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Anti-HBV agents. Part 1: Synthesis of alisol A derivatives: A new class of hepatitis B virus inhibitors

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ABSTRACT

A series of alisol A derivatives were synthesized and evaluated for their anti-hepatitis B virus (HBV) activities and cytotoxicities in vitro. The preliminary investigation demonstrates that simple modifications of the parent structure of alisol A can produce a number of potentially important derivatives against HBV. The most active anti-HBV compound **6a** showed high activities against the secretion of HBV surface antigen (IC_{50} = 0.024 mM), HBV e antigen (IC_{50} = 0.028 mM) and remarkable selective indices (SI_{HBsAg} > 108, SI_{HBeAg} > 93), which was selected for further evaluation as a novel HBV inhibitor.

Hepatitis B virus (HBV) infection is still a major world health problem despite the availability of an effective vaccine.[1] Approximately 350 million people are chronically infected with the virus worldwide, including 1.25 million in the U.S. and more than 200 million in China. HBV infection can persist for the life of the host, often leading to severe consequences such as liver failure, fibrosis, cirrhosis and hepatocellular carcinoma (HCC).[2] Worldwide deaths from liver cancer caused by HBV infection probably exceed 1 million per year.[3] Currently, there is no effective treatment that completely eliminates the infection in all chronically infected patients. The approved clinical chemotherapeutic agents (i.e., lamivudine, adefovir dipivoxil, entecavir, telbivudine) inhibit the virus replication by targeting the viral DNA polymerase, and after long-term treatment development of drug-resistant virus becomes problematic.[4] Interferon-α (IFN-α) is also clinically useful for HBV infection, but it has substantial side effects as well. The side effects of interferon and the viral resistance of nucleoside analogues make the current treatment regimens far from satisfactory.[5–7] To circumvent the existing therapeutic difficulties, novel compounds with unique modes of actions are urgently needed.

Natural products as the most consistently successful sources in drug discovery may offer more opportunities to find drugs or lead compounds.[8–11] Protostane-type triterpenoids are natural product molecules mainly found in *Alisma* genus and most of them exhibited diverse biological activities, such as anti-complementary,[12,13] anti-allergic,[14] anti-HIV reverse transcriptase[15], and other activities.[16–22] Recently, we reported that protostane-type triterpenes exhibit significant in vitro antiviral activity against HBV.[23] To the best of our knowledge, it was the first report of protostane-type triterpenes possessing anti-HBV activities. In view of their novel structural template, which differs from those of all reported anti-HBV agents, we were interested to further study the structure–activity relationships of the related class of compounds. Alisol A (**1**, Fig. 1),[24] a protostane-type triterpene, comprises 0.05–0.1% of rhizomes of *Alisma orientalis* Juzep.[16] and carries four hydroxyl groups providing possibilities for more diverse library. Thus, with compound **1** as the starting substrate, we synthesized a series of

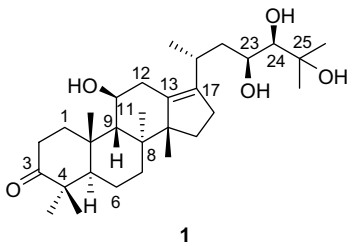

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Figure 1. Structure of compound **1**.

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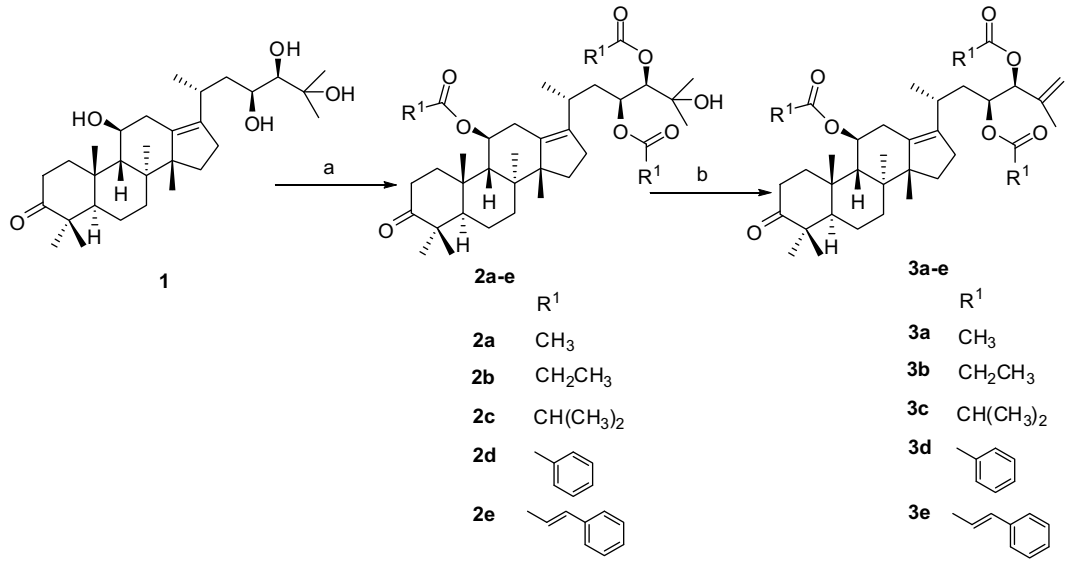
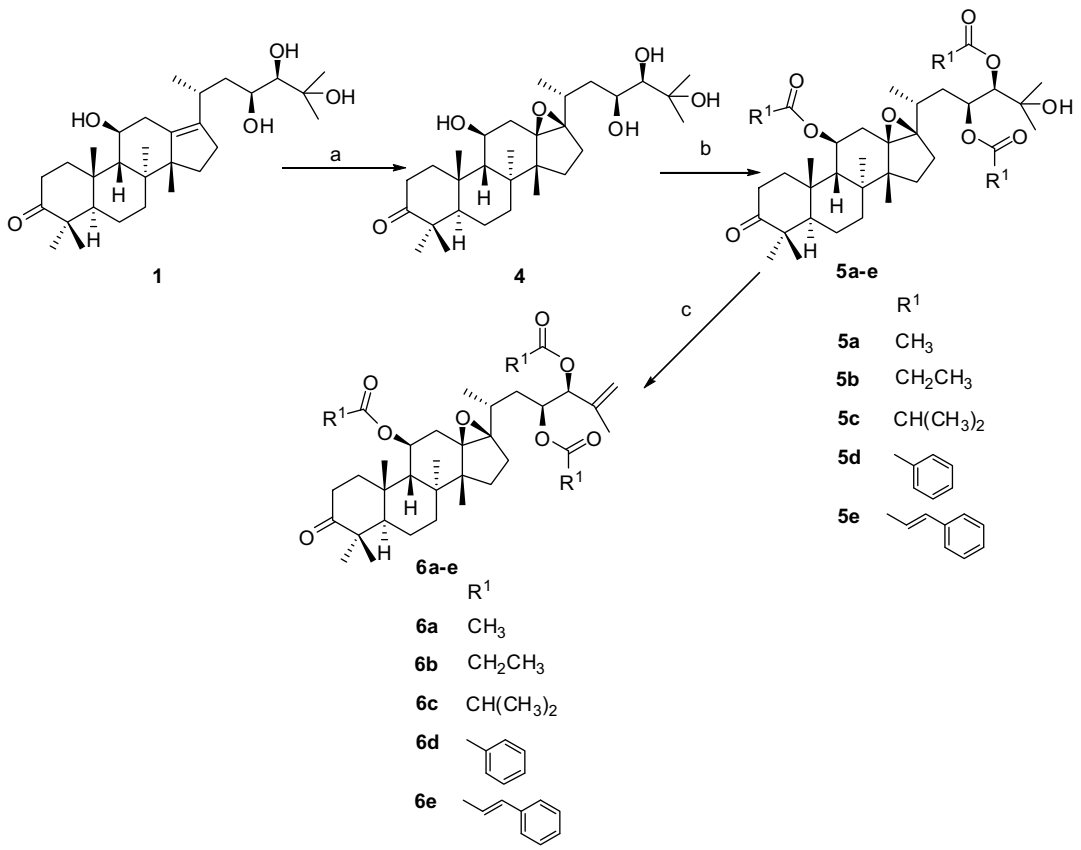
alisol A derivatives and investigated their biological activities as potential anti-HBV agents.

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 biological activities. Compound **1** was treated with acetic anhydride or with carboxylic acids in the presence of *N*,*N*′-dicyclohexylcarbodiimide (DCC) and 4-dimethylaminopyridine (DMAP) to afford compounds **2a–e**. Compounds **2a–e** were subjected to dehydration by $SOCl_2$ in pyridine to give **3a–e** in moderate yields[25] (Scheme 1).

Epoxidation of compound **1** with *m*-chloroperoxybenzoic acid (mCPBA) in CH_2Cl_2 at room temperature gave compound **4** without affecting the 3-carbonyl and hydroxyl groups[26] (Scheme 2). Compound **4** was reacted with acetic anhydride or carboxylic acids in



Scheme 1. Reagents and conditions: (a) for **2a**: $(CH_3CO)_2O$, pyridine, rt, 86%; for **2b–e**: R^1COOH, DCC, DMAP, CH_2Cl_2, rt, 68–85%; (b) $SOCl_2$, pyridine, rt, 59–75%.



Scheme 2. Reagents and conditions: (a) mCPBA, CH_2Cl_2, rt, 88%; (b) for **5a**: $(CH_3CO)_2O$, pyridine, rt, 72%; for **5b–e**: R^1COOH, DCC, DMAP, CH_2Cl_2, rt, 55–87%; (c) $SOCl_2$, pyridine, rt, 55–72%.

the presence of DCC and DMAP to give compounds **5a–e**, which were followed by dehydration with $SOCl_2$ in pyridine to afford compounds **6a–e**.

Treatment of compound **1** with 4-methylbenzenesulfonohydrazide afforded compound **7**, which was reduced with $NaBH_3CN$ to give compound **8**. Furthermore, C-3 keto was converted to C-3 thioketo for further evaluation whether a carbonyl function at C-3 was necessary for biological activity. As shown in Scheme 3, refluxing of **2a** with Lawensson's reagent in toluene did not give the C-3 thioketo derivative but gave compound **3a,** which could be confirmed by additional signals of a double bond (δ_C 140.5, C-25; δ_C 114.6, C-26) in ^{13}C NMR spectrum of **3a**. Additionally, the yield (82%) of conversion of **2a** to **3a** was better than that reported previously (75%).[25]

As a preliminary study in the developing protostane-type triterpenoids against HBV agents, a series of alisol A derivatives were prepared and tested for their cytotoxicities and potential anti-HBV activities, namely the ability 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,[27] in order to better understand the structural requirements for the biological effect. The results are summarized in Table 1.

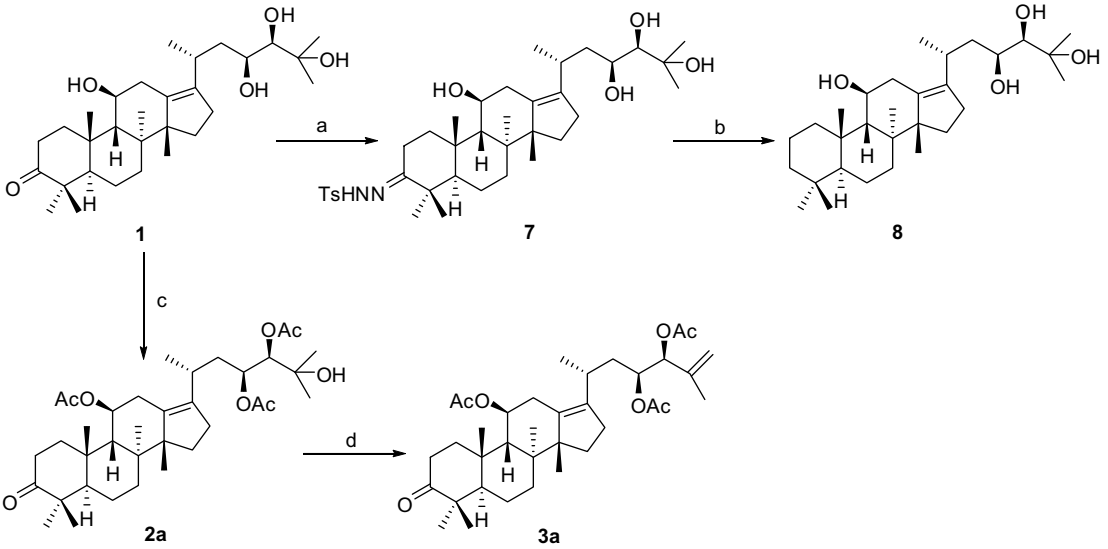
The parent compound **1** showed inhibitory potency to the secretion of HBsAg (IC_{50} = 0.039 mM), but appeared toxic (CC_{50} = 0.062 mM), which led to a relatively low selectivity index (1.6). The subseries of derivatives **2a–e** have different patterns of substitution on C-11, C-23 and C-24. As shown in Table 1, these compounds were non-cytotoxic but decreased inhibition to secretion of HBsAg. For derivatives **3a–e** that resulted from dehydration of compounds **2a–e**, compound **3a** can inhibit the secretion of HBsAg (IC_{50} = 0.028 mM, SI = 18) and HBeAg (IC_{50} = 0.029 mM, SI = 18), which indicated that the hydroxyl group at C-25 of alisol A might be a good target for further lead optimization.

Little change in inhibition to secretion of HBsAg was observed with the epoxidation of **1** (IC_{50} = 0.039 mM) to the corresponding **4** (IC_{50} = 0.045 mM), whereas compound **4** had a high SI (9.6), suggesting that the epoxy group is an important feature in the conferring relatively low cytotoxicity. Tri-acyl derivatives of compound **4** were non-cytotoxic. But unfortunately, compounds **5a–e** lost suppressant properties on the secretion of HBsAg and HBeAg. For dehydrated compounds **6a–e**, the most active compound **6a** exhibited high activities against secretion of HBsAg (IC_{50} = 0.024 mM) and HBeAg (IC_{50} = 0.028 mM), which was more potent than the positive control drug lamivudine ($IC_{50HBsAg}$ = 12 mM, $IC_{50HBeAg}$ = 26 mM). Meanwhile it had low cytotoxicity (CC_{50} > 2.6 mM), resulting in high selectivity index (SI_{HBsAg} > 108, SI_{HBeAg} > 93). Compound **6b** showed suppressant properties on the secretion of HBsAg (IC_{50} = 0.16 mM, SI > 16), while lost activity against secretion of HBeAg. Above result further demonstrated the importance of functionality at the position of C-25 to potent anti-HBV activity.

Table 1
Anti-HBV activity, cytotoxicity and selectivity index of compounds **1**–**8**[a]

Compound	CC_{50}[b] (mM)	HBsAg[c]		HBeAg[d]	
		IC_{50}[e] (mM)	SI[f]	IC_{50} (mM)	SI
1[24]	0.062	0.039	1.6	>2.4	<0.030
2a[24]	>2.0	>2.0	—	>2.0	—
2b	>1.4	>1.4	—	>1.4	—
2c	>2.2	1.7	>1.3	0.9	>2.4
2d	>1.5	>1.5	—	>1.5	—
2e	>1.4	>1.4	—	>1.4	—
3a[25]	0.52	0.028	18	0.029	18
3b	>1.2	>12	—	>12	—
3c	>1.6	>1.6	—	>1.6	—
3d	>2.3	>2.3	—	>2.3	—
3e	>1.8	>1.8	—	>1.8	—
4[25]	0.43	0.045	9.6	1.1	0.39
5a	>2.0	>2.0	—	>2.0	—
5b	>1.2	>1.2	—	>1.2	—
5c	>1.9	>1.9	—	>1.9	—
5d	>1.7	>1.7	—	1.1	>1.5
5e	>1.3	>1.3	—	1.0	>1.3
6a	>2.6	0.024	>108	0.028	>93
6b	>2.5	0.16	>16	>2.5	—
6c	>1.3	>1.3	—	>>1.3	—
6d	>1.6	>1.6	—	>1.6	—
6e	>1.6	>1.6	—	>1.6	—
7	<0.029	0.029	<1.0	0.029	<1.0
8	<0.080	0.080	<1.0	5.1	<0.016
3TC[g]	30	12	2.5	26	1.2

[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] 3TC: lamivudine, an antiviral agent used as positive control.



Scheme 3. Reagents and conditions: (a) $TsNHNH_2$, ethanol, reflux, 82%; (b) $NaBH_3CN$, TsOH, DMF/sulfolane (1:1), 120 °C, 48%; (c) $(CH_3CO)_2O$, pyridine, rt, 86%; (d) Lawensson's reagent, toluene, reflux, 82%.

Compounds **7** (IC_{50} = 0.029 mM) and **8** (IC_{50} = 0.080 mM) exhibited similar inhibitory potency to the secretion of HBsAg with that of compound **1** (IC_{50} = 0.039 mM), indicating that a carbonyl function at C-3 might not be necessary for biological activity.

In summary, a series of alisol A derivatives were synthesized and assayed for their anti-HBV activity and cytotoxicity in vitro, using lamivudine as positive control. Our investigation demonstrates that simple modifications of the parent structure of alisol A can produce a number of potentially important derivatives. Based on the above results, the following conclusions could be made: (a) the acylation of hydroxyl group at 11, 23, 24-position was found to decrease cytotoxicity; (b) epoxy group at C-13 (17) is an important feature in the conferring relatively low cytotoxicity; (c) hydroxyl group at C-25 of alisol A might be a good target for further lead optimization; and (d) a carbonyl function at C-3 might not be necessary for biological activity. However, results from more extensive investigation using a great number of derivatives are necessary for structure–activity relationship study for the design and ultimate synthesis of more effective alisol A-derived anti-HBV agents. Further investigation 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.2008.07.012.

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