Available online at www.sciencedirect.com
ScienceDirect
Bioorganic & Medicinal Chemistry Letters 18 (2008) 2351–2354
Bioorganic & Medicinal Chemistry Letters

Synthesis of novel analogues of (+)-varitriol via olefin cross-metathesis reaction

Lingaiah Nagarapu,* Venkateswarlu Paparaju and Apuri Satyender

Organic Chemistry Division-II, Indian Institute of Chemical Technology (IICT), FCL Ist Floor, Taranaka, Hyderabad, Andhra Pradhesh 500 607, India

Received 5 February 2008; revised 22 February 2008; accepted 27 February 2008
Available online 4 March 2008

Abstract—Novel analogues of (+)-varitriol have been synthesized via olefin cross-metathesis reaction using Grubb's catalyst. Newly synthesized compounds were screened for cytotoxicity and they showed mild activity against RAW264.7 and HT29 cell lines.

Cancer is currently the second common cause of death in United States, and it is likely to become the most common in the foreseeable future.[1] Hence the development and testing of novel and more selective anticancer drugs have become an important research area. Further advances in cancer therapy can be brought by chemical variation of known structural classes since it provides a means for obtaining improved drugs, increased understanding of the biochemistry of the cancer cell and novel structure–growth inhibitory relationship.

Natural products have a long history[2,3] of providing novel, clinically useful anticancer drugs and they have also served as prototypes for the development of novel analogues of clinical importance. Moreover, National Cancer Institute (NCI) has recently reemphasized the discovery of natural products potential anticancer drugs.

Among various natural products, marine natural products[4] have received increasing attention from chemists and pharmacologists during the last few decades. Natural product chemists have probed marine organisms, while synthetic chemists have targeted these novel structures for the development of new synthetic methodologies and strategies.

In 2002, Malmstrøm et al.,[5] have isolated (+)-varitriol (**1a**) (Fig. 1) from a marine derived strain of the fungus Emericella variecolor collected from the Caribbean Waters of Mochima Bay. (+)-Varitriol (**1a**) was tested in the National Cancer Institute (NCI) 60-cell line in vitro panel. It showed a more than 100-fold increased potency (over the mean toxicity) towards the RXF 393 (renal cancer, $GI_{50} = 1.63 \times 10^{-7}$ M), T-47D (breast cancer, $GI_{50} = 2.10 \times 10^{-7}$ M) and SNB-75 (CNS cancer, $GI_{50} = 2.44 \times 10^{-7}$ M) cell lines and lower potency against DU-145 (prostate cancer, $GI_{50} = 1.10 \times 10^{-6}$ M), HL-60 (TB leukaemia, $GI_{50} = 2.52 \times 10^{-5}$ M), CCRF-CEM (leukaemia, $GI_{50} = 2.60 \times 10^{-5}$ M), OVCAR-5 (ovarian cancer, $GI_{50} = 6.82 \times 10^{-5}$ M), SNB-19 (CNS cancer, $GI_{50} = 9.13 \times 10^{-5}$ M) and COLO 205 (colon cancer, $GI_{50} = 9.59 \times 10^{-5}$ M) cell lines. The combination of potent biological properties and a relatively straightforward molecular structure of (+)-varitriol (**1a**) has rekindled an interest in us to obtain novel analogues for detailed SAR studies.

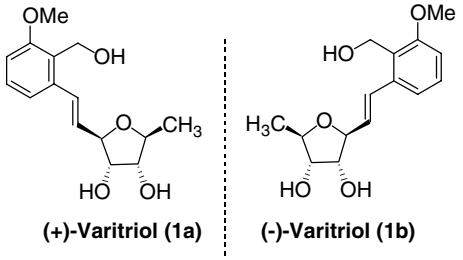


Figure 1.

Keywords: Varitriol; Analogues; Cross-metathesis reaction; Grubb's catalyst; Cytotoxicity.
* Corresponding author. Tel.: +91 40 27160123; fax: +91 40 27193382; e-mail: nagarapu2@yahoo.co.in

0960-894X/$ - see front matter © 2008 Elsevier Ltd. All rights reserved.
doi:10.1016/j.bmcl.2008.02.062

Two research groups have reported different synthetic routes to (−)-varitriol (**1b**): (i) In 2006 Jennings and Clemens[6] reported the total synthesis of (−)-varitriol from D-(−)-ribose utilizing alkene metathesis to link together the carbohydrate and aromatic moieties of the molecule. (ii) In 2007 McAllister et al.[7] reported a short and flexible route to (−)-varitriol by following Ramberg–Bäcklund reaction. Though the proposed route is operationally straightforward and shorter, the low α/β stereoselectivity is the major drawback. Of the two methods available for the synthesis of varitriol, alkene metathesis was found to be more suitable and more convenient for the synthesis of novel analogues of (+)-varitriol (**1a**).

The establishment of olefin metathesis as a powerful synthetic tool to C=C bond formation is due to the development of ruthenium-based catalysts and their derivatives introduced by Grubbs and coworkers.[8] More specifically, cross-metathesis (CM) of simple alkenes has now become one of the methods of choice to access substituted alkenes.

In continuation of our studies[9] towards synthesis of novel compounds as useful biologically active compounds, we report in this communication an efficient synthesis of novel analogues of (+)-varitriol (**1**) by utilizing CM reaction. To the best of our knowledge, in the literature there appears no report for the synthesis and screening of novel analogues of (+)-varitriol.

As a starting point for this study we have selected compound (**3**)[10] as a sugar olefin which is very closely related in structural aspects to the carbohydrate-derived olefin of (+)-varitriol (**1**). Initially compound (**3**) was allowed to react with 4-methoxy styrene (**2a**) in presence of Grubb's second-generation catalyst (5 mol %) to afford desired cross-coupled product (**4a**) with pronounced *E*-selectivity (Scheme 1).

The formation of compound (**4a**) was characterized from ^{1}H NMR by the appearance of characteristic aromatic protons at δ 7.23 ppm and δ 6.79 ppm (2d, 4H, $J = 9.0$ Hz) and olefin protons at δ 5.99 ppm (dd, H-2′, $J_{1',2'} = 15.8$ Hz, $J_{2',3'} = 8.0$ Hz) and at δ 6.47 ppm (d, H-1′, $J_{1',2'} = 15.8$ Hz). The coupling constant (*J*) values of olefinic protons confirmed the selective formation of *E*-isomer of product (**4a**). In EI-MS of compound (**4a**) the characteristic [M^+] appears at 306. In this olefin cross-metathesis reaction, undesired homocoupled products are also expected, however, the formation of such by-products has not been observed.

After establishing the method for the synthesis of (+)-varitriol analogue (**4a**), we have explored the applicability of this methodology, for the general synthesis of other novel analogues by using variously substituted styrenes (**2b–e**). Thus, reaction of compound (**2b**) and sugar olefin (**3**) in the presence of catalyst (5 mol %) resulted in the formation of compound (**4b**). In ^{1}H NMR of compound (**4b**), characteristic olefinic protons resonated at δ 6.01 ppm (dd, H-2′, $J_{2',3'} = 8.4$ Hz, $J_{1',2'} = 15.7$ Hz) and at δ 6.42 ppm (d, H-H-1′, $J_{1',2'} = 15.7$ Hz). Similarly other varitriol like compounds **4c**, **4d** and **4e** were also synthesized and the spectroscopic features fully supported the assigned structures.

The scope of the reaction was further extended to carbohydrate-derived olefin (**5**)[11] and its reaction with styrenes (**2c** and **2d**) in presence of 5 mol % catalyst gave cross-coupled products (**6a** and **6c**) (Scheme 2).

The selective formation of *E*-isomer of the products has been confirmed from the coupling constant (*J*) values of olefinic products. The formation of compounds (**6a**) and (**6c**) was further confirmed by acetylation of hydroxyl group using Ac_2O/Py to obtain acetyl derivatives (**6b**) and (**6d**), characteristic acetyl protons appeared at δ 1.92 ppm (s, 3H).

This approach was again repeated for the simple and easily available carbohydrate-derived olefin (**7**). However, the reaction of this olefin (**7**) with both styrenes (**2a** and **2d**) resulted in the formation of homocoupled dimers (**8** and **9a–b**) as products instead of the desired cross-coupled products (**10**) (Scheme 3). This homocoupling may be attributed to the absence of furanose ring in the carbohydrate-derived olefin because the presence of furanose ring may cause steric hindrance for the formation of homocoupled product. The structures of compounds **4a–e**, **6a–d**, **8** and **9a–b** were deduced from ^{1}H and ^{13}C NMR, mass spectral and elemental analysis.[12]

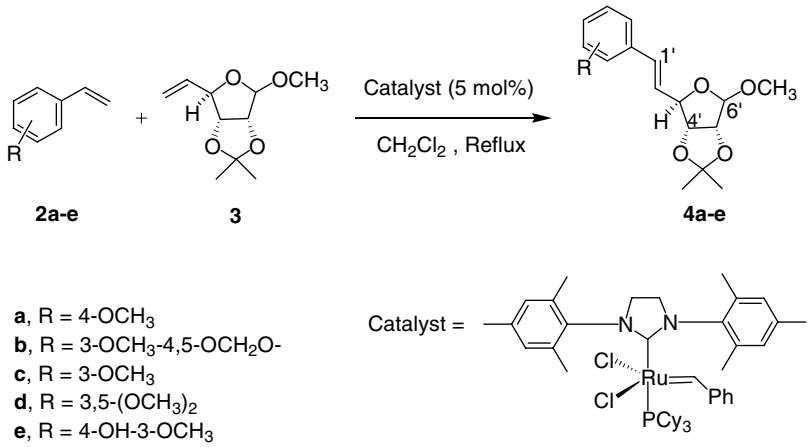
Newly synthesized compounds **4a–e**, **6a** and **6c** were screened for cytotoxicity by following a previously reported procedure.[13] All the compounds (**4a–e**, **6a** and **6c**) were tested towards RAW264.7 (mouse macrophage), A549 (human lung carcinoma) and HT29 (human colon carcinoma) cell lines. They displayed mild activity against RAW264.7 and HT29 cell lines (Table 1). In RAW264.7 cell line compounds **4a**, **4b**, **4d**, **4e** and **6a** showed activity, whereas compounds **4c** and **6c** were inactive. Similarly in HT29 cell line for compounds **4a**, **4b**, **4e**, **6a** and **6c** was active and compounds **4c** and **4d** did not show any activity.

Among the tested compounds, compounds **4b** and **4a** exhibited more activity towards RAW264.7 and HT29 cell lines, respectively. All the compounds (**4a–e**, **6a** and **6c**) were found to be inactive against A549 (human lung carcinoma) cell line.

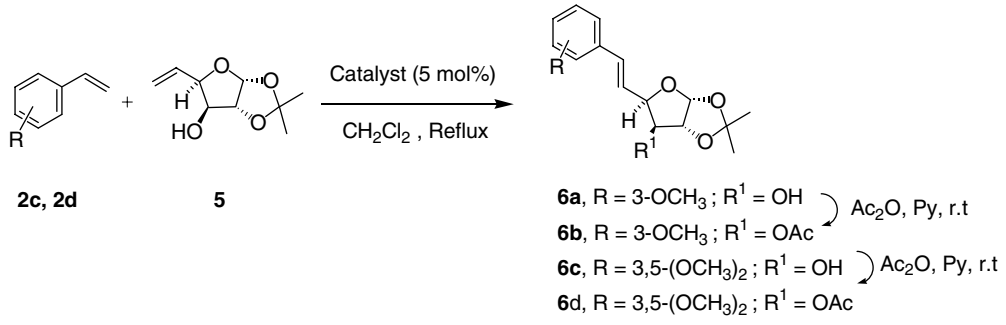
In conclusion, analogues of cytotoxic varitriol were synthesized (by utilizing CM reaction), characterized and screened for cytotoxicity against RAW264.7, A549 and HT29 cell lines. All the compounds showed mild activity and compounds **4a** and **4b** displayed more activity among the screened compounds. Though the compounds showed mild activity, the synthesis and screening of more analogues of varitriol, both protected and unprotected, would give scope for further work in this area.

Acknowledgments

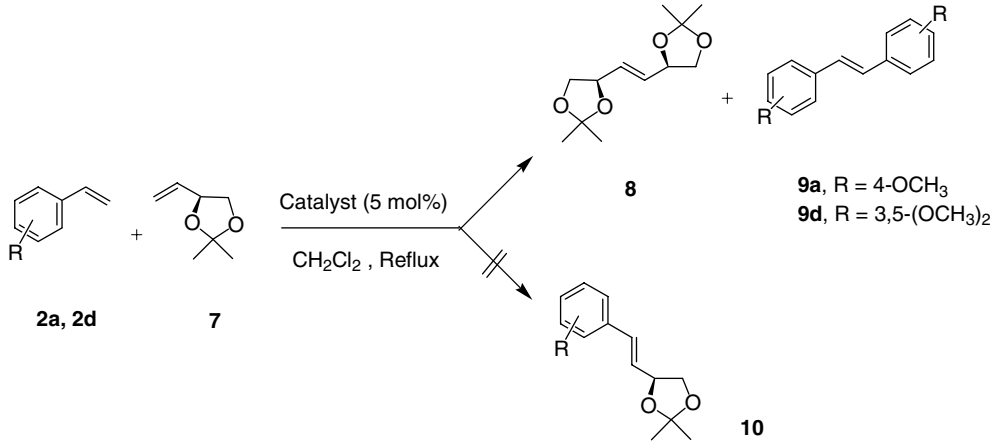
The authors thank the Head, Organic Chemistry Division-II, and Director, IICT for their support. P.V. and



Scheme 1.



Scheme 2.



Scheme 3.

Table 1. Cytotoxicity values GI_{50} (molar) for **4a–e**, **6a** and **6c**

Compound	RAW264.7 cell line (M)	HT29 cell line (M)
4a	6.96×10^{-4}	4.70×10^{-4}
4b	4.62×10^{-4}	13.97×10^{-4}
4c	Inactive	Inactive
4d	5.41×10^{-4}	Inactive
4e	8.35×10^{-4}	13.22×10^{-4}
6a	6.71×10^{-4}	17.53×10^{-4}
6c	Inactive	20.03×10^{-4}

A.S. thank CSIR, New Delhi, for financial assistance. Authors also thank Dr P. Rama Krishna, Pharmacology Division, IICT.

References and notes

1. Varmus, H. *Science* **2006**, *312*, 1162.
2. Dourous, J. D.; Suffness, M. *Cancer Chemother. Pharmacol.* **1978**, *1*, 91.

3. Cassady, J. M.; Dourous, J. D. *Anticancer agents based on natural products models*. In *Medicinal Chemistry*; Academic Press, 1980; Vol. 16.
4. Sims, J. J.; Rose, A. F.; Izac, R. R.. In *Marine Natural Products, Chemical and Biological Perspectives*; Scheuer, P. J., Ed.; Academic press: New York, 1978; Vol. 2, p 297.
5. Malmstrøm, J.; Christophersen, C.; Barrero, A. F.; Oltra, J. E.; Justicia, J.; Rosales, A. *J. Nat. Prod.* **2002**, *65*, 364.
6. Clemens, R. T.; Jennings, M. P. *Chem. Commun.* **2006**, 2720.
7. McAllister, G. D.; Robinson, J. E.; Taylor, R. J. K. *Tetrahedron* **2007**, *63*, 12123.
8. Chatterjee, A. K.; Choi, T.-L.; Sanders, D. P.; Grubbs, R. H. *J. Am. Chem. Soc.* **2003**, *125*, 11360.
9. Nagarapu, L.; Satyender, A.; Rajashaker, B.; Srinivas, K.; Rupa rani, P.; Radhika, K.; Subhashini, G. *Bioorg. Med. Chem. Lett.* **2008**, *18*, 1167.
10. Ugarkar, B. G.; DaRe, J. M.; Kopcho, J. J.; Browne, C. E.; Schanzer, J. M.; Wiesner, J. B.; Erion, M. D. *J. Med. Chem.* **2000**, *43*, 2883.
11. Hall, L.; Hough, L.; Pritchard, R. A. *J. Chem. Soc.* **1961**, 1537.
12. *Experimental:* ^{1}H NMR spectra were recorded with an AVANCE 300 Bruker at 300 MHz and Gemini 200 MHz in $CDCl_3$. Chemical shifts relative to TMS as internal standard are given as δ values in ppm. ^{13}C NMR was recorded in $CDCl_3$ on Varian (75 Hz) spectrometer. EI-MS mass spectra were measured at 70 eV (EI).
*General procedure for synthesis of compounds **4a–e**, **6a** and **6c**:* To a stirred solution of sugar olefin (0.5 mmol) and substituted styrene (0.5 mmol) in 15 mL of DCM was added Grubbs second-generation catalyst (5 mol %). The mixture was allowed to reflux at 40 °C under N_2 atmosphere for 18 h. The reaction was then cooled to room temperature and concentrated under reduced pressure. Flash chromatography (silica, 10% ethyl acetate in hexanes) afforded the cross-coupled product.
*Compound **4a**:* Yield: 66%, $[\alpha]_D$ −1.5° (*c* 0.33, $CHCl_3$), ^{1}H NMR ($CDCl_3$, 300 MHz): δ 1.30, 1.49 (2s, 6H, 2× CH_3), 3.33 (s, 3, OCH_3), 3.88 (s, 3H, Ar-OCH_3), 4.62–4.72 (m, 3H, H-5′, H-4′, H-3′), 4.93 (s, 1H, H-6′), 5.99 (dd, 1H, *H*-1′, $J_{1',2'} = 15.8$ Hz, $J_{2',3'} = 8.0$ Hz), 6.47 (d, 1H, H-6, $J_{1',2'} = 15.8$ Hz), 6.79 (d, 2H, Ar-H, $J = 9.0$ Hz), 7.23 (d, 2H, Ar-H, $J = 9.0$ Hz). ^{13}C NMR (75 MHz, $CDCl_3$): δ 25.0, 26.5, 29.5, 54.5, 55.2, 84.7, 85.6, 88.4, 109.1, 113.9, 126.4, 127.7, 132.3. EI-MS: *m*/*z* = 306 [M^+]. Anal. Calcd for $C_{17}H_{22}O_5$: C, 66.65; H, 7.24. Found: C, 66.51; H, 7.32.
*Compound **4b**:* Yield: 67%, ^{1}H NMR ($CDCl_3$, 200 MHz): δ 1.30, 1.49 (2s, 6H, 2× CH_3), 3.32 (s, 3H, Ar-OCH_3), 3.91 (s, 3H, Ar-OCH_3), 4.59–4.61 (m, 3H, H-5′, H-4′, H-3′), 4.95 (s, 1H, H-6′), 6.01 (dd, 1H, H-2′, $J_{2',3'} = 8.4$ Hz, $J_{1',2'} = 15.7$ Hz), 5.95 (s, 2H, CH_2), 6.42 (d, 1H, H-1′, $J_{1',2'} = 15.7$ Hz), 6.45 (s, 1H, Ar-H), 6.56 (s, 1H, Ar-H). ^{13}C NMR (75 MHz, $CDCl_3$): δ 25.0, 26.4, 29.6, 54.5, 56.5, 84.7, 85.6, 88.1, 100.0, 101.4, 107.2, 109.2, 127.4, 132.5. EI-MS: *m*/*z* = 350 [M^+]. Anal. Calcd for $C_{18}H_{22}O_7$: C, 61.71; H, 6.33. Found: C, 61.02; H, 6.28.
*Compound **4c**:* Yield: 65%, $[\alpha]_D$ −6.4° (*c* 0.70, $CHCl_3$), ^{1}H NMR ($CDCl_3$, 300 MHz): δ 1.30, 1.49 (2s, 6H, 2× CH_3), 3.37 (s, 3H, OCH_3), 3.81 (s, 3H, Ar-OCH_3), 4.60–4.78 (m, 3H, H-5′, H-4′, H-3′), 4.95 (s, 1H, H-6′), 6.15 (dd, 1H, *H*-2′, $J_{1',2'} = 15.8$ Hz, $J_{2',3'} = 8.6$ Hz), 6.53 (d, 1H, H-1′, $J_{2',3'} = 15.8$ Hz), 6.72–6.95 (m, 3H, Ar-H), 7.18 (m, 1H, Ar-H). ^{13}C NMR ($CDCl_3$, 75 MHz): δ 24.9, 26.4, 29.6, 54.5, 55.1, 84.6, 85.5, 88.0, 109.2, 112.0, 112.3, 113.3, 119.1, 128.9, 129.4, 132.5, 137.8, 159.7. Anal. Calcd for $C_{17}H_{22}O_5$: C, 66.65; H, 7.24. Found: C, 66.68; H, 7.28.
*Compound **4d**:* Yield: 51%, $[\alpha]_D$ −4.35° (*c* 0.39, $CHCl_3$), ^{1}H NMR ($CDCl_3$, 300 MHz): δ 1.31, 1.50 (2s, 6H, 2× CH_3), 3.35 (s, 3H, OCH_3), 3.90 (2s, merged, 6H, 2× Ar-OCH_3), 4.60–4.73 (m, 3H, H-5′, H-4′, H-3′), 4.96 (s, 1H, H-6′), 6.02 (dd, 1H, H-2′, $J_{1',2'} = 15.8$ Hz, $J_{2',3'} = 8.6$ Hz), 6.48 (d, 1H, H-1′, $J_{1',2'} = 15.8$ Hz), 6.76 (m, 1H, Ar-H), 6.85 (m, 2H, Ar-H). ^{13}C NMR ($CDCl_3$, 75 MHz): δ 24.9, 26.4, 29.6, 54.5, 55.8, 55.8, 84.7, 85.6, 88.3, 109.0, 109.1, 111.1, 112.3, 119.7, 126.6, 129.4, 132.5, 149.0, 149.0. Anal. Calcd for $C_{18}H_{24}O_6$: C, 64.27; H, 7.19. Found: C, 64.31; H, 7.11.
*Compound **4e**:* Yield: 58%, $[\alpha]_D$−8.6° (*c* 0.15, $CHCl_3$), ^{1}H NMR ($CDCl_3$, 300 MHz): δ 1.30, 1.50 (2s, 6H, 2× CH_3), 3.25 (s, 3H, OCH_3), 3.90 (s, 3H, Ar-OCH_3), 4.6–4.72 (m, 3H, H-5′, H-4′, H-3′), 4.95 (s, 1H, H-6′), 5.98 (dd, 1H, H-2′, $J_{1',2'} = 15.8$ Hz, $J_{2',3'} = 9.0$ Hz), 6.46 (d, 1H, *H*-1′, $J_{1',2'} = 15.8$ Hz), 6.78–6.90 (m, 3H, Ar-H). ^{13}C NMR (75 MHz, $CDCl_3$): δ 25.0, 26.4, 29.6, 54.5, 55.8, 84.7, 85.6, 88.3, 108.5, 109.2, 114.4, 120.3, 126.3, 132.7. Anal. Calcd for $C_{17}H_{22}O_6$: C, 63.34; H, 6.88. Found: C, 63.41; H, 6.79.
*Compound **6a**:* Yield: 61%, $[\alpha]_D$ −7.0° (*c* 0.50, $CHCl_3$), ^{1}H NMR ($CDCl_3$, 300 MHz): δ 1.33, 1.52 (2s, 6H, 2× CH_3), 3.80 (s, 3H, Ar-OCH_3), 4.10 (m, 1H, H-4′), 4.55 (d, 1H, *H*-5′, $J_{5',6'} = 3.0$ Hz), 4.86 (m, 1H, H-3′), 5.95 (d, 1H, H-6′, $J_{5',6'} = 3.0$ Hz), 6.19 (dd, 1H, H-2′, $J_{1',2'} = 15.863$, $J_{2',3'} = 5.2$ Hz), 6.8 (d, 1H, H-1′, $J_{1',2'} = 15.8$ Hz), 6.73–6.97 (m, 3H, Ar-H), 7.20 (m, 1H, Ar-H). ^{13}C NMR (75 MHz, $CDCl_3$): δ 26.074, 26.649, 29.570, 55.738, 108.5, 112.2, 119.4, 126.5, 127.9, 128.4, 130.5, 148.5, 149.0. Anal. Calcd for $C_{16}H_{20}O_5$: C, 65.74; H, 6.90. Found: C, 65.81; H, 6.85.
*Compound **6c**:* Yield: 53%, $[\alpha]_D$ −68.5° (*c* 0.48, $CHCl_3$), ^{1}H NMR ($CDCl_3$, 300 MHz): δ 1.33, 1.52 (2s, 6H, 2× CH_3), 3.88 (s, 6H, 2× Ar-OCH_3), 4.05–4.15 (m, 1H, H-4′), 4.55 (d, 1H, H-5′, $J_{5',6'} = 3.7$ Hz), 4.83 (d, 1H, H-3′, $J_{2',3'} = 5.6$ Hz), 5.92 (d, 1H, H-6′, $J_{5',6'} = 3.7$ Hz,), 6.05 (dd, 1H, *H*-2′, $J_{2',3'} = 5.6$ Hz, $J_{1',2'} = 15.8$ Hz), 6.74 (d, 1H, H-1′, $J_{1',2'} = 15.8$ Hz), 6.78 (m, 1H, Ar-H), 6.88 (m, 2H, Ar-H). ^{13}C NMR ($CDCl_3$, 75 MHz): δ 26.0, 26.6, 29.5, 55.7, 55.8, 76.1, 81.0, 84.9, 104.5, 108.8, 110.9, 111.6, 119.6, 129.1, 134.1, 148.9, 149.1. EI-MS: *m*/*z* = 322 [M^+]. Anal. Calcd for $C_{17}H_{22}O_6$: C, 63.34; H, 6.88. Found: C, 63.43; H, 6.89.
13. Kan, S.-F.; Huang, W.-J.; Lin, L.-C.; Wang, P. S. *Int. J. Cancer* **2004**, *110*, 641.