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Studies Toward the Synthesis of Luminacin D: Assembly of Simplified Analogues Devoid of the Epoxide Displaying Antiangiogenic Activity

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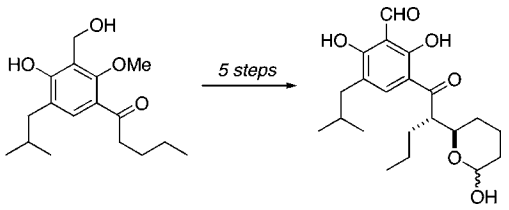
ABSTRACT



A series of structurally simplified luminacin analogues devoid of the epoxide ring are assembled in a stereocontrolled manner from 2,4-dimethoxybenzaldehyde using a syn-selective aldol reaction as the key step. The success of the approach is critically dependent on the nature and extent of the alcohol protecting groups. The synthetic analogues inhibit VEGF-stimulated angiogenesis in an in vitro assay indicating that the epoxide is not essential for biological activity in this compound class.

Angiogenesis, the process by which new blood capillaries develop from an existing blood vessel, is of fundamental importance to the development of a cancer mass.[1] Tumors can only grow to about 1 mm in diameter in the absence of new capillary growth.[2] Today, it is widely recognized that antiangiogenesis therapies hold considerable promise for the treatment of cancer and other diseases.[3] In 2000, a new class of inhibitors called the luminacins were isolated from the culture broths of *Streptomyces* sp. Mer-VD1207 by Naruse et al.[4] Wakabayashi demonstrated that they inhibit the initial stages of capillary tube formation and operate by a unique mode of action.[5] The structures of luminacins C_2 and D, two of the more potent members of the family, are depicted in Figure 1.[6] Further studies by Sharma using luminacin C_2

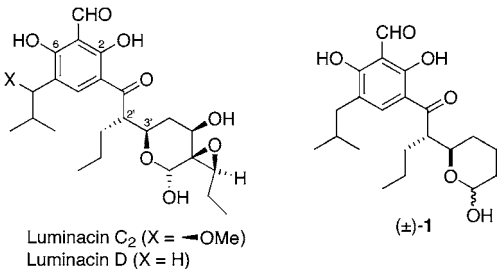


Figure 1. Structures of luminacins C_2 and D and synthetic analogue **1**.

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(1) Carmeliet, P.; Jain, R. K. *Nature* **2000**, *407*, 249.
(2) King, R. J. B. *Cancer Biology*; Longman: Edinburgh, 1996.
(3) Han, Z. C.; Liu, Y. *Int. J. Hematol.* **1999**, *70*, 68. Eatock, M. M.; Schätzlein, A.; Kaye, S. B. *Cancer Treat. Rev.* **2000**, *26*, 191. Matter, A. *Drug Discovery Today* **2001**, *6*, 1005.

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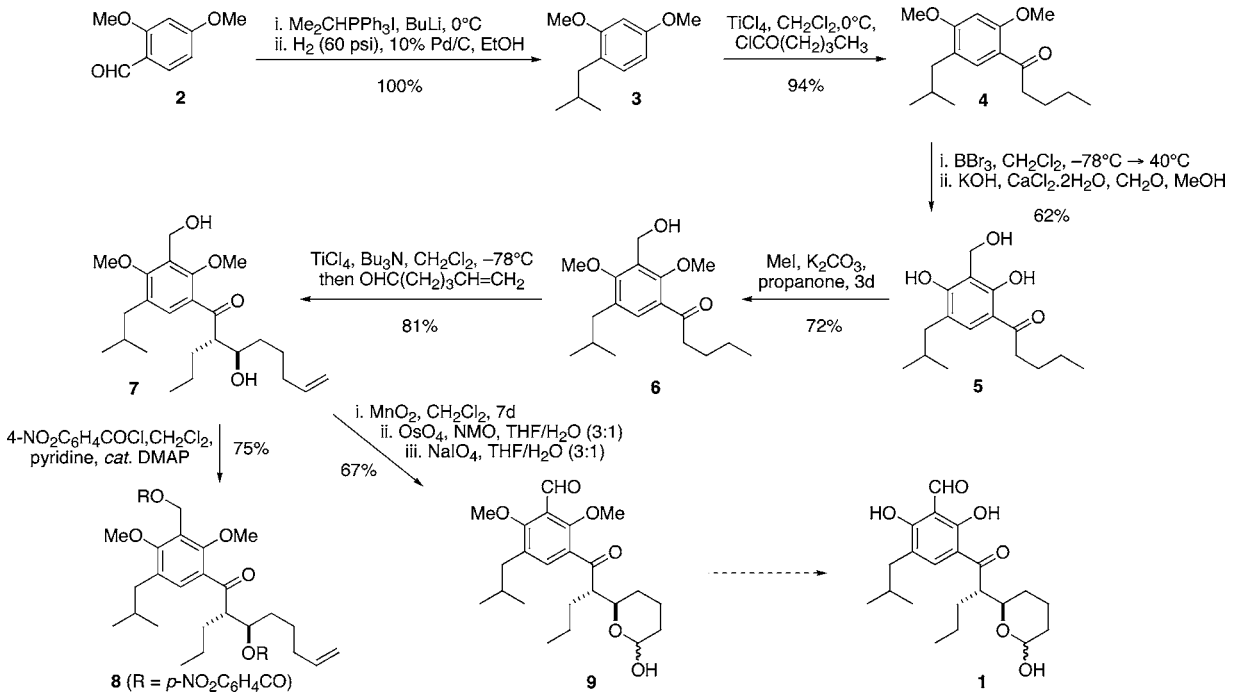
Scheme 1



reveal that it is an inhibitor of Src signal transduction, acting by disruption of proline-rich ligand-mediated protein−protein interactions rather than by direct inhibition of Src kinase activity.[7] On the basis of these observations, the luminacins have become important targets for angiogenesis research. To further probe the biological function of the luminacins, methods for the synthesis of these natural products and related structures are required. Elegant approaches to luminacins C[8] and D[9] have recently been reported. Our endeavors have focused on exploring if a syn-selective aldol reaction can be used to construct the central C-2′/C-3′ bond in a stereoselective manner. In this Letter, we report the viability of this approach by realizing the synthesis of luminacin analogue (±)-**1** containing many of the structural elements of the natural products. Furthermore, we show that compounds of this type inhibit vascular endothelial growth factor (VEGF)-stimulated angiogenesis in human umbilical vein endothelial cells (HUVECs), a discovery that establishes that the epoxide is not essential for inhibiting angiogenesis in this compound class.

Our initial synthetic approach to **1** is outlined in Scheme 1. 2,4-Dimethoxybenzaldehyde **2** was converted into trisubstituted benzene derivative **3** in quantitative yield by Wittig olefination and reduction of the resulting alkene by catalytic hydrogenation. Friedel−Crafts acylation of **3** using pentanoyl chloride gave ketone **4** as a single regioisomer. The C-1 hydroxymethyl group was introduced by removal of the methyl ethers from **4** with BBr_3, which was further reacted with formaldehyde under basic conditions to give benzyl alcohol **5**.[10] Selective methylation of the phenolic hydroxyl groups of **5** provided pentasubstituted aryl ketone **6**. Treatment of this ketone with titanium tetrachloride and tributylamine according to the Evans protocol[11] furnished the tetrachlorotitanium enolate, which was condensed with 5-hexenal to give the *syn*-aldol **7** in 81% yield. This reaction

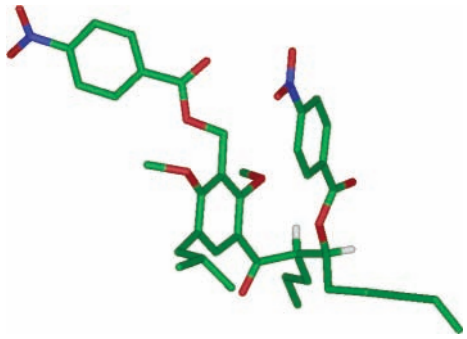


Figure 2. X-ray crystal structure of **8**.

(4) Naruse, N.; Kageyama-Kawase, R.; Funahashi, Y.; Wakabayashi, T.; Watanabe, Y.; Sameshima T.; Dobashi, K. *J. Antibiot.* **2000**, *53*, 579.

(5) Wakabayashi, T.; Kageyama-Kawase, R.; Naruse, N.; Funahashi Y.; Yoshimatsu, K. *J. Antibiot.* **2000**, *53*, 591.

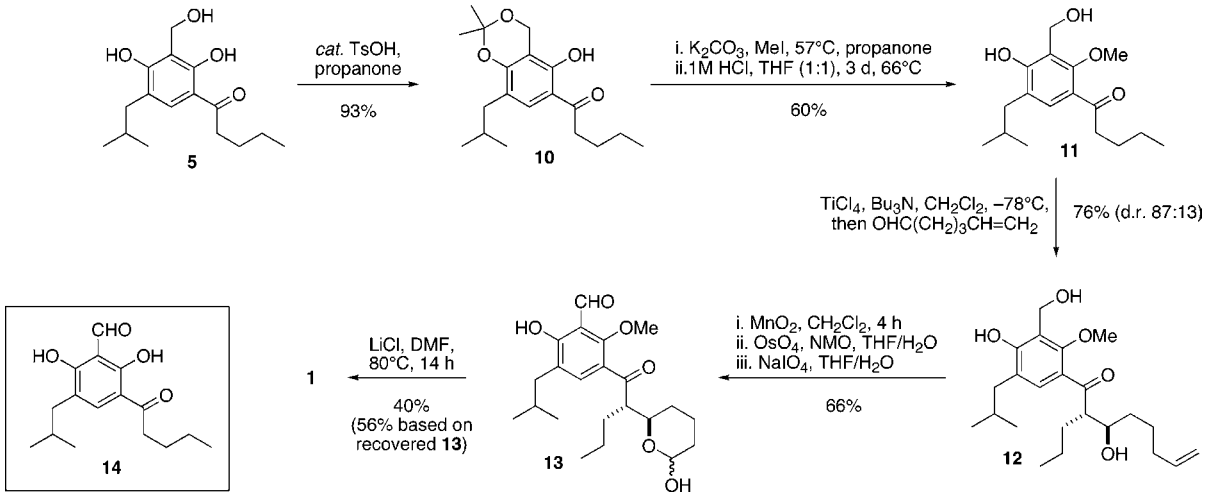
(6) Luminacin C_2 is identical to UCS15A isolated from another *Streptomyces* sp. (see ref 7a). In this article, we use the term luminacin C_2 to refer to both luminacin C_2 and UCS15A.

(7) (a) Sharma, S. V.; Oneyama, C.; Yamashita, Y.; Nakano, H.; Sugawara, K.; Hamada, M.; Kosaka N.; Tamaoki, T. *Oncogene* **2001**, *20*, 2068. (b) Oneyama, C.; Nakano H.; Sharma, S. V. *Oncogene* **2002**, *21*, 2037. (c) Oneyama, C.; Agatsuma, T.; Kanda, Y.; Nakano, H.; Sharma, S. V.; Nakano, S.; Narazaki, F.; Tatsuta, K. *Chem. Biol.* **2003**, *10*, 443.

(8) Tatsuta, K.; Nakano, S.; Narazaki, F.; Nakamura, Y. *Tetrahedron Lett.* **2001**, *42*, 7625.

(9) Shotwell, J. B.; Krygowski, E. S.; Hines, J.; Koh, B.; Huntsman, E. W. D.; Choi, H. W.; Schneekloth, J. S.; Wood J. L.; Crews, C. M. *Org. Lett.* **2002**, *4*, 3087. Fang, F.; Johannes, C.; Yao Y.; Zhu, X. Patent Appl. WO 03/057685, 2003.

Scheme 2



was highly stereoselective, with no trace of the anti diastereomer being detected in the crude reaction mixture by ^{1}H NMR spectroscopy. Esterification of diol **7** with 4-nitrobenzoyl chloride furnished dibenzoate **8**, whose relative stereochemistry was established by X-ray crystallography (Figure 2).[12] Further conversion of diol **7** into **9** was realized by oxidation of the benzylic hydroxyl group with manganese dioxide followed by cleavage of the terminal alkene to the aldehyde, which immediately resulted in lactol formation. Lactol **9** existed as a 54:46 mixture of anomers (in $CDCl_3$). Unfortunately, at this late stage efforts to effect demethylation to **1** using Lewis acids (e.g., BBr_3) met with failure.

The evidence gathered during the attempts to deprotect **9** suggested that one of the methyl ethers was much more readily cleaved than the other. Since carbonyl groups are known to assist in demethylation at adjacent sites,[13] we speculated that removal of the C-2 methyl ether was more facile than at C-6. Thus, a revised strategy was conceived in which only the phenol at C-2 would be protected.[14]

To realize this strategy, chemoselective protection of triol **5** was undertaken, which furnished phenol **10** in 93% yield (Scheme 2). The structure of **10** has been unambiguously established by X-ray crystallography (data not presented). We suggest that **10** is produced because it is thermodynamically more stable as a result of an intramolecular hydrogen bond between the ketone and the phenolic hydroxyl group. The downfield chemical shift of the −OH group in the 400 MHz ^{1}H NMR spectrum (δ 12.90 in $CDCl_3$) supports this proposal. Methylation of **10** and deprotection of the acetal provided **11** in 60% yield over the two steps. Titanium-mediated condensation between **11** and 5-hexenal furnished the aldol products in 76% yield. High diastereoselectivity was again observed (syn:anti = 87:13). By analogy with **7**, the major product was assigned as the syn stereoisomer **12**. Oxidation of the hydroxymethyl group of *syn*-**12** to the corresponding aldehyde proceeded cleanly. Interestingly, this oxidation was much more rapid than that of **7** (4 h instead of 7 days). Tetrahydropyran formation was uneventful, yielding **13** in good yield as a 57:43 mixture of anomers (in $CDCl_3$). Gratifyingly, demethylation of **13** to **1** could be achieved by heating with lithium chloride in DMF at 80 °C for 14 h. While considerable amounts of **13** were recovered under these conditions (27%), efforts to drive the reaction to completion led to degradation and the production of an inseparable impurity that we tentatively assign to be the C-2′ epimer of **1**. Thus, luminacin analogue (±)-**1** can be obtained in 13 steps from 2,4-dimethoxybenzaldehyde in 6% overall yield using this strategy.

Compounds **1**, **9**, and **13** were evaluated for their effects on the inhibition of VEGF-induced angiogenesis in HUVECs using an established fibrin matrix assay.[15] For comparison purposes in the biological assays, **14** was prepared by oxidation of **5** (100%) with manganese dioxide (Scheme 2). At a drug concentration of 50 μM, VEGF-stimulated angiogenesis was almost completely inhibited (97.6% ± 2.1%) using bismethyl ether **9**. The inhibitory effects of this compound on tube formation are clearly visible in Figure 3. Surprisingly, phenols **1** and **13**, which more closely resemble luminacin D, were less effective at this drug concentration

(10) Saimoto, H.; Yoshida, K.; Murakami, T.; Morimoto, M.; Sashiwa H.; Shigemasa, Y. *J. Org. Chem.* **1996**, *61*, 6768.

(11) Evans, D. A.; Rieger, D. L.; Bilodeau, M. T.; Urpi, F. *J. Am. Chem. Soc.* **1991**, *113*, 1047. Yoshida, Y.; Hayashi, R.; Sumihara, H.; Tanabe, Y. *Tetrahedron Lett.* **1997**, *38*, 8727.

(12) **Crystallographic Data for 8.** X-ray diffraction studies on a colorless crystal grown from methanol were performed at 125 K using a Bruker SMART diffractometer with graphite-monochromated radiation (λ = 0.71073 Å). The structure was solved by direct methods. $C_{38}H_{44}N_2O_{11}$, M = 704.75, space group P-1, triclinic, a = 8.9292(17), b = 13.439(3), c = 16.334(3) Å, α = 109.518(3), β = 99.424(4), γ = 93.348(4)°, U = 1809.0(6) Å^3, Z = 2, 10 840 reflections were measured, 6424 were unique (R_{int} = 0.0338) which were used in all calculations. The final R was 0.0732 for $I > 2\sigma(I)$, and $wR(F^2)$ was 0.1660. Some disorder was observed in the alkenyl chain, which was modeled in two equal occupancy orientations.

(13) Kocienski, P. J. *Protecting Groups*; Thieme: Stuttgart, 1994.

(14) Aldol reactions using triol **5** were unsuccessful. Other protecting groups (MOM, Bn, and allyl) for the simultaneous protection of the C-2 and C-6 phenolic groups were examined, but these proved to be less practical.

(15) Whatmore, J. L.; Swann, E.; Barraja, P.; Newsome, J. J.; Bunderson, M.; Beall, H. D.; Tooke, J. E.; Moody, C. J. *Angiogenesis* **2002**, *5*, 45.

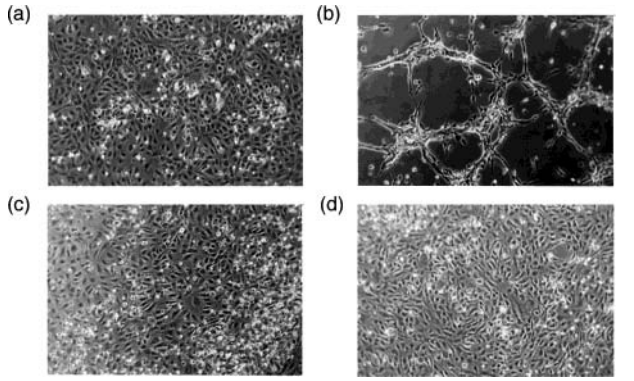


Figure 3. Inhibition of VEGF-stimulated angiogenesis of HUVECs on a fibrin matrix using synthetic luminacin analogue **9**:[15] (a) control (no added VEGF); (b) added VEGF (100 ng/mL); (c) added **9** (50 μM); (d) added VEGF (100 ng/mL) and **9** (50 μM). All experiments were conducted on at least two different cell populations.

(**1**, 27.5% ± 26.5%; **13**, 28.4% ± 31.8%). Analogue **14**, devoid of the pyran ring, failed to inhibit angiogenesis at 50 μM. Good correlation between drug concentration and percentage inhibition was witnessed with these compounds {e.g., **1**: 100% (100 μM); 53.0 ± 9.4% (80 μM); 27.5 ± 26.5% (50 μM); 2.9 ± 3.8% (20 μM)}. The cytotoxicity of the most potent analogue **9** was determined using an MTT-based colorimetric assay using HUVECs.[16] Crucially, it was not cytotoxic (IC_{50} > 100 μM) at the concentrations used in the angiogenesis assay.

These findings establish that an aldol strategy is suitable for the construction of "luminacin-like" structures, although judicious choice of protecting groups is required. Furthermore, we have determined that in vitro VEGF-stimulated angiogenesis can be efficiently inhibited by simplified analogues. These data establish that free phenolic groups at C-2 and C-6 are not essential for inhibition, and neither is the presence of the C-6′/C-8′ epoxide ring or the C-5′ hydroxyl group of the natural products. Current work is focused on applying these findings to the synthesis of more potent inhibitors of angiogenesis and to completing the total synthesis of luminacin D.

Acknowledgment. We are indebted to EPSRC (GR/S14429/01), Cancer Research UK, and FORCE Cancer Research (Exeter) for their generous financial support of this work. We thank the EPSRC Chemical Database Service at Daresbury.[17]

Supporting Information Available: Detailed experimental procedures and ^{1}H and ^{13}C NMR spectra for compounds **1**, **3**−**7**, and **9**−**14**. This material is available free of charge via the Internet at http://pubs.acs.org.

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(16) Mosmann, T. *J. Immunol. Methods* **1983**, *65*, 55.

(17) Fletcher, D. A.; McMeeking R. F.; Parkin, D. *J. Chem. Inf. Comput. Sci.* **1996**, *36*, 746.