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Anti-HBV agents. Part 3: Preliminary structure–activity relationships of tetra-acylalisol A derivatives as potent hepatitis B virus inhibitors

Quan Zhang [a], Zhi-Yong Jiang [a], Jie Luo [a], Yun-Bao Ma [a], Ji-Feng Liu [a], Rui-Hua Guo [a], Xue-Mei Zhang [a], Jun Zhou [a], Wei Niu [b], Fei-Fei Du [b], Li Li [b], Chuan Li [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] Shanghai Center for DMPK Research, Shanghai Institute of Materia Medica, Chinese Academy of Sciences, Shanghai 201203, PR China

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ABSTRACT

Thirty-two tetra-acylated derivatives of alisol A were synthesized and evaluated for their anti-hepatitis B virus (HBV) activities and cytotoxicities in vitro. Among the series of alisol A derivatives examined, five analogues were active against HBV surface antigen (HBsAg) and HBV e antigen (HBeAg) secretion in HepG 2.2.15 cells. These results also provide interesting structure–activity relationships of tetra-acylalisol A derivatives. Compounds tetra-acetyl alisol A (**A1**), tetra-methoxyacetyl alisol A (**A23**), and tetra-ethoxyacetyl alisol A (**A24**) exhibited high activities against secretion of HBsAg with IC_{50} values of 0.0048, 0.0044, and 0.014 mM, respectively, HBeAg with IC_{50} values of 0.011, 0.012, and 0.018 mM, respectively, and remarkable selective index values SI_{HBsAg} >333, SI_{HBeAg} >145; SI_{HBsAg} = 209, SI_{HBeAg} = 77; and SI_{HBsAg} >200, SI_{HBeAg} >156, respectively. Additional studies in rats showed that compound **A1** has favorable pharmacokinetic prosperities for further development purpose, with elimination half-time ($t_{1/2}$) of 1.63 h and oral bioavailability (F) of 40.9%.

Hepatitis B virus (HBV), a member of the hepadnavirus (hepatotropic DNA viruses) family, causes acute and chronic infection of the liver.[1] HBV infection is the world's ninth leading cause of death.[2] Chronic HBV infection can lead to cirrhosis, liver failure, and hepatocellular carcinoma. An estimated 350–400 million people are chronically infected by HBV throughout the world with 0.5–1.2 million global deaths per year.[3,4] Rates of new HBV infections in developing countries continue to climb at an alarming rate. Currently, only two interferons and five nucleoside inhibitors (lamivudine, adefovir dipivoxil, entecavir, telbivudine, tenofovir disoproxil fumarate) have received FDA approval as single-agent treatments for HBV infection. Unfortunately, clinical response rates to interferon-α and peginterferon-α tend to be low (20–30%).[5] On the other hand, the required prolonged regimen of nucleoside analogues almost invariably leads to drug-resistance problems.[6–9] Although the combination therapy of lamivudine with adefovir dipivoxil, or antiviral drugs together with immunomodulators such as interferon-α might be more effective and safer in the therapeutic process,[10] it is still limited.[11] Therefore, there is an urgent need for the development of novel classes of anti-HBV agents with new structures and mechanisms of action.

Triterpenoids are natural products with 30 carbon atoms, biosynthetically derived from the cyclization of squalene. Many triterpenoids are reported to have various interesting biological, pharmacological, and medicinal activities including antiviral activity.[12–22] As a part of our continuous search for active anti-HBV leads from natural sources and synthetic compound,[23–25] we first reported that protostane-type triterpenes exhibit significant in vitro antiviral activity against HBV.[26] With alisol A (**1**, Fig. 1), a naturally occurring protostane-type triterpene, as the starting point, we began bioassay-directed design and synthesized a series of alisol A analogues,[27,28] which led us to discover tetra-acylated alisol A derivatives 11,23,24,25-tetra-*O*-2′-methoxyacetylalisol A (**2**) and 11,23,24,25-tetra-*O*-2′-ethoxyacetylalisol A (**3**) with high inhibition activity against the secretion of HBV surface antigen (HBsAg) and HBV e antigen (HBeAg), and revealed that acylation of C-25 hydroxyl enhanced anti-HBV potency.[28] As a continuation of this work, we synthesized a series of tetra-acylated alisol A analogues and evaluated their HBV inhibitory activities in HepG 2.2.15 cells. The preliminary structure–activity relationships of these protostane-type triterpenes were also discussed.

The syntheses of alisol A derivatives are summarized in Scheme 1 and Scheme 2. Treatment of compound **1** with an excess of acetic anhydride and a catalytic amount of 4-dimethylaminopyridine (DMAP) in triethylamine afforded ester **A1** (78%) as the major product, together with compound **D** (6%) as a minor product. Compound **1** was converted to tetra-acylated derivatives **A2**–**A21** (Table 1) by refluxing with carboxylic acids in the presence of *N*′,*N*′-dicyclohexylcarbodiimide (DCC) and 4-dimethylaminopyridine (DMAP) in CH_2Cl_2. Compound **A22** was obtained by treatment of compound **1**

* Corresponding author. Tel./fax: + 86 871 5223265.
E-mail address: chenjj@mail.kib.ac.cn (J.-J. Chen).

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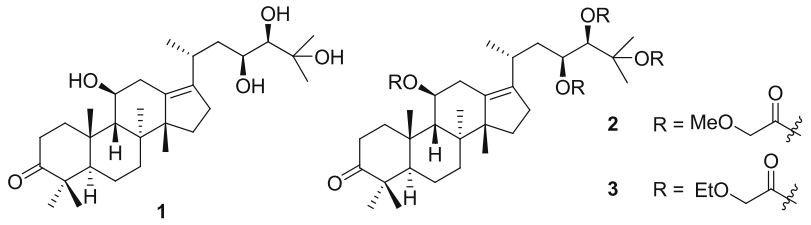
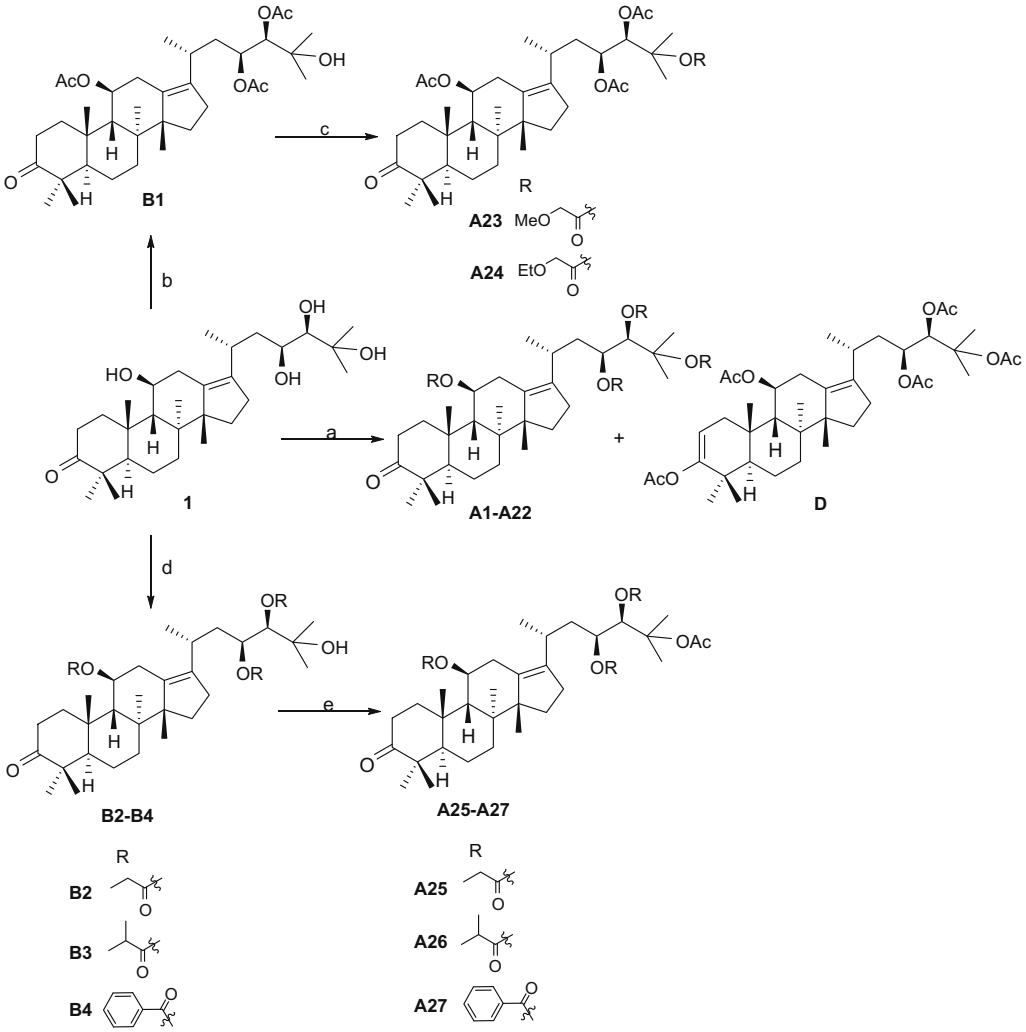


Figure 1. Structures of compounds **1**–**3**

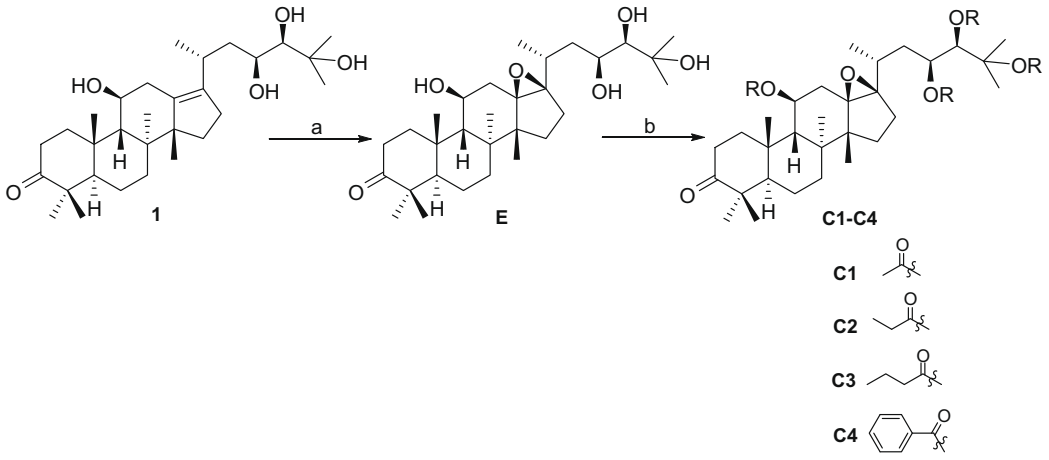
with chlorotrimethylsilane. Acetylation of compound **1** with acetic anhydride afforded tri-acetyl derivative **B1**, which was further converted to compounds **A23, A24** by reaction of compound **B1** with appropriate carboxylic acids in the presence of DCC and DMAP. Similarly, compounds **A25**–**A27** were synthesized via tri-acyl analogues **B2**–**B4** from compound **1**. Epoxidation of compound **1** with *m*-chloroperoxybenzoic acid (*m*CPBA) in CH_2Cl_2 at room temperature gave compound **E**. Treatment of compound **E** with acetic anhydride or with carboxylic acids in the presence of DCC and DMAP afforded compounds **C1**–**C4**.

The synthesized alisol A analogues **A1**–**A27**, **C1**–**C4**, and **D** were evaluated for their cytotoxicities and potential anti-HBV activities, namely the abilities to inhibit the secretion of HBsAg and HBeAg in HepG 2.2.15 cells using lamivudine (3TC, a clinically popular anti-HBV agent) as a positive control. The evaluated compounds and previously assayed compounds **1**–**3**, **B1**–**B4**, and **E** are divided into five categories: tetra-acylated derivatives (**A**); tri-acylated derivatives (**B**); combination of epoxided at C-13(17) and tetra-acylated derivatives (**C**); combination of A-ring modified and tetra-acylated derivatives (**D**); epoxided at C-13(17) derivatives (**E**). Among the tested analogues, five analogues exhibited inhibitory activity against the secretion of HBsAg and HBeAg (Table 1).

As previously assayed, the parent compound **1** showed inhibitory potency to the secretion of HBsAg (IC_{50} = 0.039 mM),

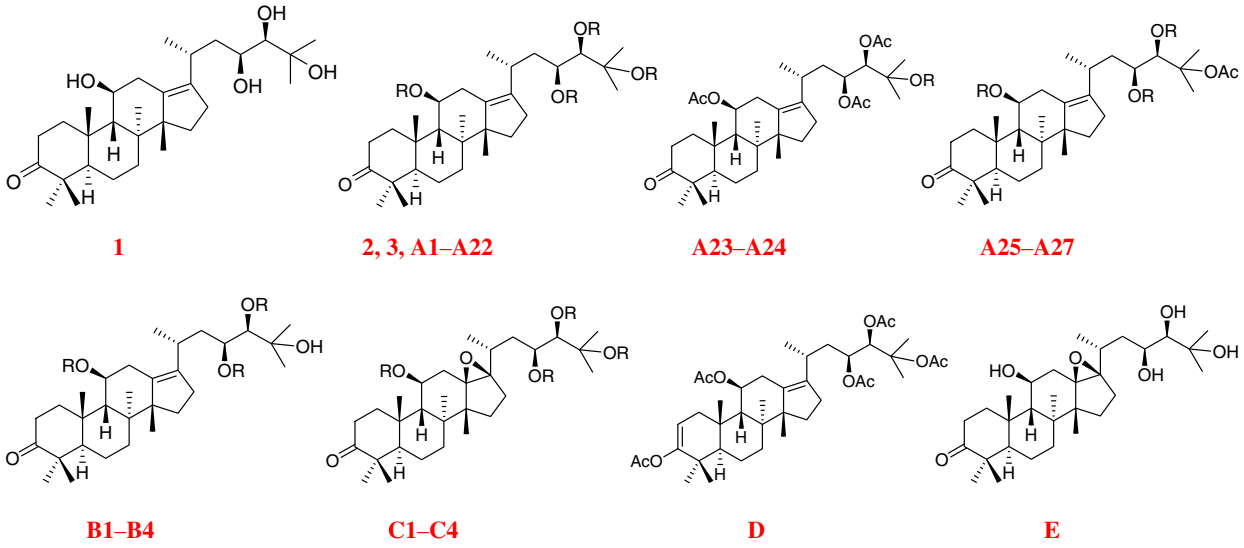


Scheme 1. Reagents and conditions: (a) for **A1** and **D**: $(CH_3CO)_2O$, DMAP, triethylamine, rt, 78% (**A1**), 6% (**D**), for **A2–A21**: RCOOH, DCC, DMAP, CH_2Cl_2, reflux, 56–92%; for **A22**: Me_3SiCl, Et_3N, CH_2Cl_2, rt, 92%; (b) $(CH_3CO)_2O$, pyridine, rt, 86%; (c) RCOOH, DCC, DMAP, CH_2Cl_2, rt, for **A23**: 92%, for **A24**: 87%; (d) RCOOH, DCC, DMAP, CH_2Cl_2, rt, 68–83%; (e) $(CH_3CO)_2O$, DMAP, triethylamine, rt, 55–82%.



Scheme 2. Reagents and conditions: (a) *m*CPBA, CH_2Cl_2, rt, 88%; (b) for **C1**: $(CH_3CO)_2O$, triethylamine, DMAP, rt, 72%, for **C2–C4**: R^1COOH, DCC, DMAP, CH_2Cl_2, reflux, 65–77%.

Table 1
Structures, anti-HBV activity, cytotoxicity and selectivity index of alisol A derivatives[a]



Compounds	R	CC50[b] (mM)	HBsAg[c]		HBeAg[d]	
			IC_{50}[e] (mM)	SI[f]	IC_{50} (mM)	SI
1[g]	/	0.062	0.039	1.6	>2.4	<0.030
2[h]	MeO	>2.5	0.028	>90	0.027	>93
3[h]	EtO	>2.3	0.20	>11	0.26	>8.7
A1[29]		>1.6	0.0048	>333	0.011	>145
A2		>1.9	0.90	>2.1	>1.9	–
A3		>1.8	>1.8	–	1.8	–
A4		>1.4	>1.4	–	>1.4	–

(*continued on next page*)

Table 1 (*continued*)

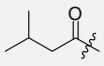
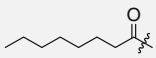
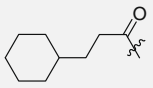
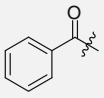
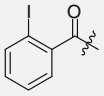
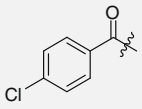
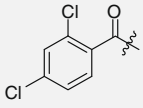
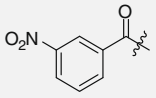
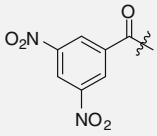
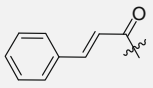
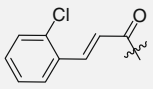
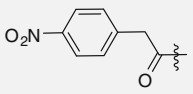
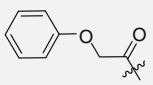
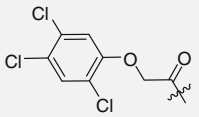
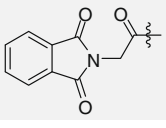
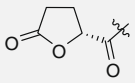
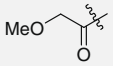
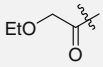
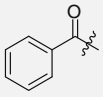
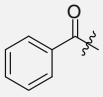
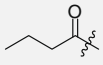
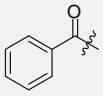
Compounds	R	CC50[b] (mM)	HBsAg[c]		HBeAg[d]	
			IC_{50}[e] (mM)	SI[f]	IC_{50} (mM)	SI
A5		>2.0	>2.0	—	>2.0	—
A6		>1.6	>1.6	—	>1.6	—
A7		>1.7	>1.7	—	>1.7	—
A8		>2.1	>2.1	—	>2.1	—
A9		>1.4	>1.4	—	>1.4	—
A10		>1.4	>1.4	—	>1.4	—
A11		>1.4	0.48	>2.9	>1.4	—
A12		>1.0	>1.0	—	>1.0	—
A13		>1.1	>1.1	—	>1.1	—
A14		>0.99	>0.99	—	>0.99	—
A15		>1.2	>1.2	—	>1.2	—
A16		>1.4	>1.4	—	>1.4	—
A17		>1.2	>1.2	—	>1.2	—
A18		>1.4	>1.4	—	>1.4	—
A19		>1.2	>1.2	—	>1.2	—
A20		>1.3	>1.3	—	>1.3	—

Table 1 (*continued*)

Compounds	R	CC50[b] (mM)	HBsAg[c]		HBeAg[d]	
			IC_{50}[e] (mM)	SI[f]	IC_{50} (mM)	SI
A21		>1.6	>1.6	—	>1.6	—
A22		0.051	0.020	2.5	>0.051	<1.0
A23		0.92	0.0044	209	0.012	77
A24		>2.8	0.014	>200	0.018	>156
A25		>1.7	0.46	>3.7	0.086	>20
A26		>1.9	>1.9	—	1.5	>1.3
A27		>1.7	>1.7	—	>1.7	—
B1[g]		>2.0	>2.0	—	>2.0	—
B2[g]		>1.4	>1.4	—	>1.4	—
B3[g]		>2.2	1.7	>1.3	0.9	>2.4
B4[g]		>1.5	>1.5	—	>1.5	—
C1		>4.0	0.046	>87	0.061	>66
C2		>2.2	>2.2	—	1.3	>1.7
C3		>1.9	>1.9	—	>1.9	—
C4		>1.7	>1.7	—	1.1	>1.5
D	/	>2.1	>2.1	—	0.28	>7.5
E[g]	/	0.43	0.045	9.6	1.1	0.39
3TC[i]	/	35	13	2.7	22	1.6

[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. 27.
[h] Data from Ref. 28.
[i] 3TC: lamivudine, an antiviral agent used as positive control.

but appeared toxic (CC_{50} = 0.062 mM), which led to a relative low selectivity index (1.6).[27] Initially, tetra-acetyl derivative **A1** was synthesized. Surprisingly, this modification resulted in an eightfold increase in inhibition to the secretion of HBsAg compared to compound **1** (IC_{50} = 0.0048 mM vs IC_{50} = 0.039 mM). Meanwhile it had lower cytotoxicity (CC_{50} >1.6 mM vs CC_{50} = 0.062 mM), resulting a higher selective index (SI >333 vs SI = 1.6). In addition, compound **A1** also showed highly inhibitory activity on HBeAg secretion with a SI value of >145 (IC_{50} = 0.011 mM). A-ring modified compound **D** (IC_{50} = 0.28 mM, SI >7.5) exhibited reduced inhibition effect on HBeAg secretion compared to compound **A1** and lost suppressant property on the secretion of HBsAg, which indicated that A-ring of alisol A derivatives is essential for potent anti-HBV activity. However, we have only one example, we can not conclude that this will be a general relationship. To further explore the role of acyloxyl at C-11,23,24,25, we performed additional structural modifications. The addition of methene groups (compound **A2**) reduced activity 187-fold of the inhibition to HBsAg secretion compared to compound **A1** (IC_{50} = 0.90 mM vs IC_{50} = 0.0048 mM). In addition, this change produced a loss of property on the secretion of HBeAg. More bulky tetra-acyl derivatives **A3**–**A21** showed loss of anti-HBV activity, except that compound **A11** was observed to possess moderate activity against the secretion of HBsAg (IC_{50} = 0.48 mM, SI >2.9). In an effort to gain more information as to the structure–activity relationships of alisol A derivatives, we probed additional structure change. Bioisosteric replacement of the acetyl groups with trimethylsilyl groups (**A22**) resulted in 4-fold decrease in inhibition to the secretion of HBsAg compared to compound **A1** (IC_{50} = 0.020 mM vs IC_{50} = 0.0048 mM), whereas compound **A22** was found toxic (CC_{50} = 0.051 mM), resulting relative low SI value (2.5).

Tri-acetyl derivative **B1** is much less potent than tetra-acetyl analogue **A1**, indicating that acetylation of the free hydroxyl at C-25 is important for potent anti-HBV activity of alisol A derivatives. To further explore this phenomenon, tri-acyl derivatives **B2**–**B4** were acetylated to give **A25**–**A27**. Of them, compound **A25** exhibited moderate activity against HBsAg secretion (IC_{50} = 0.46 mM, SI >3.7) and high potency on the secretion of HBeAg (IC_{50} = 0.086 mM, SI >20). More bulky acyls at C-11,23,24 derivatives **A26**, **A27** were inactive in inhibiting the secretion of HBsAg and HBeAg, suggesting that for C-25 acetyl alisol A derivatives, small acyloxy substituents at C-11,23,24 may be preferred for anti-HBV activity. Highly potent anti-HBV activity of compounds **2** ($IC_{50HBsAg}$ = 0.028 mM, SI_{HBsAg} >90; $IC_{50HBeAg}$ = 0.027 mM, SI_{HBeAg} >93) and **3** ($IC_{50HBsAg}$ = 0.20 mM, SI_{HBsAg} >11; $IC_{50HBeAg}$ = 0.26 mM, SI_{HBeAg} >8.7) led us to prepare compounds **A23**, **A24** which suppressed the secretion of HBsAg (IC_{50} = 0.0044 mM, SI = 209; IC_{50} = 0.014 mM, SI >200, respectively) and HBeAg (IC_{50} = 0.012 mM, SI = 77; IC_{50} = 0.018 mM, SI >156, respectively).

For derivatives **C1**–**C4** resulted from tetra-acylation of compound **E**, compound **C1** showed decreased inhibition to the secretion of HBsAg (IC_{50} = 0.046 mM, SI >87) and HBeAg (IC_{50} = 0.061 mM, SI >66) compared to compound **A1**, which indicated that, for tetra-acyl analogues, epoxide group at C-13(17) led to the decrease of suppressant property on the secretion of HBsAg and HBeAg.

Further study was carried out in male Sprague-Dawley rats to characterize the pharmacokinetic properties of compound **A1**. As shown in Table 2, following ig administration, compound **A1** was rapidly absorbed from the gastrointestinal tract demonstrating the mean time taken to attain peak concentration (T_{peak}) being 1 h, and oral bioavailability (F) was 40.9%. The mean plasma clearance (CL) of compound **A1** was 3.87 L/h/kg and steady-state distribution volume (V_{ss}) was 5.73 L/kg resulting in a reasonable elimination half-life ($t_{1/2}$) of 1.63 h after an iv administration.

In summary, a series of tetra-acylalisol A analogues were synthesized and examined for their in vitro anti-HBV activities and cytotoxicities, and nine tested compounds were active against HBV in HepG 2.2.15 cells. These results provide the following interesting structure–activity relationships: (1) acetyloxyl, methoxyacetyloxyl, and ethoxyacetyloxyl groups at C-25 of alisol A derivatives enhance potency; (2) for C-25 acetyloxyl alisol A derivatives, small acyloxyl groups at C-11,23,24 may be preferred for anti-HBV activity; (3) A-ring of alisol A derivatives might be essential for potent anti-HBV activity; (4) for tetra-acetyl analogue **A1**, epoxide functionality at C-13(17) leads to the decrease of suppressant property on the secretion of HBsAg and HBeAg. Furthermore, Compound **A1** possesses favorable pharmacokinetic properties in rats.

Table 2
Pharmacokinetic profile of compound **A1** in rats[a]

	Rat PK	
	2 mg/kg (iv)	10 mg/kg (ig)
C_0 (ng/mL)	567 ± 95.6	—
C_{max} (ng/mL)	—	383 ± 88.7
T_{peak} (h)	—	1.00 ± 0.00
$t_{1/2}$ (h)	1.63 ± 0.30	—
AUC (ng·h/mL)	510 ± 46.7	1045 ± 166
CL (L/h/kg)	3.87 ± 0.41	—
V_{ss} (L/kg)	5.73 ± 0.39	—
F (%)	—	40.9

[a] Results are expressed as mean ± SD of n = 3.

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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.2009.10.006.

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