Synthesis of amino acid derivatives of quinolone antibiotics†

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Optically pure conjugates of quinolone antibiotics with naturally occurring amino acids are synthesized in 40–98% yields.

Introduction

The broad-spectrum quinolone antibiotics act on topoisomerase II (DNA gyrase usually of Gram-negative) or on topoisomerase IV enzyme (of Gram-positive bacteria) to inhibit DNA replication and transcription.[1–2]

Porins (β-barrel proteins) mediate the entry of quinolones into cells, and quinolone antibiotics are often used to treat intracellular pathogens such as *Legionella pneumophila* and *Mycoplasma pneumoniae*.[3]

Oxolinic acid **1**, nalidixic acid **2**, cinoxacin **3**, and flumequine **4** (Fig. 1), all first-generation agents, are currently widely used to treat Gram-negative bacteria (*e.g.* urinary tract infections and psoriasis) by dermal delivery.[4] However, a major drawback is that their prolonged oral use causes gastrointestinal disturbances.

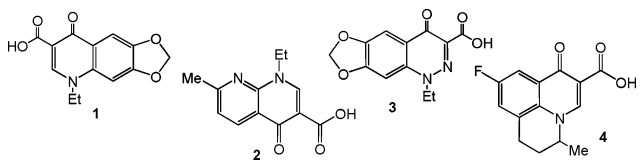
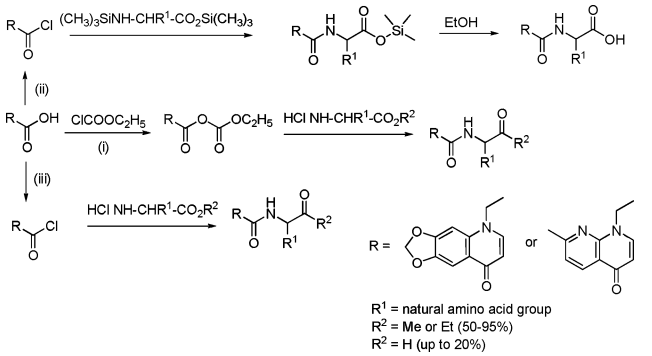


Fig. 1 Quinolone antibiotics.

Prodrugs formed from quinolone acids and amino acid esters are more lipophilic than the parent drugs,[4a,5] and show enhanced *in vivo* antibacterial properties[6,8,9] with pronounced therapeutic effects against *Pseudomonas aeruginosa*,[10–11] *Escherichia coli*,[12] *Staphylococcus aureus*[12] and *Salmonella typhi*,[9] in addition to other wide-ranging biological activities comprising anti-allergic,[7] antihypertensive,[7] bronchodilating,[7] and binding to bovine serum albumin.[4a]

Literature preparations of quinolone amino acid conjugates include the use of ethyl chloroformate,[4a,7,8,10,11] acid chlorides[5,8] and mixed anhydrides (Scheme 1).[9] Utilizing amino acid esters as coupling reagents, these methods provide quinolone–amino acid ester conjugates (yields range 50–95%) in reaction times of 5–24 h. However, coupling with free amino acids gave the target compounds in lower yields (20–50%).[8]

N-Acylbenzotriazoles[13] are efficient coupling reagents for *N*-,[14] *C*-[15] and *O*-acylation.[16] *N*-(Aminoacyl)benzotriazoles prepared from *N*-protected α-amino acids were successfully utilized for synthesis of di- and tripeptides.[17]



Scheme 1 Literature preparation of quinolone amino acid ester conjugates.

We now report syntheses of amino acid conjugates of quinolones **1–4** by coupling the free amino acids **9–23**, as well as dipeptide Gly–Gly **24** with benzotriazole-activated oxolinic acid **5**, nalidixic acid **6**, cinoxacin **7** and flumequine **8**.

Results and discussion

Preparation of benzotriazole derivatives of quinolone antibiotics

Oxolinic **1** and nalidixic **2** acids, cinoxacin **3** and flumequine **4** were converted to their corresponding benzotriazole derivatives using a standard method.[17b] Compounds **5–8** were obtained in 75–90% yields (Table 1), and are stable indefinitely at 20 °C.

Table 1 Preparation of acid benzotriazolides **5–8**

Entry	Reactant	Product	Yield (%)	Mp (°C)
1	Oxolinic acid **1**	**5**	75	229–232
2	Nalidixic acid **2**	**6**	90	169–171
3	Cinoxacin **3**	**7**	80	221–223
4	Flumequine **4**	**8**	81	218–219

Preparation of oxolinic–amino acid conjugates

The coupling of **5** with free amino acids **9–23** in aqueous MeCN in the presence of Et_3N in 3 h resulted in the formation of oxolinic–amino acid conjugates **25–39** in 58–96% yields (Table 2). Benzotriazole-activated oxolinic acid **5** reacted with free dipeptide Gly–Gly **24** giving oxolinic–dipeptide conjugate **40** in 90% yield

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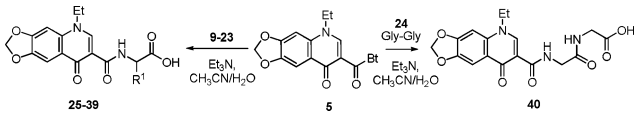
† Electronic supplementary information (ESI) available: Experimental details for compounds **6–8**, **25–29** and **31–56**. See DOI: 10.1039/b900762h

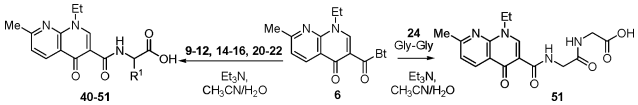
Table 2 Preparation of oxolinic–amino acid conjugates



Entry	Reactant	Product	Overall yield (%)	Mp (°C)	Lit. overall yield (%)	Lit. mp (°C)
1	Gly **9**	**25**	78	296–298	44[10]	300
2	L-Ala **10**	**26**	82	257–259	18[8]	267–269
3	DL-Ala **11**	**27**	71	257–259	25[8]	266–268
4	L-Phe **12**	**28**[b]	72	213–215	—[a]	—
5	DL-Phe **13**	**29**[c]	75	248–249	—[a]	—
6	L-Met **14**	**30**	74	193–194	—[a]	—
7	L-Leu **15**	**31**	75	219–221	40[10]	204
8	L-Ile **16**	**32**	78	225–228	—[a]	—
9	L-Trp **17**	**33**	61	167–171	—[a]	—
10	L-Ser **18**	**34**	54	246–248	42[10]	247
11	L-Cys **19**	**35**	75	218–220	—[a]	—
12	L-Asp **20**	**36**	82	238–239	40[7]	221
13	L-Val **21**	**37**	61	225–227	—[a]	—
14	L-Tyr **22**	**38**	77	292–293	—[a]	—
15	Cystine **23**	**39**	49	234–236	—[a]	—
16	Gly–Gly **24**	**40**	77	282–284	—[a]	—

[a] Compound is novel. [b] Retention time for **28** = 9.02 min. [c] Retention time for **29** = 9.16 and 9.68 min.

Table 3 Preparation of nalidixic–amino acid conjugates



Entry	Reactant	Product	Overall yield (%)	Mp (°C)	Lit. overall yield (%)	Lit. mp (°C)
1	Gly **9**	**41**	75	259–260	54[10]	276
2	L-Ala **10**	**42**	81	251–253	—[a]	—
3	DL-Ala **11**	**43**	88	252–254	50[8]	253–255
4	L-Phe **12**	**44**	86	213–215	—[a]	—
5	L-Met **14**	**45**	58	164–165	—[a]	—
6	L-Leu **15**	**46**	60	171–172	28[10]	168
7	L-Ile **16**	**47**	69	159–160	—[a]	—
8	L-Asp **20**	**48**	39	238–239	54[7]	207
9	L-Val **21**	**49**	54	182–185	—[a]	—
10	L-Tyr **22**	**50**	70	140–142	—[a]	—
11	Gly–Gly **24**	**51**	59	246–247	—[a]	—

[a] Compound is novel.

(Table 2). All novel compounds were characterized by ^{1}H-NMR, ^{13}C-NMR and elemental analysis.

HPLC (detection at 220 nm, flow rate 0.5 mL/min, and 50% MeOH as solvent) showed a single peak for **28**. By contrast two peaks were observed for the corresponding racemic mixture **29**,confirming the enantiopurity of oxolyl-L-Phe **28**.

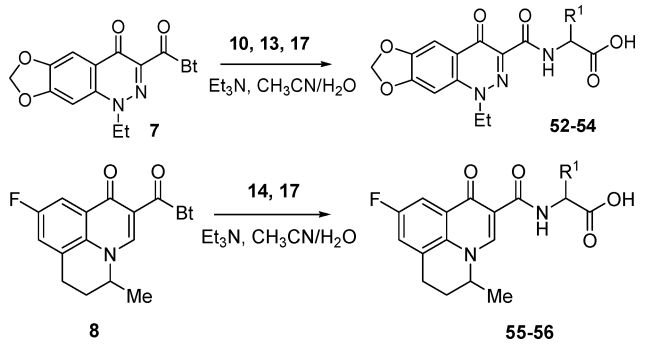
In the literature, oxolinic–amino acid conjugates were prepared either by (a) coupling of ester-activated oxolinic acid with amino acid esters (52–66%), followed by ester hydrolysis (66–90%)[7,10] or (b) reaction of oxolinic acid chloride with free amino acids (18–25%) (Table 2).[8] Our methodology allows the synthesis of oxolinic–amino acid conjugates in higher overall yields (average of 71% for 16 compounds *vs* literature average yield of 35% for 6 compounds), uses simple preparative and purification procedures, does not require anhydrous conditions, and is cost-effective.

Preparation of nalidixic–amino acid conjugates

Similarly, the coupling of **6** with **9–12**, **14–16**, and **20–22** afforded nalidixic–amino acid conjugates **41–50** in 40–98% yields (Table 3). The reaction of **6** with Gly–Gly **24** resulted in the formation of nalidixic–dipeptide conjugate **51** in 66% yield (Table 3). All products were characterized by ^{1}H-NMR, ^{13}C-NMR and elemental analysis.

Previously, nalidixic–amino acid conjugates were prepared either (a) in three steps by the active ester method[7,10] or (b) in two steps by the acid chloride method.[8] Our two-step approach provides nalidixic–amino acid conjugates in better overall yields compared to those reported in the literature (Table 3) (average of 67% for 11 compounds *vs* literature average yield of 46% for 4 compounds).

Table 4 Preparation of cinoxacin– and flumequine–amino acid conjugates[a]



Entry	Reactant	Quinolone	Product	Overall yield (%)	Mp (°C)
1	L-Ala-OH **10**	**7**	**52**	66	236–238
2	DL-Phe-OH **13**	**7**	**53**	66	266–268
3	L-Trp-OH **17**	**7**	**54**	58	179–181
4	L-Phe-OH **14**	**8**	**55**	43	175–176
5	L-Trp-OH **17**	**8**	**56**	45	200–202

[a] All compounds are novel.

Preparation of cinoxacin– and flumequine–amino acid conjugates

In order to show the generality of benzotriazole methodology, we coupled amino acids with two other quinolone antibiotics: cinoxacin **3** and flumequine **4**. Cinoxacin–amino acid conjugates **52–54** were obtained in 73–82% yields by reacting **7** with **10**, **13**, **17** in aqueous acetonitrile for 3 h (Table 4).

Under the same reaction procedure the coupling of benzotriazole-activated flumequine **8** with **14**, **17** afforded flumequine–amino acid conjugates **55–56** in 53 and 56% yields, respectively (Table 4).

Conclusions

In conclusion, we have developed a convenient and an efficient synthesis of nalidixic–, oxolinic–, cinoxacin- and flumequine–amino acid conjugates, utilizing a simple two-step route involving: (i) activation of the quinolone carboxylic acids as stable benzotriazole derivatives and (ii) coupling with free amino acids in aqueous media.

Experimental

Preparation of 7-(1*H*-benzo[*d*][1,2,3]triazole-1-carbonyl)-5-ethyl-[1,3]dioxolo[4,5-*g*]quinolin-8(5*H*)-one (5)

To a solution of 1*H*-benzotriazole (2.0 g, 16 mmol) in methylene chloride was added thionyl chloride (0.47 g, 4.0 mmol) at 25 °C. After 30 min oxolinic acid (1 g, 3.8 mmol) was added and the stirring was continued for 2 h. The precipitate was filtered off, and the filtrate was washed with water and evaporated to give a yellow solid (1.1 g, 3.0 mmol, 75%), mp 229–232 °C. ^{1}H NMR (300 MHz, DMSO-d_6) δ: 8.67 (s, 1H), 8.22 (t, $J = 8.0$ Hz, 2H), 7.79 (t, $J = 7.8$ Hz, 1H), 7.61 (t, $J = 7.6$ Hz, 1H), 7.52 (d, $J = 6.5$ Hz, 2H), 6.24 (s, 2H), 4.39 (q, $J = 6.5$ Hz, 2H), 1.40 (t, $J = 6.6$ Hz, 3H). ^{13}C NMR (75 MHz, DMSO-d_6) δ: 171.7, 164.9, 152.5, 146.8, 146.2, 145.4, 136.1, 130.9, 126.2, 123.3, 119.9, 113.6, 113.1, 102.8, 102.7, 97.0, 48.7, 14.5. $C_{19}H_{14}N_4O_4 \cdot \frac{1}{2}H_2O$, Calculated: C, 61.45; H, 4.07; N, 15.09, Found: C, 61.19; H, 3.73; N, 15.27.

General procedure for oxolinic–amino acid conjugates (25–40)

A mixture of 7-(1*H*-benzo[*d*][1,2,3]triazole-1-carbonyl)-5-ethyl-[1,3]dioxolo[4,5-*g*]quinolin-8(5*H*)-one **5** (181 mg, 0.5 mmol), amino acid (0.5 mmol) and triethylamine (101 mg, 0.13 mL, 1.0 mmol) in acetonitrile–water mixture (3.5 mL +1.5 mL) was stirred at room temperature for three hours. The acetonitrile was removed under vacuum and the residue was acidified with concentrated HCl. The precipitate was filtered, washed with cold water, dried under reduced pressure and recrystallized from aq. ethanol to gave the corresponding product.

(*S*)-2-(5-Ethyl-8-oxodihydro-[1,3]dioxolo[4,5-*g*]quinoline-7-carboxamido)-4-methylsulfanylbutanoate (30). (170 mg, 87%), mp 193–194 °C. ^{1}H NMR (300 MHz, DMSO-d_6) δ: 1.34 (t, $J = 7.2$ Hz, 3H), 1.93–2.13 (m, 5H), 2.50 (m, 2H), 4.43 (q, $J = 7.2$ Hz, 2H), 4.59–4.66 (m, 1H), 6.24 (s, 2H), 7.50 (s, 1H), 7.63 (s, 1H), 8.71 (s, 1H), 8.90 (bs, 1H), 10.53 (d, $J = 7.7$ Hz, 1H). ^{13}C NMR (75 MHz, DMSO-d_6) δ: 14.6, 29.5, 31.6, 48.9, 50.8, 96.7, 102.6, 102.8, 110.1, 123.1, 136.1, 146.2, 146.3, 152.6, 164.3, 173.2, 174.1. $C_{18}H_{20}N_2O_6S$, Calculated: C, 55.09; H, 5.14; N, 7.14, Found: C, 54.89; H, 5.06; N, 6.75.

Acknowledgements

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