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Synthesis, structure–activity relationships and biological evaluation of dehydroandrographolide and andrographolide derivatives as novel anti-hepatitis B virus agents

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ABSTRACT

Dehydroandrographolide and andrographolide, two natural diterpenoids isolated from *Andrographis paniculata* possessed activity against HBV DNA replication with IC_{50} values of 22.58 and 54.07 μM and low SI values of 8.7 and 3.7 in our random assay. Consequently, 48 derivatives of dehydroandrographolide and andrographolide were synthesized and evaluated for their anti-HBV properties to yield a series of active derivatives with lower cytotoxicity, including 14 derivatives against HBsAg secretion, 19 derivatives against HBeAg secretion and 38 derivatives against HBV DNA replication. Interestingly, compound **4e** could inhibit not only HBsAg and HBeAg secretions but also HBV DNA replication with SI values of 20.3, 125.0 and 104.9. Furthermore, the most active compound **2c** with SI value higher than 165.1 inhibiting HBV DNA replication was revealed with the optimal log*P* value of 1.78 and log*D* values. Structure–activity relationships (SARs) of the derivatives were disclosed for guiding the future research toward the discovery of new anti-HBV drugs.

Diseases caused by hepatitis B virus (HBV) are serious problems worldwide, which have affected more than 2 billion people. There are about 360 million chronically infected individuals suffering from liver cirrhosis and hepatocellular carcinoma.[1] The current therapies including vaccines, immunomodulators, interferon-α, polyethylene glycol interferon-α and nucleoside drugs for treating HBV are still unsatisfactory, due to high recurrence, drug resistance and inevitable side effects including influenza-like illness, myalgia, headache, reduction of neutrophilic granulocyte and blood platelet, etc.[2–5] Therefore, it is interesting to explore novel classes of drugs with different antiviral targets and mechanisms for anti-HBV purposes.

Natural products possess various skeletons and diverse bioactivities, which provide prolific candidates with new targets and mechanisms for anti-HBV drug discovery.[6–10] Our recent investigations revealed a series of natural products and their derivatives with promising anti-HBV activity, such as dihydrochelerythrine from *Corydalis saxicola*,[11] alisol F from *Alisma orientalis*,[12] swerilactones H-K from *Swertia mileensis*,[13] as well as derivatives of alisol A,[14] caudatin,[15] glycyrrhetinic acid[16] and hemslecin A.[17] *Andrographis paniculata*, a well-known Chinese medicine Chuan-Xin-Lian documented in each edition of Chinese Pharmacopoeia, is widely used for anti-inflammatory, antipyretic and detoxifying purposes.[18] Dehydroandrographolide (**1a**) and andrographolide (**1b**, Fig. 1), the main active constituents in *Andrographis paniculata*, exhibited various biological activities including antiinflammation, antivirus, antitumor, antibacterium, hepatoprotection and analgesia.[19–27] Although some derivatives of dehydroandrographolide (**1a**) and andrographolide (**1b**) were synthesized for treatment of influenza A virus, HIV, viral pneumonia and upper respiratory tract infection,[29–31] no investigation was concerned with anti-HBV activity. As our ongoing search for anti-HBV inhibitors from natural products, dehydroandrographolide (**1a**) and andrographolide (**1b**) were firstly revealed to be active against HBV DNA replication with 50% inhibitory concentration (IC_{50}) values of 22.6 and 54.1 μM but low selectivity index (SI) values of 8.7 and 3.7 in our random assay. Thus, 48 dehydroandrographolide and andrographolide analogues were synthesized by modifying on rings A, B and C to increase their anti-HBV activity and decrease cytotoxicity. Herein, we described the synthesis, structure–activity relationships (SARs) and in vitro anti-HBV activity of these derivatives, as well as the octanol–water partition coefficients of dehydroandrographolide, andrographolide and the most potent derivative **2c**.

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0960-894X/

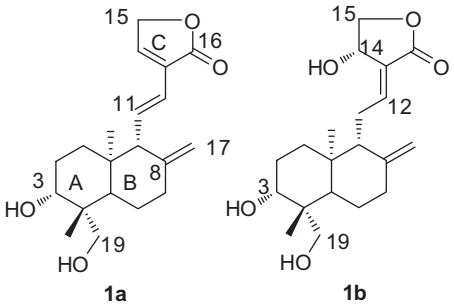


Figure 1. Compounds **1a** and **1b**.

According to molecular hybridization principle,[32] esterification of natural compounds is an effective approach for achieving promising derivatives, by which two active parts can be easily hybridized to enhance activity. For example, a series of active derivatives of caudatin[15] and hemslecin A[17] were obtained by esterification in our previous investigations, which showed obviously enhanced activity compared with the parent compounds. To increase anti-HBV activity and decrease cytotoxicity, derivatives of dehydroandrographolide and andrographolide were obtained by the Steglich esterification to incorporate cinnamoyl groups and heterocyclic rings with nitrogen, oxygen and sulfur atom, which offered appreciable inhibition of HBV DNA replication.[6,7,33] Compound **1a** was esterified with acids in the presence of 4-dimethylaminopyridine (DMAP) and *N*′,*N*′-dicyclohexylcarbodiimide (DCC) in anhydrous dichloromethane (Scheme 1). Generally, 19-*O*-subsituted derivatives were the main products; 3, 19-*O*-disubsituted derivatives were obtained with low yields at the same time. Positions of the substituents can be determined by the changes of the chemical shifts of H-3 and H-19 in ^{1}H NMR spectrum. For example, the chemical shifts of H-3 and H-19a of 19-*O*-substituted derivative **2e** appeared at δ_H 3.33 and 4.37 in contrast to δ_H 4.88 and 4.40 of 3, 19-*O*-disubstituted derivative **3a**. When compound **1b** was treated under the Steglich esterification conditions, derivatives **4a**–**6f** were obtained by losing hydroxyl group at C-14 and forming carbon–carbon double bond between C-14 and C-15 (Scheme 2).[28] Other acylation products **2j**, **3d**–**3g**, **4g**, **5g**, **6c**, **6e** and **6f** were achieved with anhydrides and catalytic amount of DMAP in anhydrous pyridine. Compounds **1a** and **1b** were treated under Pfitzner–Moffatt oxidation condition with DCC, dimethyl sulfoxide (DMSO), trifluoroacetic acid (TFA) in anhydrous pyridine and dichloromethane to reveal effects of transforming hydroxyl groups at C-3 and C-19 into carbonyl groups (Scheme 3). On the ring C, compounds **9a** and **9b** were obtained in the presence of formaldehyde or acetone under sodium carbonate (Scheme 4). To investigate the influence of lactone ring on anti-HBV activity, compound **1b** was dealt with sodium hydroxide in water to obtain product **10** with lactone ring opened. On the ring B, carbon–carbon double bond between C-8 and C-17 was epoxidized by the *m*-chloroperoxybenzoic acid (*m*-CPBA) in dichloromethane at room temperature to produce compound **11**.[34] The purity (higher than 90%) of all the derivatives was determined by HPLC (normalization method with no obvious impurity peak) or TLC with three different solvent systems (only one spot under UV detection and sprayed with 10% H_2SO_4).

Derivatives of dehydroandrographolide (**1a**) and andrographolide (**1b**) were evaluated for their anti-HBV activity, namely inhibiting the secretions of HBsAg and HBeAg, and HBV DNA replication on HepG 2.2.15 cells in vitro with tenofovir as the positive control.[13] The anti-HBV activity and cytotoxicity were summarized in Table 1.

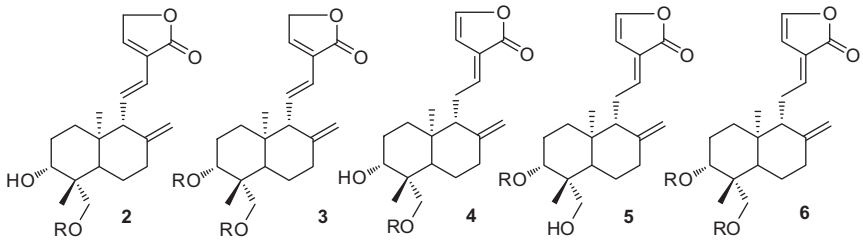
19-*O*-Cinnamoyl analogue **2a** showed high activity against HBV DNA replication with IC_{50} value of 14.6 μM and 50% cytotoxicity concentration (CC_{50}) value of 183 μM. After the introduction of methoxyl group into cinnamoyl group at the C-19 position, the cytotoxicity of analogues **2b** and **2c** decreased obviously (CC_{50} >1970 and 1706 μM) compared with compound **2a**, indicating that the methoxyl group play a crucial role in reducing cytotoxicity. The above conclusion was further supported by 19-*O*-(2′-methoxy) nicotinyl analogue **2f** with lower cytotoxicity than 19-*O*-nicotinyl analogue **2e**. Compound **2c** with 3,4,5-trimethoxycinnamic group at C-19 possessed noticeable inhibition on HBV DNA replication with the IC_{50} value of 10.3 μM and lower cytotoxicity resulting in a SI value higher than 165.1, demonstrating that both *m*-methoxyl and *p*-methoxyl groups could enhance activity against HBV DNA replication. However, 19-*O*-(3′-chloro) cinnamoyl analogue **2d** exhibited higher cytotoxicity and weaker activity due to the introduction of halogen into the substituent. The heteroatomic rings at C-19 position play an important role in suppressing HBV DNA replication and secretions of HBsAg and HBeAg in agreement with the high activities of compounds **2e**, **2g** and **2h** with the IC_{50} values of 22.1, 9.3 and 22.1 μM. In contrast to 19-*O*-substituted analogues, the 3, 19-*O*-disubstituted compounds **3a**–**3g** showed dramatic decrease of anti-HBV activity resulting from the disubstituents at C-3, and C-19.

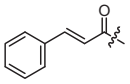
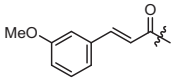
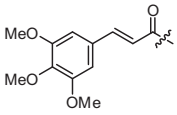
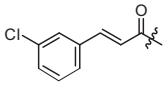
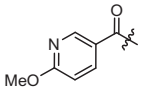
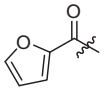
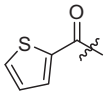
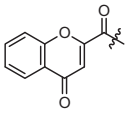
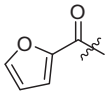
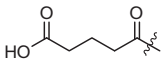
For further investigation, 23 andrographolide derivatives were obtained. 19-*O*-Cinnamoyl and 19-*O*-(3′,4′,5′-trimethoxy) cinnamoyl derivatives **4a** and **4b** exhibited increased activity with the IC_{50} values of 46.58 and 22.25 μM against HBV DNA replication. 19-*O*-Nicotinoyl (**4c**), 19-*O*-2′-furoyl (**4d**) and 19-*O*-2′-thenoyl (**4e**) derivatives showed significant inhibition on HBV DNA replication with higher SI values of 126.0, 100.5, and 104.9, and lower cytotoxicity than derivatives **2e**, **2g** and **2h** with the same substituents, inferring that the cytotoxicity of these derivatives is closely related to the conjugated double bonds between C-12 and C-15 or C-11 and C-14. According to the principle of bioisosteric replacement, 19-*O*-benzoyl analogue **4f** was prepared and exhibited similar activity against HBV DNA replication with the IC_{50} value of 20.6 μM and lower SI value compared with compounds **4c**, **4d** and **4e**, indicating that heteroatomic rings containing N, O and S are profitable for decreasing cytotoxicity. In contrast to 19-*O*-valeryl analogue **4h**, the obviously enhanced activity of 19-*O*-acetyl analogue **4g** suggested that the length of alkyl chain could influence the anti-HBV activity. Compounds **2a**–**2j** and **4a**–**4g** showed different cytotoxicity, suggesting that the conjugated double bonds are closely related to their cytotoxicity but varied with the substituents at C-19. The high cytotoxicity of 3-*O*-substituted ones (**5a**–**5h**) indicated that the substituent at C-19 and free hydroxyl group at C-3 are necessary for maintaining low cytotoxicity. Among 3, 19-disubstituted derivatives, 3, 19-*O*-disuccinyl (**6e**) and 3, 19-*O*-di (2′-carboxyl) benzoyl (**6f**) analogues exhibited the improvement of activity, which indicated the importance of free carboxyl group for anti-HBV activity.

In comparison with oxidative products **7a**, **8a** and **8b**, the IC_{50} value of compound **7b** was improved to 13.4 μM due to the presence of a free hydroxyl group at C-3. The decreased activity of derivatives **9a** and **9b** supported the importance of methylene at C-15. Derivative **10** with the lactone ring opened showed the similar activity and cytotoxicity to the parent compound **1b**, suggesting that the lactone ring plays little role in anti-HBV activity. Double bond between C-8 to C-17 in ring B was indispensable owing to the reduced inhibition on HBV DNA replication of epoxide **11**.

Although dehydroandrographolide **1a** and andrographolide **1b** were inactive against the secretions of HBsAg and HBeAg, 14 derivatives with inhibitory activity on HBsAg secretion and 19 derivatives inhibited HBeAg secretion were obtained. Compounds **2a**, **4e** and **9a** inhibited HBsAg secretion with the SI values in the range of 10.2–20.3, and compounds **2h** and **4e** suppressed HBsAg

Table 1
Anti-HBV activity and cytotoxicity of dehydroandrographolide and andrographolide derivatives in vitro[a]



Compd	R	CC_{50}[b] (μM)	HBsAg[c]		HBeAg[d]		DNA replication	
			IC_{50}[e] (μM)	SI[f]	IC_{50}[e] (μM)	SI[f]	IC_{50}[e] (μM)	SI[f]
1a		197	—[g]	—	—	—	22.6	8.7
1b		198	—	—	—	—	54.1	3.7
2a		183	18.0	10.2	28.9	6.3	14.6	15.5
2b		>1970	>1970	—	1476	—	>513	—
2c		>1706	—	—	201	>8.5	10.3	>165.1
2d		147	207	—	59.8	2.5	136	5.5
2e		113	40.0	2.8	63.5	1.8	22.1	5.1
2f		1339	>2313	—	217	6.2	>584	—
2g		92	19.2	4.6	77.5	1.2	9.3	9.9
2h		557	162	3.4	29.2	19.1	22.1	25.2
2i		171	—	—	177	1.0	36.6	4.7
2j		225	565	—	2233	—	69.5	3.2
3a		>1919	>1919	—	>1919	—	>664	—
3b		1112	1407	—	1864	—	242	4.6
3c		>1537	1448	>1.0	822	>1.8	185	>8.3
3d		757	1010	—	1390	—	455	—
3e		>1714	>1714	—	>1714	—	>429	—

(*continued on next page*)

Table 1 (*continued*)

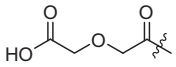
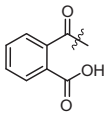
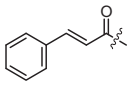
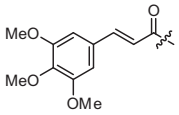
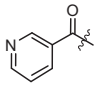
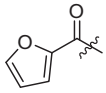
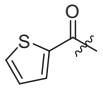
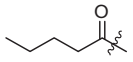
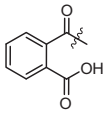
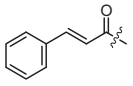
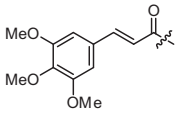
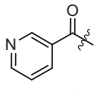
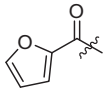
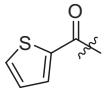
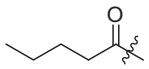
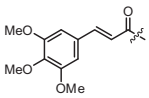
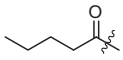
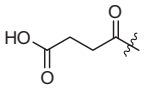
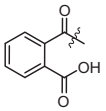
Compd	R	CC_{50}[b] (μM)	HBsAg[c]		HBeAg[d]		DNA replication	
			IC_{50}[e] (μM)	SI[f]	IC_{50}[e] (μM)	SI[f]	IC_{50}[e] (μM)	SI[f]
3f		>1933	>1933	–	>1933	–	>483	–
3g		>1624	–	–	–	–	104	>15.6
4a		271	210	–	516	–	46.6	5.8
4b		325	–	–	–	–	22.3	14.6
4c		2054	>1137	–	>974	–	16.3	126.0
4d		>2150	–	–	>949	–	21.4	>100.5
4e		2466	121	20.3	19.7	125	23.5	104.9
4f		82	880	–	123	–	20.6	4.0
4g		334	280	1.2	289	1.1	44.1	7.6
4h		225	565	–	2233	–	69.5	3.2
4i		450	1317	–	293	1.5	39.4	11.4
5a		179	466	–	1169	–	30.7	5.8
5b		211	753	–	518	1.6	53.1	3.0
5c		337	261	1.3	714	–	38.4	8.8
5d		42	777	–	586	–	25.2	1.7
5e		257	41	6.2	>2196	–	8.1	31.8
5f		103	877	–	118	–	21.6	4.8
5g		872	318	2.7	241	3.6	44.4	19.6
5h		126	625	–	208	–	69.8	1.82

Table 1 (*continued*)

Compd	R	CC_{50}[b] (μM)	HBsAg[c]		HBeAg[d]		DNA replication	
			IC_{50}[e] (μM)	SI[f]	IC_{50}[e] (μM)	SI[f]	IC_{50}[e] (μM)	SI[f]
6a		>1831	>1831	–	>253	–	>458	–
6b		1374	1641	–	1217	1.1	–	–
6c		297	251	1.2	260	1.1	39.6	7.6
6d		757	1010	–	1390	–	455	–
6e		32	1158	–	243	–	47.6	–
6f		>2042	–	–	218	>9.4	22.0	>92.8
7a		275	394	–	–	–	263	1.1
7b		122	1840	–	1948	–	13.4	9.1
8a		964	518	1.9	–	–	169	5.7
8b		155	951	–	815	–	51.3	3.0
9a		954	74	13.0	860	1.1	154	6.2
9b		122	–	–	562	–	394	–
10		182	–	–	–	–	23.2	7.8
11		1839	1294	1.4	–	–	78.0	23.6
Tf[h]		>1716	1389	>1.2	1238	>1.4	0.71	>2417.3

[a] Values are means of two independent experiments.
[b] CC_{50} is 50% cytotoxicity concentration in HepG 2.2.15 cells.
[c] HBsAg: hepatitis B surface antigen.
[d] HBeAg, hepatitis B e antigen.
[e] IC_{50} is 50% inhibitory concentration.
[f] SI (selectivity index) = CC_{50}/IC_{50}.
[g] No SI can be obtained.
[h] Tenofovir as the positive control.

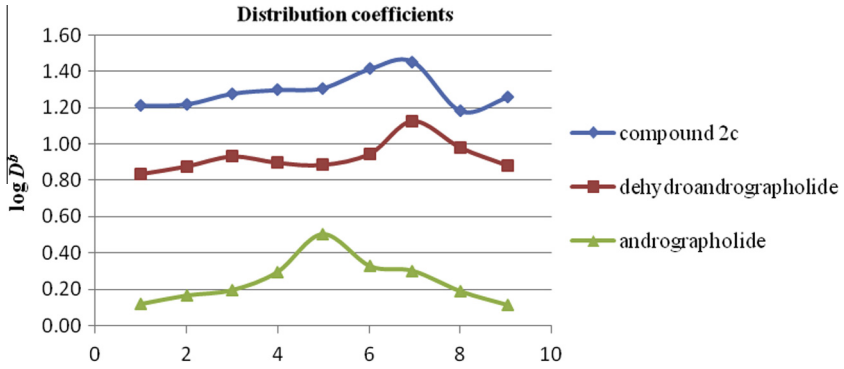
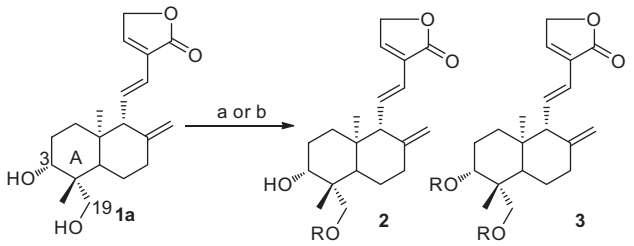


Figure 2. Octanol-buffer distribution coefficients of dehydroandrographolide, andrographolide and compound **2c**[a]. [a]Values are means of two independent experiments. Log*D* [b]is value for log octanol-buffer distribution coefficients at pH 1–9.

secretion with the SI values of 19.1 and 125.0. The most active compounds **2a**, **2h** and **4e** were all 19-*O*-substituted derivatives, suggesting that modification on C-19 is preferable for inhibiting HBsAg and HBeAg secretions.

The logarithm of the octanol–water partition coefficient (log*P*) is an important pharmaceutical parameter in evaluating solvency, absorption and transport of drugs. According to Lipinski's rule, the preferred log*P* value is less than 5, and higher log*P* will lead to poor absorption and permeation.[35] Ghose's analysis of different classes of drug molecules suggested that the qualifying range of the log*P* value is between −0.4 and 5.6, with an average of 2.52.[36] The logarithm of the octanol-buffer distribution coefficient (log*D*) is the ratio of the concentrations of all forms of the compound (ionized plus un-ionized) in each of the two phases at different pH. Compounds **1a**, **1b** and the most potent compound **2c** were measured for values of log*P* and log*D*. Solutes were equilibrates between octanol and water, or octanol and buffer using the shake-flask method.[37] The concentration of each compound in octanol was

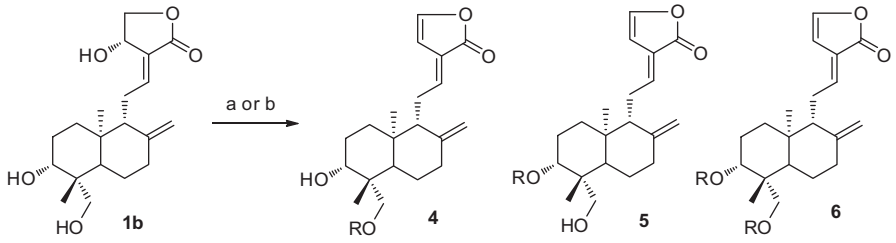


Scheme 1. Reagents and conditions: (a) corresponding acids, DMAP, DCC, CH_2Cl_2, rt, 47–62% for **2a**–**2i**, 26–28% for **3a**–**3c**; (b) anhydride, DMAP, anhydrous pyridine, reflux, 40% for **2j**, 55–57% for **3d**–**3g**.

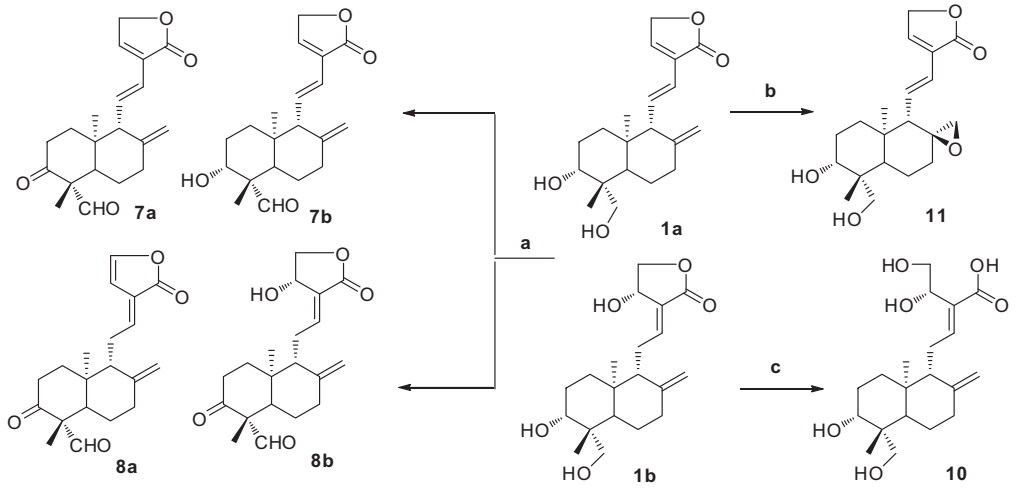
determined by HPLC method.[38,39] The log*P* values of compounds **1a**, **1b** and **2c** were determined to be 1.44, 0.52, and 1.78 at 30 °C. Among these compounds, compound **2c** may have the high absorption and permeation for its log*P* value was closed to the average value of 2.52. As shown in Figure 2, compound **2c** expressed the highest log*D* value near pH 7.0, indicated the easier absorption in the small intestine than in the stomach.

In summary, 48 derivatives of dehydroandrographolide and andrographolide were synthesized and evaluated for their anti-HBV activity, which provided 14 active derivatives inhibiting HBsAg secretion, 19 active derivatives suppressing HBeAg secretion and 38 active derivatives inhibiting HBV DNA replication. Interestingly, compound **4e** could inhibit not only HBsAg and HBeAg secretions but also HBV DNA replication with SI values of 20.3, 125.0 and 104.9. Furthermore, the most potent compound **2c** with SI value higher than 165.1 inhibiting HBV DNA replication was revealed to possess the optimal log*P* value of 1.78 and log*D* values, which is deserved to be further investigated.

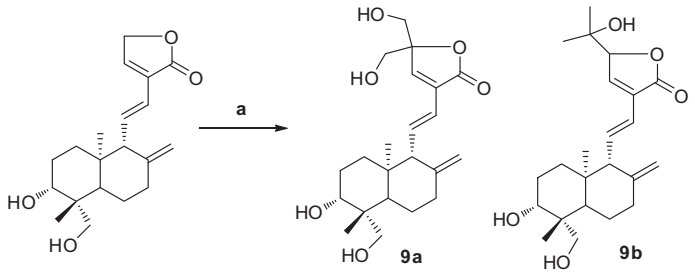
According to the results mentioned above, SARs were summarized in Figure 3 and following conclusion could be drawn: (1) 19-*O*-substituted compounds with the free hydroxyl group at C-3 could provide higher anti-HBV activity than other analogues. (2) 3,4,5-Trimethoxycinnamoyl, nicotinoyl, 2-furoyl and 2-thenoyl groups are favorable to enhance anti-HBV activity. (3) The double



Scheme 2. Reagents and conditions: (a) corresponding acids, DMAP, DCC, CH_2Cl_2, rt, 31–50% for **4a**–**4f** and **4h**, 10–12% for **5a**–**5f** and **5h**, 13–23% for **6a**, **6b** and **6d**; (b) anhydride, DMAP, anhydrous pyridine, reflux, 21–31% for **4g** and **4i**, 25% for **5g**, 20–23% for **6c**, **6e** and **6f**.



Scheme 3. Reagents and conditions: (a) DCC, DMSO, TFA, anhydrous pyridine, CH_2Cl_2, rt, 34–61%; (b) *m*-CPBA, CH_2Cl_2, rt, 63%; (c) NaOH, H_2O, reflux, 86%.



Scheme 4. Reagents and conditions: (a) methyl aldehyde or acetone, sodium carbonate, reflux, 79–80%.

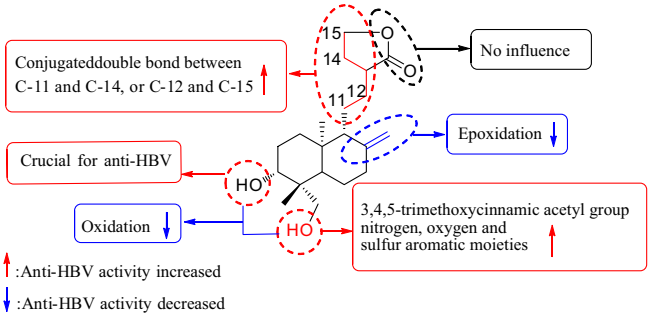


Figure 3. Structure–activity relationships of dehydroandrographolide and andrographolide derivatives anti-HBV activity.

bond between C-8 and C-17, and the conjugated double bonds between C-11 and C-14, or C-12 and C-15 are necessary for maintaining anti-HBV activity and decreasing cytotoxicity. (4) The methylene at C-15 is necessary for anti-HBV activity. This study indicated that dehydroandrographolide and andrographolide derivatives had potent anti-HBV activity, and offered valuable information for seeking non-nucleoside anti-HBV drug candidates.

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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.2014.03.060.

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