Isolation, synthesis and anti-hepatitis B virus evaluation of p-hydroxyacetophenone derivatives from Artemisia capillaris

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A B S T R A C T

p-Hydroxyacetophenone (p-HAP), as a main hepatoprotective and choleric constituent of Artemisia capillaris, was revealed with anti-hepatitis B virus (HBV) effects in recent investigation. In addition to p-HAP, four derivatives of p-HAP were also isolated from A. capillaris by various chromatographic methods. Subsequent structural modification on p-HAP and its glycoside led to the synthesis of 28 additional derivatives, of which 13 compounds showed activity inhibiting hepatitis B surface antigen (HBsAg) secretion; and 18 compounds possessed inhibition on HBV DNA replication. The primary structure–activity relationships (SARs) suggested that the conjugated derivatives of p-HAP glycoside and substituted cinnamic acids (2a–2i) obviously enhanced the activity against HBV DNA replication with IC50 values ranged from 5.8 to 74.4 μM.

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Hepatitis B virus (HBV) infection, causing a series of acute and chronic liver diseases like hepatitis, liver cirrhosis and hepatocellular carcinoma (HCC), has a worldwide distribution, especially in China.1 Currently, anti-viral therapy with nucleoside analogues and immunomodulatory agents is the preferred option to control and prevent the progression of diseases in chronic HBV infected patients.2 However, the present therapeutic drugs are still inadequate due to inevitable side effects and drug tolerance. Therefore, new kinds of non-nucleoside anti-HBV agents with novel antiviral mechanisms are urgently in need.

Recently, natural products with enormous molecular complexity and diversity have been returning to a prominent position in the prospection of anti-HBV active leading compounds, in light of which many new synthetic and semisynthetic derivatives provide high potential for the discovery of new drug candidates.3 p-Hydroxyacetophenone (p-HAP) is a main hepatoprotective and choleric constituent of Artemisia capillaris which is well-known as ‘Yin Chen’ and widely used for the treatment of icterohepatitis in traditional Chinese medicine (TCM).4 Previous investigation showed that several liver polypeptides in the liver cytosolic fraction specifically bound to the p-HAP matrix suggesting the pharmacological potential for the application of p-HAP derivatives in liver diseases.5 Meanwhile, a series of p-HAP derivatives were revealed with diverse pharmacological activities including stimulated bile secretion, lipid-lowering,6,7 anti-inflammatory,8 anti-cancer,9 anti-pathogenic microorganism10 and antipsychotics.11 Currently, p-HAP isolated from Artemisia morrisonensis was also revealed with inhibitory activity on HBV, the mechanism of which might involve the regulation of viral surface gene expression and block virion secretion by interference with the endoplasmic reticulum (ER) stress signaling pathway.12

Our previous investigation showed the crude extract of A. capillaris possessed antiviral activity against HBV, from which several naturally occurring anti-HBV active components had been obtained.13,14 In our continuing search for bioactive constituents from this traditional herb, five p-HAP derivatives including p-hy-
droxyacetophenone (1),15 p-hydroxyacetophenone-4-O-β-D-glucopyranoside (2),15 4-O-β-D-glucopyranosyl-2-hydroxy-6-methoxyac
etophenone (3),16 asterbatanoside A (4),17 and 6'-O-caffeoyl-p-hy-
droxy-acetophenone-4-O-β-D-glucopyranoside (5)18 (Fig. 1) were
isolated from the active part with manifold chromatographic
methods. According to our previous bioassay, p-HAP showed mod-
erate inhibitory activity on hepatitis B surface antigen (HBsAg)
secretion and HBV DNA replication with 50% inhibitory concentra-
tion (IC50) values of 785.7 and 306.4 μM, as well as a slight inhibi-
tion on hepatitis B e antigen (HBeAg) secretion. The glycosyl
derivatives (2–4) showed obviously enhanced inhibitory effect on
HBV DNA, whilst the activity against HBsAg and HBeAg secretions
vanished. In addition, the number of the glycosyl residues appears
to play an important role for that compound

<table>
<thead>
<tr>
<th>Compd</th>
<th>CC50 (μM)</th>
<th>HBsAg</th>
<th>HBeAg</th>
<th>HBV DNA</th>
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<td>290.8</td>
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The obvious enhanced antiviral activity of compound 3 might be
ascribed to the hydroxy group at C-2 and the methoxyl unit at
C-6. Compound 5, as a hybrid of caffeic acid and p-HAP glucopyra-
noside possessed the most significantly inhibitory activity on HBV
dNA replication with an IC50 value of 8.0 μM, as well as activity
against HBsAg and HBeAg secretions. Considering all of the five
p-HAP derivatives showed anti-HBV activity and the naturally
occurring hybridization of p-HAP glucopyranoside with caffeic acid
(5) possessed obviously enhanced anti-HBV activity, a series of
hybrids of p-HAP (1a–1n) (Scheme 1) and p-HAP glucopyranoside
(2a–2n) (Scheme 2) with different aromatic acids were also
designed and evaluated for anti-HBV activity in this Letter.

p-HAP esters (1a–1n) were synthesized in two steps by conver-
sion of the acids into the corresponding acyl chloride by reaction
with thionyl chloride for 2–3 h, followed by reaction with p-HAP
and 4-dimethylaminopyridine (DMAP) in anhydrous pyridine
at room temperature for 8 hours (Scheme 1). The yields of ester
derivatives were all over 90%. Due to the limited source of natural
occurring p-HAP glucopyranoside form A. capillaris, this reaction
substrate were synthesized through a reaction sequence of three
steps in an overall yield of 68% with p-HAP as a starting material
(Scheme 2). Glycosylation of p-HAP with x-D-glucopyranosyl bro-
mide tetaacetate in the presence of sodium hydroxide and tetra-
butyln ammonium bromide on the ice-bath condition afforded the
intermediate 2 and further deacetylated in sodium methoxide-
methanol solution, affording target compound 2. The aromatic
acids were incorporated to the C-6 of p-HAP glucopyranoside (2)
under Mitsunobu conditions19 and provided the final hybrids
2a–2n in 37–48% yields. The structure characterizations of these

Scheme 1. Synthesis of compounds 1a–1n. Reagents and conditions: (a) SOCl2, rt, 2–3 h; (b) DMAP, anhydrous pyridine, rt, 8 h.

Scheme 2. Synthesis of compounds 2a–2n. Reagents and conditions: (a) HBr, CH2Cl2, ice-bath, 6 h; (b) Bu4NBr, NaOH, CHCl3, H2O, rt, 2 h; (c) NaOMe, MeOH, rt, 8 h. (d) Ph3P, DIAD, anhydrous THF, overnight.


Table 2

Anti-HBV activity and cytotoxicity of compounds 1a–1n

<table>
<thead>
<tr>
<th>Compd</th>
<th>R</th>
<th>CC₅₀ (µM)</th>
<th>HBsAg IC₅₀ (µM)</th>
<th>SI</th>
<th>HBeAg IC₅₀ (µM)</th>
<th>SI</th>
<th>HBV DNA IC₅₀ (µM)</th>
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(continued on next page)
Table 2 (continued)

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<th>Compd R</th>
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<th>HBsAg IC50 (µM)</th>
<th>SI</th>
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<th>SI</th>
<th>HBV DNA IC50 (µM)</th>
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Table 3

Anti-HBV activity and cytotoxicity of compounds 2a-2n

![Diagram of molecule 2a-2n]

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<th>Compd</th>
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<th>CC50 (µM)</th>
<th>HBsAg IC50 (µM)</th>
<th>SI</th>
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<th>SI</th>
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compounds were conducted using ¹H NMR, ¹³C NMR and HRESIMS. Positions of the substituents were determined by the obvious down-field shift of C-6' in ¹³C NMR spectrum, as well as the marked splitting of H-6' protons in ¹H NMR spectrum. The anti-HBV activities of the compounds were evaluated on HepG 2.2.15 cell lines in vitro according to our previous report, with tenofovir (Jiangxi Chenyang Pharmaceutical Co., Ltd, China, purity >97.6%) as the positive control (Table 1). Reduction of HBsAg and HBeAg secretions was measured by ELISA method, inhibition of HBV DNA replication was monitored by real-time quantitative PCR, and cellular toxicity was assessed by MTT method.

The anti-HBV activities of p-HAP derivatives (1a–1k) were shown in Table 2. It was revealed that 4-O-(3',4'-dimethoxyxycinnamoyl)-(1d), 4-O-(3',4',5'-trimethoxycinnamoyl)-(1f), 4-O-(3',4',5'-trimethoxybenzoyl)-(1k) derivatives exhibited enhanced activity against HBV DNA replication, with IC₅₀ values in the range of 88.8–114.9 μM, indicating that more methoxyl groups was favorable to enhance the anti-HBV activity in this series of compounds. The 4-O-(nicotinoyl)-(11) derivative (11) showed moderate activity against HBV DNA replication with IC₅₀ value of 154.4 μM as well. However, the other compounds were devoid of inhibitory activities on HBsAg secretion and HBV DNA replication, suggesting that the hydroxyl group at C-4 position might contribute to the anti-HBV activity (Table 3).

In contrast to p-HAP derivatives (1a–1n), hybrids of p-HAP glucopyranoside esterified with substituted cinnamic acids or aromatic acids containing hetero atoms (2a–2n) obviously enhanced their anti-HBV activity. The above analysis suggested that glucosyl moiety at C-4 was an important determinant of anti-HBV activity. Especially, the incorporation of substituted cinnamic acids to C-6' position (2a–2i) significantly increased the inhibitory activity with IC₅₀ values ranging from 5.8 to 74.4 μM. In addition, the number of methoxyl groups appeared to influence the anti-HBV activity since the trimethoxy substituted analogue (2f) possessed lower potency inhibitory effect with an IC₅₀ value of 5.8 μM (SI = 160.3) whilst the unsubstituted analogue (2a) showed the weakest activity against HBV DNA replication with an IC₅₀ value of 67.3 μM. Similarly, compound 2e (IC₅₀ = 74.4 μM) exhibited weaker inhibitory activity than 2d (IC₅₀ = 8.8 μM) after the methoxyl groups on the cinnamoyl moiety were replaced by methylenedioxy group (–OCH₂O–). It was interesting to note that the inhibitory activity on HBV DNA replication of compound 2d was retained when the hydroxyl groups in the natural analogue 5 (IC₅₀ = 8.0 μM) were substituted by methoxyl groups, whereas its activity against HBsAg and HBeAg obviously decreased, inferring only free hydroxy substituents were tolerated on the cinnamoyl moiety for the activity against HBsAg and HBeAg secretions.

The introduction of halogen atoms is an effective strategy in the discovery of new antiviral drugs, for the halogens in drug–target complexes influence several processes (the formation of halogen bonds in ligand–target complexes, e.g.) rather than sterics alone. Currently, a significant number of drugs and drug candidates in clinical development have halogenated structures. In our investigation, the introduction of fluoride atoms in C-4' position on the cinnamoyl moiety of p-HAP glucopyranosides derivatives (2g and 2i) could enhance their anti-HBV activity (2g, IC₅₀ = 22.8 μM; 2i, IC₅₀ = 22.5 μM) in contrast to their unsubstituted analogue 2a. Compared with the methoxyl substituted derivatives (2b–2d), the fluorine atom substituted derivatives (2g–2i) showed similar activity but slightly increased cytotoxicity.

To investigate the influence of carbon-carbon double bond on the cinnamoyl moiety of p-HAP glucopyranosides derivatives, compounds 2j and 2k were prepared and evaluated for anti-HBV activity. The result showed that derivatives 2j and 2k without double bond slightly decreased the anti-HBV activity compared with derivatives 2a and 2f, since compounds 2j and 2k possessed lower antiviral activity against HBV DNA replication with IC₅₀ values of 93.7 and 20.7 μM.

In an attempt to develop compounds with improved in vitro anti-HBV activity, aromatic acids containing hetero atoms (nitrogen, oxygen and sulfur) were also incorporated to p-HAP glucopyranoside, yielded derivatives 2l–2n. However, only the hybrid of thienoyl acid (2n, IC₅₀ = 31.4 μM) showed improvement as to activity against HBV DNA replication compared with derivatives 2j (IC₅₀ = 93.7 μM), whereas the introduction of aromatic rings with nitrogen and oxygen atoms did not provide appreciable inhibition on HBV.

Collectively, five naturally occurring p-HAP derivatives (1–5) were isolated from the active part of A. capillaris, and 28 additional derivatives were further synthesized based on the preliminary SARs. It was disclosed that the conjugated derivatives of p-HAP glucoside and substituted cinnamic acids (2a–2i) obviously enhanced the activity against HBV DNA replication. Compound 2f with three methoxyl groups on cinnamic acid possessed the best inhibitory activity on HBV DNA replication (IC₅₀ = 5.8 μM, SI = 160.3). This research might provide valuable information for the discovery of...
new kind of non-nucleoside anti-HBV candidates from the TCM of A. capillaris.

Acknowledgements

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Supplementary data

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References and notes