Rhazinilam and Quebrachamine Derivatives from Yunnan *Kopsia arborea*

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Received August 8, 2008

Three new rhazinilam-derived alkaloids, kopsiyunnanines C1, C2, and C3, and a new quebrachamine-type alkaloid, kopsiyunnanine D, which possess an unusual methoxymethyl or ethoxymethyl function, were isolated from the aerial parts of Yunnan *Kopsia arborea.* This is the first report of the presence of these functions in natural alkaloids. The structures and absolute configurations of the alkaloids were determined by spectroscopic methods and confirmed by semisynthesis.

The rich variety of molecular skeletons and the diverse biological uses of *Kopsia* alkaloids have continuously intrigued and challenged chemists who are attempting both structure elucidation[1] and synthesis.[2] As a part of our project focusing on bioactive indole alkaloids,[3] we have undertaken the chemical investigation of *Kopsia officinalis* Tsiang et P. T. Li, a species growing in Yunnan Province in the southwest of China. This plant had been used historically in folk medicine for the treatment of rheumatoid arthritis, dropsy, and tonsillitis.[4] Recently, Middleton revised the scientific name to *Kopsia arborea* Blume.[5] Malayan *K. arborea* was confirmed to contain several interesting alkaloids, such as arbophylline,[1g] valparicine,[1i] mersicarpine,[6] and arboloscine.[1f] The latter two were also isolated from Yunnan *K. arborea* in our present study. Recently, we reported two new indole alkaloids from the same plant:[7] kopsiyunnanine A belongs to a new class of bisindole alkaloids composed of vallesiachotamine and aspidospermatan-type alkaloids and possesses a dihydropyran moiety; kopsiyunnanine B appears to be the first Corynanthe-type oxindole alkaloid with a rearranged D ring. In this report, we present the isolation, structure determination, and cytotoxicity evaluation of three new rhazinilam analogues, viz., kopsiyunnanine C1 (**1**), kopsiyunnanine C2 (**2**), and kopsiyunnanine C3 (**3**), and a new (−)-quebrachamine derivative, kopsiyunnanine D (**5**) (Figure 1).

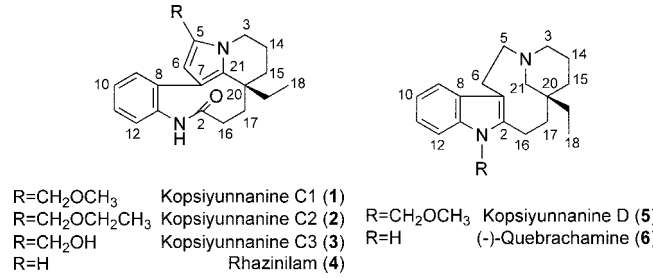

Figure 1. Alkaloids isolated from Yunnan *Kopsia arborea*.

Results and Discussion

The HREIMS spectrum of kopsiyunnanine C1 (**1**) exhibited an $[M]^+$ ion at *m*/*z* 338.1988, corresponding to the molecular formula $C_{21}H_{26}N_2O_2$ (calcd 338.1994). The UV spectrum exhibited absorption maxima at 277.5, 225.0 (sh), and 206.0 nm, suggesting a typical rhazinilam chromophore.[8] ^{1}H and ^{13}C NMR data (Table 1) showed the presence of NH as a broad singlet at δ 6.58, four aromatic protons attributed to the 1,2-disubstituted benzene ring, an amide carbonyl carbon characteristic of C-2 of rhazinilam alkaloids at δ 177.3, and an ethyl side chain [δ_H 1.45, 1.21 and δ_C 30.2 for the methylene function, and δ_H 0.70 (3H) and δ_C 8.2 for the methyl function]. Comparison of the ^{1}H and ^{13}C NMR data of **1** with those of rhazinilam (**4**),[9] which was also isolated in this study, suggested that alkaloid **1** is a derivative of **4**. Moreover, signals representative of a methoxy group [δ_H 3.66 (3H), δ_C 57.1] and an oxymethylene group [δ_H 4.31 (2H), δ_C 66.1] suggested that **1** possesses a methoxymethyl (MOM) group at C-5 of **4**, because the proton signal assigned to H-5 (δ 6.50 for **4**, in $CDCl_3$) in the pyrrole ring disappeared while the remaining H-6 was observed as a singlet at δ 5.76 (δ 5.76 for **4**, in $CDCl_3$). Full assignment of the ^{1}H and ^{13}C NMR data of **1** was carried out using 2D NMR techniques (HMQC, ^{1}H−^{1}H COSY, and HMBC). Key HMBC correlations from methoxy protons to C-22, from H_2-22 to C-6, from H-3 to C-5, and from H-6 to C-8 clearly demonstrated the linkage of an MOM function of C-5 in the pyrrole ring (Figure 2). Therefore, the structure of kopsiyunnanine C1 was proposed to be **1**, i.e., 5-methoxymethylrhazinilam.

Kopsiyunnanine C2 (**2**) was shown to have the molecular formula $C_{22}H_{28}N_2O_2$ as deduced from the observed $[M]^+$ ion at *m*/*z* 352.2159 (calcd 352.2151) in the HREIMS, revealing 14 mass units more than **1**. UV absorptions at 275.5, 224.5 (sh) and 204.0 nm indicated a typical rhazinilam nucleus. By comparing the ^{1}H NMR data (Table 1) with those of **1**, it was observed that the structures of these two compounds are closely related. The only difference was an ethoxymethyl function at C-5 of **2** in place of the MOM function of **1**, which was suggested by the appearance of a downfield methylene signal at δ_H 3.48 (2H, qd, J = 7.0, 1.5 Hz) with a related methyl signal at δ_H 1.21 (3H, t, J = 7.0 Hz) instead of the methoxy signal at δ_H 3.66 (3H, s) in **1**. In the HMBC spectrum (Figure 2), correlations from H-22 to C-23, C-5, and C-6 and from oxymethylene protons H_2-23 to the methyl function verified the above deduction. Therefore, the structure of kopsiyunnanine C2 was suggested to be **2**, i.e., 5-ethoxymethylrhazinilam.

Kopsiyunnanine C3 (**3**) possessed the molecular formula $C_{20}H_{24}N_2O_2$, as determined by HREIMS, *m*/*z* 324.1836 $[M]^+$ (calcd 324.1838), with one CH_2 unit less than **1**. Characteristic absorptions in the UV spectrum implied that **3** might also be a rhazinilam derivative. ^{1}H and ^{13}C NMR spectra (Table 1) were similar in all respects to those of **1**, showing the presence of an oxymethylene resonance at C-5; the only difference was that the methoxy function disappeared. By analyzing HMBC and ^{1}H−^{1}H COSY spectra

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10.1021/np800489e CCC: $40.75
Published on Web 01/09/2009

Table 1. ^{1}H (500 MHz, *J* in Hz) and ^{13}C (125 MHz) NMR Data for Compounds **1**−**3** in $CDCl_3$

	1		**2**		**3**	
position	δ_H (mult, Hz)	δ_C	δ_H (mult, Hz)	δ_C	δ_H (mult, Hz)	δ_C
NH	6.58 (brs)		6.55 (brs)		6.60 (brs)	
2		177.3		177.2		177.3
3	4.07 (dd, 12.5, 5.0)	43.3	4.07 (dd, 12.5, 5.5)	43.3	4.20 (dd, 12.5, 5.5)	43.3
	3.68 (ddd, 12.5, 12.5, 5.0)		3.71 (m)		3.72 (ddd, 12.5, 12.5, 5.0)	
5		126.3		126.6		129.6
6	5.76 (s)	111.8	5.74 (s)	111.4	5.73 (s)	110.4
7		116.5		116.4		116.6
8		140.3		140.3		140.1
9	7.40 (dd, 7.5, 2.0)	131.5	7.40 (dd, 7.5, 1.5)	131.5	7.40 (dd, 7.5, 1.5)	131.4
10	7.29 (ddd, 7.5, 7.5, 2.0)	127.3	7.30 (ddd, 7.5, 7.5, 1.5)	127.3	7.30 (ddd, 7.5, 7.5, 1.5)	128.0
11	7.34 (ddd, 7.5, 7.5, 2.0)	128.1	7.33 (ddd, 7.5, 7.5, 1.5)	128.0	7.34 (ddd, 7.5, 7.5, 1.5)	127.3
12	7.20 (br d, 7.5)	126.9	7.20 (br d, 7.5)	126.8	7.20 (br d, 7.5)	126.9
13		137.9		137.9		137.9
14	2.26 (dd, 14.0, 5.0)	19.1	2.20 (m)	19.1	2.22 (m)	19.0
	1.90 (dd, 14.0, 7.0)		1.90 (br d, 13.5)		1.90 (overlapped)	
15	1.69 (overlapped)	32.5	1.69 (ddd, 13.5, 13.5, 3.5)	32.5	1.71 (ddd, 13.5, 13.5, 3.0)	32.5
	1.52 (overlapped)		1.51 (overlapped)		1.52 (overlapped)	
16	2.38 (overlapped)	28.2	2.38 (overlapped)	28.1	2.38 (overlapped)	28.1
	1.96 (dd, 12.5, 7.5)		1.96 (dd, 13.5, 7.0)		1.96 (dd, 13.0, 6.5)	
17	2.45 (overlapped)	36.7	2.44 (overlapped)	36.6	2.46 (overlapped)	36.6
	1.45 (overlapped)		1.43 (overlapped)		1.48 (overlapped)	
18	0.70 (3H, t, 7.5)	8.2	0.70 (3H, t, 7.0)	8.2	0.72 (3H, t, 7.0)	8.2
19	1.45 (overlapped)	30.2	1.48 (overlapped)	30.2	1.46 (overlapped)	30.1
	1.21 (m)		1.25 (overlapped)		1.26 (m)	
20		39.1		39.0		39.1
21		132.1		131.9		132.2
22	4.31 (2H, s-like)	66.1	4.35 (2H, s)	64.3	4.51 (2H, s-like)	56.9
OCH_3	3.66 (3H, s)	57.1				
$O\underline{CH_2}CH_3$			3.48 (2H, qd, 7.0, 1.5)	64.9		
$OCH_2\underline{CH_3}$			1.21 (3H, t, 7.0)	15.2		

together with all the data above, kopsiyunnanine C3 (**3**) was deduced to be 5-hydroxymethylrhazinilam.

Ring-opened indole alkaloid rhazinilam (**4**), whose tetracyclic structure possesses a nine-membered lactam ring system bearing a pyrrole subunit, has been isolated from various Apocynaceae plants.[8b,10,11] Considered to be an artifact arising from the rearrangement of aspidosperma precursors, the absolute configuration at unique chiral center C-20 was deduced as *R* by means of biogenetic considerations[9a,12] and confirmed by X-ray crystallographic analysis.[13] Comparison of the CD spectra of **1**, **2**, and **3** with that of **4** gave remarkably similar curves, leading us to conclude that all of these alkaloids possess the same absolute configuration.

To confirm the structures, including the absolute configuration inferred by spectroscopic analyses above, we performed the semisynthesis of these alkaloids from rhazinilam (**4**) (Scheme 1). The synthesis began with Cbz protection of **4**. Next, Vilsmeier formylation of the pyrrole moiety with $POCl_3$ and DMF in ether gave **8** in 83% yield. Subsequent deprotection of the Cbz group afforded rhazinal (**9**), which is another natural rhazinilam derivative isolated from *K. teoi*.[14,15] Then, **3** was obtained quantitatively by $NaBH_4$ reduction of **9**. To our delight, mesylation of the hydroxy group of **3** followed by solvolysis with methanol or ethanol afforded **1** or **2** in 43% or 73% yield, respectively. All of the spectroscopic data including the CD spectra of synthetic compounds **1**, **2**, and **3** were identical with those of the natural products. Therefore, the structures of kopsiyunnanines C1, C2, and C3 including the absolute configurations were confirmed.

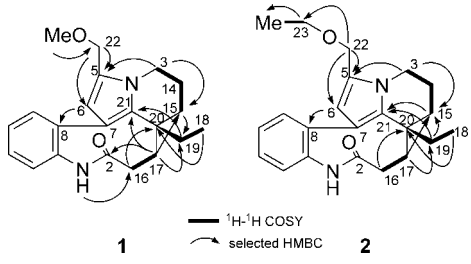
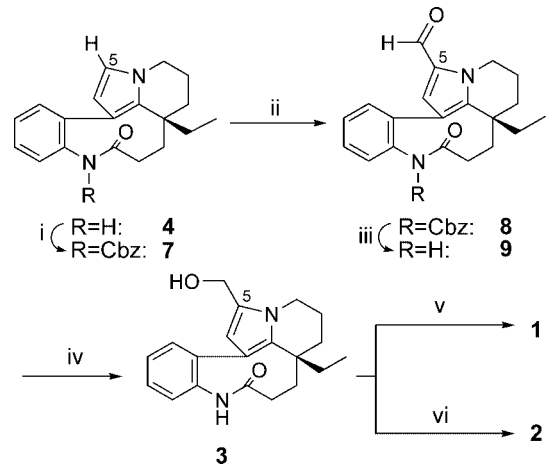


Figure 2. HMBC and ^{1}H−^{1}H COSY correlations of **1** and **2**.

Scheme 1[a]



[a] Reagents and conditions: (i) CbzCl, NaH, THF, rt, 2 h, quant.; (ii) $POCl_3$, DMF, ether, rt, 1.5 h, 83%; (iii) Pd/H_2, EtOAc, rt, 1 h, 78%; (iv) $NaBH_4$, EtOH, rt, 1 h, quant.; (v) (1) MsCl, Et_3N, CH_2Cl_2, 2 h; (2) MeOH, 2 h, 43%; (vi) (1) MsCl, Et_3N, CH_2Cl_2, 2 h; (2) EtOH, 1 h, 73%.

New alkaloid **5**, named kopsiyunnanine D, showed a $[M]^+$ ion at *m/z* 326.2364 in the HREIMS spectrum, suggesting the molecular formula $C_{21}H_{30}N_2O$ (calcd 326.2358). Significant fragments in the EIMS were detected at *m/z* 311 $[M - CH_3]^+$, 295 $[M - CH_3 - O]^+$, and 281 $[M - CH_3 - O - CH_2]^+$, reminiscent of an MOM group similar to that in alkaloid **1**. The UV spectrum was characteristic of an indole chromophore with absorption maxima at 293.0, 284.0, 229.0, and 203.0 nm. ^{1}H and ^{13}C NMR data (Table 2) revealed an α,β-disubstituted indole system, an ethyl side chain [δ_H 1.21, 1.05 and δ_C 31.8 for the methylene function and δ_H 0.80 (3H) and δ_C

Table 2. ^{1}H (500 MHz, *J* in Hz) and ^{13}C (125 MHz) NMR Data for Compound **5** in $CDCl_3$

position	δ_H (mult, Hz)	δ_C
2		141.8
3	2.32 (dd, 13.0, 5.5)	55.2
	2.18 (ddd, 13.0, 13.0, 3.5)	
5	2.40 (ddd, 13.0, 4.5, 1.5)	53.0
	2.22 (ddd, 13.0, 13.0, 4.5)	
6	2.87 (ddd, 15.0, 13.0, 4.5)	22.3
	2.77 (overlapped)	
7		110.2
8		128.3
9	7.41 (d, 7.5)	117.4
10	7.03 (ddd, 7.5, 7.5, 1.5)	119.2
11	7.08 (ddd, 7.5, 7.5, 1.5)	120.5
12	7.33 (d, 7.5)	108.8
13		136.7
14	1.20 (2H, overlapped)	22.6
15	1.09 (overlapped)	34.7
	1.18 (overlapped)	
16	2.79 (overlapped)	18.5
	2.55 (ddd, 15.0, 6.5, 1.5)	
17	1.74 (dd, 13.0, 6.0)	32.4
	1.55 (overlapped)	
18	0.80 (3H, t, 7.5)	7.9
19	1.21 (overlapped)	31.8
	1.05 (overlapped)	
20		37.5
21	3.28 (br d, 12.0)	56.5
	1.41 (overlapped)	
22	5.39 (d, 11.5)	73.8
	5.35 (d, 11.5)	
OMe	3.19 (3H, s)	55.6

7.9 for the methyl function], and seven methylene groups, showing similarity to (−)-quebrachamine (**6**),[16] which coexists in this plant. However, an oxymethylene resonance [δ_H 5.39 (1H, d, J = 11.5 Hz), 5.35 (1H, d, J = 11.5 Hz)] and a methoxy signal at δ_H 3.19 (3H, s) were observed, while the broad singlet due to the indolic N−H in **6** in the ^{1}H NMR spectrum disappeared. Moreover, HMBC correlations (Figure 3) from the methoxy protons to C-22 as well as from H_2-22 to C-2 and C-13 demonstrated that **5** is a new quebrachamine analogue with an MOM function associated with the indolic nitrogen. Other 2D NMR data led to the complete elucidation of the structure of **5** as the new alkaloid kopsiyunnanine D.

To confirm the structure of kopsiyunnanine D (**5**), we designed its semisynthesis from a known alkaloid, tabersonine (**10**), with *R* absolute configuration at C-20. Following a literature procedure,[17] (+)-quebrachamine (**6**) was prepared in good yield from **10** by sequential decarboxymethylation, acid-mediated ring opening, and reduction. Finally, treatment of (+)-**6** with MOMCl and NaH in THF completed the semisynthesis of (+)-kopsiyunnanine D (**5**) (Scheme 2).

Synthetic (+)-kopsiyunnanine D (**5**) was identical to the natural product in all aspects except for its optical activity. The specific rotation {$[\alpha]^{23}_D$ +84.4 (*c* 0.09, $CHCl_3$)} and the CD spectrum of the synthetic product showed opposite signs of those of the natural product (Figure 4). Therefore, the structure of kopsiyunnanine D including the absolute configuration was determined as shown in **5**.

Natural products possessing MOM or ethoxymethyl functions are rare. A literature search led to only a few examples having the former function, i.e., the lignan virgatusin[18] isolated from *Phyllanthus virgatus* and several phenylpropanoids from the rhizomes of smaller galanga,[19] while no compounds have been reported to have the latter function. Importantly, the MOM or ethoxymethyl function has never been detected in natural alkaloids. We did not observe any transformation of kopsiyunnanine C3 (**3**) into compound **1** or **2** when treating it with MeOH or EtOAc under acidic condition. Therefore, kopsiyunnanines C1 (**1**) and C2 (**2**) seem not to be artifacts of **3**.

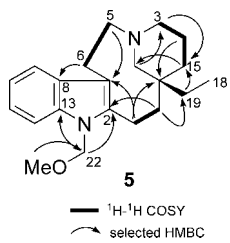
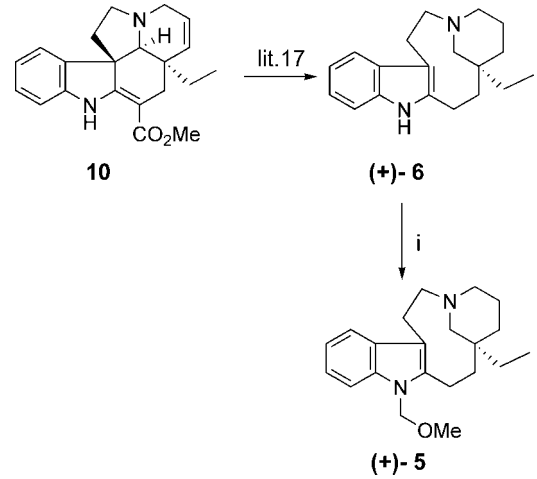


Figure 3. HMBC and ^{1}H−^{1}H COSY correlations of **5**.

Scheme 2[a]



[a] Reagents and conditions: (i) NaH, MOMCl, THF, rt, 1.5 h, 23%.

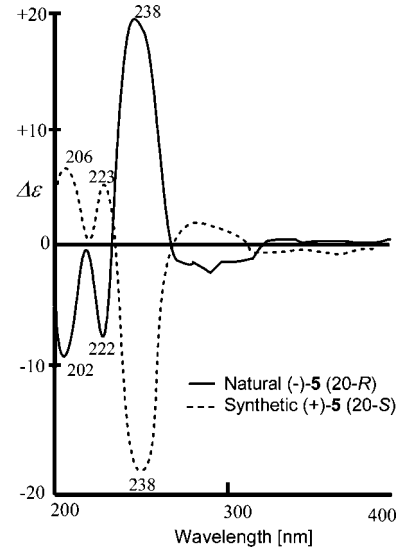


Figure 4. CD spectra (MeOH, 24 °C) of natural (−)-kopsiyunnanine D (**5**) and synthetic (+)-**5**.

Cytotoxic effects of alkaloids **1**−**6** on the A549 human lung adenocarcinoma cell line and the HT29 human colon adenocarcinoma grade II cell line were evaluated. Rhazinilam (**4**) strongly inhibited the examined tumor cell lines, while new rhazinilam alkaloids kopsiyunnanines C (**1**−**3**) exhibited moderate activities (Table 3). Rhazinilam (**4**) has received much attention for its potential use in cancer chemotherapy, acting to inhibit microtubule disassembly.[9b−e] Neither kopsiyunnanine D (**5**) nor (−)-quebrachamine (**6**) showed any activity (>30 μM).

Experimental Section

General Experimental Procedures. Optical rotations were measured with a JASCO P-1020 polarimeter. CD was recorded on a JASCO J-720WI spectrometer. NMR spectroscopic data were recorded on a

Table 3. Cytotoxicity (IC_{50}) of Compounds **1**−**6** to A549 and HT29 Tumor Cell Lines

compound	IC_{50} (μM) to A549	IC_{50} (μM) to HT29
1	5.38	4.67
2	7.44	6.39
3	8.21	8.89
4	0.35	0.35
5	>30	30
6	>30	30
docetaxel[a]	4.95×10^{-4}	3.34×10^{-4}

[a] Positive control.

JNM A-400 or a JNM A-500 spectrometer, where *J* values are given in Hz. EIMS and HREIMS were recorded on a JEOL JMS GC-mate spectrometer with direct probe insertion at 70 eV. FABMS was recorded on a JEOL AX-500 spectrometer and a JEOL JMS-HX110 spectrometer, respectively. TLC was done on precoated silica gel 60 F254 plates (Merck, 0.25 mm thick) or precoated amino-silica gel plates (Fuji Silysia Chemical Ltd.). Column chromatography was carried out over silica gel 60 (Merck, 70−230 mesh), Sephadex LH-20 (GE Healthcare), or Chromatorex NH (Fuji Silysia Chemical Ltd.). Medium-pressure liquid column chromatography was carried out over a silica gel prepacked column CPS-HS-221-05 (Kusano Kagakukikai, SiO_2).

Plant Material. *Kopsia arborea* Blume (*Kopsia officinalis* Tsiang et P. T. Li) was collected from Xishuangbanna, Yunnan Province, China, and identified by one of the authors, R.Z. A voucher specimen (no. 20060401) was deposited at the Faculty of Pharmaceutical Sciences, Kunming Medical College.

Extraction and Isolation. The aerial part of *K. arborea* Blume (*K. officinalis* Tsiang et P. T. Li) (9.0 kg, dry weight) was extracted with MeOH (109.5 L, twice at room temperature and twice under reflux) to give the extract (1.2 kg). The MeOH extract (1.2 kg) was dissolved in 1 M HCl (15 L × 3) and extracted with EtOAc (19 L × 2) to give the EtOAc extract (200 g). The aqueous layer was basified with Na_2CO_3 to pH 9−10 and successively extracted with $CHCl_3$ (15 L × 3). The $CHCl_3$ extract was dried over $MgSO_4$ and evaporated *in vacuo* to give the crude base (60 g). The aqueous layer was extracted with *n*-BuOH (15 L × 3) to give the *n*-BuOH extract (330 g).

The $CHCl_3$ extract (60 g) was separated by SiO_2 open column chromatography with $CHCl_3$/MeOH gradient to give nine fractions: fr.1: 0−10% MeOH/$CHCl_3$ (2.21 g); fr.2: 10−20% MeOH/$CHCl_3$ (1.21 g); fr.3: 20−30% MeOH/$CHCl_3$ (0.35 g); fr.4: 30−40% MeOH/$CHCl_3$ (17.82 g); fr.5: 40−50% MeOH/$CHCl_3$ (5.47 g); fr.6: 50−60% MeOH/$CHCl_3$ (10.27 g); fr.7: 70−80% MeOH/$CHCl_3$ (3.77 g); fr.8: 80−90% MeOH/$CHCl_3$ (6.61 g); fr.9: MeOH (1.20 g).

Fr.4 was separated into six fractions (A−F) by SiO_2 open column chromatography with gradient MeOH/$CHCl_3$ elution: fr.4A: 0−1% MeOH/$CHCl_3$ (0.255 g); fr.4B: 1−2.5% MeOH/$CHCl_3$ (1.21 g); fr.4C: 2.5−5% MeOH/$CHCl_3$ (11.09 g); fr.4D: 5−10% MeOH/$CHCl_3$ (1.83 g); fr.4E: 10−20% MeOH/$CHCl_3$ (1.00 g); fr.4F: 100% MeOH/$CHCl_3$ (0.71 g).

Fr.4B was eluted with EtOAc/$CHCl_3$ solvent system in gradient from 3% EtOAc/$CHCl_3$ to 5%, 10%, 20%, 50%, and 100%, and from 50% EtOAc/MeOH to MeOH. The fraction eluted with 10% EtOAc/$CHCl_3$ gave rhazinilam (**4**, 15.1 mg) as colorless needles. The fraction eluted with 10−20% EtOAc/$CHCl_3$ was purified by MPLC (10% EtOAc/$CHCl_3$) to yield rhazinilam (**4**, 19.4 mg). The fraction eluted with 5−10% EtOAc/$CHCl_3$ was purified by MPLC (10% EtOAc/$CHCl_3$) to give kopsiyunnanine D (**5**, 3.5 mg).

Fr.4C was separated by SiO_2 open column chromatography (MeOH/$CHCl_3$ in gradient) to give five fractions: fr.4C1: 0−20% MeOH/$CHCl_3$ (110.7 mg); fr.4C2: 20−50% MeOH/$CHCl_3$ (8.36 g); fr.4C3 50% MeOH/$CHCl_3$ (1.14 g); fr.4C4: 50% MeOH/$CHCl_3$ (228.4 mg); fr.4E: MeOH (158.8 mg).

Successive separation of fr.4C2 by SiO_2 open column chromatography with 10% MeOH/$CHCl_3$ gave fractions 4C2A−4C2H. Fr.4C2C (515.9 mg) was separated into eight fractions subsequently by SiO_2 open column chromatography with 3%, 5%, 10%, 15%, 25%, and 50% acetone/$CHCl_3$ in gradient. The fraction eluted with 15−25% acetone/$CHCl_3$ was purified by amino-silica open column chromatography (*n*-hexane/EtOAc in gradient) to afford kopsiyunnanine C1 (**1**, 4.1 mg) and kopsiyunnanine C2 (**2**, 0.6 mg).

Fr.4C2D (1.73 g) was separated by amino-silica open column chromatography with *n*-hexane/EtOAc in gradient to gave fractions 4C2D1−4C2D9. The fraction eluted with 70% *n*-hexane/EtOAc was purified by SiO_2 open column chromatography with the same solvent system to give quebrachamine (**6**, 4.0 mg). The fraction eluted with 30−20% *n*-hexane/EtOAc also gave kopsiyunnanine C1 (**1**, 2.5 mg) and kopsiyunnanine C2 (**2**, 0.9 mg) when purified by MPLC (EtOAc) and subsequent SiO_2 open column chromatography in EtOAc or 2% MeOH/$CHCl_3$, respectively.

Fr.4C2E (1.68 g) was eluted by MPLC (EtOAc, 90%, 80%, 50% EtOAc/MeOH, and MeOH in gradient) to obtain nine fractions. After amino-silica open column chromatography (2% MeOH/$CHCl_3$), the fraction purified by elution with 80% EtOAc/MeOH gave kopsiyunnanine C3 (**3**, 3.4 mg).

The structures of the known compounds were identified by comparing their spectroscopic data with literature values. It is worthy to note that all the rhazinilam derivatives mentioned in this paper, including rhazinilam itself, exhibited pink spots when sprayed with 10% $Ce(SO_4)_2 \cdot H_2SO_4$ reagent in SiO_2 TLC, and the color lasted for a long time.

Kopsiyunnanine C1 (1): colorless, amorphous solid; $[\alpha]^{24}_D$ −289.6 (*c* 0.07, $CHCl_3$); UV (MeOH) λ_{max} (log ϵ) 277.5 (3.33), 225.0 (4.31), 206.0 (4.58) nm; EIMS *m/z* (%) 338 [M^+, 23], 277 (Bp); HREIMS *m/z* 338.1988 ([M^+], calcd for $C_{21}H_{26}N_2O_2$, 338.1994); CD (*c* 0.31 mmol/L, MeOH, 24 °C) $\Delta\epsilon$ (λ nm) −23.9 (212), −20.0 (224), −4.2 (260), 0 (290), +0.3 (321), 0 (350); ^{1}H and ^{13}C NMR data, see Table 1.

Kopsiyunnanine C2 (2): colorless, amorphous solid; $[\alpha]^{25}_D$ −163.1 (*c* 0.06, $CHCl_3$); UV (MeOH) λ_{max} (log ϵ) 275.5 (3.01), 224.5 (4.03), 204.0 (4.25) nm; EIMS *m/z* (%) 352 [M^+, 10], 323 (8), 307 (5), 277 (Bp); HREIMS *m/z* 352.2159 ([M^+], calcd for $C_{22}H_{28}N_2O_2$, 352.2151); CD (*c* 0.31 mmol/L, MeOH, 24 °C) $\Delta\epsilon$ (λ nm) −25.9 (212), −28.2 (220), −5.2 (261), 0 (288), +3.3 (322), 0 (350); ^{1}H and ^{13}C NMR data, see Table 1.

Kopsiyunnanine C3 (3): colorless, amorphous solid; $[\alpha]^{19}_D$ −433 (*c* 0.05, $CHCl_3$); UV (MeOH) λ_{max} (log ϵ) 275.0 (3.06), 226.0 (4.14), 204.5 (4.39) nm; FABMS *m/z* (%) 347 [M + Na]$^+$; HREIMS *m/z* 324.1836 ([M^+], calcd for $C_{20}H_{24}N_2O_2$, 324.1838); CD (*c* 0.58 mmol/L, MeOH, 18 °C) $\Delta\epsilon$ (λ nm) −20.9 (212), −18.1 (229), −5.7 (260), 0 (304), +0.5 (345), 0 (400); ^{1}H and ^{13}C NMR data, see Table 1.

Kopsiyunnanine D (4): light yellow, amorphous solid; UV (MeOH) λ_{max} (log ϵ) 293.0 (3.71), 284.0 (3.79), 229.0 (4.36), 203.0 (4.32) nm; EIMS *m/z* (%) 326 [M^+, 96], 311 (23), 295 (39.7), 281 (16), 201 (Bp); HREIMS *m/z* 326.2364 ([M^+], calcd for $C_{21}H_{30}N_2O$, 326.2358); $[\alpha]^{23}_D$ −72.8 (*c* 0.05, $CHCl_3$); CD (*c* 0.3 mmol/L, MeOH, 24 °C) $\Delta\epsilon$ (λ nm) −6.4 (202), −4.7 (222), 0 (227), +17.7 (238), 0 (260), −1.3 (288), 0 (320); ^{1}H and ^{13}C NMR data, see Table 2.

Cbz Protection of Rhazinilam (4). To a solution of rhazinilam (**4**, 2.1 mg, 0.007 mmol) in anhydrous THF (3 mL) was added NaH (0.5 mg, 50−72% in mineral oil, >0.010 mmol) at 0 °C. After 5 min, the reaction mixture was warmed to rt and stirred for 1 h. The solution was then cooled to 0 °C, and CbzCl (1.20 μL, 0.008 mmol) was added. The reaction mixture was warmed slowly to rt and stirred for 2 h. The resulting solution was extracted with $CHCl_3$ (3 × 10 mL). The organic layers were combined, dried ($MgSO_4$), filtered, and evaporated *in vacuo*. The residue was filtered through a pad of SiO_2 (EtOAc) and then purified by MPLC (EtOAc) to give Cbz-rhazinilam (**7**, 4.5 mg, 0.0105 mmol, quant) as a colorless, amorphous solid: ^{1}H NMR ($CDCl_3$, 400 MHz) 7.40−7.11 (9H, overlapped, aromatic-H), 6.39 (1H, d, J = 2.8 Hz, H-5), 5.63 (1H, d, J = 2.8 Hz, H-5), 5.09 (2H, s, $-CO_2CH_2Ph$), 3.93 (1H, dd, J = 13.6, 6.0 Hz, H-3b), 3.67 (1H, ddd, J = 13.6, 13.6, 5.2 Hz, H-3a), 2.45 (1H, overlapped), 2.35 (1H, overlapped), 2.11 (1H, m), 1.96 (1H, ddd, J = 14.4, 7.2, 1.6 Hz), 1.41 (1H, m), 1.39−1.28 (2H, overlapped), 1.08 (1H, dq, J = 14.4, 7.2 Hz), 0.67 (3H, t, J = 7.2 Hz, Me); UV (MeOH) λ_{max} (log ϵ) 222.0 (4.04), 204.5 (4.34) nm; EIMS *m/z* (%) 428 ([M^+], 24), 399 (21), 91 (Bp); HREIMS *m/z* 428.2100 ([M^+], calcd for $C_{27}H_{28}N_2O_3$, 428.2100).

Preparation of Cbz-rhazinal (8). A mixture of DMF (0.05 mL, 0.6 mmol) and $POCl_3$ (4.8 μL, 0.052 mmol) was briefly stirred at 0 °C in order to form the Vilsmeier salt. The mixture was warmed to rt, after which a solution of Cbz-rhazinilam (**7**, 5.1 mg, 0.012 mmol) in dry ether (0.4 mL) was added. The reaction mixture was stirred for 1.5 h at the same temperature and quenched with aqueous Na_2CO_3 solution. The resulting solution was extracted with $CHCl_3$ (3 × 10 mL). The organic layers were combined, dried ($MgSO_4$), filtered, and evaporated under reduced pressure. The residue was purified by SiO_2 open column chromatography (EtOAc) to give Cbz-rhazinal (**8**, 4.6 mg, 0.010 mmol, 83%) as a colorless, amorphous solid: ^{1}H NMR

($CDCl_3$, 400 MHz) 9.23 (1H, s, C*H*O), 7.47−7.25 (9H, overlapped, aromatic-H), 6.36 (1H, s, H-6), 5.19 (1H, d, J = 12.4 Hz, $-CO_2CH_2Ph$), 5.15 (1H, d, J = 12.4 Hz, $-CO_2CH_2Ph$), 4.74 (1H, dd, J = 13.6, 5.2 Hz, H-3b), 3.92 (1H, ddd, J = 13.6, 13.6, 5.2 Hz, H-3a), 2.50 (1H, overlapped), 2.44 (1H, overlapped), 2.09−2.15 (2H, overlapped), 1.94 (1H, m), 1.76 (1H, td, J = 13.6, 2.8 Hz), 1.48 (2H, overlapped), 1.16 (2H, overlapped), 0.73 (3H, t, J = 7.2 Hz, Me); UV (MeOH) λ_{max} (log ϵ) 302.5 (4.01), 256 (3.72), 206.5 (4.45) nm; EIMS m/z (%) 456 [M^+, 16], 91 (Bp); HREIMS m/z 456.2045 ([M^+], calcd for $C_{28}H_{28}N_2O_4$, 456.2049).

Preparation of Rhazinal (9). A mixture of **8** (1.8 mg, 0.004 mmol) and 10% Pd/C (1.8 mg) in EtOAc (0.3 mL) was stirred at rt for 1.5 h under H_2 atmosphere. The solution was filtered through a pad of SiO_2 (EtOAc). The residue was evaporated under reduced pressure and then purified by SiO_2 open column chromatography (3% MeOH/$CHCl_3$) to give rhazinal (**9**, 1.0 mg, 0.003 mmol, 78%) as a colorless, amorphous solid: UV (MeOH) λ_{max} (log ϵ) 303.0 (4.10), 253.5 (3.72), 204.5 (4.27) nm; $[\alpha]^{22}_D$ −493 (c 0.03, $CHCl_3$); CD (c 0.27 mmol/L, MeOH, 24 °C) $\Delta\epsilon$ (λ nm) −31.4 (209), −15.3 (224), −3.4 (270), −5.2 (317), 0 (340). The MS data and the ^{1}H NMR spectrum of compound **9** were in agreement with reported data.[14,20]

Synthesis of Kopsiyunnanine C3 (3). To a solution of **9** (1.3 mg, 0.004 mmol) in dry EtOH (0.35 mL) at 0 °C was added $NaBH_4$ (2.6 mg, 0.069 mmol). The reaction mixture was stirred for 1 h at rt and then quenched with aqueous NaOH solution. The resulting solution was extracted with $CHCl_3$ (3 × 10 mL). The organic layers were combined, dried ($MgSO_4$), filtered, and evaporated under reduced pressure. The residue was filtered through a pad of SiO_2 ($CHCl_3$) and then purified by MPLC (5% MeOH/$CHCl_3$) to give kopsiyunnanine C3 (**3**, 1.8 mg, 0.005 mmol, quant) as a colorless, amorphous solid. All of the data (UV, ^{1}H NMR, ^{13}C NMR, MS, $[\alpha]_D$, and CD) were identical with those of natural **3**.

Synthesis of Kopsiyunnanine C1 (1). To a solution of **3** (3.0 mg, 0.009 mmol) in dry CH_2Cl_2 (0.7 mL) was added MsCl (0.86 μL, 0.011 mmol) at rt. The reaction mixture was stirred for 2 h at 0 °C, upon which dry MeOH (0.5 mL) was added. After the mixture was allowed to reach rt, the reaction was stirred at the same temperature for 1 h. The resulting solution was extracted with $CHCl_3$ (3 × 10 mL). The organic layers were combined, dried ($MgSO_4$), filtered, and evaporated under reduced pressure. The residue was filtered through a pad of SiO_2 (10% MeOH/$CHCl_3$) and then purified by MPLC (50% EtOAc/$CHCl_3$) to give kopsiyunnanine C1 (**1**, 1.3 mg, 0.004 mmol, 43%) as a colorless, amorphous solid. All of the data (UV, ^{1}H NMR, ^{13}C NMR, MS, $[\alpha]_D$, and CD) were identical with those of natural **1**.

Synthesis of Kopsiyunnanine C2 (2). To a solution of **3** (3.0 mg, 0.009 mmol) in dry CH_2Cl_2 (0.7 mL) was added MsCl (0.86 μL, 0.011 mmol) at rt. The reaction mixture was stirred for 2 h at 0 °C, upon which dry MeOH (0.5 mL) was added. After the mixture was allowed to reach rt, the reaction was stirred at the same temperature for 1 h. The resulting solution was extracted with $CHCl_3$ (3 × 10 mL). The organic layers were combined, dried ($MgSO_4$), filtered, and evaporated under reduced pressure. The residue was filtered through a pad of SiO_2 (10% MeOH/$CHCl_3$) and then purified by MPLC (50% EtOAc/$CHCl_3$) to give kopsiyunnanine C2 (**2**, 2.3 mg, 0.0065 mmol, 73%) as a colorless, amorphous solid. All of the data (UV, ^{1}H NMR, ^{13}C NMR, MS, $[\alpha]_D$, and CD) were identical with those of natural **2**.

Synthesis of (+)-Kopsiyunnanine D (5). To a solution of synthetic (+)-quebrachamine (**6**, 14.0 mg, 0.050 mmol), prepared according to the reported procedure,[17] in dry THF (0.5 mL) was added NaH (2.4 mg, 50−72% in mineral oil, >0.060 mmol) at 0 °C. After stirring at rt for 20 min, MOMCl (4.5 μL, 0.060 mmol) was added. The reaction mixture was continuously stirred for 1.5 h, and this was followed by the addition of H_2O to quench the reaction. The resulting solution was extracted with $CHCl_3$ (3 × 10 mL). The organic layers were combined, dried ($MgSO_4$), filtered, and evaporated under reduced pressure. The residue was purified by amino-silica column chromatography (0−3% EtOAc/*n*-hexane) to give (+)-kopsiyunnanine D (**5**, 3.7 mg, 0.0113 mmol, 23%) as a colorless, amorphous solid: $[\alpha]^{23}_D$ +84.4 (c 0.09, $CHCl_3$); UV (MeOH) λ_{max} (log ϵ) 294.0 (3.60), 284.5 (3.69), 229.5 (4.26), 203.0 (4.21) nm; EIMS m/z (%) 326 [M^+, 63], 311 (18), 295 (24), 281 (12), 201 (Bp); HREIMS m/z 326.2364 ([M^+], calcd for $C_{21}H_{30}N_2O$, 326.2358); CD (c 0.3 mmol/L, MeOH, 24 °C) $\Delta\epsilon$ (λ nm) +4.0 (206), +2.8 (223), 0 (228), +14.1 (238), 0 (260), +1.3 (289), 0 (320). ^{1}H and ^{13}C NMR data were identical with those of natural (−)-kopsiyunnanine D (**4**).

Cell Culture. Human lung and colorectal cancer cell lines, A549 and HT29, respectively, were obtained from ATC. A549 and HT29 cells were maintained in Dulbecco's modified Eagle's medium (D-MEM) (D6046, D6046) and D-MEM/F-12 medium (D8062, Sigma) with 10% heat-inactivated fetal bovine serum (FBS) and 5 mg/mL gentamicin, respectively, at 37 °C in a humidified atmosphere containing 5% CO_2.

Growth Inhibition Assay. A 190 μL volume of an exponentially growing cell suspension (1 × 10^4 cells/1.9 mL) was seeded into a 96-well microtiter plate, and 10 μL of each drug at various concentrations was added 24 h after seeding of the tumor cells. After incubation for 96 h at 37 °C, 10 μL of TetraColor ONE (Seikagaku Biobusiness Corporation, Tokyo, Japan) was added to each well, and the plates were incubated for a further 1 h at 37 °C. After incubation, optical density was measured at 450 nm with a microplate reader (SpectraMax Plus, Molecular Devices, CA), and the concentration causing 50% inhibition of cell proliferation (IC_{50}) was calculated by linear regression analysis of the linear portion of the growth curves.

Acknowledgment. This work was supported by a Grant-in-Aid for Scientific Research from the Ministry of Education, Culture, Sports, Science, and Technology, Japan, The Astellas Foundation for Research on Metabolic Disorders, and The Uehara Memorial Foundation.

Supporting Information Available: 1D and 2D NMR data of kopsiyunnanines C1 (**1**), C2 (**2**), C3 (**3**), and D (**5**). This material is available free of charge via the Internet at http://pubs.acs.org.

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NP800489E