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Anti-HBV agents. Part 2: Synthesis and in vitro anti-hepatitis B virus activities of alisol A derivatives

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ABSTRACT

Chemical modifications were performed on hydroxyl groups at C-11,23,24,25 positions and C-13(17) double bond of alisol A for structure–activity relationship study. Forty-one derivatives of alisol A were synthesized and assayed for their in vitro anti-hepatitis B virus (HBV) activities and cytotoxicities. Of them, 14 compounds were active against HBV surface antigen (HBsAg) and HBV e antigen (HBeAg) secretion in HepG 2.2.15 cells, and the most promising compound **25** exhibited high activities against secretion of HBsAg (IC_{50} = 0.028 mM), HBeAg (IC_{50} = 0.027 mM) and remarkable selective indices (SI_{HBsAg} >90, SI_{HBeAg} >93).

Various efforts are currently underway to develop therapeutic agents to arrest the replication of the hepatitis B virus (HBV). Although several nucleoside inhibitors (i.e., lamivudine, adefovir dipivoxil, entecavir, telbivudine) have been approved by the FDA and used clinically, adverse side effects and development of drug resistant virus have been reported.[1] Therefore, compounds possessing potent anti-HBV activity with novel modes of action are urgently needed to add to the existing anti-HBV therapies. Nowadays, the development of new anti-HBV agents is focused on discovering diverse compounds with either novel structures or a new mechanism of action. So far, non-nucleosides have also been reported to inhibit HBV infection.[2–11] Nevertheless, the search for compounds with novel anti-HBV target and mechanism is still needed.

Discovery of novel plant-derived natural products as potential new lead compounds for anti-HBV agents as well as the modification of these new lead compounds is continuing goals of our laboratory. Previously, we first reported protostane-type triterpenes possess anti-HBV activities.[12] Our efforts to improve the anti-HBV activity of alisol A (**1**, Fig. 1), a naturally occurring protostane-type triterpene, led us to discover 11,23,24-tri-*O*-acetyl-25-anhydroalisol A (**2**) and its related compounds [for example, 11,23,24-tri-*O*-acetyl-13β,17β-epoxy-25-anhydroalisol A (**3**)] with high activities against secretion of HBsAg and HBeAg, and our

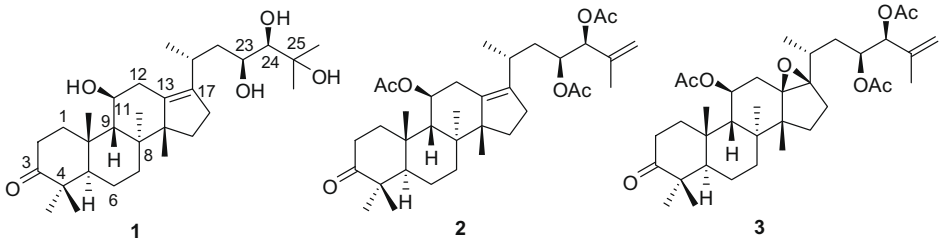

Figure 1. Structures of compounds **1**–**3**.

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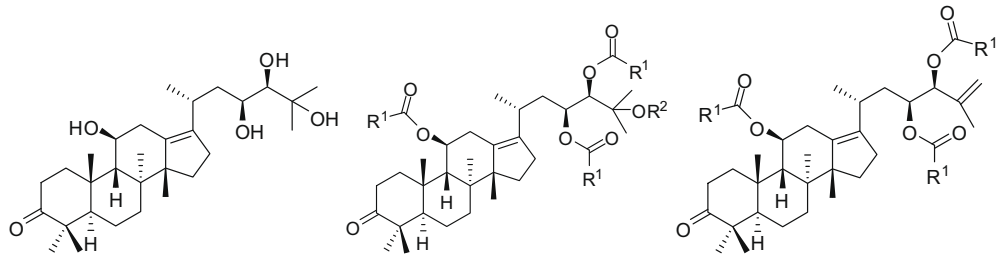
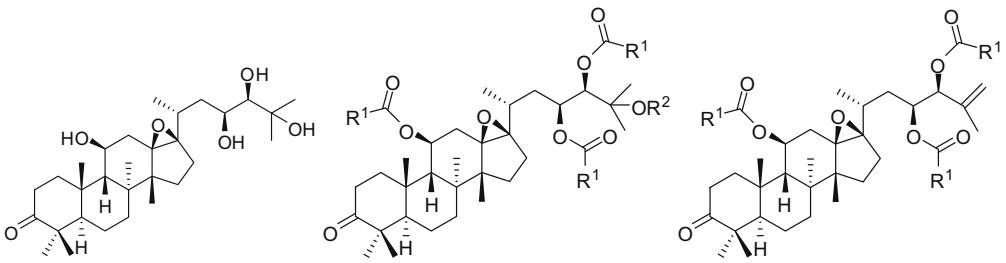
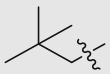
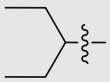
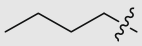
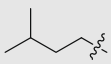
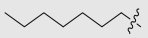
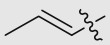
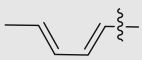
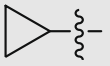
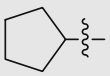
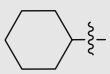
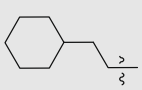
Table 1
Structures, anti-HBV activity, cytotoxicity and selectivity index of compounds **1–45** [a]



1 **4–31** **2, 32–36**



37 **3, 38–42** **43–45**

	Compounds		CC_{50}[b] (mM)	HBsAg[c]		HBeAg[d]	
	R^1	R^2		IC_{50}[e] (mM)	SI[f]	IC_{50}(mM)	SI
1[g]	/	/	0.062	0.039	1.6	>2.4	<0.030
2[g]	Me	/	0.52	0.028	18	0.029	18
3[g]	Me	/	>2.6	0.024	>108	0.028	>93
4		H	>1.2	>1.2	–	>1.2	–
5		H	>1.2	>1.2	–	>1.2	–
6		H	>1.1	>1.1	–	>1.1	–
7		H	>1.3	>1.3	–	>1.3	–
8		H	>0.90	>0.90	–	>0.90	–
9		H	>0.6	>0.6	–	>0.6	–
10		H	>0.85	>0.85	–	>0.85	–
11		H	>0.90	>0.90	–	>0.90	–
12		H	>1.0	>1.0	–	>1.0	–
13		H	>1.1	>1.1	–	>1.1	–
14		H	>0.8	>0.80	–	>0.8	–
15		H	>0.81	>0.81	–	>0.81	–

(*continued on next page*)

Table 1 (*continued*)

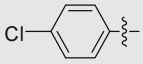
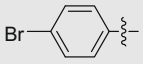
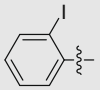
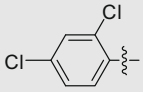
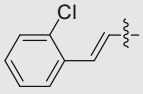
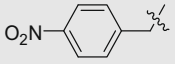
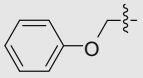
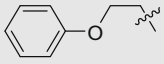
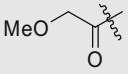
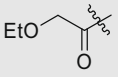
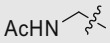
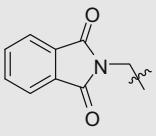
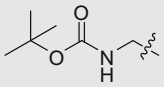
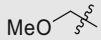
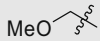
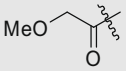
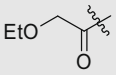
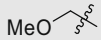
	Compounds		CC_{50}[b] (mM)	HBsAg[c]		HBeAg[d]	
	R^1	R^2		IC_{50}[e] (mM)	SI[f]	IC_{50}(mM)	SI
16		H	>0.90	>0.90	—	>0.9	—
17		H	0.65	>0.65	<1.0	>0.65	<1.0
18		H	>1.5	>1.5	—	>1.5	—
19		H	>1.2	>1.2	—	>1.2	—
20		H	>1.3	0.95	>1.3	>1.3	—
21		H	>1.0	>1.0	—	>1.0	—
22		H	>1.3	>1.3	—	>1.3	—
23		H	>0.95	>0.95	—	>0.95	—
24		H	0.40	0.14	2.9	>0.4	—
25			>2.5	0.028	>90	0.027	>93
26		H	>2.6	0.40	>6.5	2.0	>1.3
27			>2.3	0.20	>11	0.26	>8.7
28		H	0.051	<0.038	>1.3	>0.051	<1.0
29		H	0.27	0.29	<1.0	0.88	<1.0
30		H	>1.5	1.2	>1.2	0.14	>11
31		H	>1.5	0.32	>4.7	0.094	>16
32		/	>1.2	>1.2	—	>1.2	—
33		/	>0.92	>0.92	—	>0.92	—
34		/	>2.8	0.068	>42	0.12	>24

Table 1 (*continued*)

	Compounds		CC_{50}[b] (mM)	HBsAg[c]		HBeAg[d]	
	R^1	R^2		IC_{50}[e] (mM)	SI[f]	IC_{50}(mM)	SI
35		/	>1.2	0.020	>60	0.03	>40
36		/	<0.032	<0.032	—	<0.032	—
37[g]	/	/	0.43	0.045	9.6	1.1	<1.0
38		H	1.1	0.90	1.2	>1.1	<1.0
39			>0.80	0.11	>7.3	>1.6	—
40		H	0.25	0.13	1.9	>0.25	<1.0
41			>1.4	0.25	>5.6	0.32	>4.4
42		H	0.25	0.12	2.1	>0.25	<1.0
43		/	>1.3	0.044	>29	>1.3	—
44		/	>1.8	0.020	>90	>1.8	—
45		/	>1.6	0.051	>31	>1.6	—
3TC[h]	/	/	31	18	1.7	38	0.82

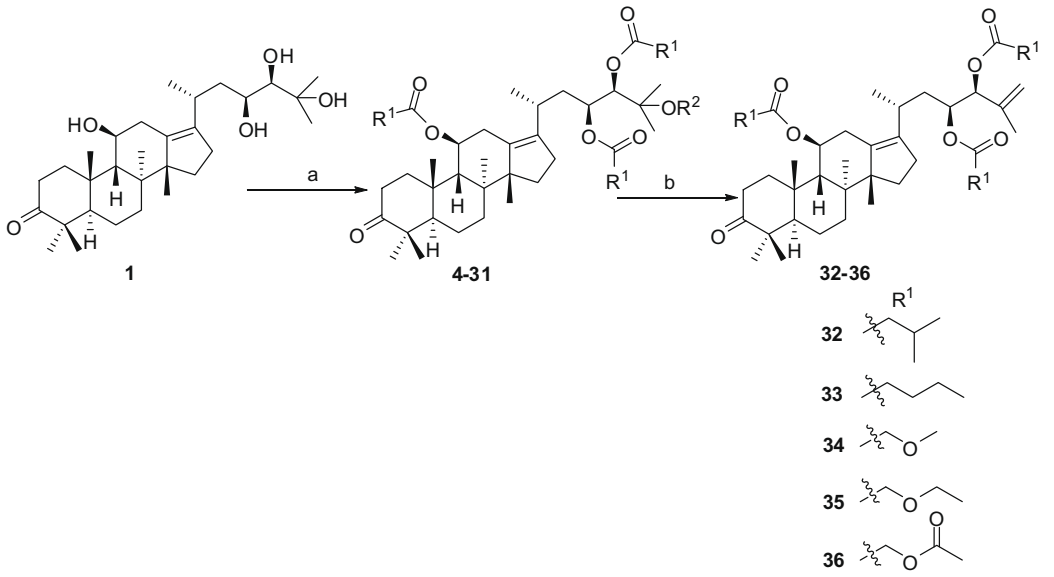
[a] All values are the mean of two independent experiments.
[b] CC_{50}: 50% cytotoxic concentration.
[c] HBsAg: HBV surface antigen.
[d] HBeAg: HBV e antigen.
[e] IC_{50}: 50% effective concentration.
[f] SI (selective index) = CC_{50}/IC_{50}.
[g] Data from Ref. 13.
[h] 3TC: Lamivudine, an antiviral agent used as positive control.

investigations revealed two important structure–activity relationships (SARs). Both acylation of hydroxyl groups at 11,23,24-position and epoxidation of C-13(17) double bond are important feature in the conferring relatively low cytotoxicity of compound **3**, and a double bond at C-25(26) is crucial for the high potency of compounds **2** and **3**,[13] which encouraged us to synthesize additional analogues. Herein, we report the synthesis, anti-HBV activity, and SARs of these protostane-type triterpenes in detail.

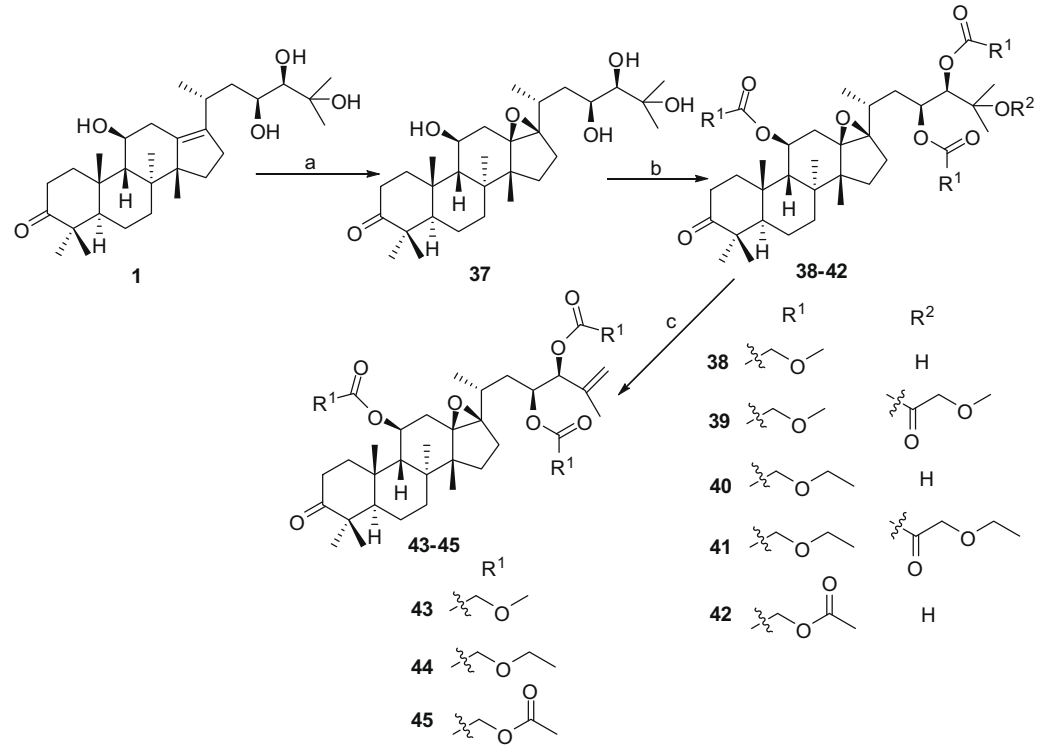
Previous study showed that acyl groups at C-11,23,24 hydroxyls of alisol A derivatives could decrease cytotoxicity.[13] A series of ester derivatives of compound **1** were synthesized to further evaluate the influence of ester side chains on the biological properties. Compound **1** was treated with carboxylic acids in the presence of *N*′,*N*′-dicyclohexylcarbodiimide (DCC) and 4-dimethylaminopyridine (DMAP) to afford acyl derivatives **4–31** (Table 1). Compounds **4, 7, 24, 26, 28** were subjected to dehydration by refluxing with Lawesson's reagent, commonly used in organic synthesis as a thiation agent,[14–16] in toluene to give compounds **32–36** in good yields without affecting the 3-carbonyl (Scheme 1).[13] Epoxidation of compound **1** with *m*-chloroperoxybenzoic acid (*m*CPBA) in CH_2Cl_2 at room temperature gave compound **37**. Treatment of compound **37** with carboxylic acids in the presence of DCC and DMAP afforded compounds **38–42**. Compounds **38**, **40**, **42** were further converted to corresponding dehydrated compounds **43–45** by refluxing with Lawesson's reagent in toluene in good yields (Scheme 2).

The synthesized alisol A derivatives were tested for their cytotoxicities and potential anti-HBV activities, namely the abilities to inhibit the secretion of HBV surface antigen (HBsAg) and HBV e antigen (HBeAg) in HBV-infected 2.2.15 cells using lamivudine (3TC, a clinically popular anti-HBV agent) as a positive control.[17] The anti-HBV activity of each compound was expressed as the concentration of compound that achieved 50% inhibition (IC_{50}) to the secretion of HBsAg and HBeAg. The cytotoxicity of each compound was expressed as the concentration of compound required to kill 50% (CC_{50}) of HepG 2.2.15 cells. The selectivity index (SI), a major pharmaceutical parameter that estimates possible future clinical development, was determined as the ratio of CC_{50} to IC_{50}. The bioactivity of each compound was evaluated by the combination of its IC_{50} and SI. The results were summarized in Table 1.

The parent compound **1** showed inhibitory potency to the secretion of HBsAg (IC_{50} = 0.039 mM), but appeared toxic (CC_{50} = 0.062 mM), which led to a relative low SI (1.6). The subseries of derivatives **4–27** had different patterns of substitution on C-11, C-23, C-24 and C-25. As shown in Table 1, these compounds were non-cytotoxic, which revealed that the acylation of hydroxyl groups at 11,23,24-position decreased cytotoxicity, and



Scheme 1. Reagents and conditions: (a) R^1COOH, DCC, DMAP, CH_2Cl_2, rt, 54–95%; (b) Lawesson's reagent, toluene, reflux, 63–83%.



Scheme 2. Reagents and conditions: (a) *m*CPBA, CH_2Cl_2, rt, 88%; (b) R^1COOH, DCC, DMAP, CH_2Cl_2, rt, 66%–82%; (c) Lawesson's reagent, toluene, reflux, 72–84%.

most of these compounds showed a significant reduction of anti-HBV activity, except that compounds **24** (IC_{50} = 0.14 mM, SI = 2.9) and **26** (IC_{50} = 0.40 mM, SI >6.5) exhibited moderate activity against the secretion of HBsAg suggesting that short straight chains with 2'-oxygen atom at C-11, C-23 and C-24 groups appeared to improve inhibitory activities against the secretion of HBsAg compared to saturated carbon side chains (**7** vs **26**). An important advance was obtained when the C-25 hydroxyl groups of compounds **24** and **26** were acylated with corresponding carboxylic acids. The tetra-methoxyacetyl derivative **25** and the tetra-ethoxyacetyl analogue **27** showed more highly potent activities against secretion of HBsAg (IC_{50} = 0.028 mM, 0.20 mM, respectively), HBeAg (IC_{50} = 0.028 mM, 0.26 mM, respectively) and much higher SI values (SI_{HBsAg} >90, SI_{HBeAg} >93; SI_{HBsAg} >11, SI_{HBeAg} >8.7, respectively) than those of corresponding triacyl derivatives (**24** vs **25**, **26** vs **27**). Compound **25** was more potent against the secretion of HBsAg and HBeAg than compound **27**, which indicated that the longer terminal alkyl chain reduced anti-HBV activity. To our surprise, compounds **39** and **41** obtained by the epoxidation from compounds **25** and **27**, respectively, showed a significant decrease in inhibition to the secretion of HBsAg and HBeAg.

To further explore the influence of side chains with 2'-heteroatom at C-11, C-23 and C-24 on anti-HBV activity. Compounds **28**–**31** were synthesized and evaluated for their anti-HBV activities and cytotoxicities. Compound **28** showed high activity against the secretion of HBsAg (IC_{50} <0.038 mM), whereas an increase in cytotoxicity was also noted (CC_{50} = 0.051 mM). As reported previously, epoxidation of C-13(17) double bond decrease cell cytotoxicity.[13] Compound **28** was converted to compound **42** which showed lower cell cytotoxicity (CC_{50} = 0.25 mM). However, activity against the secretion of HBsAg also decreased (IC_{50} = 0.12 mM, SI = 2.1). For 2'-*N*-substituted derivatives **29**–**31**, compounds **30** and **31** exhibited highly inhibitory potency to the secretion of HBeAg (IC_{50} = 0.14 mM, SI >11; IC_{50} = 0.094 mM, SI >16, respectively). But unfortunately, both compounds showed low activity against the secretion of HBsAg.

Among dehydrated compounds **2** and **32**–**36**, compound **2** showed IC_{50} of 0.028 and 0.029 mM on HBsAg and HBeAg secretion, respectively, which led to high SI values (SI_{HBsAg} = 18, SI_{HBeAg} = 18). Replacement of the acetyl moieties with more bulky substituents (**32**, **33**) led to loss of anti-HBV activity. Interestingly, potent inhibitory activities against the secretion of HBsAg and HBeAg were found to be retained with alkyloxyacetyl (or acetoxyacetyl) derivatives (**34**–**36**). The results indicated that chain length may not be the only one factor for potent anti-HBV activity. Compounds **43–45** that resulted from dehydration of compounds **38, 40**, **42** showed low cell cytotoxicities, and their IC_{50} values on inhibition of HBsAg secretion were 0.044, 0.020, and 0.051 mM, respectively (SI >29, >90, >31, respectively). Noteworthy is that an epoxide functionality at C-13(17) of compounds **43–45** caused the loss of suppressant property on the secretion of HBeAg.

In summary, a series of new alisol A analogues were synthesized and examined for their in vitro anti-HBV activities and cytotoxicities and 14 tested compounds were active against HBV in HepG 2.2.15 cells. Based on the above structure and activity relationship results, the following conclusion can be drawn: (1) the present investigation indicates that the double bond at C-25(26) of alisol A analogues is crucial for potent anti-HBV activity; (2) for dehydrated derivatives, the anti-HBV activity largely depends on the size and character of the substituents on the 11,23,24-ester moieties. As the alkyl chain is lengthened, anti-HBV activity of the derivatives decreased. However, chain length may not be the only one factor, because the alkyloxyacetyl (or acetoxyacetyl) derivatives (**34**–**36** and **43**–**45**) exhibited high potent anti-HBV activity; (3) acylation of the hydroxyl at C-25 of alisol A is important for the high potency of compounds **25** and **27;** (4) the role of epoxide functionality at C-13(17) of alisol A derivatives is ambiguous. In some analogues, epoxide group retains the inhibitory potency to the secretion of HBsAg and HBeAg: compounds **2** and **3** show similar activity. For other analogues, epoxide functionality retains the inhibitory potency to the secretion of HBsAg, but causes the loss of suppressant property on the secretion of HBeAg: compounds **43–45** show high activity against the secretion of HBsAg, but can not inhibit the secretion of HBeAg. Finally, epoxide group leads to the decrease of suppressant property on the secretion of HBsAg and HBeAg: compounds **39** and **41** are less potent than compounds **25** and **27**, respectively.

Based on these structure–activity relationships, further optimization and biological evaluation on protostane-type triterpene compounds as promising HBV inhibitors are ongoing in our laboratory and the results will be reported in due course.

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Supplementary data

Supplementary data associated with this article can be found, in the online version, at doi:10.1016/j.bmcl.2009.02.122.

References and notes

1. Lim, Y. S.; Suh, D. J. *J. Korean Med. Sci.* **2004**, *19*, 489.
2. Perni, R. B.; Conway, S. C.; Ladner, S. K.; Zaifert, K.; Otto, M. J.; King, R. W. *Bioorg. Med. Chem. Lett.* **2000**, *10*, 2687.
3. Lee, J. S.; Shim, H. S.; Park, Y. K.; Park, S. J.; Shin, J. S.; Yang, W. Y.; Lee, H. D.; Park, W. J.; Chung, Y. H.; Lee, S. W. *Bioorg. Med. Chem. Lett.* **2002**, *12*, 2715.
4. Deres, K.; Schroder, C. H.; Paessens, A.; Goldmann, S.; Hacker, H. J.; Weber, O.; Kramer, T.; Niewohner, U.; Pleiss, U.; Stoltefuss, J. *Science* **2003**, *299*, 893.
5. 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.
6. Li, Y.; Fu, L.; Yeo, H.; Zhu, J. L.; Chou, C. K.; Kou, Y. H.; Yeh, S. F.; Gullen, E.; Austin, D.; Cheng, Y. C. *Antiviral Chem. Chemother.* **2005**, *16*, 193.
7. Cheng, Y. C.; Ying, C. X.; Leung, C. H.; Li, Y. *J. Clin. Virol.* **2005**, *34*, S147.
8. Ding, P. L.; Liao, Z. X.; Huang, H.; Zhou, P.; Chen, D. F. *Bioorg. Med. Chem. Lett.* **2006**, *16*, 1231.
9. Zhao, W. G.; Wang, J. G.; Li, Z. M.; Yang, Z. *Bioorg. Med. Chem. Lett.* **2006**, *16*, 6107.
10. Ying, C. X.; Li, Y.; Leung, C. H.; Robek, M. D.; Cheng, Y. C. *Proc. Natl. Acad. Sci. U.S.A.* **2007**, *104*, 8526.
11. Cheng, P.; Zhang, Q.; Ma, Y. B.; Jiang, Z. Y.; Zhang, X. M.; Zhang, F. X.; Chen, J. J. *Bioorg. Med. Chem. Lett.* **2008**, *18*, 3787.
12. Jiang, Z. Y.; Zhang, X. M.; Zhang, F. X.; Liu, N.; Zhao, F.; Zhou, J.; Chen, J. J. *Planta Med.* **2006**, *72*, 951.
13. 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.
14. Cherkasov, R. A.; Kutyrev, G. A.; Pudovik, A. N. *Tetrahedron* **1985**, *41*, 2567.
15. Jesberger, M.; Davis, T. P.; Barner, L. *Synthesis* **2003**, *13*, 1929.
16. Cava, M. P.; Levinson, M. I. *Tetrahedron* **1985**, *41*, 5061.
17. 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**, *19*, 5316.