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Synthesis of celastrol derivatives as potential non-nucleoside hepatitis B virus inhibitors

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Running Head

Celastrol derivatives as anti-hepatitis B virus agents

Abstract

A series of para-quinone methide (*p*QM) moiety and C-20 modified derivatives of celastrol were synthesized and evaluated for their inhibitory effect on the secretion of HBsAg and HBeAg as well as the inhibitory effect against HBV DNA replication. The results suggested that amidation of C-20 carboxylic group could generate derivatives with good anti-HBV profile, among them compound **14** showed the best inhibitory activity on the secretion of HBsAg (IC_{50} = 11.9 μM) and HBeAg (IC_{50} = 13.1 μM) with SI of 3.3 and 3.0, respectively. In addition, **14** also showed potent inhibitory effect against HBV DNA replication (48.5 ± 15.1%, 25μM). This is, to our knowledge, the first report of celastrol derivatives as potential non-nucleoside HBV inhibitors.

Keywords

Tripterygium wilfordii Hook, F., Celastrol, Hepatitis virus B, Inhibitor

1. Introduction

Viral hepatitis type B is a serious infectious disease caused by hepatitis B virus (HBV) (Dienstag, 2008). It is reported by the World Health Organization (WHO) that more than 2 billion people have been infected with HBV in their lives (Shepard, et al., 2006). Due to its characters of high incidence, long course and difficulty to cure,

HBV infection has become a major threat to the public health. Especially in China, HBV infection has become a serious issue with about 28 million people have chronic hepatitis B (CHB), and 90 million HBV carriers (Jia, et al., 2017). Worse yet, long-term development of Hepatitis B can lead to acute or chronic viral hepatitis, severe hepatitis, liver cirrhosis (LC) and hepatocellular carcinoma (HCC) (Rehermann, et al., 2005). Every year, more than 1 million people death from HBV infection relating diseases such as liver failure, cirrhosis, and hepatocellular carcinoma. Currently, drugs that used for the treatment of HBV infection include interferon, immunomodulators, and DNA polymerase inhibitors. Although, interferon has dual effects of immunomodulation and antivirus, the need for parenteral administration, the effective only to 30%-40% people, and the adverse side effects greatly limited its clinical application (Chan, et al., 2005; Liu, et al., 2014). Mechanistically, nucleotide HBV DNA polymerase inhibitors such as lamivudine, adefovir, entecavir and tenofovir exert their antivirus activity though targeting viral DNA polymerase, and thus would result in the development of drug-resistant virus after long-term treatment (Zhang, et al., 2014), which make them not ideal. Therefore, the therapies for HBV remain unsatisfactory, and the development of anti-HBV agents with novel structures and mechanisms of action is of the top priority

Tripterygium wilfordii Hook, F. (TWHF), also known as *Lei Gong Teng* or *Thunder God Vine*, is a vine-like medicinal plant whose extracts have been used to treat autoimmune and inflammatory diseases such as rheumatoid arthritis (RA) for centuries in traditional Chinese medicine (TCM) (Goldbach-Mansky, 2009; Zhou, et al., 2018). It is reported that celastrol (Hou, et al., 2020a), triptolide (Kupchan, et al., 1972), triptonide (Kupchan, et al., 1972), 15-hydroxytriptolide (Niu, et al., 2015), triptriolide (Wang, et al., 2019; Yang, et al., 2018; Yang, et al., 2019) and triptophenolide (He, et al., 2016) are the major bioactive component of TWHF (Figure 1). From a structural point of view, celastrol is a pentacyclic triterpenoid that decorated with a bioactive *para*-quinone methide (*p*QM) moiety. Previous studies have reported that *p*QM could interact with DNA (Huang, et al., 2016) or target proteins residues by π-π stacking (Duan, et al., 2013), hydrophobic interactions, hydrogen bonds and/or covalent addition (Zhao, et al., 2015). Therefore, celastrol has been shown to be effective against various human diseases via interacting with many different cellular targets. It is reported that celastrol could suppress the NF-κB activation by interacting with IKK and showed anti-inflammatory and anti-cancer activities (Lee, et al., 2006). Additionally, it could disrupt the interaction of Hsp90 and CDC37 through binding to the C-terminal domain of Hsp90. It could also induce apoptosis in multiple cancer cells by activating c-Jun N-terminal kinase and suppressing PI3K/Akt signaling pathways (Kannaiyan, et al., 2011). Recently, celastrol was reported to have the ability to increase leptin sensitivity, thus has the potential to be developed as a anti-obesity agent (Kyriakou, et al., 2011; Liu, et al., 2011). Taken together, celastrol should be a promising bioactive natural product for new drug discovery. However, unlike its relative, triptolide, on which lots of total synthesis and structural modifications have conducted in the past two decades (Chen, et al., 2012; Hou, et al., 2019b; Kaloun, et al., 2016; Liu, et al., 2018; Ning, et al., 2018; Patil, et al.,2015; Wang, et al., 2017; Xu, et al., 2014a; Xu, et al., 2014b; Xu, et al., 2014c; Xu, et al., 2014d; Xu, et al., 2019; Xu, et al., 2017; Zhang, et al., 2019; Zhou, et al., 2012), and some triptolide derivatives have already entered clinic for the treatment of challenging cancer and/or rheumatoid arthritis (RA) (Carter, et al.,

2012; Pao, et al., 2019; Patil, et al., 2019; Wang, et al., 2012;; Zhou et al., 2005). So far, there are only one total synthesis (Camelio, et al., 2015) and several chemical modifications of celastrol have been reported (Figueiredo, et al., 2017a; Jiang, et al., 2016; Kyriakou, et al., 2018; Li, et al., 2015; Li, et al., 2018; Pang, et al., 2018; Shan, et al., 2017; Tang, et al., 2015; Zhang, et al., 2018; Zhu, et al., 2017), which are largely focused on its anticancer activity. In order to further explore the promising multiple biological activities of celastrol and with the aim to find new anti-HBV agents with novel chemical structure and mechanism of action, herein we reported the synthesis and anti-HBV activity evaluation of a series of *p*QM moiety and C-20 modified celastrol derivatives (Figure 2).

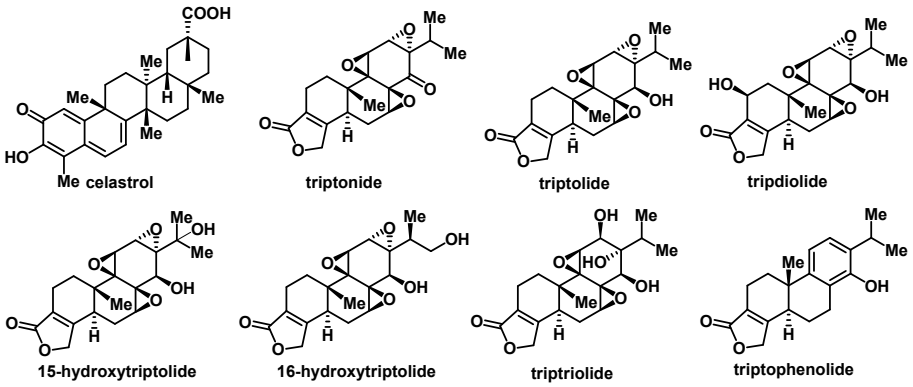


Figure 1. Representative natural products isolated form TWHF

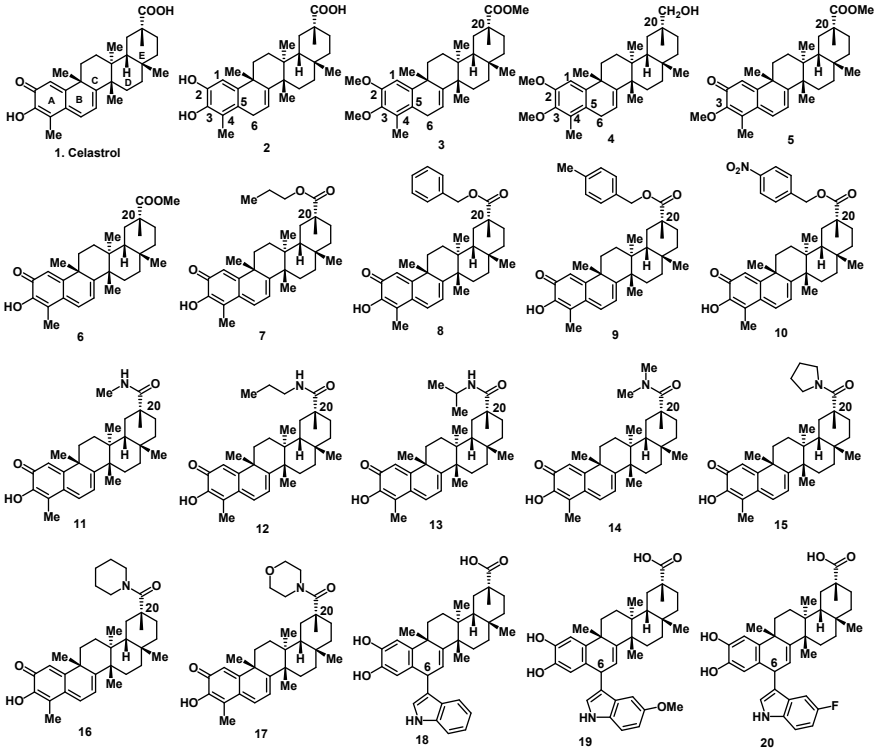
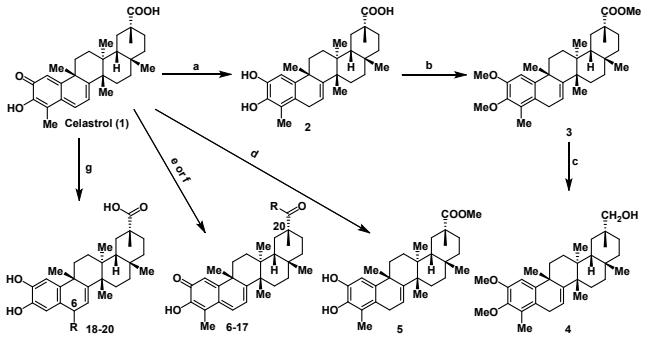


Figure 2. Celastrol derivatives **2-20**.

2. Result and discussion

The syntheses to access the target celastrol derivatives **2-20** was depicted in Scheme 1. Firstly, in order to

probe the effect of the para-quinone methide (*p*QM) moiety on anti-HBV activity, A-ring aromatic derivative **1**-**4** were synthesized by reducing of celastrol **1** in the presence of sodium borohydride ($NaBH_4$), and thus provided derivative **2** in almost quantitative yield (Figueiredo, et al., 2017b). Methylation of the phenolic C-3 hydroxyl group along with the C-20 carboxylic acid group gave ester **3**, which was reduced by lithium aluminum hydride (LAH) provided C-20 hydroxymethyl substituted derivative **4**. Directly methylation of the C-3 hydroxyl group and the C-20 carboxylic acid group of celastrol in the presence of sodium hydride and methyl iodide afforded derivative **5**. $AlCl_3$-Catalyzed Friedel-Crafts alkylation of the C-6 moiety of celastrol with various indoles afforded derivatives **18**-**20** in moderate yield. These derivatives can be used to probe the influence of the *p*QM moiety on the anti-HBV activity as well as the substituent effect of C-6. Finally, in order to probe the substituent properties of the C-20 carboxylic acid group, esters derivatives **6-10** and amide derivatives **11-17** were synthesized via alkylation of the C-20 carboxylic acid group with various alkyl halides, and/or condensation of the C-20 carboxylic acid group with various amines, respectively.



Scheme 1. Synthesis of celastrol derivatives. a) $NaBH_4$, MeOH, r.t.; b) MeI, K_2CO_3, DMF, r.t.; c) LAH, THF, r.t.; d) MeI, NaH, DMF, r.t.; e) alkyl iodides, $NaHCO_3$, DMF, r.t; f) amines, HATU, DIPEA, DMF, r.t.; g) indoles, $AlCl_3 \cdot 6H_2O$ (5 mol%), DCM, r.t..

All the synthesized celastrol derivatives were tested for their potential anti-HBV activity, namely inhibiting the secretion of HBsAg (HBV surface antigen, which indicates current hepatitis B infection), and HBeAg (HBV e antigen, which is an indicator of active viral replication. It means the person infected with Hepatitis B can likely transmit the virus onto another person), and also their inhibitory effect on HBV DNA replication in HepG 2.2.15 cells using lamivudine (3 TC, a clinically popular anti-HBV agent) as a positive control. The anti-HBV activity of the celsatrol derivatives was expressed as the concentration of derivative required for 50% inhibition (IC_{50}) of HBsAg or HBeAg secretion. The cytotoxicity of each celastrol derivatives was defined as the concentration of derivative required to kill 50% (CC_{50}) of the HepG 2.2.15 cells. While, the selectivity index (SI), a major pharmaceutical parameter that indicates possible future clinical development, was defined as the ratio of CC_{50} to IC_{50}.

As shown in Table 1, celastrol **1** was more effective on the inhibiting the secretion of HBsAg and HBeAg than that of positive control 3TC with the IC_{50} values of 12.7 and 15.1 μM, respectively. However, it showed very poor selectivity index (SI = 0.4 and 0.3 for HBsAg and HBeAg, respectively) and inhibitory rate against HBV DNA replication (25.9 ± 3.8%). After aromatization of the ring-A, the related derivatives **2**-**4** all lost their

inhibitory activity on the secretion of HBsAG and HBeAg as well as their cytotoxic activity, except for compound **4**, which only showed moderate inhibitory activity against the secretion of HBsAg and HBeAg with IC_{50} values of 68.4 μM and 72.5 μM, respectively. Esterification of the C-20 carboxylic group, the resulting derivatives (**6**-**10**) generally showed comparable inhibitory activity and cytotoxic activity as celastrol, except of compound **5** (SI of 1.1 and 1.2) which showed a little increased selectivity than celastrol. These results suggested that esterification of the C-20 carboxylic group could retain the cytotoxic activity of celastrol, however, as listed in Table 1, the antivirus activity are poor. Gratefully, amidation of the C-20 carboxylic group with either primary amines or secondary amines, the resulting derivative generally showed increased selectivity (SI). Among all the tested amide derivatives, C-20 dimethylcarbamoyl substituted compound **14** showed the best inhibitory profile on the secretion of HBsAg (IC_{50} = 9.2 μM) and HBeAg (IC_{50} = 10.0 μM) with SI of 3.3 and 3.0, respectively. In addition, compound **14** also showed highest inhibition rate against HBV DNA replication (48.5 ± 15.1%) among all the tested derivatives. These results suggested that amidaiton of the C-20 carboxylic group could generate celastrol derivative with good inhibitory activity and selectivity for the anti-HBV activity. This is probably due to the introduction of the basic nitrogen atom, which could interaction with the relative protein target the responsible for anti-HBV activity. Disappointedly, the introduction of the indoles at C-6 position of celastrol destroyed dramatically the inhibitory activity on the secretion of HBsAg and HBeAg, among the tested derivative, only derivative **19** showed moderate inhibitory activity, this is probably due to the aromatization of the A-ring that lead to the destroy of the *p*QM moiety, which is generally recognized as the key bioactive structural element for the various bioactivity of celastrol.

According to the experimental results mentioned above, preliminary structure-activity relationship (SAR) were summarized as followed: (1) the *para*-quinone methide (*p*QM) moiety of celastrol play a key role in retaining its cytotoxicity as well anti-HBV activity, aromatization of the ring-A or introduction of substituents on C-6 would exert negative effect on anti-HBV activity; (2) Esterification of the C-20 carboxylic group could retain the cytotoxic activity of celastrol, but poor anti-HBV activity; (3) Amidation of the C-20 carboxylic group could generated derivatives with good anti-HBV activity. In general, these preliminary SAR results would provide valuable information for further modification of celastrol to find more potent anti-HBV derivatives.

Table 1. Anti-HBV activity of derivatives **2-20**.

Compd	CC_{50}[a] (μM)	HBsAg		HBeAg		Inhibition rate (%)[d] (25 μM)
		IC_{50}[b](μM)	SI[c]	IC_{50}[b](μM)	SI[c]	
1	5.2±0.9	12.7±2.2	0.4	15.1±3.0	0.3	25.9±3.8%
2	>100	>100	---	>100	---	---
3	>100	91±	---	>100	---	---
4	79.0±17.4	68.4±21.3	---	72.5±12.3	---	---
5	23.5±4.7	22.1±6.2	1.1	19.9±5.5	1.2	10.5±4.1%
6	17.1±1.8	19.4±5.1	0.9	29.8±3.7	0.3	9.8±1.5%
7	6.3±2.3	23.1±5.4	0.3	25.4±5.0	0.2	---
8	4.1±1.2	11.5±3.1	0.4	17.3±4.4	0.2	---
9	3.6±0.6	9.2±1.7	0.4	10.0±3.5	0.4	---
10	7.7±3.9	15.1±3.5	0.5	14.4±4.4	0.5	---
11	15.6±4.4	18.4±5.1	0.8	20.3±3.9	0.8	13.2±5.0%
12	12.9±4.5	21.4±5.4	0.6	29.7±6.0	0.4	17.2±7.4%
13	29.3±6.3	15.9±2.3	1.8	17.2±3.8	1.7	35.2±8.7%
14	39.1±8.6	11.9±2.6	3.3	13.1±3.3	3.0	48.5±15.1%
15	17.1±9.0	24.1±6.1	0.7	21.7±7.7	0.8	25.9±9.4%
16	12.4±3.3	33.1±6.3	0.4	25.2±5.9	0.5	---
17	11.3±1.1	15.7±3.2	0.7	14.6±1.7	0.8	26.0±8.6%
18	74.0±14.9	>100	---	>100	---	---
19	69.0±8.8	67.3±5.4	---	64.4±9.6	---	---
20	91.0±12.5	>100	---	97.9±11.9	---	---
3TC[e]	493.5±65.4	177.4±33.3	2.8	192.1±45.1	2.7	89.1±22.4%

[a] *CC_{50}: CC_{50} is 50% cytotoxicity concentration in HepG2 2.2.15 cells.*

[b] *IC_{50}: Concentration of compound required for 50% inhibition of HBsAg (HBV surface antigen) or HBeAg (HBV e antigen) secretion.*

[c] *SI: Selective index, the ratio of CC_{50}/IC_{50}.*

[d] *Inhibitory effect of celastrol on HBV DNA level.*

[e] *Lamivudine (3TC) was used as positive control.*

3. Conclusion

In summary, we have designed and synthesized a series of *para*-quinone methide (*p*QM) moiety and C-20 modified celastrol derivatives and evaluated their inhibitory activity on the secretion of HBsAg and HBeAg for the first time. The present results suggested that the aromatization of the A-ring would exert negative influence on the inhibitory activity. Esterification of the C-20 carboxylic group could generate derivatives with increasing inhibitory activity, but decreasing the selectivity. Amidation of the C-20 carboxylic group could give derivatives with increased inhibitory profile. Among all the tested derivatives, **14** showed the best inhibitory activity on the secretion of HBsAg (IC_{50} = 11.9 μM) and HBeAg (IC_{50} = 13.1 μM) with SI of 3.3 and 3.0, respectively. In addition, **14** also showed potent inhibitory activity against HBV DNA replication (48.5 ± 15.1%, 25 μM). Over

all, these results indicated that derivative **14** might be used as a starting point for the discovery of non-nucleoside anti-HBV agents. It is also important to address the exact molecular target, through which derivative **14** exerts its anti-HBV activity, the related studies are currently ongoing in our laboratory and will be disclosed in the due course.

Conflict of interest

The authors of this manuscript declare no conflict of interests.

SUPPORTING INFORMATION

Additional supporting information may be found online in the Supporting Information section at the end of the article.

Data Availability Statement

No additional data are available.

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