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

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A B S T R A C T
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 IC50 values of 2.8–11.6 μM, comparable to that of the positive control, tenofovir. Compounds A1–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 mechanisms 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 (IC50 = 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• 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 equiv m-chloroperbenzoic acid (mCPBA) yielded derivative C without affecting the carbonyl and hydroxyl groups, which was further converted to derivatives D1–D5. The epoxy group was

Figure 1. Structure of hemslecin A.

1

HO
H
H
HO
HO
H
OAc
O
25
HO
HO
H
HO
H
HO
HO
H

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The anti-HBV results were summarized in Table 1. The anti-HBV drug olovir, a clinical anti-HBV agent, was used as positive control. 

Derivatives F1–F3 were obtained by epoxidation of triacylated compounds B1, B4 and B5 with 2.5 equiv mCPBA in CH2Cl2. 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 shown 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 hemiscele 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 required to decrease 50% inhibition (IC50) of HBsAg, HBeAg, and HBV DNA replication. The cytotoxicity of each compound was evaluated by the combination of its IC50 value and SI.

Compound 1 showed moderate activity against HBV DNA replication (IC50 = 11.4 μM), with obvious toxicity (CC50 = 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 IC50 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 (IC50 = 2.8, 3.1, 4.7 μM, respectively).

Acetylated derivative A1 (CC50 = 253.5 μM, SI = 89.7) and propionylated derivative A4 (CC50 = 100.3 μM, SI = 31.9) showed low cytotoxicity. Replacement of acyl moiety with butyryl (compound A7, CC50 = 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 (IC50 = 18.4 μM, SI = 5.5) and HBeAg (IC50 = 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 (IC50 = 4.7 μM, 6.1 μM, respectively). Propionyl derivative A3 appeared to be less toxic compared to butyryl derivative A6 (CC50 = 126.0 μM vs CC50 = 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 (IC50 = 15.6 μM, SI = 8.1) and HBeAg (IC50 = 14.4 μM, SI = 8.7). Dia-acylated compounds A2 and A5 exhibited high activities against HBV DNA replication with IC50 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 (IC50 = 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: (CH3)2O, pyridine, rt, 8–30%; for A3–A5: (CH3CH2CO)2O, pyridine, rt, 7–31%; for A6 and A7: (CH3CH2CH2CO)2O, pyridine, rt, 7–51%; for B1–B3: (RO)2O, DMAP, pyridine, rt, 84–92%; for B4–B6: ROOH, DCC, DMAP, CH2Cl2, rt, 90–93.
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 IC50 values of 17.3 µM (SI >46.4), 19.0 µM (SI >34.2), 114.9 µM (SI >4.5), 10.0 µM (SI >55.8), 51.2 µM (SI >14.0), but inactive to the secretion of HBsAg and HBeAg, except for compound B3 which.

Scheme 2. Reagents and conditions: (a) mCPBA (1.2 equiv), CH2Cl2, rt, 75%; (b) for D1–D5: (RCO)2O, DMAP, pyridine, rt, 81–90%; for D4, D5: RCOOH, DCC, DMAP, CH2Cl2, rt, 90–93%; (c) mCPBA (2.5 equiv), CH2Cl2, rt, 89%; (d) for B1: (CH3CO)2O, DMAP, pyridine, rt, 88%; for B4, B5: RCOOH, DCC, DMAP, CH2Cl2, rt, 93%; (e) mCPBA (2.5 equiv), CH2Cl2, rt, 55–82%.

Scheme 3. Reagents and conditions: (a) C2H5OCH2COOH, DCC, DMAP, CH2Cl2, rt, 24–80%.
lost the activity against HBV DNA replication but showed suppressant potency against the secretion of HBsAg (IC50 = 18.0 μM, SI >33.8).

Compound C derived from epoxidation of compound 1 (IC50 = 11.4 μM) showed obviously decreased cytotoxicity, and similar activity against HBV DNA replication (IC50 = 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 (IC50 = 24.0 μM, SI = 15.3), HBeAg (IC50 = 30.9 μM, SI = 11.9) and the replication of HBV DNA (IC50 = 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 (IC50 = 11.4 μM) to corresponding compound 2 (IC50 = 31.4 μM) reduced the activity against HBV DNA replication. Compounds G (IC50 = 84.6 μM) and H (IC50 = 81.4 μM) showed a considerable reduction of activity against HBV DNA replication compared with compound B5 (IC50 = 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 IC50 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.

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**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**