Yeast Glycogen Synthase Kinase-3β Pathway Inhibitors from an Organic Extract of *Streptomyces* sp.

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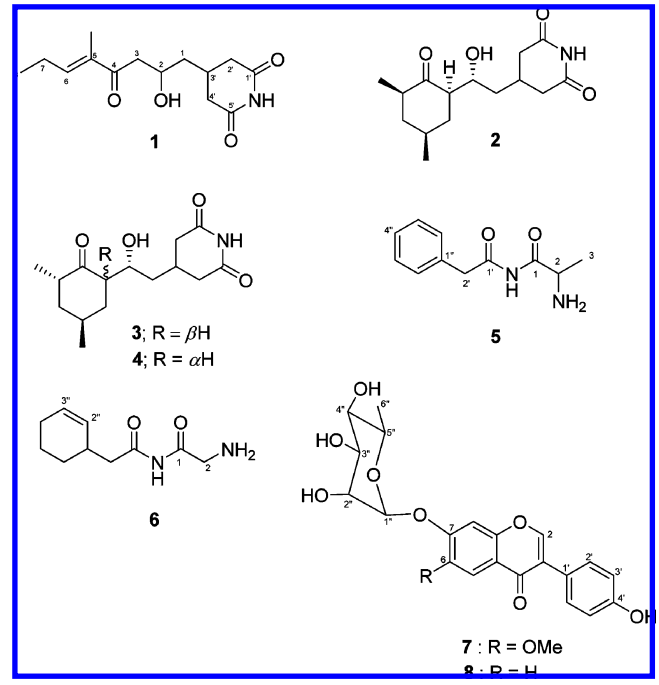
Investigation of a microbial fermentation organic extract of *Streptomyces* sp. H7667 led to the isolation of three new imides, 3-[(5*E*)-5-methyl-4-oxo-2-hydroxy-5-octenyl]glutarimide (**1**), 2-amino-*N*-2′-(phenylacetyl)propanimide (**5**), and 2-amino-*N*-(2′-(cyclohex-2″-enyl)acetyl)acetimide (**6**), and one new isoflavonoid glycoside, 6-*O*-methyl-7-*O*-α-rhamnopyranosyldaidzein (**7**), along with four known compounds. Their structures were elucidated by HRESIMS, ^{1}H and ^{13}C NMR, COSY, HMQC, HMBC, and NOESY spectra. Compounds **1**−**8** were evaluated for their inhibitory activities in the yeast glycogen synthase kinase-3β assay.

The phosphorylation of proteins on serine/threonine and tyrosine residues by protein kinases is one of the major regulatory mechanisms in biological processes including apoptosis, cell proliferation, cell differentiation, and metabolism in signal transduction pathways. Oncogenic signaling pathways induced by growth factor receptors have been shown to be deregulated in many different forms of cancer. Protein kinases, the key factors in these signaling events, are therefore promising and attractive drug targets for anticancer therapy.[1,2] Glycogen synthase kinase-3β (GSK-3β) is a serine/threonine kinase found to be distributed throughout the body and to play a central role in many cellular and physiological events.[3] Inhibition of the GSK-3β pathway has emerged as a potential therapeutic approach for a number of pathologies.[4] In our continuing efforts to identify new protein kinase inhibitors for potential use as anticancer agents, a yeast glycogen synthase kinase-3β assay was adapted to rapidly screen and identify candidate compounds that target serine/threonine and/or tyrosine kinase activities.

Actinomycetes and fungi are known to be prolific producers of bioactive secondary metabolites with great structural diversity. Some actinomycetes have also proven to be essential sources of cell cycle inhibitors. Trichostatin A from *Streptomyces hygroscopicus* causes cell cycle arrest in synchronous cultures of normal rat fibroblasts in both the G_1 and G_2 phases by acting as a potent inhibitor of histone deacetylase.[5] *Streptomyces* sp. H7667 is a novel strain isolated from Imbak Valley, Sabah, Borneo. Incidentally, Borneo is listed as a hot spot of biodiversity with an estimated 20 000−25 000 plant species. Imbak Valley, among other areas, is a preserved lowland tropical dipterocarp rain forest. Actinomycetes isolated from soil samples collected from just beneath the leaf litter of identified trees were used in an initial attempt to study the possible coevolution of the metabolic pathways of secondary metabolites of actinomycetes and plants.[6] Using this approach, *Streptomyces* sp. H7667 was isolated and identified. The crude organic extract of fermentation cultures of *Streptomyces* sp. H7667 was shown to inhibit growth of a yeast transformant (with cloned human GSK-3β) at 37 °C, but showed no inhibition at 25 °C, a characteristic of the GSK-3β screening system. This preliminary result encouraged us to further study fermentation of the microbial extract of *Streptomyces* sp. H7667. Herein we report the isolation, structure elucidation, and biological activities of new compounds **1** and **5**−**7** along with four known compounds, isocycloheximide (**2**),[7] cycloheximide (**3**),[7] naramycin B (**4**),[8] and 7-*O*-α-L-rhamnopyranosyldaidzein (**8**)[9] from *Streptomyces* sp. H7667.



Compound **1** was assigned the molecular formula $C_{14}H_{21}NO_4$ from its HRESIMS (*m*/*z* 268.1540 $[M + H]^+$, calcd 268.1555) and ^{13}C NMR data. The UV absorption bands at λ_{max} 208 and 238 nm were characteristic of an α,β-unsaturated ketone. The molecular formula of **1** indicated five degrees of unsaturation, one of which was due to a carbon−carbon double bond, three due to carbonyl carbons, and the remaining one was ascribed to a ring. The ^{13}C NMR and DEPT spectra showed one conjugated ketone (δ 202.0) and two amide carbonyls (δ 172.1 and 172.0). In the ^{1}H NMR spectrum of **1**, signals indicating one allylic methyl group [δ 1.77 (3H, s)], one olefinic proton [(δ 6.62, 1H, t, J = 7.8 Hz)], and one

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amide proton [δ 8.01 (1H, s)] were observed. The COSY spectrum displayed a methine proton coupled to three methylene groups. The ^{1}H NMR and COSY spectra indicated that the methine at δ 2.52 (1H, m), two sets of methylene protons α to a carbonyl [δ 2.80 (1H, m), 2.32, (1H, dd, J = 10.3, 6.6 Hz); δ 2.80 (1H, m), 2.36 (1H, dd, J = 10.3, 6.9 Hz)], and the third pair of methylene protons [δ 1.67 (1H, m); 1.38 (1H, ddd, J = 14.0, 9.3, 2.5 Hz)] were connected as a $-CH_2-CH-(CH_2)_2$ unit. The third pair of methylene protons also showed correlation to an oxymethine proton (δ 4.16, 1H, br t, J = 9.6, H-2), which, in turn, showed a cross-peak with a methylene group (δ 2.86, 1H, dd, J = 17.4, 2.5 Hz and 2.71, 1H, dd, J = 17.4, 9.6 Hz) adjacent to a carbonyl. This evidence indicated that compound **1** was a glutarimide derivative.[10] Additionally, the olefinic proton (δ 6.62, 1H, t, J = 7.8 Hz, H-6) displayed an interaction with methylene protons (δ 2.27, 2H, qn, J = 7.8 Hz, H-7) and further coupling to a methyl group (δ 1.08, 3H, t, J = 7.8 Hz, H-8) in the COSY spectrum. HMBC analysis supported the proposed structure for **1**. In particular, correlations of CH_3-8 (δ 1.08) with C-6 and C-7, of an allylic methyl proton (Me-5) at δ 1.77 with C-4, C-5, and C-6, of an oxymethine proton at δ 2.86 and 2.71 (CH_2-3) with C-2 and C-4, and of a methine proton at δ 2.52 (H-3′) with C-1′, C-2′, C-5′, and C-1 confirmed the connectivity of **1**. In the NOESY spectrum, an allylic methyl proton 5-Me (δ 1.77) showed cross-peaks with the two methylene protons, H-7 (δ 2.27), confirming that the double bond at C-5 had the *E*-configuration. Therefore, compound **1** was elucidated as 3-[(5*E*)-5-methyl-4-oxo-2-hydroxy-5-octenyl]glutarimide.

Compound **5** was isolated as a white powder with the molecular formula $C_{11}H_{14}N_2O_2$, determined from HRESIMS ($[M + H]^+$ m/z 207.1130, calcd 207.1154), indicating six degrees of unsaturation. The IR spectrum of **5** showed an absorption band for a carbonyl group at 1626 cm^{-1}. The ^{13}C NMR and DEPT spectra confirmed the presence of 11 carbons, including one methyl (δ_C 19.2), one methylene (δ_C 44.4), one sp^3 methine (δ_C 51.4), five sp^2 methines (δ_C 131.1, 131.1, 130.5, 130.5, and 128.8), and three quaternary carbons (δ_C 178.6, 174.6, and 137.7). The ^{1}H NMR spectrum displayed signals characteristic of a monosubstituted benzene ring at δ_H 7.30−7.23 (m, 5H), one methylene (δ_H 3.56, s, 2H), one methine (δ 4.34, q, J = 7.0 Hz, 1H), and a secondary methyl (δ_H 1.34, d, J = 7.0 Hz, 3H). In the HMBC spectrum of **5**, the δ_H 4.34 signal of H-2 exhibited a cross-peak with the carbon at δ_C 178.6 (C-1). Additional correlations were observed between δ_H 1.34 (CH_3-3) and δ_C 178.6 (C-1) and 51.1 (C-2). The methylene protons at δ_H 3.56 (H-2′) showed correlations with the signals of δ_C 174.6 (C-1′), 137.7 (C-1″), and 131.1 (C-2″/C-6″), indicating that the propanimide group and the monosubstituted benzene ring were connected through a methylene group. Thus, compound **5** was assigned as 2-amino-*N*-(2′-(phenylacetyl)propanimide.

Compound **6** was obtained as a white powder. Its molecular formula was determined as $C_{10}H_{16}N_2O_2$ on the basis of HRESIMS ($[M + H]^+$ m/z 197.1296, calcd for 197.1310), indicating four degrees of unsaturation. The IR spectrum of **6** exhibited carbonyl absorption at 1654 cm^{-1}. The ^{13}C NMR and DEPT spectra indicated 10 carbon signals, including two carbonyls (δ_C 175.7 and 174.4), two sp^2 methines (δ_C 131.6 and 129.0), one sp^3 methine (δ_C 34.2), and five sp^3 methylenes (δ_C 43.6, 43.2, 30.1, 26.2, and 22.3). Comparison of its ^{1}H and ^{13}C NMR data with values for **5** indicated that **6** and **5** were analogous, except for the presence of a cyclohexenyl substituent (δ_C 131.6, 129.0, 34.2, 30.1, 26.2, and 22.3) in **6**. This was supported in the ^{1}H NMR spectrum by signals at δ_H 5.70 (1H, ddd, J = 10.0, 6.0, 3.5 Hz, H-3″), 5.57 (1H, br dd, J = 10.0, 2.0 Hz, H-2″), 2.56 (1H, m, H-1″), 1.98 (2H, m, H-4″), 1.82 (1H, m, H-6″a), 1.75 (1H, m, H-5″a), 1.55 (1H, m, H-5″b), and 1.30 (1H, dddd, J = 12.6, 10.6, 8.0, 3.0 Hz, H-6″b). The connectivities of H-2′/H-1′, H-1″/H-2″/H-6″, H-2″/H-3″, H-3″/H-4″, H-4″/H-5″, and H-5″/H-6″ in the COSY spectrum confirmed that a cyclohexenyl group was connected to C-2′. In addition, a singlet signal at δ 3.84 (1H, s) was assigned to H-2. In the HMBC spectrum, correlations of methylene protons at δ 2.50 and 2.21 (CH_2-2′) with the carbons at δ 174.4 (C-1′), 131.6 (C-2″), 34.2 (C-1″), and 30.1 (C-6″) confirmed the location of the cyclohexenyl group at C-2′. Correlations from H-2″ (δ 5.57), H-3″ (δ 5.71), H-5″ (δ 1.75, 1.55), and H-6″ (δ 1.82, 1.30) to C-1″ (δ 34.2) and C-4″ (δ 26.2) in HMBC spectra were also observed. In addition, correlations from H-2 (δ 3.84) to C-1 (δ 175.7) and from H-2′ (δ 2.21, 2.50) to C-1′ (δ 174.4), C-1″ (δ 34.2), C-2″ (δ 131.6), and C-6″ (δ 30.1) indicated that the cyclohexenyl and the acetimide groups were connected through C-2′. Accordingly, compound **6** was assigned as 2-amino-*N*-(2′-(cyclohex-2″-enyl)acetyl)acetimide.

Table 1. Biological Activity of Tested Compounds on the Yeast Glycogen Synthase Kinase-3β

compound	temperature (°C)	zone of inhibition observed (mm)[a,b] 20 μg/disk	10 μg/disk	5 μg/disk	2.5 μg/disk
1	25	10	NA[c]	NA	NA
	37	38	34	30	28
3	25	36	33	30	25
	37	55	51	48	42
4	25	22	16	12	NA
	37	33	25	15	NA
GSK-3β inhibitor[d]	25	10	9	8	NA
	37	11	10	9	NA

[a] Diameter of disk alone is 7 mm. Stock solutions were prepared in either DMSO or methanol. No zones of inhibition were observed with MeOH or DMSO as negative controls. [b] All compounds were tested at 20 μg/disk. Active compounds were retested at lower concentrations (10−2.5 μg/disk). [c] NA indicates not active. Staurosporine gave a 13 mm zone of inhibition at 80 μg/disk at 37 °C and no ZOI at 25 °C. [d] GSK-3β inhibitor: 4-benzyl-2-methyl-1,2,4-thiadiazolidine-3,5-dione.

The molecular formula of **7** was established as $C_{22}H_{22}O_9$ by HRESIMS ($[M + H]^+$ m/z 431.1345 calcd 431.1335), indicating 12 degrees of unsaturation. Absorption bands at λ_{max} 203, 261, and 318 nm were characteristic of an isoflavone moiety.[11] The IR spectrum of **7** showed the presence of OH (3435 cm^{-1}) and carbonyl (1654 cm^{-1}) functionalities. Comparison of the ^{1}H and ^{13}C NMR data of **7** with those of daidzein 7-*O*-α-L-rhamnoside (**8**)[9] suggested they were closely related isoflavones, with the only difference being the presence of an OMe group [δ 3.94 (3H, s)] in **7**. The relative location of the OMe of **7** was confirmed by an HMBC experiment in which a correlation was observed for the OMe proton with the carbon at δ 150.3. In addition, OMe showed a cross-peak with H-5 (δ 7.63) in a 2D NOESY experiment. The relative configuration of **7** was determined on the basis of coupling constants and a NOESY experiment. The anomeric proton at δ 5.61 showed a cross-peak with the oxymethine proton at δ 4.10 (H-2″) and 7.36 (H-8) from the NOESY spectrum, indicating that the sugar was α-rhamnose. Therefore, the structure of **7** was assigned as 6-*O*-methyl-7-*O*-α-rhamnopyranosyldaidzein.

All isolated compounds were evaluated for their inhibitory activities in the yeast GSK-3β assay at both 25 and 37 °C. Only compounds **1**, **3**, and **4** exhibited significant inhibitory activities against yeast GSK-3β plates (Table 1) and gave 33−55 mm zones of inhibition (ZOI) at 20 μg/disk. All other isolated compounds were inactive. The clear ZOI at both 37 and 25 °C indicated that the agents are nonselective GSK-3β inhibitors. In this regard, compound **1** gave a clear ZOI at 10 μg/disk at 37 °C and no ZOI at 25 °C, indicating that **1** inhibited the GSK-3β pathway. Compounds **3** and **4**, a pair of regioisomers, showed different potency in the GSK-3β assay. Compound **3** exhibited potent inhibitory activity and gave a 42 mm ZOI at 2.5 μg/disk at 37 °C and 25 mm ZOI at 25 °C. Compound **3** (H-6 at the β-position of the cyclohexanone ring) appeared to be more potent as compared

to compound **4** (H-6 at the α-position of the cyclohexanone ring). Furthermore, it appeared that the configuration at C-2 and C-6 of the cyclohexanone ring (cf. **2**, **3**, and **4**) contributed to the size of the inhibition zone. Compound **3** was also isolated from the cultures of *Streptomyces griseus* and has been demonstrated to inhibit protein synthesis in mammalian systems.[12]

Experimental Section

General Experimental Procedures. UV spectra were measured on a HP 8453 UV−visible spectrophotometer. IR spectra were recorded on a Perkin-Elmer BX FT-IR spectrophotometer. Mass spectra and high-resolution mass spectra were taken with a BioTOF II ESI mass spectrometer. 1D and 2D NMR spectra were recorded in methanol-d_4 on an INOVA Unity (500 MHz) Varian spectrometer equipped with an xyz-shielded gradient triple-resonance probe. Reversed-phase HPLC was carried out on a Beckman Coulter Gold-168 system equipped with a photodiode array detector using an Alltech semipreparative Econosil C_{18} column (10 μm, 10 × 250 mm) run with a flow rate of 2.0 mL/min. Chromatographic fractions and pure compounds were monitored by TLC, detected by absorption of UV light at 254 nm and a color reaction by spraying with a solution of vanillin/perchloric acid/EtOH followed by 5 min heating at 100 °C. Column chromatography was carried out with Merck Si gel 100 (230−400 mesh).

Producing Organism and Its Fermentation. The organism was isolated from soil samples (EL-16) that were collected below the leaf litter (*Mallotus* sp.) on the right side of the ridge facing Imbak River (altitude ~91.4−121.9 m, primary lowland dipterocarp forest, base camp area, Imbak Valley, Sabah, Malaysia) in June 2000. The strain was isolated by suspending 0.1 g of soil into 10 mL of sterile distilled water and later diluting this up to 10^{-3}. The suspension (100 μL) was spread on humic-acid (HV)-vitamin B plus cycloheximide[13] at pH 7.2. Isolated strains were transferred from HV medium onto similar pH oatmeal agar plates and incubated at 28 °C for 14 days until sporulation. The strain *Streptomyces* sp. H7667 was characterized by brownish-gray aerial mycelium color and a diffusible pale orange-colored pigment on the oatmeal agar. It is Gram-positive and filamentous, with a chain of spores on the apex of aerial hyphae. The strain contained LL-diaminopimelic acid (LL-DAP) in the cell wall and was identified as a species belonging to the genus *Streptomyces*. The 16S rRNA gene was sequenced by using filamentous actinomycetes universal primers.[14] The phylogenetic position of H7667 was determined using MEGA4.[15] [A phylogenetic tree (Figure S1) of H7667 is provided in the Supporting Information.] Multiple alignment of the 16S rRNA gene sequence of *Streptomyces* sp. H7667 and other reference strains and calculation of levels of sequences homology were carried out using CLUSTAL W 1.81.[16] On the basis of the nature of the 16S rRNA gene, the strain most closely related to H7667 was identified as *Streptromyces aburaviensis* NRRL-2218 (98.20% DNA homology). The sequence of the 16S rRNA gene has been deposited in GenBank with accession number EU871644. Frozen spore stock of *Streptomyces* sp. H7667 was stored in 20% glycerol at −78 °C. The vial was thawed and spores were spread on an ISP4 plate for complete sporulation. It was then ready to inoculate to a seed medium.

Seed Medium. The composition of the seed medium (in g/L) was as follows: D-mannitol (20), peptone (20), and dextrose (10). The seed medium was prepared with distilled water, and the pH was adjusted to 7.0 prior to sterilization. The medium was dispensed at 50 mL per 250 mL in Belco baffled shaker flasks. A single colony from the agar plate was used as inoculum into each flask of mannitol-peptone media, cultured at 30 °C at 250 rpm for 2 days.

Production Medium. An aliquot (1%) from the seed medium was inoculated into the production medium. The composition of the production medium was similar to that of the seed medium. The production cultures were incubated at 30 °C at 250 rpm for 7 days and were then harvested. The supernatants were filtered and partitioned with *n*-BuOH three times. Both the *n*-BuOH-soluble and aqueous layers were tested using the yeast GSK-3β screen.

Extraction and Isolation. The fermentation broth (90 L) was centrifuged, and the supernatant was extracted with *n*-BuOH. The organic extract (123.0 g) was suspended in H_2O (1:1), then partitioned successively with hexanes, $CHCl_3$, and EtOAc (3 × 250 mL each). The $CHCl_3$-soluble partition of *n*-BuOH extract of fermented *Streptomyces* sp. H7667, which inhibited significantly the growth of the yeast GSK-3β transformant at a concentration of 80 μg/disk with a 25 mm zone of inhibition, was subjected to bioassay-guided fractionation. This extract (9.3 g) was chromatographed using silica gel CC, eluting with 100% hexanes, a gradient of hexanes−EtOAc, and then EtOAc−MeOH, respectively, to afford nine fractions (F1−F9). Fraction 3 [eluted with *n*-hexane−acetone (20:1)] was chromatographed sequentially on silica gel eluting with $CHCl_3$−MeOH mixtures of increasing polarity to obtain 10 subfractions (F3A−F3J). Compound **5** (4.2 mg) was purified by HPLC eluting with MeOH−H_2O (15:85) from subfraction F3D. Compound **6** (20.1 mg) was obtained from subfraction F3E by HPLC eluting with MeOH−H_2O (50:50). Fraction F5 (582.9 mg) was further purified by CC eluting with 100% $CHCl_3$ and an increasing polarity with MeOH to afford three subfractions (F5a−F4c). Subfractions F5a and F5b were subjected to C_{18} RP-HPLC eluting isocratically with MeOH−H_2O (55:45) to give pure compounds **1** (4.7 mg), **2** (154.6 mg), **3** (13.3 mg), and **4** (6.0 mg). Fraction F7 was chromatographed over a Sephadex LH-20 column and eluted with MeOH−H_2O (60:40) to yield 12 subfractions (F7A−F7L). Compounds **7** (2.4 mg) and **8** (3.8 mg) were isolated by HPLC eluted with MeCN−H_2O (20:80) from subfraction F7L.

3-[(5*E*)-5-Methyl-4-oxo-2-hydroxy-5-octenyl]glutarimide (1): colorless, viscous oil; UV (MeOH) λ_{max} (log ε) 208 (4.19), 238 (4.16) nm; IR (neat) ν_{max} 3400, 1705, 1685, 1275, 1260, 1125 cm^{-1}; ^{1}H NMR ($CDCl_3$, 500 MHz) δ 8.01 (1H, s, NH), 6.62 (1H, t, J = 7.8 Hz, H-6), 4.16 (1H, br t, J = 9.6 Hz, H-2), 2.86 (1H, dd, J = 17.4, 2.5 Hz, H-3a), 2.80 (1H, m, H-2′a), 2.71 (1H, dd, J = 17.4, 9.6 Hz, H-3b), 2.70 (1H, m, H-4′a), 2.52 (1H, m, H-3′), 2.36 (1H, dd, J = 10.3, 6.9 Hz, H-4′b), 2.32 (1H, dd, J = 10.3, 6.6 Hz, H-2′b), 2.27 (2H, qn, J = 7.8 Hz, H-7), 1.77 (3H, s, 5-Me), 1.67 (1H, m, H-1a), 1.38 (1H, ddd, J = 14.0, 9.3, 2.5 Hz, H-1b), 1.08 (3H, t, J = 7.8 Hz, H-8); ^{13}C NMR ($CDCl_3$, 125 MHz) δ 202.0 (C-4), 172.1 (C-1′), 172.0 (C-5′), 146.1 (C-6), 136.7 (C-5), 65.1 (C-2), 43.6 (C-3), 40.8 (C-1), 38.4 (C-4′), 37.1 (C-2′), 27.1 (C-3′), 22.5 (C-7), 13.0 (C-8), 10.9 (5-Me); HRESIMS m/z 268.1540 [M + H]$^+$ (calcd for $C_{14}H_{22}NO_4$, 268.1555).

2-Amino-*N*-(2′-(phenylacetyl)propanimide (5): white powder; UV (MeOH) λ_{max} (log ε) 208 (3.68) nm; IR (KBr) ν_{max} 3401, 1669, 1626, 1540, 1457, 669 cm^{-1}; ^{1}H NMR (CD_3OD, 500 MHz) δ 7.30−7.23 (5H, m, H-2″-H-6″), 4.34 (1H, q, J = 7.0 Hz, H-2), 3.56 (2H, s, H-2′), 1.34 (3H, d, J = 7.0 Hz, H-3); ^{13}C NMR (CD_3OD, 125 MHz) δ 178.6 (C-1), 174.6 (C-1′), 137.7 (C-1″), 131.1 (2C-2″/6″), 130.5 (2C-3″/5″), 128.8 (C-4″), 51.1 (C-2), 44.4 (C-2′), 19.2 (C-3); HRESIMS m/z 207.1130 [M + H]$^+$ (calcd for $C_{11}H_{15}O_2N_2$, 207.1154).

2-Amino-*N*-(2′-(cyclohex-2″-enyl)acetyl)acetimide (6): white powder; UV (MeOH) λ_{max} (log ε) 204 (3.47) nm; IR (KBr) ν_{max} 3329, 2927, 1654, 1534, 1437, 1409, 1337, 1279, 1095, 681 cm^{-1}; ^{1}H NMR (CD_3OD, 500 MHz) δ 5.70 (1H, ddd, J = 10.0, 6.0, 3.5 Hz, H-3″), 5.57 (1H, br dd, J = 10.0, 2.0 Hz, H-2″), 3.84 (2H, s, H-2), 2.56 (1H, m, H-1″), 2.50 (1H, dd, J = 14.0, 7.5 Hz, H-2′a), 2.21 (1H, dd, J = 14.0, 8.0 Hz, H-2′b), 1.98 (2H, m, H-4″), 1.82 (1H, m, H-6″a), 1.75 (1H, m, H-5″a), 1.55 (1H, m, H-5″b), 1.30 (1H, dddd, J = 12.6, 10.6, 8.0, 3.0 Hz, H-6″b); ^{13}C NMR (CD_3OD, 125 MHz) δ 175.7 (C-1), 174.4 (C-1′), 131.6 (C-2″), 129.0 (C-3″), 43.2 (C-2), 43.6 (C-2′), 34.2 (C-1″), 30.1 (C-6″), 26.2 (C-4″), 22.3 (C-5″); HRESIMS m/z 197.1296 [M + H]$^+$ (calcd for $C_{10}H_{17}O_2N_2$, 197.1310).

6-*O*-Methyl-7-*O*-α-rhamnopyranosyldaidzein (7): white solid; UV (MeOH) λ_{max} (log ε) 203 (4.23), 261 (4.12), 318 (3.64) nm; IR (KBr) ν_{max} 3435, 2950, 1654, 1637, 1560, 1508, 1260 cm^{-1}; ^{1}H (CD_3OD, 500 MHz) δ 8.20 (1H, s, H-2), 7.63 (1H, s, H-5), 7.39 (2H, d, J = 8.5 Hz, H-2′/H-6′), 7.36 (1H, s, H-8), 6.85 (2H, d, J = 8.5 Hz, H-3′/H-5′), 5.61 (1H, d, J = 2.0 Hz, H-1″), 4.10 (1H, dd, J = 5.0, 2.0 Hz, H-2″), 3.94 (3H, s, 6-OMe), 3.91 (1H, dd, J = 10.0, 5.0 Hz, H-3″), 3.64 (1H, m, H-5″), 3.50 (1H, t, J = 10.0 Hz, H-4″), 1.25 (3H, d, J = 6.5 Hz, H-6″); ^{13}C NMR (CD_3OD, 125 MHz) δ 178.0 (C-4), 158.9 (C-4′), 155.0 (C-2), 153.6 (C-8a), 152.8 (C-7), 150.3 (C-6), 131.6 (C-2′/C-6′), 125.8 (C-3), 124.4 (C-1′), 120.0 (C-4a), 116.4 (C-3′/C-5′), 106.3 (C-8), 105.7 (C-5), 100.8 (C-1″), 73.7 (C-4″), 72.3 (C-3″), 71.9 (C-2″), 71.5 (C-5″), 56.9 (6-OMe), 18.2 (C-6″); HRESIMS [M + H]$^+$ m/z 431.1345 (calcd for $C_{22}H_{23}O_9$, 431.1335).

Glycogen Synthase Kinase-3β Assay. Disruption of four yeast GSK-3 homologues (MCK1, MDS1, MRK1, and YOL128C) in *gsk-3* null mutant confers a temperature-sensitive phenotype (growth defect at 37 °C), which can be suppressed by the expression of cloned mammalian GSK-3β.[17] Inhibition of GSK-3β should therefore mimic the phenotype of the *gsk-3* null mutant, indicated by growth inhibition of the transformant of *gsk-3* null with pKT10-GSK-3β only at 37 °C. A genetically modified yeast strain, H10075 with genotype {*MAT*α

his3 leu2 ura3 trp1 ade2 mck1::TRP1 mds1::HIS3 mrk1 yol128c::LEU2 [pKT10-GSK-3β], was grown in 5 mL of liquid media of SC minus uracil, shaking, at 37 °C for 2 days. Yeast culture (400 μL) was added to 100 mL of SC minus uracil agar [0.67% yeast nitrogen base without amino acids and ammonium sulfate, 0.5% ammonium sulfate, 2% D-(+)-glucose anhydrate, 1 mL each of 0.03 mg/mL adenine (hemisulfate salt), 0.03 mg/mL L-tryptophan, 0.03 mg/mL L-leucine, 0.03 mg/mL L-histidine, and 1.5% bacteriological agar, pH 5.6]. To perform the assay, compounds and extracts at a concentration of 80 μg per disk were dispensed on filter disks and placed on plates containing yeast seeded into SC minus uracil media. The Petri dishes were incubated at both 25 and 37 °C for 72 h. Three phenotypes were observed on yeast plates. A clear ZOI at 37 °C and no ZOI at 25 °C indicated that the agent tested inhibits the GSK-3β pathway. Clear zones of inhibition at both 37 and 25 °C of equal width indicated that the agents are cytotoxic. Plates that had a negative phenotype were plates that had no observable ZOI. Active compounds were then tested at lower concentrations (20, 10, 5, 2.5 μg per disk). The assays were performed in duplicate. GSK-3β inhibitor (4-benzyl-2-methyl-1,2,4-thiadiazolidine-3,5-dione) and staurosporine were used as positive controls.

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Supporting Information Available: The phylogenetic tree of *Streptomyces* strain H7667, experimental procedures of **9**−**13**, and ^{1}H and ^{13}C NMR spectra of **1** and **5**−**7** are available free of charge via the Internet at http://pubs.acs.org.

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