcipain [16];

[d]Itg2 strain [16]; ND, not determined.

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Table 2

IC_{50} values of compounds **6a**, **6e** and **6f**, chloroquine and artemisinin against the W2, FCR3,3D7, VI/S and D6 *P. falciparum* strains.

Compd	IC_{50}/nM				
	W2	**FCR3**	**3D7**	**VI/S**	**D6**
Chloroquine	78.1±6.91	51.1±0.1	11.6±1.1	65.0±4.1	16.9±2.1
Artemisinin	12.0±1.97	5.38±0.54	9.71±4.18	4.24±0.54	14.5±1.2
6a	4.09±0.13	1.75±0.32	3.11±0.72	1.59±0.11	4.71±0.12
6e	4.81±0.12	2.00±0.02	2.50±1.51	2.25±0.24	4.75±0.37
6f	4.21±0.56	1.89±0.10	2.48±0.41	1.65±0.11	4.96±0.66