Total synthesis of luotonin A and 14-substituted analogues†

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A new and concise synthesis of luotonin A was achieved from the previously described compounds ethyl 4-oxo-3,4-dihydroquinazoline-2-carboxylate and 1-(2-nitrophenyl)prop-2-en-1-one. New 14-substituted luotonin A analogues were prepared using 14-chloroluotonin A as the key intermediate.

Introduction

The natural product luotonin A (**1**) was first described a decade ago by Ma and Nomura. It was extracted from the aerial parts of *Peganum nigellasterum* (Chinese Bunge), commonly known as "*luo-tuo-hao*", giving the name to this group of compounds.[1] Since then, many alternative approaches to the preparation of luotonin A have been reported.[2] Several reviews have summarized these synthetic developments as the number of synthetic procedures in this field has grown.[3]

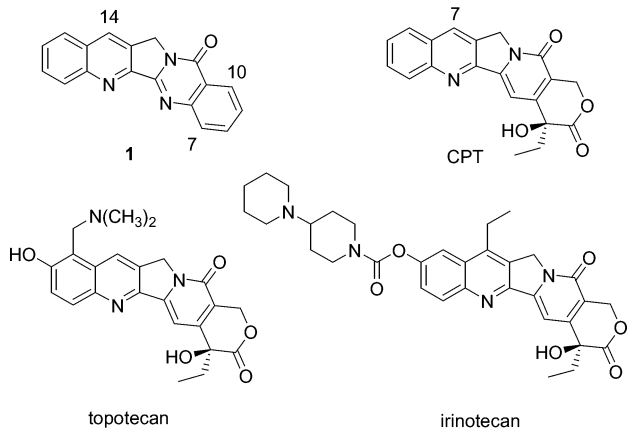

Fig. 1 Structures of luotonin A (**1**), 20(*S*)-camptothecin, topotecan and irinotecan.

Structural similarities between **1** and camptothecin (CPT) have been stated since the discovery of **1**, but the biological associations, albeit at a lower inhibitory extent, were confirmed in an elegant experiment by Hecht and co-workers.[4] Like CPT, luotonin A has been described as a selective (class I) human DNA-topoisomerase I (Top I) covalent binary complex stabilizer,[5] although **1** has been reported as having a lower Top I selectivity than CPT, with regard to Top I–Top II inhibitory effects.[6]

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The most commonly stated inadequacies of **1** are associated with poor solubility and an approximately ten-fold lower biological effect compared to CPT. This has led to the development of analogues, in an attempt to improve these undesirable qualities. These analogues were also tested as potential alternatives for camptothecin-resistant tumour strains. Only a few of the presently described analogues have shown to be as effective or slightly more effective than **1**. The majority of existing analogues of **1** have modifications to the E-ring, but alterations to positions in other rings have also been described.[2q,5–7]

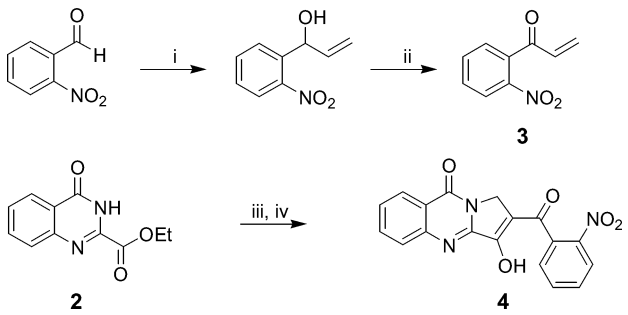
Several camptothecin analogues have emerged as approved therapeutic antineoplastic agents, namely the topotecan and irinotecan hydrochlorides (Fig. 1), while others are in various stages of clinical trials. These compounds have been shown to be effective in treatments for breast, colon, ovarian and small-cell lung cancer as well as malignant melanomas and leukaemia.[8] It is hoped that analogues of luotonin A can serve as models, or in some cases as substitutes, for camptothecin-based anti-cancer agents.

The method described herein provides a new and concise synthetic route to luotonin A and analogues with substituents at the C-14 position. There are several different numbering schemes reported for **1**. We chose to adopt the numbering system from Curran's work,[2q] where the assignment is in agreement with other 14-substituted analogues of **1**. The same numbering scheme can also be found in the work by Dallavalle.[7]

Results and discussion

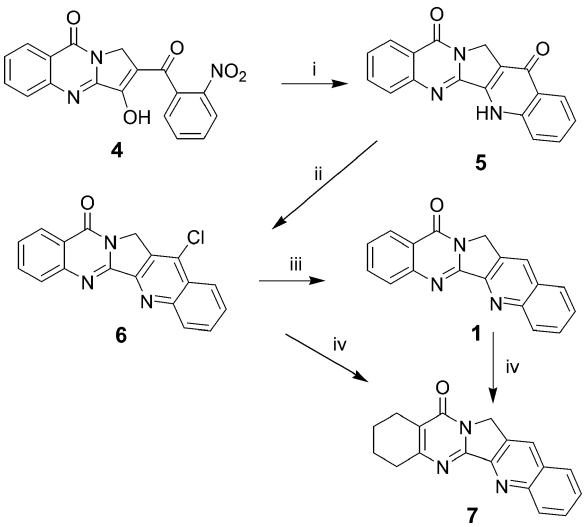
The synthetic route devised involved the sequential formation of the B, C and D rings of luotonin A. The A and E rings were derived from 2-nitrobenzaldehyde and ethyl 4-oxo-3,4-dihydroquinazoline-2-carboxylate (**2**), respectively.

The preparation of ethyl 4-oxo-3,4-dihydroquinazoline-2-carboxylate (**2**) was modified from previously described procedures.[9] These were reported as giving yields of 57%[9a] and 90%.[9b] In our procedure an excess of diethyl oxalate was added to a melt of 2-aminobenzamide, giving yields consistently above 90%. Formation of 1-(2-nitrophenyl)propenone (**3**) was achieved *via* a method described by Danishefsky,[10] using 2-nitrobenzaldehyde as the starting material in 86% over two steps. The Michael addition of **2** with the propenone compound **3** occurs rapidly, and an intramolecular Claisen condensation followed to produce compound **4** (Scheme 1).



Scheme 1 *Reagents and conditions*: i) THF, −70 °C, vinylmagnesium bromide. ii) Jones reagent, 86% over 2 steps. iii) DMF, *t*-BuOK, 60 °C. iv) **3**, 83%.

Catalytic hydrogenation of **4** with Pd/C in DMF produced compound **5** in quantitative yield. Chlorination of **5** using phosphorus oxychloride gave 14-chloroluotonin A (**6**). Reduction of **6** to the target molecule **1** (84%) was found to work best using freshly activated Raney nickel catalyst at room temperature (Scheme 2).



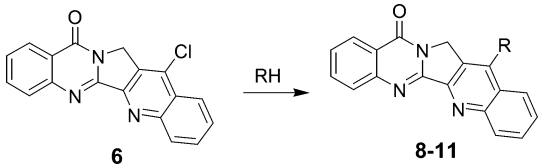
Scheme 2 *Reagents and conditions*: i) Pd/C, H_2, DMF, 1 h, 96%. ii) $POCl_3$, reflux, 1 h, 99%. iii) Raney nickel, dioxane, 1 h, 84%. iv) Raney nickel, dioxane, reflux, 1 h (or rt, >4 h).

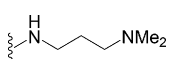
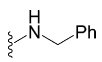
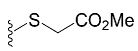
Furthermore, reduction of **6** or luotonin A with Raney nickel generated compound **7** (Scheme 2). The structure was confirmed as 7,8,9,10-tetrahydroluotonin A, using HMBC analysis. A correlation was found between the C-11 carbonyl and the protons associated with the C-10 carbon. Compound **7** has been previously reported as 16,17,18,19-tetrahydroluotonin A,[5] and biological screening returned a lower Top I inhibitory response than **1**.[11]

The halo-substituent of **6** can undergo various modifications. An equivalent CPT analogue, 7-chlorocamptothecin, has been described.[12] Substituents in this position have been shown to influence the solubility and biological effect of the CPT analogues.[13]

To test the reactivity of **6**, a small group of established reactions were performed. Initially, nucleophilic substitutions with amines and a sulfur compound were investigated, since analogues of CPT with alkyl, alkyl amino and alkyl imino chains have been synthesized, and some of these compounds displayed superior Top I inhibition compared to CPT.[14]

Table 1 Nucleophilic substitutions of 14-chloroluotonin A

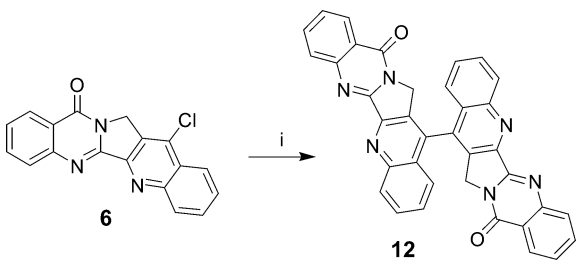


R	Conditions	Product	Yield (%)
	Neat, excess amine	**8**	81
	Neat, excess amine	**9**	79
	DMF, K_2CO_3	**10**	87
	DMF, K_2CO_3	**11**	86

Nucleophilic substitutions of **6** were performed under two different sets of conditions, depending on the nature of the reactant. The more volatile amines were refluxed neat (*N*,*N*-dimethyl-1,3-diaminopropane and piperidine), producing compounds **8** and **9** respectively. The other substitution conditions used benzylamine or methyl mercaptoacetate, in DMF with K_2CO_3, giving compounds **10** and **11** (Table 1).

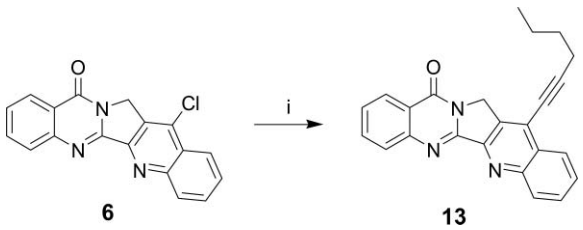
The structure of **8** is strikingly reminiscent of other groups of polycyclic quinoline-based structures such as nitracrines[15] and some quindoline derivatives.[16] The nitracrines are a group of hypoxia-selective cytotoxic compounds that display *in vitro* activity, while the quindoline derivatives have shown activity towards stabilizing G-quadruplex DNA of certain oncogenes.

Two different reactions involving palladium catalysts demonstrated the potential of **6** in transition-metal-catalyzed reactions. Attempted dechlorination of **6** with Pd/C in acetic acid produced the coupled product, 14,14′-bisluotonin A (**12**), quantitatively (Scheme 3). This is presumed to occur *via* an acidic modification of the Wurtz-type coupling between the heteroaromatic halide subunits.[17]



Scheme 3 *Reagents and conditions*: i) Pd/C, HOAc, 40 °C, 3 h, 100%.

Coupling **6** with 1-hexyne using a copper-free Sonogashira reaction produced compound **13** (Scheme 4).[18]



6 13

Scheme 4 *Reagents and conditions*: i) $Pd(OAc)_2$, *rac*-BINAP, K_2CO_3, benzene, reflux, 18 h, 87%.

Conclusions

A new method for the total synthesis of luotonin A was successful using straightforward and cost-effective operations. More importantly, several new 14-substituted analogues of luotonin A were synthesized and a technique for generating novel and diverse libraries of luotonin A analogues has been described.

Experimental

General

Solvents and reagents were commercially sourced and used without further purification or preparation, with the exception of THF, which was freshly distilled over sodium and benzophenone. Melting points were taken using a Büchi Melting Point B-545 capillary apparatus in open capillary tubes and are uncorrected. IR spectra were acquired (neat) using a Thermo Nicolet Avatar 330 FT-IR instrument. NMR data was recorded at 300.13 MHz for ^{1}H, and 75.5 MHz for ^{13}C, or at a frequency of 500.16 MHz for ^{1}H, and 125.8 MHz for ^{13}C, for 2D analyses with the specified deuterated solvents. Elemental analyses and mass spectrum services were externally sourced.

Preparation of ethyl 4-oxo-3,4-dihydroquinazoline-2-carboxylate (2)

Diethyl oxalate (410 ml, 3.0 mol) was carefully poured, in a portionwise fashion, into a melt of 2-aminobenzamide (136.15 g, 1.0 mol). The mixture was stirred at reflux for 8 h, and monitored by TLC [DCM–Et_2O (1 : 1)]. The solution was allowed to cool to room temperature and the excess diethyl oxalate was removed under vacuum. The remaining solid was suspended in EtOH and filtered. Recrystallization from EtOH produced **2** (200 g, 92%) as fine white needles, mp 190–191 °C (lit.,[9a] 179–180 °C); IR ν_{max}/cm^{-1} 1639 and 1609; ^{1}H NMR (300 MHz, DMSO-d_6) δ_H 1.33–1.38 (3H, t, *J* 7.1), 4.35–4.42 (2H, q, *J* 7.1), 7.61–7.66 (1H, m), 7.80–7.91 (2H, m) 8.16–8.19 (1H, dd, *J* 7.9, 1.1), 12.61 (1H, s); ^{13}C NMR (75 MHz, DMSO-d_6) δ_C 13.9, 62.6, 122.9, 126.0, 128.3, 128.6, 134.8, 143.4, 147.2, 160.2, 161.0; MS(ESI) *m*/*z* 219 $[M + H]^+$.

Preparation of 1-(2-nitrophenyl)prop-2-en-1-ol and 1-(2-nitrophenyl)prop-2-en-1-one (3)

Under an atmosphere of argon, 2-nitrobenzaldehyde (12.09 g, 80 mmol) was dissolved in freshly distilled THF (400 ml) and cooled to −70 °C. Vinyl magnesium bromide in THF (1.0 M, 100 mmol) was added, so that the internal temperature did not exceed −60 °C. After 1 h the solution was quenched with HCl (0.01 M, 100 ml), concentrated under vacuum to 200 ml, extracted with Et_2O–diethyl ether (3 × 300 ml), dried over $MgSO_4$ and evaporated, giving a dark oil, 1-(2-nitrophenyl)prop-2-en-1-ol (14.33 g, assumed quantitative yield). This was used immediately in the oxidation reaction to the corresponding ketone.

A solution of Jones reagent (2.67 M) was prepared and 20 ml was added dropwise to 1-(2-nitrophenyl)prop-2-en-1-ol (14.30 g, 79.8 mmol) in acetone (200 ml) at 3 °C, such that the reaction did not exceed 8 °C. The reaction was complete within 1 h. The solution was treated with *i*-PrOH (10 ml) and stirred at room temperature for 10 minutes. This was then passed through a pad of Celite and the solvent was evaporated to give **3**, as a dark oil (11.92 g, 67.3 mmol, 86%). This substance was used immediately after isolation in the preparation of **4**. Spectral data were in agreement with previously published results.[10]

Preparation of compound 4

The ester **2** (13.093 g, 60.0 mmol) was dissolved in DMF (200 ml) and warmed to 60 °C. Potassium *tert*-butoxide (7.407 g, 66.0 mmol) was dissolved in DMF (50 ml) and added to the solution. After 30 minutes the acrylic nitro-compound **3** (11.70 g, 66 mmol) was added and stirred at 60 °C for 2 hours. The solvent was removed under vacuum to relative dryness, treated with water (300 ml) and stirred for 2 hours at room temperature. The solution was filtered and treated with 1 M HCl (*ca.* 65 ml) to pH 4. This gave a precipitate that was filtered and collected. Concentration of the liquor to 50 ml gave a further 10% of precipitate. The solid collected was dissolved in refluxing 95% ethanol (600 ml), cooled and collected. The filtrate was washed with absolute ethanol (2 × 100 ml) and Et_2O (2 × 50 ml) to give the desired compound **4** as a white powder (17.53 g, 50.2 mmol, 83%), mp 215 °C (dec.); IR ν_{max}/cm^{-1} 1661, 1604, 1518, 1432, 1342 and 1275; Anal. calcd. for $C_{18}H_{11}N_3O_5$: C, 61.9; H, 3.2; N, 12.0. Found: C, 61.8; H, 3.3; N, 12.0; ^{1}H NMR (300 MHz, DMSO-d_6) δ_H 4.72 (2H, s), 7.59–7.64 (2H, m), 7.75–7.93 (4H, m) 8.22–8.26 (2H, m); ^{13}C NMR (75 MHz, DMSO-d_6) δ_C 46.3, 115.3, 120.8, 124.0, 126.1, 126.9, 127.4, 128.8, 130.9, 134.5, 134.8, 136.7, 146.0, 148.1, 153.4, 154.9, 158.6, 186.4; HRMS-FAB: *m*/*z* calcd for $C_{18}H_{11}N_3O_5$ + H, 350.0771; found 350.0773 $[M + H]^+$.

Preparation of 5*H*,12*H*-5,6,11a-triazadibenzo[*b*,*h*]fluoren-11,14-dione (5)

The pyrroloquinazolinone **4** (6.986 g, 20.0 mmol) was suspended in DMF (100 ml) and reduced using Pd/C 10% (0.6 g) and hydrogen (20 bar). The reaction proceeded for 1 h at 50 °C. The solution was filtered through a pad of Celite and the product was washed out with hot DMF until the solution was clear of any UV-active compound by TLC. The solvent was evaporated to relative dryness and boiled in 95% ethanol (200 ml). The cooled solution was filtered, and the collected solid was washed with absolute ethanol (2 × 50 ml), then Et_2O (2 × 50 ml) to give the desired compound **5** (5.79 g, 96%) as a white powder, mp >400 °C; IR ν_{max}/cm^{-1} 1675, 1584 and 1461; ^{1}H NMR (300 MHz, DMSO-d_6, 90 °C) δ_H 4.97 (2H, s), 7.39–7.44 (1H, m), 7.61–7.67 (1H, m), 7.72–7.77 (1H, m), 7.87–7.96 (3H, m), 8.24–8.27 (1H, dd, *J* 0.9, 8.1), 8.30–8.33 (1H, dd, *J* 0.9, 8.1), 12.74 (1H, s); ^{13}C NMR (75 MHz, D_2SO_4) δ_C 50.9, 118.1, 119.5, 121.0, 121.2, 121.6, 124.5, 128.7, 132.5, 133.5, 136.0,

136.1, 140.2, 140.2, 141.1, 149.1, 158.9, 166.9; HRMS-FAB: *m/z* calcd for $C_{18}H_{11}N_3O_2$ + H, 302.0924; found 302.0921 $[M + H]^+$.

Preparation of 14-chloroluotonin A (6)

The dione **5** (5.5 g, 18.25 mmol) was refluxed in $POCl_3$ (30 ml) for 1 h. The solvent was removed under vacuum and the remaining compound was treated with ice-water (20 ml). The mixture was neutralized with aq. $NaHCO_3$ (50 ml) and the solid product filtered. This was washed with water (2 × 30 ml), absolute ethanol (2 × 25 ml) and diethyl ether (2 × 25 ml). The dried substance was recrystallized with copious amounts of chloroform to give **6** (5.80 g, 99%) as a white powder, mp 287 °C (dec.); IR ν_{max}/cm^{-1} 1684, 1626, 1605, 1466 and 1329; ^{1}H NMR (300 MHz, $CDCl_3$) δ_H 5.37 (2H, s), 7.57–7.62 (1H, m), 7.78–7.94 (3H, m), 8.12–8.15 (1H, dd, *J* 0.5, 8.3), 8.36–8.39 (1H, dd, *J* 1.0, 8.4), 8.45–8.48 (1H, dd, *J* 1.2, 8.4), 8.51–8.54 (1H, dd, *J* 0.6, 8.5); ^{13}C NMR (75 MHz, D_2SO_4, DMSO-d_6) δ_C 57.2, 124.2, 126.9, 127.7, 131.6, 134.1, 135.9, 138.4, 138.7, 140.2, 140.8, 141.5, 144.9, 145.4, 146.5, 153.9, 159.3, 163.0; HRMS-FAB: *m/z* calcd for $C_{18}H_{10}ClN_3O$ + H, 320.0585; found 320.0581 $[M + H]^+$.

Preparation of luotonin A (1)

Compound **6** (96 mg, 300 μmol) was suspended in 1,4-dioxane (50 ml) at room temperature. Activated Raney nickel (0.5 g) was added and the vessel sealed whilst being stirred vigorously. The reaction was monitored via TLC [hexane–DCM (1 : 15)] and was found to be complete after approximately 1 hour. Filtration of the mixture through a pad of Celite and evaporation to dryness gave the crude compound, which was then redissolved in DCM and purified by chromatography using a gradient system of hexane–DCM (1 : 20) → DCM. The fractions containing the major component were combined and evaporated to dryness giving **1** as a fine white powder (72 mg, 84%), mp 255–257 °C (dec.) from ($CHCl_3$–acetone), (lit.,[1] 252 °C (dec.)); ^{1}H NMR (300 MHz, DMSO-d_6) δ_H 5.30 (2H, s), 7.59–7.65 (1H, m), 7.72–7.77 (1H, dd, *J* 7.6 and 7.3 Hz), 7.87–7.93 (3H, m), 8.13–8.16 (1H, d, *J* 8.1 Hz), 8.24–8.29 (2H, m), 8.74 (1H, s); ^{13}C NMR (75 MHz, DMSO-d_6) δ_C 47.5, 121.0, 125.9, 127.1, 128.0, 128.2, 128.4, 128.4, 129.6, 130.4, 130.9, 131.7, 134.4, 148.3, 149.0, 151.4, 153.0, 159.6; HRMS-FAB: *m/z* calcd for $C_{18}H_{11}N_3O$ + H, 286.0975; found 286.0991 $[M + H]^+$.

Preparation of 7,8,9,10-tetrahydroluotonin A (7)

Method A. The procedure for the formation of **1** was used but the reaction time was increased to 4 hours. TLC showed the starting material was no longer luotonin A (R_f 0.5) and was completely transformed. The solution was passed through a pad of Celite and evaporated to dryness, giving **7** (84 mg, 94%) as a fine granular substance, mp 272–273 °C (dec.), (lit.,[5] 275 °C (dec.)); IR ν_{max}/cm^{-1} 2940, 1667, 1625, 1609, 1468, 1456, 1377 and 1286; ^{1}H NMR (300 MHz, DMSO-d_6, 90 °C) δ_H 1.78–1.95 (4H, m), 2.86–2.90 (2H, m), 2.96–3.00 (2H, m), 5.05 (2H, s), 7.52–7.57 (1H, m), 7.79–7.88 (3H, m), 8.22–8.25 (1H, d, *J* 7.8 Hz); ^{13}C NMR (75 MHz, DMSO-d_6, 90 °C) δ_C 21.4, 21.8, 28.4, 31.8, 46.7, 120.2, 125.3, 125.9, 127.1, 131.7, 132.6, 133.6, 135.0, 146.8, 148.8, 153.1, 158.8, 159.0; HRMS-FAB: *m/z* calcd for $C_{18}H_{15}N_3O$ + H, 290.1293; found 290.1295 $[M + H]^+$.

Method B. Reaction conditions and treatment were as for the formation of **1** (above) but the solution was heated to reflux, giving **7** (80 mg, 92%).

Method C. A sample of **1** (50 mg) was reacted using method A and isolated, giving **7** (33 mg, 65%).

Preparation of compound 8

To a sealed vessel **6** (96 mg, 300 μmol) was dissolved in 3-*N*-(dimethylamino)propylamine (1.0 ml) and heated at 145 °C for 2 hours. TLC: DCM–MeOH (99 : 1). The solution was evaporated to dryness under vacuum, dissolved in DCM (30 ml), treated with aq. $NaHCO_3$ (10 ml), and brine (10 ml). The organic phase was dried over $MgSO_4$, filtered, evaporated to dryness, and recrystallized from acetonitrile, giving **8** (94 mg, 81%), mp 258 °C (dec.); IR ν_{max}/cm^{-1} 2818, 1677, 1572, 1466, 1433, 1367 and 1336; ^{1}H NMR (300 MHz, DMSO-d_6) δ_H 1.80-1.89 (2H, m), 2.20 (6H, s), 2.39–2.43 (2H, m), 3.67–3.72 (2H, m), 5.41 (2H, s), 7.49–7.59 (2H, m), 7.67–7.77 (2H, m), 7.84–7.91 (2H, m), 7.94–7.98 (1H, dd, *J* 8.4, 1.0 Hz), 8.15–8.18 (1H, d, *J* 8.4), 8.22–8.25 (1H, m); ^{13}C NMR (75 MHz, DMSO-d_6) δ_C 25.2, 40.9, 42.3, 42.3, 47.9, 54.5, 109.0, 119.0, 120.8, 122.4, 125.4, 125.8, 126.7, 127.9, 129.8, 129.9, 134.4, 147.0, 149.1, 149.3, 151.6, 153.7, 159.5; HRMS-FAB: *m/z* calcd for $C_{23}H_{23}N_5O$ + H, 386.1975; found 386.1973 $[M + H]^+$.

Preparation of compound 9

In piperidine (1 ml), **6** (65 mg, 200 μmol) was refluxed for 4 hours. The solution was evaporated to dryness, extracted with DCM (3 × 20 ml), washed with aq. $NaHCO_3$ (10 ml) and brine (10 ml). The organic extracts were dried with $MgSO_4$, filtered, evaporated to dryness, and triturated in diethyl ether to give **9** (58 mg, 79%), mp 306–308 °C (dec.); IR ν_{max}/cm^{-1} 2925, 1672, 1606, 1566, 1504, 1465, 1385, 1335 and 1102; ^{1}H NMR (300 MHz, DMSO-d_6, 50 °C) δ_H 1.73–1.88 (6H, m), 3.46–3.49 (4H, m), 5.50 (2H, s), 7.58–7.69 (2H, m), 7.78–7.84 (1H, m), 7.91–7.94 (2H, m), 8.12–8.16 (2H, m), 8.28–8.31 (1H, m); ^{13}C NMR (75 MHz, DMSO-d_6, 50 °C) δ_C 23.5, 25.9, 25.9, 47.51, 52.1, 52.1, 120.3, 120.7, 124.2, 124.3, 125.7, 126.6, 126.7, 127.8, 129.7, 129.9, 134.2, 149.1, 150.0, 151.7, 152.7, 153.0, 159.3; HRMS-FAB: *m/z* calcd for $C_{23}H_{20}N_4O$ + H, 369.1710; found 369.1712 $[M + H]^+$.

Preparation of compound 10

In DMF, **6** (96 mg, 300 μmol) was refluxed with benzylamine (48 mg, 450 μmol) and K_2CO_3 (125 mg) for 18 hours. The solution was evaporated to dryness and washed with water (3 × 5.0 ml). The compound was filtered and washed with hot ethanol, giving **10** (102 mg, 87%) as an amorphous solid, mp 300 °C (dec.); IR ν_{max}/cm^{-1} 3267, 1683, 1634, 1567, 1466, 1453, 1423, 1390 and 1348; ^{1}H NMR (300 MHz, DMSO-d_6) δ_H 4.92–4.94 (2H, d, *J* 6.3 Hz), 5.24 (2H, s), 7.25–7.29 (1H, m), 7.34–7.42 (4H, m), 7.55–7.63 (2H, m), 7.75–7.80 (1H, m), 7.87–7.88 (2H, m), 8.03–8.05 (1H, d, *J* 8.0 Hz), 8.14–8.21 (2H, m), 8.47–8.50 (1H, d, *J* 8.5 Hz); ^{13}C NMR (75 MHz, DMSO-d_6) δ_C 46.4, 47.6, 108.6, 119.0, 120.8, 122.2, 125.7, 125.8, 125.9, 125.9, 126.7, 127.1, 127.9, 128.8, 128.8, 129.9, 130.0, 134.3, 140.4, 147.3, 149.2, 149.3, 151.6, 153.5, 159.4; HRMS-FAB: *m/z* calcd for $C_{25}H_{18}N_4O$ + H, 391.1553; found 391.1562 $[M + H]^+$.

Preparation of compound 11

In DMF (3.0 ml) K_2CO_3 (210 mg) was added followed by **6** (160 mg, 500 μmol) and refluxed with methyl mercaptoacetate (80 mg, 750 μmol) for 18 h. The solution was evaporated to dryness and partitioned between DCM and water. The organic phase was dried with $NaSO_4$, filtered and evaporated to dryness, giving **11** (167 mg, 86%), mp 236–237 °C (dec.); IR ν_{max}/cm^{-1} 1657, 1607, 1547, 1498, 1468, 1408, 1385, 1342, 1310 and 1257; ^1H NMR (300 MHz, DMSO-d_6, 60 °C) δ_H 3.54 (3H, s), 4.03 (2H, s), 5.40 (2H, s), 7.62–7.67 (1H, m), 7.85–7.99 (4H, m), 8.30–8.35 (2H, m), 8.55–8.58 (1H, d, *J* 8.3 Hz); ^{13}C NMR (75 MHz, DMSO-d_6, 60 °C) δ_C 35.8, 47.6, 52.0, 120.9, 124.8, 125.6, 127.0, 127.8, 128.9, 129.0, 130.4, 130.4, 134.2, 135.6, 137.4, 148.7, 148.8, 150.6, 152.6, 159.2, 168.9; (ESI) *m/z* 390 $[M + H]^+$; HRMS-FAB: *m/z* calcd for $C_{21}H_{15}N_3O_3S$ + H, 390.0907; found 390.0908 $[M + H]^+$.

Preparation of 14,14′-bisluotonin A (12)

Under argon, **6** (160 mg, 500 μmol) was dissolved in acetic acid (5.0 ml). 10% Pd/C (20 mg) was added to the solution, which was warmed to 40 °C for 3 hours. Filtration through Celite, evaporation of the solvent to dryness and recrystallization from ethanol gave **12** (142 mg, 100%), mp >400 °C; IR ν_{max}/cm^{-1} 1681, 1584, 1531, 1463, 1402, 1383 and 1336; ^1H NMR (300 MHz, DMSO-d_6) δ_H 4.67–4.73 (2H, d, *J* 17.9 Hz), 5.10–5.16 (2H, d, *J* 17.9 Hz), 7.57–7.69 (6H, m) 7.92–8.03 (6H, m), 8.22–8.25 (2H, dd, 8.0, 1.0 Hz), 8.48–8.51 (2H, d, *J* 8.4 Hz); ^{13}C NMR (75 MHz, DMSO-d_6) δ_C 47.3, 121.0, 125.3, 125.9, 126.0, 127.4, 128.2, 129.3, 130.6, 130.9, 131.0, 134.7, 135.6, 148.8, 149.0, 152.0, 153.3, 159.5; HRMS-FAB: *m/z* calcd for $C_{36}H_{20}N_6O_2$ + H, 569.1721; found 569.1723 $[M + H]^+$.

Preparation of 14-(hex-15-ynyl)luotonin A (13)

Under an argon atmosphere, a solution of hexyne (2.0 mmol) in degassed benzene (20.0 ml) was added to a mixture of 7-chloroluotonin A **6** (160 mg, 500 μmol), $Pd(OAc)_2$ (0.05 mmol), *rac*-BINAP (0.1 mmol) and potassium carbonate (1.0 mmol). The reaction mixture was heated to reflux for 18 h. The solvent was evaporated and the residue was purified by column chromatography [hexane–chloroform–acetone (30 : 10 : 1)], giving **13** (159 mg, 87%) as a white substance, mp 226–227 °C (dec.). ^1H NMR (300 MHz, $CDCl_3$) δ_H 1.02–1.07 (3H, t, *J* 7.3 Hz), 1.54–1.67 (2H, m), 1.73–1.83 (2H, m), 2.68–2.73 (2H, t, *J* 7.1 Hz), 5.34 (2H, s), 7.55–7.60 (1H, m), 7.69–7.74 (1H, m), 7.80–7.88 (2H, m), 8.10–8.13 (1H, d, *J* 8.1 Hz), 8.33–8.36 (1H, d, *J* 8.3 Hz), 8.42–8.46 (2H, m). ^{13}C NMR (75 MHz, $CDCl_3$) δ_C 13.8, 19.9, 22.3, 30.7, 47.8, 74.0, 107.8, 121.5, 125.9, 126.6, 127.2, 127.6, 128.7, 128.8, 128.9, 130.8, 131.2, 131.7, 134.7, 149.4, 149.5, 150.6, 153.0, 160.7. HRMS-FAB: *m/z* calcd for $C_{24}H_{19}N_3O$ + H, 366.1601; found 366.1603 $[M + H]^+$.

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