Isolation, Structure Elucidation, and Synthesis of Cytotoxic Tryptanthrin Analogues from *Phaius mishmensis*

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Bioassay-guided chromatographic separation of the cytotoxic MeOH extract of *Phaius mishmensis* led to the isolation of two known and six new indoloquinazolinones, phaitanthrins A−E (**1**−**5**) and methylisatoid (**6**). The structures of the new compounds were elucidated by spectroscopic analysis. Phaitanthrin A (**1**) and tryptanthrin (**7**) showed moderate cytotoxicity against MCF-7, NCI-H460, and SF-268 cell lines. A series of ketone adducts of tryptanthrin were prepared and tested initially for anticancer activity *in vitro* against MCF-7, NCI-H460, and SF-268 human cancer cell lines. The 3-pentanone adduct **13** showed activity similar to tryptanthrin.

Tryptanthrin (indolo[2,1-*b*]quinazoline-6,12-dione) is a weak basic indoloquinazoline alkaloid formed in a number of plants such as *Isatis*,[1] *Calanthe*,[2] *Wrightia*,[3] *Couroupota*,[4] and *Strobilanthus*[5] species. Tryptanthrin can also be produced by *Candida lipolytica* when grown in media containing an excess of tryptophan, hence the name tryptanthrin.[6] It has been reported to have various biological activities, such as antibacterial, antifungal, and anti-leishmanial,[3,5,7] and found to be a potent dual inhibitor of COX-2 and 5-LOX.[8] In recent years, tryptanthrin has attracted much attention as an aryl hydrocarbon receptor agonist and anticancer agent.[9,10] In our continuous chemical and pharmacological studies of natural alkaloids we have been interested in compounds possessing the tryptanthrin skeleton due to the aforementioned biological activities. We started with the investigation of the alkaloidal constituents of *Phaius mishmensis* (Orchidaceae), which is a native orchid of Taiwan.[11] This represents the first chemical and pharmacological investigation of this species.

In our preliminary biological screening, the crude MeOH extract of *P. mishmensis* showed significant cytotoxicity against human breast carcinoma (MCF-7), lung carcinoma (NCI-H460), and central nervous system carcinoma (SF-268) cell lines. Thus, successive column and preparative TLC separations of the active $CHCl_3$ solubles yielded eight indoloquinazolinones, including the new phaitanthrins A−E (**1**−**5**) and methylisatoid (**6**) and the known tryptanthrin (**7**)[12] and candidine (**8**).[13] In this paper, we report the structure elucidation of these new alkaloids by means of spectroscopic analysis, the synthesis of ketone adducts of tryptanthrin, and their cytotoxicities toward a panel of human cancer cell lines.

Phaitanthrin A (**1**), isolated as an optically active amorphous powder, has the molecular formula $C_{18}H_{14}N_2O_3$ based on the molecular ion peak at *m/z* 306.1006 in HREIMS. A broad IR band at 3320 cm^{-1} and two strong IR absorptions at 1710 and 1643 cm^{-1} indicated the presence of hydroxy, carbonyl, and amido functionalities, respectively. The ^1H NMR spectrum in combination with the data from COSY experiment revealed the presence of two *ortho*-disubstituted benzene rings, one at δ 7.56 (1H, t, $J = 7.8$ Hz, H-2), 7.78 (2H, m, H-3 and -4), 8.36 (1H, d, $J = 7.8$ Hz, H-1) and the other at δ 7.28 (1H, t, $J = 8.0$ Hz, H-8), 7.43 (1H, t, $J = 8.0$ Hz, H-9), 7.54 (1H, d, $J = 8.0$ Hz, H-7), 8.50 (1H, d, $J = 8.0$ Hz, H-10). From HMBC and HMQC experiments, it was apparent that carbons corresponding to two benzene rings as well as an amido (C-12) and an imino (C-5a) group at δ 159.7 and 159.8 were very close to tryptanthrin (**7**), except for the C-6 carbonyl signal, which was replaced by a saturated quaternary carbon resonating at δ 75.5 (Tables 1 and 2). A ^1H NMR signal at δ 5.68 (1H, s), which showed no HMQC correlation, was assignable to a hydroxy group. Additional ^1H and ^{13}C signals at δ_H 2.18 (3H, s, H-3′) and 3.34, 3.54 (each 1H, d, $J = 17.5$ Hz, H-1′); δ_C 30.8 (C-3′), 51.1 (C-1′), and 206.1 (C-2′) were characteristic of a $-CH_2COCH_3$ group. Thus the hydroxy and 2-oxopropyl groups were attached to C-6 in agreement with the HMBC correlations from H-7 to C-6, from H-3′ to C-1′ and C-2′, and from both H-1′ and 6-OH to C-5a, C-6, and C-6a as well as the NOE correlations between H-1′ and H-7 and between H-1′ and H-3′. On the basis of the above evidence, the structure of **1** was determined as 6-hydroxy-6-(2-oxopropyl)tryptanthrin, named phaitanthrin A.

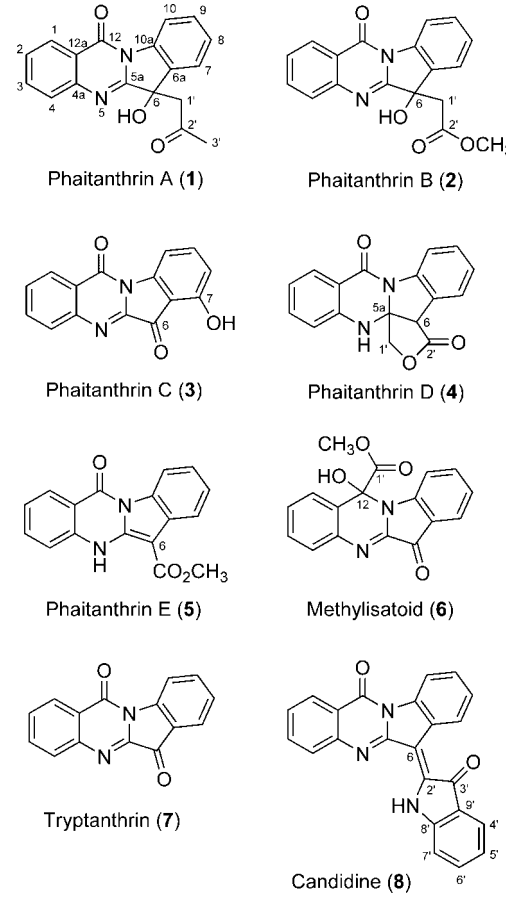

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Table 1. ^{1}H NMR Data (300 MHz, $CDCl_3$) for Phaitanthrins A−E (**1**−**5**), Methylisatoid (**6**), and Tryptanthrin (**7**) (*J* in Hz)

	1	2	3	4	5	6	7
H-1	8.36 d (7.8)	8.28 d (7.8)	8.45 d (8.0)	7.92 d (7.7)	8.11 d (7.6)	7.47 d (7.8)	8.42 d (7.9)
H-2	7.56 t (7.8)	7.50 m	7.69 t (8.0)	7.04 t (7.7)	7.39 t (7.6)	7.37 t (7.8)	7.66 t (7.9)
H-3	7.78 m	7.76 m	7.86 t (8.0)	7.46 t (7.7)	7.70 t (7.6)	7.43 t (7.8)	7.84 t (7.9)
H-4	7.78 m	7.76 m	8.03 d (8.0)	6.99 d (7.7)	8.13 d (7.6)	7.63 d (7.8)	8.02 d (7.9)
H-5				5.20 s	7.96 br s		
H-6				4.03 s			
H-7	7.54 d (8.0)	7.56 d (8.0)		7.69 d (7.7)	7.56 d (8.0)	7.78 d (7.8)	7.90 d (8.1)
H-8	7.28 t (8.0)	7.22 t (8.0)	6.88 d (8.2)	7.32 t (7.7)	7.15 t (8.0)	7.18 t (7.8)	7.42 t (8.1)
H-9	7.43 t (8.0)	7.32 t (8.0)	7.66 t (8.2)	7.46 t (7.7)	7.43 t (8.0)	7.59 t (7.8)	7.78 t (8.1)
H-10	8.50 d (8.0)	8.39 d (8.0)	8.02 d (8.2)	7.64 d (7.7)	6.88 d (8.0)	7.10 d (7.8)	8.62 d (8.1)
H-1′	3.34 d (17.5)	3.26 d (16.1)		4.10 d (9.6)			
	3.54 d (17.5)	3.35 d (16.1)		4.15 d (9.6)			
H-3′	2.18 s						
OCH_3		3.58 s (2′-OCH_3)			3.60 s (1′-OCH_3)	3.75 s (1′-OCH_3)	
OH	5.68 s (6-OH)	4.83 s (6-OH)	8.24 br s (7-OH)			5.14 br s (12-OH)	

Phaitanthrin B (**2**) was obtained as an optically active amorphous powder and analyzed for $C_{18}H_{14}N_2O_4$ by HREIMS, one oxygen atom more than that of **1**. Accordingly, the ^{1}H and ^{13}C NMR spectra of **2** showed resemblance to compound **1** with signals attributable to a 6-substituted 6-hydroxytryptanthrin moiety. The remaining ^{1}H and ^{13}C signals at δ_H 3.26 and 3.35 (d, J = 16.1 Hz, CH_2), 3.58 (s, OCH_3) and δ_C 42.7 (CH_2), 52.0 (OCH_3), 170.5 (C=O) were assigned to a $-CH_2CO_2CH_3$ group. The connectivity between aromatic and aliphatic moieties was revealed by analysis of the HMBC and NOESY spectra. For example, H-7 showed HMBC correlations with C-6, H-1′ and 6-OH with C-5a, C-6, and C-6a. Consequently, the structure of 6-hydroxy-6-(methoxycarbonyl)-methyltryptanthrin was confirmed for phaitanthrin B.

Phaitanthrin C (**3**) was isolated as an amorphous powder. The HREIMS showed a molecular ion at *m*/*z* 264.0533, consistent with the molecular formula $C_{15}H_8N_2O_3$. The ^{1}H and ^{13}C spectra of **3** showed a set of *ortho*-disubstituted benzene ring signals at δ 7.69 (1H, t, J = 8.0 Hz, H-2), 7.86 (1H, t, J = 8.0 Hz, H-3), 8.03 (1H, d, J = 8.0 Hz, H-4), and 8.45 (1H, d, J = 8.0 Hz, H-1) together with an amido carbon (C-12) at δ 158.0 and an imino carbon (C-5a) at δ 144.8 for a quinazolinone moiety as in tryptanthrin. The ^{1}H NMR spectrum also contained a set of three coupled aromatic resonances at δ 6.88 (1H, d, J = 8.2 Hz, H-8), 7.66 (1H, t, J = 8.2 Hz, H-9), and 8.02 (1H, d, J = 8.2 Hz, H-10) attributable to a trisubstituted benzene, along with a carbonyl signal (C-6) at δ 183.7 indicating an indolinone moiety. A hydroxy group at δ 8.24 was placed at C-7 on the basis of HMBC correlation from H-9 to an oxygenated carbon at δ 157.9 (C-7) and no HMBC correlation to C-6. This is also strongly supported by an upfield shift of C-6a to δ 108.9. Thus, the structure of 7-hydroxytryptanthrin was established for phaitanthrin C (**3**).

Phaitanthrin D (**4**) was isolated as an optically active amorphous powder. The HRFABMS showed a pseudomolecular ion at *m*/*z* 293.0927 $[M + H]^+$, corresponding to the molecular formula $C_{17}H_{12}N_2O_3$. A set of *ortho*-disubstituted benzene ring signals in the ^{1}H and ^{13}C NMR spectra [δ_H 6.99 (1H, d, J = 7.7 Hz, H-4), 7.04 (1H, t, J = 7.7 Hz, H-2), 7.46 (1H, t, J = 7.7 Hz, H-3), 7.92 (1H, d, J = 7.7 Hz, H-1); δ_C 112.5 (C-4), 121.3 (C-2), 122.4 (C-12a), 123.7 (C-1), 134.8 (C-3), 154.7 (C-4a)] and an aliphatic quaternary carbon at δ 66.2 (C-5a) combined with the HMBC correlations from H-1 to an amido C-12 (δ 164.6) and the NOE correlation between H-4 and NH (δ 5.20, H-5) indicated the presence of a dihydroquinazolinone unit in **4**. A second set of four mutually coupled aromatic protons resonated at δ 7.32 (1H, t, J = 7.7 Hz, H-8), 7.46 (1H, t, J = 7.7 Hz, H-9), 7.64 (1H, d, J = 7.7 Hz, H-10), and 7.69 (1H, d, J = 7.7 Hz, H-7). A methine group at δ_H 4.03 (s, H-6); δ_C 47.0 (C-6) as well as the HMBC correlations from H-6 to C-5a, C-6a (δ 113.8), C-7 (δ 127.1), and C-10a (δ 143.3) indicated the existence of an indoline unit fused to a dihydroquinazolinone to form a 5a,6-disubstituted tryptanthrin structure for **4**. The HMBC correlations from the diasterotropic methylene H-1′ at δ 4.10 and 4.15 to C-5a, C-6, and an ester carbonyl C-2′ (δ 176.2) and the HMBC correlations from H-6 to C-1′ and C-2′, together with the NOE correlation between NH and H-6, suggested a γ-lactone ring *cis*-fused to the tryptanthrin at C-5a and C-6. Therefore, the structure of phaitanthrin D is as depicted in **4**.

Phaitanthrin E (**5**) was isolated as an amorphous powder with the molecular formula $C_{17}H_{12}N_2O_3$ and a pseudomolecular ion peak at *m*/*z* 293.0923 $[M + H]^+$ in the HRFABMS. The aromatic region of the ^{1}H NMR and COSY spectra suggested two *ortho*-disubstituted benzene rings as in tryptanthrin. H-1 (δ 8.11) showed HMBC correlation with an amido C-12 (δ 160.6) and H-4 (δ 8.13) showed NOE correlation with an amino NH (δ 7.96, H-5), indicating the existence of a hydroquinazolinone unit in **5**. However, H-7 (δ 7.56) exhibited HMBC correlation with olefinic C-6 at δ 90.4 instead of carbonyl in tryptanthrin, indicating the presence of an indole unit. A methoxycarbonyl group (δ_H 3.60; δ_C 54.2 and 167.6) was placed at C-6 on the basis of a downfield shift of C-5a (δ 147.7) and an upfield shift of C-6 (δ 90.4) by assuming that the double bond between C-5a and C-6 is in conjugation with a carbonyl group and two nitrogen atoms. Consequently, the structure of 6-methoxycarbonyl-5-hydroindolo[2,1-*b*]quinazolin-12-one was assigned to phaitanthrin E (**5**).

Compound **6** was obtained as an optically active amorphous powder and confirmed to have the molecular formula $C_{17}H_{12}N_2O_4$ by HREIMS data. By comparison of ^{1}H and ^{13}C NMR spectra of **6** with those of tryptanthrin, it was evident that an oxygenated quaternary carbon (δ 81.7) replaced the carbonyl group at C-12. Two substituents, a hydroxy (δ 5.14) and a methoxycarbonyl (δ_H 3.75; δ_C 54.5 and 170.7), were attached to C-12 on the basis of the HMBC correlations from H-1 (δ 7.47) to C-12 (δ 81.7) and from 12-OH to C-1′ (δ 170.7) and the NOE correlations from both H-1 and H-10 to 1′-OCH_3 and 12-OH. Thus, the structure of **6** was determined to be 12-hydroxy-12-methoxycarbonyltryptanthrin, which is known as methylisatoid. Although methylisatoid (**6**) has been synthesized by Cornforth,[14] this represents the first isolation as a pure compound from a natural source.

The absolute configuration of compounds **1**, **2**, **4**, and **6** has not been determined. Our attempts to synthesize a pair of diastereomeric esters by acylating **1** with (+)-α-methoxy-α-trifluoromethylphenylacetyl chloride [(+)-MTPACl],[15] even with simple acetyl chloride, were unsuccessful. This most likely is due to steric inhibition of the tertiary alcohol, which is present in the tryptanthrin skeleton. We tried unsuccessfully to analyze the C-6 chemical shift behaviors using the chiral shift reagent tris[3-trifluoroacetyl-*d*- and *l*-camphorato]europium(III) [(*R*)- and (*S*)-Eu(tfc)$_3$].[16]

The eight indoloquinazolinones isolated from a $CHCl_3$ extract of *P. mishmensis* were subjected to cytotoxic evaluation against MCF-7, NCI-H460, and SF-268 cell lines (Table 3). Tryptanthrin (**7**) showed moderate cytotoxicity against MCF-7, NCI-H460, and SF-268 cell lines with IC_{50} values of 11.1, 9.0, and 24.4 μM,

Table 2. ^{13}C NMR Data (75 MHz, $CDCl_3$) for Phaitanthrins A−E (**1**-**5**), Methylisatoid (**6**), and Tryptanthrin (**7**)

	1	2	3	4	5	6	7
C-1	126.9	127.0	127.6	123.7	129.9	125.5	127.5
C-2	127.3	127.5	130.4	121.3	126.5	129.1	130.2
C-3	134.3	134.4	135.2	134.8	134.7	131.0	135.1
C-4	127.6	127.8	130.7	112.5	124.4	129.4	130.7
C-4a	147.1	147.1	146.5	154.7	138.6	139.6	146.6
C-5a	159.7[a]	159.3	144.8	66.2	147.7	143.5	144.3
C-6	75.5	75.3	183.7	47.0	90.4	183.7	182.6
C-6a	132.1	131.4	108.9	113.8	113.2	120.4	121.9
C-7	123.2	123.6	157.9	127.1	126.9	125.5	125.4
C-8	126.9	127.0	115.6	128.1	123.7	123.7	127.2
C-9	130.3	130.7	141.2	129.8	132.0	137.5	138.3
C-10	116.7	117.0	109.6	128.1	114.5	111.8	118.0
C-10a	138.9	139.0	144.2	143.3	134.2	148.7	146.3
C-12	159.8[a]	159.6	158.0	164.6	160.6	81.7	158.1
C-12a	121.6	121.9	123.5	122.4	119.6	123.9	123.7
C-1′	51.1	42.7		76.0	167.6	170.7	
C-2′	206.1	170.5		176.2			
C-3′	30.8						
OCH_3		54.5			54.2	54.5	

[a] Assignments are interchangeable.

Table 3. Cytotoxicity of Tryptanthrins **1**−**13** toward Three Cancer Lines[a]

	IC_{50} (μM)		
compound	MCF-7	NCI-H460	SF-268
1	33.8 ± 3.3	27.0 ± 1.6	43.9 ± 1.0
1 (synthesized)	28.2 ± 0.3	31.4 ± 5.9	45.4 ± 1.1
2	>50	>50	>50
3	>50	>50	>50
4	>50	>50	>50
5	>50	>50	>50
6	>50	>50	>50
7	11.1 ± 0.8	9.0 ± 0.8	24.4 ± 0.5
8	>50	>50	>50
9	40.5 ± 6.2	42.8 ± 1.5	>50
10	>50	>50	>50
11	19.7 ± 4.4	19.1 ± 1.6	34.8 ± 2.2
12	20.7 ± 0.5	20.6 ± 0.3	33.9 ± 1.3
13	15.8 ± 0.6	13.9 ± 1.7	24.8 ± 1.7

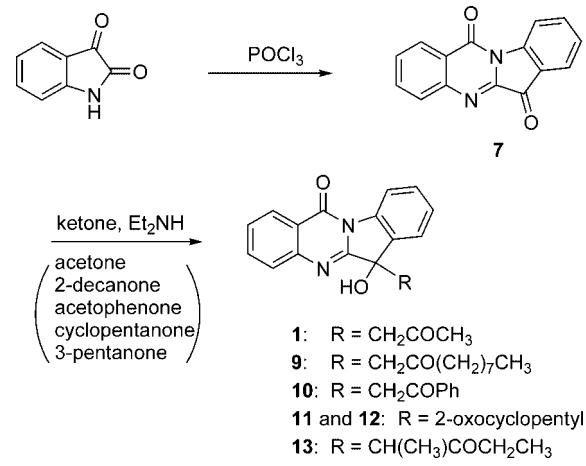
[a] Values were mean ± SD (n = 3−8). MCF-7 = human breast tumor cell line. NCI-H460 = human lung tumor cell line. SF-268 = human central nervous system tumor cell line.

respectively, whereas phaitanthrin A (**1**) showed activity with IC_{50} values of 33.8, 27.0, and 43.9 μM, respectively. Hence, the carbonyl functionality at C-6 and C-12 might be important for activity. However, the antimigratory effects of these compounds have not yet been analyzed.[17]

Tryptanthrin (**7**) has been reported to possess *in vitro* cytotoxicity against human cancer lines.[3,10] It has limited solubility in commonly used solvents. In the case of phaitanthrin A (**1**), an aldol adduct of tryptanthrin with acetone, **1** has better solubility but slightly lower activity than **7**. Therefore, attempts were made to synthesize a series of ketone adducts for the evaluation of their cytotoxicity.

We synthesized tryptanthrin from commercially available isatin and $POCl_3$. The mixed aldol reaction between a variety of ketones and tryptanthrin in the presence of base produced the adducts **9**−**13** (Scheme 1). Their cytotoxic results are shown in Table 3. The synthetic racemic **1** exhibited almost the same activity as the naturally isolated phaitanthrin A (**1**), indicating that the cytotoxicity is independent of the stereogenicity at C-6. Compounds **11** and **12** were racemic diastereomers obtained from tryptanthrin and cyclopentanone and were separated by Si gel column. Compound **11** was crystallized from CH_2Cl_2−MeOH. Its complete structure and the relative configuration were determined as an enantiomeric mixture of (6*R*,1′*S*)- and (6*S*,1′*R*)-6-hydroxy-6-(2-oxocyclopentyl)-indolo[2,1-*b*]quinazolin-12-one by single-crystal X-ray diffraction study (Figure 1). Consequently, compound **12** was the other

Scheme 1



enantiomeric mixture of (6*R*,1′*R*) and (6*S*,1′*S*). Almost identical activity of pure **11** and **12** was observed. Compound **13**, a mixture of two diastereomeric adducts of tryptanthrin and 3-pentanone, showed cytotoxicity very close to tryptanthrin. This suggested that the presence of tertiary carbon C-1′ increased the anticancer activity. The steric hindrance of diisopropyl ketone inhibited the addition with tryptanthrin. Therefore, no study of the effect of a quaternary carbon at C-1′ on the cytotoxicity was made.

Experimental Section

General Experimental Procedures. Melting points were recorded on a Yanaco MP-3 melting point apparatus and are not corrected. Optical rotations were measured on a Jasco DIP-370 digital polarimeter. UV spectra were recorded on an Agilent 8453 spectrophotometer. IR spectra were recorded on a Nicolet Magna FT-IR spectrophotometer. NMR spectra were recorded on Bruker Avance-300, AMX-400, and Avance-500 FT-NMR spectrometers; all chemical shifts were given in ppm from TMS as an internal standard. Mass spectra were obtained on a VG 70-250S spectrometer by a direct inlet system.

Plant Material. Whole plants of *Phaius mishmensis* were collected from Nantou Hsien, Taiwan, in October 2003. The collection was authenticated by Professor C. S. Kuoh, Department of Life Sciences, National Cheng Kung University, Tainan, Taiwan. A voucher specimen (No. PLW-0304) was deposited in the Herbarium of National Cheng Kung University, Tainan, Taiwan.

Extraction and Isolation. The dried *P. mishmensis* plants (3.5 kg) were extracted with MeOH (7 × 8 L) under reflux. The combined extract was concentrated under reduced pressure to give a dark brown syrup. The syrup was suspended in H_2O and then partitioned succes-

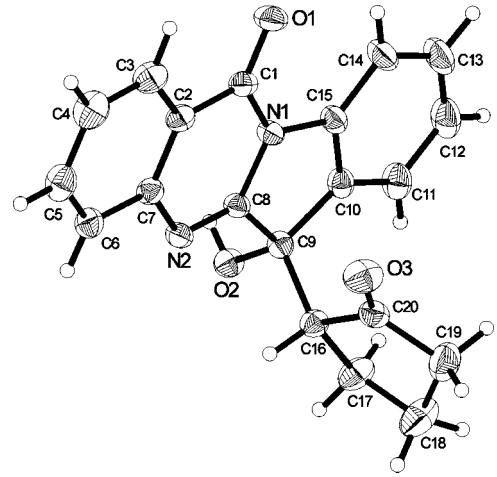


Figure 1. ORTEP plot of molecule of **11** drawn with the thermal ellipsoids at the 30% probability level. Small circles represent hydrogen atoms.

sively with hexanes, $CHCl_3$, and EtOAc. The $CHCl_3$ extract showed strong activity against MCF-7, NCI-H460, and SF-268 cell lines. Therefore the $CHCl_3$ extract (14.7 g) was chromatographed on a Si gel column by eluting with a gradient of $CHCl_3$−MeOH (20:1) to MeOH to yield seven fractions. Fraction 2 was chromatographed on Si gel eluting with pure $CHCl_3$ to yield **1** (30 mg) and **8** (10 mg). Fraction 3 was chromatographed on Si gel using hexanes−acetone (4:1) as eluent to obtain **6** (2 mg), **4** (12 mg), and **5** (2 mg). Fractions 4 and 5 were separately chromatographed on Si gel using $CHCl_3$−MeOH (50:1) as eluent to give **3** (3 mg) and **2** (4 mg), respectively. Owing to the low solubility of **7** in organic solvents, it appeared in each fraction, and the combined amount was 0.35 g.

Phaitanthrin A (1): white, amorphous powder; $[\alpha]_D$ −25.1 (*c* 0.03, $CHCl_3$); UV ($CHCl_3$) λ_{max} (log ϵ) 263 (3.18), 302 (2.92), 316 (3.03), 330 (3.01) nm; IR (KBr) ν_{max} 3320, 1710, 1643, 1602 cm^{-1}; EIMS *m/z* 306 $[M]^+$ (16), 262 (10), 249 (100), 220 (15), 192 (9), 149 (8), 130 (10), 57 (23); HREIMS *m/z* 306.1006 $[M]^+$ (calcd for $C_{18}H_{14}N_2O_3$, 306.1004); ^1H and ^{13}C NMR data, Tables 1 and 2.

Phaitanthrin B (2): white, amorphous powder; $[\alpha]_D$ −3.0 (*c* 0.21, $CHCl_3$); UV ($CHCl_3$) λ_{max} (log ϵ) 259 (2.77), 334 (2.57) nm; IR (KBr) ν_{max} 3356, 1738, 1685, 1663, 1604 cm^{-1}; EIMS *m/z* 322 $[M]^+$ (20), 294 (13), 249 (100); HREIMS *m/z* 322.0951 $[M]^+$ (calcd for $C_{18}H_{14}N_2O_4$, 322.0954); ^1H and ^{13}C NMR data, Tables 1 and 2.

Phaitanthrin C (3): orange, amorphous powder; UV ($CHCl_3$) λ_{max} (log ϵ) 220 (4.11), 242 (4.08), 320 (3.43), 420 (3.38) nm; IR (KBr) ν_{max} 3440, 1698, 1674, 1597 cm^{-1}; EIMS *m/z* 264 $[M]^+$ (11), 247 (15), 117 (100); HREIMS *m/z* 264.0533 $[M]^+$ (calcd for $C_{15}H_8N_2O_3$, 264.0534); ^1H and ^{13}C NMR data, Tables 1 and 2.

Phaitanthrin D (4): yellow, amorphous powder; $[\alpha]_D$ −6.9 (*c* 0.26, $CHCl_3$); UV (MeOH) λ_{max} (log ϵ) 204 (3.32), 236 (3.22), 284 (2.85), 402 (2.85) nm; IR (KBr) ν_{max} 3349, 1780, 1633, 1614, 1592 cm^{-1}; FABMS *m/z* 293 $[M + H]^+$ (3), 167 (17), 149 (100); HRFABMS *m/z* 293.0927 $[M + H]^+$ (calcd for $C_{17}H_{13}N_2O_3$, 293.0927); ^1H and ^{13}C NMR data, Tables 1 and 2.

Phaitanthrin E (5): white, amorphous powder; UV (CH_3OH) λ_{max} (log ϵ) 216 (4.59), 288 (3.64) nm; IR (KBr) ν_{max} 3307, 1756, 1713, 1650, 1611 cm^{-1}; FABMS *m/z* 293 $[M + H]^+$ (7), 167 (18), 154 (100); HRFABMS *m/z* 293.0923 $[M + H]^+$ (calcd for $C_{17}H_{13}N_2O_3$, 293.0926); ^1H and ^{13}C NMR data, Tables 1 and 2.

Methylisatoid (6): orange, amorphous powder; $[\alpha]_D$ −8.0 (*c* 0.07, $CHCl_3$); UV ($CHCl_3$) λ_{max} (log ϵ) 201 (3.11), 261 (3.18), 356 (2.99), 450 (3.02) nm; IR (KBr) ν_{max} 3176, 1760, 1725, 1644, 1606, 1593 cm^{-1}; EIMS *m/z* 308 $[M]^+$ (3), 249 (100), 221 (55), 146 (19), 130 (23), 102 (14); HREIMS *m/z* 308.0796 $[M]^+$ (calcd for $C_{17}H_{12}N_2O_4$, 308.0797); ^1H and ^{13}C NMR data, Tables 1 and 2.

Tryptanthrin (7): yellow needles ($CHCl_3$); mp 260−261 °C (lit.[18] 260−261 °C); UV ($CHCl_3$) λ_{max} (log ϵ) 253 (3.69), 313 (0.49), 335 (0.46), 399 (0.42) nm; IR (KBr) ν_{max} 1725, 1684 cm^{-1}; EIMS *m/z* 248 $[M]^+$ (100), 220 (20), 192 (25); HREIMS *m/z* 248.0587 $[M]^+$ (calcd for $C_{15}H_8N_2O_2$, 248.0586); ^1H and ^{13}C NMR data, Tables 1 and 2.

Candidine (8): purple needles ($CHCl_3$);mp 267−268 °C (lit.[19] 269−270 °C); UV ($CHCl_3$) λ_{max} (log ϵ) 250 (4.16), 284 (4.00), 574 (3.88) nm; IR (KBr) ν_{max} 3225, 1688, 1675, 1627, 1606 cm^{-1}; EIMS *m/z* 363 $[M]^+$ (100), 335 (43), 259 (24); HREIMS *m/z* 363.1006 $[M]^+$ (calcd for $C_{23}H_{13}N_3O_2$, 363.1008); ^1H NMR ($CDCl_3$, 300 MHz) δ 7.06 (1H, t, J = 7.4 Hz, H-5′), 7.10 (1H, d, J = 7.4 Hz, H-7′), 7.41 (1H, t, J = 7.6 Hz, H-8), 7.49 (1H, t, J = 7.6 Hz, H-9), 7.53 (1H, t, J = 7.9 Hz, H-2), 7.55 (1H, t, J = 7.4 Hz, H-6′), 7.77 (1H, d, J = 7.4 Hz, H-4′), 7.82 (1H, m, H-3 and -4), 8.44 (1H, d, J = 7.9 Hz, H-1), 8.68 (1H, d, J = 7.6 Hz, H-10), 9.24 (1H, d, J = 7.6 Hz, H-7), 11.73 (1H, br s, H-1′); ^{13}C NMR ($CDCl_3$, 75 MHz) δ 107.7 (C-6), 112.2 (C-7′), 116.5 (C-10), 120.4 (C-9′), 120.7 (C-12a), 122.0 (C-5′), 124.0 (C-6a), 125.4 (C-4′ and −7), 126.5 (C-8), 127.2 (C-1, −2, and −4), 129.6 (C-9), 134.3 (C-3), 136.7 (C-6′), 137.7 (C-10a), 138.5 (C-5a), 146.8 (C-4a), 150.8 (C-8′), 154.4 (C-2′), 159.1 (C-12), 187.4 (C-3′).

Synthesis of Ketone Adduct of Tryptanthrin. The tryptanthrin was prepared according to Moskovkina's method.[18] Then, 5 mL of ketone (acetone, 2-decanone, acetophenone, cyclopentanone, or 3-pentanone) was added to tryptanthrin (1 mmol). Diethylamine (3 mmol) was added to the mixture. The resulting suspension was stirred at room temperature for 1 day.[20] The clear solution was directly chromatographed on a Si gel column by eluting with CH_2Cl_2 to give pure ketone adducts **9**−**13**.

6-Hydroxy-6-(2-oxodecyl)indolo[2,1-*b*]quinazolin-12-one (9, a 2-decanone adduct of tryptanthrin): colorless syrup; 66% yield; IR (film) ν_{max} 3418, 1655, 1600 cm^{-1}; EIMS *m/z* 404 $[M]^+$ (3), 263 (11), 249 (100), 220 (12), 192 (10), 130 (17), 102 (14); HRESIMS *m/z* 405.2176 $[M + H]^+$ (calcd for $C_{25}H_{29}N_2O_3$, 405.2178); ^1H NMR ($CDCl_3$, 300 MHz) δ 0.85 (3H, t, J = 7.2 Hz), 1.25 (10H, m), 1.46 (2H, quintet, J = 7.2 Hz), 2.37 (2H, t, J = 7.2 Hz), 3.36 and 3.49 (each 1H, d, J = 17.2 Hz), 5.06 (1H, br s), 7.16 (1H, t, J = 8.0 Hz), 7.25 (1H, t, J = 8.0 Hz), 7.48 (2H, m), 7.73 (2H, m), 8.24 (1H, d, J = 8.0 Hz), 8.34 (1H, d, J = 8.0 Hz); ^{13}C NMR ($CDCl_3$, 75 MHz) δ 13.9, 22.4, 23.2, 27.3, 28.7, 28.9, 29.1, 31.6, 43.2, 50.5, 75.2, 116.2, 121.1, 122.8, 126.8 (2 x C), 126.9, 127.4, 129.8, 132.2, 134.1, 138.5, 147.0, 159.8, 159.9, 207.9.

6-Hydroxy-6-(benzoylmethyl)indolo[2,1-*b*]quinazolin-12-one (10, an acetophenone adduct of tryptanthrin): yellowish, amorphous powder; 58% yield; IR (KBr) ν_{max} 3435, 1644 cm^{-1}; EIMS *m/z* 368 $[M]^+$ (6), 249 (78), 220 (18), 192 (16), 130 (30), 120 (33), 105 (92), 77 (100); HRESIMS *m/z* 369.1237 $[M + H]^+$ (calcd for $C_{23}H_{17}N_2O_3$, 369.1239); ^1H NMR ($CDCl_3$, 300 MHz) δ 3.95 and 4.16 (each 1H, d, J = 17.7 Hz), 4.70 (1H, br s), 7.23 (1H, t, J = 7.8 Hz), 7.41 (3H, m), 7.52 (3H, m), 7.73 (2H, m), 7.87 (2H, m), 8.35 (1H, d, J = 7.8 Hz), 8.52 (1H, d, J = 7.8 Hz); ^{13}C NMR ($CDCl_3$, 75 MHz) δ 46.6, 76.7, 117.2, 122.1, 123.6, 126.9, 127.0, 127.4, 127.8, 128.1, 128.7, 130.5, 132.3, 133.9, 134.4, 136.2, 139.4, 147.2, 159.7 (2 × C), 197.7.

6-Hydroxy-6-(2-oxocyclopentyl)indolo[2,1-*b*]quinazolin-12-one (11 and 12, a cyclopentanone adduct of tryptanthrin): mixture of two diastereomers in a 3:2 ratio; white, amorphous powder; 62% yield; IR (KBr) ν_{max} 3418, 1651 cm^{-1}; EIMS *m/z* 332 $[M]^+$ (6), 249 (100), 220 (19), 192 (25), 130 (49), 102 (59); HRESIMS *m/z* 333.1238 $[M + H]^+$ (calcd for $C_{20}H_{17}N_2O_3$, 333.1239). Due to the instability of the product, the mixture was quickly chromatographed on a Si gel column and eluted with CH_2Cl_2−Et_2O (20:1) to give some pure **11** and **12** for spectral and cytotoxic analyses. Enantiomeric mixture of (6*R*,1′*S*)- and (6*S*,1′*R*)-**11**: ^1H NMR ($CDCl_3$, 300 MHz) δ 1.21 (2H, m), 1.79 (2H, m), 2.40 (2H, m), 3.00 (1H, dd, J = 9.9, 9.2 Hz), 5.30 (1H, br s), 7.37 (1H, t, J = 7.8 Hz), 7.50 (1H, t, J = 7.8 Hz), 7.56 (2H, m), 7.75 (2H, m), 8.39 (1H, d, J = 7.8 Hz), 8.52 (1H, d, J = 7.8 Hz); ^{13}C NMR ($CDCl_3$, 75 MHz) δ 20.4, 25.3, 39.9, 53.1, 79.7, 117.1, 122.0, 123.9, 126.9, 127.1, 127.6, 128.0, 130.6, 130.9, 134.5, 139.3, 147.0, 159.3, 159.4, 220.9. Enantiomeric mixture of (6*R*,1′*R*)- and (6*S*,1′*S*)-**12**: ^1H NMR ($CDCl_3$, 300 MHz) δ 1.02 (1H, m), 1.80 (3H, m), 2.14 (1H, m), 2.52 (1H, dd, J = 18.5, 6.6 Hz), 3.31 (1H, dd, J = 11.6, 8.1 Hz), 5.79 (1H, br s), 7.31 (1H, t, J = 8.0 Hz), 7.36 (1H, d, J = 8.0 Hz), 7.52 (1H, t, J = 8.0 Hz), 7.57 (1H, t, J = 8.0 Hz), 7.80 (1H, t, J = 8.0 Hz), 7.87 (1H, d, J = 8.0 Hz), 8.40 (1H, d, J = 8.0 Hz), 8.58 (1H, d, J = 8.0 Hz); ^{13}C NMR ($CDCl_3$, 75 MHz) δ 20.2, 25.2, 39.7, 54.9, 79.4, 117.4, 122.0, 123.7, 127.0, 127.1, 127.8, 128.1, 130.7, 131.0, 134.6, 139.6, 147.2, 157.9, 159.5, 222.6.

X-ray Crystallographic Data of 11. Colorless single crystals of **11** suitable for X-ray diffraction study were grown by recrystallization from CH_2Cl_2−MeOH. Data were obtained on a Siemens Smart CCD 1000 diffractometer with graphite-monochromated Mo Kα radiation, operating at 50 kV and 35 mA at 296 K, over a 2θ range of 5.06−56.62°. Data were processed on a Pentium III PC using the Bruker AXS SHELXTL, NT software package. Neutral atom scattering factors were taken from Cromer and Waber. 6-Hydroxy-6-(2-oxocyclopentyl)-indolo[2,1-*b*]quinazolin-12-one (**11**): $C_{20}H_{16}N_2O_3$, M_r = 332.35; crystal size 0.30 × 0.25 × 0.25 mm^3; monoclinic, space group $P2(1)/n$; unit cell dimensions: a = 12.1325(19) Å, b = 10.4626(17) Å, c = 12.994(2) Å, α = 90°, β = 103.455(2)°, γ = 90°; volume: 1604.2(4) $Å^3$; Z = 4; D_c = 1.376 Mg/m^3. The structures were refined by full-matrix least-squares on F^2 using SHELEXL-97 (Sheldrick, 1997). Final discrepancy indices of R_1 = 0.0490, wR_2 = 0.1434 and GOOF = 1.091 for observed data with $I > 2\sigma(I)$. Crystallographic data for the structure **11** (CCDC-675761) reported in this paper have been deposited with the Cambridge Crystallographic Data Centre. Copies of the data can be obtained, free of charge, via the Internet at http://www.ccdc.cam.ac.uk/conts/retrieving.html (or from the CCDC, 12 Union Road, Cambridge CB2 1EZ, U.K.; fax: +44 1223 336033; e-mail: deposit@ccdc.cam.ac.uk).

6-Hydroxy-6-(3-oxopentan-2-yl)indolo[2,1-*b*]quinazolin-12-one (13, a 3-pentanone adduct of tryptanthrin): mixture of two diastereomers in a 2:1 ratio; white, amorphous powder; 68% yield; IR (KBr) ν_{max} 3402, 1661, 1602 cm^{-1}; EIMS *m/z* 334 $[M]^+$ (2), 249 (100), 220 (12), 192 (13), 130 (30), 102 (27); HRESIMS *m/z* 335.1397 $[M + H]^+$ (calcd

for $C_{20}H_{19}N_2O_3$, 335.1396); TLC eluting with hexanes−THF (3:1) afforded the two diastereomers. Major compound: ^{1}H NMR ($CDCl_3$, 300 MHz) δ 1.08 (3H, d, J = 7.2 Hz), 1.10 (3H, t, J = 7.2 Hz), 2.61 (2H, m), 3.46 (1H, q, J = 7.2 Hz), 4.99 (1H, s), 7.25 (1H, t, J = 7.7 Hz), 7.38 (1H, t, J = 8.1 Hz), 7.53 (1H, m), 7.62 (1H, d, J = 7.7 Hz), 7.77 (2H, m), 8.33 (1H, d, J = 7.7 Hz), 8.47 (1H, d, J = 8.1 Hz); ^{13}C NMR ($CDCl_3$, 75 MHz) δ 7.5, 12.1, 37.0, 51.5, 79.6, 116.9, 121.8, 125.5, 126.8, 127.0, 127.5, 127.9, 130.4, 130.5, 134.5, 139.2, 147.0, 159.5, 159.7, 214.8. Minor compound: ^{1}H NMR ($CDCl_3$, 300 MHz) δ 1.05 (3H, t, J = 7.0 Hz), 1.29 (3H, d, J = 7.4 Hz), 2.40 and 2.62 (each 1H, dq, J = 18.6, 7.0 Hz), 3.28 (1H, q, J = 7.4 Hz), 5.17 (1H, s), 7.32 (1H, t, J = 7.7 Hz), 7.49 (3H, m), 7.75 (2H, m), 8.38 (1H, d, J = 7.7 Hz), 8.54 (1H, d, J = 7.7 Hz); ^{13}C NMR ($CDCl_3$, 75 MHz) δ 7.4, 11.5, 35.5, 52.2, 79.6, 117.2, 122.1, 124.0, 126.9, 127.0, 127.6, 127.9, 130.6, 131.5, 134.5, 139.6, 147.0, 159.5, 159.6, 214.8.

Cytotoxicity Assay. The cytotoxicity assay was carried out according to the procedure described previously.[21]

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