HETEROCYCLES, Vol. 97, No. 1, 2018, pp. 514 - 522. © 2018 The Japan Institute of Heterocyclic Chemistry
Received, 28th November, 2017, Accepted, 18th December, 2017, Published online, 19th December, 2017
DOI: 10.3987/COM-17-S(T)6

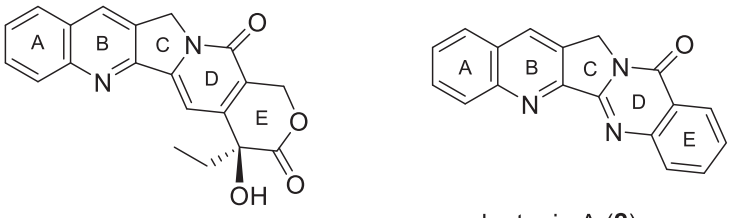
CONCISE APPROACH TO MONO- AND DISUBSTITUTED LUOTONIN A ANALOGS AND THEIR CYTOTOXICITY TEST

Natsuko Kagawa,[a]* Kimiko Nishimura,[b] Shinya Abe,[c] Takashi Masuko,[c] and Masahiro Toyota[b]*

[a]Center for Environment, Health and Field Sciences, Chiba University, 6-2-1 Kashiwa-no-ha, Kashiwa, Chiba 277-0882, Japan. [b]Department of Chemistry, Graduate School of Science, Osaka Prefecture University, Sakai, Osaka 599-8531, Japan. [c]Faculty of Pharmacy, Kindai University, 3-4-1 Kowakae, Higashiosaka City, Osaka 577-8502, Japan. E-mail: toyota@c.s.osakafu-u.ac.jp

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,



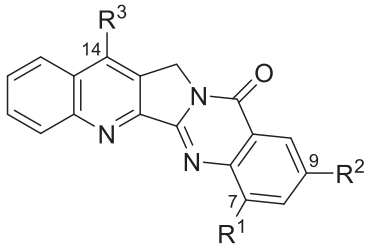
camptothecin (**1**) luotonin A (**2**)

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.



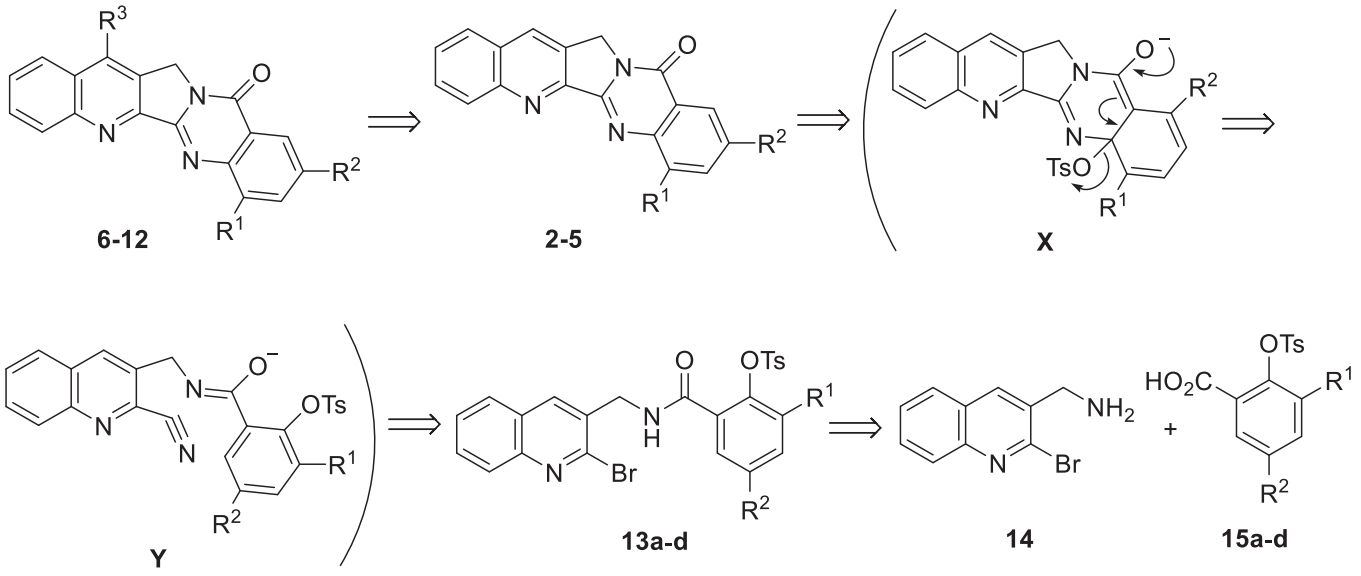
3-12

	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

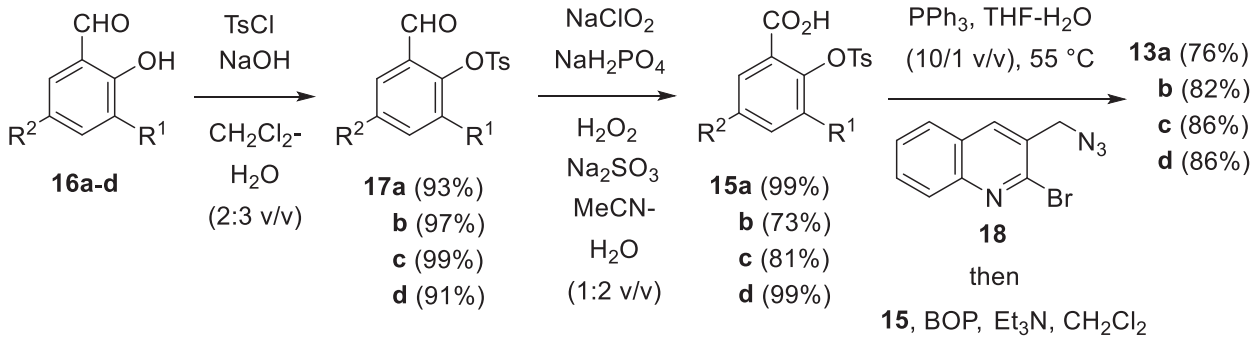
First of all, we mapped out a synthetic plan to assemble luotonin A analogs. The luotonin A analogs **3-12**, bearing a cycloalkane unit at the C-14 position, would be synthesized by means of an oxidative coupling reaction of pentacyclic compounds **2** and **4-5**. An intramolecular anion-assisted double 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).

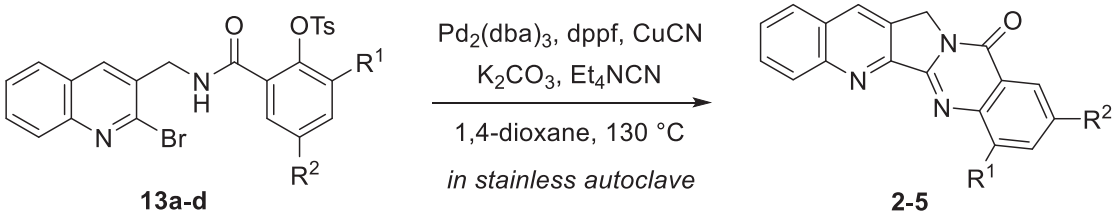
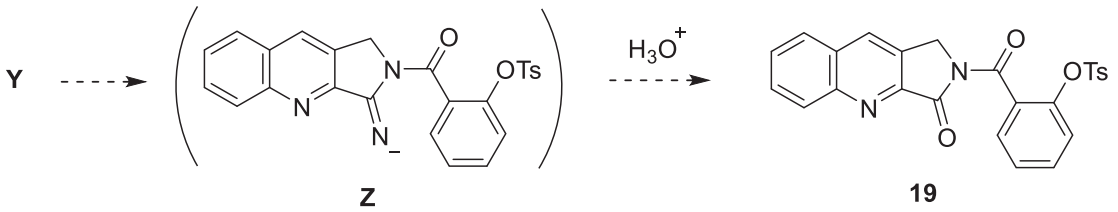


Table 1. Anion-assisted double hetero Diels–Alder reaction of **13a-d**

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.



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.

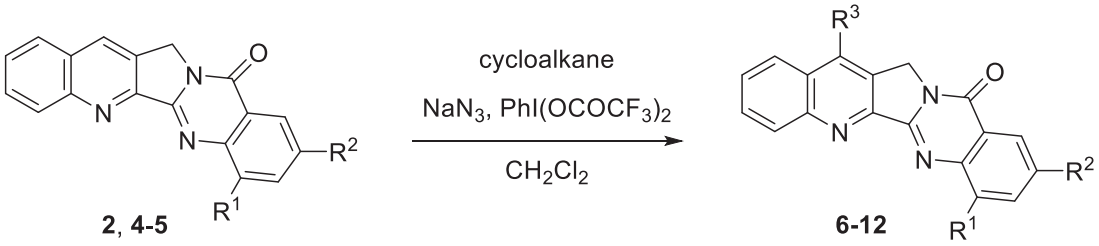
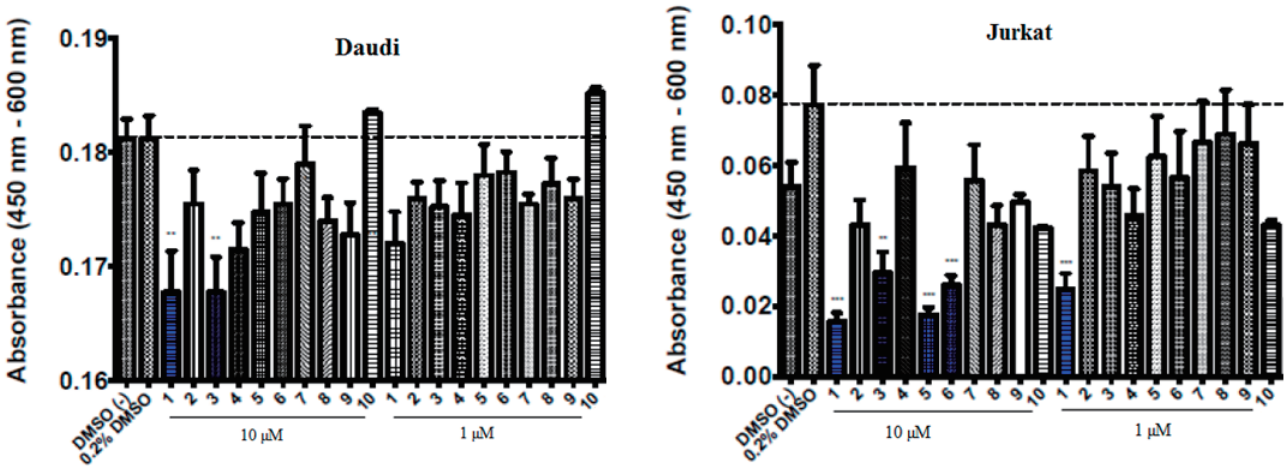


Table 2. Functionalization at the C-14 position in **2**, **4**, and **5**

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 an 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.

ACKNOWLEDGEMENT

Partial support of this work by JSPS KAKENHI (26870101) and the Japan Food Chemical Foundation is gratefully acknowledged.

REFERENCES AND NOTES

1. M. E. Wall, M. C. Wani, C. E. Cook, K. H. Palmer, A. T. McPhail, and G. A. Sim, *J. Am. Chem. Soc.*, 1966, **88**, 3888.
2. G. L. Patrick, In *An Introduction to Medicinal Chemisty*, 4th Edition, Oxford University Press, 2009, 531.

3. A selected short review, see: J. F. Pizzolato and L. B. Saltz, *Lancet*, 2003, **361**, 2235.
4. Z.-Z. Ma, Y. Hano, T. Nomura, and Y.-J. Chen, *Heterocycles*, 1997, **46**, 541.
5. A. Cagir, S. H. Jones, R. Gao, B. M. Eisenhauer, and S. M. Hecht, *J. Am. Chem. Soc*., 2003, **125**, 13628.
6. A selected review, see: J. L. Liang, H. C. Cha, and Y. Jahng, *Molecules*, 2011, **16**, 4861; For recent selected syntheses of luotonin A and luotonin A analogs, see: (a) W. R. Bowman, M. R. J. Elsegood, T. Stein, and G. W. Weaver, *Org. Biomol. Chem*., 2007, **5**, 103; (b) H.-B. Zhou, G.-S. Liu, and Z.-J. Yao, *J. Org. Chem*., 2007, **72**, 6270; (c) K. C. Jahng, S. I. Kim, D. H. Kim, C. S. Seo, J. K. Son, S. H. Lee, E. S. Lee, and Y. Jahng, *Chem. Pharm. Bull*., 2008, **56**, 607; (d) V. Sridhara, P. Ribelle, M. T. Ramos, and J. C. Menéndez, *J. Org. Chem*., 2009, **74**, 5715; (e) Y. Ju, F. Liu, and C. Li, *Org. Lett*., 2009, **11**, 3582; (f) T. M. Potewar, M. K. Kathiravan, A. S. Chothe, and K. V. Srinivasan. *Eur. J. Chem*., 2011, **2**, 235; (g) M.-C.Tseng, Y.-W. Chu, H.-P. Tsai, C.-M. Lin, J. Hwang, and Y.-H. Chu, *Org. Lett*., 2011, **13**, 920; (h) T. Boisse, L. Gavara, P. Gautret, B. Baldeyrou, A. Lansiaux, J.-F. Goossens, L.-P. Henichart, and B. Rigo, *Tetrahedron Lett*., 2011, **52**, 1592; (i) K. Natsuki, T. Shindo, and M. Toyota, *Heterocycles*, 2012, **84**, 1301; (j) N. Haider, G. Meng, S. Roger, and S. Wank, *Tetrahedron*, 2013, **69**, 7066; (k) R. Mekala, R. Kamaraju, S. Regati, N. Gudimalla, C. K. Bannoath, and J. Sarva, *Tetrahedron Lett*., 2016, **57**, 1418; (l) M. Atia, D. Bogdán, M. Brügger, N. Haider, and P. Matyus, *Tetrahedron*, 2017, **73**, 3231.
7. (a) M. Toyota, C. Komori, and M. Ihara, *Heterocycles*, 2002, **56**, 101; (b) M. Toyota, C. Komori, and M. Ihara, *ARKIVOC*, 2003, **viii**, 15.
8. J. J. Mason and J. Bergman, *Org. Biomol. Chem*., 2007, **5**, 2486.
9. B. O. Lindgren and T. Nilsson, *Acta Chim. Scand*., 1973, **27**, 888.
10. B. Castro, J. R. Dormoy, G. Evin, and C. Selve, *Tetrahedron Lett*., 1975, **16**, 1219.
11. A. P. Antonchick and L. Burgmann, *Angew. Chem. Int. Ed*., 2013, **52**, 3267.
12. K. Nacro, C. C. Zha, P. R. Guzzo, R. J. Herr, D. Peace, and T. D. Friedrich, *Bioorg. Med. Chem*., 2007, **15**, 4237.