ORGANIC LETTERS

2013
Vol. 15, No. 1
120–123

Fluevirosines A–C: A Biogenesis Inspired Example in the Discovery of New Bioactive Scaffolds from *Flueggea virosa*

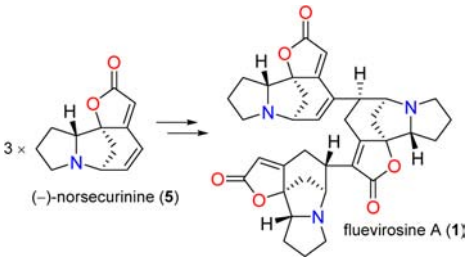
Hua Zhang, Chuan-Rui Zhang, Kong-Kai Zhu, An-Hui Gao, Cheng Luo, Jia Li, and Jian-Min Yue*

State Key Laboratory of Drug Research, Shanghai Institute of Materia Medica, Chinese Academy of Sciences, 555 Zuchongzhi Road, Zhangjiang Hi-Tech Park, Shanghai 201203, People's Republic of China

jmyue@mail.shcnc.ac.cn

Received November 15, 2012

ABSTRACT



Biogenesis inspired chemical investigation of a Chinese folk medicine, *Flueggea virosa*, returned three unprecedented C,C-linked trimeric *Securinega* alkaloids, fluevirosines A–C (1–3). Their absolute structures were characterized on the basis of spectroscopic data and computational analysis. Compounds 2 and 3 showed inhibition against the splicing of XBP1 mRNA.

The plant originated *Securinega* alkaloids are a group of secondary metabolites isolated mainly from the species of the *Securinega*, *Phyllanthus*, *Flueggea*, *Margaritaria*, and *Breynia* genera of the Euphorbiaceae family.[1] A typical structure of these alkaloids comprises an indolizidine (securinine-type)/pyrrolizidine (norsecurinine-type) heterocycle and an α,*β*,*γ*,*δ*-conjugated lactone fragment to form a highly rigid tetracyclic skeleton. Due to their fascinating structural features and significant biological properties, the *Securinega* alkaloids have attracted considerable interest from natural products, synthetic, and medicinal chemists, as well as pharmacologists in the past half century,[1,2] since securinine was initially discovered from *Securinega suffructicosa*.[3]

Flueggea virosa (Roxb. ex Willd.) Voigt, whose parts are used in Chinese folk medicine for the treatment of eczema, rheumatoid arthritis, etc.,[4] has proven to be a rich source of *Securinega* alkaloids.[5] Fluggeaine ether[5a] from *F. virosa*, the first dimer of this alkaloid family, is likely the condensation product of two norsecurinine-type cometabolites via an ether bridge. Considering the α,*β*,*γ*,*δ*-unsaturated carbonyl moiety that is prone to attack from nucleophiles, the report of fluggeaine ether is not surprising, while the exploration of more complex oligomers also appears prospective. However, despite the rapid development of

(1) For reviews, see: (a) Snieckus, V. In *The Alkaloids*; Manske, R. H. F., Ed.; Academic Press: New York, 1973; Vol. 14, pp 425–506. (b) Beutler, J. A.; Brubaker, A. N. *Drug Future* **1987**, *12*, 957–976.

(2) For reviews, see: (a) Zhang, W.; Li, J. Y.; Lan, P.; Sun, P. H.; Wang, Y.; Ye, W. C.; Chen, W. M. *J. Chin. Pharm. Sci.* **2011**, *20*, 203–217. (b) Weinreb, S. M. *Nat. Prod. Rep.* **2009**, *26*, 758–775.

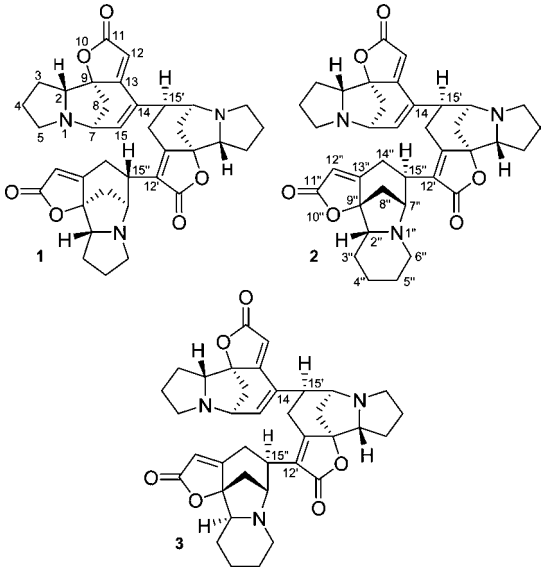
(3) Murev'eva, V. I.; Ban'kovskii, A. I. *Dokl. Akad. Nauk SSSR* **1956**, *110*, 998–1000.

(4) Li, B.; Gilbert, M. G.; Fischer, G.; Meyer, C. A. In *Flora of China*; Wu, Z. Y., Raven, P. H., Hong, D. Y., Eds.; Science Press: Beijing, Missouri Botanical Garden Press: St. Louis, 2008; Vol. 11, p 178.

(5) (a) Chen, M. J.; Hou, L. L. *Zhiwu Xuebao* **1985**, *27*, 625–629. (b) Luger, P.; Weber, M.; Dung, N. X.; Ky, P. T.; Long, P. K. *Acta Crystallogr., Sect. C* **1995**, *51*, 127–129. (c) Dehmlow, E. V.; Guntenhöner, M.; Ree, T. V. *Phytochemistry* **1999**, *52*, 1715–1716. (d) Gan, L. S.; Fan, C. Q.; Yang, S. P.; Wu, Y.; Lin, L. P.; Ding, J.; Yue, J. M. *Org. Lett.* **2006**, *8*, 2285–2288. (e) Gan, L. S.; Yue, J. M. *Nat. Prod. Commun.* **2006**, *1*, 819–823. (f) Wang, G. C.; Wang, Y.; Li, Q.; Liang, J. P.; Zhang, X. Q.; Yao, X. S.; Ye, W. C. *Helv. Chim. Acta* **2008**, *91*, 1124–1129. (g) Wang, G. C.; Liang, J. P.; Wang, Y.; Li, Q.; Ye, W. C. *Zhongguo Tianran Yaowu* **2008**, *6*, 251–253. (h) Zhao, B. X.; Wang, Y.; Zhang, D. M.; Jiang, R. W.; Wang, G. C.; Shi, J. M.; Huang, X. J.; Chen, W. M.; Che, C. T.; Ye, W. C. *Org. Lett.* **2011**, *13*, 3888–3891. (i) Zhao, B. X.; Wang, Y.; Zhang, D. M.; Huang, X. J.; Bai, L. L.; Yan, Y.; Chen, J. M.; Lu, T. B.; Wang, Y. T.; Zhang, Q. W.; Ye, W. C. *Org. Lett.* **2012**, *14*, 3096–3099.

10.1021/ol303146a © 2012 American Chemical Society
Published on Web 12/17/2012

separation and NMR techniques in the past two decades, the first C−C linked dimeric flueggenines A (**4**) and B were only isolated and characterized from *F. virosa* in 2006 by our research group, with the former showing cytotoxicity against the P-388 tumor cell line.[5d]



In the previous paper, we proposed a plausible biosynthetic pathway for flueggenines A (**4**) and B involving a key self-catalyzed step similar to that of the Baylis−Hillman reaction.[5d] Inspired by the discovery of the two dimers and the fact that they still possess a less hindered C-13′ (as marked in **4**, Scheme 1) active site, we assumed that a higher level of oligomers could also exist in a trace of amount in this herb. To challenge this hypothesis, we recollected the plant material of *F. virosa* and performed a more careful and intensive investigation. Confirming our assumption, the current project resulted in the separation and identification of three unprecedented trimeric alkaloids, fluevirosines A−C (**1**−**3**), together with their monomeric precursors (−)-norsecurinine (**5**),[5a] viroallosecurinine (**6**),[6] and virosecurinine (**7**),[5a] as well as the dimeric intermediate flueggenine A (**4**).[5d] Absolute structures were assigned to **1**−**3** based on detailed examinations of spectroscopic data, comparisons with the authentic co-metabolites **4**−**7** (for structures, see Chart S1 in Supporting Information (SI)), computational analysis, and biogenetic considerations. We herein describe the extraction, isolation, structural elucidation, and bioactivities of these intriguing alkaloids.

High resolution EIMS analysis of fluevirosine A (**1**)[7] revealed a molecular ion at *m*/*z* 609.2838 consistent with a molecular formula of $C_{36}H_{39}N_3O_6$ (calcd 609.2839) incorporating 19 double bond equivalents (DBEs). The IR spectrum displayed medium (1643 and 1624 cm^{-1}, C=C) and strong (1755 cm^{-1}, C=O) absorption bands corresponding to olefinic group(s) and α,β-unsaturated γ-lactone(s).[8] The NMR data for **1** (Table S1 in SI) showed high similarities to those for flueggenine A (**4**)[5d] and (−)-norsecurinine (**5**)[5a] (Table S2 in SI), exhibiting resonances for three lactone carbonyls (δ_C 172.6, 172.1, and 171.9), three trisubstituted double bonds (δ_C 173.9, 110.0; 168.5, 107.4; 139.0, 132.2; δ_H 6.68, 5.68, and 5.66), one tetrasubstituted double bond (δ_C 164.9 and 122.1), and 25 sp^3 carbons (15 methylenes, eight methines, and three oxygenated quaternary carbons at δ_C 91.9, 91.8, and 89.9). These data accounted for seven DBEs and suggested the presence of a 12-ring system in **1**. The above observations indicated that **1** was likely a trimeric *Securinega* alkaloid consisting of one norsecurinine and two dihydronorsecurinine substructures.

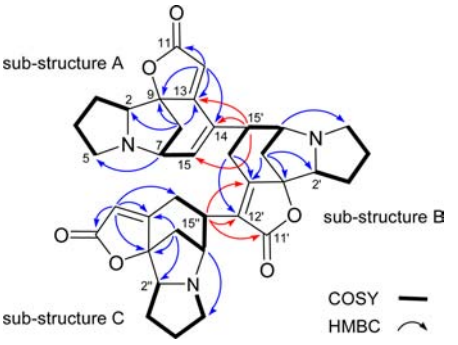


Figure 1. Key 2D NMR correlations for **1**.

Further analysis of the 2D NMR data ($^1H-^1H$ COSY, HSQC, and HMBC; Figure 1 and Figures S3−S5 in SI) enabled a full assignment of all signals and the construction of a trimeric architecture for **1** as shown. More specifically, the $^1H-^1H$ COSY data (Figure 1) revealed diagnostic structural fragments that well matched those in **5**[5a] and dihydronorsecurinine,[9] i.e., (a) H-2 to H_2-5 and H_2-8 to H-15 via H-7 for substructure A; (b) H-2′ to H_2-5′ and H_2-8′ to H-14′ via H-7′ and H-15′ for substructure B; (c) H-2″ to H_2-5″ and H_2-8″ to H-14″ via H-7″ and H-15″ for substructure C. In addition, the three structural units as one norsecurinine and two dihydronorsecurinines were also confirmed by HMBC data (Figure 1) as depicted. In particular, the critical HMBC correlations from H-15′ to C-13, C-14, and C-15, and from H-15″ to C-11′, C-12′, and C-13′, unambiguously established the connections of C-15′/C-14 and C-15″/C-12′, thus defining the planar structure of **1** as drawn.

The relative configuration of **1** was characterized by interpretation of ROESY data (Figure S6 in SI) and comparisons with model compounds **4**[5d] and flueggine B.[5h]

(6) Tatematsu, H.; Mori, M.; Yang, T. H.; Chang, J. J.; Lee, T. T. Y.; Lee, K. H. *J. Pharm. Sci.* **1991**, *80*, 325–327.

(7) Pale yellow solid; $[\alpha]_D^{23}$ +13.5 (*c* 0.855, $CHCl_3$); UV (MeOH) λ_{max} (log ε) 259 (4.43), 212 (4.78) nm; CD (MeOH) λ (Δε) 266 (−11.8) nm; IR (KBr) ν_{max} 2962, 2872, 1755, 1643, 1624, 1460, 1223, 1118, 1080, 919 cm^{-1}; ^1H and ^{13}C NMR data, see Table S1 in SI; EIMS (70 eV) *m*/*z* 609 $[M]^+$ (14), 540 (78), 500 (8), 471 (6), 350 (14), 204 (6), 190 (10), 96 (29), 70 (100); HR-EIMS *m*/*z* 609.2838 $[M]^+$ (calcd for $C_{36}H_{39}N_3O_6$, 609.2839).

(8) Pretsch, E.; Bühlmann, P.; Badertscher, M. In *Structure Determination of Organic Compounds*, 4th ed.; Springer-Verlag: Berlin and Heidelberg, 2009; pp 273−275 and 317.

(9) Han, G.; LaPorte, M. G.; Folmer, J. J.; Werner, K. M.; Weinreb, S. M. *J. Org. Chem.* **2000**, *65*, 6293–6306.

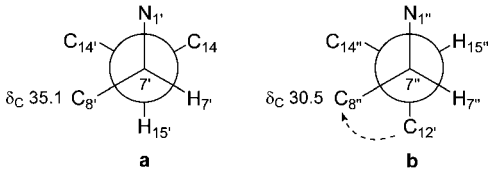


Figure 2. Newman view along bonds of C-7′−C-15′ (a) and C-7″−C-15″ (b) of **1** (dashed arrow donates the γ-gauche effect).

Except for obvious changes from C-9′ to C-14′ owing to the C-12′ substitution, excellent NMR resemblances (Table S2 in SI) indicated a common relative stereochemistry between **4** and the substructures A and B of **1**, which was further confirmed by the key ROESY correlation of H-15′/H-8′b. Furthermore, H-15″ was assigned to be β-positioned versus the α-oriented H-15′ based on the observations described below. In contrast to $J_{14'\alpha,15'}$ that showed a highly comparable magnitude (6.0 Hz) with that (5.5 Hz) of **4**, $J_{14''\alpha,15''}$ of **1** was not resolvable indicating a zero or nearly zero value, which supported a reversed substitution pattern. Moreover, the chemical shift for C-8′ (δ_C 35.1) only slightly varied from that for C-8 (δ_C 34.8) of dihydronorsecurinine,[9] whereas the signal for C-8″ (δ_C 30.5) was markedly upfield shifted due to a γ-gauche effect (Figure 2) caused by the bulky 15″α substituent, which was also in good agreement with the case of flueggine B[5h] whose H-15 was β-positioned (Table S2 in SI). The relative structure of fluevirosine A (**1**) was thus elucidated as shown.

High resolution EIMS analysis of fluevirosine B (**2**)[10] revealed a molecular ion at m/z 623.2981 consistent with a molecular formula of $C_{37}H_{41}N_3O_6$ (calcd 623.2995) which was 14 mass units higher than that of **1** indicative of a similar trimeric analogue. Analysis of the NMR data (Table S1 in SI) for **2** confirmed this hypothesis with diagnostic resonances across substructures A and B being almost superimposable with those of the counterparts in **1**. The only difference existed in the fact that the substructure C of **2** possessed a dihydrosecurinine scaffold instead of the dihydronorsecurinine moiety in **1**, which was supported by the 2D NMR data (Chart S2 and Figures S12−S14 in SI). A further comparison of the NMR data for the substructure C of **2** with those for dihydrovirosecurinine[11] and dihydroviroallosecurinine[11] (Table S3 in SI) supported fragment C was a dihydroviroallosecurinine residue. As with the case of **1**, H-15″of **2** was determined to be α-oriented by comparing the magnitude of $J_{14''\beta,15''}$ (0 Hz) with those of $J_{14\beta,15\alpha}$ (0 Hz) and $J_{14\beta,15\beta}$ (6.3 Hz) in dihydroviroallosecurinine, which suggested that H_β-15″ of **2** was substituted. The remarkably shielded C-8″ ($\Delta\delta_C$ −4.7 ppm) in **2** compared to C-8 in dihydroviroallosecurinine further confirmed the above assignment requiring the 15″-substituent to be coplanar with CH_2-8″. The relative structure of fluevirosine B (**2**) was thereby elucidated as shown.

Fluevirosine C (**3**)[12] was assigned a molecular formula of $C_{37}H_{41}N_3O_6$ by high resolution EIMS analysis at m/z 623.2986 $[M]^+$ (calcd 623.2995) suggestive of an isomeric analogue of **2**. Analysis of the NMR data (Table S1 in SI) for **3** confirmed this hypothesis with diagnostic resonances across substructures A and B being highly comparable to those of the counterparts in **2**. The only difference existed in that the dihydroviroallosecurinine moiety (Substructure C) in **2** was replaced by a dihydrovirosecurinine fragment in **3** (Table S3 in SI). Compared with dihydrovirosecurinine,[11] a 15″β-substitution pattern in **3** was also evident from the observation of the significantly shielded C-8″ ($\Delta\delta_C$ −4.0 ppm) owing to a γ-gauche effect, which required that the 15″-substituent was cofacial with CH_2-8″. The relative structure of fluevirosine C (**3**) was hence elucidated as shown.

The ^1H and ^{13}C NMR data for **1**−**3** and their C-15″ epimers were calculated using the Gauge-Independent Atomic Orbitals (GIAO) method (for more details, see Experimental Section in SI).[13] The acquired data for H-15″ were in accord with the experimental data ($\Delta\delta_H \leq 0.15$ ppm), while those for the counterparts of their C-15″ epimers varied significantly ($\Delta\delta_H > 0.85$ ppm). Additionally, the impact of the 15″-substituent (α or β substitution) on the resonance of C-8″, as reflected by the calculated chemical shifts, also matched the factual data which were explained by the γ-gauche effect.

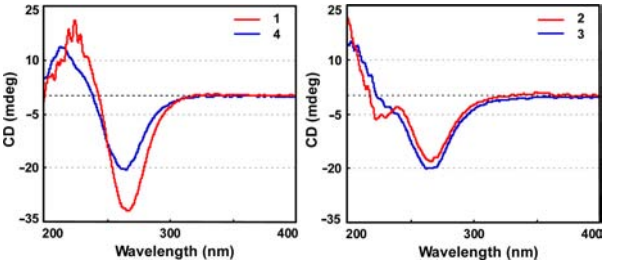


Figure 3. CD spectra of alkaloids **1**−**4**.

Given the co-occurrence of compounds **1**−**7**, it was self-evident that **1**−**3** were biogenetically derived from the monomers **5**−**7** with the dimer **4** being the key intermediate

(10) Pale yellow solid; $[\alpha]_D^{23}$ +24.0 (*c* 0.235, $CHCl_3$); UV (MeOH) λ_{max} (log ε) 259 (4.19), 212 (4.58) nm; CD (MeOH) λ ($\Delta\varepsilon$) 266 (−6.8) nm; IR (KBr) ν_{max} 2948, 2866, 1757, 1626, 1454, 1233, 1111, 1080, 912 cm^{-1}; ^1H and ^{13}C NMR data, see Table S1 in SI; EIMS (70 eV) m/z 623 $[M]^+$ (5), 554 (12), 510 (3), 419 (4), 350 (16), 204 (24), 106 (25), 84 (51), 70 (100); HR-EIMS m/z 623.2981 $[M]^+$ (calcd for $C_{37}H_{41}N_3O_6$, 623.2995).

(11) Dihydrovirosecurinine and dihydroviroallosecurinine were obtained from $NaBH_4$ reduction of virosecurinine and viroallosecurinine, respectively; see Experimental Section in SI.

(12) Pale yellow solid; $[\alpha]_D^{23}$ −3.0 (*c* 0.305, $CHCl_3$); UV (MeOH) λ_{max} (log ε) 259 (4.29), 213 (4.68) nm; CD (MeOH) λ 266 ($\Delta\varepsilon$ −7.6) nm; IR (KBr) ν_{max} 2931, 2852, 1755, 1637, 1624, 1458, 1227, 1080, 912 cm^{-1}; ^1H and ^{13}C NMR data, see Table S1 in SI; EIMS (70 eV) m/z 623 $[M]^+$ (21), 554 (27), 510 (12), 419 (10), 350 (29), 204 (39), 136 (56), 106 (18), 84 (23), 70 (100); HR-EIMS m/z 623.2986 $[M]^+$ (calcd for $C_{37}H_{41}N_3O_6$, 623.2995).

(13) (a) Cheeseman, J. R.; Trucks, G. W.; Keith, T. A.; Frisch, M. J. *J. Chem. Phys.* **1996**, *104*, 5497–5509. (b) Blanco, F.; Alkorta, I.; Elguero, J. *Magn. Reson. Chem.* **2007**, *45*, 797–800.

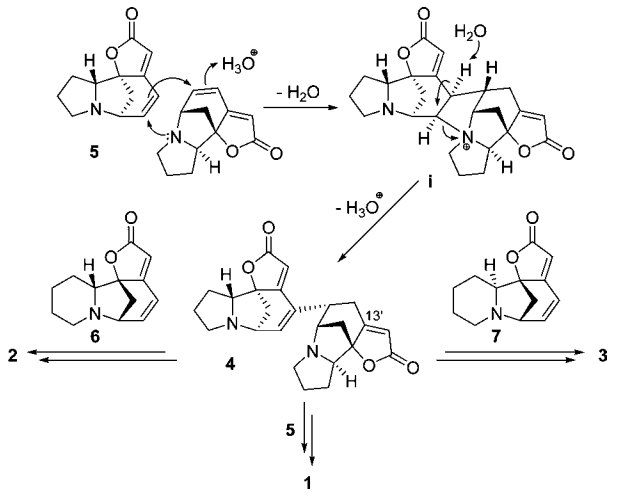
product that was luckily isolated and identified, which indicated the absolute configurations of **1**–**3** as drawn. Meanwhile, CD experiments (Figure 3) provided additional evidence in support of this assignment. Consistent with the biogenetic proposal, compounds **1**–**3** all showed comparable negative Cotton effects [266 ($\Delta\varepsilon$ −11.8), 266 ($\Delta\varepsilon$ −6.8), and 266 ($\Delta\varepsilon$ −7.6) nm, respectively] with those of **4** [265 ($\Delta\varepsilon$ −10.9) nm] and **5** [257 ($\Delta\varepsilon$ −9.8) nm][5d] corresponding to the α,β,γ,δ-conjugated lactone chromophore.

Scheme 1 illustrates the proposed biosynthetic pathway for **1**–**3** from their precursors **5**–**7** via two sequentially self-catalytic key steps similar to that of the Baylis–Hillman reaction,[5d,14] which further confirmed the aforementioned structural assignment. The possibility that they were the handling artifacts produced during purification was excluded by stirring the monomeric alkaloids with silica gel in different solvents (petroleum ether, EtOAc, $CHCl_3$, and MeOH), which mimicked the separation conditions, and no oligomers were detected.

All the isolates were assessed *in vitro* for their cytotoxicity against the tumor cell lines HL-60 (leukemia) and A-549 (lung), along with inhibition against the XBP1 (X-box binding protein 1) mRNA splicing. While **1**–**7** exhibited weak to no cytotoxicities (Figure S34 in SI) toward the tested cell lines, **2** and **3** displayed inhibition (37% and 35% at 20 μM, respectively; Figure S35 in SI) against the splicing activity of XBP1 mRNA which is a novel and hot therapeutic target in cancer treatment.[15]

In conclusion, despite the continuous and intensive endeavor of natural products chemists in the past half century, fluevirosines A–C (**1**–**3**) were not discovered until now, likely owing to their low natural yields (0.00004% to 0.0003%) and the complex nature of their NMR data, which could also have prevented them from being characterized. Our report of **1**–**3** from *F. virosa* presents a nice story of the biogenesis directed discovery of novel bioactive molecules from natural sources, while the proposed biosynthetic pathway paves the way for future biomimetic syntheses of these fascinating structures. This study also highlights the probability of the exploration into a much

Scheme 1. Proposed Biosynthetic Pathway for Fluevirosines A–C (**1**–**3**)



scarcer and higher level of oligomers, which remains a project in due course. It is noteworthy that *Securinega* alkaloid oligomers are hitherto only found in *F. virosa*, which might transmit some chemotaxonomic information that deserves further investigation. All in all, as hot a research topic as the *Securinega* alkaloids have always been, the present work has extended our current knowledge of them beyond the recently focused synthetic studies[2] and calls for more attention from related scientific communities.

Acknowledgment. This project was supported by the National Natural Science Foundation (81021062 and 20902095) and the Foundation (2012CB721105) from the Ministry of Science and Technology of P. R. China. We thank Prof. Y. K. Xu of Xishuangbanna Tropical Botanical Garden, for the identification of the plant material.

Supporting Information Available. Full experimental procedures, tabulated NMR data, and 1D and 2D NMR spectra of new and/or known compounds, as well as bioassay results, were provided. This material is available free of charge via the Internet at http://pubs.acs.org.

The authors declare no competing financial interest.

(14) Basavaiah, D.; Rao, A. J.; Satyanarayana, T. *Chem. Rev.* **2003**, *103*, 811–891.

(15) (a) Shajahan, A. N.; Riggins, R. B.; Clarke, R. *Drug News Perspect.* **2009**, *22*, 241–246. (b) Koong, A. C.; Chauhan, V.; Romero-Ramirez, L. *Cancer Biol. Ther.* **2006**, *5*, 756–759.