Total Synthesis of Luotonin and a Small Library of AB-Ring Substituted Analogues by Cascade Radical Annulation of Isonitriles

Raghuraman Tangirala,[a] Smitha Antony,[b] Keli Agama,[b] Yves Pommier,[b] Dennis P. Curran*[a]

[a] Department of Chemistry, University of Pittsburgh, Pittsburgh, PA 15260, USA
Fax +1(412)6249861; E-mail: curran@pitt.edu

[b] Laboratory of Molecular Pharmacology, Center for Cancer Research, NCI, Bethesda, MD 20892, USA

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Dedicated to the memory of Dr. Anne Ghosez-Giese, 1960–2004.

Abstract: A four-step total synthesis of luotonin is deployed to make a small library of AB-ring substituted analogues. These analogues show weak activity in a standard topoisomerase I mediated DNA cleavage assay.

Key words: camptothecin, topoisomerase I inhibitor, luotonin, isonitrile radical annulation

Nomura and coworkers recently reported the isolation of the pentacyclic alkaloid luotonin A (**1**) from the Chinese medicinal plant *Peganum nigellastrum*.[1] Luotonin can be considered as an analogue of the potent anti-cancer agent 20(*S*)-camptothecin (**2**, Figure 1).[2] These two molecules share a pyrroloquinoline ABC ring system, while camptothecin's pyridone-hydroxylactone DE ring is replaced by a quinazolone system in luotonin A.

Interest in luotonin A has increased considerably following the recent report by Hecht and coworkers that it mediates topoisomerase-dependent cytotoxicity.[3] Like camptothecin, luotonin A is a 'topoisomerase I poison' that stabilizes the topoisomerase I DNA complex by forming a ternary complex. These observations are important because it has been thought until very recently that the E-ring hydroxylactone of camptothecin was indispensable for anti-cancer activity.[4]

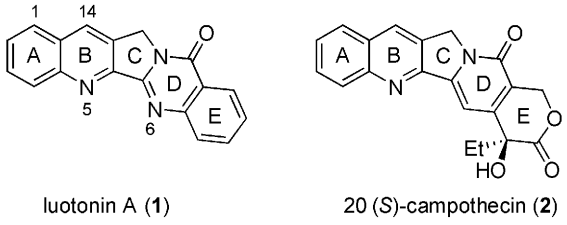

Figure 1

Luotonin's structural and biological features make it an attractive synthetic target, and a number of total syntheses have been completed.[5] Most of these have parallels in the camptothecin area.[2b] Unfortunately, luotonin A is not nearly as potent as camptothecin, and it is not likely to be a drug candidate for cancer chemotherapy. However, it is a good lead compound, and Hecht,[6] Dallavalle[7] and their coworkers recently reported the synthesis of an assortment of substituted E-ring analogues[6,7] along with a few A-ring analogues.[7]

We report herein a four-step total synthesis of luotonin A that features a cascade radical annulation of phenyl isonitrile in the key step.[8] The generality of the synthesis is shown by making a score of AB-ring analogues. About a dozen of these analogues were tested in a topoisomerase I DNA cleavage assay, but they exhibited only modest activity.

The synthesis of luotonin A starts from benzoyleneurea **3** and is shown in Scheme 1.[9] Conversion of **3** to the dibromide **4** followed by monohydrolysis with 1 N NaOH provided bromoquinazolone **5** as a single regioisomer in 52% overall yield. Propargylation of **5** by a standard procedure for pyridones[10] provided **6a** in 66% yield. The O-propargylated isomer (not shown), a common side product in pyridone progargylations, was not observed in this reaction. Photoirradiation of **6a** and phenyl isonitrile with a sunlamp in the presence of hexamethylditin provided luotonin A (**1**) in 47% yield after purification by flash chromatography.

The synthesis of luotonin is concise and modular, making it ideal for rapid synthesis of analogues. To illustrate the potential, we conducted a matrix of 24 reactions starting from three propargyl quinazolones and eight isonitriles, as summarized in Table 1. The propargyl quinazolones **6b** and **6c** were prepared by the same procedure as **6a** (see Scheme 1), while the isonitriles are well-known compounds.[11]

Stock solutions of the quinazolones and isonitriles were mixed in small vials to make the reaction mixtures. These were then irradiated simultaneously, and each crude product was purified by a quick filtration through silica gel followed by evaporation to remove tin and isonitrile-derived side products. The yields of the resulting crude products are shown in Table 1. The desired products were identified as the major components of 18 of the 24 reaction mixtures (75–95% HPLC purity), but six reactions were failures (little or no product isolated). Given the generality of the cascade annulation, it seems unlikely that these reactions should fail, so we suspect instead that there

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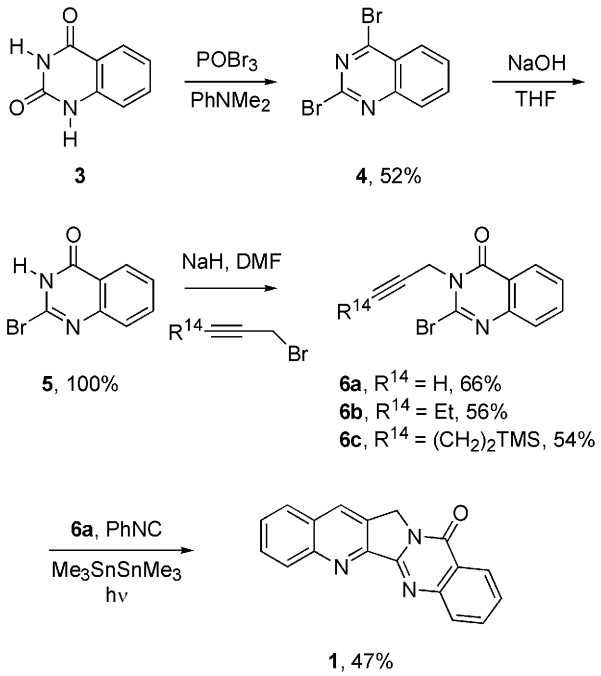


Scheme 1 Four-step total synthesis of luotonin A (**1**).

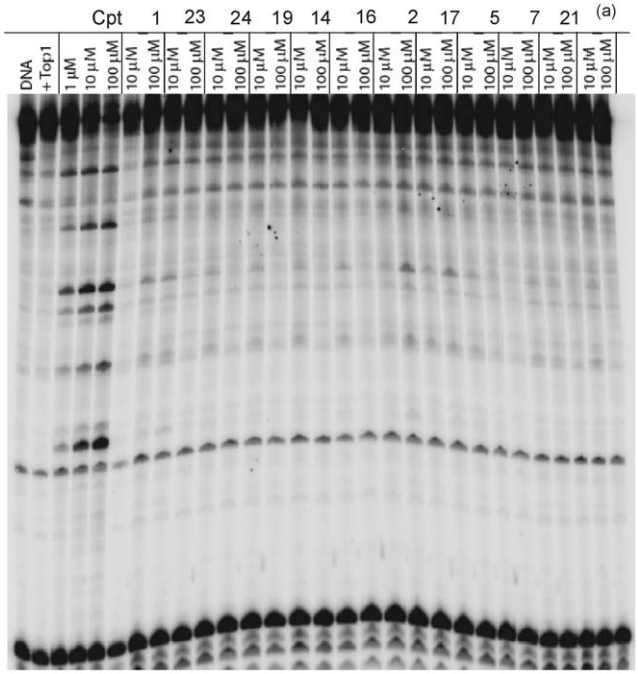


Figure 2 Topoisomerase cleavage assays of luotonin analogues. Header numbers correspond to entries in Table 1. (a) 2-Acetoxy-14-*tert*-butyldimethylsilyl luotonin.

was a problem with the parallel irradiation procedure. Single products were obtained from phenyl isonitrile and the *para*-substituted isonitriles, while 3,4-ethylenedioxyisonitrile gave separable mixtures of two regioisomeric products (entries 23, 24). Prior to testing, samples of the luotonin analogues were purified by reverse-phase preparative HPLC with a Novapak column.

The detailed procedures for the topoisomerase I assays have been described elsewhere.[12] Briefly, labeled DNA was incubated with recombinant topo I with and without drug. After 20 minutes, the reaction was stopped and the samples were denatured and analyzed for cleavage on a polyacrylimide gel.

The results of the topoisomerase assay of eleven representative luotonin analogues are shown in Figure 2. Lanes 1 and 2 are controls with no drug, while lanes 3–5 are the camptothecin (cpt) standard at 1, 10 and 100 μm. Lanes 6–29 are the luotonin samples at 10 and 100 μM. As expected from prior results, luotonin A itself is only weakly active in this assay. Most of the analogues have activity that is comparable to or weaker than luotonin A. However, 7-ethylluotonin and perhaps 7-ethyl-10-hydroxyluotonin appear to be slightly more active than the parent. One of the goals of this study was to determine whether luotonin shared a similar SAR to camptothecin in the AB ring. Unfortunately, the activity of the analogues in this assay is low enough that detailed SAR comparisons are probably not meaningful.

In conclusion, luotonin A- and AB-ring substituted analogues are readily available by a four-step synthesis from benzoyleneurea **3** that features a cascade radical annulation of isonitriles. Although two of the eleven analogues tested are more active than luotonin A in a topoisomerase I DNA cleavage assay, none of the compounds is close to the potency of the natural product camptothecin. Accordingly, the identification of more potent analogues of luotonin remains a worthwhile goal, and the synthesis herein provides a very flexible and general route to such analogues.

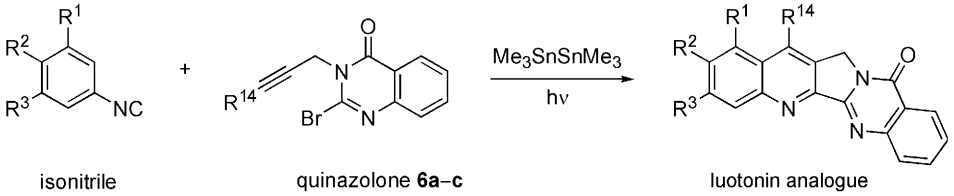
2,4-Dibromoquinazoline (4)

A two-necked round-bottomed flask charged with benzoyleneurea (1.9 g, 12 mmol) was fitted with an overhead mechanical stirrer. Addition of phosphorus oxybromide (23 g, 80 mmol) was followed by a dropwise addition of *N*,*N*-dimethylaniline (0.75 mL, 5.9 mmol) via syringe at r.t. This mixture was heated to 105 °C in an oil bath and vigorously stirred for 4 h. At this temperature, the reaction mixture forms a pale yellow slurry, which turns bright yellow when the reaction is complete. The mixture was then cooled to 0 °C in an ice bath and carefully quenched with chilled H_2O. The resulting mixture was extracted with CH_2Cl_2. The combined organic extracts were dried over $MgSO_4$ and concentrated under reduced pressure. The crude product was immediately purified by column chromatography using basic alumina as the stationary phase (gradient elution 15:85 to 3:2 EtOAc–hexane) to afford **4** as a white solid (1.8 g, 52%). ^{1}H NMR (300 MHz, CD_3OD): δ = 7.78 (ddd, *J* = 1.2, 7.0, 8.3 Hz, 1 H), 7.89 (dd, *J* = 0.5, 8.4 Hz, 1 H), 8.01 (ddd, *J* = 1.4, 6.9, 8.4 Hz, 1 H), 8.19 (dd, *J* = 1.3, 8.4 Hz, 1 H). ^{13}C NMR (75 MHz, $CDCl_3$): δ = 124.8, 128.0, 128.3, 129.5, 136.0, 145.7, 151.8, 157.6. HRMS (EI): *m/z* calcd for $C_8H_4{}^{79}Br_2N_2$ [M^+ – 2]: 285.8741; found: 285.8750. LRMS (EI): *m/z* (%) = 286 (55) [M^+ – 2], 288 (85) [M^+], 290 (54) [M^+ + 2], 207 (100), 209 (100), 128 (75), 102 (75), 75 (50).

2-Bromoquinazolin-4(3*H*)-one (5)

To a solution of **4** (1.8 g, 6.1 mmol) in THF (12 mL) was added aq NaOH (1 N, 37 mL, 37 mmol) at r.t. and the reaction mixture was stirred for 2 h. The reaction mixture was then acidified (pH = 5) with

Table 1 Analogues of Luotonin from Cascade Radical Annulations of Isonitriles and Quinazolones **6a–c**



isonitrile quinazolone **6a–c** luotonin analogue

Entry	Isonitrile	Quinazolone	R^{14}	R^1	R^2	R^3	Yield (%)
1	C_6H_5NC	**6a**	H	H	H	H	47
2	C_6H_5NC	**6b**	Et	H	H	H	68
3	C_6H_5NC	**6c**	CH_2CH_2TMS	H	H	H	–[a]
4	*p*-FC_6H_5NC	**6a**	H	H	F	H	65
5	*p*-FC_6H_5NC	**6b**	Et	H	F	H	75
6	*p*-FC_6H_5NC	**6c**	CH_2CH_2TMS	H	F	H	–[a]
7	*p*-$CF_3C_6H_5NC$	**6a**	H	H	CF_3	H	71
8	*p*-$CF_3C_6H_5NC$	**6b**	Et	H	CF_3	H	62
9	*p*-$CF_3C_6H_5NC$	**6c**	CH_2CH_2TMS	H	CF_3	H	–[a]
10	*p*-$CH_3C_6H_5NC$	**6a**	H	H	CH_3	H	57
11	*p*-$CH_3C_6H_5NC$	**6b**	Et	H	CH_3	H	33
12	*p*-$CH_3C_6H_5NC$	**6c**	CH_2CH_2TMS	H	CH_3	H	–[a]
13	*p*-$OCH_3C_6H_5NC$	**6a**	H	H	OCH_3	H	63
14	*p*-$OCH_3C_6H_5NC$	**6b**	Et	H	OCH_3	H	86
15	*p*-$OCH_3C_6H_5NC$	**6c**	CH_2CH_2TMS	H	OCH_3	H	74
16	*p*-$OAcC_6H_5NC$	**6a**	H	H	OAc	H	62
17	*p*-$OAcC_6H_5NC$	**6b**	Et	H	OAc	H	39
18	*p*-$OAcC_6H_5NC$	**6c**	CH_2CH_2TMS	H	OAc	H	–[a]
19	*p*-$NHBocC_6H_5NC$	**6a**	H	H	NHBoc	H	52
20	*p*-$NHBocC_6H_5NC$	**6b**	Et	H	NHBoc	H	94
21	*p*-$NHBocC_6H_5NC$	**6c**	CH_2CH_2TMS	H	NHBoc	H	37
22	3,4-$[O(CH_2)O]$–C_6H_4NC	**6a**	H	–$[O(CH_2)O]$–			–[a]
23	3,4-$[O(CH_2)O]$–C_6H_4NC	**6b**	Et	–$[O(CH_2)O]$–			86[b]
24	3,4-$[O(CH_2)O]$–C_6H_4NC	**6c**	CH_2CH_2TMS	–$[O(CH_2)O]$–			94[b]

[a] Product not isolated.
[b] Mixture of 1,2- and 2,3-regioisomers.

glacial acetic acid and extracted with CH_2Cl_2. The combined organic extracts were concentrated under reduced pressure. Small amounts of H_2O were azeotropically removed by coevaporation with MeOH to afford **5** as a tan solid (1.4 g, 100%). ^{1}H NMR (300 MHz, CD_3OD): δ = 7.46 (ddd, *J* = 1.1, 7.2, 8.1 Hz, 1 H), 7.54 (d, *J* = 8.1 Hz, 1 H), 7.73 (ddd, *J* = 1.5, 7.1, 8.5 Hz, 1 H), 8.13 (dd, *J* = 1.5, 8.0 Hz, 1 H). ^{13}C NMR (75 MHz, CD_3SOCD_3): δ = 114.4, 115.5, 122.5, 127.0, 135.1, 141.0, 150.4, 163.0. IR (CH_2Cl_2, NaCl): 3385, 2909, 2847, 1700, 1669, 1593, 1449, 1296 cm^{-1}. HRMS (EI): *m/z* calcd for $C_8H_5{}^{79}BrN_2O$ [M^+ – 2]: 223.9585; found: 223.9582. LRMS (EI): *m/z* (%) = 224 (27) [M^+ – 2], 226 (27) [M^+], 203 (37), 183 (10), 145 (60), 91 (47), 71 (100).

2-Bromo-3-prop-2-ynylquinazolin-4(3*H*)-one (6)

NaH [95% in mineral oil, (13 mg, 0.51 mmol)] was added to a solution of **5** (0.11 g, 0.47 mmol) in DMF (2.3 mL) at 0 °C under argon. After stirring this mixture for 10 min at 0 °C, propargyl bromide (80% w/w in toluene, 62 μL, 0.56 mmol) was then added

via a syringe and the reaction mixture was stirred at r.t. for 6.5 h. The final mixture was poured into brine (10 mL) and extracted with EtOAc (3 × 10 mL). The combined organic extracts were dried over $MgSO_4$ and concentrated under reduced pressure. The crude product was purified by flash chromatography (1:4 EtOAc–hexane) to give *N*-propargylated quinazolinone **6** (81 mg, 66%). ^{1}H NMR (300 MHz, $CDCl_3$): δ = 2.35 (t, *J* = 2.5 Hz, 1 H), 5.08 (d, *J* = 2.5 Hz, 2 H), 7.50 (ddd, *J* = 1.2, 7.4, 8.1 Hz, 1 H), 7.62 (dd, *J* = 0.6, 8.2 Hz, 1 H), 7.75 (ddd, *J* = 1.6, 7.2, 8.3 Hz, 1 H), 8.24 (dd, *J* = 1.2, 8.0 Hz, 1 H). ^{13}C NMR (75 MHz, $CDCl_3$): δ = 38.2, 72.9, 76.8, 120.3, 126.9, 127.4, 127.7, 134.6, 135.1, 147.0, 160.5. IR (CH_2Cl_2, NaCl): 3267, 2980, 2361, 2125, 1690, 1577, 1557, 1332, 1152 cm^{-1}. HRMS (EI): *m/z* calcd for $C_{11}H_7{}^{79}BrN_2O$ [M^+ – 1]: 261.9742; found: 261.9747. LRMS (EI): *m/z* (%) = 262 (46) [M^+ – 1], 262 (46) [M^+ +1], 183 (100), 155 (20), 129 (31), 102 (19), 63 (14).

General Procedure for the Synthesis of Luotonins

A solution of N-propargylated quinazolinone **6** in benzene was added to a 15 × 45 mm cylindrical screw-cap glass vial. The appropriate isonitrile followed by hexamethylditin were added at r.t. The vial was capped and the reaction mixture was irradiated with a 275 W GE sunlamp for 8 h. The solvent was then evaporated and the residue was purified by column chromatography (1:9 acetone–CH_2Cl_2 or 1:9 EtOAc–CH_2Cl_2) to give luotonins as pale yellow to tan solids.

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(9) Representative experiments are provided at the end of the paper. Complete details and characterization data are in: Tangirala, R. *PhD Thesis*; University of Pittsburgh: Pittsburgh, **2005**.

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