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CONCISE APPROACH TO MONO- AND DISUBSTITUTED LUOTONIN A ANALOGS AND THEIR CYTOTOXICITY TEST

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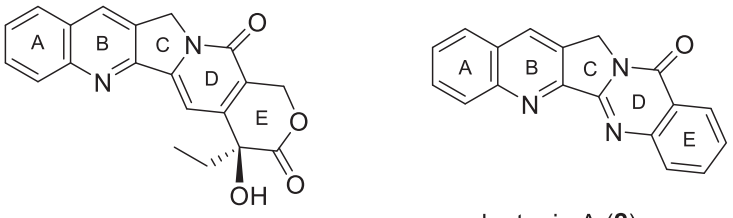
Abstract – A concise approach for the preparation of luotonin A analogs has been developed. The new synthetic route contains an anion-assisted intramolecular double hetero Diels−Alder reaction and a direct oxidative cross coupling reaction. Some synthetic luotonin A analogs show cytotoxic activities against Daudi and Jurkat human cancer cells as potent as camptothecin.

Camptothecin (**1**) is a naturally occurring cytotoxic alkaloid which was extracted from the Asian tree *Camptotheca acuminata* in 1966, and the US National Cancer Institute screening programme identified camptothecin (**1**) as a drug with potential antitumor activity.[1] In 1985, topoisomerase I was found to be the target of camptothecin (**1**).[2] The lactone moiety (E ring part) of **1** is crucial for its antitumor activity,



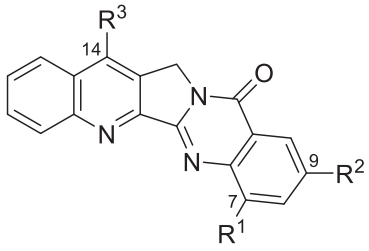
camptothecin (**1**) luotonin A (**2**)

Figure 1

but at blood pH it is in equilibrium with the corresponding less active ring-opened structure.[3] It is considered that such a structural conversion brings serious side effects for patients. Luotonin A (**2**), whose structure is similar to **1**, was isolated from *Peganum nigellastrum* Bunde in 1997, and shows potent

cytotoxic activity against mouse leukemia P-388 cells.[4] Although the E ring unit of **1** is replaced by benzene ring, luotonin A (**2**) acts as a poison to topoisomerase I in a similar mechanism as camptothecin (**1**).[5] Compared to camptothecin (**1**), luotonin A (**2**) has no an acid and/or base sensitive lactone and a tertiary alcohol moieties in the E ring system, so the access to **2** seems to become easier than that of **1** (Figure 1). Such an intriguing biological activitity and its unique structure of **2** have led to interest in structural modifications for improving the biological properties.[6] We have been involved in the development of novel approach to luotonin A (**2**) using an intramolecular anion-assisted double hetero Diels−Alder reaction.[7] Herein, we describe the synthesis of luotonin A derivatives and their cytotoxic activities.

Since little detailed studies on the biological activities on C-14 functionalized luotonin A derivativies were reported,[8] we became interested in the development of novel approach to them. Additionally, a couple of disubstituted luotonin A derivatives were selected as target molecules for their biological tests as shown in Figure 2.



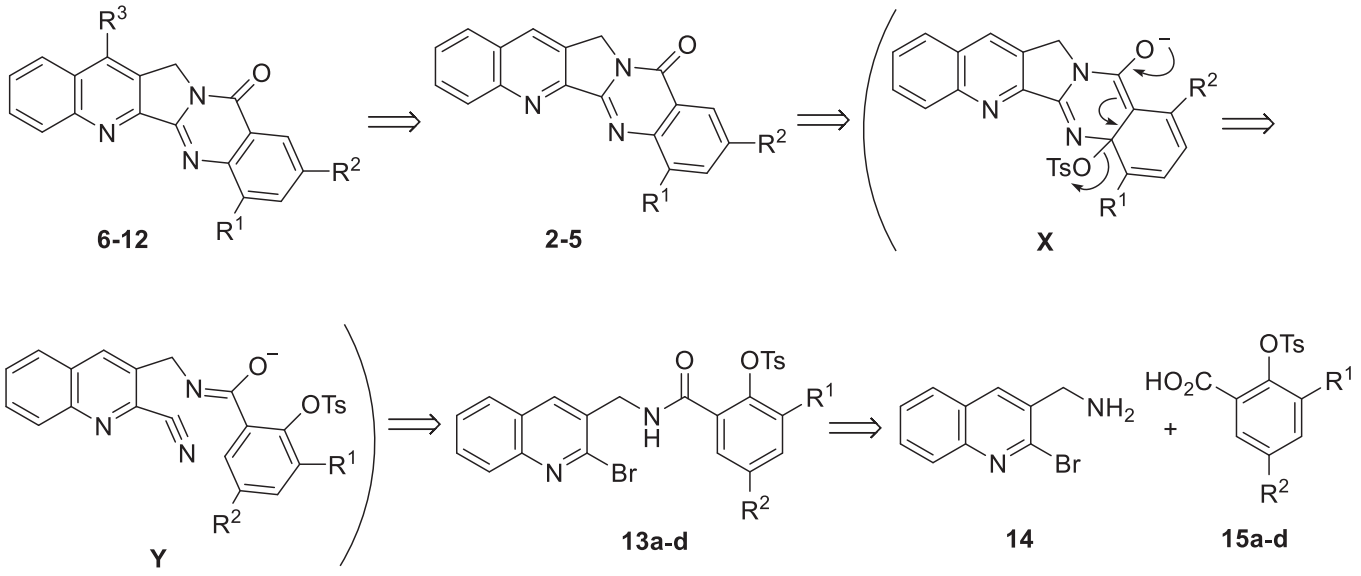
3-12

	R^1	R^2	R^3
3	H	Me	H
4	H	OMe	H
5	OMe	H	H
6	H	H	Cyp^1
7	H	H	Cyo^2
8	H	OMe	Cyh^3
9	OMe	H	Cyh^3
10	OMe	H	Cyo^2
11	OMe	H	Cyp^1
12	H	H	Cyh^3

1) cyclopentyl; 2) cyclooctyl
3) cyclohexyl

Figure 2

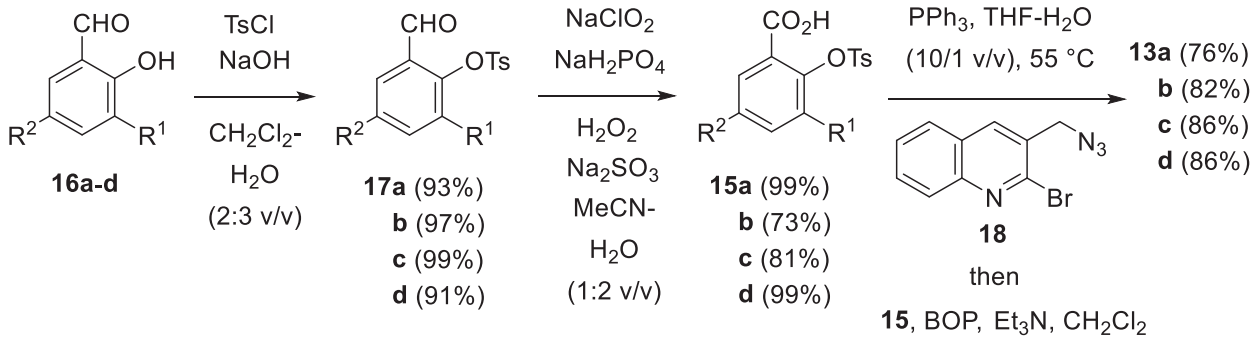
First of all, we mapped out a synthetic plan to assemble luotonin A analogs. The luotonin A analogs **3-12**, bearing a cycloalkane unit at the C-14 position, would be synthesized by means of an oxidative coupling reaction of pentacyclic compounds **2** and **4-5**. An intramolecular anion-assisted double hetero Diels−Alder reaction of amides **13a-d** would produce the pentacyclic compounds **2-5** through the imidate anion intermediates **X** and **Y**. The amides **13a-d** would be prepared by condensation of amine **14** with carboxylic acids **15a-d** as shown in Scheme 1.



a: R^1=R^2=H; **b**: R^1=H, R^2=Me; **c**: R^1=H, R^2=OMe; **d**: R^1=OMe, R^2=H.

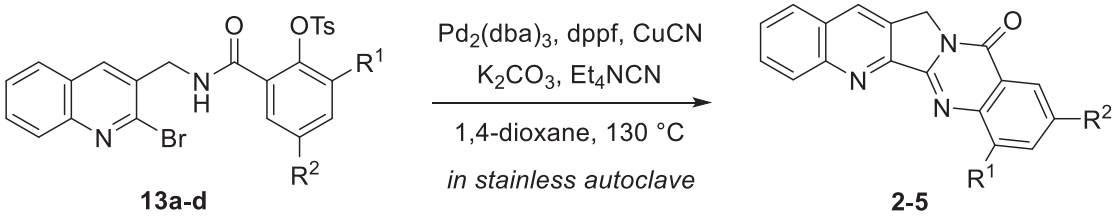
Scheme 1

Commercially available aldehydes **16a-d** were transformed into the tosylates **17a-d** in good yields, which were oxidized with sodium chlorite to give the corresponding carboxylic acids **15a-d**.[9] Condensation of the carboxylic acids **15a-d** with (2-bromoquinolin-3-yl)methanamine (**14**) derived from the azide **18** in the presence of (benzotriazol-1-yloxy)tris(dimethylamino)phosphonium hexafluorophosphate (BOP) reagent provided the amides **13a-d** in reasonable yields (Scheme 2).[10]



Scheme 2

With the requisite substrates **13a-d** in hand, an intramolecular anion-assisted double hetero Diels−Alder reaction was performed using **13a**. The reaction was conducted at 130 °C for 12 hours in 1,4-dioxane with $Pd_2(dba)_3$ (7 mol%), 1,1'-bis(diphenylphosphino)ferrocene (DPPF) (21 mol%), CuCN (4 equiv), K_2CO_3 (1 equiv) and Et_4NCN (2 equiv) in a stainless autoclave to afford luotonin A (**2**) in 81% yield. Similarly, the pentacyclic compounds **3-5** were obtained in good yields. It is obvious that methyl or methoxy group on the benzene ring of **13** did not affect the reaction yields (Table 1).

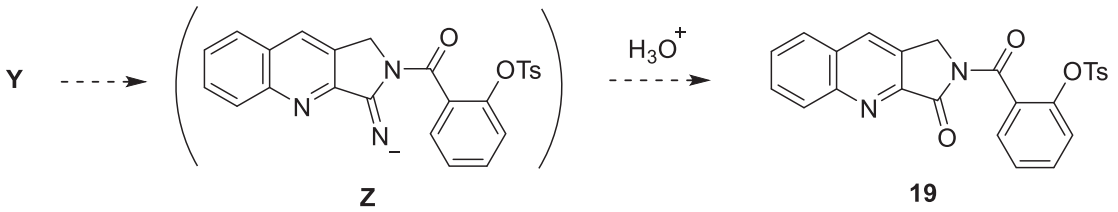


13a-d **2-5**

Table 1. Anion-assisted double hetero Diels–Alder reaction of **13a-d**

entry	substrate	product	yield (%)
1	**13a**	**2**	81
2	**13b**	**3**	68
3	**13c**	**4**	72
4	**13d**	**5**	82

In order to clarify the reaction mechanism, the reaction was quenched when the staring material **Y** was still left. Attempts to isolate the expected compound **19**, which might be hydrolysis product of mono Michael addition intermediate **Z**, were unsuccessful (Scheme 3). Although an intramolecular double Michael reaction mechanism cannot be ruled out, we believe that the formation of pentacyclic compounds **2-5** proceeds via an intramolecular anion-assisted double hetero Diels−Alder reaction.



Z **19**

Scheme 3

We next focused on functionalization at C-14 position in the pentacyclic compounds **2** and **4-5**. Since it seems to be mild reaction conditions, we decided to apply Antonchick's oxidative cross-coupling reaction protocol (Table 2).[11] Although alkylation of luotonin A (**2)** or the pentacyclic compounds **4-5** with *n*-hexane or 1,4-dioxane met with failure, introduction of cyclopentane ring at the C-14 position of **2** furnished the compound **6** in 40% yield (entry 1). A cyclooctane ring was next installed at the C-14 position of **2** in 58% yield (entry 2). To evaluate the biological activity of dually substituted luotonin A analogs, alkylation at the C-14 position of **4-5** using *n*-hexane, 1,4-dioxane, and cycloalkanes was investigated. As a result, only a cyclohexane ring was introduced in **4** in 43% yield (entry 3). On the other hand, a direct oxidative cross coupling reaction of **5** with cyclohexane, cyclooctane, and cyclopentane gave rise to **9** (61%), **10** (79%), and **11** (20%), respectively (entries 4-6). A cyclohexane ring was introduced in **2** in 58% yield (entry 7). The yields of the above oxidative cross-coupling reaction were

turned out to be slightly less than fantastic, and considerable quantities of the starting materials remained in most cases.

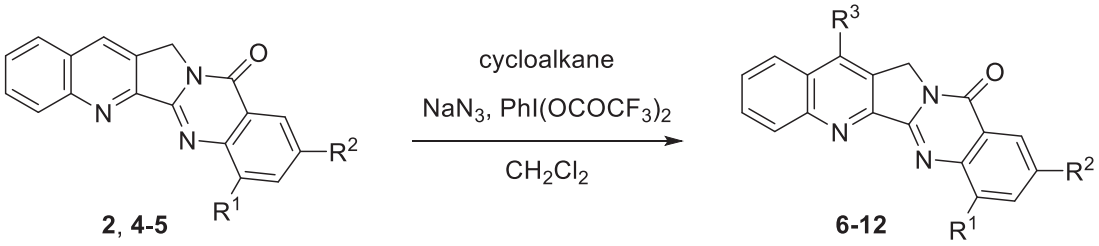
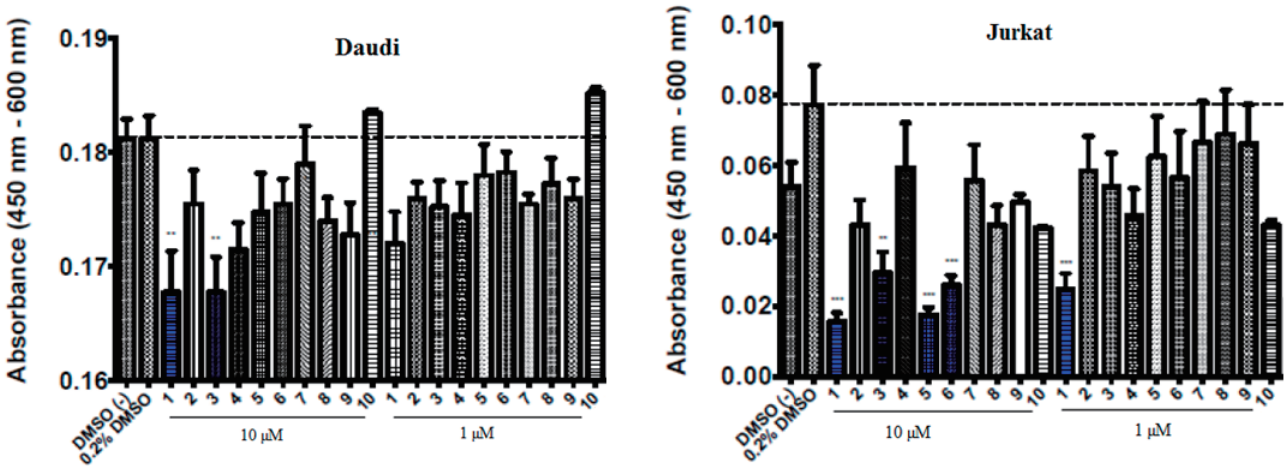


Table 2. Functionalization at the C-14 position in **2**, **4**, and **5**

entry	substrate	cycloalkane	product	yield (%)
1	**2**	cyclopentane	**6**	40
2	**2**	cyclooctane	**7**	58
3	**4**	cyclohexane	**8**	43
4	**5**	cyclohexane	**9**	61
5	**5**	cyclooctane	**10**	79
6	**5**	cyclopentane	**11**	20
7	**2**	cyclohexane	**12**	58

Although the mode of action of luotonin A (**2**) is known as a DNA topoisomerase I inhibitor, it is not revealed which human cancer cells luotonin A analogs are effective for. Therefore, we examined cytotoxicity tests of camptothecin (**1**), luotonin A (**2**), and synthetic compounds **3**-**10** using BT20, LS-174T, HCT116, Daudi, Jurkat, and OSC19 human cancer cells. As a result, camptothecin (**1**) showed strong cytotoxicity for those cancer cells except Daudi and Jurkat cancer cells (*see* Supplementary Material). Compared with camptothecin (**1**), the synthetic compounds **3**-**10** are less effective, however, it should be noted that the compound **3** showed cytotoxic activity against Daudi cancer cell as potent as **1**, and the compound **5** was effective against Jurkat cancer cell as much as **1**.

In summary, the novel synthesis of mono- and disubstituted luotonin A analogs by a combination of an intramolecular anion-assisted double hetero Diels−Alder reaction and a oxidative cross-coupling reaction has been accomplished. As a result of their cytotoxic tests using several human cancers cell, it was turned out that a couple of mono-substituted luotonin A analogs showed cytotoxic activity as potent as camptothecin (**1**). Further work related to structure-activity relationships in this series of compounds is in progress and will be reported at a later time.



EXPERIMENTAL

All reactions were run in oven-dried glassware under an argon atmosphere. All reactions were monitored by thin-layer chromatography (TLC) on Merck silica gel 60 F_{254} plates, visualized by UV florescence quenching (254 nm), *p*-anisaldehyde (in EtOH), phosphomolybdic acid (in EtOH), ammonium molybdate (in 10% H_2SO_4), potassium permanganate (in water containing NaOH and K_2CO_3), or Hanessian's staining solution. Ambient temperature refers to 18–26 °C. Flash column chromatography (EtOAc/Hexanes or EtOAc/CH_2Cl_2 or EtOAc/$CHCl_3$) was performed on Cica 60 (spherical/ 63–210 μm) silica gel. NMR spectra were measured on Varian 400 MR or JEOL AL–400 spectrometers at 400 MHz for ^{1}H NMR spectra and 100 MHz for ^{13}C NMR spectra, or a JEOL JX–500 spectrometer at 500 MHz for ^{1}H NMR spectra, or JEOL JNM–ECA600 or JNM–ECZ600 spectrometers at 600 MHz for ^{1}H NMR spectra and 150 MHz for ^{13}C NMR spectra. ^{1}H NMR spectra were calibrated from internal standard TMS (δ 0.0) or solvent resonance ($CHCl_3$: 7.26, $(CD_3)_2SO$: 2.49). ^{13}C NMR spectra were calibrated from solvent resonance ($CHCl_3$: 77.0, $(CD_3)_2SO$: 39.5). NMR data are reported as: chemical shift (parts per million, ppm), multiplicity (s = singlet, d = doublet, t = triplet, q = quartet, m = multiplet, br = broad signal), coupling constant (Hz), and integration. Infrared spectra were recorded on a SHIMADZU FT–IR 8300 spectrophotometer and reported in frequency of absorption (cm^{-1}). High-resolution mass spectra using fast atom bombardment (FAB) was reported on a JEOL MStaion JMS-700.

Materials

Anhydrous THF and methylene chloride (CH_2Cl_2) were purchased from Kanto Chemical Co., Inc. Dioxane was distilled from CaH_2 prior to use. DMF and DMSO were distilled from CaH_2 under reduced pressure. $POCl_3$ and PBr_3 were distilled and used immediately. TsCl was recrystallized from $CHCl_3$ prior to use. Unless otherwise mentioned, commercially obtained reagents were used as received.

9-Methylquinolino[2',3':3,4]pyrrolo[2,1-*b*]quinazolin-11(13*H*)-one (**3**): A mixture of amide **13b** (42.3 mg, 81.0 μmol), CuCN (31.6 mg, 0.350 mmol), $Pd_2(dba)_3$ (4.70 mg, 5.00 μmol), DPPF (9.70 mg, 17.0

μmol), K_2CO_3 (15.3 mg, 0.110 mmol), and Et_4NCN (41.8 mg, 0.270 mmol) in 1,4-dioxane (2.00 mL) was heated at 130 ºC for 14 h. The resulting mixture was diluted with CH_2Cl_2, and the precipitates were filtered off through Celite. The filtrate was washed with saturated aqueous $NaHCO_3$ solution, saturated aqueous NaCl solution, dried over $MgSO_4$, and evaporated to provide a crude product. The crude was purified by flash column chromatography (SiO_2, 75% EtOAc/Hexanes) to afford 9-methylluotonin A (**3**) (16.4 mg, 68%) as a yellow solid; mp 250–254 ºC; IR (NaCl): 3374, 2921, 1674, 1481, 1198, 1027, 832, 747 cm^{-1}; ^1H NMR (600 MHz, $CDCl_3$): δ 2.56 (s, 3H), 5.35 (s, 2H), 7.68 (d, *J* = 7.2 Hz, 1H), 7.70 (d, *J* = 7.8 Hz, 1H), 7.85 (t, *J* = 7.8 Hz, 1H), 7.96 (d, *J* = 7.8 Hz, 1H), 8.02 (d, *J* = 7.8 Hz, 1H), 8.23 (s, 1H), 8.45 (s, 1H), 8.48 (d, *J* = 8.4 Hz, 1H); ^{13}C NMR (125 MHz, $CDCl_3$): δ 160.7, 151.8, 151.3, 149.4, 147.3, 137.9, 136.1, 131.5, 130.7, 130.6, 130.0, 129.4, 128.6, 128.4, 127.9, 125.9, 121.0, 47.3, 21.5; HRMS (FAB) calcd for $C_{19}H_{14}ON_3$ $[M+H]^+$: 300.1137. Found: 300.1155.

9-Methoxyquinolino[2',3':3,4]pyrrolo[2,1-*b*]quinazolin-11(13*H*)-one (**4**): A mixture of amide **13c** (163 mg, 0.300 mmol), CuCN (111 mg, 1.24 mmol), $Pd_2(dba)_3$ (14.3 mg, 16.0 μmol), DPPF (29.8 mg, 54.0 μmol), K_2CO_3 (44.8 mg, 0.320 mmol), and Et_4NCN (70.8 mg, 0.450 mmol) in 1,4-dioxane (6.00 mL) was heated at 130 ºC for 14 h. The resulting mixture was diluted with CH_2Cl_2, and the precipitates were filtered off through Celite. The filtrate was washed with saturated aqueous $NaHCO_3$ solution, saturated aqueous NaCl solution, dried over $MgSO_4$, and evaporated to provide a crude product. The crude was purified by flash column chromatography (SiO_2, 67% CH_2Cl_2/EtOAc) to afford 9-methoxyluotonin A (**4**) (68.0 mg, 72%) as a yellow solid; mp 225–230 ºC; IR (NaCl): 3419, 2918, 1673, 1629, 1435, 1359, 1168, 832, 761 cm^{-1}; ^1H NMR (600 MHz, $CDCl_3$): δ 3.98 (s, 3H), 5.34 (s, 2H), 7.45 (dd, *J* = 3.0, 9.0 Hz, 1H), 7.67 (dt, *J* = 1.2, 6.6 Hz, 1H), 7.78 (d, *J* = 3.0 Hz, 1H), 7.84 (ddd, *J* = 1.8, 6.0, 8.4 Hz, 1H), 7.94 (d, *J* = 7.8 Hz, 1H), 8.04 (d, *J* = 9.0 Hz, 1H), 8.43 (s, 1H), 8.45 (d, *J* = 8.4 Hz, 1H); ^{13}C NMR (125 MHz, $CDCl_3$): δ 160.5, 159.1, 151.3, 150.6, 149.4, 143.9, 131.4, 130.6, 130.6, 130.3, 129.2, 128.6, 128.3, 127.9, 124.8, 122.3, 106.0, 55.9, 47.3; HRMS (FAB) calcd for $C_{19}H_{14}O_2N_3$ $[M+H]^+$: 316.1086. Found: 316.1087.

7-Methoxyquinolino[2',3':3,4]pyrrolo[2,1-*b*]quinazolin-11(13*H*)-one (5): A mixture of amide **13d** (39.6 mg, 73.0 mmol), CuCN (27.5 mg, 0.310 mmol), $Pd_2(dba)_3$ (5.30 mg, 5.80 μmol), DPPF (8.70 mg, 1.60 μmol), K_2CO_3 (11.0 mg, 80.0 mmol), and Et_4NCN (22.3 mg, 0.140 mmol) in 1,4-dioxane (2.00 mL) was heated at 130 ºC for 14 h. The resulting mixture was diluted with CH_2Cl_2, and the precipitates were filtered off through Celite. The filtrate was washed with saturated aqueous $NaHCO_3$ solution, saturated aqueous NaCl solution, dried over $MgSO_4$, and evaporated to provide a crude product. The crude was purified by flash column chromatography (SiO_2, 67% CH_2Cl_2/EtOAc) to afford 7-methoxyluotonin A (**5**) (18.9 mg, 82%) as a pale yellow solid; mp 289–291 ºC (lit.[12] mp 290–292 ºC); IR (NaCl): 2917, 1669, 1568, 1433, 1122, 617 cm^{-1}; ^1H NMR (600 MHz, $CDCl_3$, 50 ºC): δ 4.09 (s, 3H), 5.34 (s, 2H), 7.29 (d, *J* =

8.4 Hz, 1H), 7.51 (t, *J* = 7.8 Hz, 1H), 7.68 (t, *J* = 7.8 Hz, 1H), 7.83 (t, *J* = 8.4 Hz, 1H), 7.94 (d, *J* = 8.4 Hz, 1H), 8.01 (d, *J* = 8.4 Hz, 1H), 8.41 (s, 1H), 8.45 (d, *J* = 8.4 Hz, 1H); ^{13}C NMR (150 MHz, $CDCl_3$, 50 ºC): δ 160.5, 155.7, 151.7, 151.2, 149.5, 140.1, 131.2, 130.9, 130.4, 129.5, 128.7, 128.3, 127.9, 127.8, 122.5, 117.6, 114.4, 56.1, 47.3; HRMS (FAB) calcd for $C_{19}H_{14}O_2N_3$ $[M+H]^+$: 316.1086. Found: 316.1087.

14-Cyclopentylquinolino[2',3':3,4]pyrrolo[2,1-*b*]quinazolin-11(13*H*)-one (6): To a solution of cyclopentane (0.250 mL, 2.68 mmol) and luotonin A (**2**) (16.1 mg, 56.0 μmol) in CH_2Cl_2 (0.500 mL) were added NaN_3 (60.0 mg, 0.923 mmol) and PIFA (102 mg, 0.238 mmol) at ambient temperature. The reaction mixture was stirred for 2 h, and then NaN_3 (59.1 mg, 0.900 mmol) and PIFA (99.3 mg, 0.231 mmol) were added to the reaction mixture. The resulting mixture was stirred overnight and quenched with saturated aqueous $Na_2S_2O_3$. The mixture was extracted three times with CH_2Cl_2. The combined organic layers were washed with saturated $NaHCO_3$, saturated NaCl, dried over $MgSO_4$, and evaporated to provide a crude product. The crude was purified by flash column chromatography (SiO_2, 83% toluene/acetone) to afford 14-cyclopentylluotonin A (**6**) (7.90 mg, 40%) as a yellow solid; mp >300 ºC; IR (NaCl): 3383, 2919, 1673, 1624, 1466, 1253, 1046, 876, 757 cm^{-1}; ^{1}H NMR (600 MHz, $CDCl_3$): δ 1.89–1.98 (m, 2H), 2.06–2.14 (m, 4H), 2.25–2.32 (m, 2H), 3.89–3.95 (m, 1H), 5.42 (s, 2H), 7.58 (t, *J* = 7.8 Hz, 1H), 7.69 (t, *J* = 7.2 Hz, 1H), 7.82 (t, *J* = 7.5 Hz, 1H), 7.86 (dt, *J* = 1.2, 8.4 Hz, 1H), 8.13 (d, *J* = 7.8 Hz, 1H), 8.27 (d, *J* = 9.0 Hz, 1H), 8.45 (dt, *J* = 1.8, 7.2 Hz, 1H), 8.50 (d, *J* = 8.4 Hz, 1H); ^{13}C NMR (100 MHz, $CDCl_3$): δ 160.7, 150.8, 150.0, 149.5, 148.1, 134.5, 131.9, 129.9, 128.8, 128.0, 127.9, 127.2, 126.4, 124.1, 121.3, 47.6, 41.5, 32.6, 26.5; HRMS (FAB) calcd for $C_{23}H_{20}ON_3$ $[M+H]^+$: 354.1606. Found: 354.1628.

Supplementary Material

Supplementary material associated with this article can be found, in the online version.

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