

Activity of Macrocyclic Jatropha Diterpenes from *Euphorbia kansui* in a TrkA Fibroblast Survival Assay

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Three new macrocyclic diterpenes, kansuinins F (**1**), G (**2**), and H (**3**), together with four known jatropha diterpenes, kansuinins D (**4**), E (**5**), and A (**6**) and $3\beta,5\alpha,7\beta,15\beta$ -tetraacetoxy-9 α -nicotinoyloxyjatropha-6(17)-11 E -dien-14-one, were isolated from the roots of *Euphorbia kansui*. Compounds **1** and **2** were assigned as 6(17)-en-11,12-epoxy-14-one-type jatropha diterpenes, and compound **3** as a 6(17)-en-11,14-epoxy-12-one jatropha diterpene. The structures of compounds **1–3** and the relative configurations of compounds **4** and **5** were determined by spectral data analysis. Kansuinin E (**5**) exhibited a specific survival effect on fibroblasts that expressed TrkA, a high-affinity receptor for nerve growth factor.

Euphorbia kansui L. (Euphorbiaceae) is a well-known Chinese herbal medicine that is poisonous due to the presence of ingenane esters. After decreasing the toxicity of these diterpenes using traditional methods, it is utilized as a remedy for ascites and edema in mainland China.^{1,2} In 1975, Hirata and Uemura reported the presence of the macrocyclic diterpenes kansuinins A and B from the roots of *E. kansui*.^{3,4} Recently, the structure of kansuinin A (**6**) has been revised using HMBC NMR spectra.^{5,6} Three jatropha diterpenes, kansuinins C–E, 13 ingenol diterpenes, and four euphane-type triterpenes were reported from this plant.^{5,7,8} In the present work, we have isolated three new jatropha diterpenes from the petroleum ether extract of *E. kansui*, namely, kansuinins F (**1**), G (**2**), and H (**3**), along with four known jatropha diterpenes, kansuinins D (**4**), E (**5**), and A (**6**) and $3\beta,5\alpha,7\beta,15\beta$ -tetraacetoxy-9 α -nicotinoyloxyjatropha-6(17),11 E -dien-14-one.⁹

Nerve growth factor (NGF) is one of the neurotrophins that supports the survival and differentiation of a variety of neurons, such as the cholinergic neurons of the basal forebrain. In Alzheimer's disease patients, the basal forebrain cholinergic neurons have been reported to undergo severe degeneration.¹⁰ Promising results have been obtained for NGF as the prime candidate for its neuroprotective effects in the animal models of neurodegenerative diseases. However, difficulties encountered in the delivery of NGF protein to the brain and poor bioavailability at the desired target sites have severely hampered the progress of clinical trials involving neurotrophic factors.¹¹ Therefore, studies on small molecules that mimic or induce NGF activity may shed light on the potential treatment for this disease. Kansuinin A (**6**) has previously been reported to induce NGF production in L-M cells.¹² The actions of NGF are mediated through its high-affinity receptor, TrkA. To investigate whether the compounds isolated from *E. kansui* can activate TrkA signaling, their survival effects on TrkA-expressing fibroblasts were studied.

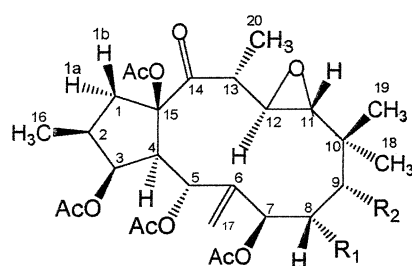
Results and Discussion

Compound **1**, obtained as colorless needles, showed a sodiated molecular ion $[M + Na]^+$ at m/z 799.2938 (calcd

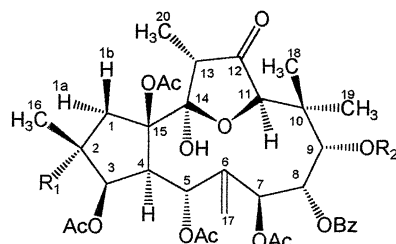
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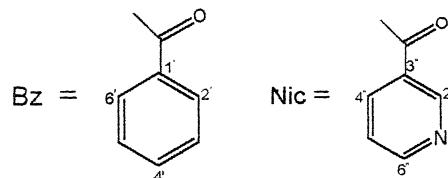
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	R ₁	R ₂
1	OBz	OBz
2	H	ONic
5	OBz	ONic



	R ₁	R ₂
3	OH	Ac
4	H	Nic
6	H	Ac



for 799.2936) in the HRFTICRMS, corresponding to the molecular formula $C_{42}H_{48}O_{14}$. The 1H NMR and DEPT spectra showed the presence of two benzoyl groups [δ_H 8.04 (2H), 7.41 (2H), 7.53 (1H), δ_C 164.6 (CO), 129.7, 130.0, 130.0, 128.4, 128.4, 133.7 and δ_H 8.02 (2H), 7.52 (2H), 7.57 (1H), δ_C 164.9 (CO), 129.5, 130.0, 130.0, 128.7, 128.7, 133.7] and four acetyl groups (δ_H 2.09, 1.56, 2.06, 2.06). According to the ^{13}C NMR, DEPT, and HSQC spectra, the remaining signals consisted of 20 carbons: four methyls, one methyl-

ene, one exocyclic methylene (δ_{H} 5.06, 4.89; δ_{C} 111.6, 143.4), 10 methines, three quaternary carbons, and one carbonyl (δ_{C} 216.2). Analysis of the ^1H - ^1H COSY spectrum (Table S1, Supporting Information) showed the structural fragment $\text{CH}_2\text{CH}(\text{CH}_3)\text{CH}(\text{OR})\text{CHCH}(\text{OR})\text{C}=\text{CH}_2$. In the HMBC spectrum (Table S2, Supporting Information), H-1a correlated to C-2, C-3, C-14, and C-15; H-1b correlated to C-15; H-2 correlated to C-16; and H-3 correlated to C-1 and C-15, which helped reveal the presence of a pentacyclic carbon skeleton.

The ^1H - ^1H COSY spectrum of **1** revealed a further partial structural fragment, $\text{CH}(\text{CH}_3)\text{CH}(\text{O})\text{CH}(\text{O})\text{C}(\text{CH}_3)_2$, and in the HMBC spectrum, H-9 correlated to C-10, C-11, C-18, and C-19; H-11 correlated to C-10, C-12, C-13, C-18, and C-19; H-12 correlated to C-13 and C-20; and H-13 correlated to C-11, C-12, C-14, and C-20, respectively. The chemical shift values of H-11 [δ_{H} 3.36 (1H, d, $J = 2.0$ Hz)] and H-12 [δ_{H} 3.28 (1H, dd, $J = 7.6, 2.0$ Hz)] showed that the epoxy group must be between C-11 and C-12.^{8,13} From the above data, it was deduced that the carbon skeleton of kansuinin F (**1**) is the same as that of several known compounds from *Euphorbia salicifolia*, which were assigned as 6(17)-en-11,12-epoxy-14-one jatrophone-type diterpenes.¹³ While these compounds from *E. salicifolia* have an isobutanoyl group, kansuinin F (**1**) has two benzoyl groups that were assigned to C-8 and C-9, respectively. The correlations between the acetate carbonyl [CO-3 (δ_{C} 169.7), CO-5 (δ_{C} 168.7), CO-7 (δ_{C} 169.9)] and H-3 (δ_{H} 5.46), H-5 (δ_{H} 5.57), and H-7 (δ_{H} 4.84) demonstrated that these three acetyl groups could be placed at C-3, C-5, and C-7, respectively. The stereochemistry of C-4 and C-15 was also established from the observation that all jatrophone diterpenes subjected to X-ray analysis exhibited a *trans* ring junction and because no NOE was observed between H-4 and OAc-15.¹⁴ A convenient point of reference was H-4, with an α configuration.¹⁵ The zero coupling constant ($J_{4,5} = 0$ Hz) and the observation of a NOE between H-4 and H-5 required that H-5 has a β configuration.^{13,14} In the NOESY spectrum (Table S3, Supporting Information), the NOE correlations between H-5 and H-8, H-11, and H-13 revealed that H-8, H-11, and H-13 are β -oriented. H-8 showed a NOE correlation with H-9, indicating that H-9 was also in the β configuration. In turn, H-4 had NOE correlations with H-2 and H-3, suggesting that H-3 was in an α configuration, while H-7 had a NOE correlation with H-4, implicating an α configuration for H-7. Therefore, the structure of compound **1** was characterized as 3 β ,5 α ,7 β ,15 β -tetraacetoxy-8 α ,9 α -dibenzoyloxy-11 α ,12 β -epoxyjatropha-6(17)-en-14-one.

Compound **2**, obtained as colorless needles, showed a molecular ion $[\text{M} + \text{Na}]^+$ at m/z 680.2714 (calcd for 680.2717) in the HRFTICRMS, corresponding to the molecular formula $\text{C}_{34}\text{H}_{43}\text{O}_{12}\text{N}$. The ^1H NMR and DEPT spectra showed the presence of one nicotinoyl group [δ_{H} 9.17 (1H), 8.28 (1H), 7.41 (1H), 8.77 (1H); δ_{C} 163.2 (CO), 150.1, 126.2, 123.6, 137.4, 153.0] and four acetyl groups (δ_{H} 2.07, 2.15, 1.47, 2.06). According to the ^{13}C NMR, DEPT, and HSQC spectra, the remaining signals again consisted of 20 carbons: four methyls, two methylenes, one exocyclic methylene (δ_{H} 5.28, 5.22; δ_{C} 114.5, 138.9), nine methines, three quaternary carbons, and one ketone (δ_{C} 216.2). The carbon skeleton of compound **2** was assigned as the same as that of compound **1** [i.e., as a 6(17)-en-11,12-epoxy-14-one type of jatrophone diterpene]. While compound **1** has two benzoyl groups, compound **2** has one nicotinoyl group and no substituent at C-8 [δ_{H} 2.13 (1H, m, H-8a), 1.55 (1H, dd, $J = 16.4, 4.4$ Hz, H-8b)]. In the HMBC spectrum (Table

S2, Supporting Information), the correlations between H-8a and C-6, C-7, and C-9 and that between H-8b and C-10 demonstrated the presence of a methylene group at C-8. ^1H - ^1H COSY correlations supported this observation (Table S1, Supporting Information). The $^3J_{\text{CH}}$ correlation between H-9 (δ_{H} 5.40) and the nicotinoate carbonyl (δ_{C} 163.2) indicated that the nicotinoyl group was situated at C-9. The stereochemistry was determined from the NOESY spectrum (Table S3, Supporting Information). Accordingly, compound **2** was characterized as 3 β ,5 α ,7 β ,15 β -tetraacetoxy-9 α -nicotinoyloxy-11 α ,12 β -epoxyjatropha-6(17)-en-14-one.

Compound **3**, obtained as colorless needles, showed a molecular ion $[\text{M} + \text{Na}]^+$ at m/z 769.2666 (calcd for 769.2678) in the HRFTICRMS, corresponding to the molecular formula $\text{C}_{37}\text{H}_{46}\text{O}_{16}$. The ^1H NMR, ^{13}C NMR, and 2D NMR spectral data were almost identical with those of kansuinin A (**6**),^{3,4,6} and the presence of the partial structure 11 β ,14 β -epoxyjatropha-6(17)-en-12-one could be suggested. However, compound **6** has five acetyls, one hydroxyl, and one benzoyl group, while kansuinin H (**3**) has five acetyls, two hydroxyls, and one benzoyl group. The signal at δ_{C} 107.4 was assigned at C-14 from the HSQC spectrum. The chemical shift values of δ_{C} 107.4 (quaternary carbon) and δ_{H} 8.14 (OH) indicated that one hydroxyl group was situated at C-14.⁶ In the HMBC spectrum (Table S2, Supporting Information), the signal of δ_{C} 78.9 showed 2J correlations with H-1, H-3, and CH_3 -16 (δ_{H} 1.51), indicating that C-2 was attached to a hydroxyl group (δ 9.21). The correlation of the benzoyl carbonyl (δ_{C} 166.1) and H-8 (δ_{H} 6.48) demonstrated that one benzoyl group was located at C-8. In addition, the correlations between H-3 (δ_{H} 5.88), H-5 (δ_{H} 6.78), H-7 (δ_{H} 7.03), H-9 (δ_{H} 5.55), and the acetate carbonyl (δ_{C} 169.8, 169.3, 169.9, 169.8) revealed that four acetyl groups were attached at C-3, C-5, C-7, and C-9, respectively. From the NOESY spectrum, OH-2 had a NOE correlation with H-4, showing that OH-2 was in the α configuration. The H-8 proton showed NOE correlations with H-5, H-9, and CH_3 -18, indicating a β configuration (Table S3, Supporting Information). In conclusion, the structure of kansuinin H (**3**) was established as 3 β ,5 α ,7 β ,9 α ,15 β -pentaacetoxy-8 α -benzoyloxy-2 α ,14 α -dihydroxy-11 β ,14 β -epoxyjatropha-6(17)-en-12-one.

Recently, Wang et al.⁸ have isolated kansuinins D (**4**) and E (**5**) from *E. kansui*. However, no 2D NMR data were provided and the relative configuration of kansuinin E (**5**) was not determined. When the 2D NMR spectra of kansuinins D and E were measured in CDCl_3 and pyridine- d_5 at ambient temperature, it was observed that in CDCl_3 the ^1H and ^{13}C NMR signals of the nicotinoyl group and other signals (H-4, H-5, H-17a/b) were broadened or overlapped, compared to those measured in pyridine- d_5 . The structures and stereochemistry of kansuinins D and E were established as 3 β ,5 α ,7 β ,15 β -tetraacetoxy-8 α -benzoyloxy-9 α -nicotinoyloxy-14 α -hydroxy-11 β ,14 β -epoxyjatropha-6(17)-en-12-one (**4**) and 3 β ,5 α ,7 β ,15 β -tetraacetoxy-8 α -benzoyloxy-9 α -nicotinoyloxy-11 α ,12 β -epoxyjatropha-6(17)-en-14-one (**5**), respectively. The ^1H , ^{13}C , and NOESY NMR spectra of **4** and **5** in pyridine- d_5 are shown Table S4 (Supporting Information). Compound **6** (kansuinin A) and 3 β ,5 α ,7 β ,15 β -tetraacetoxy-9 α -nicotinoyloxyjatropha-6(17),11 E -dien-14-one are known compounds, whose structures were identified by comparison with the literature.^{6,9}

The ability of different kansuinins to activate TrkA signaling was examined by delineating the survival effects of these compounds in fibroblasts that express TrkA. The survival of these cells is solely dependent on NGF treat-

Table 1. ¹H NMR and ¹³C NMR Spectral Data of Compounds **1**, **2**, and **3** [¹H 400 MHz, ¹³C 100 Hz, TMS, δ (ppm) (*J* = Hz)]

position	1^a		2^b		3^b	
	¹ H	¹³ C ^c	¹ H	¹³ C ^c	¹ H	¹³ C ^c
1a	2.89 dd (14.0, 7.6)	45.9 t	27.8 dd (14.4, 7.6)	46.9 t	3.09 d (15.2)	46.0 t
1b	1.88 m		1.73 dd (14.4, 12.4)		2.97 d (15.2)	78.9 s
2	2.29 m	38.7 d	2.18 m	39.1 d		78.9 s
3	5.46 br s	75.8 d	5.42 br s	75.8 d	5.88 br d (3.6)	77.8 d
4	3.21 br s	54.6 d	2.24 br s	54.6 d	4.31 br d (3.6)	50.2 d
5	5.57 br s	68.2 d	5.56 br s	68.3 d	6.78 br s	70.5 d
6		143.4 s		138.9 d		147.7 s
7	4.84 br s	70.9 d	5.35 br s	69.5 d	7.03 br s	69.5 d
8a	5.96 br s	69.5 d	2.13 m	31.7 t	6.48 br s	72.6 d
8b			1.55 dd (16.4, 4.4)			
9	5.33 d (2.4)	79.3 d	5.40 br s (4.4)	73.0 d	5.55 s	82.6 d
10		38.9 s		39.8 d		41.8 s
11	3.36 d (2.0)	61.8 d	3.22 d (2.0)	63.7 d	4.52 s	77.4 d
12	3.28 dd (7.6, 2.0)	57.8 d	2.90 dd (8.8, 2.0)	58.8 d		215.5 s
13	2.72 m	43.7 d	2.50 dq (8.8, 6.8)	43.3 d	2.67 q (7.2)	51.5 d
14		216.2 s		216.6 s		107.4 s
15		93.3 s		92.8 s		90.5 s
16	0.88 d (6.4)	13.3 q	0.86 d (6.8)	13.1 q	1.51 s	21.4 q
17a	5.06 br s	111.6 t	5.28 br s	114.5 t	5.43 br s	108.1 t
17b	4.89 br s		5.22 br s		5.41 br s	
18	0.70 s	18.0 q	0.68 s	12.8 q	1.60 s	19.2 q
19	1.31 s	25.6 q	0.98 s	23.8 q	1.27 s	22.5 q
20	1.34 d (6.8)	17.5 q	1.25 d (6.8)	16.9 q	1.59 d (7.2)	9.94 q
			Acetyls			
CO-3		169.7 s		169.4 s		169.8 s
COMe-3	2.09 s	21.3 q	2.07 s	21.2 q	2.07 s	20.4 q
CO-5		168.7 s		169.2 s		169.3 s
COMe-5	1.56 s	20.1 q	2.15 s	20.9 q	2.01 s	20.9 q
CO-7		169.9 s		169.1 s		169.9 s
COMe-7	2.06 s	21.0 q	1.47 s	20.4 q	2.25 s	20.9 q
CO-9						169.8 s
COMe-9					2.12 s	21.8 q
CO-15		169.7 s		169.7 s		169.7 s
COMe-15	2.06 s	21.1 q	2.06 s	21.1 q	2.18 s	21.4 q
			Benzoyls			
CO-8		164.6 s				166.1 s
COPh-1'-8		129.7 s				131.0 s
2',6'	8.04 m	130.0 d			8.31 d (7.6)	130.2 d
3',5'	7.41 m	128.4 d			7.33 t (7.6)	128.8 d
4'	7.53 t (7.6)	133.7 d			7.42 t (7.6)	133.3 d
CO-9		164.9 s				
9-COPh-1'-9		129.5 s				
2',6'	8.02 m	130.0 d				
3',5'	7.52 m	128.7 d				
4'	7.57 dd (8.4, 7.6)	133.7 d				
			Nicotinoyl			
CO-9				163.2 s		
CONic-2''-9			9.17 br s	150.1 d		
3''				126.2 s		
4''			8.28 d (7.6)	137.4 d		
5''			7.41 m	123.6 d		
6''			8.77 br s	153.0 d		
OH-10					9.21 s	
OH-14					8.14 s	

^a Measured in CDCl₃. ^b Measured in pyridine-*d*₅. ^c Assignment made by a combination of HMQC and HMBC NMR data.

ment, and they normally die in the absence of NGF. TrkA-expressing fibroblasts were incubated with different concentrations of selected kansuinins for 2 days, and their survival was quantified by a MTT assay. To determine the specificity of survival effects of these compounds, their effects on fibroblasts that express TrkB were also studied for comparison. Unlike the TrkA-expressing cells, the survival of TrkB-expressing cells is dependent on the presence of brain-derived neurotrophic factor (BDNF), a member of the neurotrophin family related to NGF.¹⁶ Of all the kansuinins examined, only kansuinin E (**5**) showed a specific effect on the survival of TrkA fibroblasts, when compared with TrkB cells, with an ED₅₀ value of 0.23 μg/mL (Table 2). In contrast, kansuinins A (**6**), D (**4**), and F (**1**) enhanced the survival of both TrkA- and TrkB-expressing fibroblasts.

Experimental Section

General Experimental Procedures. Melting points were determined on a Kofler micro-melting apparatus and were

Table 2. Survival Effect of Nerve Growth Factor Dependent Fibroblasts by Compounds Isolated from *E. kansui*

compound	TrkA	TrkB
	ED ₅₀ ^a (μg/mL)	ED ₅₀ ^a (μg/mL)
1	> 50	> 50
4	0.55 ± 0.06	0.22 ± 0.09
5	0.23 ± 0.07	> 50
6	7.92 ± 2.3	3.28 ± 1.28

^a ED₅₀ represents the sample concentration that is required to achieve 50% cell viability. Fibroblasts stably expressing TrkA and TrkB were treated with various concentrations (0.001–10 μg/mL) of five compounds for 2 days. Cell viability was detected using MTT assay and compared to that of solvent (DMSO) control. Each data point represents average ± SEM, *n* = 3. The ED₅₀ values for nerve growth factor in TrkA cells and brain-derived neurotrophic factor in TrkB cells were 25 and 10 ng/mL, respectively.

uncorrected. Optical rotations were taken in CHCl₃ on a Perkin-Elmer PE241 polarimeter. The UV spectra were determined in MeOH on a Shimadzu UV1600 spectrophotometer.

The IR spectra were obtained on a Perkin-Elmer 930 spectrophotometer. The NMR spectra were recorded with a Bruker AV400 or DPX500 spectrometer, with TMS as an internal standard. The mass spectra were obtained with a Bruker APEX-II or a JEOL FABMate spectrometer. Column chromatography was carried out with silica gel (Qingdao) and Sephadex LH-20 (Pharmacia). HPLC was performed on a Waters 515 apparatus equipped with a Waters UV2487 and Alltech 2000 ELSD detector. Hypersil C₁₈ and C₈ columns (20 × 200 mm) were used for preparative purposes with a Waters UV 2487 detector. TLC was performed on HPTLC plates (Alltech), with compounds visualized by spraying with 10% sulfuric acid followed by heating.

Plant Material. The dried roots of *Euphorbia kansui* L. were collected from Shanxi Province of the People's Republic of China in October 2000 and identified by Prof. Min-Jian Qin (Department of Natural Medicinal Resources, China Pharmaceutical University, People's Republic of China). A voucher specimen (No. 20-93-39-1) has been deposited at the Department of Natural Medicinal Chemistry, China Pharmaceutical University.

Extraction and Isolation. The dried roots of *E. kansui* (20 kg) were extracted with 95% EtOH under reflux three times. The solvent was evaporated under reduced pressure at 50–60 °C to obtain an extract (1.2 kg). This extract was dissolved and partitioned with petroleum ether, ethyl acetate, and butanol, respectively, to afford 280, 72, and 53 g, respectively, of each extract. The petroleum ether extract was subjected to passage over a silica gel column eluted with petroleum ether–ethyl acetate (6:4). The column chromatographic fractions (500 mL each) were combined into 95 portions. Fraction 37 was subjected to silica gel with petroleum ether–acetone (7:3), then purified by preparative HPLC (85% MeOH) to afford 3 β ,5 α ,7 β ,15 β -tetraacetoxy-9 α -nicotinoyloxy-jatropha-6(17),11 E -dien-14-one (13 mg). Fraction 38 was chromatographed over silica gel with petroleum ether–ethyl ether (5:5) to give compound **3** (40 mg). Fractions 42–43 were recrystallized from petroleum ether–ethyl acetate to yield compound **6** (1 g). Fractions 49–52 were recrystallized from petroleum ether–ethyl acetate to afford compound **4** (200 mg), and the mother liquor was subjected repeatedly to silica gel column chromatography eluted with petroleum ether–acetone (6:4) to give compounds **1** (30 mg) and **2** (18 mg). Fraction 57 was subjected to silica gel column chromatography eluted with chloroform and purified further by preparative HPLC (Hypersil C₈, 20 × 200 mm, MeOH–H₂O, 60:40, Waters UV 2487 detector) to obtain compound **5** (80 mg).

Kansuinin F (1): colorless needles (petroleum ether–ethyl acetate); mp 222–225 °C; [α]_D²⁵ +41.0° (*c* 0.322, CHCl₃); UV (MeOH) λ_{\max} (log ϵ) 201 (4.62), 230 (4.72), 275 (3.66) nm; IR (KBr) ν_{\max} 3446, 1745, 1728, 1600, 1452, 1373, 1037 cm⁻¹; ¹H and ¹³C NMR data, see Table 1; HRFTICRMS *m/z* 799.2938 [M + Na]⁺ (calcd for C₄₂H₄₈O₁₄Na, 799.2936).

Kansuinin G (2): colorless needles (petroleum ether–ethyl acetate); mp 220–223 °C; [α]_D²⁵ –107.8° (*c* 0.372, CHCl₃); UV (MeOH) λ_{\max} (log ϵ) 201 (4.83), 220 (4.72), 264 (3.71) nm; IR (KBr) ν_{\max} 3024, 1739, 1703, 1592, 1521, 1456, 1283, 1067, 1041 cm⁻¹; ¹H and ¹³C NMR data, see Table 1; HRFTICRMS *m/z* 680.2714 [M + Na]⁺ (calcd for C₃₄H₄₃O₁₂Na, 680.2717).

Kansuinin H (3): colorless needles (petroleum ether–acetone); mp 206–208 °C; [α]_D²⁵ +28.4° (*c* 0.081, CHCl₃); UV (MeOH) λ_{\max} (log ϵ) 202 (4.18), 226 (4.17), 265 (3.10) nm; IR (KBr) ν_{\max} 3410, 3320, 1740, 1600, 1380, 1240, 1050, 760 cm⁻¹; ¹H and ¹³C NMR data, see Table 1; HRFTICRMS *m/z* 769.2666 [M + Na]⁺ (calcd for C₃₇H₄₆O₁₆Na, 769.2678).

TrkA Fibroblast Survival Assay. NIH-3T3 mouse fibroblast cells (ATCC cell line) were stably transfected with neuro-

trophin receptor (TrkA or TrkB), as previously described,¹⁷ and were maintained in DMEM supplemented with 12% heat-inactivated newborn calf serum (Invitrogen). Cells (2 × 10⁴/mL) were seeded onto 96-well plates, and various concentrations (0.001–10 μ g/mL) of compounds were added to these cells in defined medium without serum.¹⁸ Since these cells survive only in the presence of NGF or BDNF, the extent of cell survival indicates the activation of the cognate receptor, TrkA or TrkB, by the compounds added to the culture. The number of viable cells was quantified using a MTT (3-[4,5-dimethylthiazol-2-yl]-2,5-diphenyl tetrazolium bromide) assay, after incubation for 2 days. Briefly, 10 μ L of MTT solution (1 mg/mL) was added to each well containing 100 μ L of culture medium, for 4 h at 37 °C. Viable cells will cleave MTT into a formazan dye, and solubilization buffer (1% SDS/0.1 N HCl, 100 μ L) was added to each well and incubated overnight at 37 °C. The optical density of the resulting solution was determined colorimetrically at 570 nm using a multiwell spectrophotometer (Dynex Technologies). Dose–response curves were prepared and the ED₅₀ values were calculated using Sigmaplot 8.0 software. Cell survival was calculated compared to solvent control (dimethyl sulfoxide, DMSO) as previously described.¹⁸ The final concentration of DMSO was equal to or less than 0.1% and showed no effect on cell survival.

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Supporting Information Available: Tables of COSY, HMBC, and NOESY NMR data for compounds **1–3** and a table of ¹H, ¹³C, and NOESY NMR spectral data for compounds **4** and **5**. This information is available free of charge via the Internet at <http://pubs.acs.org>.

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