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Total Synthesis of Schizocommunin and Revision of Its Structure

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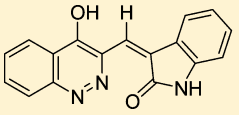
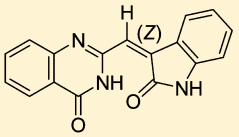
Supporting Information

ABSTRACT: A proposed structure for schizocommunin (*Z*)-**1**$_{hydroxy}$ and its geometric isomer (*E*)-**1**$_{hydroxy}$, which exist in a keto form, has been synthesized. However, the spectroscopic data of (*Z*)-**1**$_{keto}$ and (*E*)-**1**$_{keto}$ were not consistent with those reported for natural schizocommunin. After reinvestigating the spectral data for natural schizocommunin, we synthesized the quinazolinone derivative (*Z*)-**2** as a revised structure for schizocommunin. All of the spectral data of (*Z*)-**2** were completely identical to those reported for natural schizocommunin. (*Z*)-**2** showed moderate antiproliferative activity.



Proposed Structure



Revised Structure (schizocommunin)

Schizocommunin was first reported in 1999 from the liquid culture medium of *Schizophyllum commune*, strain IFM 46788 (monokaryon), which was isolated from mucous plugs obstructing the left upper lobe bronchus of a patient with human allergenic bronchopulmonary mycosis.[1] Schizocommunin showed strong cytotoxic activity against murine lymphoma cells, and this cytotoxicity might be attributable to one of the pathogenic factors in human allergenic bronchopulmonary mycosis induced by *S. commune*.[1] Further biological studies on schizocommunin were prevented by the very limited supply of schizocommunin available from natural sources, and there have been no reports of the total synthesis of schizocommunin.[2] Although a spectroscopic analysis revealed that the structure of schizocommunin included both 4-hydroxycinnnoline and oxindole skeletons that were connected by exomethylene, as shown in (*Z*)-**1**$_{hydroxy}$ in Figure 1, the configuration of the C(3)−C(9′) olefin was not discussed in detail.[1] It is possible that **1** has either geometric structures (*Z*)- and (*E*)-**1**$_{hydroxy}$ or tautomeric structures (*Z*)- and (*E*)-**1**$_{keto}$ (Figure 1). However, on the basis of our evidence presented here, natural schizocommunin does not have the cinnoline structure **1**. We report here a total synthesis of both the putative structure of schizocommunin **1** and a revised structure of natural schizocommunin ((*Z*)-**2**) (Figure 2).

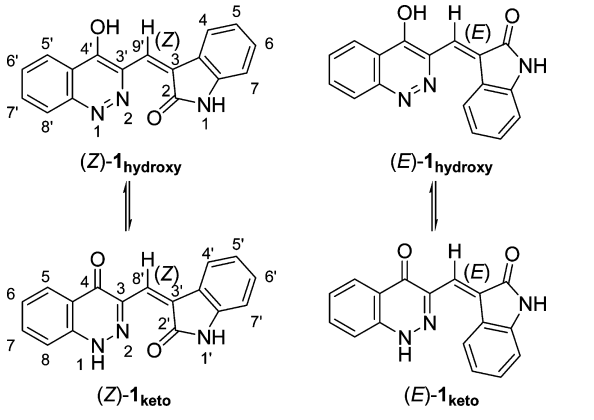


Figure 1. Putative structures of schizocommunin (*Z*)-**1**$_{hydroxy}$ and its isomers.

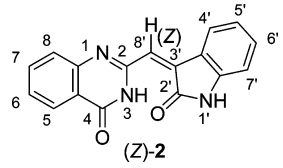
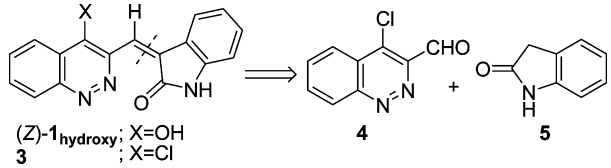


Figure 2. Revised structure (*Z*)-**2** for natural schizocommunin.

RESULTS AND DISCUSSION

A retrosynthetic analysis of (*Z*)-**1**$_{hydroxy}$ (X = OH) is shown in Scheme 1. Since interconversion between a 4-chloro- and 4-hydroxycinnoline has been reported,[3,4] we planned to prepare

Scheme 1. Retrosynthetic Analysis of Putative Schizocommunin ((*Z*)-1$_{hydroxy}$)



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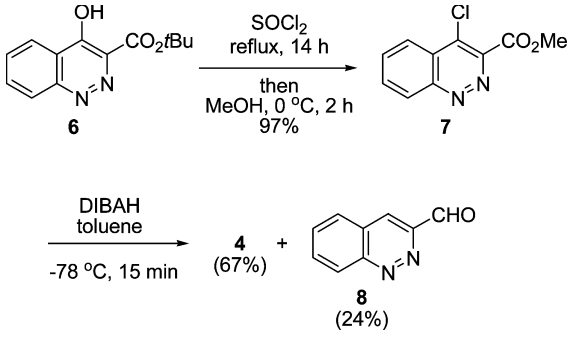
(*Z*)-$\mathbf{1}_{\mathbf{hydroxy}}$ from chloride **3**, which would be synthesized by the aldol condensation of **4** and oxindole **5**.

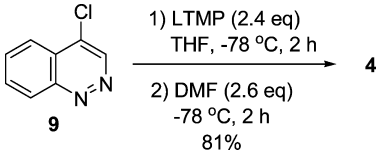
Known ester **6**[5] was treated with $SOCl_2$ and then MeOH to give 4-chlorocinnoline ester **7** in 97% yield (Scheme 2). Reduction of **7** with DIBAH at −78 °C provided 4-chlorocinnoline-3-carboxaldehyde **4** in 67% yield along with cinnoline-3-carboxaldehyde **8** in 24% yield.

Scheme 2. Synthesis of 4-Chlorocinnoline-3-carboxaldehyde (4)



Alternatively, **4** was also obtained in a single step by the ortho-directed formylation of known 4-chlorocinnoline **9**.[4] Treatment of **9** with 2.4 equiv of lithium tetramethylpiperidide in THF at −78 °C followed by formylation with *N*,*N*′-dimethylformamide gave desired **4** in 81% yield (Scheme 3).

Scheme 3. Synthesis of 4 via the Ortho-Directed Formylation of 9

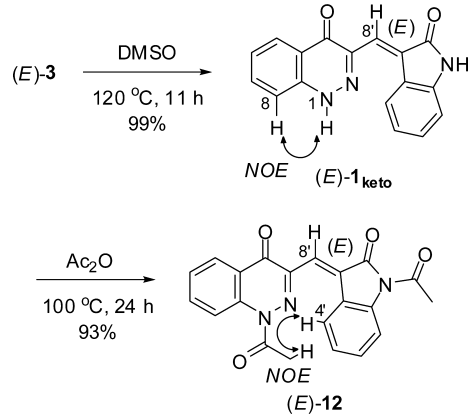


The reaction of **4** with **5** in the presence of 2 equiv of triethylamine provided (*E*)-3-((4′-chlorocinnolin-3′-yl)-methylene)indolin-2-one ((*E*)-**3**) in 96% yield as a single diastereomer (Scheme 4).

Since attempts to prepare a single crystal of (*E*)-**3** to determine its configuration have not yet been successful, (*E*)-**3** was converted to (*E*)-**10** by *N*-acetylation with acetic anhydride (Scheme 4). The configuration of (*E*)-**10** was confirmed to be *E* by X-ray crystallographic analysis.[6,7] Deacetylation of (*E*)-**10** gave (*E*)-**3** quantitatively. In addition, treatment of (*E*)-**3** with NaOMe afforded (*E*)-**11** as a single diastereomer (Scheme 4). X-ray crystallographic analysis also confirmed that the configuration of (*E*)-**11** was *E*.[6,7]

Next, (*E*)-**3** was quantitatively converted to (*E*)-$\mathbf{1}_{\mathbf{keto}}$ as a single diastereomer under heating conditions in DMSO (reagent-grade; H_2O content 0.051%),[8] as shown in Scheme 5. The observation of a NOESY correlation between 8-H (7.73 ppm) in the aromatic ring and 1-NH (14.23 ppm) suggested that the tautomeric structure (*E*)-$\mathbf{1}_{\mathbf{keto}}$ exists in DMSO-d_6, as shown in Scheme 5.[4]

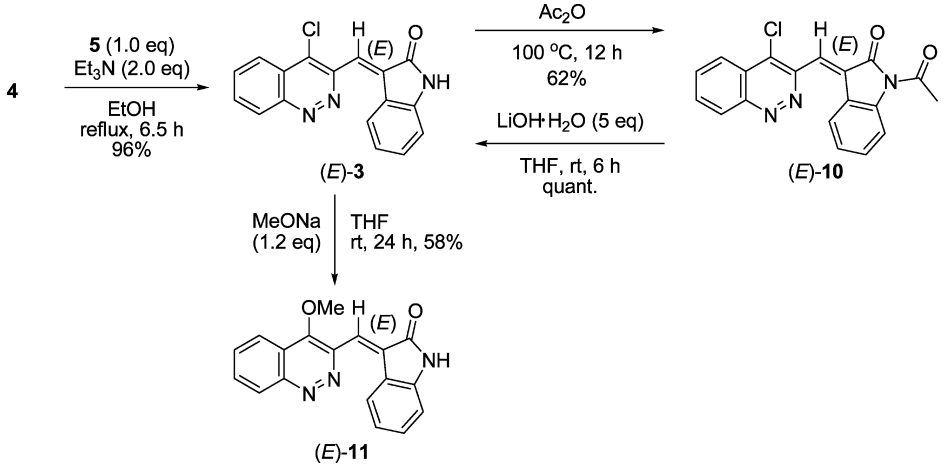
Scheme 5. Synthesis of (*E*)-$\mathbf{1}_{\mathbf{keto}}$ and (*E*)-12



N,*N*-Diacetylation of (*E*)-$\mathbf{1}_{\mathbf{keto}}$ with acetic anhydride gave (*E*)-**12** as a single diastereomer (Scheme 5). The observation of NOE enhancement between a methyl proton of the acetyl group on cinnolone and 4′-H on the oxindole skeleton of (*E*)-**12**,[7] but not between the vinyl proton (8′-H) and 4′-H of (*E*)-**12**,[9] proved that the configuration of (*E*)-**12** was *E* (Scheme 5). All of these results suggested that the configuration of $\mathbf{1}_{\mathbf{keto}}$ should also be *E*.

A small amount of red crystals that were suitable for X-ray crystallographic analysis were obtained by slow crystallization

Scheme 4. Synthesis of (*E*)-3, (*E*)-10, and (*E*)-11



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(for three months) from a saturated acetonitrile solution of (*E*)-$\mathbf{1}_{keto}$ at −10 °C. The configuration of the obtained red crystals was determined to be an unexpected *Z*-form ((*Z*)-$\mathbf{1}_{keto}$) by X-ray crystallographic analysis (Figure 3).[6]

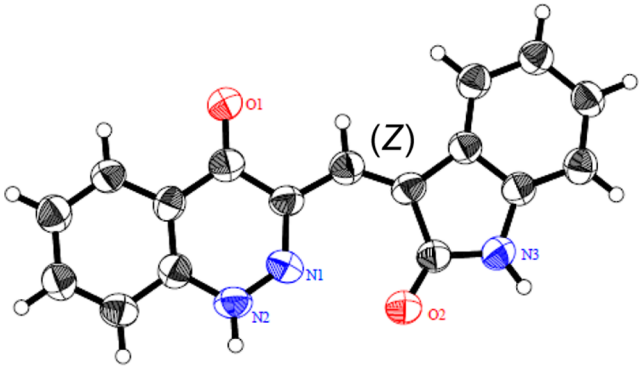


Figure 3. X-ray crystallographic analysis of (*Z*)-$\mathbf{1}_{keto}$.

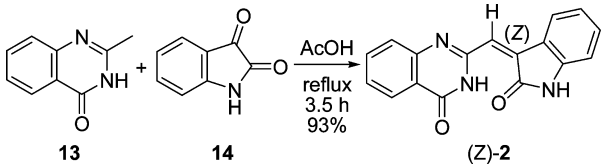
Isolation of pure (*Z*)-$\mathbf{1}_{keto}$ by recrystallization was very difficult, as described above. However, a quantity of (*Z*)-$\mathbf{1}_{keto}$ that was adequate for NMR analysis was obtained as a 1:2 mixture of (*E*)-$\mathbf{1}_{keto}$ and (*Z*)-$\mathbf{1}_{keto}$ by the scale-up recrystallization of (*E*)-$\mathbf{1}_{keto}$. Importantly, we found that the ^{1}H NMR spectra of natural schizocommunin were not identical to those of either (*E*)- or (*Z*)-$\mathbf{1}_{keto}$.[7]

Notably, (*Z*)-$\mathbf{1}_{keto}$ in DMSO-d_6 was transformed into (*E*)-$\mathbf{1}_{keto}$ over 2 weeks at room temperature in an NMR tube.[7] These results suggested that the minor (*Z*)-$\mathbf{1}_{keto}$ was preferentially crystallized in the presence of an equilibrium between (*E*)-$\mathbf{1}_{keto}$ and (*Z*)-$\mathbf{1}_{keto}$ because of the greater crystallizability of (*Z*)-$\mathbf{1}_{keto}$ than (*E*)-$\mathbf{1}_{keto}$.[9−11]

Reinvestigation of the original IR data (1685 and 1675 cm^{-1}) and the HMBC correlation from the aromatic proton at 5-H (8.17 ppm) in the aromatic ring to the amide carbon at 4-C (160.8 ppm) in natural schizocommunin[1] led us to propose a new quinazolinone structure **2** for schizocommunin, as shown in Figure 2. Although **2** is a known compound,[12] spectroscopic data and a synthetic route of **2** have not been reported, and we decided to synthesize this compound.

When commercially available 2-methyl-4(3*H*)-quinazolinone **13** was reacted with isatin **14** in acetic acid,[13] the orange powder (*Z*)-**2** was obtained in 93% yield as a single diastereomer (Scheme 6). The *Z*-configuration of quinazolinone (*Z*)-**2** was established unambiguously, for the first time, by X-ray crystallographic analysis (Figure 4).[6] To our delight, the ^{1}H and ^{13}C NMR spectra of synthetic (*Z*)-**2** were completely identical to those reported for natural schizocommunin.[7]

Scheme 6. Synthesis of Quinazolinone (*Z*)-2



We assumed that the synthetic cinnolines $\mathbf{1}_{keto}$, **3**, **10**, **11**, and **12** afforded *E*-configured compounds due to unfavorable electrostatic repulsion between the lone pair electron of nitrogen in the cinnoline rings and the oxygen atom of the indolin-2-one ring, respectively.[11,14] A minor (*Z*)-$\mathbf{1}_{keto}$ would be isomerized to (*E*)-$\mathbf{1}_{keto}$ in DMSO-d_6 due to unfavorable repulsion. Calculations (B3LYP 6-31G* level with Spartan 06) also suggested that (*E*)-$\mathbf{1}_{keto}$, (*E*)-**3**, (*E*)-**10**, (*E*)-**11**, and (*E*)-**12** are more stable than their respective *Z*-isomers.

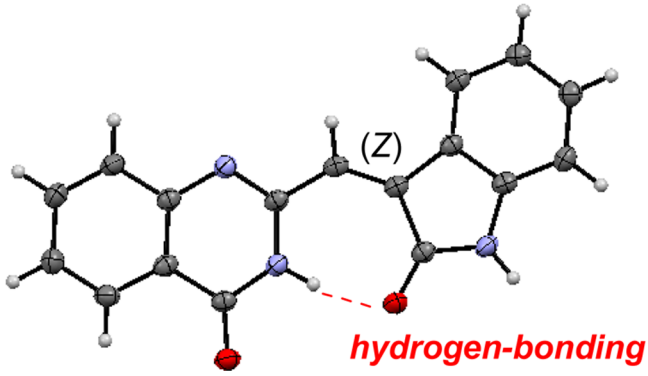


Figure 4. X-ray crystallographic analysis of (*Z*)-**2**, showing intramolecular hydrogen bonding.

On the other hand, in the case of quinazolinone (*Z*)-**2**, the *Z*-isomer was also preferentially obtained because of hydrogen bonding, as suggested by X-ray crystallographic analysis (Figure 4).[6,9,15]

To further investigate the properties of schizocommunin and its derivatives, compounds (*E*)-$\mathbf{1}_{keto}$, (*Z*)-**2**, (*E*)-**3**, (*E*)-**10**, and (*E*)-**11** were examined with regard to their antiproliferative activities against HeLa human cervical cancer cells and A549 human lung cancer cells (Table 1). The results showed that synthetic schizocommunin (*Z*)-**2** as well as (*E*)-**3** and (*E*)-**11** had antiproliferative activity against HeLa cells.

Table 1. Antiproliferative Activities of Compounds (*E*)-$\mathbf{1}_{keto}$, (*Z*)-2, (*E*)-3, (*E*)-10, and (*E*)-11 against HeLa and A549 Cells

	IC$_{50}$ (μM)	
compound	HeLa	A549
(*E*)-$\mathbf{1}_{keto}$	>100	>100
(*Z*)-**2**	15.2	>100
(*E*)-**3**	7.8	93.3
(*E*)-**10**	28.8	>100
(*E*)-**11**	19.6	>100
SAHA[a]	2.6−17.0	3.7−4.3
cisplatin	6.1−28.0	8.0−23.4

[a]Suberoylanilide hydroxamic acid.

In summary, we achieved the total synthesis of the putative structures of schizocommunin (*Z*)-$\mathbf{1}_{hydroxy}$ and its geometric isomer (*E*)-$\mathbf{1}_{hydroxy}$, which both exist in a keto form. However, the ^{1}H NMR spectra for synthetic (*Z*)- and (*E*)-$\mathbf{1}_{keto}$ were not identical to those reported for natural schizocommunin. A reinvestigation of the NMR and IR results for natural schizocommunin led us to propose a revised structure, quinazolinone **2**, which was synthesized in a single step. All of the spectral data of (*Z*)-**2** were identical to those reported for natural schizocommunin. Studies to determine the bioactivities of the synthetic compounds are currently under way.

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EXPERIMENTAL SECTION

General Experimental Procedures. All reactions involving air- or moisture-sensitive reagents or intermediates were performed under an inert atmosphere of argon or nitrogen in glassware. All reagents were purchased from Aldrich, Wako Chemicals, and Kanto Chemical. Unless otherwise noted, solvents and reagents were reagent-grade and used without purification. Reactions were monitored by thin-layer chromatography carried out on 0.25 mm Merck silica gel plates (60F-254). Column chromatography was performed with silica gel (Fuji Silysia, PSQ-60B) or DIOL-silica (Fuji Silysia, MB100-40/75). Melting points were determined on a Yanagimoto micro melting point apparatus. Infrared (IR) spectra were recorded on a Shimadzu IRAffinity-1. ^{1}H NMR spectra were obtained on 400 or 600 MHz instruments (JEOL JNM-ECS 400, JEOL JNM-ECA 600) in the indicated solvent at 25 °C unless otherwise stated. ^{13}C NMR spectra were obtained at 100 or 150 MHz in the indicated solvent. Mass spectra were measured by JEOL, JMS-HX 110, or JMS-T100LP for HRMS. X-ray crystallographic analyses were performed on a Bruker SMART APEX II and a Rigaku VariMax with a RAPID system. Crystallographic data have been deposited with the Cambridge Crystallographic Data Centre. Copies of the data can be obtained free of charge via http://www.ccdc.cam.ac.uk/conts/retrieving.html (or from the Cambridge Crystallographic Data Centre, 12 Union Road, Cambridge, CB2 1EZ, UK; fax: +44 1223 336033; e-mail: deposit@ccdc.cam.ac.uk).[6]

Chemistry. Compounds **6**[5] and **9**[4] were prepared as described in the literature.

*Methyl 4-chlorocinnoline-3-carboxylate (**7**).* *tert*-Butyl 4-hydroxycinnoline-3-carboxylate (**6**[5]) (36 mg, 0.15 mmol) in $SOCl_2$ (1.5 mL) was refluxed for 14 h. The reaction mixture was concentrated under vacuum, and then the residue was diluted with 1.5 mL of cold MeOH (0 °C). After being stirred for 12 h at 0 °C, the reaction mixture was concentrated under vacuum. The residue was purified by flash column chromatography (*n*-hexane/AcOEt = 1:1) to give **7** (31 mg, 0.14 mmol, 97%) as a white solid: ^{1}H NMR ($CDCl_3$, 400 MHz) δ 4.15 (s, 3H), 7.95−8.04 (m, 2H), 8.37 (d, J = 8.3 Hz, 1H), 8.66 (d, J = 8.3 Hz, 1H); ^{13}C NMR ($CDCl_3$, 100 MHz) δ 53.4, 124.0, 124.7, 130.3, 132.7, 132.9, 135.1, 144.5, 150.9, 164.4; IR (neat) ν 1730, 1168, 768 cm^{-1}; HRMS (EI) calcd for $C_{10}H_7N_2O_2{}^{35}Cl$ [M]$^+$ 222.0196, found 222.0191.

*4-Chlorocinnoline-3-carboxaldehyde (**4**). Reduction of Ester **7** (Scheme 2).* A 1.0 M solution of DIBAH in toluene (60 μL, 0.060 mmol) was added dropwise to a solution of **7** (12 mg, 0.055 mmol) in toluene (0.55 mL) at −78 °C. After being stirred for 15 min at the same temperature, the reaction was quenched by the addition of saturated potassium sodium tartrate (2.0 mL). The mixture was stirred for 3 h at rt and extracted with 5 mL of AcOEt (3 times). The combined organic extracts were washed with brine and dried over Na_2SO_4. The resulting solution was filtered and concentrated under vacuum. The residue was purified by flash column chromatography on silica gel using a mixture of *n*-hexane and AcOEt (2:1) as a solvent to give **4** (7.1 mg, 0.037 mmol, 67%) along with cinnoline-3-carboxaldehyde **8** (2.1 mg, 0.013 mmol, 24%).

*Ortho-Directed Formylation of Cinnoline **9** (Scheme 3).* A solution of 2,2,6,6-tetramethylpiperidine (5.2 g, 36 mmol) in anhydrous THF (76 mL) was treated with 23 mL of 1.6 M *n*-butyllithium (36 mmol) in *n*-hexane for 15 min at −78 °C. The mixture was warmed to 0 °C and stirred for 30 min at the same temperature. A solution of 4-chlorocinnoline **9**[4] (2.5 g, 15 mmol) in anhydrous THF (76 mL) was added to the resulting solution of lithium tetramethylpiperidide for 30 min at −78 °C, and the mixture was stirred for 2 h at −78 °C. *N*,*N*′-dimethylformamide (2.8 mL, 36 mmol) was added to the resulting solution at −78 °C, and the mixture was stirred for 30 min at the same temperature. The reaction was then quenched with 2 M HCl in ether (60 mL, 0.12 mol) at −78 °C, and the solution was gradually warmed to −10 °C. Water was added dropwise to the solution while the temperature was maintained between 0 and 5 °C. The resulting mixture was extracted with 100 mL of AcOEt (3 times), and the combined organic layers were washed with brine, dried, and evaporated under vacuum. The residue was purified by flash column chromatography on silica gel using a mixture of AcOEt and *n*-hexane (1:4 to 1:2) as the solvent to give 4-chlorocinnoline-3-carboxaldehyde, **4** (2.4 g, 12 mmol, 81%), as a yellow powder: mp 190−192 °C (AcOEt); ^{1}H NMR ($CDCl_3$, 400 MHz) δ 8.01 (dd, J = 8.0, 8.0 Hz, 1H), 8.10 (dd, J = 8.0, 8.0 Hz, 1H), 8.47 (d, J = 8.0 Hz, 1H), 8.70 (d, J = 8.0 Hz, 1H), 10.80 (s,1H); ^{13}C NMR ($CDCl_3$, 100 MHz) δ 124.4, 124.9, 130.2, 133.0, 133.7, 136.9, 144.3, 150.7, 190.1; IR (neat) ν 2832, 1719, 1698, 1340, 766 cm^{-1}; HRMS (EI) calcd for $C_9H_5N_2{}^{35}Cl$ [M]$^+$ 192.0090, found 192.0082.

*(E)-3-((4′-Chlorocinnolin-3′-yl)methylene)indolin-2-one ((E)-**3**).* To the mixture of 4-chlorocinnoline-3-carboxaldehyde **4** (19 mg, 0.10 mmol) and oxindole **5** (13 mg, 0.10 mmol) was added triethylamine (20 mg, 0.20 mmol) in ethanol (1.0 mL). The solution was heated at 80 °C for 6.5 h. After the solution was allowed to cool to rt, the precipitate was collected by filtration, washed with *n*-hexane, and dried to afford (*E*)-3-((4′-chlorocinnolin-3′-yl)methylene)indolin-2-one, (*E*)-**3** (30 mg, 0.096 mmol, 96%), as an orange powder: mp 258−261 °C (acetonitrile); ^{1}H NMR (DMSO-d_6, 600 MHz) δ 6.92 (d, J = 7.3 Hz, 1H), 6.98 (dd, J = 8.3, 7.3 Hz, 1H), 7.34 (dd, J = 8.3, 7.3 Hz, 1H), 8.06 (s, 1H), 8.11−8.17 (m, 2H), 8.37 (d, J = 8.3 Hz, 1H), 8.44 (d, J = 8.3 Hz, 1H), 8.68 (d, J = 8.3 Hz, 1H), 10.81 (br, 1H); ^{13}C NMR (DMSO-d_6, 150 MHz) δ 110.1, 120.9, 121.5, 123.6, 124.0, 126.2, 127.2, 129.8, 131.8, 131.8, 132.9, 133.7, 135.3, 144.3, 147.6, 149.5, 168.7; IR (neat) ν 3146, 1718, 1615, 1461, 1325 cm^{-1}; HRMS (ESI) m/z calcd for $C_{17}H_{11}ClN_3O$ [M + H]$^+$ 308.0591, found 308.0592.

*(E)-1-Acetyl-3-((4′-chlorocinnolin-3′-yl)methylene)indolin-2-one ((E)-**10**).* A mixture of (*E*)-**3** (18 mg, 0.060 mmol) and 2 mL of Ac_2O (21 mmol) was heated for 15 h at 100 °C. After the mixture was allowed to cool to rt, the reaction was quenched with water and extracted with 3 mL of AcOEt (3 times). The combined organic extracts were washed with saturated aqueous Na_2CO_3 and dried over anhydrous magnesium sulfate. The resulting solution was filtered and concentrated under vacuum. The residue was purified by flash column chromatography on DIOL-silica gel using a mixture of chloroform and methanol (40:1) as a solvent to give (*E*)-1-acetyl-3-((4′-chlorocinnolin-3′-yl)methylene)indolin-2-one, (*E*)-**10** (13 mg, 0.037 mmol, 62%), as a yellow powder: mp 206 °C (AcOEt); ^{1}H NMR (600 MHz, DMSO-d_6) δ 2.71 (s, 3H), 7.24 (dd, J = 8.3, 7.3 Hz, 1H), 7.49 (dd, J = 8.3, 7.3 Hz 1H), 8.13 (dd, J = 8.3, 7.3 Hz, 1H), 8.17 (dd, J = 8.3, 7.3 Hz, 1H), 8.21 (s, 1H), 8.25 (d, J = 8.3 Hz, 1H), 8.37 (d, J = 8.3 Hz, 1H), 8.55 (d, J = 8.3 Hz, 1H), 8.68 (d, J = 8.3 Hz, 1H); ^{13}C NMR (150 MHz, DMSO-d_6) δ 26.3, 115.5, 121.2, 123.4, 123.8, 124.5, 126.2, 128.0, 129.4, 129.6, 131.5, 132.9, 133.6, 135.6, 141.1, 147.0, 149.5, 167.8, 170.2; IR (neat) ν 1743, 1699, 1366, 1325, 1173 cm^{-1}; HRMS (ESI) m/z calcd for $C_{19}H_{12}ClN_3NaO_2$ [M + Na]$^+$ 372.0516, found 372.0517.

*Deacetylation of (E)-**10** (Scheme 4).* A mixture of (*E*)-**10** (3.5 mg, 0.010 mmol) and $LiOH·H_2O$ (2.1 mg, 0.050 mmol) in THF (1.0 mL) was stirred for 6 h at rt. The reaction mixture was quenched with a 0.1 M HCl solution (1 mL). The resulting mixture was extracted with 2 mL of AcOEt (3 times), and the combined organic layers were washed with brine and dried over anhydrous magnesium sulfate. Subsequent filtration and concentration in vacuo gave (*E*)-**3** (3.1 mg, 0.010 mmol, quant.).

*(E)-3-((4′-Methoxycinnolin-3′-yl)methylene)indolin-2-one ((E)-**11**).* A solution of 28 w/w% MeONa in MeOH (23 mg, 0.12 mmol) was added to a solution of (*E*)-**3** (31 mg, 0.10 mmol) in THF (4 mL) at rt. The mixture was stirred for 24 h at rt. The resulting solution was diluted with water (2 mL) and extracted with 5 mL of AcOEt (3 times). The combined organic extracts were washed with brine and dried over anhydrous magnesium sulfate. The resulting solution was filtered and concentrated under vacuum. The residue was purified by flash column chromatography on DIOL-silica gel using a mixture of AcOEt and *n*-hexane (1:2) as a solvent to give (*E*)-3-((4′-methoxycinnolin-3′-yl)methylene)indolin-2-one, (*E*)-**11** (18 mg, 0.058 mmol, 58%), as an orange solid: mp >300 °C (acetonitrile); ^{1}H NMR (600 MHz, DMSO-d_6) δ 4.22 (s, 3H), 6.91 (d, J = 7.3 Hz 1H), 6.98 (dd, J = 7.3, 7.3 Hz, 1H), 7.32 (dd, J = 7.3, 7.3, 1H), 7.97 (dd, J = 8.3,

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7.3 Hz, 1H), 8.03 (s, 1H), 8.07 (dd, J = 8.3, 7.3 Hz, 1H), 8.35 (d, J = 8.3 Hz, 1H), 8.55 (d, J = 7.3 Hz, 1H), 8.59 (d, J = 8.3 Hz, 1H), 10.74 (br, 1H); ^{13}C NMR (150 MHz, DMSO-d_6) δ 64.0, 109.8, 120.2, 121.3, 121.3, 121.9, 125.3, 126.8, 129.4, 129.8, 131.1, 131.5, 132.1, 143.2, 143.7, 151.1, 154.2, 168.9; IR (neat) ν 3065, 1705, 1609, 1462 cm^{-1}; HRMS (ESI) m/z calcd for $C_{18}H_{14}N_3O_2$ $[M + H]^+$ 304.1086, found 304.1076.

*(E)-3-((2′-Oxoindolin-3′-ylidene)methyl)cinnolin-4(1H)-one ((E)-**1**$_{keto}$).* A solution of (*E*)-**3** (31 mg, 0.10 mmol) in DMSO (6 mL; H_2O content 0.051%) was stirred for 11 h at 120 °C. The reaction mixture was cooled to rt, diluted with AcOEt (200 mL), and then washed with 60 mL of water (3 times). The organic layer was concentrated under vacuum to afford (*E*)-3-((2′-oxoindolin-3′-ylidene)methyl)cinnolin-4(1*H*)-one, (*E*)-**1**$_{keto}$ (29 mg, 0.099 mmol, 99%), as an orange powder: mp >300 °C (acetonitrile); ^{1}H NMR (DMSO-d_6, 600 MHz) δ 6.88 (d, J = 7.3 Hz, 1H), 6.97 (dd, J = 8.3, 7.3 Hz, 1H), 7.29 (dd, J = 8.3, 7.3 Hz, 1H), 7.53 (dd, J = 7.3, 7.3 Hz, 1H), 7.72 (d, J = 8.3 Hz, 1H), 7.87 (dd, J = 8.3, 7.3 Hz, 1H), 8.02 (s, 1H), 8.17 (d, J = 7.3 Hz, 1H), 8.65 (d, J = 7.3 Hz, 1H), 10.64 (br, 1H), 14.27 (br, 1H); ^{13}C NMR (DMSO-d_6, 150 MHz) δ 109.7, 117.4, 121.0, 121.7, 123.0, 124.7, 125.6, 126.1, 126.6, 127.3, 130.6, 134.2, 140.5, 143.3, 143.4, 169.2, 169.7; IR (neat) ν 3075, 1705, 1612, 1576, 1464 cm^{-1}; HRMS (ESI) m/z calcd for $C_{17}H_{11}N_3NaO_2$ $[M + Na]^+$ 312.0749, found 312.0742.

*(E)-1-Acetyl-3-((1′-acetyl-2′-oxoindolin-3′-ylidene)methyl)-cinnolin-4(1H)-one ((E)-**12**).* A mixture of (*E*)-**1**$_{keto}$ (22 mg, 0.074 mmol) and 2.5 mL of Ac_2O (26 mmol) was heated for 24 h at 100 °C. After the mixture was allowed to cool to rt, the reaction was quenched with water and extracted with 3 mL of AcOEt (3 times). The combined organic extracts were washed with saturated aqueous Na_2CO_3 and dried over anhydrous magnesium sulfate. The resulting solution was filtered and concentrated under vacuum. The residue was purified by flash column chromatography on DIOL-silica gel using a mixture of chloroform and methanol (40:1) as a solvent to give (*E*)-1-acetyl-3-((1′-acetyl-2′-oxoindolin-3′-ylidene)methyl)cinnolin-4(1*H*)-one, (*E*)-**12** (26 mg, 0.069 mmol, 93%), as an orange-brown powder: mp 290 °C (AcOEt/*n*-hexane); ^{1}H NMR (600 MHz, $CDCl_3$) δ 2.76 (s, 3H), 2.78 (s, 3H), 7.12 (dd, J = 7.8, 7.3 Hz, 1H), 7.41 (dd, J = 8.3, 7.3 Hz 1H), 7.60 (dd, J = 7.8, 7.3 Hz, 1H), 7.86 (ddd, J = 8.8, 7.3, 1.5 Hz, 1H), 7.98 (d, J = 7.8 Hz, 1H), 8.05 (s, 1H), 8.36 (d, J = 8.3 Hz, 1H), 8.39 (dd, J = 7.8, 1.5 Hz, 1H), 8.99 (d, J = 8.8 Hz, 1H); ^{13}C NMR (600 MHz, $CDCl_3$) δ 24.9, 26.9, 117.0, 120.1, 121.5, 123.8, 124.0, 124.3, 126.1, 127.2, 127.5, 130.1, 131.7, 135.3, 139.4, 141.4, 144.5, 167.9, 170.6, 170.7, 173.9; IR (neat) ν 1732, 1715, 1653, 1599, 1456, 1371, 1258, 1179, 1146, 760 cm^{-1}; HRMS (ESI) m/z calcd for $C_{21}H_{15}N_3NaO_4$ $[M + Na]^+$ 396.0960, found 396.0948.

*(Z)-2-((2′-Oxoindolin-3′-ylidene)methyl)quinazolin-4(3H)-one (schizocommunin; (Z)-**2**).* A mixture of 2-methyl-4(3*H*)-quinazolinone **13** (0.16 g; 1.0 mmol) and isatin **14** (0.15 g; 1.0 mmol) in glacial acetic acid (1 mL) was refluxed for 3.5 h. After the mixture was allowed to cool to rt, the precipitate was filtered, washed with methanol, and dried to afford (*Z*)-2-((2′-oxoindolin-3′-ylidene)-methyl)quinazolin-4(3*H*)-one, (*Z*)-**2** (0.27 g, 0.93 mmol, 93%), as an orange powder: mp 298−300 °C (acetone), 284−286 °C ($CHCl_3$/MeOH) [mp 271−273 °C ($CHCl_3$/MeOH)];[1] ^{1}H NMR (600 MHz, DMSO-d_6) δ 6.94 (d, J = 7.3 Hz 1H), 7.09 (dd, J = 7.3, 7.3 Hz, 1H), 7.37 (dd, J = 7.3, 7.3, 1H), 7.58 (s, 1H), 7.61 (dd, J = 7.3, 7.3 Hz, 1H), 7.80 (d, J = 8.3 Hz, 1H), 7.89 (dd, J = 8.3, 7.3 Hz, 1H), 7.95 (d, J = 7.3 Hz 1H), 8.19 (d, J = 7.3 Hz 1H), 11.49 (br, 1H), 14.40 (br, 1H); ^{13}C NMR (150 MHz, DMSO-d_6) δ 110.5, 121.5, 121.9, 122.6, 123.2, 126.0, 127.9, 128.0, 130.0, 131.6, 134.3, 134.7, 141.6, 148.9, 150.4, 160.8, 168.8; IR (neat) ν 3187, 3057, 1647, 1636, 1558, 1541, 1457 cm^{-1}; HRMS (ESI) m/z calcd for $C_{17}H_{11}N_3NaO_2$ $[M + Na]^+$ 312.0749, found 312.0759.

Cytotoxicity Determination. Cells were seeded into 96-well microplates at 4000 cells per well and allowed to attach for 24 h. HeLa human cervical cancer cells and A549 human lung cancer cells were then incubated in Dulbecco's modified Eagle's medium (Invitrogen Co., Ltd., Carlsbad, CA, USA) supplemented with 10% fetal bovine serum, penicillin G (100 U/mL), streptomycin (100 μg/mL), and amphotericin B (0.25 μg/mL). Media were supplemented with the indicated concentrations of compounds (*E*)-**1**$_{keto}$, (*Z*)-**2**, (*E*)-**3**, (*E*)-**10**, and (*E*)-**11** for 48 h. Cell proliferation was measured using a cell counting kit (Dojindo, Kumamoto, Japan) to count living cells for 2 h. The cell number was determined by scanning with a Bio-Rad model 550 microplate reader at 450 nm (630 nm as a reference).

■ ASSOCIATED CONTENT

Ⓢ Supporting Information

The spectroscopic data and further details of the structure assignment are available free of charge via the Internet at http://pubs.acs.org.

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Notes

The authors declare no competing financial interest.

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(11) Isomerization of (*Z*)-**1**$_{keto}$ to (*E*)-**1**$_{keto}$ was explained by calculations (B3LYP 6-31G* level with Spartan 06) that suggested that (*E*)-**1**$_{keto}$ was 3.7 kcal/mol more stable than (*Z*)-**1**$_{keto}$.

(12) Quinazolinone **2** is a known compound (CAS No. 397880-61-4) that is commercially available from AKos Screening Library (http://www.akosgmbh.de/AKosSamples/index.html), Ambinter Stock Screening Collection (http://www.freewebs.com/quyblvant/ambinter-stock-screening-collection.html), Aurora Screening Library (http://www.aurorafinechemicals.com/), Otava Stock Chemicals

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(15) NOE enhancement was also observed between a vinyl proton (8′-H: 7.58 ppm) and 4′-H (7.94 ppm) on the oxindole skeleton of synthetic (*Z*)-**2**.