Available online at www.sciencedirect.com
SCIENCE DIRECT®
Bioorganic & Medicinal Chemistry Letters 15 (2005) 163–166
Bioorganic & Medicinal Chemistry Letters

Cytotoxic neolignans: an SAR study

Zwe-Ling Kong, Shin-Cheng Tzeng and Yeuk-Chuen Liu*

Food Science Department, National Taiwan Ocean University, Keelung, Taiwan, ROC

Received 15 September 2004; revised 1 October 2004; accepted 5 October 2004
Available online 26 October 2004

Abstract—The neolignans, magnolol **1** and honokiol **2** have been reported to inhibit the growth of several tumor cell lines in vitro and in vivo. The chemical structure of magnolol and honokiol consists of biphenyl skeleton with phenolic and allylic functionalities. Analogs of **1** and **2** containing different substitution have been studies for their effect on the growth of Hep-G2 and their structure–activity relationships were reported in this work.

Magnolol and honokiol are significant bioactive constituents isolated from the bark of *Magnoliae officinalis*, or *M. obovata* which have been used in traditional Chinese medicine for the relief of flu symptoms, treatment of anxiety and stroke. Recently, magnolol has been demonstrated to possess potent anti-oxidative activity,[1–3] anti-anxiety,[4] anti-inflammatory,[5] and anti-cancer[6] activities. Magnolol and honokiol have cytotoxic activities against A459 cell line (human nonsmall lung cell cancer), SK-MEL-2 (human melanoma), SK-OV-3 (ovarian cancer), XF498 (CNS cell line), HCT-15 (colon cancer), and Hep-G2 (liver cancer).[6–9]

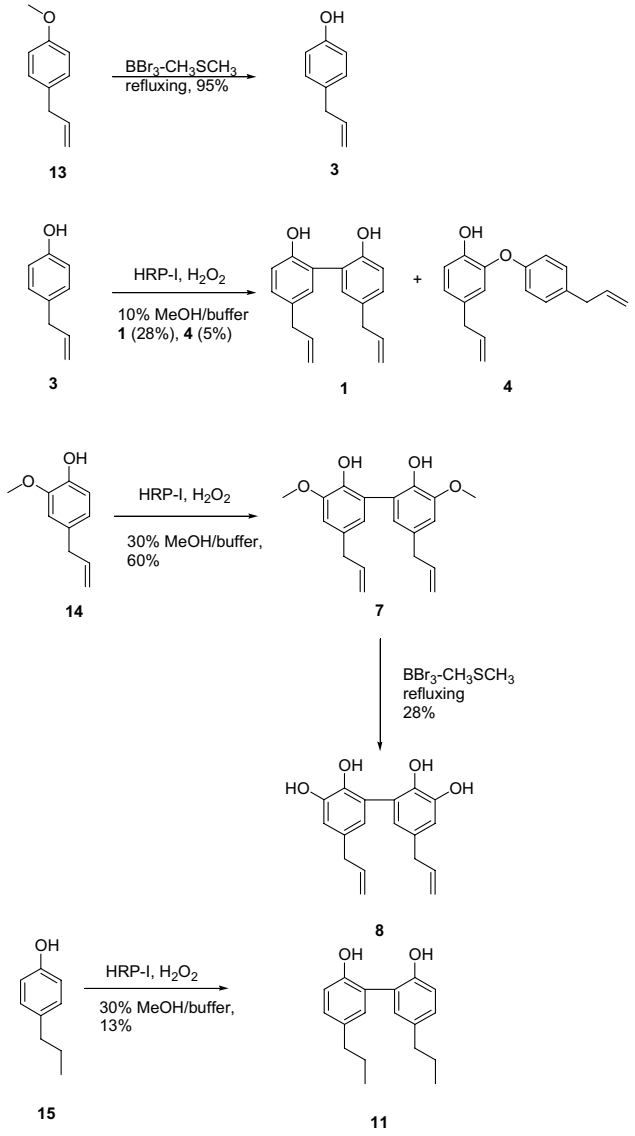
Magnolol at low concentrations (3–10 μM) inhibited DNA synthesis and human cancer cell growth (COLO-205 and Hep-G2), but did not inhibit the growth of human untransformed cells such as keratinocytes, fibroblasts, and human umbilical vein endothelial cells.[7] Apoptosis was observed in mouse melanoma B16-BL6, human acute monocytic leukemia THP-1, fibrosarcoma HT-1080, COLO-205, and Hep-G2 cells when magnolol concentration was increased to 100 μM, but not in human untransformed gingival fibroblasts and human umbilical vein endothelial cells. The mechanism of apoptosis induced by magnolol has also been studied.[7–9] Several cellular phenomena related to apoptosis after the treatment of magnolol have been observed including: (1) intracellular calcium concentration increased, translocation of cytochrome *c* from mitochondria to cytosol; (2) activation of caspase 3, caspase 8, and caspase 9; and (3) downregulation of bcl-2 protein.[7–9] If the COLO cell line was incubated with magnolol in the presence of a phospholipase C inhibitor, no apoptosis was observed. Honokiol was reported to possess higher activity in the induction of apoptosis than magnolol.[10] Honokiol induced apoptosis by inhibition of Akt and MAPK phosphorylation.[10]

The chemical structure of magnolol is an *ortho,ortho*-C–C dimer of 4-allyl-phenol which consists a biphenyl skeleton with phenolic functionalities and two *p*-allyl groups as side chains. Since the molecular target of anti-cancer activities of magnolol was not clear, we envisioned that further modifications of magnolol functionalities would provide us more information concerning the basic structure–activity relationship of magnolol. Magnolol and its analogs described in the present study were illustrated in Schemes 1 and 2.

Magnolol and the ether derivative **4** were prepared by horseradish peroxidase-catalyzed phenolic coupling of 4-allyl-phenol **3** in the presence of H_2O_2 (Scheme 1). Briefly, the hydrogen peroxide was added slowly (0.03 equiv/min) to the mixture of 4-allyl-phenol in buffer solution (pH 6.0, 10% methanol v/v) in the presence of horseradish peroxidase (Sigma, Type-1, 1000 units/mmol of substrate) at room temperature for 18 min. Bis-eugenol **7** and tetrahydromagnolol **11** were also prepared by phenolic oxidative coupling of eugenol and propylphenol catalyzed by horseradish peroxidase, respectively (Scheme 1) as previous described condition except 30% methanol in buffer solution being employed. The

Keywords: Neolignans; Cytotoxicities; Hep-G2; SAR.
* Corresponding author: Tel.: +886 2 24622192x5142; fax: +886 2 24634203; e-mail: york@mail.ntou.edu.tw

0960-894X/$ - see front matter © 2004 Elsevier Ltd. All rights reserved.
doi:10.1016/j.bmcl.2004.10.011

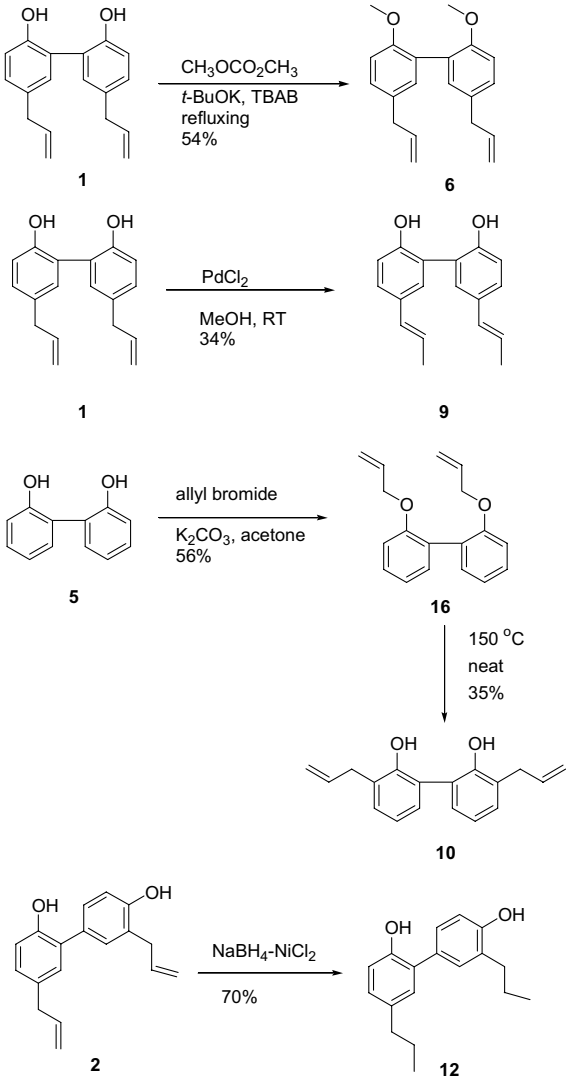


Scheme 1. Synthesis of neolignan analogs.

magnolol analogs, including dimethyl **6**, propenyl **9**, 3,3′-diallyl **10** derivatives and tetrahydrohonokiol were prepared by the methods as illustrated in Scheme 2. Briefly, dimethyl derivative of magnolol **6** was prepared by heating magnolol in dimethylcarbonate with base.[11] Demethylated derivative of bis-eugenol **8** was obtained by heating bis-eugenol **7** with boron tribromide.[12] Propenyl derivative **9** was prepared by isomerization of double bond of magnolol catalyzed with $PdCl_2$.[13] 3,3′-Diallyl derivative **10** was obtained by thermal Claisen rearrangement of allyl ether of 2,2′-biphenol **5**.[14] Tetrahydrohonokiol **12** was obtained by reduction of honokiol with sodium borohydride in the presence of $NiCl_2$.[15] All the chemical structures and purities of synthetic chemicals described in this work were confirmed by NMR.[16]

The chemical structure of magnolol consists of biphenyl skeleton with phenolic and *p*-allylic functionalities. Since liver cancer was the leading cause of death in Asia, human liver tumor cell line was chosen for the present study. Twelve analogs (Schemes 1 and 2) were tested for cytotoxicity against a human liver tumor cell line (Hep-G2) and the results were summarized in Table 1.

Substitution of the biphenol was required for activity since the 2,2′-dihydroxybiphenyl **5** (IC_{50}: >200 μM) had no cytotoxic activity against Hep-G2. For the sake of investigating the role of phenolic group contributing to the anti-cancer activities, methoxyl analog **6** was synthesized to diminish the possibility of the participation of phenolic ionic bonding to its biomolecular target. Cytotoxicity of methylated derivative **6** (IC_{50}: >200 μM) decreased significantly which demonstrated that free phenolic functionalities were essential for the activities. Introducing methoxyl groups at the 3 and 3′-positions as in bis-eugenol (3,3′-dimethoxy-5,5′-di-2-propenyl-1,1′-biphenyl-2,2′-diol) **7** or hydroxyl groups at 3 and 3′-positions as in dihydroxyl magnolol **8** diminished the cytotoxicity (IC_{50} of **7** and **8**: 117 and 104 μM, respectively). It might be due to the formation of intra-molecular hydrogen



Scheme 2. Synthesis of neolignan analogs.

Table 1. Cytotoxicities of neolignan analogs against Hep-G2

Compounds	IC_{50} (μM)
1	32.0
2	16.5
3	>200
4	51.8
5	>200
6	>200
7	116.8
8	103.5
9	38.0
10	42.1
11	23.4
12	13.1

bonding between the hydroxyl groups and methoxyl groups in the bis-eugenol molecule **7** and the catechol groups in the dihydroxyl-magnolol moiety **8**. The intramolecular hydrogen bonding could prohibit the formation of intermolecular ionic or hydrogen bonding between the phenoxyl groups of bis-eugenol analogs and the biomolecular target. Since the cytotoxicity of *ortho-O*-ether **4** (IC_{50}: 52 μM) was comparable to magnolol or honokiol, the significant activity of the derivative **4** demonstrated that at least one free hydroxyl group was essential to the induction of cytotoxicity. Although the highly conjugated derivative was known to be pro-oxidants and prone to form DNA-adduct, oxidative stress might not play an important role of highly conjugated derivative of magnolol for the cytotoxic activity since the cytotoxicity of highly conjugated magnolol analog **9** (IC_{50}: 38 μM) was close to the activity of its parent compound **1** (IC_{50}: 32 μM). No significant differences of cytotoxicities of saturated allyl analogs (**11** and **12**) comparing to the corresponding parent compounds might imply that the double bonds did not contribute π–π-interaction with the biomolecular target. The position and nature of substituents on the benzene rings also modulated anti-tumor activity of magnolol derivatives. Honokiol is also one of a significant bioactive neolignans other than magnolol isolated from Magnolia bark. The structure of honokiol consists of *para*-allyl-phenol and an *ortho*-allyl-phenol which link together through *ortho,para*- C–C-coupling. According to the previous report,[6] the cytotoxicity of honokiol was comparable to magnolol against different cell lines. The cytotoxic activity of honokiol was also close to magnolol against Hep-G2 in the present study. The 3′-allyl group of honokiol (both allyl groups of magnolol are *para* to the phenolic-OH groups) might play an important role to the cytotoxicity since the ether derivative and 3,3′-diallyl derivative **10** (IC_{50}: 42 μM) also have significant activities against Hep-G2.

Acknowledgements

This work was partly supported by the National Science Council of the Republic of China under Grant NSC 91-2113-M-019-001.

References and notes

1. Chan, P.; Cheng, J. T.; Tsao, C. W.; Niu, C. S.; Hong, C. Y. *Life Sci.* **1996**, *59*, 2067–2073.
2. Lee, Y. M.; Hsiao, G.; Chen, H. R.; Chen, Y. C.; Sheu, J. R.; Yen, M. H. *Eur. J. Pharmacol.* **2001**, *422*, 159–167.
3. Haraguchi, H.; Ishikawa, H.; Shirataki, N.; Fukuda, A. *J. Pharm. Pharmacol.* **1997**, *49*, 209–212.
4. Maruyama, Y.; Kuribara, H.; Morita, M.; Yuzurihara, M.; Weintraub, S. T. *J. Nat. Prod.* **1998**, *61*, 135–138.
5. Wang, J. P.; Ho, T. F.; Chang, L. C.; Chen, C. C. *J. Pharm. Pharmacol.* **1995**, *47*, 857–860.
6. Kim, Y. K.; Ryu, S. Y. *Planta Med.* **1999**, *65*, 291–292.
7. Lin, S. Y.; Chang, Y. T.; Liu, J. D.; Yu, C. H.; Hp, Y. S.; Lee, Y.-H.; Lee, W.-S. *Mol. Carcinog.* **2001**, *32*, 73–83.
8. Ikeda, K.; Nagase, H. *Biol. Pharm. Bull.* **2002**, *25*, 1546–1549.
9. Yang, S. E.; Hsieh, M. T.; Tsai, T. H.; Hsu, S. L. *Br. J. Pharmacol.* **2003**, *138*, 193–201.
10. Bai, X. H.; Cerimele, F.; Ushio-Fukai, M.; Waqas, M.; Campbell, P. M.; Govindarajan, B.; Der, C. J.; Battle, T.; Frank, D. A.; Ye, K.; Murad, E.; Dubiel, W.; Soff, G.; Arbiser, J. L. *J. Biol. Chem.* **2003**, *278*, 35501–35507.
11. Ouk, S.; Thiebaud, S.; Borredon, E.; Legars, P.; Lecomte, L. *Tetrahedron Lett.* **2002**, *43*, 2661–2663.
12. Agharahimi, M. R.; LeBel, N. A. *J. Org. Chem.* **1995**, *60*, 1856–1863.
13. Jing, X. B.; Gu, W. X.; Ren, X. F.; Bie, P. Y.; Pan, X. F. *J. Chin. Chem. Soc.* **2001**, *48*, 59–63.
14. Li, C. Y.; Wang, Y.; Hu, M. K. *Bioorg. Med. Chem.* **2003**, *11*, 3665–3671.
15. Narisada, M.; Horibe, I.; Watanabe, F.; Takeda, K. *J. Org. Chem.* **1989**, *54*, 5808.
16. Structures were confirmed with a 500 MHz NMR spectrometer (Bruker, Rheinstetten, Germany) spectrometer with TMS as an internal reference. The purities of all the synthetic compounds are >98% according to the NMR analysis.
^1H NMR of magnolol **1** δ_H (acetone-d_6): 7.09 (2H, d, J = 2.04 Hz), 7.04 (2H, dd, J = 8.2, 2.1 Hz), 6.90 (2H, d, J = 8.2 Hz), 5.97 (2H, m), 5.07 (2H, dd, J = 17.0, 1.8 Hz), 4.99 (2H, dd, J = 10.1, 1.8 Hz), 3.33 (4H, d, J = 6.7 Hz) 4-allyl-phenol **3** δ_H (acetone-d_6): 6.97 (2H, d, J = 8.35 Hz), 6.73 (2H, d, J = 8.45 Hz), 5.92 (1H, m), 5.02 (1H, dd, J = 18.7, 1.8 Hz), 4.97 (1H, dd, J = 9.8, 1.8 Hz), 3.26 (2H, d, J = 6.15 Hz).
^1H NMR of isomagnolol **4** δ_H (acetone-d_6): 8.1 (1H), 7.14 (2H, d, J = 8.5 Hz, H-3′/5′), 6.93 (1H, d, J = 8.2 Hz), 6.88 (1H, dd, J = 8.2, 1.6 Hz), 6.83 (2H, d, J = 8.5 Hz), 6.77 (1H, d, J = 1.6 Hz), 5.95 (2H, m), 5.05 (4H, m), 3.32 (2H, dd, J = 6.7 Hz), 3.27 (2H, d, J = 6.6 Hz).
^1H NMR of dimethoxymagnolol **6** ($CDCl_3$): 6.90 (2H, d, J = 8.3 Hz), 7.06 (2H, d, J = 2.15 Hz), 7.13 (2H, dd, J = 8.3, 2.15 Hz), 5.90 (2H, m), 5.08 (2H, dd, J = 17.0, 1.65 Hz), 5.05 (2H, d, J = 9.9 Hz), 3.75 (6H, s), 3.36 (4H, d, J = 6.7 Hz).
^1H NMR of bis-eugenol **7** δ_H ($CDCl_3$): 6.79 (2H, d, J = 1.85 Hz), 6.68 (2H, J = 1.9 Hz), 5.99 (2H, m), 5.11 (2H, dd, J = 17.0, 1.75 Hz), 5.00 (2H, dd, J = 10.05, 0.8 Hz), 3.85 (6H, s), 3.33 (4H, d, J = 6.70 Hz).
^1H NMR of demethylated bis-eugenol **8** δ_H ($CDCl_3$): 6.80 (2H, d, J = 1.80 Hz), 6.71 (2H, J = 1.7 Hz), 6.11 (2H, s), 5.96 (2H, m), 5.57 (2H, s), 5.11 (2H, dd, J = 18.28, 1.55 Hz), 5.07 (2H, d, J = 10.55 Hz), 3.33 (4H, d, J = 6.70 Hz).
^1H NMR of highly conjugated magnolol derivative **9** δ_H ($CDCl_3$): 7.29 (2H, dd, J = 8.35, 2.05 Hz), 7.23 (2H, d,

J = 1.95 Hz), 6.96 (2H, d, J = 8.35 Hz), 6.35 (2H, dd, J = 15.7, 1.05 HZ), 6.13 (2H, m), 1.87 (6H, dd, J = 6.6, 1.35 Hz).

^{1}H NMR of 3,3′-diallyl-biphenol **10** δ_H ($CDCl_3$): 7.20 (2H, d, J = 6.7, 0.7 Hz), 7.14 (2H, J = 7.5, 1.4 Hz), 7.00 (2H, t, J = 7.55 Hz), 6.06 (2H, m), 5.48 (2H, s), 5.17 (2H, dd, J = 17.2, 0.55 Hz), 5.12 (2H, dd, J = 11.5, 1.3 Hz), 3.48 (4H, d, J = 6.55 Hz).

^{1}H NMR of tetrahydromagnolol **11** δ_H ($CDCl_3$): 7.13 (2H, dd, J = 8.25, 2.05 Hz), 7.07 (2H, dd, J = 2.0 Hz), 6.94 (2H, d, J = 8.25 Hz), 5.48 (2H, s), 2.56 (4H, t, J = 7.4 Hz), 1.64 (4H, m), 0.95 (6H, d, J = 7.3 Hz).

^{1}H NMR of tetrahydronokiol **12** δ_H ($CDCl_3$): 7.21 (1H, d, J = 2.05 Hz), 7.04 (1H, d, J = 8.15, 2.05 Hz), 7.01 (1H, J = 2.10 Hz), 6.88 (1H, d, J = 7.95 Hz), 6.87 (1H, J = 7.95 Hz), 5.09 (1H, s), 4.90 (1H, s), 2.63 (2H, t, 7.55 Hz), 2.54 (1H, s, 7.50 Hz), 1.67 (4, m), 1.0 (3H, t, J = 7.35 Hz), 0.95 (3H, t, J = 7.35 Hz).