Synthesis and Biological Properties of Luotonin A Derivatives

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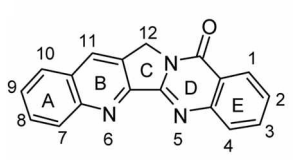
A series of new derivatives on the ring A of luotonin A were prepared by Friedländer condensation of 6,7,8,10-tetrahydropyrrolo[2,1-*b*]quinazoline-6,10-dione and suitably substituted 2-aminobenzaldehydes and 2-aminoacetophenones. Their inhibitory activities on topoisomerases and cytotoxicities against selected human cancer cell lines were evaluated. Among the compounds tested, 8-fluoroluotonin A showed similar inhibitory activity on topoisomerase I comparable to camptothecin while luotonin A and 9-hydroxyluotonin A showed 1.37 and 0.94 times stronger inhibitory activity, respectively, on topoisomerase II compared to etoposide. Some derivatives of luotonin A showed moderate cytotoxicity. The possible relationship between the inhibitory activity on Topo II and the cytotoxicity of luotonin A and its analogues, thus, cannot be ruled out.

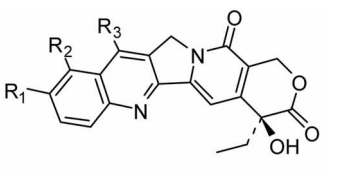
Key Words : Luotonin A, Topoisomerase I, Topoisomerase II, Cytotoxicity, Friedländer reaction

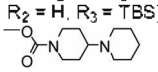
Introduction

Luotonin A is an alkaloid isolated from *Peganum nigellastrum* Bunge (Zygophyllaceae) which has been used as a traditional oriental medical practice for the treatment of rheumatism, abscess, and inflammation.[1] The basic fractions of *P. nigellastrum* showed anti-tumor activtity,[2] and the origin of such an activity was recently revealed by identifying its constituent luotonin A which inhibited the growth of leukemia P-388 cells (IC_{50} = 1.8 μg/mL).[3] Very recently, Hecht *et al.*[4] have demonstrated that despite the lack of lactone ring functionality, luotonin A stabilizes the human DNA topoisomerase I (Topo I)-DNA covalent binary complex and mediates Topo I-dependent cytotoxicity in intact cells (IC_{50} = 5.7-12.6 μm/mL), like camptothecin (CPT) and its analogues.[4,5] Such intriguing properties of luotonin A not only led developments of efficient methods for total synthesis,[6] but also introduced a variety of its derivatives.[7] Studies on the derivatives of luotonin A were focused on the introduction of substituents on the ring E[7] and adjustment of the ring size on the ring C.[8] To the best of our knowledge, only very limited numbers of compounds with (a) substituent(s) on the ring A and B were reported and shown promising biological properties.[6p,7d]



Luotonin A



Camptothecin (R_1 = R_2 = R_3 = H)
Topotecan (R_1 = OH, R_2 = -CH_2NMe_2, R_3 = H)
DB-67 (R_1 = OH, R_2 = H, R_3 = TBS)
Irinotecan (R_1 = 
R_2 = H, R_3 = -CH_2CH_3)

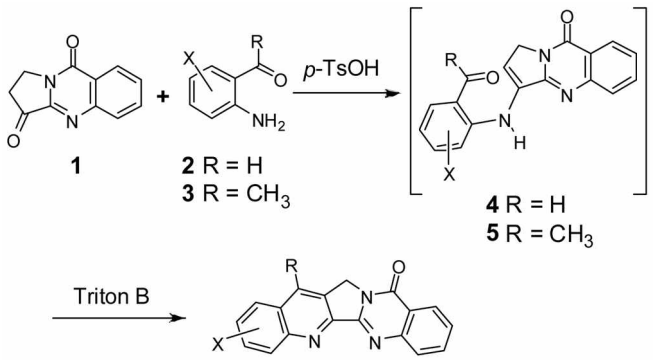
Although early crystal structure of the CPT-stabilized DNA-Topo I cleavage complex has revealed that the crucial 20-OH of CPT is hydrogen bonded with Asp533 as well as ester moiety with Arg364 of Topo I,[9] luotonin A, has shown similar interaction mode and shown promising cytotoxicity even though the functionalities in CPT are lacking.[4,5]

Our continuing interest in the search for biologically active agents derived from natural sources[10] spurred us to design a series of luotonin A derivatives and to evaluate their biological properties.

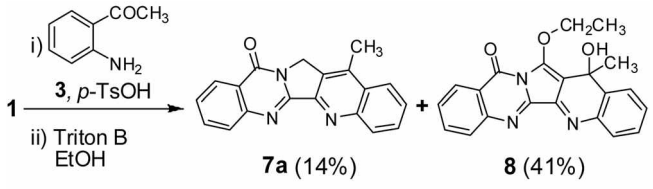
Results and Discussion

Chemistry. Synthesis of the luotonin A derivatives **6** and **7** was straightforward as shown. Reactions of **1** with (substituted) 2-aminobenzaldehydes (**2**) were carried out under classical acidic Friedländer condition to afford the corresponding luotonin A derivatives (**6**) in fair yields. Hydrogenolysis of 9-benzyloxyluotonin A (**6d**) gave 9-hydroxyluotonin A (**6h**) in quantitative yield. Although most of the Friedländer condensation employed basic reaction condition,[11] reactions of (substituted) 2-aminophenyl ketones (**3**) in the presence of 10-20% KOH in EtOH were too sluggish to give the desired products. We, thus, pursued a step-wise reaction. The ketone **1a** was treated with 2-aminoacetophenone as well as substituted 2-aminoacetophenones in the presence of *p*-TsOH to afford corresponding Schiff's bases (**5**) in excellent yields (over 90% in most cases). ^{1}H NMR spectra of **5**, in fact, showed that more stable enamine-forms were the only forms. The resulting enamines were then subjected to Friedländer condition in the presence of Triton B to lead the desired compounds in good yields except **7a**. The prerequisite ketone (**1**),[9] suitably substituted benzaldehydes (**2**),[12] and 2-aminoacetophenones (**3**)[13] were prepared by employing previously reported methods.

In some cases, preparations of 2-aminoaldehydes are suffered from polymerization of resulting aminoaldehydes during reduction of 2-nitroaldehydes, which limits the scope of Friedländer reaction in the introduction of more substituents on Ring A.



It should be noted that Triton-B catalyzed cyclization of an intermediate enamine **5a** resulted in **7a** (14%) and unexpected compound **8** (41%), of which the structure was confirmed by spectroscopic methods. The reaction mechanism for the formation of **8** remained to be explored.



DNA topoisomerase inhibitory activity. Topo I and II inhibitory activities of the compounds prepared were measured by assessing the relaxation of supercoiled pBR 322 plasmid DNA employing the method previously described.[14] As shown in Table 1, 9-hydroxyluotonin A (**6h**) and 8-fluoroluotonin A (**6k**) showed 54.1% and 81.1% inhibition at the concentration of 100 μM which are comparable to 83.9% of CPT. In DNA Topo II assay, luotonin A (**6a**) and **6h** are the most active inhibitors to show 73.5 and 52.4% inhibition comparable to 55.8% of etoposide at the concentration of 100 uM. It should be noted that the compounds (**7a-d**) with a methyl group at C11 did not show any promising inhibitory activity against Topo I and Topo II (data not shown). Although luotonin A has long been known as a Topo I inhibitor, present data implies that luotonin A can also be a good Topo II inhibitor.

Cytotoxicity. Cytotoxicities of compounds prepared were screened by the method previously reported[15] against selected human cancer cell lines: HeLa (human cervix tumor), HCT-116 (human colon tumor), DU-145 (human prostate tumor), MDA-MB231 (human breast tumor), and HL-60 (human myeloid leukemic tumor) cell lines. As shown in Table 2, IC_{50} values of compound prepared were ranged 19-86 μM against HCT-116, DU-145, MDA-MB231, and HL-60 while no activity was observed for HeLa up to 100 μM (Table 2).

At the concentration of 100 μM, **6k** showed strong inhibitory activity on DNA Topo I compared with DNA Topo II with medium cytotoxicities against DU-145, MDA-MB231, and HL-60. On the other hand, compounds **6a** and **6h** showed stronger inhibitory activity against Topo II than against Topo I with relatively low, but selective cytotoxi-

Table 1. Inhibition of CPT, etoposide and selected luotonin A derivatives against Topo I and II

	Reference	6a	6e	6f	6g	6h	6j	6k	6n
Topo I Inhibition[a]	83.9	33.7	16.7	11.2	12.9	54.1	17.4	81.1	19.2
Rel. potency	1	0.40	0.20	0.13	0.15	0.65	0.21	0.97	0.23
Topo II Inhibition[b]	55.8	73.5	0	22.2	0	52.4	0	2.7	3.0
Rel. potency	1	1.32	–	0.40	–	0.94	–	0.05	0.06

[a]Data were taken with 0.2 unit of Topo I and 100 μM of CPT or compounds. [b]Data were taken with 0.2 unit of Topo II and 100 μM of etoposide or compounds

Table 2. Cytotoxic activity of luotonin A and its derivatives determined by cell proliferation assay

Compounds	cell lines (IC_{50}, μM)[a]				
	HeLa	HCT-116	DU-145	MDA-MB231	HL-60
6a	33.83 ± 3.45	51.11 ± 1.07	23.25 ± 5.95	56.07 ± 3.35	> 100
6h	> 100	56.57 ± 6.60	> 100	47.41 ± 0.25	66.29 ± 5.31
6k	> 100	> 100	86.26 ± 3.04	49.95 ± 0.02	56.65 ± 6.01
6n	> 100	19.36 ± 0.73	27.83 ± 2.24	43.91 ± 0.95	21.78 ± 2.49
Dauxorubicin	7.46 ± 0.41	2.31 ± 0.91	4.78 ± 0.21	2.75 ± 0.02	0.72 ± 0.08
Etoposide	12.13 ± 0.36	14.94 ± 1.59	39.00 ± 6.82	22.15 ± 0.32	2.23 ± 0.77
CPT	7.84 ± 0.27	2.17 ± 0.18	5.51 ± 1.14	15.03 ± 0.30	0.06 ± 0.02

[a]Each data point represents mean ± S.D. from three different experiments performed in triplicate. Cell lines used are HeLa, human cervix tumor cell line; HCT 116, human colon tumor cell line; DU 145, human prostate tumor cell line; MDA-MB231, human breast tumor cell line; HL-60, human myeloid leukemic tumor cell line.

cities against HCT-116, MDA-MB231 and HL-60. It should be noted that **3n** is not a good inhibitor of Topo I and II, but showed stronger cytotoxicities against HCT-116 and HL-60 with IC_{50}'s at the 20 μM level. These results indicate that obvious correlation cannot be described between the cytotoxicity and the inhibitory activity of DNA relaxation and decatenation by DNA Topo I and II.

In conclusion, a series of new derivatives of luotonin A on the ring A were prepared by Friedländer condensation of 6,7,8,10-tetrahydropyrrolo[2,1-*b*]quinazoline-6,10-dione and suitably substituted 2-aminobenzaldehydes and 2-aminoacetophenones. Their inhibitory activities on topoisomerases and cytotoxicities against selected human cancer cell lines were evaluated to show that 8-fluoroluotonin A showed similar inhibitory activity on Topo I comparable to camptothecin while luotonin A and 9-hydroxyluotonin A 1.37 and 0.94 times stronger inhibition, respectively, on Topo II compared to etoposide. Some derivatives of luotonin A showed moderate cytotoxicity. The possible relationship between the inhibitory activity on Topo II and the cytotoxicity of luotonin A and its analogues, thus, cannot be ruled out.

Experimental Section

Melting points were determined using a Fischer-Jones melting points apparatus and are not corrected. IR spectra were taken from a Perkin-Elmer 1330 spectrophotometer. NMR spectra were obtained using a Bruker-250 spectrometer 250 MHz or 300 MHz for ^{1}H NMR and 62.5 MHz or 75 MHz for ^{13}C NMR and are reported as parts per million (ppm) from the internal standard tetramethylsilane (TMS). The starting materials **1a**,[8] **2**,[12] and **3**[13] were prepared by employing previously reported method. Chemicals and solvents were commercial reagent grade and used without further purification. Elemental analyses were taken on a Hewlett-Packard Model 185B elemental analyzer.

Luotonin A (6a) (General Procedure). A mixture of ketone (**1**) (48 mg, 0.24 mmol), 2-aminobenzaldehyde (29 mg, 1.2 equiv.), and *p*-TsOH (20 mg) in toluene (20 mL) was refluxed for 12 h, cooled to room temperature, and diluted with hexane:ether (1:1). Resulting precipitate was collected and washed with hexane:ether (1:1) to afford **6a** as a solid which was recrystallized from EtOH to give spectroscopically pure compound: mp 280-281 °C. The spectral data were identical to those of literature values.

9-Chloroluotonin A (6b): Pale yellow needles (78%): mp: 233-234 °C. ^{1}H NMR ($CDCl_3$, 250 MHz) δ 8.42 (d, 1H, J = 8.3 Hz, H1), 8.38 (d, 1H, J = 8.3 Hz, H4), 8.36 (s, 1H, H11), 8.09 (d, 1H, J = 8.3 Hz, H7), 7.93 (d, 1H, J = 2.1 Hz, H10), 7.85 (td, 1H, J = 8.3, 1.0 Hz, H2), 7.77 (dd, 1H, J = 9.0, 2.1 Hz, H8), 7.58 (td, 1H, J = 8.0, 1.0 Hz, H3), 5.34 (s, 2H). MS (ESI) Cacld. for $C_{18}H_{11}ClN_3O$ $[M+H]^-$ 320. Found 320. *Anal.* Calcd for $C_{18}H_{10}ClN_3O{\cdot}H_2O$: C, 64.01; H, 3.58; N, 12.44. Found: C, 63.98; H, 3.60; N, 14.43.

9-Fluoroluotonin A (6c): Pale yellow needles (78%): mp: 273 °C. ^{1}H NMR ($CDCl_3$, 250 MHz) δ 8.45 (dd, 1H, $J_{H\text{-}F}$ = 9.0 Hz, J_{ortho} = 8.0 Hz, H10), 8.42 (d, 1H, J = 8.1 Hz, H1), 8.39 (s, 1H, H11), 8.18 (d, 1H, J = 8.1 Hz, H4), 7.84 (td, 1H, J = 8.0, 1.2 Hz, H2), 7.65-7.54 (m, 3H), 5.33 (s, 2H). MS (ESI) cacld. For $C_{18}H_{11}FN_3O$ $[M+H]^+$ 304. Found 304. *Anal.* Calcd for $C_{18}H_{10}FN_3O{\cdot}H_2O$: C, 67.29; H, 3.76; N, 13.08. Found: C, 67.28; H, 3.83; N, 13.02.

9-Bromoluotonin A (6d): Pale yellow needles (78%): mp: > 350 °C. ^{1}H NMR ($CDCl_3$, 250 MHz) δ 8.46 (s, 1H, H11), 8.42 (dd, 1H, J = 8.3, 0.8 Hz, H1), 8.18 (dd, 1H, J = 8.3, 0.8 Hz, H4), 8.09 (d, 1H, J = 8.3 Hz, H7), 7.92 (d, 1H, J = 7.5 Hz, H10), 7.85 (td, 1H, J = 8.3, 1.0 Hz, H3), 7.60-7.50 (m, 2H), 5.36 (s, 2H). MS (ESI) Cacld. For $C_{18}H_{11}BrN_3O$ $[M+H]^+$ 364. Found 364. *Anal.* Calcd for $C_{18}H_{10}BrN_3O{\cdot}0.5H_2O$: C, 57.93; H, 2.97; N, 11.26. Found: C, 57.95; H, 2.99; N, 11.26.

9-Methoxyluotonin A (6e): Pale yellow needles (45%): mp: 282 °C. ^{1}H NMR ($CDCl_3$, 300 MHz) δ 8.38 (dd, 1H, J = 8.0, 1.2 Hz, H1), 8.30 (d, 1H, J = 9.3 Hz, H4), 8.27 (s, 1H, H14), 8.06 (d, 1H, J = 8.1 Hz, H7), 7.81 (td, 1H, J = 7.8, 1.5 Hz, H2) 7.53 (td, 1H, J = 7.6, 1.0 Hz, H3), 7.45 (dd, 1H, J = 9.3, 2.7 Hz, H8), 7.11 (d, 1H, J = 2.7 Hz, H10), 5.62 (s, 2H), 3.94 (s, 3H, -OCH_3). ^{13}C NMR ($CDCl_3$, 62.5 MHz) δ 160.70, 159.35, 152.77, 149.43, 148.59, 145.69, 134.52, 132.04, 130.31, 130.02, 129.72, 128.58, 127.16, 126.40, 123.97, 121.14, 104.99, 55.69 and 47.30. ESI MS Calcd for $C_{19}H_{14}N_3O_2$ $[M+H]^+$ 316.3. Found 316.10 *Anal.* Calcd for $C_{19}H_{13}BrN_3O_2$: C, 72.37; H, 4.16; N, 13.33. Found: C, 72.39; H, 4.15; N, 13.32.

9-Benzyloxyluotonin A (6f): Pale yellow needles (55%): mp: 311 °C. ^{1}H NMR ($CDCl_3$, 250 MHz) δ 8.41 (dd, 1H, J = 8.0, 1.2 Hz, H1), 8.36 (d, 1H, J = 9.4 Hz, H4), 8.28 (s, 1H, H11), 8.09 (d, 1H. J = 8.2 Hz, H7), 7.83 (td, 1H, J = 7.7, 1.3 Hz, H2), 7.58-7.33 (m, 8H), 5.29 (s, 2H), 5.22 (s, 2H). MS (ESI) Calcd for $C_{25}H_{18}N_3O_2$ $[M+H]^+$. 392. Found 392. *Anal.* Calcd for $C_{25}H_{17}N_3O_2{\cdot}0.5H_2O$: C, 74.99; H, 4.53; N, 10.49. Found: C, 75.05; H, 4.49; N, 10.52.

9-Acetyloxyluotonin A (6g): Pale yellow needles (47%): mp: 212 °C. ^{1}H NMR (CD_3OD, 250 MHz) δ 8.45 (d, 1H, J = 7.8 Hz, H1), 8.39-8.37 (m, 2H, H4 and H11), 8.07 (d, 1H, J = 8.0 Hz, H7), 7.83 (td, 1H, J = 8.0. 1.0 Hz, H2), 7.78 (d, 1H, J = 2.5 Hz, H10), 7.64 (dd, 1H, J = 8.1, 0.8 Hz, H8), 7.56 (t, 1H, J = 7.8 Hz, H3), 5.32 (s, 2H), 3.96 (s, 3H). MS (ESI) Calcd for $C_{20}H_{14}N_3O_3$ $[M+H]^+$ 344. Found 344 *Anal.* Calcd for $C_{25}H_{13}N_3O_3{\cdot}0.5H_2O$: C, 68.18; H, 4.00; N, 11.93. Found: C, 68.18; H,3.97; N, 11.93.

9-Hydroxyluotonin A (6h): A mixture of 9-benzyloxyluotonin A (39.1 mg, 0.1 mmol) and Pd/C (5%, 50 mg) in acetic acid (5 mL) was stirred under H_2 atmosphere for 6 h and work-up as usual to give 30 mg (99%) of white solid: mp 279 °C. ^{1}H NMR (CD_3OD, 250 MHz) δ 8.36 (s, 1H, H11), 8.30 (d, 1H, J = 8.0 Hz, H1), 8.10 (d, 1H, J = 8.0 Hz, H4), 7.93-7.84 (m, 2H, H7 and H2), 7.57 (td, 1H, J = 8.1, 0.8 Hz, H3), 7.43 (dd, 1H, J = 7.8, 1.5 Hz, H8), 7.18 (d, 1H, J = 1.0 Hz, H10), 5.28 (s, 2H). MS (ESI) Calcd for $C_{18}H_{12}N_3O_2$ $[M+H]^+$ 302. Found 302 *Anal.* Calcd for $C_{18}H_{11}N_3O_2{\cdot}H_2O$: C, 67.71; H, 4.10; N, 13.16. Found: C, 67.78; H, 4.13; N, 13.13.

8-Chloroluotonin A (6i): Pale yellow needles (36%): mp: 286 °C. ^{1}H NMR ($CDCl_3$, 250 MHz) δ 8.43 (s, 2H, H7 & H11), 8.42 (d, 1H, J = 8.3 Hz, H1), 8.11 (d, 1H, J = 8.3 Hz, H4), 7.92-7.82 (m, 2H), 7.65-7.55 (m, 2H), 5.32 (s, 2H). MS (ESI) Cacld for $C_{18}H_{11}ClN_3O$ $[M+H]^+$ 320. Found 320. *Anal.* Calcd for $C_{18}H_{10}ClN_3O{\cdot}0.5H_2O$: C, 65.76; H, 3.37; N, 12.78. Found: C, 65.78; H, 3.36; N, 12.77.

10-Chloroluotonin A (6j): Pale yellow needles (78%): mp: 262 °C. ^{1}H NMR ($CDCl_3$, 250 MHz) δ 8.90 (s, 1H, H11), 8.43 (d, 1H, J = 8.3 Hz, H1), 8.40 (d, 1H, J = 8.3 Hz, H4), 8.11 (d, 1H, J = 8.3 Hz, H7), 7.86 (t, 1H, J = 7.5 Hz, H2), 7.78-7.72 (m, 2H, H8 and H9), 7.59 (td, 1H, J = 8.0, 1.0 Hz, H3), 5.39 (s, 2H). MS (ESI) Cacld for $C_{18}H_{11}ClN_3O$ $[M+H]^+$ 320. Found 320. *Anal.* Calcd for $C_{18}H_{10}ClN_3O{\cdot}H_2O$: C, 64.01; H, 3.58; N, 12.44. Found: C, 63.98; H, 3.60; N, 14.43.

8-Fluoroluotonin A (6k): Pale yellow needles (39%): mp: 269 °C. ^{1}H NMR ($CDCl_3$, 250 MHz) δ 8.45 (s, 1H, H11), 8.42 (dd, 1H, J = 8.1, 1.5 Hz, H1), 8.12-8.05 (m, 2H, H4 and H7), 7.96 (dd, 1H, $J_{H\text{-}F}$ = 9.0, J = 8.0 Hz, H10), 7.86 (td, 1H, J = 8.0, 1.0 Hz, H3), 7.58 (td, 1H, J = 8.0, 1.0 Hz, H3), 7.49 (ddd, 1H, $J_{H\text{-}F}$ = 9.0, J = 8.0, 1.0 Hz, H9), 5.30 (s, 2H). MS (ESI) Cacld for $C_{18}H_{11}FN_3O$ $[M+H]^-$ 304. Found 304. *Anal.* Calcd for $C_{18}H_{10}FN_3O{\cdot}H_2O$: C, 67.29; H, 3.76; N, 13.08. Found: C, 67.32; H, 3.69; N, 13.12.

8,9-Dimethoxyluotonin A (6l): Pale yellow needles (49%): mp 294 °C. ^{1}H NMR ($CDCl_3$, 250 MHz) δ 8.41 (d, 1H, J = 7.5 Hz, H1), 8.25 (s, 1H, H11), 8.06 (d, 1H, J = 8.0 Hz, H4), 7.82 (t, 1H, J = 8.0 Hz, H3), 7.75 (s, 1H, H7), 7.58 (td, 1H, J = 7.8, 1.3 Hz, H2), 7.13 (s, 1H, H10), 5.27 (s, 2H), 4.05 (s, 6H). MS (ESI) Cacld. for $C_{20}H_{16}N_3O_3$ $[M+H]^+$ 346. Found 346. *Anal.* Calcd for $C_{20}H_{15}N_3O_3{\cdot}H_2O$: C, 66.11; H, 4.72; N, 11.56. Found: C, 66.17; H, 4.69; N, 11.53.

8,9-Difluoroluotonin A (6m): Pale yellow needles (43%): mp 245 °C. ^{1}H NMR ($CDCl_3$, 250 MHz) δ 8.35 (s, 1H, H11), 8.33 (d, 1H, J = 7.5 Hz, H1), 8.17 (ddd, 1H, $J_{H\text{-}F}$ = 10.0, $J_{H\text{-}F}$ = 8.0 Hz, J_{para} = 1.0 Hz, H7), 8.06 (d, 1H, J = 8.0 Hz, H4), 7.83 (t, 1H, J = 8.0 Hz, H2), 7.67 (dd, 1H, $J_{H\text{-}F}$ = 10.0 Hz, $J_{H\text{-}F}$ = 8.0 Hz, H10), 7.56 (t, 1H, J = 7.8 Hz, H3), 5.30 (s, 2H). MS (ESI) Cacld. for $C_{18}H_{10}F_2N_3O$ $[M+H]^+$ 322. Found 322. *Anal.* Calcd for $C_{18}H_{10}F_2N_3O{\cdot}H_2O$: C, 63.72; H, 3.27; N, 11.20. Found: C, 63.67; H, 2.29; N, 11.19.

8,9-Methylenedioxyluotonin A (6n): Pale yellow needles (88%): mp: 336 °C. ^{1}H NMR ($CDCl_3$, 250 MHz) δ 8.38 (d, 1H, J = 8.0 Hz, H1), 8.18 (s, 1H, H11), 8.05 (d, 1H, J = 8.0 Hz, H4), 7.81 (td, 1H, J = 7.5, 1.5 Hz, H2), 7.66 (s, 1H, H7), 7.53 (td, 1H, J = 7.7, 1.3 Hz, H3), 7.09 (s, 1H, H10), 6.16 (s, 2H), 5.28 (s, 2H). ^{13}C NMR ($CDCl_3$, 62.5 MHz) δ 160.58, 152.79, 151.79, 149.62, 149.33, 148.55, 148.07, 134.46, 129.85, 128.58, 128.46, 127.04, 126.73, 126.31, 121.02, 106.35, 102.61, 102.36, 47.18. MS (ESI) Cacld. for $C_{19}H_{12}N_3O_3$ $[M+H]^+$ 330. Found 330. *Anal.* Calcd for $C_{20}H_{11}N_3O_3{\cdot}H_2O$: C, 66.70; H, 3.77; N, 12.10. Found: C, 66.67; H, 3.79; N, 12.09.

11-Methylluotonin A (7a): To a solution of ketone **1** (48 mg, 0.24 mmol) and 2-aminoacetophenone (**3**, 0.40 mg, 1.2 equiv.) in toluene (20 mL) was added *p*-TsOH (10 mg). Resulting mixture was refluxed stirred for 12 h, cooled to room temperature, and diluted with hexane:ether (1:1). Resulting precipitate was collected and washed with hexane: ether (1:1) to afford the corresponding enamine (**5**) as a solid, which was recrystallized, from EtOH to give spectroscopically pure compound. Into the solution of the compound obtained in absolute EtOH (10 mL) was added triton B (0.1 mL) and resulting reaction mixture was refluxed for 12 h. Cooling the reaction mixture resulted in precipitation which was collected and washed with cold EtOH to give yellow solid. The crude product was chromatographed on silica gel eluting with hexane:EtOAc (1:1). The early fractions (R_f = 0.4) give 30 mg (41%) of 14-hydroxy-14-methylquino[2',3':3,4]pyrrolo[2,1-*b*]quinazolin-13-ethyloxy-11-one (**8**) as a violet powder: mp 220-222 °C. ^{1}H NMR ($CDCl_3$, 250 MHz) δ 8.35 (d, 1H, J = 8.0 Hz, H1), 8.15 (d, 1H, J = 8.3 Hz, H4), 8.10 (dd, 1H, J = 8.3, 1.0 Hz, H7), 7.83 (ddd, 1H, J = 8.3, 7.8, 1.0 Hz, H2), 7.78-7.67 (m, 3H), 7.52 (ddd, 1H, J = 8.0, 7.5, 0.8 Hz, H9), 4.62 (q, J = 7.5 Hz, 2H), 2.76 (s, 3H), 1.40 (t, J = 7.5 Hz, 3H). ^{13}C NMR ($CDCl_3$, 62.5 MHz) δ 168.42, 161.31, 148.34, 147.83, 145.68, 143.90, 143.37, 134.57, 130.97, 130.29, 128.89, 128.16, 128.04, 127.77, 126.79, 126.47, 124.22, 122.61, 61.84, 15.55, 14.18. ESI MS $[M+H]^+$ 360. Mass spectrum m/z (rel. intensity) 360.1 (M+1, 100), 314.3 (30), 286.2 (30). The latter fractions (R_f = 0.2) afforded 10 mg (14%) of **7a** as a yellow powder: mp 298 °C. ^{1}H NMR ($CDCl_3$, 250 MHz) δ 8.44 (d, 1H, J = 8.3 Hz, H1), 8.38 (d, 1H, J = 8.3 Hz, H4), 8.07 (d, 1H, J = 8.3 Hz, H7), 8.06 (d, 1H, J = 8.3 Hz, H10), 7.82 (td, 1H, J = 8.3, 1.0 Hz, H2), 7.79 (td, 1H, J = 8.3, 1.0 Hz, H3), 7.66 (td, 1H, J = 8.0, 0.8 Hz, H8), 7.54 (t, 1H, J = 8.0 Hz, H9), 5.30 (s, 2H), 2.84 (s, 3H). ^{13}C NMR ($CDCl_3$, 62.5 MHz): δ 160.56, 153.02, 150.12, 149.18, 140.52, 134.60, 131.26, 130.31, 128.70, 128.58, 128.28, 127.42, 126.43, 123.50, 121.22, 47.04 and 15.13. Mass spectrum, m/z (rel. intensity) 300.4 (M+1, 35), 285 (100), 272 (80). MS (ESI) Cacld. for $C_{19}H_{14}N_3O$ $[M+H]^+$ 300, found 300. *Anal.* Calcd for $C_{19}H_{13}N_3O{\cdot}H_2O$: C, 72.367; H, 4.795; N, 12.691. Found: C, 70.37; H, 4.80; N, 12.69.

8,9-Difluoro-11-methylluotonin A (7b): Pale yellow needles (55%): mp: 320 °C. ^{1}H NMR ($CDCl_3$, 250 MHz) δ 8.43 (d, 1H, J = 7.8 Hz, H1), 8.19 (dd, 1H, $J_{H\text{-}F}$ = 10.0 Hz, J_{ortho} = 8.0 Hz, H7), 8.10 (d, 1H, J = 8.0 Hz, H4), 7.88-7.80 (m, 2H), 7.58 (td, 1H, J = 7.8, 1.3 Hz, H2), 5.29 (s, 2H), 2.79 (s, 3H). MS (ESI) Cacld for $C_{19}H_{12}F_2N_3O$ $[M+H]^-$ 336. Found 336. *Anal.* Calcd for $C_{19}H_{11}F_2N_3O$: C, 68.06; H, 3.31; N, 11.33. Found: C, 67.97; H, 3.34; N, 11.29.

9-Bromo-11-methylluotonin A (7c): Pale yellow needles (37%): mp: 268 °C. ^{1}H NMR ($CDCl_3$, 250 MHz) δ 8.41 (d, 1H, J = 8.2 Hz, H1), 8.30 (d, 1H, J = 9.1 Hz, H4), 8.25, (d, 1H, J = 1.96 Hz, H10), 8.08 (d, 1H, J = 8.6 Hz, H7) 7.89-7.80 (m, 2H, H3 and H8), 7.57 (t, 1H, J = 7.3 Hz, H9), 5.29 (s, 2H), 2.79 (s, 3H). ^{13}C NMR ($CDCl_3$, 62.5 MHz): δ MS (ESI) Cacld for $C_{19}H_{13}Br_2N_3O$ $[M+H]^+$ 378. Found 378. *Anal.* Calcd for $C_{19}H_{12}BrN_3O$: C, 60.34; H, 3.20; N, 11.11. Found: C, 60.37; H, 3.24; N, 11.07.

DNA relaxation assay of Topo I. The test compounds

were dissolved in DMSO at 10 mM as stock solution. The activity of DNA Topo I was determined by assessing the relaxation of supercoiled DNA pBR322. The mixture of 100 ng of plasmid pBR322 DNA and 0.2 units of calf thymus DNA Topo I (Fermentas, USA) was incubated without and with the prepared compounds at 37 °C for 30 minutes in the relaxation buffer (35 mM Tris-HCl (pH 8.0), 72 mM KCl, 5 mM $MgCl_2$, 5 mM dithiothreitol, 2 mM spermidine, 0.01% bovine serum albumin). The reaction in the final volume of 10 μL was terminated by adding 2.5 μL of the stop solution containing 10% SDS, 0.2% bromophenol blue, 0.2% xylene cyanol and 30% glycerol. DNA samples were then electrophoresed on a 1% agarose gel at 15 V for 7 h with a running buffer of TAE. Gels were stained for 30 min in an aqueous solution of ethidium bromide (0.5 μg/mL). DNA bands were visualized by transillumination with UV light and were quantitated using AlphaImager™ (Alpha Innotech Corporation).

DNA relaxation assay of Topo II. The mixture of 100 ng of supercoiled pBR322 plasmid DNA and 0.2 units of human DNA Topo IIα (Amersham, USA) was incubated without and with the prepared compounds in the assay buffer (10 mM Tris-HCl (pH 7.9) containing 50 mM NaCl, 5 mM $MgCl_2$, 1 mM EDTA, 1 mM ATP, and 15 μg/mL bovine serum albumin) for 30 min at 37 °C. The reaction in a final volume of 10 μL was terminated by the addition of 3 μL of 7 mM EDTA. Reaction products are analyzed on a 1% agarose gel at 25 V for 4 h with a running buffer of TAE. Gels were stained for 30 min in an aqueous solution of ethidium bromide (0.5 μg/mL). DNA bands were visualized by transillumination with UV light and supercoiled DNA was quantitated using AlphaImager™ (Alpha Innotech Corporation).

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