Bioorganic & Medicinal Chemistry Letters 23 (2013) 1201–1205

Contents lists available at SciVerse ScienceDirect

Bioorganic & Medicinal Chemistry Letters

journal homepage: www.elsevier.com/locate/bmcl

Synthesis of hemslecin A derivatives: A new class of hepatitis B virus inhibitors

Rui-Hua Guo [a], Chang-An Geng [a], Xiao-Yan Huang [a], Yun-Bao Ma [a], Quan Zhang [a], Li-Jun Wang [a], Xue-Mei Zhang [a], Rong-Ping Zhang [b,*], Ji-Jun Chen [a,*]

[a] State Key Laboratory of Phytochemistry and Plant Resources in West China, Kunming Institute of Botany, Chinese Academy of Sciences, Kunming 650204, PR China
[b] Kunming Medical University, Kunming, Yunnan 650500, PR China

ARTICLE INFO

Article history:
Received 22 August 2012
Revised 23 December 2012
Accepted 7 January 2013
Available online 16 January 2013

Keywords:
Hemslecin A
Derivatives
Hepatitis B virus

ABSTRACT

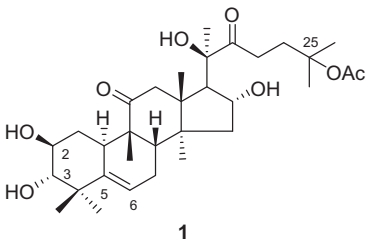
A series of hemslecin A derivatives were synthesized and evaluated for their anti-hepatitis B virus (HBV) activities, namely, inhibiting the secretion of hepatitis B surface antigen (HBsAg), hepatitis B e antigen (HBeAg), and HBV DNA replication on HepG 2.2.15 cells. Most of the derivatives showed enhanced anti-HBV activities, of which compounds **A1**–**A7**, **B5**, **C** and **E** exhibited significant activities inhibiting HBV DNA replication with IC_{50} values of 2.8–11.6 μM, comparable to that of the positive control, tenofovir. Compounds **A1**–**A3**, **A5**, **B5**, and **C** displayed low cytotoxicities, which resulted in high SI values of 89.7, 55.6, 77.8, >83.4, >55.8, and >150.5, respectively.

Hepatitis B virus (HBV) infection is a major health problem worldwide. It is estimated that more than 2 billion people have been infected with HBV, of which 350 million live with HBV chronic infection.[1,2] Chronic HBV infection concomitant with liver damage, cirrhosis of liver, and hepatocellular carcinoma leads to 1 million deaths per year.[3] HBV vaccines have made great achievements in preventing new infections, but are ineffective for HBV carriers.[4] Currently, interferons and HBV reverse transcriptase inhibitors (lamivudine, adefovir dipivoxil, entecavir, telbivudine, tenofovir disoproxil fumarate) are the main treatment agents for HBV infection.[5,6] However, their usage is limited due to low response rate, serious side effects or drug resistance.[7,8] Thus, the current therapies for HBV remain unsatisfactory, and new anti-HBV agents are urgently needed.

Natural products with various skeletons and diverse biological activities are considered as important sources in drug discovery. Presently, many naturally originated compounds have been reported with promising anti-HBV activities and different mechanism of action compared to nucleoside analogs.[9–18]

Many plants of the genus *Hemsleya* are traditionally used for treating hepatitis, sore throat, pelvic inflammatory disease, enteritis, etc. in China.[19] Hemslecin A (**1**, Fig. 1), a cucurbitane-type terpene, widely present in this genus has been used to cure inflammatory diseases in clinical practice.[20] Our recent investigation revealed that hemslecin A exhibited activity against HBV DNA replication (IC_{50} = 11.2 μM, SI = 5.8) based on anti-HBV assay on HepG 2.2.15 cell line in vitro. Thus, with hemslecin A as the starting substrate, a series of derivatives via chemical modification on hydroxyl groups at 2, 3, 16-position, C-5(6) double bond, and C-25 were synthesized and evaluated for their anti-HBV activity in order to further study the structure–activity relationships.

The presence of free hydroxyl groups allowed us to prepare ester derivatives of compound **1** in order to evaluate the influence of ester side chain on their anti-HBV activities. Treatment of compound **1** with various anhydrides in pyridine at room temperature (rt) gave mono- or di-acylated derivatives **A1**–**A7**. Compound **1** was treated with various anhydrides or carboxylic acids in the presence of 4-dimethylaminopyridine (DMAP) to afford triacylated compounds **B1**–**B5** (Scheme 1). Epoxidation of compound **1** with 1.2 equiv *m*-chloroperoxybenzoic acid (*m*CPBA) yielded derivative **C** without affecting the carbonyl and hydroxyl groups, which was further converted to derivatives **D1**–**D5**. The epoxy group was



1

Figure 1. Structure of hemslecin A.

* Corresponding authors. Tel./fax: +86 871 5223265.
E-mail addresses: zhrpkm@163.com (R.-P. Zhang), chenjj@mail.kib.ac.cn (J.-J. Chen).

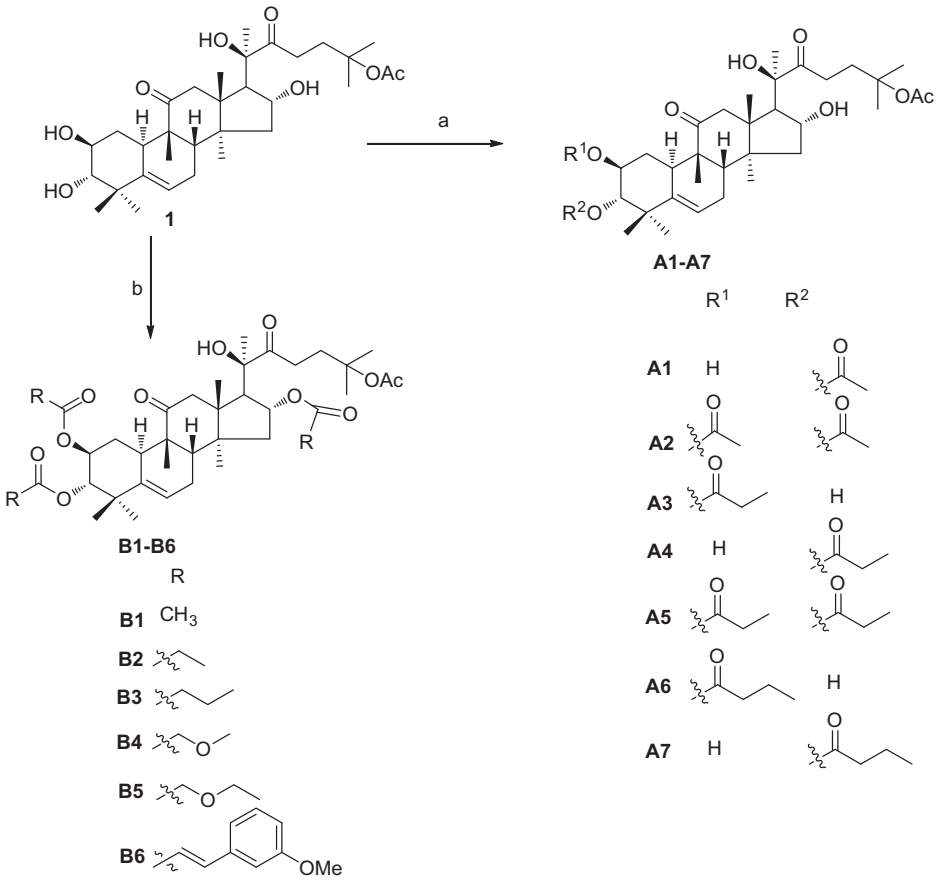
0960-894X/$ - see front matter Crown Copyright © 2013 Published by Elsevier Ltd. All rights reserved.
http://dx.doi.org/10.1016/j.bmcl.2013.01.024

determined as β-oriented by ROESY experiment, in which no correlation between δ_H 2.48 [H-6] and 2.28 [H-8, β] was observed. However, treatment of compound **1** with 2.5 equiv *m*CPBA afforded compound **E**. Derivatives **F1**–**F3** were obtained by epoxidation of triacylated compounds **B1**, **B4** and **B5** with 2.5 equiv *m*CPBA in CH_2Cl_2. Connections among 3-chlorobenzoyl and C-5 was determined by HMBC experiment, in which no correlation between H-6 and carbonyl group was detected. The ROESY spectrum showed no correlation between H-6 and H-8. Thus, the structures of compounds **E** and **F1**–**F3** were assigned as showed in Scheme 2. 25-Deacylated compound **2** was isolated from dried rhizomes of *Hemsleya chinensis*, which was treated with ethoxyacetic acid to produce derivatives **G** and **H** in order to assay the influence of acylation at C-25 on their anti-HBV activity (Scheme 3).

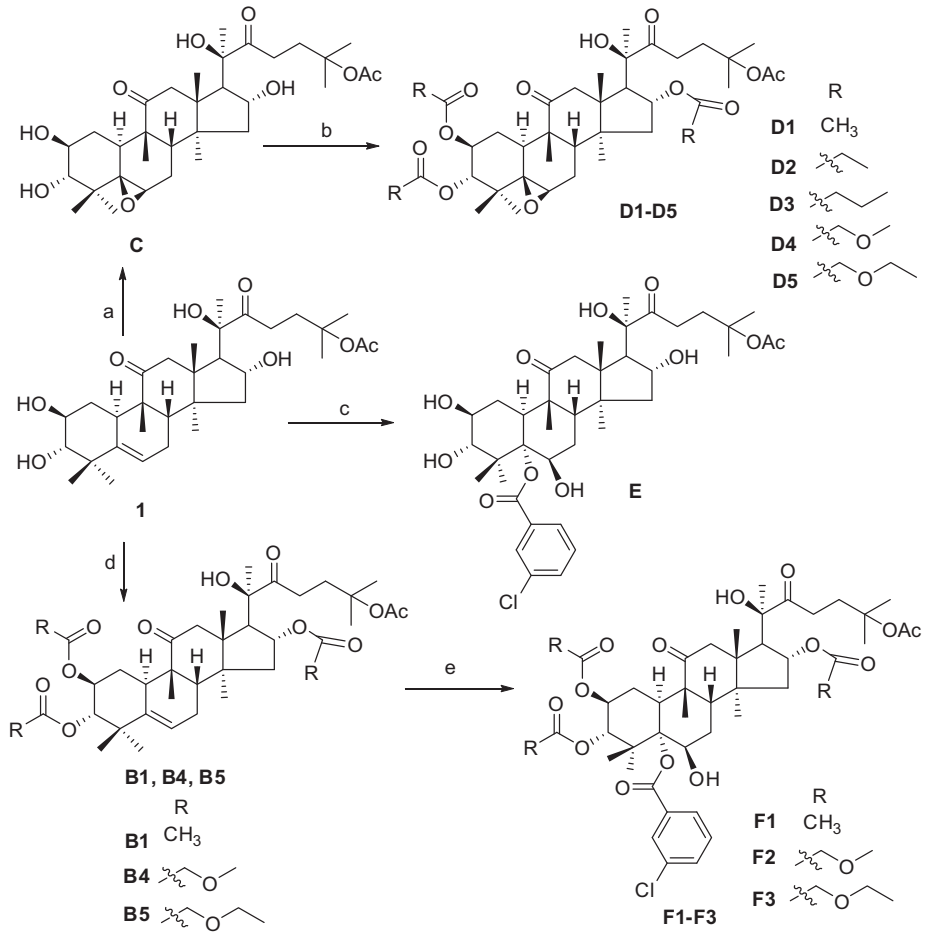
The anti-HBV activity and cytotoxicity of the synthesized hemslecin A derivatives were evaluated in HepG 2.2.15 cells, and tenofovir, a clinical anti-HBV agent, was used as positive control. The anti-HBV results were summarized in Table 1. The anti-HBV activity of each compound was expressed as the concentration of compound that achieved 50% inhibition (IC_{50}) of HBsAg, HBeAg, and HBV DNA replication. The cytotoxicity of each compound was expressed as the concentration of compound required to destroy 50% (CC_{50}) of the HepG 2.2.15 cells. The selectivity index (SI), one of the important pharmaceutical parameter, was determined as the ratio of CC_{50} value to IC_{50} value. The bioactivity of each compound was evaluated by the combination of its IC_{50} values and SI.

Compound **1** showed moderate activity against HBV DNA replication (IC_{50} = 11.4 μM), with obvious toxicity (CC_{50} = 66.2 μM), which led to a low SI value (SI = 5.8). To explore the influence of hydroxyl groups of compound **1** on anti-HBV activity, compounds **A1**–**A7** were synthesized and tested for their anti-HBV activities and cytotoxicities, and the results were shown in Table 1. Derivatives **A1**–**A7** exhibited significant inhibition against HBV DNA replication with IC_{50} values in the range of 2.8–9.8 μM. Compounds **A1**, **A4**, and **A7** with acylation of hydroxyl group at C-3 position exhibited significant inhibition against HBV DNA replication (IC_{50} = 2.8, 3.1, 4.7 μM, respectively).

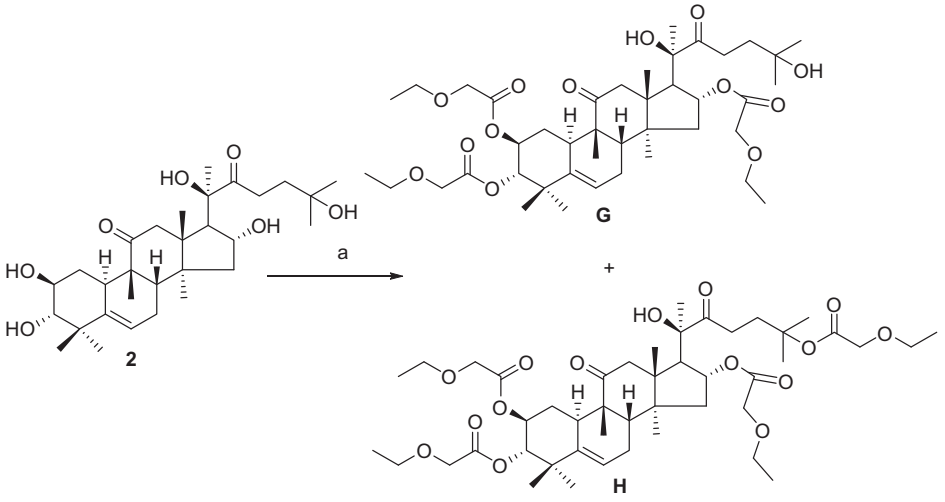
Acetylated derivative **A1** (CC_{50} = 253.5 μM, SI = 89.7) and propionylated derivative **A4** (CC_{50} = 100.3 μM, SI = 31.9) showed low cytotoxicity. Replacement of acyl moiety with butyryl (compound **A7**, CC_{50} = 6.3 μM) led to high cytotoxicity, which demonstrated that the cytotoxicity of the derivatives increased with the acyl chain lengthened. Meanwhile, compound **A4** showed moderate activity against the secretion of HBsAg (IC_{50} = 18.4 μM, SI = 5.5) and HBeAg (IC_{50} = 17.0 μM, SI = 5.9). Compounds **A3** and **A6** with acylation of hydroxyl group at C-2 position exhibited high activity against HBV DNA replication (IC_{50} = 4.7 μM, 6.1 μM, respectively). Propionyl derivative (**A3**) appeared to be less toxic compared to butyryl derivative (**A6**) (CC_{50} = 126.0 μM vs CC_{50} = 12.4 μM), resulting in a relatively high SI value (77.8 vs 2.0). Moreover, compound **A3** showed inhibitory potency to the secretion of HBsAg (IC_{50} = 15.6 μM, SI = 8.1) and HBeAg (IC_{50} = 14.4 μM, SI = 8.7). Diacylated compounds **A2** and **A5** exhibited high activities against HBV DNA replication with IC_{50} values of 9.8 μM (SI = 55.6), 7.2 μM (SI >83.4). Furthermore, compound **A5** showed the highest activity against the secretion of HBsAg (IC_{50} = 9.9 μM). These results indicated that mono- or di-acylation of hydroxyl group(s) at C-2 or (and) C-3 position resulted in enhancement of anti-HBV activity.



Scheme 1. Reagents and conditions: (a) for **A1** and **A2**: $(CH_3CO)_2O$, pyridine, rt, 8–30%; for **A3**–**A5**: $(CH_3CH_2CO)_2O$, pyridine, rt, 7–51%; for **A6** and **A7**: $(CH_3CH_2CH_2CO)_2O$, pyridine, rt, 14–77%; (b) for **B1**–**B3**: $(RCO)_2O$, DMAP, pyridine, rt, 84–92%; for **B4**–**B6**: RCOOH, DCC, DMAP, CH_2Cl_2, rt, 90–93%.



Scheme 2. Reagents and conditions: (a) *m*CPBA (1.2 equiv), CH_2Cl_2, rt, 75%; (b) for **D1**–**D5**: $(RCO)_2O$, DMAP, pyridine, rt, 81–90%; for **D4**, **D5**: RCOOH, DCC, DMAP, CH_2Cl_2, rt, 90–93%; (c) *m*CPBA (2.5 equiv), CH_2Cl_2, rt, 89%; (d) for **B1**: $(CH_3CO)_2O$, DMAP, pyridine, rt, 88%; for **B4**, **B5**: RCOOH, DCC, DMAP, CH_2Cl_2, rt, 93%; (e) *m*CPBA (2.5 equiv), CH_2Cl_2, rt, 55–82%.



Scheme 3. Reagents and conditions: (a) $C_2H_5OCH_2COOH$, DCC, DMAP, CH_2Cl_2, rt, 24–80%.

The subseries of triacylated compounds **B1**–**B6** were linked with different ester chains at C-2, C-3, and C-16 of compound **1**. As shown in Table 1, these compounds were non-cytotoxic, which revealed that the acylation of hydroxyl groups at C-2, C-3, C-16 decreased cytotoxicity, exhibited inhibitory activity on HBV DNA replication with IC_{50} values of 17.3 μM (SI >46.4), 19.0 μM (SI >34.2), 114.9 μM (>4.5), 10.0 μM (>55.8), 51.2 μM (>14.0), but inactive to the secretion of HBsAg and HBeAg, except for compound **B3** which

Table 1
Anti-HBV activity, cytotoxicity, and selectivity index of compounds **1**, **2** and **A**–**H**

No.	CC_{50}[a] (μM)	HBsAg		HBeAg		HBV DNA	
		IC_{50}[b] (μM)	SI[c]	IC_{50} (μM)	SI	IC_{50} (μM)	SI
1	66.2	>66.2	<1.0	>66.2	<1.0	11.4	5.8
A1	253.5	189.5	1.3	>253.5	<1.0	2.8	89.7
A2	546.2	22.4	24.3	46.7	11.7	9.8	55.6
A3	126.0	15.6	8.1	14.4	8.7	4.7	77.8
A4	100.3	18.4	5.5	17.0	5.9	3.1	31.9
A5	>596.4	9.9	>60.0	596.4	>1.0	7.2	>83.4
A6	12.4	>12.4	<1.0	>12.4	<1.0	6.1	2.0
A7	6.3	>6.3	<1.0	>6.3	<1.0	4.7	1.3
B1	>807.1	>807.1	—	>807.1	—	17.3	>46.6
B2	>649.9	>649.9	—	>649.9	—	19.0	>34.2
B3	>608.0	18.0	>33.8	>608.0	—	>608.0	—
B4	>522.4	>522.4	—	>522.4	—	114.9	>4.5
B5	>560.3	>560.3	—	>560.3	—	10.0	>55.8
B6	>718.9	>718.9	—	>718.9	—	51.2	>14.0
C	>1745.0	>1745.0	—	>1745.0	—	11.6	>150.5
D1	>960.9	>960.9	—	>960.9	—	>960.9	—
D2	>1667.0	768	>2.2	>1667.0	—	>1667.0	—
D3	>617.6	>617.6	—	401.3	>2.0	>617.6	—
D4	>1111.3	>1111.3	—	591.7	>1.9	>1111.3	—
D5	>633.3	>633.3	—	>633.3	—	>633.3	—
E	366.5	24.0	15.3	30.9	11.9	7.2	50.9
F1	>1047.1	178.0	>5.9	327.2	>3.2	>1047.1	—
F2	>1744.8	>1744.8	—	>1744.8	—	>1744.8	—
F3	>710.4	>710.4	—	>710	—	>710.4	—
2	60.6	>60.6	<1.0	>60.6	<1.0	31.4	1.9
G	184.5	101.3	1.8	>184.5	<1.0	84.6	2.2
H	>1452.1	225.4	>6.4	>1452.1	—	81.4	>17.8
Tenofovir[d]	>1740.0	1572.1	>1.1	1160.2	>1.5	0.49	>3571.4

[a] CC_{50}: 50% cytotoxic concentration.
[b] IC_{50}: 50% inhibitory concentration.
[c] SI (selectivity index) = CC_{50}/IC_{50}.
[d] Tenofovir: an antiviral agent used as positive control.

lost the activity against HBV DNA replication but showed suppressant potency against the secretion of HBsAg (IC_{50} = 18.0 μM, SI >33.8).

Compound **C** derived from epoxidation of compound **1** (IC_{50} = 11.4 μM) showed obviously decreased cytotoxicity, and similar activity against HBV DNA replication (IC_{50} = 11.6 μM), leading to a high SI value (>150.5). This suggested that the epoxy group was an important feature in conferring low cytotoxicity. Unfortunately, triacylated derivatives **D1**–**D5** lost anti-HBV activity. After opening the epoxide ring, compound **E** possessed inhibitory potency to the secretion of HBsAg (IC_{50} = 24.0 μM, SI >15.3), HBeAg (IC_{50} = 30.9 μM, SI = 11.9) and the replication of HBV DNA (IC_{50} = 7.2 μM, SI = 50.9). Compounds **F1**–**F3** were non-cytotoxic but showed a significant reduction of anti-HBV activity.

The effects of different kinds of substituents to the C-25 were also studied. Deacetylation of compound **1** (IC_{50} = 11.4 μM) to corresponding compound **2** (IC_{50} = 31.4 μM) reduced the activity against HBV DNA replication. Compounds **G** (IC_{50} = 84.6 μM) and **H** (IC_{50} = 81.4 μM) showed a considerable reduction of activity against HBV DNA replication compared with compound **B5** (IC_{50} = 10.0 μM). These findings suggested that the presence of acetyl at C-25 played an important role in maintaining anti-HBV activity.

In summary, a series of derivatives were synthesized via chemical modifications on hydroxyl groups at C-2, C-3, C-16, C-5(6) double bond and C-25 position of hemslecin A and evaluated for their anti-HBV activity and cytotoxicities in vitro. Fifteen derivatives showed potent activity against HBV DNA replication. Ten compounds (**A1**–**A7**, **B5**, **C**, and **E**) showed significant activity against HBV DNA replication with IC_{50} values in the range of 2.8–11.6 μM. Among them, compounds **A1**–**A3**, **A5**, **B5**, and **C** had low cytotoxicity, resulting in high SI values of 89.7, 55.6, 77.8, >83.4, >55.8, and >150.5, respectively. Based on the above results, the following conclusions could be made: (a) Mono- or di-acylation of hydroxyl group(s) at C-2 or (and) C-3 resulted in enhancement of anti-HBV activity. (b) The acylation of hydroxyls at C-2, C-3, C-16 decreased cytotoxicity. (c) Epoxide of double bond at C-5(6) decreased the cytotoxicity and maintained activity against HBV DNA replication. (d) Acylation of the hydroxyl group at C-25 of hemslecin A was an important feature in conferring anti-HBV activity.

Acknowledgments

The work was supported by grants from the National Natural Science Foundation of China for Distinguished Young Scholars (No. 81025023). The authors are grateful to the staff of the analytical group of the State Key Laboratory of Phytochemistry and Plant Resources in West China, Kunming Institute of Botany, Chinese Academy of Sciences, for measurements of all spectra.

Supplementary data

Supplementary data associated with this article can be found, in the online version, at http://dx.doi.org/10.1016/j.bmcl.2013.01.024.

References and notes

1. Torres, C.; Leone, F. G. P. Y.; Pezzano, S. C.; Mbayed, V. A.; Campos, R. H. *Mol. Phylogenet. Evol.* **2011**, *59*, 114.
2. Qadri, I.; Fatima, K.; AbdeL-Hafiz, H. *BMC Microbiol.* **2011**, *11*, 48.
3. Mu, S. C.; Wang, G. M.; Jow, G. M.; Chen, B. F. *J. Med. Virol.* **2011**, *83*, 783.
4. Kumar, R.; Nath, M.; Tyrrell, D. L. *J. Med. Chem.* **2002**, *45*, 2032.
5. Ocama, P.; Opio, C. K.; Lee, W. M. *Am. J. Med.* **2005**, *118*, 1413.e15.
6. Ayoub, W. S.; Keeffe, E. B. *Aliment. Pharmacol. Ther.* **2008**, *28*, 167.
7. Tipples, G. A.; Ma, M. M.; Fischer, K. P.; Bain, V. G.; Kneteman, N. M.; Tyrrell, D. L. J. *Hepatology* **1996**, *24*, 714.

8. Wong, D. K.; Cheung, A. M.; O'Rourke, K.; Naylor, C. D.; Detsky, A. S.; Heathcote, J. *Ann. Intern. Med.* **1993**, *119*, 312.
9. Yan, M. H.; Cheng, P.; Jiang, Z. Y.; Ma, Y. B.; Zhang, X. M.; Zhang, F. X.; Yang, L. M.; Zheng, Y. T.; Chen, J. J. *J. Nat. Prod.* **2008**, *71*, 760.
10. Cheng, P.; Ma, Y. B.; Yao, S. Y.; Zhang, Q.; Wang, E. J.; Yan, M. H.; Zhang, X. M.; Zhang, F. X.; Chen, J. J. *Bioorg. Med. Chem. Lett.* **2007**, *17*, 5316.
11. Zhang, Q.; Jiang, Z. Y.; Luo, J.; Cheng, P.; Ma, Y. B.; Zhang, X. M.; Zhang, F. X.; Zhou, J.; Chen, J. J. *Bioorg. Med. Chem. Lett.* **2008**, *18*, 4647.
12. Zhang, Q.; Jiang, Z. Y.; Luo, J.; Liu, J. F.; Ma, Y. B.; Guo, R. H.; Zhang, X. M.; Zhou, J.; Chen, J. J. *Bioorg. Med. Chem. Lett.* **2009**, *19*, 2148.
13. Zhang, Q.; Jiang, Z. Y.; Luo, J.; Ma, Y. B.; Liu, J. F.; Guo, R. H.; Zhang, X. M.; Zhou, J.; Niu, W.; Du, F. F.; Li, L.; Li, C.; Chen, J. J. *Bioorg. Med. Chem. Lett.* **2009**, *19*, 6659.
14. Geng, C. A.; Jiang, Z. Y.; Ma, Y. B.; Luo, J.; Zhang, X. M.; Wang, H. L.; Shen, Y.; Zuo, A. X.; Zhou, J.; Chen, J. J. *Org. Lett.* **2009**, *11*, 4120.
15. Geng, C. A.; Wang, L. J.; Zhang, X. M.; Ma, Y. B.; Huang, X. Y.; Luo, J.; Guo, R. H.; Zhou, J.; Shen, Y.; Zuo, A. X.; Jiang, Z. Y.; Chen, J. J. *Chem. Eur. J.* **2011**, *17*, 3893.
16. Ma, W. H.; Huang, H.; Zhou, P.; Chen, D. F. *J. Nat. Prod.* **2009**, *72*, 676.
17. Ma, W. H.; Lu, Y.; Huang, H.; Zhou, P.; Chen, D. F. *Bioorg. Med. Chem. Lett.* **2009**, *19*, 4958.
18. Yeo, H.; Li, Y.; Fu, L.; Zhu, J. L.; Gullen, E. A.; Dutschman, G. E.; Lee, Y.; Chung, R.; Huang, E. S.; Austin, D. J.; Cheng, Y. C. *J. Med. Chem.* **2005**, *48*, 534.
19. Nie, R. L.; Chen, B. L. *Acta Bot. Yunnan.* **1986**, *8*, 115.
20. Tian, R. R.; Chen, J. C.; Zhang, G. H.; Qiu, M. H.; Wang, Y. H.; Du, L.; Shen, X.; Liu, N. F.; Zheng, Y. T. *Chin. J. Nat. Med.* **2008**, *6*, 214.