Organic & Biomolecular Chemistry

Cite this: *Org. Biomol. Chem.*, 2012, **10**, 8985

www.rsc.org/obc

PAPER

Quinine bis-conjugates with quinolone antibiotics and peptides: synthesis and antimalarial bioassay†

Siva S. Panda,[a] Kiran Bajaj,[a] Marvin J. Meyers,[b] Francis M. Sverdrup[b] and Alan R. Katritzky*[a,c]

Received 23rd July 2012, Accepted 14th September 2012
DOI: 10.1039/c2ob26439k

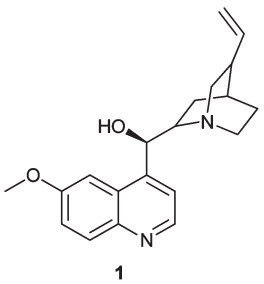
Benzotriazole-mediated syntheses led to novel bis-conjugates of quinine with quinolone antibiotics and amino acid linkers which were successfully prepared by two alternative routes with excellent yields and retention of chirality. These bis conjugates retain *in vitro* antimalarial activity with IC_{50} values ranging from 12 to 207 nM, similar to quinine itself.

Introduction

Quinolines derivatives occur in numerous natural products, however quinoline and quinolone derivatives possess interesting biological and pharmaceutical properties.[1] Originating from the bark of cinchona tree and first brought to Europe from Peru in the 17th century, quinine (**1**) has a unique therapeutic heritage; its exceptional pharmacological efficacy as an antimalarial agent led to the claim that it is "the drug that has relieved more human suffering than any other in history" (Fig. 1).[2] Quinine analogs act as calcitonin gene-related peptide receptor antagonists.[3] Quinine derivatives have also been used successfully as chiral catalysts in a wide variety of synthetic transformations.[4,5]

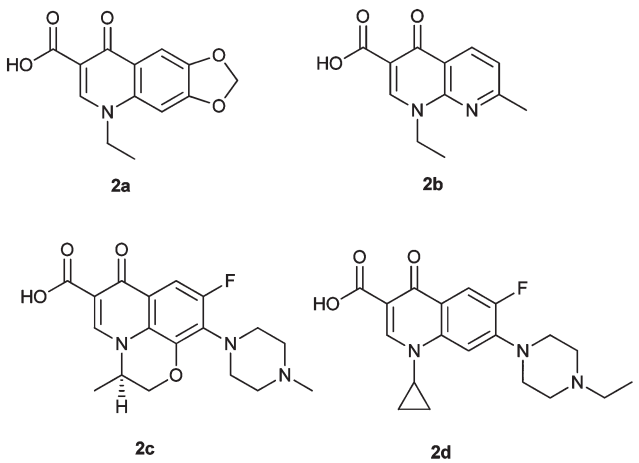
Synthetic quinolone-derived antimicrobial agents include oxolinic acid **2a** and nalidixic acid **2b** (a naphthyridine), which have found widespread clinical use in the treatment of urinary tract infections.[6,7] Levofloxacin **2c** (a highly potent antibacterial) and enrofloxacin **2d** are fluoroquinolones used for the treatment of cystic fibrosis and bacterial infections. These antibiotics are prescribed worldwide for the treatment of life-threatening bacterial infections especially those that have failed to respond to other classes of antibiotic.[8] Enrofloxacin **2d** is used for the treatment of pets and domestic animals and poultry (Fig. 2).[9] Quinolones and fluoroquinolones have also been proposed for treatment of malaria since these drugs have *in vitro* antimalarial activity against chloroquine-sensitive and chloroquine-resistant *P. falciparum*.[10–12] Activities of 25 quinolones and fluoroquinolones studied *in vitro* against erythrocytic and liver stages of *Plasmodium falciparum* showed all to inhibit both chloroquine-sensitive and chloroquine-resistant *P. falciparum* grown in red blood cells.[13]

Dangerous deterioration of the efficacy of the classical antimalarial drugs has resulted in increasing resistance of the malaria parasite *Plasmodium falciparum*. Considerable concomitant bacteraemia, septicaemia or localized bacterial infections was



1

Fig. 1 Quinine.



2a **2b** **2c** **2d**

Fig. 2 Quinolone antibiotics.

[a]*Center for Heterocyclic Compounds, Department of Chemistry, University of Florida, Gainesville, FL 32611-7200, USA. E-mail: katritzky@chem.ufl.edu*
[b]*Center for World Health & Medicine, Saint Louis University, Saint Louis, MO 63104, USA*
[c]*Department of Chemistry, King Abdulaziz University, Jeddah, 21589, Saudi Arabia*

†Electronic supplementary information (ESI) available: ^{1}H, ^{13}C NMR, CHN/HRMS for all compounds. Antimalarial bioassay graphical data for **8a–k** are provided. See DOI: 10.1039/c2ob26439k

revealed in patients with severe malaria[14] but the administration of combination therapy was often successful. The potential value of malaria therapy using combinations of drugs[15,16] is identified as a strategic and viable option to improve the efficacy and delaying the development and selection of resistant parasites. Combination therapy (CT) drugs utilize the simultaneous administration of two or more blood schizontocidal antimalarial drugs with independent modes of action and different biochemical targets in the parasite. Successful treatment with a high cure rate has been realized with combined use of the antibiotic tetracycline and quinine.[17] However, due to side effects, this combination cannot be used for children or pregnant women. Over 90% of adult Gabonese patients with *Plasmodium falciparum* malaria were cured by using quinine-clindamycin and quinine-doxycycline.[18]

Several reports show that quinine amino acid conjugates can exhibit significant antimalarial activity[19] and that quinolone antibiotic conjugates with amino acids can possess anti-allergic[20] and antibacterial activities.[21,22] Since amino acids have been used as carriers for drugs because of their ability to transplant into mammalian tissue, we have synthesized novel conjugates of quinine linked by amino acid residues to quinolone antibiotics as potential enhancers of the antimalarial activity.

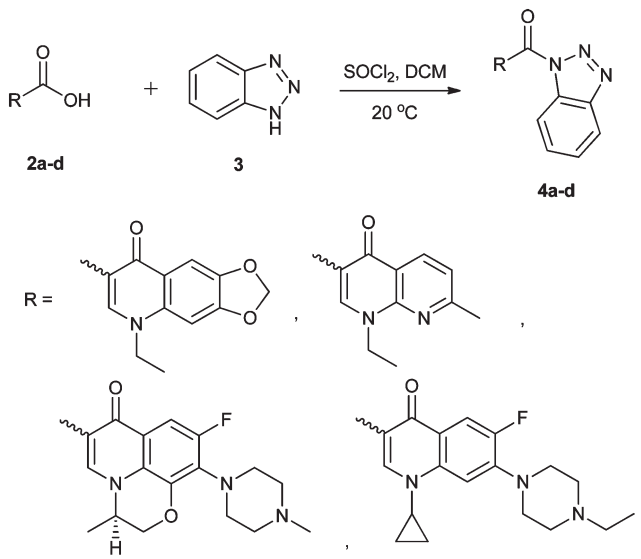
Results and discussion

Preparation of quinolone antibiotic benzotriazolides 4a–d

The carboxylic groups of quinolone antibiotics were activated in high yields (76–96%) by benzotriazole **3** using previously reported methods (Scheme 1, Table 1).[23]

Syntheses of quinine bis-conjugates with quinolone antibiotic and a peptidic fragment

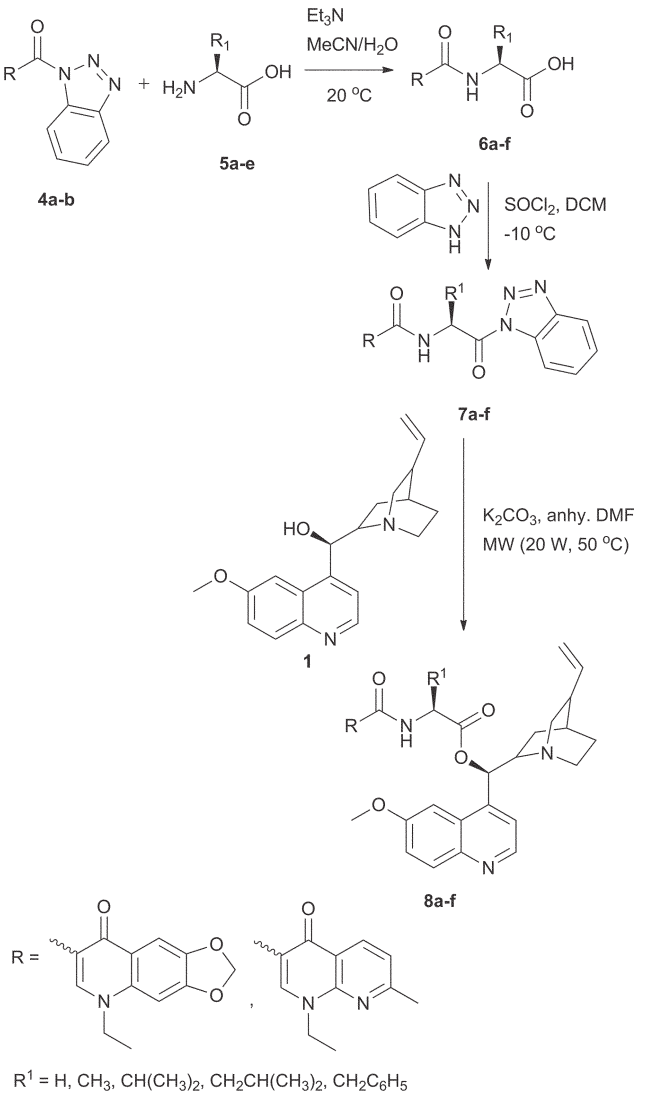
Quinine bis-conjugates with antibiotics and amino acid as linker were prepared by using two routes depending on the nature of antibiotics.



Scheme 1 Preparation of quinolone antibiotic benzotriazolides **4a–d**.

Table 1 Preparation of quinolone antibiotic benzotriazolides **4a–d**

Entry	Product **4**	Yield (%)	Mp (°C)	Lit Mp (°C)
1	Oxolinic-Bt **4a**	96	230–231	229–232[23]
2	Nalidixic-Bt **4b**	82	171–172	171–172[23]
3	Levofloxacin-Bt **4c**	79	231–233	Novel
4	Enrofloxacin-Bt **4d**	76	210–212	Novel



Scheme 2 Synthesis of quinine bis-conjugates with quinolone antibiotics and peptides **8a–f** (Route I).

Route I: Route I utilizes amino acid–antibiotic conjugates **6a–f** prepared by our previously reported method (Scheme 2).[23] The carboxyl groups of amino acid–antibiotic conjugates **6a–f** were activated by conversion to the corresponding acyl-benzotriazolides **7a–f** in high yields (76–90%) (Scheme 2, Table 2).

The reactive intermediates **7a–f** were utilized for *O*-acylation of quinine **1** to give target compounds **8a–f** in the presence of potassium carbonate in anhydrous DMF under microwave irradiation at 50 °C and 20 W for 5–10 min in good yields (68–72%). Initially, compounds **8b** and **8e** were synthesized in

Table 2 Preparation of antibiotics-amino acyl benzotriazolides **7a–f**

Entry	Product **7**	Yield (%)	Mp (°C)
1	Oxolinic-Phe-Bt **7a**	89	248–249
2	Oxolinic-Ala-Bt **7b**	86	199–200
3	Oxolinic-Val-Bt **7c**	78	240–242
4	Nalidixic-Gly-Bt **7d**	90	255–257
5	Nalidixic-Leu-Bt **7e**	76	92–93
6	Nalidixic-Ala-Bt **7f**	80	208–210

Table 3 Preparation of quinine bis-conjugates **8a–f** with antibiotics and peptidic fragment

		Yield (%)		Time		
Entry	Product **8**	Conv.	MW	Conv. (h)	MW (min)	Mp (°C)
1	**8a**	—	70	—	5	125–127
2	**8b**	51	72	7	5	224–226
3	**8c**	—	68	—	5	160–162
4	**8d**	—	70	—	10	173–175
5	**8e**	50	69	8	10	109–111
6	**8f**	—	70	—	10	112–113

the presence of potassium carbonate in anhydrous DMF at room temperature for 8 h and then switched to microwave irradiation, which showed better yields in less reaction time (Scheme 2, Table 3).

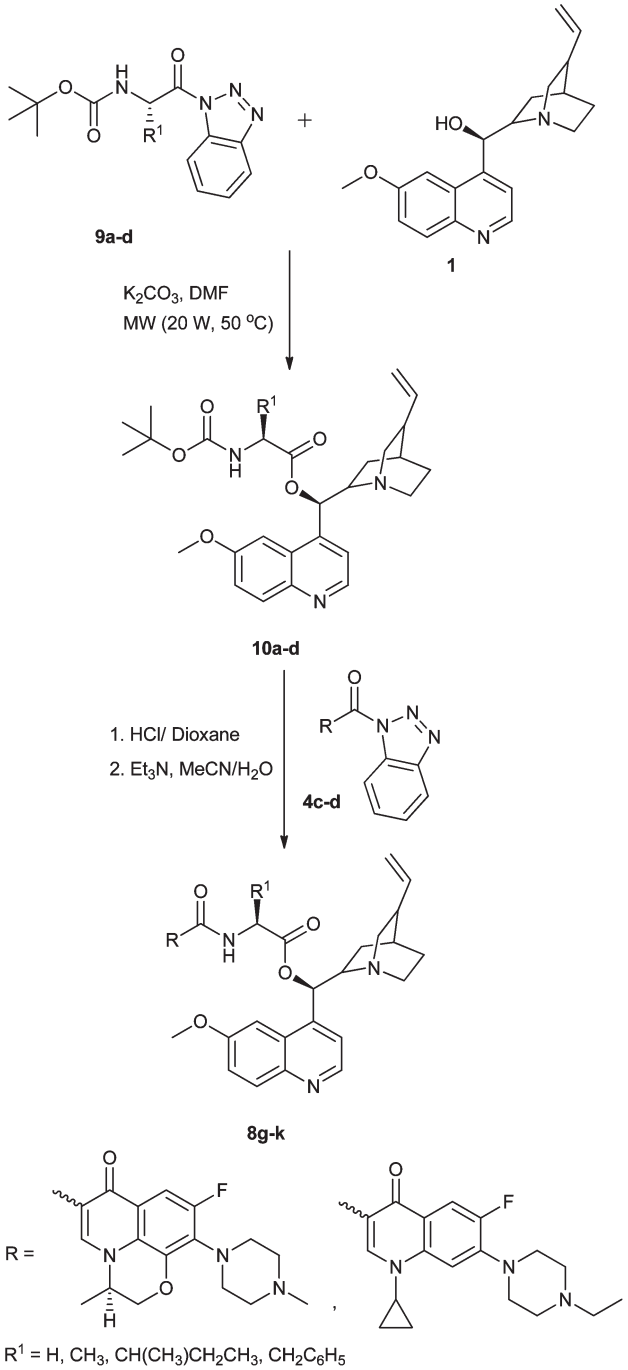
Route II: an alternative method to the preparation of quinine bis-conjugates with antibiotics and peptide fragment. Route I failed to prepare the amino acid–levofloxacin/enrofloxacin conjugates due to the presence of the piperazine nucleus in levofoxacin and enrofloxacin, which leads to the formation of water soluble hydrochloride salts. To overcome this problem we synthesized the Boc-protected amino acid–quinine conjugates **10a–d** by *O*-acylation of quinine **1** with Boc-aminoacylbenzotriazoles **9a–d** using similar conditions used in route I for *O*-acylation. (Scheme 3, Table 4) Boc group deprotection with dioxane/HCl mixture at 20 °C for 1 h gave unprotected amino acid–quinine conjugates, which were further used for the next step without characterization. The target compounds **8g–k** were

Table 4 Preparation of Boc-protected amino acid–quinine conjugates **10a–d**

Entry	Product **10**	Yield (%)	Mp (°C)
1	Boc-Gly-quinine **10a**	86	74–76
2	Boc-Ala-quinine **10b**	81	110–112
3	Boc-Phe-quinine **10c**	80	166–168
4	Boc-Ile-quinine **10d**	90	68–70

Table 5 Preparation of quinine bis-conjugates **8g–k** with antibiotics and peptidic fragments

Entry	Product **8**	Yield (%)	Time (h)	Mp (°C)
1	**8g**	88	2.5	115–117
2	**8h**	78	2.5	145–146
3	**8i**	72	2.5	161–163
4	**8j**	83	2	104–106
5	**8k**	71	2	128–129



Scheme 3 Synthesis of quinine bis-conjugates **8g–k** with quinolone antibiotics and peptides (Route II).

prepared by coupling unprotected amino acid–quinine conjugates with antibiotic–benzotriazolides **4c–d** in the presence of triethylamine at 20 °C for 2 h in good yields (71–88%) (Scheme 3, Table 5).

Antimalarial bioassay

***In vitro* activity of compounds against *Plasmodium falciparum*.** To determine if the conjugates between quinine and antibiotics

Table 6 *In vitro* antimalarial activities of compounds against the chloroquine-sensitive 3D7 strain of *P. falciparum*

Compound	IC_{50} [a] (nM)
Oxolinic-Phe-quinine **8a**	115 (65–200)
Oxolinic-Ala-quinine **8b**	29 (18–46)
Oxolinic-Val-quinine **8c**	183 (108–313)
Nalidixic-Gly-quinine **8d**	46 (19–108)
Nalidixic-Leu-quinine **8e**	50 (31–82)
Nalidixic-Ala-quinine **8f**	16 (9–27)
Levofloxacin-Gly-quinine **8g**	12 (9–16)
Levofloxacin-Ala-quinine **8h**	33 (20–53)
Levofloxacin-Phe-quinine **8i**	48 (35–65)
Enrofloxacin-Gly-quinine **8j**	28 (19–44)
Enrofloxacin-Ile-quinine **8k**	207 (163–263)
Quinine **1**	18 (11–30)
Oxolinic acid **2a**	>10 000
Naladixic acid **2b**	>20 000
Levofloxacin **2c**	>20 000

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

described here retained antimalarial activity, the bis-conjugates (compounds **8a–k**) as well as the parent compounds were tested against the blood stage of *P. falciparum* strain 3D7 *in vitro*. Table 6 gives the IC_{50} values determined 72 h after compound addition. Quinine was extremely potent (IC_{50} = 18 nM) as expected. The parent antibiotics were less active (IC_{50}s > 10 μM), consistent with previous work.[13] The bis-conjugates were all active with IC_{50}s ranging from 12 to 207 nM, indicating that conjugation did not interfere with antimalarial activity (Table 6).

Experimental section

General

Melting points were determined on a capillary point apparatus equipped with a digital thermometer. NMR spectra were recorded in $CDCl_3$ 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, NC). 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 s; Power Max-cooling mode).

General preparation for benzotriazole derivatives of quinolone antibiotics (4a–d)

Compounds **4a–d** were synthesized by a reported procedure[23] (Table 1).

(*S*)-6-(1*H*-Benzo[*d*][1,2,3]triazole-1-carbonyl)-9-fluoro-3-methyl-10-(4-methyl-piperazin-1-yl)-2*H*-[1,4]oxazino[2,3,4-*ij*]quinolin-7(3*H*)-one (4c). Light yellow microcrystals (79%); mp 231–233 °C; ^1H NMR ($CDCl_3$): δ 8.26 (s, 1H), 8.13 (d, J = 8.1 Hz, 1H), 8.03 (d, J = 7.8 Hz, 1H), 7.55 (t, J = 7.5 Hz, 1H), 7.47–7.39 (m, 2H), 4.54 (d, J = 9.0 Hz, 2H), 4.39 (d, J = 11.1 Hz, 1H), 3.38 (m, 4H), 2.58 (m, 4H), 2.39 (s, 3H), 1.61 (d, J = 6.3 Hz, 3H); ^{13}C NMR ($CDCl_3$): δ 172.7, 164.1, 157.3, 154.0, 146.0, 145.4, 139.7, 132.4, 132.2, 131.7, 130.1, 126.0, 123.9, 122.3, 120.0, 114.3, 112.2, 105.6, 105.2, 68.3, 55.9, 55.1, 50.8, 46.6, 18.5. Anal. calcd for $C_{24}H_{23}FN_6O_3$: C, 62.33; H, 5.01; N, 18.17. Found: C, 62.19; H, 5.15; N, 18.21.

3-(1*H*-Benzo[*d*][1,2,3]triazole-1-carbonyl)-1-cyclopropyl-7-(4-ethylpiperazin-1-yl)-6-fluoroquinolin-4(1*H*)-one (4d). Light yellow microcrystals (76%); mp 210–212 °C; ^1H NMR ($CDCl_3$): δ 8.42 (s, 1H), 8.25 (d, J = 7.5 Hz, 1H), 8.06 (d, J = 8.1 Hz, 1H), 7.99–7.90 (m, 1H), 7.61 (t, J = 6.9 Hz, 1H), 7.46 (t, J = 7.5 Hz, 1H), 7.31–7.28 (m, 1H), 3.61–3.45 (m, 1H), 3.36 (br s, 4H), 2.75 (br s, 4H), 2.62–2.55 (m, 2H), 1.35–1.30 (m, 2H), 1.25–1.16 (m, 5H); ^{13}C NMR ($CDCl_3$): δ 173.0, 164.3, 155.1, 151.8, 147.8, 146.1, 145.0, 144.9, 138.2, 131.8, 130.2, 126.1, 120.1, 114.5, 113.3, 105.2, 52.6, 52.5, 49.8, 35.0, 12.0, 8.4. Anal. calcd for $C_{25}H_{25}FN_6O_2$: C, 65.20; H, 5.47; N, 18.25. Found: C, 64.95; H, 5.90; N, 18.17.

General procedure for the preparation of quinoline antibiotic–amino acid conjugates 6a–f

Compounds **6a–f** were synthesized by the established procedure.[23]

General procedure for the synthesis of antibiotic–aminoacylbenzotriazolides 7a–f

1-*H*-Benzotriazole (4.0 eq.) was dissolved in anhydrous methylene chloride. Thionyl chloride (1.2 eq.) was added and stirred for 30 min, and then the solution was cooled down to −15 °C. Antibiotic–amino acid conjugate (1.0 eq.) was added and the reaction mixture was stirred for 4 h at −15 °C. Upon completion of the reaction, 5% solution of sodium bicarbonate was added and the organic layer was extracted twice with the alkaline solution, washed with brine and dried over anhydrous magnesium sulfate. The solvent was evaporated and crystallized from diethyl ether to yield antibiotic–aminoacylbenzotriazolides.

(*S*)-*N*-(1-(1*H*-Benzo[*d*][1,2,3]triazol-1-yl)-1-oxo-3-phenylpropan-2-yl)-5-ethyl-8-oxo-5,8-dihydro-[1,3]dioxolo[4,5-*g*]quinoline-7-carboxamide (7a). While microcrystals (89%); mp 248–249 °C; ^1H NMR ($CDCl_3$): δ 10.97 (d, J = 6.9 Hz, 1H), 8.36 (s, 1H), 8.30 (d, J = 8.4 Hz, 1H), 8.14 (d, J = 8.1 Hz, 1H), 7.76 (s, 1H), 7.65 (t, J = 7.8 Hz, 1H), 7.52 (t, J = 7.8 Hz, 1H), 7.40 (d, J = 7.8 Hz, 2H), 7.31–7.16 (m, 3H), 6.66 (s, 1H), 6.45–6.37 (m, 1H), 6.10 (d, J = 10.5 Hz, 2H), 4.16–4.03 (m, 2H), 3.57 (dd, J = 13.5, 4.2 Hz, 1H), 3.31 (dd, J = 13.7, 9.5 Hz, 1H), 1.43 (t, J = 7.1 Hz, 3H); ^{13}C NMR ($CDCl_3$): δ 175.1, 171.3, 165.6, 153.0, 146.9, 146.2, 145.4, 136.3, 136.0, 131.4, 131.4, 130.7, 129.7, 128.8, 127.3, 126.5, 123.9, 120.4, 114.8, 110.4, 104.0, 102.9, 95.4, 54.9, 49.8, 38.7, 14.6. Anal. calcd for $C_{28}H_{23}N_5O_5$: C, 66.00; H, 4.55; N, 13.74. Found: C, 65.78; H, 4.40; N, 13.64.

(*S*)-*N*-(1-(1*H*-Benzo[*d*][1,2,3]triazol-1-yl)-1-oxopropan-2-yl)-5-ethyl-8-oxo-5,8-dihydro-[1,3]dioxolo[4,5-*g*]quinoline-7-

carboxamide (7b). Beige powder (86%); mp 199–200 °C; ^{1}H NMR ($CDCl_3$): δ 10.91 (d, J = 6.0 Hz, 1H), 8.58 (s, 1H), 8.33 (d, J = 8.4 Hz, 1H), 8.15 (d, J = 8.1 Hz, 1H), 7.86 (s, 1H), 7.66 (t, J = 7.6 Hz, 1H), 7.52 (t, J = 7.8 Hz, 1H), 6.92 (s, 1H), 6.15 (s, 2H), 4.23 (q, J = 7.2 Hz, 2H), 1.84 (m, 4H), 1.51 (t, J = 7.2, 3H); ^{13}C NMR ($CDCl_3$): δ 175.2, 172.3, 165.4, 153.1, 146.8, 146.1, 145.7, 136.2, 131.5, 130.7, 126.5, 124.1, 120.3, 114.7, 110.7, 104.2, 102.8, 95.4, 50.0, 49.2, 18.6, 14.6. HRMS (+ESI-TOF) m/z for $C_{22}H_{19}N_5O_5$ $[M + Na]^+$ calcd 456.1278, found 456.1289.

(*S*)-*N*-(1-(1*H*-Benzo[*d*][1,2,3]triazol-1-yl)-3-methyl-1-oxobutan-2-yl)-5-ethyl-8-oxo-5,8-dihydro-[1,3]dioxolo[4,5-*g*]quinoline-7-carboxamide (7c). White solid (78%); mp 240–242 °C; ^{1}H NMR ($CDCl_3$): δ 11.01 (d, J = 6.9 Hz, 1H), 8.59 (s, 1H), 8.34 (d, J = 8.1 Hz, 1H), 8.15 (d, J = 8.4 Hz, 1H), 7.91 (s, 1H), 7.65 (t, J = 7.5 Hz, 1H), 7.52 (t, J = 7.7 Hz, 1H), 6.91 (s, 1H), 6.15 (s, 2H), 6.14–6.10 (m, 1H), 4.22 (q, J = 7.1 Hz, 2H), 2.67–2.63 (m, 1H), 1.51 (t, J = 7.1 Hz, 3H), 1.22 (d, J = 6.9 Hz, 3H), 1.18 (d, J = 6.9 Hz, 3H); ^{13}C NMR ($CDCl_3$): δ 175.4, 171.6, 166.0, 153.0, 146.9, 146.2, 145.7, 136.1, 130.6, 126.4, 125.6, 124.0, 114.8, 110.9, 104.3, 102.8, 95.4, 48.1, 50.0, 31.4, 20.3, 17.9, 14.7. HRMS (+ESI-TOF) m/z for $C_{24}H_{23}N_5O_5$ $[M + Na]^+$ calcd 484.1591, found 484.1604.

***N*-(2-(1*H*-Benzo[*d*][1,2,3]triazol-1-yl)-2-oxoethyl)-1-ethyl-7-methyl-4-oxo-1,4-dihydro-1,8-naphthyridine-3-carboxamide (7d).** Beige powder (90%); mp 255–257 °C; ^{1}H NMR ($CDCl_3$): δ 10.70–10.64 (m, 1H), 8.91 (s, 1H), 8.67 (d, J = 8.1 Hz, 1H), 8.26 (d, J = 7.8 Hz, 1H), 8.12 (d, J = 8.4 Hz, 1H), 7.63 (t, J = 7.8 Hz, 1H), 7.49 (t, J = 7.7 Hz, 1H), 7.29 (d, J = 8.1 Hz, 1H), 5.34 (d, J = 5.8 Hz, 2H), 4.54 (q, J = 7.2 Hz, 2H), 2.68 (s, 3H), 1.48 (t, J = 7.1 Hz, 3H); ^{13}C NMR ($CDCl_3$): δ 179.1, 177.2, 168.3, 165.8, 163.6, 148.8, 147.8, 146.2, 136.6, 131.4, 130.8, 126.6, 121.6, 120.5, 114.5, 112.4, 47.1, 43.6, 25.5, 15.5. Anal. calcd for $C_{20}H_{18}N_6O_3$: C, 61.53; H, 4.65; N, 21.53. Found: C, 61.16; H, 4.65; N, 21.25.

(*S*)-*N*-(1-(1*H*-Benzo[*d*][1,2,3]triazol-1-yl)-4-methyl-1-oxopentan-2-yl)-1-ethyl-7-methyl-4-oxo-1,4-dihydro-1,8-naphthyridine-3-carboxamide (7e). Yellow microcrystals (76%); mp 92–93 °C; ^{1}H NMR ($CDCl_3$): δ 10.64 (d, J = 6.3 Hz, 1H), 8.88 (s, 1H), 8.70 (d, J = 8.4 Hz, 1H), 8.31 (d, J = 7.8 Hz, 1H), 8.15 (d, J = 8.1 Hz, 1H), 7.66–7.62 (m, 1H), 7.54–7.48 (m, 1H), 7.33 (d, J = 8.4 Hz, 1H), 6.26–6.18 (m, 1H), 4.53 (q, J = 7.2 Hz, 2H), 2.71 (s, 3H), 2.08–1.96 (m, 3H), 1.49 (t, J = 7.1 Hz, 3H), 1.15 (d, J = 6.0 Hz, 3H), 1.04 (d, J = 6.0 Hz, 3H); ^{13}C NMR ($CDCl_3$): δ 177.1, 172.2, 165.1, 163.5, 148.6, 147.6, 146.1, 136.4, 131.4, 130.5, 126.3, 121.5, 120.5, 120.3, 114.7, 112.3, 52.0, 47.0, 41.3, 25.7, 25.4, 23.6, 21.5, 15.4. HRMS (+ESI-TOF) m/z for $C_{24}H_{26}N_6O_3$ $[M + Na]^+$ calcd 469.1959, found 469.1970.

(*S*)-*N*-(1-(1*H*-Benzo[*d*][1,2,3]triazol-1-yl)-1-oxopropan-2-yl)-1-ethyl-7-methyl-4-oxo-1,4-dihydro-1,8-naphthyridine-3-carboxamide (7f). Beige powder (80%); mp 208–210 °C; ^{1}H NMR ($CDCl_3$): δ 10.68 (d, J = 5.1 Hz, 1H), 8.88 (s, 1H), 8.71 (d, J = 8.1 Hz, 1H), 8.33 (d, J = 8.4 Hz, 1H), 8.16 (d, J = 7.8 Hz, 1H), 7.67 (t, J = 7.7 Hz, 1H), 7.53 (t, J = 7.8 Hz, 1H), 7.34 (d, J = 8.1 Hz, 1H), 6.20–6.11 (m, 1H), 4.58–4.53 (m, 2H), 2.71 (s, 3H), 1.85 (d, J = 6.9 Hz, 3H), 1.49 (t, J = 7.1 Hz, 3H); ^{13}C NMR ($CDCl_3$): δ 177.1, 172.2, 164.9, 163.6, 148.7, 146.2, 136.5, 131.5, 130.7, 126.5, 121.6, 120.6, 120.4, 114.8, 112.3, 49.3, 47.1, 25.4, 18.6, 15.4. Anal. calcd for $C_{21}H_{20}N_6O_3$: C, 62.37; H, 4.98; N, 20.78. Found: C, 62.03; H, 5.02; N, 20.67.

General procedure for the synthesis of quinine bis-conjugates 8a–f

A dried heavy-walled Pyrex tube containing a small stir bar was charged with benzotriazole intermediate (1.0 eq.) and quinine (1.0 eq.) dissolved in DMF along with anhydrous potassium carbonate (2.0 eq.). The reaction mixture was exposed to microwave irradiation (20 W) at a temperature of 50 °C for specified times. Each mixture was allowed to cool through an inbuilt system until the temperature had fallen 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.

Conventional method. Quinine (1.0 eq.) dissolved in DMF along with anhydrous potassium carbonate (2.0 eq.) in a round bottom flask. Benzotriazole intermediate (1.0 eq.) was added to the solution and the mixture was stirred at room temperature for 6–8 h. After completion of the reaction, the 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.

(2*S*)-(1*R*)-(6-Methoxyquinolin-4-yl)(5-vinylquinuclidin-2-yl)-methyl 2-(5-ethyl-8-oxo-5,8-dihydro-[1,3]dioxolo[4,5-*g*]quinoline-7-carboxamido)-3-phenylpropanoate (8a). White solid (70%); mp 125–127 °C; ^{1}H NMR ($CDCl_3$): δ 10.98 (d, J = 6.9 Hz, 1H), 8.68–8.65 (m, 1H), 8.53 (s, 1H), 8.35–8.28 (m, 1H), 8.02–7.96 (m, 1H), 7.90–7.85 (m, 1H), 7.67–7.62 (m, 1H), 7.54–7.49 (m, 1H), 7.45–7.35 (m, 2H), 7.24–7.17 (m, 2H), 7.09 (d, J = 9.3 Hz, 2H), 6.87 (d, J = 14.7 Hz, 1H), 6.44–6.36 (m, 1H), 6.14 (s, 2H), 5.84–5.77 (m, 1H), 5.58–5.56 (m, 1H), 5.04–5.86 (m, 2H), 4.21–4.15 (m, 2H), 3.89 (s, 3H), 3.61–3.57 (m, 1H), 3.39–3.16 (m, 4H), 2.60–2.45 (m, 2H), 2.57–1.71 (m, 4H), 1.52–1.39 (m, 4H); ^{13}C NMR ($CDCl_3$): δ 175.1, 171.3, 165.3, 158.0, 152.9, 147.7, 146.7, 145.5, 143.3, 142.0, 136.1, 136.0, 131.7, 129.5, 129.4, 128.6, 127.0, 124.1, 122.0, 114.6, 110.9, 104.4, 102.7, 101.6, 95.3, 70.5, 59.3, 56.7, 55.8, 54.4, 49.8, 42.5, 40.0, 38.1, 27.7, 24.5, 14.7. HRMS (+ESI-TOF) m/z for $C_{42}H_{42}N_4O_7$ for $[M + 1]^+$ calcd 715.3126, found 715.3130.

(2*S*)-(1*R*)-(6-Methoxyquinolin-4-yl)(5-vinylquinuclidin-2-yl)-methyl 2-(5-ethyl-8-oxo-5,8-dihydro-[1,3]dioxolo[4,5-*g*]quinoline-7-carboxamido)propanoate (8b). White solid (72%); mp 224–226 °C; ^{1}H NMR ($CDCl_3$): δ 10.69 (d, J = 7.2 Hz, 1H), 8.81 (s, 1H), 8.68 (s, 1H), 8.30 (d, J = 9.3 Hz, 1H), 7.68 (s, 1H), 7.99 (s, 1H), 7.51–7.46 (m, 2H), 6.93 (s, 1H), 6.13 (s, 2H), 5.69–5.53 (m, 1H), 5.07–5.00 (m, 2H), 4.75 (br s, 1H), 4.30–4.26 (m, 3H), 4.19 (s, 3H), 4.05–4.01 (m, 1H), 3.61–3.42 (m, 2H), 3.29–3.22 (m, 1H), 3.20–3.11 (m, 1H), 2.76–2.36 (m, 1H), 2.31–2.14 (m, 2H), 1.99–1.83 (m, 1H), 1.75–1.68 (m, 1H), 1.61 (d, J = 6.6 Hz, 3H), 1.58–1.49 (m, 3H); ^{13}C NMR ($CDCl_3$): δ 175.1, 170.8, 165.8, 160.8, 153.1, 146.8, 146.0, 145.2, 143.2, 140.0, 137.0, 136.2, 128.1, 127.0, 126.3, 123.9, 118.1, 117.8, 110.4, 104.1, 102.8, 101.7, 95.6, 70.2, 58.5, 58.4,

54.9, 50.2, 48.8, 43.7, 36.9, 27.1, 24.4, 19.9, 17.4, 14.8. HRMS (+ESI-TOF) *m/z* for $C_{36}H_{38}N_4O_7$ $[M + 1]^+$ calcd 639.2813, found 639.2815.

(2*S*)-(1*R*)-(6-Methoxyquinolin-4-yl)(5-vinylquinuclidin-2-yl)-methyl 2-(5-ethyl-8-oxo-5,8-dihydro-[1,3]dioxolo[4,5-*g*]quinoline-7-carboxamido)-3-methylbutanoate (8c). White powder (68%); mp 160–162 °C; ^{1}H NMR ($CDCl_3$): δ 10.74 (d, J = 7.5 Hz, 1H), 8.70 (d, J = 4.2 Hz, 1H), 8.49 (s, 1H), 7.96 (d, J = 9.0 Hz, 1H), 7.81 (d, J = 4.2 Hz, 1H), 7.46–7.39 (m, 2H), 6.89 (s, 1H), 6.56–6.52 (m, 1H), 6.12 (s, 2H), 5.89–5.80 (m, 1H), 5.03 (d, J = 4.2 Hz, 1H), 4.98 (s, 1H), 4.73–4.70 (m, 1H), 4.19–4.17 (m, 2H), 3.92 (s, 3H), 3.47–3.41 (m, 1H), 3.22–2.97 (m, 2H), 2.70–2.55 (m, 3H), 2.39–2.20 (m, 3H), 2.02–1.92 (m, 1H), 1.80–1.75 (m, 1H), 1.61–1.40 (m, 4H), 1.30–1.20 (m, 1H), 1.01 (d, J = 5.7 Hz, 3H), 0.90 (d, J = 5.7 Hz, 3H); ^{13}C NMR ($CDCl_3$): δ 175.2, 171.4, 165.6, 157.9, 152.9, 147.5, 146.6, 145.5, 144.8, 143.7, 141.9, 136.0, 131.6, 127.2, 124.0, 121.9, 114.6, 111.0, 104.3, 102.7, 101.6, 95.3, 59.4, 58.1, 56.6, 55.8, 49.8, 42.5, 39.9, 30.8, 27.8, 27.6, 24.8, 19.7, 18.0, 14.6. Anal. calcd for $C_{38}H_{42}N_4O_7$: C, 68.45; H, 6.35; N, 8.40. Found: C, 68.05; H, 6.67; N, 8.23.

(1*R*)-(6-Methoxyquinolin-4-yl)(5-vinylquinuclidin-2-yl)methyl 2-(1-ethyl-7-methyl-4-oxo-1,4-dihydro-1,8-naphthyridine-3-carboxamido)acetate (8d). White microcrystals (70%); mp 173–175 °C; ^{1}H NMR ($CDCl_3$): δ 10.41 (t, J = 5.3 Hz, 1H), 8.84 (s, 1H), 8.74 (d, J = 4.8 Hz, 1H), 8.64 (d, J = 7.8 Hz, 1H), 7.99 (d, J = 9.0 Hz, 1H), 7.43–7.40 (m, 2H), 7.36–7.25 (m, 2H), 6.57 (d, J = 6.9 Hz, 1H), 5.90–5.76 (m, 1H), 5.03–4.94 (m, 2H), 4.60–4.47 (m, 2H), 4.42–4.20 (m, 2H), 3.94 (s, 3H), 3.45–3.35 (m, 1H), 3.17–2.98 (m, 2H), 2.69 (s, 3H), 2.65–2.55 (m, 2H), 2.30–2.20 (m, 1H), 1.95–1.80 (m, 4H), 1.78–1.67 (m, 1H), 1.61–1.56 (m, 1H), 1.49 (t, J = 6.9, 7.2 Hz, 3H); ^{13}C NMR ($CDCl_3$): δ 177.0, 169.4, 165.4, 163.5, 158.1, 148.7, 147.7, 147.6, 144.9, 143.4, 142.0, 136.6, 132.0, 127.2, 122.0, 121.5, 120.3, 119.2, 114.6, 112.4, 101.6, 74.9, 59.4, 56.8, 55.9, 47.0, 42.7, 41.8, 39.9, 27.9, 27.7, 25.4, 24.4, 15.4. HRMS (+ESI-TOF) *m/z* for $C_{34}H_{37}N_5O_5$ $[M + Na]^+$ calcd 618.2687, found 618.2694.

(2*S*)-(1*R*)-(6-Methoxyquinolin-4-yl)(5-vinylquinuclidin-2-yl)-methyl-2-(1-ethyl-7-methyl-4-oxo-1,4-dihydro-1,8-naphthyridine-3-carboxamido)-4-methylpentanoate (8e). White microcrystals (69%); mp 109–111 °C; ^{1}H NMR ($CDCl_3$): δ 10.22 (d, J = 6.9 Hz, 1H), 8.68 (s, 1H), 8.61 (d, J = 4.2 Hz, 1H), 8.56 (dd, J = 8.1, 1.2 Hz, 1H), 7.87 (d, J = 9.0 Hz, 1H), 7.36–7.30 (m, 2H), 7.26–7.16 (m, 3H), 6.44 (d, J = 7.5 Hz, 1H), 5.82–5.70 (m, 1H), 4.94 (dd, J = 7.2, 1.2 Hz, 1H), 4.89 (s, 1H), 4.74–4.68 (m, 1H), 4.52–4.30 (m, 2H), 3.83 (s, 3H), 3.40–3.28 (m, 1H), 3.16–3.00 (m, 1H), 2.98–2.85 (m, 1H), 2.62 (s, 3H), 2.58–2.42 (m, 2H), 2.22–2.12 (m, 1H), 1.93–1.80 (m, 1H), 1.75–1.48 (m, 6H), 1.41 (t, J = 7.2 Hz, 3H), 0.86–0.81 (m, 6H); ^{13}C NMR ($CDCl_3$): δ 177.0, 172.2, 164.9, 163.5, 157.9, 148.7, 147.6, 147.5, 144.9, 143.6, 142.1, 136.4, 131.8, 127.2, 121.9, 121.5, 120.5, 119.6, 114.6, 112.4, 101.7, 74.3, 59.4, 56.8, 55.8, 51.5, 47.0, 42.6, 40.8, 40.0, 27.9, 27.7, 25.4, 25.2, 24.8, 23.2, 21.9, 15.5. Anal. calcd for $C_{38}H_{45}N_5O_5$: C, 70.02; H, 6.96; N, 10.74. Found C, 69.72; H, 7.15; N, 10.54.

(2*S*)-(1*R*)-(6-Methoxyquinolin-4-yl)(5-vinylquinuclidin-2-yl)-methyl-2-(1-ethyl-7-methyl-4-oxo-1,4-dihydro-1,8-naphthyridine-3-carboxamido)propanoate (8f). White power (70%); mp 112–113 °C; ^{1}H NMR ($CDCl_3$): δ 10.38 (d, J = 6.6 Hz, 1H), 8.78 (s, 1H), 8.71 (d, J = 3.6 Hz, 1H), 8.62 (d, J = 7.8 Hz, 1H), 7.97 (d, J = 9.3 Hz, 1H), 7.43–7.35 (m, 2H), 7.30–7.26 (m, 2H), 6.54–6.51 (m, 1H), 5.90–5.73 (m, 1H), 4.99 (d, J = 7.8 Hz, 1H), 4.95 (s, 1H), 4.86–4.70 (m, 1H), 4.60–4.40 (m, 2H), 3.91 (s, 3H), 3.48–3.32 (m, 1H), 3.20–3.08 (m, 1H), 3.01 (t, J = 12.0 Hz, 1H), 2.68 (s, 3H), 2.65–2.50 (m, 2H), 2.30–2.20 (m, 1H), 1.96–1.62 (m, 5H), 1.50–1.46 (m, 6H); ^{13}C NMR ($CDCl_3$): δ 176.9, 172.2, 164.6, 163.5, 158.0, 148.6, 147.5, 147.4, 144.8, 143.6, 141.9, 136.4, 131.8, 127.1, 122.0, 121.5, 120.5, 119.1, 114.6, 112.3, 101.5, 74.9, 59.4, 56.7, 55.8, 48.6, 47.0, 42.6, 39.9, 27.8, 27.7, 25.4, 24.5, 17.9, 15.4. HRMS (+ESI-TOF) *m/z* for $C_{35}H_{39}N_5O_5$ $[M + Na]^+$ calcd 632.2843, found 632.2850.

General procedure for the preparation of *N*-(Boc-aminoacyl)-benzotriazoles 9a–d

Compounds **9a–d** were synthesized by irradiating an equimolar amount of Boc protected amino acid with 1-(methylsulfonyl)-1*H*-benzo[*d*][1,2,3]triazole ($BtSO_2Me$) in the presence of 2.0 eq. of triethylamine for 2 min run time and 30 min hold time at 70 °C and 50 W irradiation power. Completion of the reaction was monitored by TLC. After completion of the reaction, the mixture was quenched with water. The precipitate obtained was washed with saturated solution of sodium carbonate and water to afford compound **9a–d** (Table 7).

Table 7 Preparation of *N*-(Pg-aminoacyl)benzotriazoles **9a–d**

Entry	Products	Yield (%)	Mp (°C)	Lit Mp (°C)
1	Boc-Gly-Bt **9a**	75	68–70	68–69[24]
2	Boc-Ala-Bt **9b**	54	145–146	144–145[24]
3	Boc-Phe-Bt **9c**	59	86–88	85–86[24]
4	Boc-Ile-Bt **9d**	85	100–101	Novel

***tert*-Butyl ((2*S*,3*S*)-1-(1*H*-benzo[*d*][1,2,3]triazol-1-yl)-3-methyl-1-oxopentan-2-yl)carbamate (9d).** White solid (85%); mp 100–101 °C; ^{1}H NMR ($CDCl_3$): δ 8.30 (d, J = 8.1 Hz, 1H), 8.15 (d, J = 8.1 Hz, 1H), 7.68 (t, J = 7.7 Hz, 1H), 7.53 (t, J = 7.7 Hz, 1H), 5.67 (br s, 1H), 5.36 (br s, 1H), 2.20 (br s, 1H), 1.61–1.42 (m, 2H), 1.45 (s, 9H), 1.07 (d, J = 6.6 Hz, 3H), 0.90 (t, J = 7.4 Hz, 3H); ^{13}C NMR ($CDCl_3$): δ 172.3, 155.8, 146.2, 131.2, 130.7, 126.6, 120.5, 114.6, 80.4, 59.0, 38.3, 28.5, 24.4, 16.2, 11.5. Anal. calcd for $C_{17}H_{24}N_4O_3$: C, 61.43; H, 7.28; N, 16.85. Found: C, 61.22; H, 7.34; N, 16.73.

General procedure for the synthesis of quinine–aminoacid conjugates 10a–d

A dried heavy-walled Pyrex tube containing a small stir bar was charged with *N*-(Boc-aminoacyl)benzotriazoles (1.0 eq.) and quinine (1.0 eq.) dissolved in DMF along with anhydrous potassium carbonate (2.0 eq.). 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.

(1*R*)-(6-Methoxyquinolin-4-yl)(5-vinylquinuclidin-2-yl)methyl 2-((*tert*-butoxycarbonyl)amino)acetate (10a). White microcrystals (86%); mp 74–76 °C; ^{1}H NMR ($CDCl_3$): δ 8.72 (d, J = 4.8 Hz, 1H), 8.07 (d, J = 9.3 Hz, 1H), 7.78 (d, J = 2.1 Hz, 1H), 7.45–7.41 (m, 3H), 5.72–5.64 (m, 1H), 5.64–5.52 (m, 1H), 5.09 (s, 1H), 5.04 (d, J = 4.8 Hz, 1H), 4.17 (s, 3H), 4.09 (dd, J = 9.4, 6.0 Hz, 2H), 3.85–3.75 (m, 1H), 3.66–3.57 (m, 1H), 3.51–3.44 (m, 1H), 3.33–3.28 (m, 1H), 3.14–3.07 (m, 1H), 2.78–2.72 (m, 1H), 2.21–2.10 (m, 3H), 1.99–1.90 (m, 1H), 1.70–1.60 (m, 1H), 1.43 (s, 9H); ^{13}C NMR ($CDCl_3$): δ 169.2, 159.7, 156.3, 146.9, 144.2, 140.4, 137.1, 131.6, 125.9, 123.9, 118.0, 117.8, 101.1, 80.4, 70.9, 58.4, 58.1, 54.9, 43.8, 43.3, 36.9, 28.6, 27.1, 24.4, 20.0. HRMS (+ESI-TOF) m/z for $C_{27}H_{35}N_3O_5$ $[M + 1]^+$ calcd 482.2649, found 482.2655.

(2*S*)-(1*R*)-(6-Methoxyquinolin-4-yl)(5-vinylquinuclidin-2-yl)-methyl 2-((*tert*-butoxycarbonyl)amino)propanoate (10b). White microcrystals (81%); mp 110–112 °C; ^{1}H NMR ($CDCl_3$): δ 8.73 (d, J = 4.8 Hz, 1H), 8.01 (d, J = 8.7 Hz, 1H), 7.39–7.30 (m, 4H), 6.50–6.47 (m, 1H), 5.89–5.76 (m, 1H), 5.04–4.95 (m, 3H), 4.43–4.39 (m, 1H), 3.95 (s, 3H), 3.40–3.31 (m, 1H), 3.10–2.99 (m, 2H), 2.70–2.57 (m, 2H), 2.56–2.39 (m, 1H), 2.32–2.27 (m, 1H), 1.90–1.47 (m, 3H), 1.43 (s, 9H), 1.33 (d, J = 7.2 Hz, 3H); ^{13}C NMR ($CDCl_3$) δ 172.7, 158.1, 155.2, 147.6, 144.9, 143.2, 141.8, 132.0, 127.1, 122.1, 119.0, 114.8, 101.4, 80.2, 75.1, 59.4, 56.8, 55.9, 49.4, 42.7, 39.8, 28.5, 28.0, 27.7, 24.5, 18.4. HRMS (+ESI-TOF) m/z for $C_{28}H_{37}N_3O_5$ $[M + 1]^+$ calcd 496.2806, found 496.2815.

(2*S*)-(1*R*)-(6-Methoxyquinolin-4-yl)(5-vinylquinuclidin-2-yl)-methyl 2-((*tert*-butoxycarbonyl)amino)-3-phenylpropanoate (10c). Off white microcrystals (80%); mp 166–168 °C; ^{1}H NMR ($CDCl_3$): δ 8.67 (t, J = 4.7 Hz, 1H), 8.08–8.00 (m, 1H), 7.40–7.26 (m, 2H), 7.20–7.03 (m, 4H), 6.87 (d, J = 6.9 Hz, 2H), 6.60–6.54 (m, 1H), 5.86–5.68 (m, 1H), 5.06–4.94 (m, 3H), 4.72–4.63 (m, 1H), 3.92 (s, 3H), 3.42–3.28 (m, 1H), 3.08–2.80 (m, 5H), 2.68–2.53 (m, 2H), 2.34–2.22 (m, 1H), 1.86–1.60 (m, 3H), 1.49–1.38 (m, 10H); ^{13}C NMR ($CDCl_3$): δ 171.5, 158.3, 155.3, 147.6, 144.9, 142.5, 141.3, 135.5, 132.0, 129.3, 128.7, 127.3, 127.0, 122.2, 119.0, 115.1, 101.4, 80.4, 74.6, 58.8, 56.3, 56.0, 54.6, 42.5, 39.5, 38.1, 28.5, 27.6, 23.7. HRMS (+ESI-TOF) m/z for $C_{34}H_{41}N_3O_5$ $[M + Na]^+$ calcd 594.2938, found 594.2939.

(1*R*)-(6-Methoxyquinolin-4-yl)(5-vinylquinuclidin-2-yl)methyl 2-((*tert*-butoxycarbonyl)amino)-3-methylpentanoate (10d). White solid (90%); mp 68–70 °C; ^{1}H NMR ($CDCl_3$): δ 8.73 (d, J = 4.5 Hz, 1H), 8.01 (d, J = 9.3 Hz, 1H), 7.43 (s, 1H), 7.39–7.35 (m, 2H), 6.51–6.49 (m, 1H), 5.87–5.79 (m, 1H), 5.04 (d, J = 4.8 Hz, 1H), 4.99 (s, 1H), 4.95–4.90 (m, 1H), 4.37–4.32 (m, 1H), 3.96 (s, 3H), 3.41–3.38 (m, 1H), 3.06–2.98 (m, 2H), 2.68–2.55 (m, 2H), 2.28–2.25 (m, 1H), 2.00–1.86 (m, 3H), 1.77–1.68 (m, 1H), 1.53–1.47 (m, 2H), 1.46–1.36 (m, 10H), 0.94–0.92 (m, 2H), 0.83 (d, J = 10.5 Hz, 3H), 0.67–0.65 (m, 2H); ^{13}C NMR ($CDCl_3$): δ 172.2, 158.1, 155.8, 147.4, 145.0, 143.4, 141.9, 132.0, 127.1, 122.0, 119.1, 114.7, 101.5, 80.1, 75.0, 59.4, 58.3, 56.8, 55.8, 42.6, 39.8, 37.8, 28.5, 27.9, 27.6, 25.0, 24.5, 15.8, 11.5. HRMS (+ESI-TOF) m/z for $C_{31}H_{43}N_3O_5$ $[M + 1]^+$ calcd 538.3275, found 538.3258.

General procedure for the synthesis of quinine bis-conjugates 8g–k

Quinine–amino acid conjugate was stirred in HCl gas saturated dioxane for 1 h. Dioxane was evaporated under reduced pressure and the residue was treated with diethyl ether. The resultant sticky solid was treated without further purification with benzotriazole derivatives of quinolone antibiotic in the presence of triethylamine (2.0 eq.) in acetonitrile–water mixture (3.5 mL + 1.5 mL) and stirred at room temperature for 3 h. Acetonitrile was removed under vacuum and the residue quenched in ice cold water. The reaction was then extracted with ethyl actetae (3 × 20 mL). The organic layer was dried over anhydrous $MgSO_4$ and subjected to column chromatography in a methanol/dichloromethane gradient to obtain the desired product.

(1*R*)-(6-Methoxyquinolin-4-yl)(5-vinylquinuclidin-2-yl)methyl 2-((*S*)-9-fluoro-3-methyl-10-(4-methylpiperazin-1-yl)-7-oxo-3,7-dihydro-2*H*-[1,4]oxazino[2,3,4-*ij*]quinoline-6-carboxamido)-acetate (8g). White microcrystals (88%); mp 115–117 °C; ^{1}H NMR ($CDCl_3$): δ 10.49 (t, J = 5.4 Hz, 1H), 8.75 (d, J = 4.8 Hz, 1H), 8.46 (s, 1H), 8.00 (d, J = 9.3 Hz, 1H), 7.57 (d, J = 12.6 Hz, 1H), 7.45–7.32 (m, 3H), 6.56 (d, J = 6.9 Hz, 1H), 5.89–5.77 (m, 1H), 5.03–5.00 (m, 2H), 4.35–4.28 (m, 4H), 3.94 (s, 3H), 3.40–3.29 (m, 5H), 3.13–2.99 (m, 3H), 2.69–2.62 (m, 2H), 2.57–2.52 (m, 4H), 2.35 (s, 3H), 1.95–1.85 (m, 2H), 1.76–1.68 (m, 1H), 1.63–1.52 (m, 3H), 1.45–1.42 (m, 3H); ^{13}C NMR ($CDCl_3$): δ 175.2, 169.4, 165.5, 158.0, 157.4, 154.2, 147.6, 144.8, 144.0, 143.4, 141.8, 139.5, 132.1, 131.9, 127.0, 124.3, 122.3, 122.2, 122.0, 119.1, 114.6, 110.3, 105.3, 105.0, 101.4, 74.9, 68.2, 59.4, 56.8, 55.8, 55.0, 50.7, 46.6, 42.7, 41.7, 39.9, 27.8, 27.6, 24.3, 18.3. HRMS (+ESI-TOF) m/z for $C_{40}H_{45}FN_6O_6$ $[M + Na]^+$ calcd 747.3277, found 747.3286.

(2*S*)-(1*R*)-(6-Methoxyquinolin-4-yl)(5-vinylquinuclidin-2-yl)-methyl 2-((*S*)-9-fluoro-3-methyl-10-(4-methylpiperazin-1-yl)-7-oxo-3,7-dihydro-2*H*-[1,4]oxazino[2,3,4-*ij*]quinoline-6-carboxamido)-propanoate (8h). White microcrystals (78%); mp 145–146 °C; ^{1}H NMR ($CDCl_3$): δ 10.49 (d, J = 6.9 Hz, 1H), 8.70 (d, J = 4.5 Hz, 1H), 8.53 (s, 1H), 7.98 (d, J = 9.3 Hz, 1H), 7.70 (d, J = 12.6 Hz, 1H), 7.43–7.38 (m, 2H), 7.32 (dd, J = 9.3, 2.7 Hz, 1H), 6.54 (d, J = 6.9 Hz, 1H), 5.90–5.77 (m, 1H), 5.03–5.00 (m, 1H), 4.97 (d, J = 0.6 Hz, 1H), 4.78 (p, J = 7.1 Hz, 1H), 4.42–4.25 (m, 3H), 3.93 (s, 3H), 3.44–3.32 (m, 5H), 3.20–3.09 (m, 1H), 3.07–2.98 (m, 1H), 2.69–2.60 (m, 2H), 2.57–2.53 (m, 4H), 2.36 (s, 3H), 2.39–2.20 (m, 3H), 1.98–1.88 (m, 1H), 1.87–1.82 (m, 1H), 1.80–1.65 (m, 1H), 1.55 (d, J = 6.9 Hz, 3H), 1.50 (d, J = 7.2 Hz, 3H); ^{13}C NMR ($CDCl_3$): δ 175.4, 172.2, 164.9, 158.0, 157.9, 154.3, 147.5, 144.8, 143.9, 143.7, 142.0, 139.6, 131.9, 131.8, 127.1, 124.4, 122.4, 122.0, 119.1, 114.6, 110.5, 105.6, 105.2, 101.5, 74.5, 68.3, 59.5, 56.8, 55.9, 55.1, 50.8, 48.6, 46.6, 42.4, 40.0, 27.9, 27.7, 24.5, 18.4, 17.9. HRMS (+ESI-TOF) m/z for $C_{41}H_{47}FN_6O_6$ $[M + 1]^+$ calcd 739.3614, found 739.3613.

(2*S*)-(1*R*)-(6-Methoxyquinolin-4-yl)(5-vinylquinuclidin-2-yl)-methyl 2-((*S*)-9-fluoro-3-methyl-10-(4-methylpiperazin-1-yl)-7-oxo-3,7-dihydro-2*H*-[1,4]oxazino[2,3,4-*ij*]quinoline-6-carboxamido)-3-phenylpropanoate (8i). White microcrystals (72%); mp 161–163 °C; ^{1}H NMR ($CDCl_3$): δ 10.62 (d, J = 7.2 Hz, 1H), 8.72–8.62 (m, 1H), 8.50 (s, 1H), 7.98 (d, J = 9.0 Hz, 1H), 7.65 (d, J = 12.6 Hz, 1H), 7.43–7.26 (m, 2H), 7.22 (d, J = 7.5 Hz, 1H), 7.14–7.06 (m, 5H), 6.53–6.48 (m, 1H), 5.88–5.40 (m, 1H), 5.18–4.88 (m, 3H), 4.38–4.21 (m, 3H), 3.89 (s, 3H), 3.43–3.28 (m, 5H), 3.23–2.94 (m, 4H), 2.66–2.48 (m, 6H), 2.36 (s, 3H), 1.80–1.66 (m, 4H), 1.50 (overlapped d, J = 6.6 Hz, 3H), 1.50–1.41 (m, 2H); ^{13}C NMR ($CDCl_3$): δ 175.2, 171.1, 165.0, 157.9, 157.4, 154.2, 147.5, 144.8, 143.9, 143.1, 141.8, 139.5, 136.1, 132.1, 131.9, 131.7, 129.3, 128.5, 127.1, 127.0, 124.3, 122.3, 121.9, 119.4, 114.6, 110.4, 105.4, 105.1, 101.5, 74.4, 68.2, 59.2, 56.5, 55.8, 54.9, 54.2, 50.6, 46.5, 42.4, 39.8, 38.3, 38.0, 27.6, 24.4, 18.3. HRMS (+ESI-TOF) m/z for $C_{47}H_{51}FN_6O_6$ $[M + Na]^+$ calcd 837.3746, found 837.3738.

(1*R*)-(6-Methoxyquinolin-4-yl)(5-vinylquinuclidin-2-yl)methyl 2-(1-cyclopropyl-7-(4-ethylpiperazin-1-yl)-6-fluoro-4-oxo-1,4-dihydroquinoline-3-carboxamido)acetate (8j). White microcrystals (83%); mp 104–106 °C; ^{1}H NMR ($CDCl_3$): δ 10.53 (t, J = 7.2 Hz, 1H), 8.74 (d, J = 4.5 Hz, 1H), 8.73 (s, 1H), 8.01 (d, J = 5.1 Hz, 1H), 7.98 (s, 1H), 7.41 (s, 1H), 7.40 (s, 1H), 7.36–7.30 (m, 2H), 6.56 (d, J = 7.2 Hz, 1H), 5.91–5.75 (m, 1H), 5.01 (d, J = 9.3 Hz, 1H), 4.33 (dd, J = 18.0, 5.7 Hz, 1H), 4.24 (dd, J = 17.9, 5.6 Hz, 1H), 3.93 (s, 3H), 3.48–3.38 (m, 2H), 3.32 (t, J = 4.3 Hz, 4H), 3.18–2.99 (m, 2H), 2.68 (t, J = 4.8 Hz, 4H), 2.56–2.48 (m, 2H), 2.32–2.21 (m, 2H), 1.90–1.83 (m, 2H), 1.61–1.49 (m, 2H), 1.35–1.25 (m, 4H), 1.17–1.12 (m, 5H); ^{13}C NMR ($CDCl_3$) δ 175.0, 169.4, 165.6, 158.0, 155.1, 152.1, 147.7, 146.8, 145.3, 144.9, 143.4, 142.0, 138.6, 131.9, 127.1, 122.0, 119.2, 114.6, 112.9, 112.6, 110.7, 104.9, 101.5, 74.8, 59.4, 56.8, 55.9, 52.7, 52.5, 50.2, 42.7, 41.8, 40.0, 34.9, 27.9, 27.7, 24.5, 12.2, 8.4. HRMS (+ESI-TOF) m/z for $C_{41}H_{47}FN_6O_5$ $[M + 1]^+$ calcd 723.3665, found 723.3663.

(2*S*,3*S*)-(1*R*)-(6-Methoxyquinolin-4-yl)(5-vinylquinuclidin-2-yl)-methyl 2-(1-cyclopropyl-7-(4-ethylpiperazin-1-yl)-6-fluoro-4-oxo-1,4-dihydroquinoline-3-carboxamido)-3-methylpentanoate (8k). White solid (71%); mp 128–129 °C; ^{1}H NMR ($CDCl_3$): δ 10.57 (d, J = 7.8 Hz, 1H), 8.74–8.66 (m, 2H), 8.06–7.92 (m, 2H), 7.45–7.30 (m, 4H), 6.60–6.42 (m, 1H), 5.92–5.80 (m, 1H), 5.03–5.01 (m, 1H), 4.98 (s, 1H), 3.96–3.95 (m, 1H), 3.91 (s, 3H), 3.47–3.40 (m, 2H), 3.34–3.26 (m, 4H), 3.25–2.95 (m, 2H), 2.70–2.60 (m, 4H), 2.51 (q, J = 7.2 Hz, 2H), 2.31–2.18 (m, 1H), 2.08–1.92 (m, 2H), 1.84–1.67 (m, 2H), 1.60–1.43 (m, 3H), 1.31–1.20 (m, 5H), 1.17–1.12 (m, 5H), 0.98–0.94 (m, 4H), 0.85–0.74 (m, 2H); ^{13}C NMR ($CDCl_3$): δ 175.5, 171.5, 171.3, 165.3, 157.8, 155.2, 151.9, 147.4, 146.7, 145.1, 145.0, 144.8, 143.7, 141.9, 141.8, 138.5, 131.6, 127.2, 121.9, 119.3, 114.5, 112.8, 112.5, 110.7, 104.7, 101.6, 74.2, 59.3, 57.6, 56.6, 55.7, 52.6, 52.4, 50.0, 42.4, 39.9, 37.7, 37.2, 34.8, 27.8, 27.6, 25.0, 16.1, 12.1, 11.5, 8.2. HRMS (+ESI-TOF) m/z for $C_{41}H_{47}FN_6O_6$ $[M + 1]^+$ calcd 779.4291, found 779.4286.

Antimalarial activity assay

P. falciparum strain 3D7 was cultured according to the method of Trager and Jensen[25] with minor modifications. Parasites were grown in human erythrocytes (2% hematocrit) in an atmosphere of 5% CO_2, 5% O_2, 90% N_2 in RPMI1640 medium (Gibco) supplemented with 25 mM Hepes buffer (Sigma), 25 mg L^{-1} gentamicin (Gibco), 1 mM Sodium pyruvate (Sigma), 50 mg L^{-1} hypoxanthine (Sigma), 2 g L^{-1} glucose (Sigma), 2.52 g L^{-1} sodium bicarbonate (Sigma) and 5 g L^{-1} Albumax 1 (Gibco). *In vitro* antimalarial activity was determined by the SYBR Green I method described by Smilkstein *et al.*[26] with modifications.[27] Stock solutions of each compound were prepared in DMSO at a concentration 10 mM and 3-fold serial dilutions prepared in DMSO. Drugs were then diluted 250-fold into culture medium in 96-well storage plates to create 2X drug solutions. Drug solutions (50 µl per 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^{-1} 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) 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).

Conclusion

In conclusion, we have developed convenient benzotriazole-mediated efficient syntheses of quinine bis conjugates incorporated with quinolone (or fluoroquinolone) antibiotic and peptide fragments *via* two alternative routes and have demonstrated that some of these bis-conjugates retain antimalarial activity similar to quinine (IC_{50} values ranging from 12 to 207 nM).

Acknowledgements

We thank the University of Florida, The Kenan Foundation, King Abdulaziz University, Jeddah, Saudi Arabia. We also thank Dr Hall for English checking.

Notes and references

1 (*a*) L. V. Faro, J. M. de Almeida, C. C. Cirne-Santos, V. A. Giongo, L. R. Castello-Branco, I. de B. Oliveira, J. E. F. Barbosa, A. C. Cunha, V. F. Ferreira, M. C. de Souza, I. C. N. P. Paixão and M. C. B. V. de Souza, *Bioorg. Med. Chem. Lett.*, 2012, **22**, 5055–5058; (*b*) F. da C. Santos, P. Abreu, H. C. Castro, I. C. P. P. Paixão, C. C. Cirne-Santos, V. Giongo, J. E. Barbosa, B. R. Simonetti, V. Garrido, D. C. Bou-Habib, D. de O. Silva, P. N. Batalha, J. R. Temerozo, T. M. Souza, C. M. Nogueira, A. C. Cunha, C. R. Rodrigues, V. F. Ferreira and M. C. B. V. de Souza, *Bioorg. Med. Chem.*, 2009, **17**, 5476–5481.
2 D. Greenwood, *J. Antimicrob. Chemother.*, 1992, **30**, 417–427.
3 R. A. Daines, K. K. Sham, J. J. Taggart, W. D. Kingsbury, J. Chan, A. Breen, J. Disa and N. Aiyar, *Bioorg. Med. Chem. Lett.*, 1997, **7**, 2673–2676.
4 B. Kolesinska, K. Kasperowicz, M. Sochacki, A. Mazur, S. Jankowski and Z. J. Kaminski, *Tetrahedron Lett.*, 2010, **51**, 20–22.

5 H. Chen, Q. F. Wang, X. L. Sun, J. Luo and R. Jiang, *Mendeleev Commun.*, 2010, **20**, 104–105.
6 T. Hogberg, I. Khanna, S. D. Drake, L. A. Mitscher and L. L. Shen, *J. Med. Chem.*, 1984, **27**, 306–310.
7 L. A. Mitscher, H. E. Gracey, G. W. Clark III and T. Suzuki, *J. Med. Chem.*, 1978, **21**, 485–489.
8 H. Liu and S. G. Mulholland, *Am. J. Med.*, 2005, **118**, 14S–20S.
9 P. M. Vancutsem, J. G. Babish and W. S. Schwark, *Cornell Vet.*, 1990, **80**, 173–186.
10 A. A. Divo, A. C. Sartorelli, C. L. Patton and F. J. Bia, *Antimicrob. Agents Chemother.*, 1988, **32**, 1182–1186.
11 B. Pradines, C. Rogier, T. Fusai, J. Mosnier, W. Daries, E. Barret and D. Parzy, *Antimicrob. Agents Chemother.*, 2001, **45**, 1746–1750.
12 A. E. Yeo and K. H. Rieckmann, *J. Parasitol.*, 1994, **80**, 158–160.
13 N. Mahmoudi, L. Ciceron, J.-F. Franetich, K. Farhati, O. Silvie, W. Eling, R. Sauerwein, M. Danis, D. Mazier and F. Derouin, *Antimicrob. Agents Chemother.*, 2003, **47**, 2636–2639.
14 J. Prada, S. A. Alabi and U. Bienzle, *Lancet*, 1993, **342**, 1114.
15 N. White, *Phil. Trans. R. Soc. London B.*, 1999, **354**, 739–749.
16 N. J. White, *Drug Resist. Update.*, 1998, **1**, 3–9.
17 N. J. White, *J. Antimicrob. Chemother.*, 1992, **30**, 571–585.
18 W. Metzger, B. Mordmueller, W. Graninger, U. Bienzle and P. G. Kremsner, *J. Antimicrob. Chemother.*, 1995, **39**, 245–262.
19 R. Hubel, T. Jelinek and W. Beck, *Z. Naturforsch., B: Chem. Sci.*, 2000, **55**, 821–833.
20 C. Laruelle, M. Lepant and B. Raynier, *Fr. Demande*, FR 2564832 A1, 1985, **18**.
21 T. S. Leonova, E. N. Padeiskaya and V. G. Yashunskii, *Khim.-Farm. Zh.*, 1987, **21**, 692–696.
22 J. P. Sanchez, *Eur. Pat. Appl.*, EP 304087 A2, 1989.
23 A. T. Katritzky, A. M. Munawar, J. Kovacs and L. Khelashvili, *Org. Biomol. Chem.*, 2009, **7**, 2359–2362.
24 A. R. Katritzky, P. Angrish and E. Todadze, *Synlett*, 2009, **15**, 2392–2411.
25 W. Trager and J. B. Jensen, *Science*, 1976, **193**, 673–675.
26 M. Smilkstein, N. Sriwilaijaroen, J. X. Kelly, P. Wilairat and M. Riscoe, *Antimicrob. Agents Chemother.*, 2004, **48**, 1803–1806. PMCID: 400546.
27 R. W. Winter, J. X. Kelly, M. J. Smilkstein, R. Dodean, G. C. Bagby, R. K. Rathbun, J. I. Levin, D. Hinrichs and M. K. Riscoe, *Exp. Parasitol.*, 2006, **114**, 47–56.