Advances in structural modifications of celastrol

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Abstract

Celastrol, a quinone methide triterpenoid from the Chinese medicinal root bark of *Tripterygium wilfordii*, possesses beneficial therapeutic properties and affects numerous cellular pathways. Recently, structure and activity relation studies based on celastrol semi-synthetic derivatives have contributed greatly to our understanding of its mechanism of action and cellular targets. These modifications have resulted in better activity and improvement of the pharmacokinetic properties of celastrol. This review will mainly cover advances in the structural modifications of celastrol at the C-20 carboxylic acid functionality, alterations of the A ring, and modifications at C-6.

Keywords: Celastrol, triterpenoids, antitumor activity

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1. Introduction

Over the past few decades the high activity of extracts from the medicinal herb *Tripterygium wilfordii* have attracted intensive attention. Celastrol (**1**), a quinone methide triterpenoid extracted from the root bark of the traditional Chinese medicine “Thunder of God”, has been used traditionally for the treatment of various diseases such as inflammation, joint pain, edema,

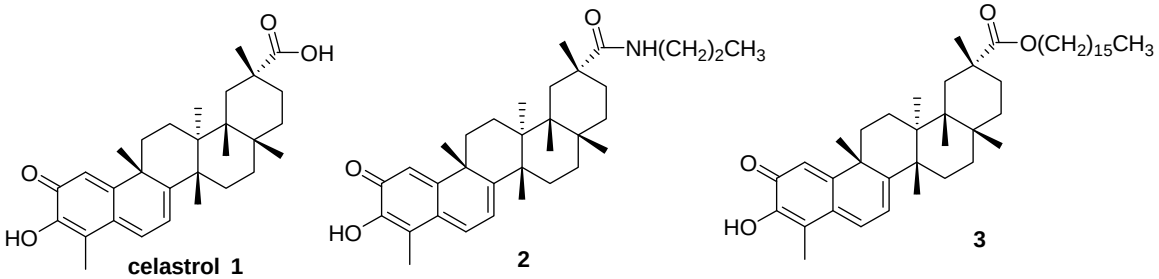
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chills, and rheumatoid arthritis.[1-6] Celastrol shows a great variety of biological effects such as anti-oxidant,[7,8] anti-tumor,[9-12] anti-obesity,[13,14] cardiac protection,[15,16] anti-inflammatory[17-19] and antiangiogenic[20,21] activities, in both *in vitro* and *in vivo* models. It exhibits astonishing clinical potential as a therapeutic and chemopreventive agent toward various cancers. Celastrol has also been reported to inhibit growth,[22,23] induce apoptosis,[24-27] and suppress invasion and metastasis[28-30] of tumor cells. It is extremely effective towards many molecular targets, but there are some inherent deficiencies including high toxicity, poor stability and water solubility which impede its further application.[31]

The screening and assignment of active new derivatives from synthetic or semi-synthetic strategies has attracted intense interest from medicinal scientists. This review provides a summary of the recent advances in celastrol structural modifications of functionality of the C-20 carboxylic acid, alteration of the A ring at C-2, and modification of the B ring at C-6.

2. Modification of the C-20 Carboxylic Acid Group Functionality

In order to improve solubility, reduce toxicity, and develop new activities of celastrol analogues, simple modifications strategies are still the esterification or amidification of the 20-carboxylic acid group,[32] for example, the *N*-propylamide (**2**) and cetyl ester (**3**), which showed antiproliferation activity similar to or better than that of celastrol in various cancer cell line assays (Scheme 1).[33,34]

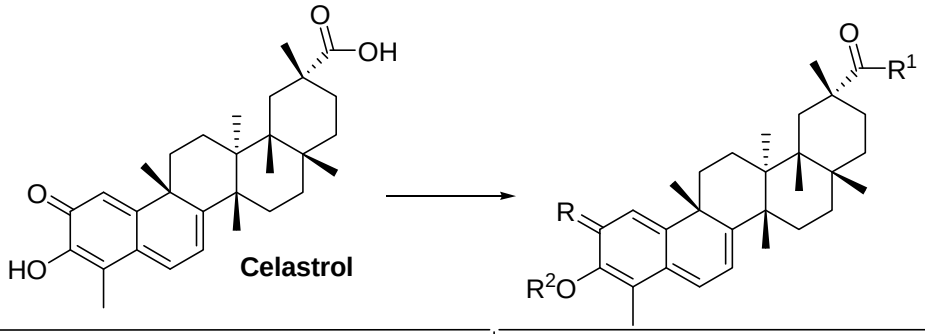


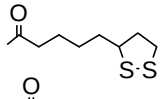
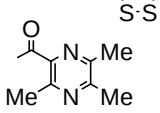
Scheme 1

In 2010, Xu and coworkers prepareded a series of celastrol analogues (**4-16**) and investigated their neuroprotective effect against t-BHP-induced cell damage in neuronal PC12 cells (Scheme 2).[35] Cells treated with celastrol and compound **15** were subjected to Western blot analysis to check Hsp70 protein expression. Celastrol enormously increased the Hsp70 expression at 0.4 and 1.6 μM. Compound **15** improved Hsp70 expression at a concentration as low as 0.1 μM, but its neuroprotective effect was not evident until its concentration reached 0.4 μM. Compared with celastrol, **15** is more effective in protecting cells from t-BHP-induced cell damage and against t-BHP-induced cytotoxicity. Compound **15** up-regulated Hsp70 protein

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expression dose-dependently and probably helped to strengthen the cellular capability to resist oxidative stress, and eventually promote cell survival. These results suggest that **15** may be used as a potential candidate for intervention in neurodegenerative diseases.



Compound	R	R^1	R^2	Compound	R	R^1	R^2
4	O	OCH_3	H	**12**	O	OH	COC_3H_7
5	O	OCH_3	H	**13**	O	OH	$COCH(CH_3)_2$
6	O	OC_3H_7	H	**14**	O	OH	
7	O	OC_4H_9	H				
8	O	OCH_2Ph	H	**15**	O	OH	
9	O		H				
10	O	OH	$COCH_3$	**16**	NOH	OH	H
11	O	OH	COC_2H_5				

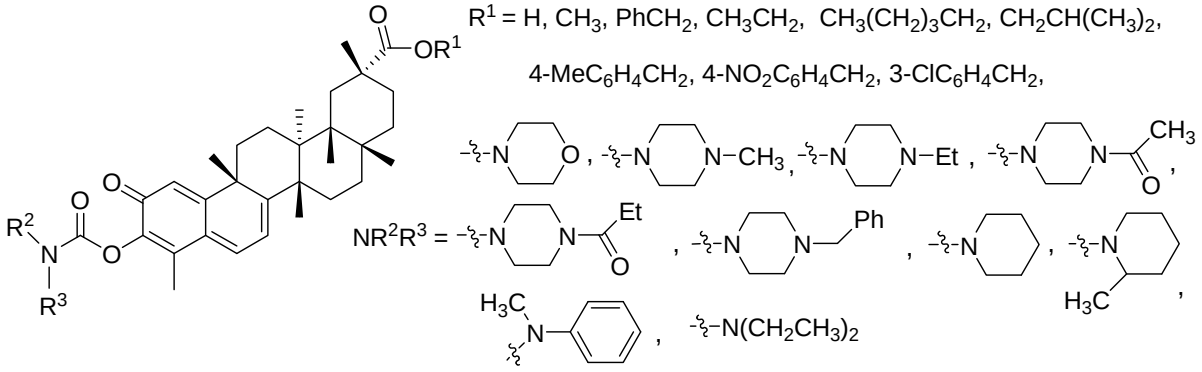
Scheme 2

To improve the aqueous solubility of celastrol, in 2012 San *et al.* designed and synthesized a series of celastrol derivatives of amino carbamic acid ester at C-3 of the A ring and esterification of the 20-carboxylic acid (Scheme 3).[36] Although the aqueous solubility of derivatives was significantly improved, these compounds were less active than celastrol in inhibiting the proliferation activity of Bel and A-549 tumor cells.

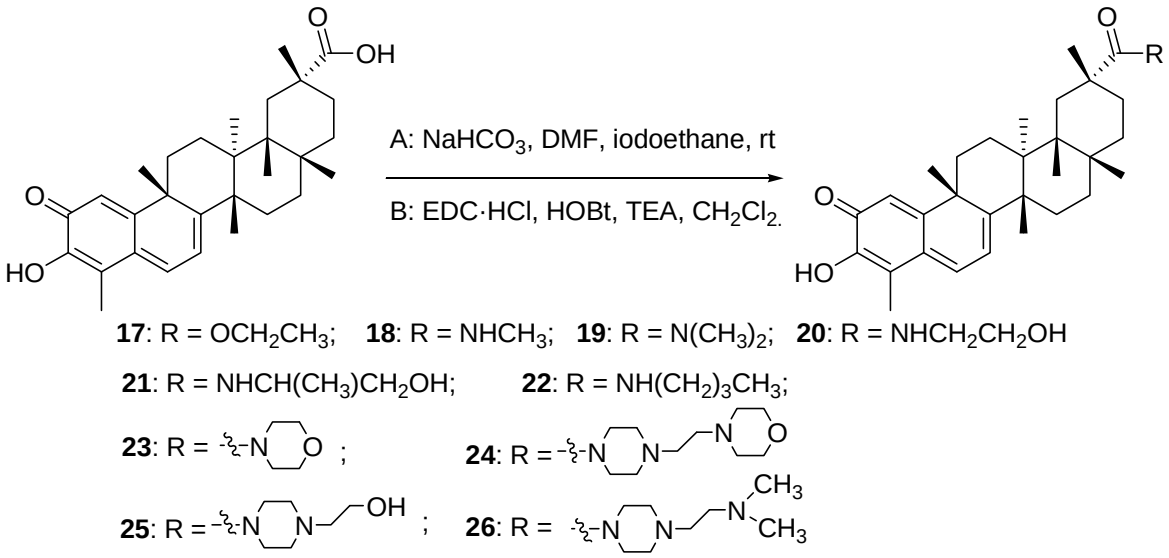
Recently, Liu and coworkers designed and prepared a series of celastrol derivatives (**17-26**) as potential telomerase inhibitors (Scheme 4).[37] All these compounds, including the positive control 5-fluorouracil and doxorubicin (AMD), were evaluated for their anticancer activities toward SGC-7901 (human gastric cancer cells), SMMC-7721 (human hepatoma cells), MGC-803 (human gastric cancer cells) and HepG-2 (human hepatoma cells) cell lines in *in vitro* bioassays. Celastrol and its derivatives demonstrated remarkably potent anticancer activities with SGC-7901, SMMC-7721, MGC-803 and HepG-2 cell lines. For example, the higher activities of compounds **20** and **21** displayed excellent action against cell lines SGC-7901(IC_{50} 0.14-0.18 μM, IC_{50} 0.08-0.12 μM), SMMC-7721 (IC_{50} 0.24-0.36 μM, IC_{50} 0.54-0.68 μM), MGC-803(IC_{50} 0.38-0.40 μM, IC_{50} 1.14-1.30 μM) and HepG2 (IC_{50} 0.50-0.72 μM, IC_{50} 0.21-0.35 μM). The IC_{50} of

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celastrol against SGC-7901, SMMC-7721, MGC-803 and HepG2 cell lines were 0.08-0.22 μM, 0.40-0.76 μM, 1.30-1.80 μM and 2.80-5.22 μM, respectively.



Scheme 3

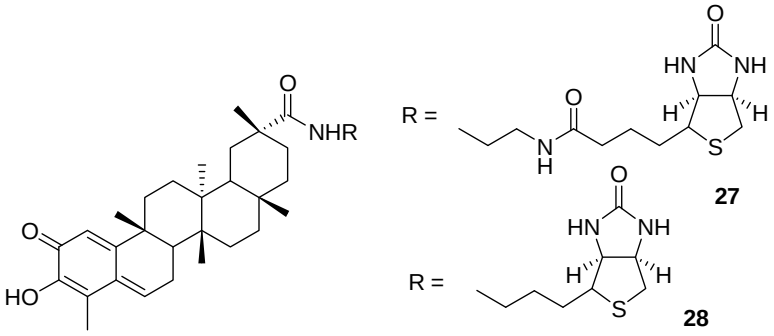


Scheme 4

The results of telomerase assay showed a direct correlation between telomerase inhibition and anti-proliferative inhibition of SMMC-7721 cells. In particular, compounds **20** and **21** with hydrophilic moieties exhibited high anti-proliferative activities with IC_{50} values at 0.11 and 0.34 μM. A molecular docking study indicated that the highly active compound **20** interlinked nicely with telomerase hTERT via two hydrogen bonds and intermolecular interactions. A preliminary mechanism of antitumor action demonstrated that compound **20** could induce significant SMMC-7721 cell apoptosis. These results may provide an effective approach for the development of novel telomerase inhibitors based on celastrol.

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Celastrol from the natural source possesses numerous beneficial therapeutic properties and influences diverse cellular pathways. In order to understand the mechanism of action and cellular target(s) of celastrol, Morimoto and coworkers designed and synthesized a family of celastrol derivatives **27** and **28** for the identification of protein targets and investigated them as inducers toward heat shock response (Scheme 5).[38] In the celastrol structure, conversion of the carboxylic acid functionality into amides or long-chain analogues leads to improvement in its potency and yields bioactive compounds that induce the heat shock response (HSR) and antioxidant response, and inhibit Hsp90 activity. The biotinylated conjugates of celastrol **27** and **28** were used as affinity reagents in extracts of human Panc-1 cells to identify Annexin II, eEF1A, and β-tubulin. The plausible results confirmed a previous suggestion that β-tubulin is another potential target of celastrol and celastrol targets Hsp90 indirectly by invoking a redox imbalance.

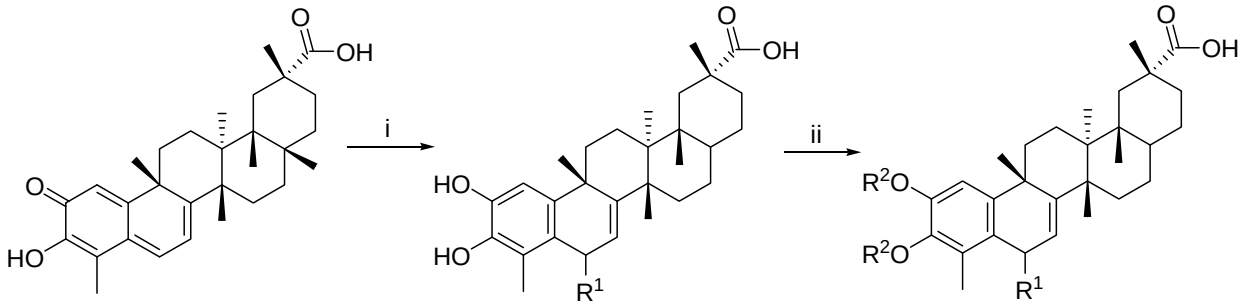


Scheme 5

3 Alteration of the A ring and modification at C6

In 2014, Pan and coworkers synthesized a series of new C-6-modified celastrol derivatives and evaluated them for their cytotoxic activity toward seven human cancer cell lines (BGC-823, H4, Bel7402, H522, Colo 205, HepG2 and MDA-MB-468) *in vitro* (Scheme 6).[31] Most of the compounds exhibited higher potent inhibition against BGC823 (IC_{50} = 0.42–0.77 μM), H4 (IC_{50} = 0.35–1.37 μM), and Bel7402 (IC_{50} = 0.45–1.55 μM) than that of celastrol (IC_{50} = 3.73 μM, IC_{50} = 2.09 μM and IC_{50} = 1.55 μM). It was clear that the C-6 functionalized celastrol derivatives possessed increased cytotoxicity potency *in vitro* against the human cancer cell BGC823, H4, Bel7401. In particular, 2,3-acetylation was more effective than propionylation, but the C(6)–carbonated derivatives dramatically dropped in cytotoxicity potency. The compound **29-A** displayed excellent inhibition of tumor growth *in vivo* antitumor assay on nude mice bearing Colo 205 xenografts with a 0.06 μM IC_{50} value. The results indicate that C-6 functionalized celastrol derivatives may be regarded as potential drug candidates for treating cancer.

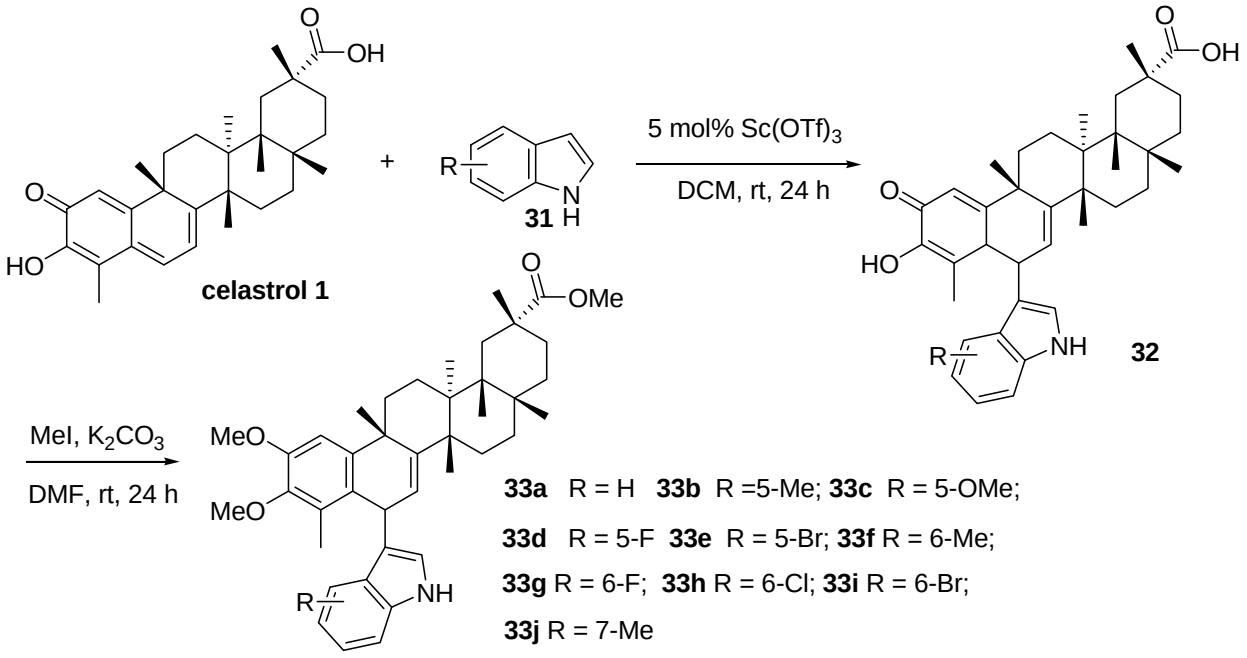
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Derivatives	R^1	R^2	Derivatives	R^1	R^2
29	SO_3Na	H	**30-A**	CH_2COCH_3	$COCH_3$
29A	SO_3Na	$COCH_3$	**30-B**	$S(p\text{-}C_6H_4)CH_3$	$COCH_3$
29B	SO_3Na	$COCH_2CH_3$	**30-C**	$S(p\text{-}C_6H_4)NHCOCH_3$	$COCH_3$
30	CH_2COCH_3	H	**30-D**	$SCH_2CH(COOH)NHCOCH_3$	$COCH_3$

Reagents and conditions: (i) For **29**: $NaHSO_3$, MeOH, rt, 1.5 h, N_2; For **30-A**: acetone, 1 N HCl (cat.), rt, N_2; For **30-B~D**: RSH, MeOH, rt, N_2; (ii) Ac_2O, Py, rt, N_2; or $(CH_3CH_2CO)_2O$, Py, rt, N_2.

Scheme 6



Scheme 7

In a later work, Pan and coworkers have reported a series of C-6-indole-substituted celastrol derivatives and evaluated their cytotoxic activity against human hepatocellular carcinoma Bel7402 and human glioblastoma cell line H4 (Scheme 7).[39] The active compounds

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exhibited inhibition activities against Bel7402 (IC_{50} = 0.01–23.91 μM) and H4 (IC_{50} = 2.03–14.56 μM), and the IC_{50} of celastrol as positive control against Bel7402 and H4 were 2.09 μM, 1.55 μM, respectively. The results from their structure-activity relationships indicated that the analogues **33f** and **33h** showed significant antiproliferative activities against Bel7402 cancer cells *in vitro* (IC_{50} 0.02 μM and 0.01 μM, respectively). Celastrol derivatives at C-6 position bearing a C–C bond still displayed potent *in vitro* anticancer activities. In fact, the addtion of a C–C bond at C-6 inevitably influences the Michael acceptor action and formation of a protein complex.[42-42] At the same time, the study strongly suggested that the Michael-acceptor action of celastrol is not necessary for its antitumor activity.

4. Summary and Outlook

Celastrol, as a representative of the natural quinone methide triterpenoids, displays wide anti-inflammatory and anticancer activity and produces multiple biological effects at the molecular level. In particular, it has excellent prospects as an anti-cancer therapeutic drug in view of its perceived beneficial effects on a variety of cancers *in vitro* and *in vivo*. At present, modifications of celastrol have chiefly been focused on the C-20 carboxylic acid functionality, and alteration at the A ring and C-6-modification at the B ring. Some authors have stated that the quinone methide moiety is required for its antitumor activity;[32,38] however, while the A ring is transformed into a benzene ring with an alkoxy group, the corresponding derivatives still exhibit significant activities against certain tumor cell such as, BGC823, H4 and Bel7402. Some more recent studies have indicated that the C(6)-position of celastrol was suitable for modification and have shown that the Michael acceptor property is not necessary for its anti-tumor activity.[31,39] Therefore, the modifications of celastrol should focus on developing new biological activities, improving its pharmacokinetic properties, and exploring novel modifications, strategies and location points. With better understanding of the mechanism of action of celastrol, we believe that more and better celastrol derivatives will be brought about in the future.

5. Acknowledgements

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