ORGANIC LETTERS

2007
Vol. 9, No. 25
5183−5186

New Lactimidomycin Congeners Shed Insight into Lactimidomycin Biosynthesis in *Streptomyces amphibiosporus*

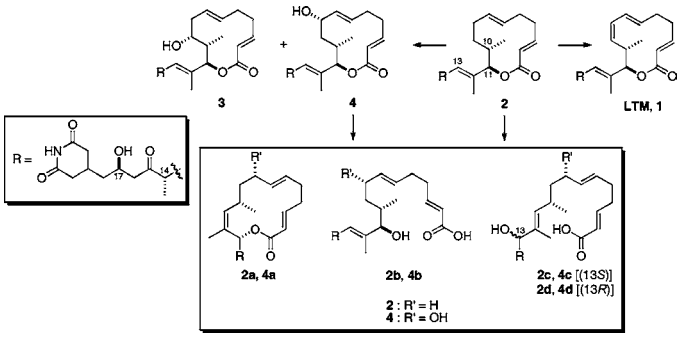
Jianhua Ju,[†] Jeong-Woo Seo,[†] Yeng Her,[‡] Si-Kyu Lim,[†] and Ben Shen*,[†,§,‡]

Division of Pharmaceutical Sciences, University of Wisconsin National Cooperative Drug Discovery Group, and Department of Chemistry, University of Wisconsin−Madison, Madison, Wisconsin 53705-2222

bshen@pharmacy.wisc.edu

Received September 13, 2007

ABSTRACT



Lactimidomycin (LTM, 1) is a macrolide antitumor antibiotic with a glutarimide side chain from *Streptomyces amphibiosporus* ATCC53964. To further develop LTM and related analogues as drug candidates we have (i) improved LTM production by ~20 fold, (ii) identified three new metabolites (2–4) possibly involved in the LTM biosynthetic pathway; (iii) found 3 to be identical with a previously identified isomigrastatin precursor, (iv) determined the absolute stereochemistry of LTM, and (v) produced new LTM rearrangement products 2a–d and 4a–d.

Lactimidomycin (LTM, **1**), a macrolide antibiotic with a glutarimide side chain containing an unsaturated 12-membered lactone ring, was first discovered in 1992 from the fermentation broth of *Streptomyces amphibiosporus* ATCC-53964, with its stereochemistry undetermined.[1] LTM exhibited strong in vitro cytotoxicity against a number of human cell lines (IC_{50} = 3.0−65 nM) and in vivo antitumor activity in mice.[1] In addition, it showed antifungal activity and inhibited both DNA and protein syntheses.[1] LTM belongs to a small group of glutarimide-containing polyketide natural products; others include streptimidone,[2] cycloheximide,[3] migrastatin (MGS, **5**),[4] dorrigocin (DGN) A (**6a**) and B (**7**),[5] NK30424A/B (**8**),[6] and iso-MGS, **9**)[7] (Figure 1). We previously found that **5**−**7** are shunt metabolites of

[†] Division of Pharmaceutical Sciences.
[§] University of Wisconsin National Cooperative Drug Discovery Group.
[‡] Department of Chemistry.

(1) Sugawara, K.; Nishiyama, Y.; Toda, S.; Komiyama, N.; Hatori, M.; Moriyama, T.; Sawada, Y.; Kamei, H.; Konishi, M.; Oki, T. *J. Antibiot.* **1992**, *45*, 1433−1441.

(2) Kim, B. S.; Moon, S. S.; Hwang, B. K. *J. Agric. Food Chem.* **1999**, *47*, 3372−3380 and references cited therein.

(3) (a) Allen, M. S.; Becker, A. M.; Rickards, R. W. *Aust. J. Chem.* **1976**, *29*, 673−679. (b) Jeffs, P. W.; McWilliams, D. *J. Am. Chem. Soc.* **1981**, *103*, 6185−6192.

(4) (a) Nakae, K.; Yoshimoto, Y.; Sawa, T.; Homma, Y.; Hamada, M.; Takeuchi, T.; Imoto, M. *J. Antibiot.* **2000**, *53*, 1130−1136. (b) Takemoto, Y.; Nakae, K.; Kawatani, M.; Takahashi, Y.; Naganawa, H.; Imoto, M. *J. Antibiot.* **2001**, *54*, 1104−1107. (c) Nakamura, H.; Takahashi, Y.; Naganawa, H.; Nakae, K.; Imoto, M.; Shiro, M.; Matsumura, K.; Watanabe, H.; Kitahara, T. *J. Antibiot.* **2002**, *55*, 442−444.

(5) (a) Hochlowski, J. E.; Whittern, D. N.; Hill, P.; McAlpine, J. B. *J. Antibiot.* **1994**, *47*, 870−874. (b) Kadam, S.; McAlpine, J. B. *J. Antibiot.* **1994**, *47*, 875−880.

(6) (a) Takayasu, Y.; Tsuchiya, K.; Aoyama, T.; Sukenaga, Y. *J. Antibiot.* **2001**, *54*, 1111−1115. (b) Takayasu, Y.; Tsuchiya, K.; Sukenaga, Y. *J. Antibiot.* **2002**, *55*, 337−340.

10.1021/ol702249g CCC: $37.00
Published on Web 11/13/2007

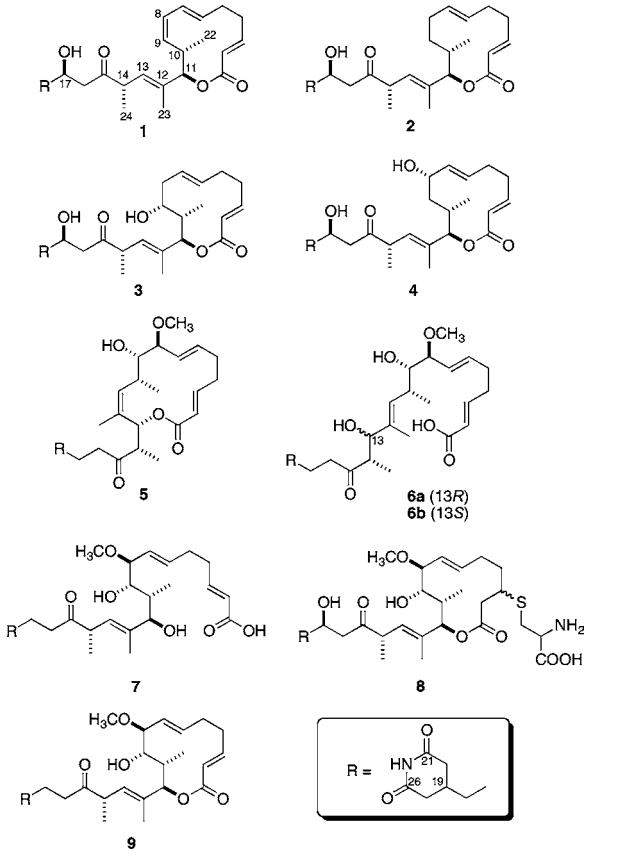


Figure 1. Representative glutarimide-containing polyketide natural products.

9, and that congeners of **9** undergo H_2O-mediated rearrangements to produce the linear dorrigocin and ring-expanded migrastatin scaffolds.[8,9] Iso-MGS and its congeners also undergo facile thermal rearrangements to afford the migrastatin scaffold exclusively.[10] We also optimized the fermentation conditions of *Streptomyces platensis*, the producer of **9**, which led to the isolation and characterization of numerous biosynthetic intermediates, shedding light on the post-polyketide synthase (PKS) tailoring steps for biosynthesis of **9**.[9]

Since **1** and **9** share several structural features, we further examined the fermentation behavior of *S. amphibiosporus* to elucidate LTM biosynthesis and to possibly identify new analogues of **1**. Such analogues might provide clues to the biosynthetic relationship between **9** and **1** in addition to possessing useful bioactivities of their own.

Here we report the following: (i) fermentation optimization of *S. amphibiosporus* leading to dramatically improved yields of LTM (isolated yield: 80 mg/L), (ii) isolation and structure determination of three structurally related homologues of LTM (Figure 1), namely, 8,9-dihydro-LTM (**2**), 8,9-dihydro-9-hydroxy-LTM (**3**), and 8,9-dihydro-8-hydroxy-LTM (**4**) that provide valuable clues to the post-PKS tailoring steps in LTM biosynthesis, (iii) the structure of **3** is identical with that of a previously reported precursor to **9**, (iv) establishment of the absolute stereochemistry for **1** and its three congeners, and (v) H_2O-mediated rearrangements of **2** and **4** resulting in semi-synthesis of linear dorrigocin and ring-expanded migrastatin scaffolds; related rearrangement products for **3** have been previously reported.[9]

It is well-known that alteration of the cultivation parameters (for example, media composition, aeration, culture vessel, pH value, etc.) can affect the metabolic profile of various microorganisms.[11] We started with fermentation of *S. amphibiosporus* using a modified medium composition (Supporting Information). After fermentation, the culture broth was extracted with EtOAc. HPLC−UV and LC−MS analyses of the crude extract revealed the presence of **1** as the only metabolite related to biosynthesis of **1** (Figure 2A).

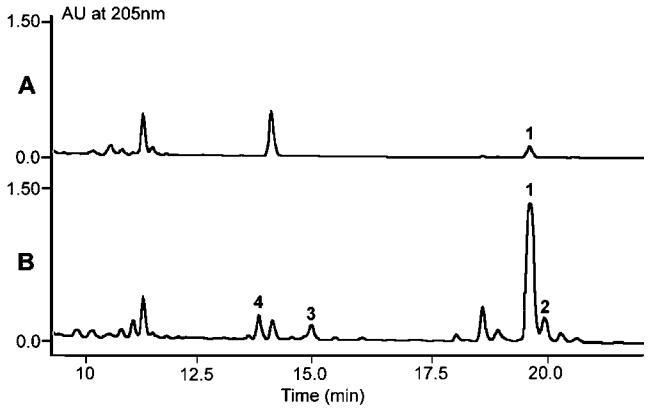


Figure 2. HPLC profiles of (A) EtOAc fermentation extract in the absence of resin and (B) EtOH eluent of XAD-16 adsorbent resin harvested from *S. amphibiosporus* fermentation broth. Peak assignments correlate to compounds shown in Figure 1. Other major peaks are metabolites unrelated to **1** biosynthesis.

Scale-up fermentation (3 L) gave an isolated yield of approximately 3.0 mg/L, which is comparable to the isolated yield reported previously.[1] We then introduced adsorbent resin to the production medium because of its ability to sequester, stabilize, and control the distribution of the secondary metabolites.[8] Not surprisingly, HPLC−UV and LC−MS analyses of the resin extract suggested the titer of LTM was dramatically improved when 2.0−3.5% (w/v) XAD-16 adsorbent resin was supplemented in the production medium. Moreover, three additional LTM analogues were observed (Figure 2B). High percentages of resin (>4%) in the culture medium of *S. amphibiosporus* led to reduced or no production of LTM, suggesting that the strain is intolerant of high resin percentages. Large-scale fermentation of *S. amphibiosporus* (20 L) in the presence of 3% XAD-16 resin

(7) Woo, E. J.; Starks, C. M.; Carney, J. R.; Arslanian, R.; Cadapan, L.; Zavala, S.; Licari, P. *J. Antibiot.* **2002**, *55*, 141−146.
(8) Ju, J.; Lim, S.-K.; Jiang, H.; Shen, B. *J. Am. Chem. Soc.* **2005**, *127*, 1622−1623.
(9) Ju, J.; Lim, S.-K.; Jiang, H.; Seo, J.-W.; Shen, B. *J. Am. Chem. Soc.* **2005**, *127*, 11930−11931.
(10) Ju, J.; Lim, S.-K.; Jiang, H.; Seo, J.-W.; Her, Y.; Shen, B. *Org. Lett.* **2006**, *8*, 5865−5868.
(11) Bode, H. B.; Bethe, B.; Hofs, R.; Zeeck, A. *ChemBioChem*. **2002**, *3*, 619−627.

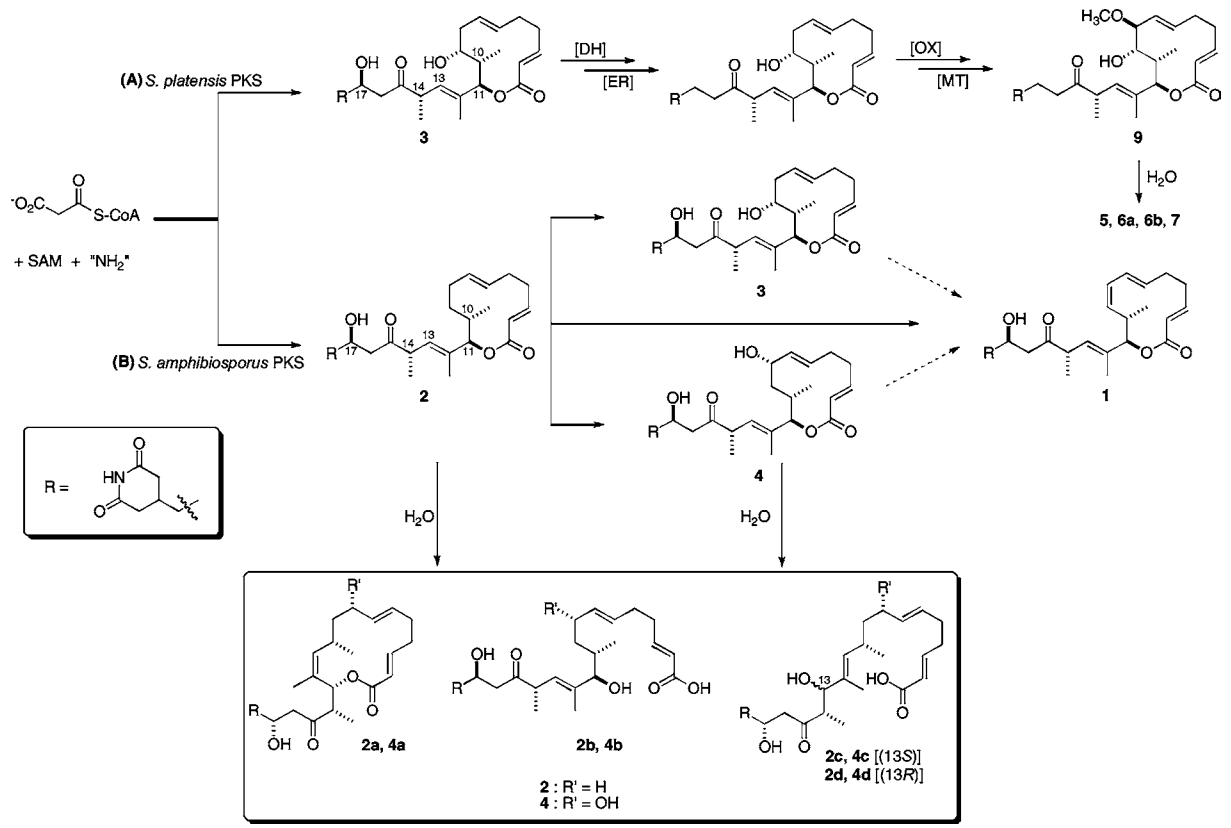


Figure 3. Compounds **2** and **3** en route to **1**, **5**−**7** biosynthesis. (A) **3** as the nascent polyketide product of the *S. platensis* PKS and precursor to **9** via dehydration, enoyl reduction, oxidation, and methylation. Iso-MGS (**9**) is a precursor to **5** and DGNs **6a**, **6b**, and **7**. The timing of steps involved in **9** production from **3** is not yet determined. (B) **2** as the nascent polyketide product of the *S. amphibiosporus* PKS and precursor to **1**, **3**, and **4**. Both **3** and **4** could be potential precursors to **1**. Intermediate **3** is also a precursor to **9** as noted in pathway A, and as such, **3** is an important branch point that co-relates compounds **1**, **5**−**7**, and **9**. Compounds **2** and **4** rapidly undergo H_2O-mediated rearrangements to provide **2a**−**d** and **4a**−**d** as has been observed for **3**, **9**, and other analogues.[8,9] SAM, (*S*)-adenosylmethionine; NH_2, an unspecified amino donor; DH, dehydratase; ER, enoyl reductase; MT, methyltransferase; OX, oxidase.

was conducted. After fermentation, the resin was collected by filtration with cheesecloth, air-dried, and then eluted with absolute ethanol. Solvent removal followed by silica gel chromatography with $CHCl_3$/MeOH and EtOAc/hexane afforded compounds **1**−**4** in isolated yields of approximately 80, 3, 5, and 5 mg/L, respectively.

Compound **3** was obtained as a colorless oil with MS, ^{1}H, and ^{13}C NMR spectra (measured in $CDCl_3$) and optical rotation identical with those of 17-hydroxy-8-desmethoxy-iso-MGS isolated recently by us from *S. platensis*, whose absolute stereochemistry (9*S*,10*S*,11*R*,14*S*,17*R*) has been established (Supporting Information).[9] We have previously shown that **3** can undergo H_2O-mediated rearrangement to the corresponding MGS and DGN analogues.[9] Isolation of **3** from both *S. platensis* and *S. amphibiosporus* suggests that **9** and **1** share similar biosynthetic machineries for construction of C-10, C-11, C-14, and C-17 (Figure 3).

The NMR spectral data of **1** were originally reported in DMSO-d_6, leaving its relative and absolute stereochemistry unsolved. We recently established the absolute stereochemistry of **9** and its congeners. It therefore seemed reasonable that the absolute stereochemistry of **1** at C-10, C-11, C-14, and C-17 might be the same as that observed for analogues of **9**. To verify this hypothesis, we acquired 1D and 2D NMR spectral data of **1** in $CDCl_3$, which enabled complete accounting for all ^{1}H and ^{13}C NMR signals (Supporting Information). The relevant ^{1}H and ^{13}C NMR chemical shifts and proton splitting centers at C-10, C-11, C-14, and C-17 are as found in **3** and 17-hydroxy-iso-MGS.[9] Thus, the absolute stereochemistry of **1** was assigned as 10*S*,11*R*,14*S*,17*R*.

Compound **2** was purified as a colorless oil having a molecular formula of $C_{26}H_{37}O_6N$. HR-MALDI-MS reveals that **2** has a mass 2 amu greater than **1**, corresponding to a saturated double bond equivalent. The ^{13}C NMR spectrum of **2** displayed 26 carbon signals, 6 of which were salient olefinic carbons, suggesting **2** as the dihydro-derivative of **1**. Analyses of its 1D and 2D NMR (^{1}H−^{1}H COSY, TOCSY, HMQC, and gHMBC) spectra allowed full assignments of its ^{1}H and ^{13}C signals (see the Supporting Information), verifying saturation of the C8/C9 in **2**. The COSY correlations between H-10/H_2-9/H_2-8/H-7, and gHMBC correlations from H-11/C-9, H-22/C-9, H-10/C-9/C-8, and H-7/C-9/C-8, confirmed the above conclusion. Other 1D and 2D NMR spectral data of **2** agreed with the rest of the structure and confirm that the stereochemistry of **2** is identical to that of **1** and **3**.

Compound **4** was obtained as a white powder and was found to have the same molecular formula, $C_{26}H_{37}O_7N$, as **3** provided by high-resolution MALDI-MS. The ^1H and ^{13}C NMR (in $CDCl_3$) spectra of **4** showed significant similarities to those of **3**. To clarify this situation, a series of 2D NMR ($^1H-^1H$ COSY, TOCSY, HMQC, and gHMBC) spectra of **4** were acquired, which allowed full assignment of all ^1H and ^{13}C signals (Supporting Information). Analysis of ^1H and ^{13}C NMR data for **4** revealed the presence of the same C-11 side chain found in **1**−**3**. The COSY correlations between H_2-16/H-17 (δ 4.05)/H_2-18 confirmed the hydroxy group at C-17. In the gHMBC spectrum of **4**, the signal due to H-11 (δ 5.19) showed correlations with C-1 (δ 167.4), C-12 (δ 134.9), C-13 (δ 128.5), C-9 (δ 39.4), C-10 (δ 32.5), C-22 (δ 19.8), and C-23 (δ 15.2), establishing the 12-membered lactone structure. The COSY correlations between H-7 (δ 5.27)/H-8 (δ 4.45)/H_2-9 (δ 1.51, 1.97) and gHMBC correlations between H-7/C-8 (δ 73.4) and H-9 (δ 1.51)/C-8 placed another hydroxyl group at C-8. Additional examination of the 1D and 2D NMR data permitted complete structural elucidation of **4** to be made with the exception of stereochemistry (Figure 1).

The absolute stereochemistry of **4** was assigned by comparing its ^1H and ^{13}C NMR chemical shifts and proton splitting patterns with those of **3** and iso-MGS congeners. The configuration of the two double bonds in the lactone ring was assigned as *trans* due to the large coupling constant of 15.5−16.0 Hz. As in the case of **1**, **4** has the same stereochemistry at C-10, C-11, C-14, and C-17 on the basis of the relevant proton chemical shifts, coupling constants (e.g., $J_{H10,H11} = 5.0$ Hz, $J_{H13,H14} = 9.5$ Hz), and the close ^{13}C NMR chemical shifts from C-10 to C-19. The coupling constant between H-7 and H-8 ($J = 8.0$ Hz) is significantly different from that of **9** ($J = 3.5$ Hz), which has an 8β-OCH_3 configuration, indicating an 8α-OH configuration in **4**. This scenario is also consistent with the $J_{H7,H8\beta}$ value (7.0−9.5 Hz) observed in 8-desmethoxy-iso-MGS and its analogues.[9] The 8-OH configuration in **4** was further supported by the observation of the dramatic ^1H and ^{13}C NMR signal downfield shifts of 22-CH_3 (Δ0.14 ppm and Δ11.4 ppm, respectively) when compared with those of **3**, due to a *syn*-parallel disposition. Hence, the absolute stereochemistry of **4** was assigned as 8*S*,10*S*,11*R*,14*S*,17*R*.

Iso-MGS congeners including **3** are relatively stable in most organic solvents.[9] However, in water, they undergo H_2O-mediated rearrangement into their corresponding 14-membered migrastatin and ring-opened dorrigocin analogues (Figure 3).[4,5,8,9] Compounds **2** and **4** also possess similar chemical properties; incubation of **2** and **4** in H_2O−DMSO (9:1) at 37 °C for 12 h irreversibly resulted in a series of new substances (Figure 3). Rearrangement products **2a**−**d** and **4a**−**d** (Figure 3) correspond to migrastatin/dorrigocin counterparts and are the overwhelmingly predominant products observed by HPLC, LC-MS, and NMR analyses (Supporting Information). Similar, but reversible, rearrangements have been observed in a recent example describing the ring-opened form of pseudotrienic acids A and B from *pseudomonas sp.* MF381-IODS. These acids were found to readily interconvert with their respective macrolide forms.[12] Under identical conditions, **1** was found to be stable and remained unchanged. These results provide experimental evidence supporting the idea that the C8/C9 double bond changes the 12-membered-ring configuration thereby influencing the propensity to undergo ring-opening and ring-expansion rearrangements.

Feeding studies with ^{13}C-labeled sodium acetate and methionine have shown that LTM is of polyketide origin.[1] The isolation of **2**−**4** implies that **2** is the nascent polyketide product assembled by the PKS and that **3** and **4** may be shunt metabolites formed during the desaturation of **2** en route to **1** (Figure 3). Desaturation has been elaborately involved in natural products biosynthesis and drug metabolism. For instance, desaturation and hydroxylation of valproic acid has been demonstrated by using a purified and reconstituted microsomal cytochrome P-450 in vitro.[13,14] Similarly, testosterone is known to undergo P-450-catalyzed oxidation.[15] In both cases, substrate oxidation provides one olefinic product and two hydroxylated products. We believe that LTM provides another example of this unique pathway in which a fully saturated C−C bond (in **2**) is converted to two hydroxylated congeners **3** and **4** and the diene-containing LTM (**1**). This notion is promoted by our ability to detect only one stereoisomer of each hydroxylated regioisomer. The stereospecificity of hydroxylation suggests that this process is enzyme-catalyzed. This is significant since the chemistry carried out appears to occur following the completion of all PKS orchestrated steps. Such post-PKS tailoring events have not yet been noted in the biosynthesis of glutarimide-containing polyketide natural products. Thus, our disclosure of compounds **2**−**4** here sheds new insight into LTM biosynthesis.

Acknowledgment. We thank the Analytical Instrumentation Center of the School of Pharmacy, UW-Madison for support in obtaining MS and NMR data and Dr. Scott Rajski (UW-Madison) for assistance in manuscript preparations. This work is supported in part by NIH grants CA106150 and CA113297.

Supporting Information Available: Experimental procedures, MS and ^1H and ^{13}C NMR data of **1**, **2**, **4**, **2a**−**c**, and **4a**−**c**, copies of ^1H and ^{13}C NMR spectra of **2** and **4**, optical rotation data for **2**−**4**, and MS data for **3**. This material is available free of charge via the Internet at http://pubs.acs.org.

OL702249G

(12) Pohanka, A.; Broberg, A.; Johansson, M.; Kenne, L.; Levenfors, J. *J. Nat. Prod.* 2**005**, *68*, 1380−1385.

(13) Rettie, A. E.; Rettenmeier, A. W.; Howald, W. N.; Baillie, T. A. *Science* **1987**, *235*, 890−893.

(14) (a) Rettie, A. E.; Boberg, M.; Rettenmeier, A. W.; Baillie, T. A. *J. Biol. Chem.* **1988**, *263*, 13733−13738. (b) Fisher, M. B.; Thompson, S. J.; Ribeiro, V.; Lechnier, M. C.; Rettie, A. E. *Arch. Biochem. Biophys.* **1998**, *356*, 63−70.

(15) (a) Nagata, K.; Liberato, D. J.; Gillette, J. R.; Sasame, H. A. *Drug Metab. Dispos.* **1986**, *14*, 559−565. (b) Aoyama, T.; Korzekwa, K.; Nagata, K.; Gillette, J.; Gelboin, H. V.; Gonzalez, F. J. *Eur. J. Biochem.* **1989**, *181*, 331−336. (c) Korzekwa, K. R.; Trager, W. F.; Nagata, K.; Parkinson, A.; Gillette, J. R. *Drug Metab. Dispos.* **1990**, *18*, 974−979.