Bioorganic & Medicinal Chemistry Letters 25 (2015) 1509–1514

Contents lists available at ScienceDirect

Bioorganic & Medicinal Chemistry Letters

journal homepage: www.elsevier.com/locate/bmcl

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

Yong Zhao, Chang-An Geng, Hao Chen, Yun-Bao Ma, Xiao-Yan Huang, Tuan-Wu Cao, Kang He, Hao Wang, Xue-Mei Zhang, Ji-Jun Chen *

State Key Laboratory of Phytochemistry and Plant Resources in West China, Kunming Institute of Botany, Chinese Academy of Sciences, Kunming 650201, PR China

ARTICLE INFO

Article history:
Received 15 December 2014
Revised 31 January 2015
Accepted 10 February 2015
Available online 18 February 2015

Keywords:
Artemisia capillaris
p-Hydroxyacetophenone derivatives
Anti-HBV activity
Structure–activity relationships

ABSTRACT

p-Hydroxyacetophenone (*p*-HAP), as a main hepatoprotective and choleretic 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 IC_{50} values ranged from 5.8 to 74.4 μM.

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 choleretic 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 bounded 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 microorganism[10] 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]

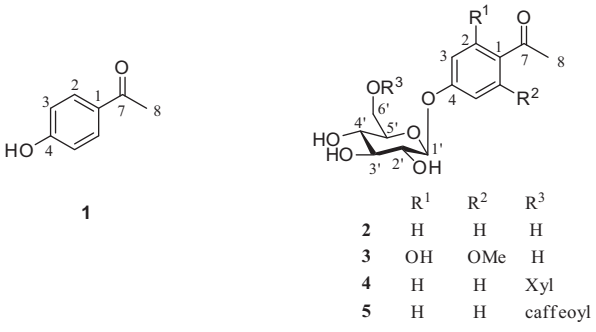
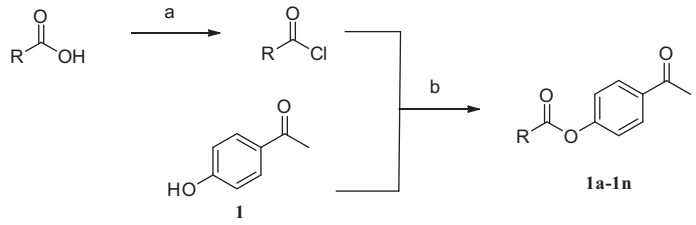


Figure 1. Structures of compounds **1**–**5**.

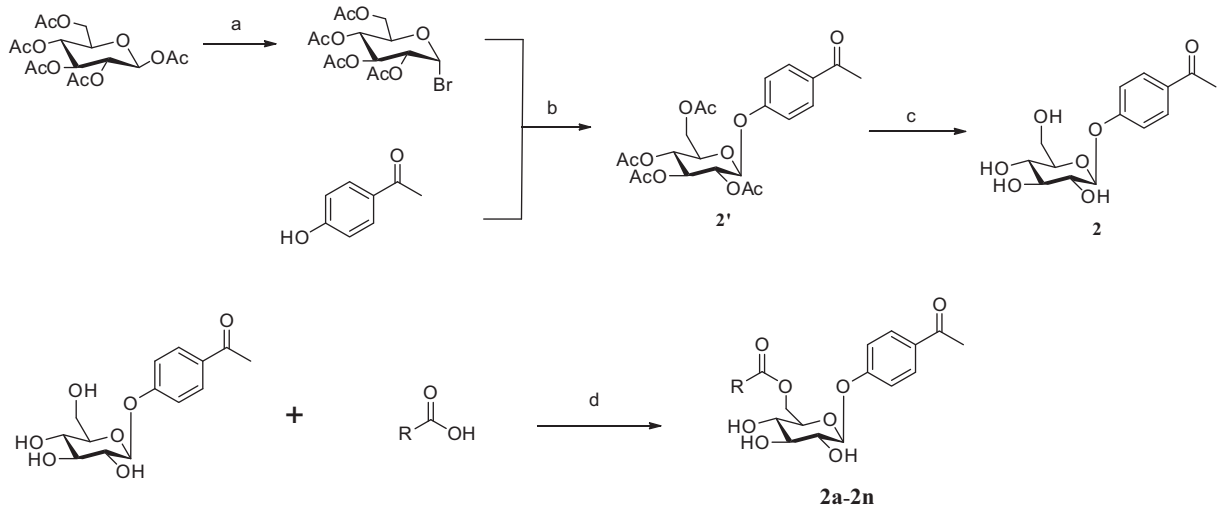
Our pervious investigation showed the crude extract of *A. capillaris* possessed antiviral activity against HBV, from which several naturally occurring anti-HBV active components had been obtained.[13,14] In our continuing search for bioactive constituents from this traditional herb, five *p*-HAP derivatives including *p*-hy-

* Corresponding author. Tel.: +86 871 65223265; fax: +86 871 65227197.
E-mail address: chenjj@mail.kib.ac.cn (J.-J. Chen).

http://dx.doi.org/10.1016/j.bmcl.2015.02.024
0960-894X/



Scheme 1. Synthesis of compounds **1a**–**1n**. Reagents and conditions: (a) $SOCl_2$, rt, 2–3 h; (b) DMAP, anhydrous pyridine, rt, 8 h.



Scheme 2. Synthesis of compounds **2a**–**2n**. Reagents and conditions: (a) HBr, CH_2Cl_2, ice-bath, 6 h; (b) Bu_4NBr, NaOH, $CHCl_3$, H_2O, rt, 2 h; (c) NaOMe, MeOH, rt, 8 h. (d) Ph_3P, DIAD, anhydrous THF, overnight.

droxyacetophenone (**1**),[15] *p*-hydroxyacetophenone-4-*O*-β-D-glucopyranoside (**2**),[15] 4-*O*-β-D-glucopyranosyl-2-hydroxy-6-methoxyacetophenone (**3**),[16] asterbatanoside A (**4**),[17] and 6′-*O*-caffeoyl-*p*-hydroxy-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 moderate inhibitory activity on hepatitis B surface antigen (HBsAg) secretion and HBV DNA replication with 50% inhibitory concentration (IC_{50}) values of 785.7 and 306.4 μM, as well as a slight inhibition 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 **4** with two glycosides have more potent activity against HBV DNA replication (IC_{50} = 38.5 μM) compared with compound **2** (IC_{50} = 227.8 μM). 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 glucopyranoside possessed the most significantly inhibitory activity on HBV DNA replication with an IC_{50} 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 conversion 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 α-D-glucopyranosyl bromide tetraacetate in the presence of sodium hydroxide and tetrabutyl 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 conditions[19] and provided the final hybrids **2a**–**2n** in 37–48% yields. The structure characterizations of these

Table 1
Anti-HBV activity and cytotoxicity of natural occurring *p*-HAP derivatives **1**–**5**[a]

Compd	CC_{50} (μM)	HBsAg		HBeAg		HBV DNA	
		IC_{50} (μM)	SI[b]	IC_{50} (μM)	SI	IC_{50} (μM)	SI
1	2388.2	785.7	3.0	1854.7	1.3	306.4	7.8
2	>3947.9	>3947.9	—[c]	>3947.9	—	227.8	>17.3
3	>2365.5	>2365.5	—	>2365.5	—	92.0	>25.7
4	>2599.2	>2599.2	—	>2599.2	—	38.5	>67.5
5	1583.0	290.8	5.4	1277.1	1.2	8.0	>197.9
Tenofovir[d]	>1742.2	>1742.2	—	>1742.2	—	2.9	>600.8

[a] Values are means of two independent experiments.
[b] SI (selectivity index) = CC_{50}/IC_{50}.
[c] SI values are not obtained.
[d] Tenofovir was used as the positive control.