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Synthesis and topoisomerase poisoning activity of A-ring and E-ring substituted luotonin A derivatives

Kassoum Nacro,[a] Conxiang (Charles) Zha,[a] Peter R. Guzzo,[a] R. Jason Herr,[b,*] Denise Peace[c] and Thomas D. Friedrich[c]

[a]*Discovery Research & Development, Albany Molecular Research Inc., 21 Corporate Circle, PO Box 15098, Albany, NY 12213-5098, USA*
[b]*Department of Medicinal Chemistry, Albany Molecular Research Inc., 21 Corporate Circle, PO Box 15098, Albany, NY 12213-5098, USA*
[c]*Center for Immunology and Microbial Disease, Albany Medical College, 47 New Scotland Avenue, Albany, NY 12208, USA*

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Abstract—A series of A-ring and E-ring analogues of the natural product luotonin A, a known topoisomerase I poison, was evaluated for growth inhibition in human carcinoma and leukemia cell lines. Rational design of structures was based on analogues of the related alkaloid camptothecin, which has been demonstrated to exert cytotoxic effects by the same mechanism of action. When compared to luotonin A, several compounds exhibited an improved topoisomerase I-dependent growth inhibition of a human leukemia cell line.

1. Introduction

Luotonin A (**1a**, Fig. 1) is a pyrroloquinazolinoquinoline alkaloid isolated from *Peganum nigellastrum*, a Chinese herbal medicinal plant.[1] It was first reported as a cytotoxic agent due to its activity against the murine leukemia P-388 cell line (IC_{50} 1.8 μg/mL or 6.3 μM), although at that time the inhibitory mechanism of action was not determined.[1,2] In late 2003 it was demonstrated by Hecht and co-workers that luotonin A was able to stabilize the covalent 'cleavable complex' between the DNA phosphodiester backbone and the nuclear enzyme topoisomerase I.[3] In a side-by-side comparison, this mode of action was demonstrated to be identical to the means by which the structurally analogous alkaloid camptothecin (CPT) interacts with DNA and topoisomerase I to induce cellular apoptosis.

Topoisomerase I (topo I) represents a family of omnipresent biological protein isoforms that catalyze the relaxation of supercoiled DNA during a number of critical cellular processes (e.g., during replication, transcription, and repair).[4] Since intracellular levels of topoisomerase I are elevated in a number of human solid tumors relative to the respective normal tissues, an intense interest continues toward the development of drugs which can affect the DNA replication process by selectively interfering with topo I function.

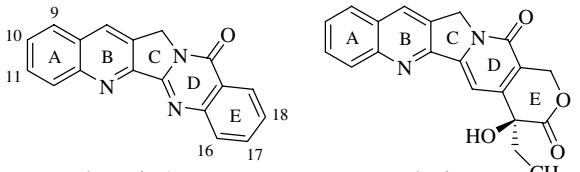

Figure 1. Structures of luotonin A and camptothecin.

Early enzymology studies uncovered the mechanism by which the natural product alkaloid camptothecin exerts a cytotoxic effect through interference with the topoisomerase I-catalyzed DNA unraveling process.[5] However, it was the better understanding of molecular binding interactions described by the resolved X-ray crystal structures of camptothecin/topo I/DNA noncovalent complexes[6] that has led to a renewed recent interest in DNA topoisomerase I inhibitors as oncologics.[7,8]

Keywords: Luotonin A; Topoisomerase; Camptothecin; Growth inhibition.
* Corresponding author. Tel.: +1 518 867 3954; e-mail: rjason.herr@albmolecular.com

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The aforementioned report by Hecht and co-workers[3] described the ability of luotonin A to stabilize a DNA/topo I binary complex by the same mechanism of action as camptothecin. In this same study, the potencies for the growth inhibition of an *Saccharomyces cerevisiae* strain by these two natural products were measured at 5–12 μM for luotonin A and ~0.80 μM for camptothecin.[9] While the difference in these two IC_{50} values represents close to an order of magnitude difference in activity, we were intrigued by the challenge of creating a more drug-like analogue of luotonin A through a rational design approach. Our goal was to optimize the binding activity of the luotonin A scaffold through substitution to take advantage of the reported key hydrogen-bonding interactions identified by the resolved CPT/DNA/topoisomerase I ternary complex X-ray crystal structures[6] and through molecular modeling experiments[7] (some shown in Fig. 2). Another aim was to increase water solubility of luotonin A analogues by capitalizing on substitution patterns reported for the potent (and water-soluble) camptothecin analogues now in commercial use. The recent research reports by other research groups working concurrently in this same arena[10,11] have prompted us to report here our efforts toward the preparation of A-ring and E-ring analogues of luotonin A and the results from their evaluation for in vitro cytotoxicity in three tumor cell lines.

2. Chemistry

To date there have been more than a dozen publications describing the total (or formal) synthesis of luotonin A, all more or less convergent routes that make devising analogue syntheses amenable at various stages.[1c,12] We chose to utilize the route devised by Harayama and co-workers,[12m,q] in which we coupled appropriately substituted 2-chloro-3-(bromomethyl)quinolines **2** with substituted 4(3*H*)-quinazolinones **3**, followed by a palladium-mediated cyclization of the 3-amidomethylquinoline adducts **4** to provide a variety of luotonin A analogues **1** (Scheme 1). In this way we were able to make compounds which were substituted on either the A-ring or the E-ring, as well as to prepare an analogue containing a modification on both A- and E-rings, in a convergent fashion. It was our hypothesis that substituents incorporated onto the E-ring would directly interact with the topoisomerase I 'camptothecin E-ring binding pocket' to provide increased binding affinity, whereas A-ring substitution would fine-tune the SAR as well as to enhance pharmacokinetic properties through increased aqueous solubility.

2.1. E-ring analogue syntheses

The ability of luotonin A to interact with the topoisomerase I/DNA complex in the same way as camptothecin[3] led us to propose a series of compounds in which substituents on the E-ring could interact with the hydrogen bond donors and acceptors on the topoisomerase I enzyme and DNA backbone in the same fashion as the camptothecin C-20 hydroxyl moiety (i.e., Fig. 2).[6–8] We hypothesized that any increase in binding affinity would lead to the effect of greater potency (and therefore greater tumor cell toxicity).[13]

Thus, luotonin A (**1a**) as well as five E-ring analogues **1b–f** were prepared by the convergent route shown in Scheme 2. In order to construct compounds incorporating functionality on the E-ring of luotonin A, 4(3*H*)-quinazolinones **3a–f** were either obtained from commercial sources or were prepared in a single step from the corresponding anthranilic acids.[14] Condensation reactions between 4(3*H*)-quinazolinones **3a–f** and

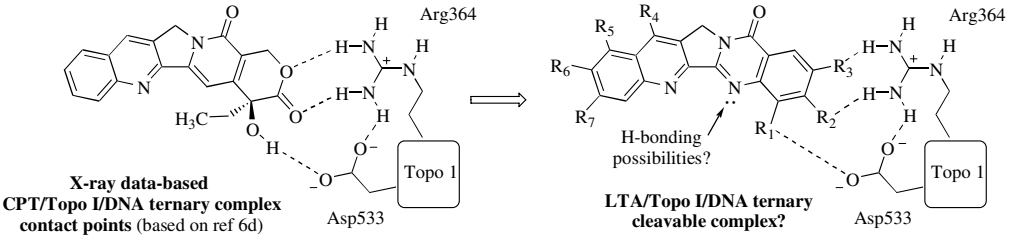
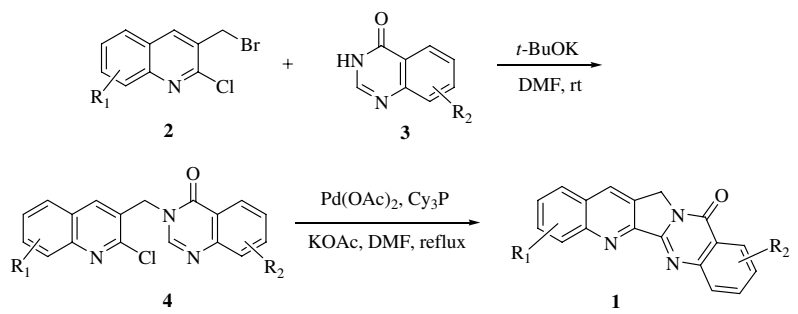
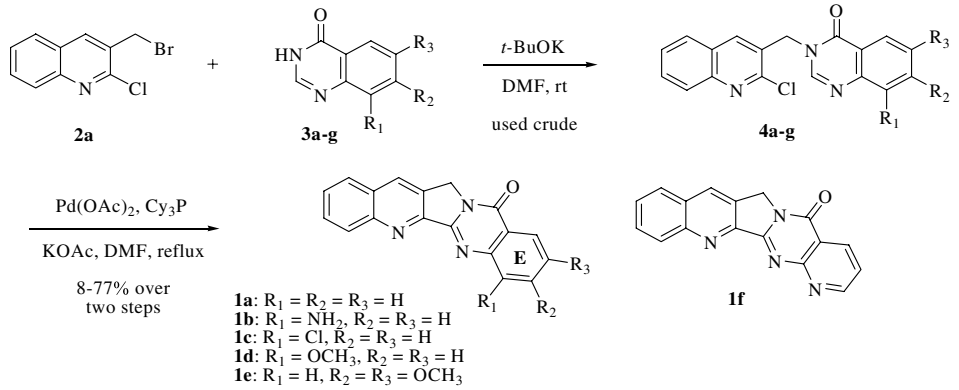


Figure 2. Some key binding interactions reported for the DNA/topo I/camptothecin ternary cleavable complex.



Scheme 1. Convergent synthesis of A-ring and E-ring analogues **1**.



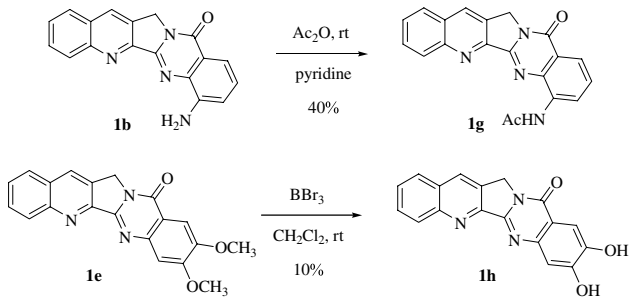
Scheme 2. Synthesis of luotonin A analogues substituted on the E-ring.

3-(chloromethyl)quinoline **2a**[15] were achieved using potassium *tert*-butoxide in DMF to provide C-3-amidomethylquinolines **4a**–**f**. These adducts **4** were not purified but subjected to palladium-catalyzed cyclization conditions to provide the luotonin A analogues, albeit in poor to good yields for the process. Thus, novel analogues **1a**–**c** and **1f** were prepared by this two-step process, as well as the compounds **1d** and **1e**, previously prepared by Hecht and co-workers[10a,b] by a different synthetic procedure.[16]

Two additional E-ring analogues were also prepared by one-step elaborations (Scheme 3). The 16-amino analogue **1b** ($R_1 = NH_2$) was acylated to provide the corresponding acetanilide analogue **1g** ($R_1 = NHAc$). Bis-demethylation of the 17,18-dimethoxy analogue **1e** (R_2, $R_3 = OCH_3$) provided the corresponding diol analogue **1h** (R_2, $R_3 = OH$). In both cases the yields were low due to significantly poor solubility of the starting materials in almost all organic solvents.

2.2. A-ring analogue syntheses

The bulk of camptothecin analogues reported include relatively subtle (but powerful) changes by substitution onto the camptothecin A and B rings.[5] The most notable examples show that addition of polar functionality at camptothecin C-10 can increase binding affinity and molecule stability,[17] such as the dialkylaminomethyl substitution on the commercial CPT analogue topotecan.[18] Given the overall structural similarity between luotonin A and camptothecin, we intended to prepare 'CPT-like' A-ring analogues of **1**, with the hope that there would be a measurable increase in observed cytotoxicity, either through enhanced binding interactions or increased aqueous solubility in the cell culture media.



Scheme 3. Synthesis of additional E-ring luotonin A analogues.

Thus, 4(3*H*)-quinazolinone (**3a**) was coupled with 3-(chloromethyl)quinoline **2b**,[19] which was cyclized under palladium catalysis to provide the 10-methoxy compound **1i** (Scheme 4), previously prepared by Curran and co-workers[10d] by a different method. Demethylation of **1i** provided 10-hydroxy analogue **1j**, which was then converted to two dialkylaminoethyl analogues **1k** and **1l** by simple displacement of the corresponding alkyl chlorides. Phenol **1j** was also subjected to aminomethylation conditions described by Kingsbury and co-workers[18a] to provide the 'topotecan-like' analogues **1m** and **1n**.

2.3. A/E-ring analogue synthesis

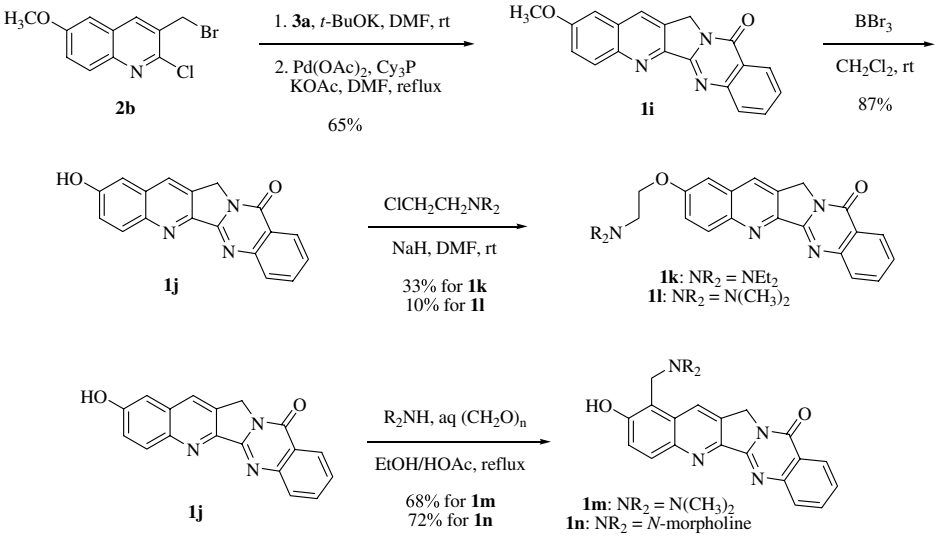
We also prepared one analogue **1o** in which both the A- and E-rings were incorporated with a methoxy substituent (Scheme 5). Thus, the methoxy-substituted 4(3*H*)-quinazolinone **3d** was coupled with bromomethyl quinoline **2b**, followed by palladium-catalyzed cyclization to prepare the 10,16-bis-methoxy luotonin A analogue **1o**.

3. Assay results and discussion

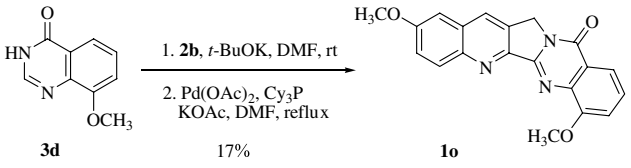
3.1. In vitro antiproliferative activities

Luotonin A (**1a**) and the seven E-ring analogues **1b**–**h** were subjected to testing in three cell lines: cervical carcinoma (HeLa), breast carcinoma (MCF7), and adriamycin-resistant breast carcinoma (ADR-Res). These results are shown in Table 1. In all cases growth inhibition was measured at five concentration points versus a control, and the growth inhibition was expressed in GI_{50} values (defined as the concentrations corresponding to 50% growth inhibition). Luotonin A and its analogues were also directly compared to the in vitro activity of camptothecin as well as the two marketed camptothecin derivatives topotecan and irinotecan.

Luotonin A (**1a**) was shown to have micromolar cytotoxic activity (GI_{50} av 3.7 μM across all three cell lines),



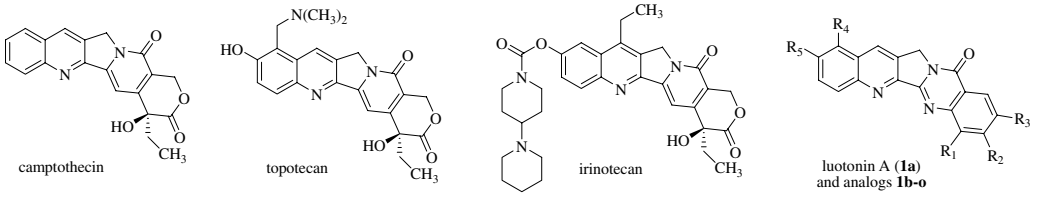
Scheme 4. Synthesis of luotonin A analogues substituted on the A-ring.



Scheme 5. Synthesis of a luotonin A analogue substituted on both the A- and E-rings.

but the only E-ring analogue to show broad moderate activity was the 16-amino derivative **1b** (GI_{50} av 2.7 μM across all three cell lines). The 16-chloro derivative **1c** was found to be weakly active against the ADR-Res and MCF7 cell lines (GI_{50} av 11.5 μM) and the analogue **1h**, bearing a dihydroxy moiety at carbons C-17 and C-18, was found to be weakly active against the HeLa and MCF7 cell lines (GI_{50} av 8.0 μM). Interestingly, the oxygenated compounds **1d** and **1e** provided results that mirrored the experiments reported by Hecht and co-workers for the same compounds in their complementary research. We found compound **1d** to be only moderately active in HeLa (GI_{50} 12 μM) and inactive in ADR-Res and MCF7 cell lines, which parallels the weak cytotoxicity found for the same compound in the Hecht

Table 1. GI_{50} Data for camptothecin standards, luotonin A (**1a**), and luotonin A analogues **1b–p**



Compound	R_1	R_2	R_3	R_4	R_5	ADR-Res GI_{50} (μM)	HeLa GI_{50} (μM)	MCF7 GI_{50} (μM)
Camptothecin	—	—	—	—	—	0.01	0.01	0.01
Topotecan	—	—	—	—	—	0.04	0.03	0.05
Irinotecan	—	—	—	—	—	>1	>1	>1
Luotonin A (**1a**)	H	H	H	H	H	5	3	3
1b	NH_2	H	H	H	H	3	2	2
1c	Cl	H	H	H	H	13	>10	10
1d	OCH_3	H	H	H	H	>20	12	>20
1e	H	OCH_3	OCH_3	H	H	>20	>10	>20
1f	(Pyridyl)	H	H	H	H	>20	>20	>20
1g	NHAc	H	H	H	H	>20	>20	>20
1h	H	OH	OH	H	H	>20	6	10
1i	H	H	H	H	OCH_3	>20	>10	>20
1j	H	H	H	H	OH	>20	>10	>20
1k	H	H	H	H	$O(CH_2)_2NEt_2$	4	2	3
1l	H	H	H	H	$O(CH_2)_2N(CH_3)_2$	4	2	2
1m	H	H	H	$CH_2N(CH_3)_2$	OH	>20	10	13
1n	H	H	H	CH_2(morpholine)	OH	>20	>20	>20
1o	OCH_3	H	H	H	OCH_3			

team's *S. cerevisiae* strain.[9,10a,b]Likewise, compound **1d** was found to show only weak cytotoxic activity (11 μM) against the lung carcinoma cell line H460 in the hands of Dallavalle and co-workers.[10c] We observed the compound **1e** to be completely inactive in our three cell lines, which is what the Hecht team found in their *S. cerevisiae* assay.[9,10a,b]

Results of the in vitro evaluation of the seven A-ring luotonin A analogues **1i**–**n** are also shown in Table 1. From this data set, two compounds were observed to be as potent as luotonin A: **1k**, bearing a 2-(diethylamino)ethoxy substituent at C-10, and **1l**, bearing a 2-(dimethylamino)ethoxy substituent at C-10. Both compounds exhibited a GI_{50} av of ~2.8 μM across all three cell lines. Analogue **1m**, bearing a C-9/10 substitution pattern analogous to topotecan, was also found to be weakly active against the HeLa and MCF7 cell lines (GI_{50} av 11.5 μM). The single A/E-ring-substituted luotonin A analogue **1o** did not show measurable activity in the assays.

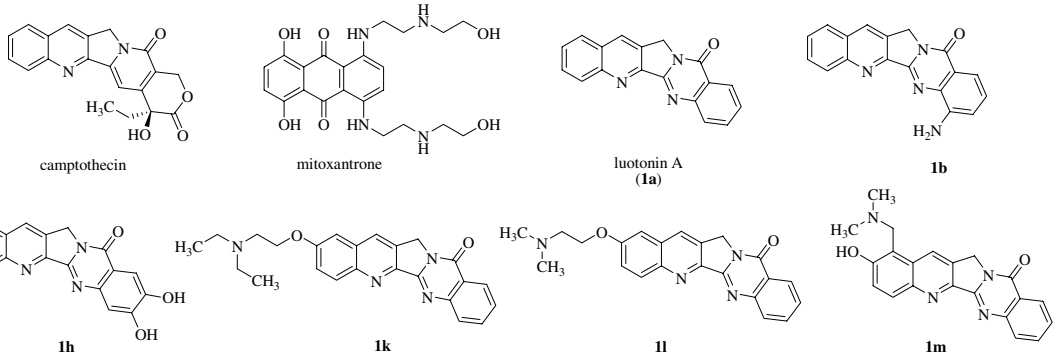
3.2. Growth inhibition in a topoisomerase I mutant cell line

Camptothecin, luotonin A, and some of the most potent analogues were evaluated in two mutant cell lines to assess the role of topoisomerase. In the first set of experiments, camptothecin, luotonin A (**1a**), and the analogues **1b**, **1k**, **1l**, and **1m** were assayed in the human leukemic CEM cell line as a control system (where cytotoxicity is mediated by inhibition of topoisomerase I catalysis). At the same time, the compounds were introduced to the C2 cell line, a CEM cell derivative that expresses a mutated topoisomerase I which confers resistance to camptothecin. The GI_{50} results of both assays are shown in Table 2, along with a calculated relative resistance index for each compound.

As expected, camptothecin did not inhibit growth of the C2 cells (topo I mutant) at concentrations that were effective on the control CEM cell population. The relative resistance was roughly calculated as >500-fold. Luotonin A did show weak growth inhibition of both cell lines, but not to the extent that a resistance ratio could be calculated. The 16-amino E-ring derivative **1b** and C-17/C-18 dihydroxy E-ring congener **1h** both show a difference between the two cell lines, suggesting a dependence on topoisomerase I. This may be attributed to the ability of the amino and hydroxy moieties to interact in a hydrogen bond donor fashion with the Asp533/Arg364 residue pair in the topoisomerase I 'E-ring binding pocket' in the same way as the camptothecin C-20 hydroxyl moiety (i.e., Fig. 2). The poor cytotoxic activity of the E-ring analogues **1c**–**g** and A-ring analogues **1i** and **1j** and **1n**–**1o** versus luotonin A in the HeLa, MCF7, and ADR-Res cell lines, however, may be explained in part by a negative electrostatic interaction between the E-ring substituents with the topo I binding pocket residues or may simply lead to unfavorable interactions due to sterics.

The A-ring topotecan-like analogue **1m** showed almost no meaningful cell growth inhibition in either the CEM or C2 (mutant) cell lines. Interestingly, the two C-10 (dialkylamino)ethyloxy analogues **1k** and **1l** were potent in both CEM strains, suggesting that their cytotoxic effects are not dependent solely on topoisomerase I

Table 2. GI_{50} data for luotonin A analogues in topoisomerase mutant cell lines versus control systems



Compound	CEM[a] GI_{50} (μM)	C2[b] GI_{50} (μM)	Relative resistance	HL60[c] GI_{50} (μM)	MX2[d] GI_{50} (μM)	Relative resistance
Camptothecin	0.002	>1	>500	0.03	0.02	—
Mitoxantrone	—	—	—	0.01	0.13	13
Luotonin A (**1a**)	⩾40	>40	—	—	—	—
1b	6	>40	>7	15	>40	>2.5
1h	9	>40	>4	>40	>40	—
1k	3	7	2	4	10	2.5
1l	3	7	2	4	9	2
1m	30	>40	>1.5	20	>40	>2

[a] Human leukemic CEM cell line (parental control).
[b] C2 cell line (camptothecin-resistant mutant CEM cell line).
[c] Human leukemic HL60 cell line (parental control).
[d] MX2 cell line (mitoxantrone-resistant mutant HL60 cell line).

and therefore cannot be classified simply as topo I poisons. It should be noted that the analogues **1k** and **1l** were much more soluble in the assay media than any of the other analogues tested (including luotonin A itself), which may have conferred an advantage to these two compounds in terms of local concentration (and therefore reflected in apparent increased cytotoxic potency). On the other hand, the C-10 methoxy and C-10 hydroxy A-ring analogues **1i** and **1j** and the A/E-ring analogue **1o** were some of the most insoluble compounds to be put into the HeLa, MCF7, and ADR-Res cell line assays, and in each of these cases were ineffective as growth inhibitors.

3.3. Growth inhibition in a topoisomerase II mutant cell line

In another set of experiments, camptothecin, luotonin A analogues **1b**, **1h**, **1k**, and **1l** were evaluated against the human leukemic HL60 cell line as well as against the mutant MX2 cell line, an HL60 derivative that expresses a mutated topoisomerase II. As a result of this mutation, the MX2 cells are resistant to mitoxantrone (aka Novantrone), an antineoplastic agent known to induce cell death through the DNA/topo II ternary complex. The results of both assays are also shown in Table 2, along with the calculated relative resistance index for each compound.

As expected, camptothecin (which does not induce apoptosis by a topo II-interactive mechanism) inhibited growth equally well of both HL60 and mutant MX2 cells. However, the analysis of control compound mitoxantrone showed a 13-fold resistance when GI_{50} values were compared from HL60 versus MX2 cell colonies. Relative to MX2 cells, HL60 cells showed moderately increased susceptibility to the 16-amino analogue E-ring derivative **1b**, suggesting weak interaction with topo II. The C-17/18 dihydroxy E-ring derivative**1h** showed only minimal activity in both cell lines, such that no measure of relative cytotoxicity could be made.

Conversely, the two C-10 (dialkylamino)ethoxy compounds **1k** and **1l** showed inhibitory activity in both cell lines, but each with only about a twofold resistance in the MX2 cells. One interpretation is that these compounds are possibly weak poisons of the DNA complex with both topoisomerase I and II enzymes. This is consistent with the recent work by Ma and co-workers,[1b] who have demonstrated that luotonin A itself weakly interacts with the topoisomerase II isoform to induce apoptosis, as well as through the topo I-based mechanism. It is also a possibility that the greater toxicity of **1k** and **1l** versus the parent luotonin A template is a function of the pendant dialkylamino substituent moiety (GI_{50} av $\sim$2.8 μM across the ADR-Res, HeLa, MCF7 cell lines for **1k** and **1l** versus 5 μM for **1a**). At physiological pH, protonation of the terminal nitrogen might enhance the solubility of **1k** and **1l** in the culture media, as well as potentially facilitate the interaction with polyionic DNA itself.

Since these compounds **1k** and **1l** have appeared to be fairly consistent growth inhibitors of all of the cell lines examined, but not necessarily significantly dependent on either topo I or topo II, it may be that they are acting to promote apoptosis by a novel mechanism not addressed by these experiments. This finding would be consistent with the work of Hecht and co-workers, who found that a few of their luotonin A E-ring analogues exerted greater inhibition in the absence of topo I,[9,10b] suggesting that an alternative mechanism of action may be operating in some cases.

3.4. Conclusions

We have prepared 14 congeners of the natural product luotonin A and evaluated their antiproliferative activities in three established cancer cell lines (HeLa, MCF7, and ADR-Res) in vitro. One E-ring derivative **1b** and two A-ring analogues **1k** and **1l** were found to be equivalent to luotonin A (**1a**) in these carcinoma cells, and one E-ring analogue **1h** and one A-ring derivative **1m** showed moderate activity in the HeLa and MCF7 cell lines. These five compounds were evaluated in four human leukemia cell lines to assess the role of topoisomerases I and II. Inhibition of cell proliferation was exhibited by the 16-amino derivative **1b** and the C-17/18 dihydroxy analogue **1h** in the CEM cell lines, but not in the C2 (camptothecin-resistant) mutant cells, suggesting a topoisomerase I-mediated mechanism. Compounds **1b** and **1m** also exhibited increased inhibition of HL60 relative to MX2, suggesting partial inhibition of topoisomerase II. Compound **1h** was inactive against both HL60 and MX2 (mitoxantrone-resistant) mutant cells, such that there are no conclusions to be drawn for a mechanism influenced by the inactivation of topoisomerase II.

The two C-10 (dialkylamino)ethyloxy A-ring analogues **1k** and **1l** appear to be acting by a mechanism not solely dependent on topoisomerase I. These compounds exhibited only a twofold relative resistance between both the CEM and C2 cell lines and the HL60 and MX2 cell lines. One interpretation is that these compounds are possibly weak poisons of the DNA complex with both topoisomerase I and II enzymes, but not necessarily significantly dependent on either mode or may be acting to promote apoptosis by a novel mechanism not addressed by these experiments.

One of the major hurdles of the definition of meaningful SAR in this research effort has been the overall poor solubility of substrates for the buffered in vitro assays. While the luotonin A scaffold bears three nitrogens, none of them seemed to be sufficiently basic enough to create a salt form, which would be expected to impart better aqueous solubility. While we hope that our contribution to better evaluating the potential of luotonin A analogues as anticancer agents has been complementary to other recently published reports,[10a–c,11] any future role of this scaffold series should include the preparation of compounds substituted with solubilizing moieties to impart better aqueous solubility in the in vitro assays used.

4. Experimental

4.1. Chemistry

4.1.1. General methods. All non-aqueous reactions were performed under an atmosphere of dry nitrogen unless otherwise specified. Commercial grade reagents and anhydrous solvents were used as received from vendors and no attempts were made to purify or dry these components further. Removal of solvents under reduced pressure was accomplished with a Buchi rotary evaporator using a Teflon-linked KNF vacuum pump. Data for proton NMR spectra were obtained on a Bruker AC nuclear magnetic resonance spectrometer at 300 or 500 MHz and are reported in ppm δ values, using tetramethylsilane as an internal reference. Mass spectroscopic analyses were performed on a ThermoFinnigan aQa single quadrupole mass spectrometer utilizing electrospray ionization (EI). In a few cases, a HPLC purification was performed using an analytical instrument with multiple injections. This small-scale purification was performed on a Phenomenex Luna C18(2) reversed phase column (5 μm, 250 × 4.6 mm) at a flow rate of 1.0 mL/min. Solvents consisted of solvent A (5:95, acetonitrile/water containing 0.05% TFA) and solvent B (95:5, acetonitrile/water containing 0.05% TFA). The elution protocol started with 90% A for 5 min, followed by a linear gradient to 5% A over 20 min and held at 5% A for 5 min, and fractions were collected as the peak of interest was eluted.

4.1.2. Representative procedure for the preparation of Luotonin A derivatives. The following representative example was adapted from the procedure described by Harayama and co-workers.[12m,q] Potassium *tert*-butoxide (1.2 mmol) was added to a suspension of the 4(3*H*)-quinazolinone **3** (1.0 mmol) in DMF (5 mL) at room temperature under nitrogen. After stirring for 5 min, the mixture became a homogeneous solution. To this solution was added the 2-chloro-3-bromomethylquinoline **2** (1.0 mmol) and the resulting mixture was stirred at room temperature for 1 h. The mixture was diluted with methylene chloride (30 mL), washed sequentially with saturated aqueous ammonium chloride solution and brine (50 mL each), dried over sodium sulfate, and filtered. The solvents were removed under reduced pressure and the residue was triturated with methanol to provide the 3-(quinolinylmethyl)-quinazolin-4(3*H*)-one **4** as an off-white solid. A degassed mixture of **4** (0.7 mmol), palladium(II) acetate (10 mol %), tricyclohexylphosophine (20 mol %) and potassium acetate (1.4 mmol) in DMF (7 mL) was heated at 160 °C for 30 min. The cooled mixture was diluted with methylene chloride (30 mL), washed sequentially with water, saturated aqueous ammonium chloride solution, and brine (50 mL each), dried over sodium sulfate, and filtered. The solvents were removed under reduced pressure and the residue was purified by trituration with methanol/chloroform to provide the luotonin A derivative **1** as an off-white solid.

4.1.3. Luotonin A (1a).[12] Compound **1a** was prepared as described by the general procedure from commercially available 4(3*H*)-quinazolinone (**3a**) and 2-chloro-(3-bromomethyl)quinoline (**2a**)[15] as an off-white solid. Yield: 45% (over two steps). The spectral data were consistent with the reported data.

4.1.4. 16-Aminoluotonin A (1b). Compound **1b** was prepared as described by the general procedure from 8-aminoquinazolin-4(3*H*)-one (**3b**)[20] and 2-chloro-(3-bromomethyl)quinoline (**2a**)[15] as a yellow solid. Yield 62% (over two steps): mp > 300 °C; ^{1}H NMR (DMSO-d_6, 300 MHz) δ 8.75 (s, 1H), 8.28 (d, J = 8.5 Hz, 1H), 8.18 (d, J = 7.6 Hz, 1H), 7.96–7.90 (m, 1H), 7.80–7.71 (m, 1H), 7.42 (dd, J = 7.8, 1.3 Hz, 1H), 7.31 (t, J = 7.8 Hz, 1H), 7.10 (dd, J = 7.7, 1.3 Hz, 1H), 5.98 (br s, 2H), 5.31 (s, 2H); ESI MS m/z 301 $[M+H]^+$.

4.1.5. 16-Chloroluotonin A (1c). Compound **1c** was prepared as described by the general procedure from 8-chloroquinazolin-4(3*H*)-one (**3c**)[14c,21] and 2-chloro-(3-bromomethyl)quinoline (**2a**)[15] as a yellow solid. Yield 18% (over two steps): mp 290 °C; ^{1}H NMR (DMSO-d_6, 300 MHz) δ 8.81 (s, 1H), 8.36 (d, J = 8.5 Hz, 1H), 8.27 (d, J = 8.0 Hz, 1H), 8.21 (d, J = 8.3 Hz, 1H), 8.11 (d, J = 7.8 Hz, 1H), 7.94 (t, J = 7.6 Hz, 1H), 7.80 (t, J = 7.8 Hz, 1H), 7.61 (t, J = 7.8 Hz, 1H), 5.34 (s, 2H); ESI MS m/z 320 $[M+H]^+$.

4.1.6. 16-Methoxyluotonin A (1d).[10a–c] Compound **1d** was prepared as described by the general procedure from 8-methoxyquinazolin-4(3*H*)-one (**3d**)[14a,c] and 2-chloro-(3-bromomethyl)quinoline (**2a**)[15] as a yellow solid. Yield 77% (over two steps): mp 290–292 °C; ^{1}H NMR (DMSO-d_6, 300 MHz) δ 8.74 (s, 1H), 8.29 (d, J = 14.1 Hz, 1H), 8.16 (d, J = 13.3 Hz, 1H), 7.96–7.87 (m, 1H), 7.85–7.72 (m, 2H), 7.56 (d, J = 7.9 Hz, 1H), 7.46 (dd, J = 7.9, 1.2 Hz, 1H), 5.30 (s, 2H), 4.02 (s, 3H); ESI MS m/z 316 $[M+H]^+$. These data are in accord with the spectral data reported in Ref. 10a,c.

4.1.7. 17,18-Dimethoxyluotonin A (1e).[10a,b] Compound **1e** was prepared as described by the general procedure from 6,7-dimethoxyquinazolin-4(3*H*)-one (**3e**)[14a,c] and 2-chloro-(3-bromomethyl)quinoline (**2a**)[15] as a yellow solid. Yield 42% (over two steps): mp > 300 °C; ^{1}H NMR (DMSO-d_6, 500 MHz) δ 8.72 (s, 1H), 8.23 (d, J = 8.5 Hz, 1H), 8.15 (d, J = 7.7 Hz, 1H), 7.92–7.87 (m, 1H), 7.77–7.72 (m, 1H), 7.58 (s, 3H), 7.43 (s, 3H); ESI MS m/z 346 $[M+H]^+$. These data are in accord with the spectral data reported in Ref. 10a.

4.1.8. 16-Azaluotonin A (1f). Compound **1f** was prepared as described by the general procedure from pyrido[2,3-*d*]pyrimidin-4(3*H*)-one (**3f**)[14b,c] and 2-chloro-(3-bromomethyl)quinoline (**2a**)[15] as a tan solid. Yield 12% (over two steps): mp > 300 °C; ^{1}H NMR (DMSO-d_6, 500 MHz) δ 9.09 (dd, J = 4.4, 2.0 Hz, 1H), 8.80 (s, 1H), 8.68 (dd, J = 7.8, 2.0 Hz, 1H), 8.30 (d, J = 8.6 Hz, 1H), 8.20 (d, J = 7.5 Hz, 1H), 7.98–7.89 (m, 1H), 7.83–7.75 (m, 1H), 7.65 (dd, J = 7.8, 4.4 Hz, 1H), 5.33 (s, 2H); ESI MS m/z 287 $[M+H]^+$.

4.1.9. 16-Acetamidoluotonin A (1g). Acetyl chloride (0.06 mL, 0.81 mmol) was added dropwise to a solution

of 16-aminoluotonin A (**1b**, 100 mg, 0.33 mmol) in pyridine (5 mL) at room temperature under nitrogen and the mixture was stirred for 24 h. The solvent was removed under reduced pressure and the residue was triturated with methanol/chloroform to provide compound **1g** as a yellow solid (45 mg, 40% yield): mp > 300 °C; ^{1}H NMR (DMSO-d_6, 300 MHz) δ 9.85 (s, 1H), 8.80 (s, 1H), 8.66 (d, J = 7.6 Hz, 1H), 8.33 (d, J = 8.5 Hz, 1H), 8.21 (d, J = 7.5 Hz, 1H), 8.09–7.88 (m, 2H), 7.84–7.75 (m, 1H), 7.59 (t, J = 7.9 Hz, 1H), 5.35 (s, 2H), 2.34 (s, 3H); ESI MS m/z 343 $[M+H]^+$.

4.1.10. 17,18-Dihydroxyluotonin A (1h). Boron tribromide (0.49 mL, 5.21 mmol) was added dropwise to a suspension of 17,18-dimethoxyluotonin A (**1e**, 300 mg, 0.87 mmol) in methylene chloride (20 mL) at room temperature under nitrogen, and the resulting mixture was stirred for 18 h. The mixture was diluted with methanol (5 mL), stirred for 5 min, and the solvents were removed under reduced pressure. The residue was triturated with methanol/chloroform to produce compound **1h** as a yellow solid (26 mg, 10% yield): mp > 300 °C; ^{1}H NMR (DMSO-d_6, 300 MHz) δ 8.72 (s, 1H), 8.23 (d, J = 8.4 Hz, 1H), 8.15 (d, J = 7.8 Hz, 1H), 7.91–7.85 (m, 1H), 7.83–7.71 (m, 1H), 7.54 (s, 1H), 7.23 (s, 1H), 5.25 (s, 2H); ESI MS m/z 318 $[M+H]^+$.

4.1.11. 10-Methoxyluotonin A (1i).[10d] Compound **1i** was prepared as described by the general procedure from commercially available 4(3*H*)-quinazolinone (**3a**) and 2-chloro-(3-bromomethyl)-6-methoxy-quinoline (**2b**)[22] as a yellow solid. Yield 65% (over two steps): mp > 300 °C; ^{1}H NMR (DMSO-d_6, 300 MHz) δ 8.62 (s, 1H), 8.28 (d, J = 7.9 Hz, 1H), 8.18 (m, 1H), 7.93–7.92 (m, 2H), 7.63–7.55 (m, 3H), 5.30 (s, 2H), 3.97 (s, 3H); ESI MS m/z 316 $[M+H]^+$.

4.1.12. 10-Hydroxyluotonin A (1j). Boron tribromide (5 mL, 5 mmol, 1.0 M in methylene chloride) was added dropwise to a suspension of 10-methoxyluotonin A (**1i**, 73 mg, 0.23 mmol) in methylene chloride (10 mL) at −78 °C under nitrogen. The resulting mixture was warmed to room temperature, stirred for 12 h, and then heated at reflux for 30 min. The cooled mixture was diluted with water (5 mL), stirred for 30 min, and then diluted with methylene chloride (50 mL). The biphasic mixture was basified to pH 11 with 2 N sodium hydroxide solution and the aqueous layer was collected and acidified to pH 2 with concentrated hydrochloric acid. The resulting precipitate was collected by vacuum filtration, washed with water (20 mL), and dried to produce compound **1j** as a gray solid (60 mg, 87% yield): mp > 300 °C; ^{1}H NMR (DMSO-d_6, 300 MHz) δ 10.50 (s, 1H), 8.52 (s, 1H), 8.28 (d, J = 7.9 Hz, 1H), 8.13 (d, J = 9.0 Hz, 1H), 7.93–7.92 (m, 2H), 7.63–7.57 (m, 1H), 7.52–7.48 (m, 1H), 7.33–7.32 (m, 1H), 5.27 (s, 2H); ESI MS m/z 302 $[M+H]^+$.

4.1.13. 10-[2-(*N*,*N*-Diethylamino)ethyl]oxy-luotonin A (1k). Sodium hydride (16 mg, 0.66 mmol) was added to a solution of 10-hydroxyluotonin A (**1j**, 100 mg, 0.33 mmol) in DMF (5 mL) at room temperature under nitrogen and the mixture was stirred for 10 min. To this solution was added a mixture of 2-chloroethyl diethylamine hydrochloride (63 mg, 0.39 mmol) and potassium *tert*-butoxide (41 mg, 0.39 mmol) in DMF (1 mL), and the resulting mixture was stirred at room temperature for 44 h. The reaction mixture was diluted with methylene chloride (30 mL), washed sequentially with saturated aqueous sodium bicarbonate and brine solutions (20 mL each), dried over sodium sulfate, and filtered. The solvents were removed under reduced pressure and the residue was purified by HPLC chromatography (iterative injections on an analytical instrument) to provide compound **1k** as a yellow solid (44 mg, 33% yield): mp 250 °C; ^{1}H NMR (DMSO-d_6, 500 MHz) δ 9.36 (b, 1H), 8.64 (s, 1H), 8.30 (d, J = 7.8 Hz, 1H), 8.24(d, J = 9.1 Hz, 1H), 7.94–7.92 (m, 2H), 7.65–7.60 (m, 3H), 5.32 (s, 2H), 4.53 (t, J = 4.8 Hz, 2H), 3.66 (m, 2H), 3.34–3.24 (m, 4H), 1.27 (t, J = 7.2 Hz, 6H); ESI MS m/z 401 $[M+H]^+$.

4.1.14. 10-[2-(*N*,*N*-Dimethylamino)ethyl]oxy-luotonin A (1l). Compound **1l** was prepared by the same procedure as described for the preparation of **1k** from 10-hydroxyluotonin A (**1j**) and 2-chloroethyl dimethylamine hydrochloride as a yellow solid. Yield 10%: mp 200 °C; ^{1}H NMR (DMSO-d_6, 300 MHz) δ 9.68 (b, 1H), 8.65 (s, 1H), 8.31–8.22 (m, 2H), 7.94–7.93(m, 2H), 7.64–7.60 (m, 3H), 5.32 (s, 2H), 4.54 (m, 2H), 3.64 (m, 2H), 2.92 (s, 6H); ESI MS m/z 373 $[M+H]^+$.

4.1.15. 9-(*N*,*N*-Dimethylamino)methyl-10-hydroxy-luotonin A (1m). A mixture of 10-hydroxyluotonin A (**1j**, 100 mg, 0.33 mmol), 37% aqueous formaldehyde (0.2 mL, 2.7 mmol), and 40% aqueous methylamine (0.2 mL, 2.3 mmol) in acetic acid (6 mL) was stirred at room temperature for 2 d. The solvents were removed under reduced pressure and the residue was purified by HPLC chromatography (iterative injections on an analytical instrument) to produce compound **1m** as a yellow solid (80 mg, 68% yield): mp 230 °C; ^{1}H NMR (DMSO-d_6, 500 MHz) δ 8.98 (s, 1H), 8.31 (s, 1H), 8.29 (s, 1H), 7.98–7.91 (m, 2H), 7.69 (d, J = 9.2 Hz, 1H), 7.67–7.60 (m, 1H), 5.27 (s, 2H), 4.75 (s, 2H), 2.87 (s, 6H); ESI MS m/z 359 $[M+H]^+$.

4.1.16. 10-Hydroxy-9-(*N*-morpholino)methyl-luotonin A (1n). Compound **1n** was prepared by the same procedure as described for the preparation of **1m** from 10-hydroxyluotonin A (**1j**) and morpholine as a yellow solid. Yield 72%: mp 280 °C; ^{1}H NMR (DMSO-d_6, 500 MHz) δ 8.82 (s, 1H), 8.28 (d, J = 7.6 Hz, 1H), 8.08 (d, J = 9.1 Hz, 1H), 7.96–7.89 (m, 2H), 7.65–7.58 (m, 1H), 7.50 (d, J = 9.1 Hz, 1H), 5.28 (s, 2H), 4.06 (br s, 2H), 3.59 (br s, 4H), 2.51 (m, 4H); ESI MS m/z 401 $[M+H]^+$.

4.1.17. 10,16-Dimethoxyluotonin A (1o). Compound **1o** was prepared as described by the general procedure from 8-methoxyquinazolin-4(3*H*)-one (**3d**)[14a,c] and 2-chloro-(3-bromomethyl)-6-methoxyquinoline (**2b**)[22] as a yellow solid. Yield 17% (over two steps): mp 300–305 °C; ^{1}H NMR (DMSO-d_6, 500 MHz) δ 8.60 (s, 1H), 8.19 (d, J = 8.6 Hz, 1H), 7.83 (dd, J = 7.9, 1.1 Hz, 1H), 7.58–7.52 (m, 3H), 7.46 (dd, J = 7.9, 1.1 Hz, 1H),

5.29 (s, 2H), 4.01 (s, 3H), 3.96 (s, 3H); ESI MS *m/z* 346 $[M+H]^+$.

4.2. Cell-based assays

4.2.1. Cells and cell culture. Cell lines were maintained in the following culture media: HeLa S3 (American Type Culture Collection, ATCC), MEM/7% fetal calf serum (FCS); MCF7 (ATCC), RPMI 1640/1 mM sodium pyruvate/10% FCS; ADR-Res (NCI-Frederick), RPMI/10% FCS. CEM and CEM/C2 (ATCC) RPMI1640/10 mM Hepes, 4.5 g/L glucose/1 mM sodium pyruvate/10% FCS; HL-60 (ATCC), RPMI 1640/4 mM glutamine/25 mM Hepes/4.5 g/L glucose/1 mM sodium pyruvate/20% FCS. HL60/MX2 (ATCC) RPMI1640 2 mM glutamine/10 mM Hepes/4.5 g/L glucose/1 mM sodium pyruvate/10% FCS. All media were supplemented with penicillin (100 U/mL), and streptomycin (100 μg/mL).

4.2.2. Growth inhibition assays. HeLa, MCF7, and ADR-Res cells were plated in 96-well plates at 2×10^3 cells per well in a volume of 100 μL. One day after plating, compounds were dissolved in DMSO, diluted in culture medium over a 5-point concentration range and added to each well in a volume of 100 μL. Four days after compound addition, the cells were fixed by the addition of 30 μL of 10% (v/v) glutaraldehyde for 30 min. The wells were then extensively washed in tap water and air-dried. Cells were then stained by the addition of 100 μL of 0.2% (w/v) crystal violet in 20% ethanol for 1 h. After extensive washing with tap water, the plates were air-dried and 100 μL of 10% (v/v) acetic acid was added to solubilize the crystal violet, which was quantified on a plate reader at 570 nm.

CEM, CEM/C2, HL-60, and MX2 cells were placed in 96-well plates at 1×10^4 cells per well. One day later, compounds were dissolved in DMSO, diluted in culture medium over a 5-point concentration range, and added to wells in a volume of 100 μL. Two days later, 20 μL of Alamar blue was added for 24 h and read at 570 and 600 nm.

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