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Research Article

Synthesis and Antimalarial Bioassay of Quinine – Peptide Conjugates

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Amino acid and peptide conjugates of quinine were synthesized using microwave irradiation in 52–95% yields using benzotriazole methodology. The majority of these conjugates retain *in vitro* antimalarial activity with IC_{50} values below 100 nM, similar to quinine.

Key words: amino acid, chemical biology, drug design, peptide

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Malaria is a devastating and common infectious disease, responsible for more than one million deaths each year. Quinine (Figure 1), an alkaloid isolated from the bark of the cinchona tree (1), has been the most acceptable antimalarial drug for almost 400 years. The protozoal parasite responsible for malaria, *Plasmodium falciparum*, has gained resistance to most forms of monotherapy especially in South-East Asia, South America, and East Africa (2).

However, quinine has an impressive track record considering that most antimalarial drugs encounter parasite resistance within a few years of introduction. Quinine is treated as a privileged natural structure because of its low parasite resistance, which is difficult for the parasite to circumvent.

Quinoline-containing antimalarial drugs, such as quinine, chloroquine, and mefloquine, are mainstays of chemotherapy against malaria. While the search for novel drug targets and new lead structures for the treatment of malaria is a critical objective, a complimentary strategy is to utilize and develop lead structures from nature with proven efficacious properties like quinine or artemisinin. The synthesis of quinine derivatives, a strategy that led to the discovery of chloroquine, has been largely abandoned (3) because of toxicity and reaction complexity. Artemisinin is used against chloroquine-resistant malarial parasites. The poor solubility of artemisinin coupled with its short plasma half-life led to a high rate of parasite recrudescence. Modification of the cinchona alkaloids is one of the most successful strategies for antimalarial drug development (3,4). Peptide derivatives of primaquine evidenced a comparable or greater activity of conjugates with respect of the parent drug (5).

Efficient cellular drug delivery is a severe problem for many therapeutic agents due to charge, hydrophilic character, and/or size. High drug doses, necessary to compensate for the reduced bioavailability, often cause strong adverse effects. Synthetic drug delivery vectors can sometimes solve this problem, if limitations like low-cellular uptake efficiency or cytotoxicity are overcome. Among such synthetic vectors, so-called cell-penetrating peptides have proven applicability because of their ability as drug carriers to cross cell membranes (6). Drug–peptide conjugates are also known to overcome multidrug resistance in chemotherapy and bind to specific receptors expressed on tumor cells (7–9).

Recently, Islahudin *et al.* reported that quinine can block a cell's ability to take up essential amino acids like tryptophan and tyrosine; they suggested that dietary amino acid supplements could improve the performance of quinine (10,11). Amino acids like L-alanine, L-phenylalanine, L-arginine, L-glutamic acid, and L-lysine may also mask the bitter taste of quinine (12).

We previously used benzotriazole methodology to prepare diverse peptide conjugates (13) and quinine bis-conjugates incorporating both quinolone antibiotics and amino acid units as potential antimalarial agents (14). We now expand the latter approach by coupling amino acids and peptides of varying polarity with quinine. We utilized microwave

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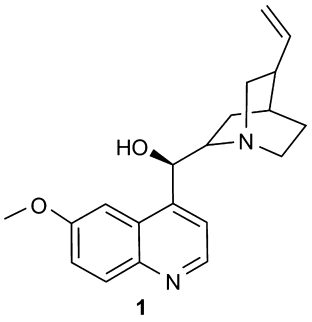

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Figure 1: Quinine.

irradiation to shorten reaction times (15) and minimize epimerization in the preparation of peptide–quinine bioconjugates. Quinine–peptide conjugates that retain activity against the malarial parasite *Plasmodium falciparum* may provide means to identify novel antimalarial agents with improved activity on resistant strains.

Experimental Section

General methods

Melting points were determined on a capillary point apparatus equipped with a digital thermometer. NMR spectra were recorded in $CDCl_3$ or DMSO-d_6, on Mercury or Gemini NMR spectrometers operating at 300 MHz for ^1H (with TMS as an internal standard) and 75 MHz for ^{13}C. Elemental analyses were performed on a Carlo Erba-EA1108 instrument. All microwave-assisted reactions were carried out with a single-mode cavity Discover Microwave Synthesizer (CEM Corporation, Matthews, NC, USA). The reaction mixtures were transferred into a 10-mL glass pressure microwave tube equipped with a magnetic stirrer bar. The tube was closed with a silicon septum, and the reaction mixture was subjected to microwave irradiation (Discover mode; run time: 60 seconds; PowerMax-cooling mode).

General procedure and experimental details for the preparation of compounds **2**–**11** are given in the Appendix S1. In addition, Tables S1–S3 that contain details for the characterization of compounds **4a**–**g** (Table S1), compounds **7a**–**g** (Table S2), and compounds **8a**–**d** and **9a**–**d** (Table S3) are also included in the Appendix S1.

General procedure for the synthesis of quinine conjugates 12a–n

A dried, heavy-walled Pyrex tube containing a small stir bar was charged with *N*-(Pg-peptidoylbenzotriazoles) (1.2 equivalent) and quinine (1 equivalent) dissolved in dimethyl formamide (DMF) along with anhydrous potassium carbonate (2 equivalent). The reaction mixture was exposed to microwave irradiation (20 W) at 50 °C for 10 min. Each mixture was allowed to cool through an inbuilt system until the temperature fell below 30 °C (ca. 10 min). Each reaction mixture was quenched with ice-cold water, and the solid obtained was filtered and washed with 10% Na_2CO_3 and water to give the desired compound.

(2S)-(1R)-(6-Methoxyquinolin-4-yl)(5-vinylquinuclidin-2-yl)methyl 2-((tert-butoxycarbonyl)amino)-3-(1-tosyl-1H-imidazol-4-yl) propanoate (Boc-L-His(Tos)-QN, 12e)

White solid (92%); mp 141–142 °C; ^1H NMR ($CDCl_3$) δ 8.72–8.71 (d, J = 4.3 Hz, 1H), 8.04–8.01 (d, J = 9.1 Hz, 1H), 7.80–7.69 (m, 3H), 7.41–7.26 (m, 6H), 6.42–6.38 (m, 1H), 5.90–5.67 (m, 2H), 5.02–4.96 (m, 2H), 4.68–4.63 (m, 1H), 3.95 (s, 3H), 3.36–3.20 (m, 1H), 3.07–2.90 (m, 3H), 2.61–2.53 (m, 1H), 2.43 (s, 3H), 2.27–2.24 (m, 1H), 1.91–1.71 (m, 7H), 1.44 (br s, 9H); ^{13}C NMR ($CDCl_3$) δ 171.0, 158.1, 155.7, 147.7, 146.5, 144.8, 143.4, 141.8, 139.9, 136.4, 134.8, 132.0, 130.6, 127.5, 126.8, 122.0, 115.0, 114.7, 101.5, 80.2, 59.3, 56.8, 55.9, 53.3, 42.6, 39.8, 29.6, 28.5, 27.8, 21.9. Anal. Calcd for $C_{38}H_{45}N_5O_7S$: C, 63.76; H, 6.34; N, 9.78. Found: C, 63.47; H, 6.40; N, 9.66.

(2S)-(1R)-(6-Methoxyquinolin-4-yl)(5-vinylquinuclidin-2-yl)methyl 3-(benzyloxy)-2-((tert-butoxycarbonyl)amino) propanoate (Boc-L-Ser(Bzl)-QN, 12f)

White solid (94%); mp 58–59 °C; ^1H NMR ($CDCl_3$) δ 8.62 (dd, J = 8.2 Hz, 4.5 Hz, 1H), 8.00 (dd, J = 9.8 Hz, 4.7 Hz, 1H), 7.41–7.06 (m, 10H), 6.54–6.48 (m, 1H), 5.84–5.73 (m, 1H), 5.47–5.40 (m, 1H), 5.26–5.18 (m, 1H), 5.02–4.96 (m, 2H), 4.53–4.48 (m, 1H), 4.44–4.43 (m, 1H), 4.30 (br s, 1H), 3.94 (br s, 1H), 3.72–3.67 (m, 1H), 3.37–3.35 (m, 1H), 3.09–2.97 (m, 2H), 2.63–2.55 (m, 2H), 2.26 (br s, 1H), 1.47–1.39 (m, 12H); ^{13}C NMR ($CDCl_3$) δ 170.4, 170.2, 158.0, 155.6, 155.3, 147.6, 144.8, 143.2, 141.9, 131.9, 128.5, 127.7, 122.0, 119.1, 114.6, 101.5, 80.3, 73.6, 70.5, 70.3, 67.3, 59.5, 59.1, 57.0, 56.7, 55.8, 54.4, 42.8, 42.5, 39.8, 28.5. Anal. Calcd for $C_{35}H_{43}N_3O_6$: C, 69.86; H, 7.20; N, 6.89. Found: C, 69.61; H, 7.41; N, 7.20.

(2S)-5-Benzyl 1-((1R)-(6-methoxyquinolin-4-yl)(6-vinylquinuclidin-2-yl)methyl) 2-((tert-butoxycarbonyl)amino) pentanedioate (Boc-L-Glu (Bz)-QN, 12g)

White solid (67%); mp 76–78 °C; ^1H NMR ($CDCl_3$) δ 8.74–8.68 (m, 1H), 8.01 (d, J = 8.8 Hz, 1H), 7.49–7.26 (m, 8H), 6.55–6.47 (m, 1H), 5.87–5.75 (m, 1H), 5.11–4.99 (m, 5H), 4.50–4.41 (m, 1H), 3.95 (s, 3H), 3.42–3.35 (m, 1H), 3.18–3.00 (m, 1H), 2.74–2.60 (m, 1H), 2.49–2.18 (m, 4H), 2.00–1.73 (m, 4H), 1.63–1.52 (m, H), 1.42 (s, 9H); ^{13}C NMR ($CDCl_3$) δ 172.6, 172.5, 171.6, 158.2, 155.5, 147.6, 144.9, 143.0, 141.5, 135.8, 132.0, 128.8, 128.5, 128.4, 122.2, 122.1, 114.9, 101.3, 80.4, 66.8, 59.3, 59.2, 56.6, 56.5, 55.89, 53.1, 42.7, 39.68, 30.35, 30.25, 28.48, 27.64, 27.54. HRMS m/z for $C_{37}H_{45}N_3O_7$ $[M + H]^+$ calcd. 644.3330, found 644.3347.

(2S)-(1R)-(6-Methoxyquinolin-4-yl)(6-vinylquinuclidin-2-yl)methyl 2,6-bis(((benzyloxy) carbonyl)amino)hexanoate (Z-L-Lys(Z)-QN, 12h)

Oil (95%);. ^{1}H NMR ($CDCl_3$) δ 8.71–8.64 (m, 1H), 7.99 (d, J = 9.1 Hz, 1H), 7.42–7.41 (m, 1H), 7.38–7.20 (m, 11H), 6.51–6.43 (m, 1H), 5.90–5.75 (m, 2H), 5.18–5.13 (m, 1H), 5.07 (s, 2H), 5.02 (s, 2H), 4.97 (s, 1H), 4.43–4.35 (m, 1H), 3.88 (s, 3H), 3.46–3.35 (m, 1H), 3.16–2.90 (m, 4H), 2.66–2.57 (m, 2H), 2.28–2.19 (m, 1H), 1.89–1.16 (m, 11H); ^{13}C NMR ($CDCl_3$) δ 171.9, 157.9, 156.6, 156.2, 154.9, 147.3, 144.6, 143.2, 141.5, 136.6, 136.1, 131.7, 128.4, 127.9, 126.7, 125.3, 121.8, 119.0, 114.9, 114.6, 114.3, 101.5, 66.9, 66.5, 59.0, 56.4, 55.6, 53.8, 52.3, 42.4, 40.1, 39.5, 31.1, 29.2, 27.6, 27.4, 24.4, 22.3, 22.1. HRMS *m/z* for $C_{42}H_{48}N_4O_7$ $[M + H]^+$ calcd. 721.3596, found 721.3629.

(2S)-4-Benzyl 1-((1R)-(6-methoxyquinolin-4-yl)(6-vinylquinuclidin-2-yl)methyl)2-(((benzyloxy) carbonyl) amino) succinate (Z-L-Asp(Bz)-QN, 12i)

Oil (68%); ^{1}H NMR ($CDCl_3$) δ 8.70–8.69 (m, 1H), 8.00 (d, J = 9.5 Hz, 1H), 7.53–7.21 (m, 13H), 6.50–6.40 (m, 1H), 5.90–5.67 (m, 2H), 5.13–4.71 (m, 7H), 3.93 (s, 3H), 3.67–2.58 (m, 8H), 2.36–2.25 (m, 1H), 1.91–1.51 (m, 4H); ^{13}C NMR ($CDCl_3$) δ 170.7, 170.3, 158.2, 156.2, 147.6, 144.9, 143.0, 141.9, 136.2, 135.4, 135.2, 132.0, 128.8, 128.6, 128.5, 128.4, 127.0, 122.1, 119.0, 118.7, 114.7, 101.4, 75.9, 67.5, 67.1, 59.3, 56.9, 55.8, 50.7, 43.5, 42.7, 39.8, 36.4, 27.9, 24.5. HRMS *m/z* for $C_{39}H_{41}N_3O_7$ $[M + H]^+$ calcd. 664.3017, found 664.3035.

(2S)-((1R)-(6-Methoxyquinolin-4-yl)(8-vinylquinuclidin-2-yl)methyl) 2-(benzylamino)-3-(benzylthio)propanoate (Z-L-Cys(Bz)-QN, 12j)

Oil (65%); ^{1}H NMR ($CDCl_3$) δ 8.74–8.68 (m, 1H), 8.02–7.97 (m, 1H), 7.37–7.14 (m, 15H), 6.52–6.42 (m, 1H), 5.86–5.75 (m, 1H), 5.49–4.45 (m, 1H), 5.12–4.99 (m, 4H), 4.65–4.58 (m, 1H), 3.93 (s, 3H), 3.64–3.53 (m, 1H), 3.38–3.29 (m, 1H), 3.08–2.62 (m, 4H), 2.27–1.46 (m, 7H); ^{13}C NMR ($CDCl_3$) δ 174.8, 162.7, 160.5, 152.1, 149.3, 147.3, 146.3, 142.0, 140.7, 136.5, 134.1, 133.6, 133.3, 133.0, 132.0, 131.6, 126.6, 123.6, 119.3, 106.0, 80.0, 71.9, 70.6, 63.8, 61.3, 60.3, 58.2, 47.1, 44.2, 41.2, 37.8, 32.1, 20.0. HRMS *m/z* for $C_{37}H_{41}N_3O_3S$ $[M + H]^+$ calcd. 652.2840, found 652.2849.

(2S)-(1R)-(6-Methoxyquinolin-4-yl)(6-vinylquinuclidin-2-yl)methyl 2-((S)-2-(((benzyloxy) carbonyl)amino)propanamido)-3-phenylpropanoate (Z-L-Ala-L-Phe-QN, 12k)

White solid (75%); mp 85–87 °C; ^{1}H NMR ($CDCl_3$) δ 8.74–8.68 (m, 1H), 8.05–7.98 (m, 1H), 7.56–6.96 (m, 17H), 6.74–6.49 (m, 1H), 5.96–5.65 (m, 1H), 5.40–4.92 (m, 6H), 4.45–4.14 (m, 1H), 3.97–3.84 (m, 2H), 3.40–2.86 (m, 4H), 2.68–2.55 (m, 1H), 2.29–2.23 (m, 1H), 1.96–1.24 (m, 7H); ^{13}C NMR ($CDCl_3$) δ 176.7, 175.6, 162.7, 152.2, 146.3, 140.7, 136.5, 135.3, 134.3, 134.2, 133.8, 133.8, 133.3, 133.1, 132.9, 132.8, 132.8, 131.0, 131.9, 131.4, 126.7, 119.4, 71.9, 69.9, 63.8, 61.1, 60.4, 57.9, 55.0, 53.9, 47.1, 44.3, 42.2, 32.4, 32.1, 24.1. HRMS *m/z* for $C_{40}H_{44}N_4O_6$ $[M + H]^+$ calcd. 677.3334, found 677.3303.

(2S)-(1R)-(6-Methoxyquinolin-4-yl)(6-vinylquinuclidin-2-yl)methyl2-((S)-2-(((benzyloxy) carbonyl)amino)-3-methyl butanamido)-4-methylpentanoate (Z-L-Val-L-Leu-QN, 12l)

White solid (93%); mp 83–84 °C; ^{1}H NMR ($CDCl_3$) δ 8.74–8.70 (m, 1H), 8.03–7.96 (m, 1H), 7.44–7.26 (m, 10H), 6.58–6.51 (m, 1H), 5.88–5.79 (m, 1H), 5.55–4.97 (m, 6H), 4.74–4.35 (m, 1H), 3.95 (s, 3H), 3.43–3.34 (m, 1H), 3.13–3.00 (m, 1H), 2.70–2.60 (m, 1H), 2.30–0.80 (m, 23H); ^{13}C NMR ($CDCl_3$) δ 172.1, 171.3, 158.1, 156.5, 147.8, 147.5, 141.8, 132.0, 131.8, 128.7, 128.2, 127.1, 126.8, 122.0, 121.7, 118.7, 114.7, 101.4, 72.0, 67.3, 60.1, 59.3, 57.1, 55.9, 51.0, 43.4, 42.6, 41.1, 40.1, 39.8, 31.2, 28.0, 27.6, 25.0, 23.0, 21.9, 19.4. HRMS *m/z* for $C_{40}H_{44}N_4O_6$ $[M + H]^+$ calcd. 671.3811, found 671.3803.

(1R)-(6-Methoxyquinolin-4-yl)(6-vinylquinuclidin-2-yl)methyl 2-((2S,3S)-2-(((benzyloxy)carbonyl)amino)-3-methyl pentanamido)acetate (Z-L-Ileu-Gly-QN, 12m)

Oil (52%); ^{1}H NMR ($CDCl_3$) δ 8.72 (d, J = 4.3 Hz, 1H), 8.02 (d, J = 9.1 Hz), 7.49–7.24 (m, 8H), 6.90–6.53 (m, 2H), 5.84–5.73 (m, 1H), 5.42–5.37 (m, 1H), 5.13–4.98 (m, 4H), 4.24–4.06 (m, 3H), 3.93 (s, 3H), 3.43–3.35 (m, 1H), 3.20–3.05 (m, 2H), 2.96–2.62 (m, 3H), 2.32–2.31 (m, 1H), 1.95–1.72 (m, 3H), 1.59–1.41 (m, 3H), 1.26–1.02 (m, 2H), 0.92–0.83 (m, 5H); ^{13}C NMR ($CDCl_3$) δ 171.9, 169.0, 158.3, 156.5, 147.5, 144.8, 142.7, 141.3, 136.2, 132.0, 128.7, 128.4, 128.2, 126.8, 122.1, 118.9, 115.1, 101.4, 67.3, 59.8, 59.1, 56.5, 55.9, 42.8, 41.5, 39.5, 37.4, 36.7, 27.5, 24.8, 24.0, 15.7, 11.6. HRMS *m/z* for $C_{36}H_{44}N_4O_6$ $[M + H]^+$ calcd. 269.3334, found 269.3348.

(8S,11S)-(1R)-(6-Methoxyquinolin-4-yl)(6-vinylquinuclidin-2-yl)methyl 8-benzyl-11-methyl-3,6,9-trioxo-1-phenyl-2-oxa-4,7,10-triazadodecan-12-oate (Z-Gly-L-Phe-L-Ala-QN, 12n)

White solid (76%); mp 91–92 °C; ^{1}H NMR ($CDCl_3$) δ 8.72–8.67 (m, 1H), 8.01–7.93 (m, 1H), 7.43–7.09 (m, 18H), 6.18–5.55 (m, 3H), 5.10–4.96 (m, 5H), 4.72–4.55 (m, 1H), 4.17–4.13 (m, 1H), 3.94–3.70 (m, 5H), 3.37–2.96 (m, 5H), 2.68–2.53 (m, 1H), 2.28–1.84 (m, 2H), 1.69–1.52 (m, 2H), 1.27 (br s, 3H);^{13}C NMR ($CDCl_3$) δ 175.9, 175.1, 174.1, 162.2, 151.6, 149.4, 148.1, 145.6, 142.6, 140.5, 135.8, 133.6, 132.8, 132.3, 131.2, 130.0, 126.2, 125.9, 123.0, 120.8, 119.0, 105.6, 105.2, 71.4, 64.0, 63.4, 60.7, 59.9, 58.8, 52.5, 48.6, 47.7, 46.8, 43.7, 41.9, 31.7, 29.2, 23.1, 21.6. HRMS *m/z* for $C_{42}H_{47}N_5O_7$ $[M + H]^+$ calcd. 734.3548, found 734.3557.

Antimalarial activity assay

Plasmodium falciparum strain 3D7 was cultured according to the method of Trager and Jensen (16) with minor modifications. Parasites were grown in human erythrocytes (2% hematocrit) in an atmosphere of 5% CO_2, 5% O_2, and 90% N_2 in RPMI 1640 medium (Gibco, Grand Island, NY, USA) supplemented with 25 mM hepes buffer (Sigma, Saint Louis, MO, USA), 25 mg/L gentamicin (Gibco), 1 mM sodium pyruvate (Sigma), 50 mg/L hypoxanthine (Sigma), 2 g/L glucose (Sigma), 2.52 g/L sodium bicarbonate (Sigma), and 5 g/L Albumax 1 (Gibco). *In vitro* antimalarial activity was determined by the SYBR Green I method described by Smilkstein *et al.* (17) with modifications (18). Stock solutions of each compound were prepared in DMSO at a concentration of 10 mM and threefold serial dilutions prepared in DMSO for an 11-point curve. Drugs were then diluted 250-fold into culture medium in 96-well storage plates to create 2× drug solutions. Drug solutions (50 μL/well) were transferred in quadruplicate to parasite cultures (50 μL) in 96-well black tissue culture plates for a total volume of 100 μL at 2% hematocrit, 0.2% parasitemia, and 0.2% DMSO final concentrations. The plates were then incubated for 72 h at 37 °C. After incubation, 100 μL of lysis buffer containing 0.2 μL/mL SYBR Green I was added to each well. After incubation for 1 h at room temperature in the dark, plates were read on a Safire2 (Tecan, Morrisville, NC, USA) plate reader with excitation and emission wavelengths of 497 and 520 nM, respectively. The 50% inhibitory concentrations (IC_{50}s) were determined by nonlinear regression using a four-parameter logistic equation (GraphPad Prism software, La Jolla, CA, USA).

Results and Discussion

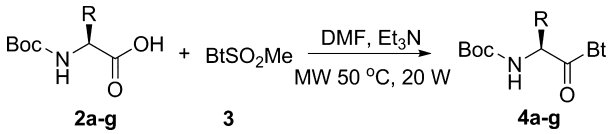
N-Pg-aminoacyl benzotriazolides **4a–g** and **7a–g**, *N*-Pg-dipeptidoylbenzotriazolides **9a–d**, and *N*-Pg-tripeptidoyl **11** were synthesized as previously reported (12,15). *N*-Pg-Peptidoylbenzotriazoles were used as active intermediates to prepare quinine–peptide bioconjugates **12a–n** under microwave irradiation.

Preparation of N-Boc-aminoacylbenzotriazoles 4a–g

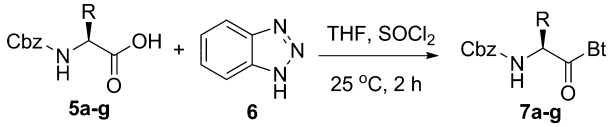
N-(Boc-α-Aminoacyl)benzotriazoles **4a–g** (Scheme 1) were prepared in 54–85% yields from the corresponding *N*-Boc-α-amino acids following our one-step procedure (19).

Preparation of N-Cbz-aminoacylbenzotriazoles 7a–g

N-(Cbz-α-Aminoacyl)benzotriazoles **7a–g** (Scheme 2) were prepared in 64–88% yields from the corresponding *N*-Cbz-α-amino acids following our published procedure (20,21).



Scheme 1: Synthesis of *N*-Boc-aminoacylbenzotriazoles **4a–g**.



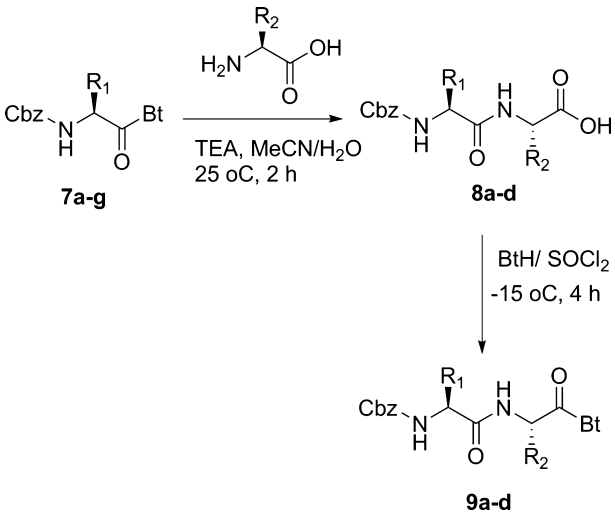
Scheme 2: Synthesis of *N*-Cbz-aminoacylbenzotriazoles **7a–g.**

Preparation of N-Cbz-dipeptidoylbenzotriazoles 9a–d

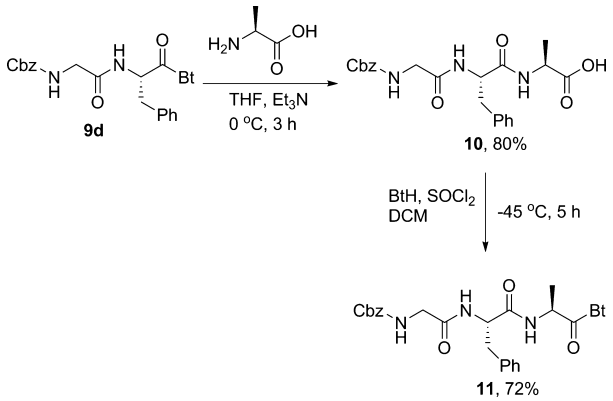
The *N*-Cbz-dipeptidoylbenzotriazoles **9a–d** were synthesized as previously reported by stepwise linking amino acids and subsequent coupling with benzotriazole-activated *N*-protected amino acid analogs in solution phase (yields, 69–79%; Scheme 3) (20).

Preparation of N-Cbz-tripeptidoyl benzotriazoles 11

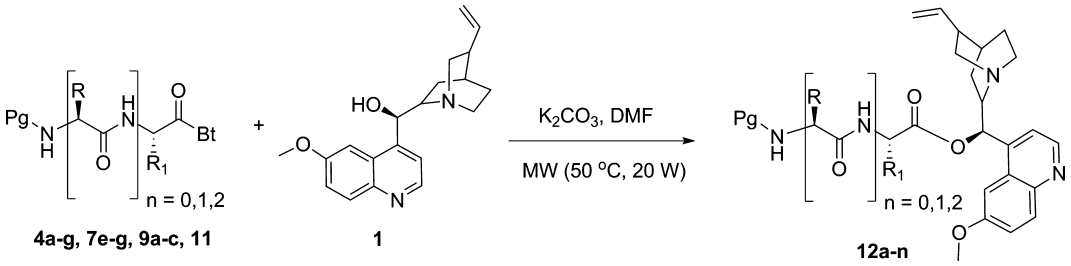
The Cbz-protected tripeptide **10** was synthesized in 80% yield by our reported method (13) of stepwise coupling of



Scheme 3: Synthesis of *N*-Cbz-dipeptidoylbenzotriazoles **9a–d.**



Scheme 4: Synthesis of *N*-Cbz-tripeptidoyl benzotriazoles **11.**



Scheme 5: Synthesis of quinine conjugates **12a–n.**

Table 1: Preparation of quinine conjugates by chiral acylation with *N*-(protected)-peptidoylbenzotriazoles **12a–n**

Entry	Reactant **4**	Product **12**	Yield%	mp (°C)	Lit.mp (°C)
1	Boc-Gly-Bt **4a**	Boc-Gly-QN **12a**	86	74–75	74–7614 (14)
2	Boc-L-Ala-Bt **4b**	Boc-L-Ala-QN **12b**	81	111–113	110–112 (14)
3	Boc-L-Phe-Bt **4c**	Boc-L-Phe-QN **12c**	80	165–167	166–168 (14)
4	Boc-L-Ile-Bt **4d**	Boc-L-Ile-QN **12d**	92	68–70	68–70 (14)
5	Boc-L-His(Tos)-Bt **4e**	Boc-L-His(Tos)-QN **12e**	92	141–142	Novel
6	Boc-L-Ser(Bzl)-Bt **4f**	Boc-L-Ser(Bzl)-QN **12f**	94	58–59	Novel
7	Boc-L-Glu(Bz)-Bt **4g**	Boc-L-Glu(Bz)-QN **12g**	67	76–78	Novel
8	Z-L-Lys(Z)-Bt **7e**	Z-L-Lys(Z)-QN **12h**	95	Oil	Novel
9	Z-L-Asp(Bz)-Bt **7f**	Z-L-Asp(Bz)-QN **12i**	68	Oil	Novel
10	Z-L-Cys(Bz)-Bt **7g**	Z-L-Cys(Bz)-QN **12j**	65	Oil	Novel
11	Z-L-Ala-L-Phe-Bt **9a**	Z-L-Ala-L-Phe-QN **12k**	75	85–87	Novel
12	Z-L-Val-L-Leu-Bt **9b**	Z-L-Val-L-Leu-QN **12l**	93	83–84	Novel
13	Z-L-Ileu-Gly-Bt **9c**	Z-L-Ileu-Gly-QN **12m**	52	Oil	Novel
14	Z-Gly-L-Phe-L-Ala-Bt **11**	Z-Gly-L-Phe-L-Ala-QN **12n**	76	91–92	Novel

amino acids with subsequent benzotriazole-activated *N*-protected amino acid analog **9d** in solution phase. Compound **10** was activated by benzotriazole to obtain **11** in 72% yields (Scheme 4).

Preparation of quinine–peptide bioconjugates 12a–n

We prepared amino acid and peptide conjugates of quinine **12a–n** by *O*-acylation of quinine **1** with *N*-protected acylbenzotriazoles **9** in the presence of K_2CO_3 in anhydrous DMF under microwave irradiation (50 °C, 20 W) for 10–30 min (Scheme 5, Table 1). Compounds **12a–n** were purified by column chromatography and characterized by ^{1}H NMR, ^{13}C NMR, and HRMS analysis.

In vitro *activity of compounds against* Plasmodium falciparum

To determine whether the quinine–peptide conjugates described retained antimalarial activity, compounds **12a–n** as well as quinine itself were tested against the blood stage of *P. falciparum* strain 3D7 *in vitro*. Table 2 gives the IC_{50} values determined 72 h after compound addition. Quinine was potent (IC_{50} = 18 nM), consistent with previous work (14). Eleven of the 14 conjugates maintained potency in line with quinine with IC_{50} values below 100 nM (**12a–c**, **12e**, **12g–j**, **12l–n**) and highest with **12i** (at 17 nm) and **12 m** (at 23 nm) showing activity comparable to that of quinine. Compounds **12f** and **12k** were somewhat less potent with IC_{50} values of 95 and 120 nM, respectively. Compound **12d** (Boc-L-Ser(Bzl)-QN) was much less active with an IC_{50} of 550 nM, approximately 30-fold higher than that of quinine. These data indicate that conjugation of small peptides to the quinine hydroxyl

Table 2: *In vitro* antimalarial activities of compounds against the chloroquine-sensitive 3D7 strain of *Plasmodium falciparum*

Compound	IC_{50} (nM)[a]
12a	27 (23–32)
12b	36 (30–43)
12c	38 (33–44)
12d	550 (490–620)
12e	42 (36–48)
12f	95 (81–110)
12g	76 (67–86)
12h	71 (62–82)
12i	17 (16–20)
12j	40 (35–46)
12k	120 (100–140)
12l	74 (60–92)
12m	23 (20–25)
12n	57 (50–64)
Quinine	18 (11–30)

[a]Mean value (95% confidence intervals).

group does not interfere with antimalarial activity and provides a handle for optimization of antimalarial and pharmacokinetic properties. Furthermore, the peptides do not appear to hinder the cell permeability of the conjugates.

Conclusion

In conclusion, we have reported convenient benzotriazole-mediated efficient syntheses for chirally pure quinine conjugates with amino acids and peptides of varying polarity. These quinine–peptide conjugates possess *in vitro* antimalarial activity similar to that of quinine.

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Supporting Information

Additional Supporting Information may be found in the online version of this article:

Appendix S1. Experimental details for compounds 2–11. ^{1}H, ^{13}C and CHN/HRMS spectra for **4f, 4g, 7e, 7f, 9b, 9c, 11 and 12a-n** and graphs for antimalarial bioassay of **12a–n** and quinine. This material is available free.