Prenylxanthones and a Bicyclo[3.3.1]nona-2,6-diene Derivative from the Fungus *Emericella rugulosa*

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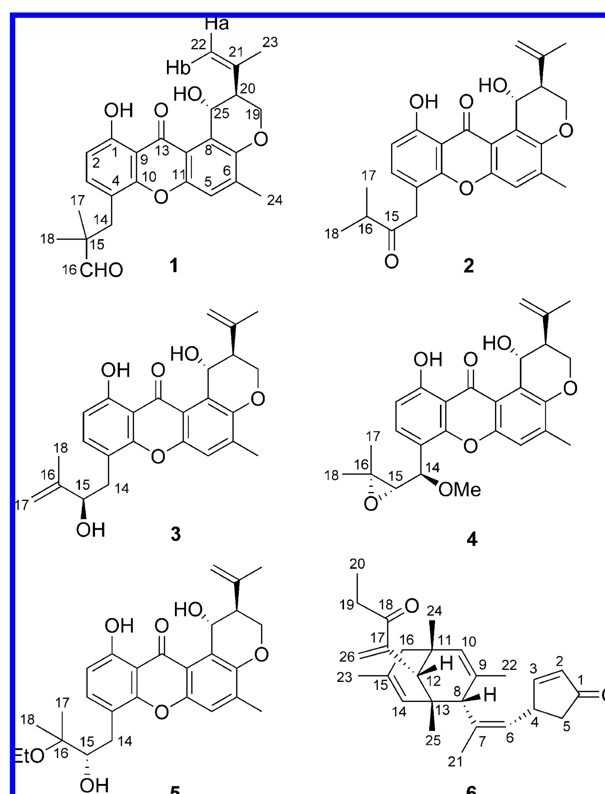
Five new prenylxanthones, ruguloxanthones A−C (**1**−**3**), 14-methoxytajixanthone (**4**), and tajixanthone ethanoate (**5**), a new bicyclo[3.3.1]nona-2,6-diene derivative, rugulosone (**6**), and seven known compounds, shamixanthone, tajixanthone, 14-methoxytajixanthone-25-acetate, tajixanthone hydrate, tajixanthone methanoate, isoemericellin, and ergosterol, were isolated from the fungus *Emericella rugulosa.* The structures of **1**−**6** were established using spectroscopic techniques. Compound **6** exhibited antimalarial and antimycobacterial activities, as well as cytotoxicity against three cancer cell lines.

Previous investigations of secondary metabolites from the fungal genus *Emericella* have given rise to the isolation of several types of compounds such as prenylated xanthones, found to be cytotoxic against cancer cell lines,[1−4] sesterterpenes with unusual tricyclic and pentacyclic skeletons,[5−8] prenylated polyketides,[9] benzophenone derivatives,[4] and a cyclopeptide.[10] *Emericella rugulosa* (Ascomycota),[11] the subject of the present investigation, has been reported to produce three diketopiperazines.[12] As part of our work on bioactive constituents from fungi, hexane and EtOAc extracts of *E. rugulosa*, a strain isolated from Thai soil, showed antimalarial activity in vitro against *Plasmodium falciparum* (IC_{50} 7.5 and 6.3 μg/mL, respectively) and antimycobacterial activity in vitro against *Mycobacterium tuberculosis* (MIC 200 μg/mL for both extracts). We report herein the isolation, structural characterization, and bioactivity of six new compounds (**1**−**6**), together with the identification of seven known compounds from *E. rugulosa*.



Results and Discussion

The dried mycelial mat of *E. rugulosa* was extracted with organic solvents, and the resulting crude extracts were fractionated by silica gel column chromatography and preparative TLC to obtain five new prenylxanthones (**1**−**5**), a new bicyclo[3.3.1]nona-2,6-diene derivative (**6**), and six known prenylxanthones,[13,14] shamixanthone, tajixanthone, 14-methoxytajixanthone-25-acetate, tajixanthone hydrate, tajixanthone methanoate, and isoemericellin,[2] as well as ergosterol.[15]

Compound **1** was obtained as yellow crystals and was assigned the molecular formula $C_{25}H_{26}O_6$, as deduced from HRESITOFMS data (observed *m*/*z* 445.1623 $[M + Na]^+$), indicating 13 degrees of unsaturation. The ^1H and ^{13}C NMR spectra (Tables 1 and 2) and DEPT experiments suggested the presence of 25 carbon signals, attributable to four methyls, three methylenes, six methines (including aromatic and aldehyde carbons), and 12 nonprotonated (including one ketone carbonyl) carbons. The IR spectrum showed the presence of hydroxy (3524 cm^{-1}), aldehyde (1718 cm^{-1}), and aromatic ketone (1637 cm^{-1}) groups. The ^1H NMR spectroscopic data gave resonances for three aromatic protons, with two (H-2 and H-3) exhibiting *ortho* coupling ($J = 8.6$ Hz) at δ 6.75 and 7.38 and the third (H-5) appearing as a singlet at δ 7.25. The aromatic methyl substituent (δ 2.36) was assigned to position C-6, as supported by a NOESY correlation between CH_3-24 and H-5 and the HMBC correlations of CH_3-24 to C-5 and C-7. A chelated hydroxy resonance at δ 12.71 was connected to the aromatic carbon atom C-1, as confirmed by HMBC correlations from OH-1 to C-1 and C-2. The $OCHCHCH_2O$ spin system, corresponding to the C-19/C-20/C-25 unit of **1**, was consistent with a dihydropyran ring fused to an aromatic ring, which was also confirmed by the NOESY and HMBC data. The HMBC spectrum showed correlations of H_2-19 to C-7, C-21, and C-25; H-20 to C-8; and H-25 to C-7, C-8, C-19, and C-21, confirming that the dihydropyran ring is fused to an aromatic unit at C-7 and C-8. The two terminal olefinic proton signals, together with a methyl singlet at δ 1.85, revealed an isopropenyl moiety connecting to the pyran ring at C-20 and was confirmed by the HMBC correlation of H_3-23 and H_2-22 to C-20 (Figure 1). The presence of a 2-methyl-2-formylpropyl moiety was deduced from the resonances at δ 9.63 (1H, s, H-16), 3.06 (1H, d, $J = 14.0$ Hz, H-14a), 2.97 (1H, d, $J = 14.0$ Hz, H-14b), 1.11 (3H, s, CH_3-17), and 1.10 (3H, s, CH_3-18). The HMBC spectrum showed

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Table 1. ^{1}H NMR Spectroscopic Data (δ, ppm) of Compounds **1−5** in $CDCl_3$

position	1	2	3	4	5
2	6.75 (d, 8.6)[a]	6.71 (d, 8.2)	6.75 (d, 8.6)	6.86 (d, 8.6)	6.77 (d, 8.4)
3	7.38 (d, 8.6)	7.35 (d, 8.2)	7.50 (d, 8.6)	7.71 (d, 8.5)	7.51 (d, 8.4)
5	7.25 (s)	7.10 (s)	7.26 (s)	7.22 (s)	7.25 (s)
14a	3.06 (d, 14.0)	3.90 (d, 16.8)	3.16 (dd, 14.0, 4.9)	4.65 (d, 8.2)	3.12 (dd, 14.2, 2.2)
14b	2.97 (d, 14.0)	3.81 (d, 16.8)	3.81 (dd, 14.0, 7.8)		2.70 (dd, 14.2, 10.1)
15			4.41 (m)	3.17 (d, 8.2)	3.76 (dd, 10.1, 2.2)
16	9.63 (s)	2.74 (hept, 7.0)			
17	1.11 (s)	1.14 (d, 6.9)	4.91 (s), 4.84 (s)	1.32 (s)	1.26 (s)
18	1.10 (s)	1.12 (d, 6.9)	1.88 (s)	1.24 (s)	1.22 (s)
19a	4.42 (dd, 10.9, 2.4)	4.35 (dd, 10.5, 2.7)	4.42 (dd, 10.9, 3.5)	4.42 (dd, 10.9, 3.5)	4.35 (dd, 10.9, 2.7)
19b	4.34 (dd, 10.9, 2.8)	4.27 (dd, 10.5, 2.7)	4.34 (dd, 10.9, 2.7)	4.34 (dd, 10.9, 3.1)	4.28 (dd, 10.9, 3.1)
20	2.73 (brm)	2.65 (brm)	2.73 (brm)	2.73 (brm)	2.67 (brm)
22a	4.81 (s)	4.73 (s)	4.80 (s)	4.81 (s)	4.51 (s)
22b	4.60 (s)	4.50 (s)	4.59 (s)	4.59 (s)	4.37 (s)
23	1.85 (s)	1.77 (s)	1.85 (s)	1.85 (s)	1.78 (s)
24	2.36 (s)	2.26 (s)	2.35 (s)	2.35 (s)	2.28 (s)
25	5.54 (brd, 2.7)	5.34 (s)	5.41 (brd, 2.0)	5.42 (s)	5.34 (brd, 2.0)
OH-1	12.71 (s)	12.58 (s)	12.65 (s)	12.87 (s)	12.60 (s)
OH-25				4.92 (brs)	4.98 (brs)
OMe-14				3.37 (s)	
OC*H*$_2$CH$_3$-16					3.43 (q, 7.0)
OCH$_2$C*H*$_3$-16					1.12 (t, 7.0)

[a] Values in parentheses are coupling constants in Hz.

Table 2. ^{13}C NMR Spectroscopic Data (δ, ppm) of Compounds **1−5** in $CDCl_3$

position	1	2	3	4	5
1	160.8 s[a]	160.9 s	160.4 s	162.0 s	160.2 s
2	109.9 d	110.1 d	109.9 d	110.9 d	109.9 d
3	139.1 d	138.2 d	138.2 d	135.5 d	138.2 d
4	114.3 s	112.3 s	115.6 s	115.9 s	117.0 s
5	119.0 d	119.0 d	119.1 d	118.9 d	119.1 d
6	138.7 s	138.5 s	138.5 s	138.9 s	138.3 s
7	149.6 s	149.6 s	149.6 s	149.8 s	149.5 s
8	121.1 s	121.1 s	121.1 s	121.3 s	121.1 s
9	109.2 s	109.3 s	109.2 s	109.0 s	109.2 s
10	153.1 s	153.0 s	153.2 s	152.6 s	153.0 s
11	151.8 s	152.0 s	152.1 s	151.8 s	152.1 s
12	116.8 s	116.9 s	116.9 s	116.8 s	116.9 s
13	184.4 s	184.3 s	184.5 s	184.4 s	184.5 s
14	36.2 t	41.0 t	35.3 t	76.0 d	31.1 t
15	47.2 s	211.1 s	75.5 d	66.7 d	76.7 d
16	205.3 d	40.3 d	146.8 s	57.7 s	77.1 s
17	21.7 q	18.4 q	111.4 t	19.8 q	21.6 q
18	21.5 q	18.5 q	18.0 q	24.8 q	19.9 q
19	64.6 t	64.5 t	64.6 t	64.6 t	64.6 t
20	44.9 d	44.9 d	44.9 d	44.9 d	44.9 d
21	142.5 s	142.5 s	142.6 s	142.5 s	142.6 s
22	112.3 t	112.3 t	112.3 t	112.3 t	112.3 t
23	22.5 q	22.5 q	22.5 q	22.5 q	22.5 q
24	17.4 q	17.4 q	17.4 q	17.5 q	17.4 q
25	63.2 d	63.2 d	63.2 d	63.2 d	63.2 d
OMe-14				56.8 q	
OC*H*$_2$CH$_3$-16					56.6 t
OCH$_2$C*H*$_3$-16					16.1 q

[a] Multiplicities were deduced from DEPT and/or HSQC experiments.

a correlation of H$_2$-14 to the aromatic carbons C-3, C-4, and C-10, which indicated that the 2-methyl-2-formylpropyl group is located at C-4.

The relative configurations of **1** at C-20 and C-25 were assigned as *S* and *R*, respectively, the same as those reported for shamixanthone and tajixanthone,[13,14] on the basis of the coupling constants and NOESY correlations of those protons (Figure 2), as well as comparison of the specific rotation [+24 (*c* 0.06, $CHCl_3$)] with the analogue, shamixanthone [+25.2 (*c* 0.33, $CHCl_3$)].[2] Thus, the structure of **1** was defined as a new xanthone as shown and has been named ruguloxanthone A.

Compound **2** was obtained as a yellow solid and was also assigned the molecular formula $C_{25}H_{26}O_6$ from the HRESITOFMS. The IR spectrum showed the presence of hydroxy (3503 cm^{-1}),

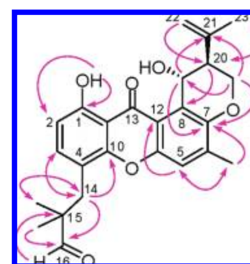

Figure 1. Selected HMBC correlations for **1**.

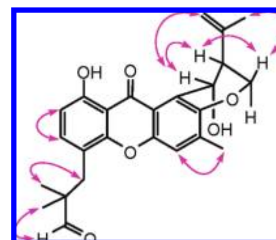

Figure 2. NOESY correlations for **1**.

ketone (1718 cm^{-1}), and aromatic ketone (1640 cm^{-1}) groups. The ^{1}H and ^{13}C NMR spectra of **2** (Tables 1 and 2) were similar to those of **1**, except for the replacement of the 2-methyl-2-formylpropyl group signals with resonances characteristic of a 3-methyl-2-oxobutyl unit. The HMBC spectrum showed correlations of H$_3$-17 and H$_3$-18 to C-15 and C-16, and of H$_2$-14 to C-3, C-4, C-10, and C-15, confirming the connectivity of this side chain. Compound **2** also exhibited a positive specific rotation in the same manner as **1**. Thus, **2** (ruguloxanthone B) was also defined as a new xanthone, as shown.

It may be suggested that compounds **1** and **2** are derived from tajixanthone via epoxide ring-opening under acid conditions, which could undergo a pinacol-type rearrangement to give the aldehyde **1** or ketone **2**. However, attempts to stir the tajixanthone in EtOAc/2.5 M H_2SO_4, CH_2Cl_2/2.5 M H_2SO_4, and EtOH/10% acetic acid, with or without silica gel for 7 days, were unsuccessful. Therefore, the potential biotransformation of tajixanthone to **1** and **2** is an interesting topic and requires further investigation.

Compound **3** was obtained as yellow crystals, and the HRESITOFMS data indicated that it is an isomer of **1** and **2**. The IR data showed the occurrence of hydroxy (3449 and 3367 cm^{-1}) and

Table 3. ^1H and ^{13}C NMR Spectroscopic Data (δ, ppm) of Compound **6** in $CDCl_3$

position	^1H	^{13}C	COSY	HMBC	NOESY
1		209.9			
2	6.13 (d, 5.5)[a]	133.4	3	1, 3, 4, 5	3
3	7.38 (dd, 5.5, 2.7)	167.1	2, 4	1, 2, 4	2
4	3.95 (brm)	40.2	3, 6, 5a, 5b	2[d], 3, 6, 7[d]	5a, 8, 25
5a	2.56 (dd, 18.9, 6.4)	41.6	4	1, 3, 4, 6	4
5b	(dd, 18.9, 1.8)				
6	5.13 (d, 9.8)	130.9	4, 8[b], 21[b]	3[d],4, 5, 8, 21	5b, 21
7		137.2			
8	3.27 (brs)	55.5	6[b], 10[b], 12[c]	6, 7, 9, 10, 13, 14, 21	4, 12, 22, 25
9		131.5			
10	5.39 (s)	136.6	8[b], 22[b]	8, 11, 12, 22, 24	16[d], 22, 24
11		35.3			
12	3.11 (s)	45.6	8[c], 14[c], 16[c], 24[c], 25[c], 26[b]	8, 10, 11, 13, 14, 16, 17, 18, 24, 26	8, 24, 25
13		39.8			
14	5.00 (s)	128.9	12[c], 16[b], 23[b]	12, 16, 23	21, 23, 25
15		132.5			
16	1.74 (brABq, 17.9)	39.9	14[b], 24[c]	10, 11, 12, 14, 15, 23, 24	23, 24, 26b
17		147.6			
18		203.6			
19	2.72 (m)	30.4	20	18, 20	20, 26a
20	1.09 (t, 7.4)	9.1	19	18, 19	19
21	1.54 (s)	22.2	6[b]	6, 7	6, 14
22	1.51 (s)	21.4	10[b]	8, 9, 10	10, 8
23	1.63 (s)	22.8	14[b]	14, 15, 16	14
24	0.76 (s)	26.7		10, 11, 12, 16	10, 12, 16
25	0.84 (s)	23.4		8, 12, 13, 14	4, 8, 12, 14, 26b
26a	6.16 (s)	124.8	12[b]	11[d], 12, 17, 18	19
26b	5.76 (s)				16, 25

[a] Values in parentheses are coupling constants in Hz. [b] Allylic coupling of these protons. [c] *W*-coupling of these protons. [d] Weak correlation.

conjugated ketone (1641 cm^{-1}) groups. The ^1H and ^{13}C NMR spectra of **3** (Tables 1 and 2) were similar to those of **1** except for the absence of a 2-methyl-2-formylpropyl side chain, which was replaced by a 2-hydroxy-3-methylbut-3-enyl group. The COSY spectrum showed correlations between H_2-14 and H-15, and H_2-17 and H_3-18, and HMBC correlations of H_2-14 to C-3, C-4, C-10, C-15, and C-16; H-15 to C-16 and C-18; H_2-17 to C-15 and C-18; and H_2-18 to C-15, C-16, and C-17 were observed, which supported the presence of this group. Compound **3** showed a positive specific rotation [+71 (*c* 0.06, $CHCl_3$)], in contrast to the related compounds tajixanthone hydrate [−72 (*c* 2.3, $CHCl_3$)][13] and tajixanthone methanoate [−76 (*c* 0.23, $CHCl_3$)],[3] which were reported to have an *S* configuration at C-15. Assuming that the configurations at C-20 and C-25 in **3** are the same as in **1** and **2**, this implies that C-15 of **3** has the *R* configuration. On the basis of this evidence, **3** (ruguloxanthone C) was defined as a new xanthone, as shown.

Compound **4** was obtained as yellow crystals and was assigned the molecular formula $C_{26}H_{28}O_7$ from the HRESITOFMS data (observed *m/z* 475.1733 $[M + Na]^+$), implying 13 degrees of unsaturation. The ^{13}C NMR and DEPT data exhibited 26 carbons attributable to five methyls, two methylenes, seven methines, and 12 nonprotonated carbons. The IR spectrum indicated the presence of hydroxy (3492 cm^{-1}) and aromatic ketone (1645 cm^{-1}) groups. The ^1H and ^{13}C NMR spectra of **4** (Tables 1 and 2) were similar to those of tajixanthone,[13] except for the signal corresponding to C-14, which showed a singlet at δ 3.37 for a methoxy group, as confirmed by the NOESY correlation between the C-14 methoxy protons and H-14 as well as an HMBC correlation from the C-14 methoxy protons to C-14. Moreover, the coupling constant ($J = 8.2$ Hz) of H-14 and H-15 revealed a *trans* relationship with a dihedral angle between the protons close to 180°.[16] The C-14 and C-15 carbons were assigned with *R* and *S* configurations, respectively, by analogy with 14-methoxytajixanthone-25-acetate [−38 (*c* 0.1, $CHCl_3$)].[3] Thus, **4** (14-methoxytajixanthone) was defined as a new tajixanthone derivative.

Compound **5** was obtained as yellow needles and shown to possess the molecular formula $C_{27}H_{32}O_7$, as deduced from its HRESITOFMS data (observed *m/z* 491.2046 $[M + Na]^+$), implying 12 degrees of unsaturation. The IR spectrum showed absorption bands for hydroxy (3434 cm^{-1}) and conjugated ketone (1638 cm^{-1}) groups. The ^1H and ^{13}C NMR data of **5** (Tables 1 and 2) were similar to those of tajixanthone methanoate,[3] except for the absence of a C-16 methoxy group signal, which was replaced by signals for an ethoxy group. The HMBC spectrum showed a correlation of the methylene protons of the ethoxy group to C-16 and confirmed the location of the ethoxy unit at C-16. The C-15 carbon was assigned with the *S* configuration, by analogy with tajixanthone methanoate, since both gave the same sign of specific rotation.[3,14] Accordingly, **5** (tajixanthone ethanoate) was proposed as a further new tajixanthone derivative, as shown.

The reaction of tajixanthone in EtOH/2.5 M H_2SO_4, with or without silica gel at room temperature for 18 h, yielded the ethanolysis and hydrolysis products, tajixanthone ethanoate (**5**) and tajixanthone hydrate, respectively. However, when tajixanthone was stirred in EtOH/glacial acetic acid with or without silica gel for 3 days, no reaction was observed. Thus, it may be concluded that **5** is naturally occurring and not an artifact produced during the isolation process.

Compound **6** was obtained as a colorless, viscous liquid and assigned the molecular formula $C_{26}H_{34}O_2$, as deduced from the HRESITOFMS (observed 401.2458 $[M + Na]^+$), implying 10 degrees of unsaturation. The ^{13}C NMR spectrum showed 26 carbon signals, attributable to six methyls, four methylenes, eight methines, and eight nonprotonated carbons. The UV and IR data of **6** suggested the presence of an α,β-unsaturated carbonyl group. The COSY spectrum of **6** showed connectivities for four proton spin-systems: H-2↔H-3↔H-4↔H_2−5, H-4↔H-6↔H_3-21 and H-8 (allylic coupling), H-10↔H-8 and H_3-22 (allylic coupling); H-14↔H_2-16 and H_3-23 (allylic coupling); H-12↔H_2-26 (allylic coupling); and H_2-19↔H_3-20. The COSY spectrum also showed *W*-coupling (^4J-coupling) correlations between H-12 and H-8, H-14, H_2-16, H_3-24, and H_3-25, which confirmed the connection of these protons (Table 3 and Figure 3). Interpretation of the HMBC spectrum of **6** led to the assembly of these four proton networks and the remaining hydrogens and nonprotonated carbons, to complete the whole structure of **6**.

The HMBC spectrum of **6** displayed correlations of H-12 to C-8, C-10, C-11, C-13, C-14, C-16, C-18, C-24, and C-26; H-8 to C-9,

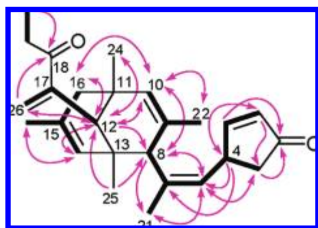

Figure 3. COSY (bold lines) and selected HMBC (arrows, H→C) correlations for **6**.

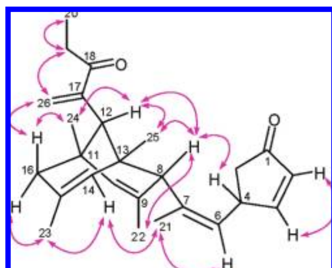

Figure 4. Selected NOESY correlations for **6**.

C-10, C-13, and C-14; H-10 to C-8, C-11, and C-12; H-14 to C-12, and C-16; and H-16 to C-10, C-11, C-12, and C-14 and revealed the presence of a bicyclo[3.3.1]nona-2,6-diene skeleton[17,18] (Table 3 and Figure 3). The side chain at C-8 was supported by correlations from H-8 to C-6 and C-7. The HMBC correlation of H-12 to C-17, C-18, and C-26 was used to establish that the pent-1-en-3-one moiety is attached to C-12. The relative configuration of the bicyclo[3.3.1]nona-2,6-diene ring in **6** was assigned on the basis of NOESY correlations (Table 3 and Figure 4). The alkenes were assigned with the *Z*-configuration on the basis of correlations between H-2 and H-3, H-6 and H_3-21, H-10 and H_3-22, and H-14 and H_3-23. Correlations between H-8 and H-4, H-12, and H_3-25 indicated that these protons are all on the same side of the molecule. Moreover, the correlation between H-26 and H-16 confirmed the location of these groups. On the basis of the above evidence, compound **6** was assigned as a new bicyclo[3.3.1]nona-2,6-diene derivative, and it has been named rugulosone.

Recently, the bicyclo[3.3.1]nonane core has been found in compounds such as gymnastatin F[17] and isariotins A−D,[18] isolated from fungi, and a plausible biosynthetic pathway of this core skeleton was also proposed.[17] Therefore, the bicyclo[3.3.1]nona-2,6-diene core of **6** might occur via the same pathway. It should be noted that this is the first bicyclo[3.3.1]nona-2,6-diene derivative obtained from a natural source.

Compound **6** showed both in vitro antimalarial activity against *Plasmodium falciparum*, with an IC_{50} value of 1.9 μg/mL, and antimycobacterial activity against *Mycobacterium tuberculosis*, with a MIC value of 12.5 μg/mL. Compound **6** also exhibited cytotoxicity against the BC1, KB, and NCI-H187 cancer cell lines, with IC_{50} values of 1.3, 2.6, and 1.3 μg/mL, respectively. None of the isolated xanthones were antimalarial, antimycobacterial, or cytotoxic against cancer cell lines.

Experimental Section

General Experimental Procedures. Melting points were determined using a Gallenkamp melting point apparatus and are uncorrected. Optical rotations were obtained using a JASCO DIP-1000 digital polarimeter. UV spectra were measured on an Agilent 8453 UV−visible spectrophotometer. IR spectra were recorded on a Perkin-Elmer Spectrum One spectrometer. NMR spectra were recorded in $CDCl_3$ on a Varian Mercury Plus 400 spectrometer, using residual $CHCl_3$ as internal standard. HRESITOFMS were obtained using a Micromass LCT mass spectrometer, and the lock mass calibration was applied for the determination of accurate masses. Column chromatography and preparative TLC were carried out on silica gel 60 (230−400 mesh) and PF_{254}, respectively.

Fungal Material. The fungus was collected from Plaung Village, Khao Kitchakut District, Chanthaburi Province, Thailand, in June 2007 and was identified by Assoc. Prof. K. Soytong. A voucher specimen (Er01) was deposited at the Department of Plant Pest Management, King Mongkut's Institute of Technology Ladkrabang, Bangkok, Thailand. The fungus was cultured in conical flasks (1 L, 60 flasks) with potato dextrose broth (PDB) (250 mL/flask) and incubated in standing conditions at 25−28 °C for 4 weeks. The culture broth was filtered to give a wet mycelial mat and then air-dried at room temperature.

Extraction and Isolation. The air-dried mycelial mat of *E. rugulosa* (282 g) was ground and extracted successively at room temperature with hexane (400 mL × 3), EtOAc (400 mL × 3), and MeOH (400 mL × 3), to give crude hexane (8.3 g), EtOAc (5.9 g), and MeOH (10.9 g) extracts.

The hexane extract (8.2 g) was separated initially by silica gel column chromatography, eluted with gradient systems of CH_2Cl_2/hexane and EtOAc/CH_2Cl_2, followed by EtOAc/MeOH. Each fraction (100 mL) was monitored by TLC, and fractions with similar TLC patterns were combined to yield fractions F_1−F_5. Fraction F_3 was subjected to silica gel flash column chromatography, eluted with a gradient system of CH_2Cl_2/hexane followed by EtOAc/CH_2Cl_2, to furnish five subfractions, designated as $F_{3/1}$−$F_{3/5}$. $F_{3/5}$ was further separated by column chromatography, eluted with CH_2Cl_2/hexane (1:1), to yield shamixanthone (180.5 mg) and isoemericellin (7.5 mg). Fraction F_4 was chromatographed on a silica gel column, eluted with a gradient system of CH_2Cl_2/hexane followed by EtOAc/CH_2Cl_2, to give five subfractions, $F_{4/1}$−$F_{4/5}$. Subfraction $F_{4/2}$ was purified by silica gel flash column chromatography, eluted with EtOAc/hexane (1:10), to afford an additional amount of shamixanthone (15.6 mg). Subfraction $F_{4/3}$ was subjected to silica gel flash column chromatography, eluted with EtOAc/hexane (1:10), followed by preparative TLC (EtOAc/hexane, 1:10), to yield **6** as a colorless, viscous liquid (32.6 mg), **1** as a yellow solid (10.5 mg), yellow needles of tajixanthone (20.0 mg), and **2** as a yellow solid (5.0 mg). Subfraction $F_{4/4}$ was chromatographed on a silica gel column, eluted with a gradient system of EtOAc/hexane, and further separated by preparative TLC (EtOAc/hexane, 1:10), to give yellow needles of **5** (12.6 mg), yellow crystals of **3** (17.2 mg), and yellow crystals of tajixanthone methanoate (18.8 mg). Subfraction $F_{4/5}$ was further purified by flash column chromatography, eluted with a gradient system of EtOAc/hexane, to afford an additional amount of tajixanthone (127.5 mg), **4** as a yellow solid (13.2 mg), and 14-methoxytajixanthone-25-acetate as a yellow solid (10.1 mg). Purification of fraction F_5 by flash column chromatography, eluted with a gradient system of EtOAc/CH_2Cl_2, gave tajixanthone hydrate as a yellow solid (28.0 mg).

The EtOAc extract was chromatographed on a silica gel (60 g) column, eluted with a gradient system of EtOAc/CH_2Cl_2 followed by EtOAc/MeOH, to yield fractions F'_1−F'_7. Fraction F'_4 was separated by column chromatography, eluted with a gradient system of EtOAc/CH_2Cl_2, to give colorless needles of ergosterol (15.6 mg). Fraction F'_6 was separated by column chromatography, eluted with a gradient system of EtOAc/CH_2Cl_2, to give an additional amount of tajixanthone hydrate (15.8 mg).

Ruguloxanthone A (1): yellow crystals (CH_2Cl_2/EtOH); mp 188−189 °C; $[\alpha]^{28}_D$ +24 (*c* 0.06, $CHCl_3$); UV (MeOH) λ_{max} (log ε) 204 (4.47), 241 (4.40), 254 (4.35), 274 (4.48) nm; IR (KBr) ν_{max} 3524, 3078, 2974, 2923, 2709, 1718, 1637, 1600, 1572, 1473, 1428, 1352, 1248, 1224, 1199, 1049 cm^{-1}; ^1H and ^{13}C NMR data, see Tables 1 and 2; HRESITOFMS *m/z* 445.1623 $[M + Na]^+$ (calcd for $C_{25}H_{26}O_6$ + Na, 445.1627).

Ruguloxanthone B (2): yellow solid; mp 200−202 °C; $[\alpha]^{28}_D$ +220 (*c* 0.03, $CHCl_3$); UV (MeOH) λ_{max} (log ε) 203 (4.54), 241 (4.37), 252 (4.34), 273 (4.41) nm; IR (KBr) ν_{max} 3503, 2925, 2853, 1718, 1640, 1605, 1570, 1474, 1457, 1431, 1249, 1227, 1201, 1046 cm^{-1}; ^1H and ^{13}C NMR data, see Tables 1 and 2; HRESITOFMS *m/z* 445.1632 $[M + Na]^+$ (calcd for $C_{25}H_{26}O_6$ + Na, 445.1627).

Ruguloxanthone C (3): yellow crystals (CH_2Cl_2/EtOH); mp 156−157 °C; $[\alpha]^{29}_D$ +71 (*c* 0.06, $CHCl_3$); UV (MeOH) λ_{max} (log ε) 203 (4.43), 241 (4.35), 274 (4.44) nm; IR (KBr) ν_{max} 3449, 3367, 3304, 3086, 2922, 2855, 1641, 1606, 1578, 1482, 1472, 1430, 1350, 1246, 1230, 1196, 1084, 1049, 1018 cm^{-1}; ^1H and ^{13}C NMR data, see Tables 1 and 2; HRESITOFMS *m/z* 445.1627 $[M + Na]^+$ (calcd for $C_{25}H_{26}O_6$ + Na, 445.1627).

14-Methoxytajixanthone (4): yellow crystals (CH_2Cl_2/EtOH); mp 189−190 °C; $[\alpha]^{29}_D$ −191 (*c* 0.12, $CHCl_3$); UV (MeOH) λ_{max} 204 (4.37), 242 (4.29), 250 (4.28), 267 (4.28), 272 (4.29) nm; IR (KBr) ν_{max} 3492, 3086, 3298, 2927, 2855, 1710, 1645, 1605, 1576, 1470, 1431, 1252, 1237, 1078 cm^{-1}; ^1H and ^{13}C NMR data, see Tables 1 and 2; HRESITOFMS *m/z* 475.1733 $[M + Na]^+$ (calcd for $C_{26}H_{28}O_7$ + Na, 475.1733).

Tajixanthone Ethanoate (5): yellow needles (CH_2Cl_2/EtOH); mp 171−173 °C; $[\alpha]^{27}_D$ −58 (*c* 0.25, $CHCl_3$); UV (MeOH) λ_{max} (log ε) 202 (4.22) 241 (4.15), 274 (4.26), 296 (3.69), 393 (3.54) nm; IR (KBr) ν_{max} 3434, 3083, 2967, 2926, 1638, 1593, 1578, 1476, 1429, 1354, 1244, 1212, 1195, 1115, 1084, 1017 cm^{-1}; ^1H and ^{13}C NMR data, see Tables 1 and 2; HRESITOFMS *m/z* 491.2046 $[M + Na]^+$ (calcd for $C_{27}H_{32}O_7$ + Na, 491.2081).

Rugulosone (6): colorless, viscous liquid, $[\alpha]^{28}_D$ −10 (*c* 0.08, $CHCl_3$); UV (MeOH) λ_{max} (log ε) 211 (4.44) nm; IR (neat) ν_{max} 3586, 3326, 2963, 2921, 2862, 1714, 1647, 1584, 1455, 1411, 1371 cm^{-1}; ^1H and ^{13}C NMR data, see Table 3; HRESITOFMS *m/z* 401.2458 $[M + Na]^+$ (calcd for $C_{26}H_{34}O_2$ + Na, 401.2456).

Preparation of Tajixanthone Ethanoate (5) and Tajixanthone Hydrate. To a solution of tajixanthone (9.2 mg, 0.021 mmol) in ethanol (2 mL) was added 2.5 M H_2SO_4 (0.5 mL), and the solution was stirred at rt for 18 h. Cooled water was added to the reaction mixture and extracted with EtOAc (10 mL × 3). The organic layer was combined, washed with water and brine, and dried over anhydrous Na_2SO_4. The filtrate was vaporated to dryness, and the residue was purified by preparative TLC (10% EtOAc/hexane) to give solid **5** (4.2 mg, 44.8%) and tajixanthone hydrate (4.1 mg, 40.2%). The IR and NMR spectra were identical to those of the respective natural products, tajixanthone ethanoate (**5**) (Tables 1 and 2) and tajixanthone hydrate.[13,14]

Antimalarial Assay. Antimalarial activity was evaluated in vitro against the parasite *Plasmodium falciparum* (K1, multidrug-resistant strain), using the method of Trager and Jensen.[19] Quantitative assessment of malarial activity in vitro was determined by means of the microculture radioisotope technique based upon the method described by Desjardins et al.[20] The inhibitory concentration (IC_{50}) represents the concentration that causes 50% reduction in parasite growth, as indicated by the in vitro uptake of [^3H]-hypoxanthine by *P. falciparum*. The standard compound, artemisinin, exhibited an IC_{50} value of 1.0 ng/mL.

Antimycobacterial Assay. Antimycobacterial activity was assessed against *Mycobacterium tuberculosis* H37Ra using the microplate Alamar Blue assay (MABA).[21] The standard drugs isoniazid and kanamycin sulfate showed respective MIC values of 0.04−0.09 and 2.0−5.0 μg/mL.

Cytotoxicity Assay. Cytotoxicity assays against the human epidermoid carcinoma (KB), human breast cancer (BC1), and human small-cell lung cancer (NCI-H187) cell lines were performed employing the colorimetric method as described by Skehan and co-workers.[22] The reference substance was ellipticine, which showed IC_{50} values of 0.36, 0.32, and 0.26 μg/mL, respectively.

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Supporting Information Available: ^1H and ^{13}C NMR spectra for **1**−**6** and 2D-NMR spectra for **6**. This material is available free of charge via the Internet at http://pubs.acs.org.

References and Notes

(1) Malmstrom, J.; Chistophersen, C.; Barrero, A. F.; Oltra, J. E.; Justicia, J.; Rosales, A. *J. Nat. Prod.* **2002**, *65*, 364–366.
(2) Bringmann, G.; Lang, G.; Steffens, S.; Günther, E.; Schaumann, K. *Phytochemistry* **2003**, *63*, 437–443.
(3) Pornpakakul, S.; Liangsakul, J.; Ngamrojanavanich, N.; Roengsumran, S.; Sihanonth, P.; Piapukiew, J.; Sangvichien, E.; Puthong, S.; Petsom, A. *Arch. Pharm. Res.* **2006**, *29*, 140–144.
(4) Kralj, A.; Kehraus, S.; Krick, A.; Eguereva, E.; Kelter, G.; Maurer, M.; Wortmann, A.; Fiebig, H. H.; König, G. M. *J. Nat. Prod.* **2006**, *69*, 995–1000.
(5) Takahashi, H.; Hosoe, T.; Nozawa, K.; Kawai, K. *J. Nat. Prod.* **1999**, *62*, 1712–1713.
(6) Yoganathan, K.; Rossant, C.; Glover, R. P.; Cao, S.; Vittal, J. J.; Ng, S.; Huang, Y.; Buss, A. D.; Butler, M. S. *J. Nat. Prod.* **2004**, *67*, 1681–1684.
(7) Wei, H.; Itoh, T.; Kinoshita, M.; Nakai, Y.; Kurotaki, M.; Kobayashi, M. *Tetrahedron* **2004**, *60*, 6015–6019.
(8) Hosoe, T.; Itabashi, T.; Kobayashi, N.; Udagawa, S.; Kawai, K. *Chem. Pharm. Bull.* **2006**, *54*, 158–157.
(9) Wei, H.; Itoh, T.; Kinoshita, M.; Kotoku, N.; Aoki, S.; Kobayashi, M. *Tetrahedron* **2005**, *61*, 8054–8058.
(10) Malmstrom, J.; Ryager, A.; Anthoni, U.; Halfdan, N. *Phytochemistry* **2002**, *60*, 869–872.
(11) Domsch, K. H.; Gams, W.; Anderson, T. H. *Compendium of Soil Fungi, Vol. I*; IHW-Verlag: Exeter, UK, 1993.
(12) Trigos, A.; Martinez, L. C.; Lopez-Reyes, E. E. *Micol. Apl. Int.* **2005**, *17*, 1–4.
(13) Chexal, K. K.; Fouweather, C.; Holker, J. S. E.; Simpson, T. J.; Young, K. *J. Chem. Soc., Perkin Trans. 1* **1974**, 1584–1593.
(14) Chexal, K. K.; Fouweather, C.; Holker, J. S. E.; Simpson, T. J.; Young, K. *J. Chem. Soc., Perkin Trans. 1* **1975**, 549–554.
(15) Bok, J. W.; Lermer, L.; Chilton, J.; Klingeman, H. G.; Towers, G. H. N. *Phytochemistry* **1999**, *51*, 891–898.
(16) Pavia, D. L.; Lampman, G. M.; Kriz, G. S.; Vyvyan, J. R. *Introduction to Spectroscopy*, 4th ed.; Books/Cole, Cengage Learning: Belmont, CA, 2008; pp 241−246.
(17) Amagata, T.; Minoura, K.; Numata, A. *J. Nat. Prod.* **2006**, *69*, 1384–1388.
(18) Haritakun, R.; Srikitikulchai, P.; Khoyaiklang, P.; Isaka, M. *J. Nat. Prod.* **2007**, *70*, 1478–1480.
(19) Trager, W.; Jensen, J. B. *Science* **1967**, *193*, 673–675.
(20) Desjardins, R. E.; Canfield, C. J.; Haynes, J. D.; Chulay, J. D. *Antimicrob. Agents Chemother.* **1979**, *16*, 710–718.
(21) Collins, L.; Franzblau, S. G. *Antimicrob. Agents Chemother.* **1997**, *4*, 1004–1009.
(22) Skehan, P.; Storeng, R.; Scudiero, D.; Monks, A.; McMahon, J.; Vistica, D.; Warren, J. T.; Bokesch, H.; Kenney, S.; Boyd, M. R. *J. Natl. Cancer Inst.* **1990**, *82*, 1107–1112.

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