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Synthesis of 3- and 29-substituted celastrol derivatives and structure-activity relationship studies of their cytotoxic activities

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ABSTRACT

A series of 3-carbamate and 29-ester celastrol derivatives (compounds **1**–**26**) were designed and synthesized. These analogues were evaluated for their cytotoxic activities against several cancer cell lines. Cytotoxicity data revealed that the properties of substituents and substitution position had important influence on cytotoxic activity. Modification of C-3 hydroxyl with size-limited groups did not reduce the activity obviously. The introduction of polarity group like piperazine could improve the solubility. Compound **23** was chosen to further evaluate anti-tumor efficacy *in vivo*. It showed higher inhibition rate and better safety than celastrol during *in vivo* experiment by intragastric administration. The preliminary antitumor studies of compound **23** *in vivo* showed that it might be promising for the development of new antitumor agents.

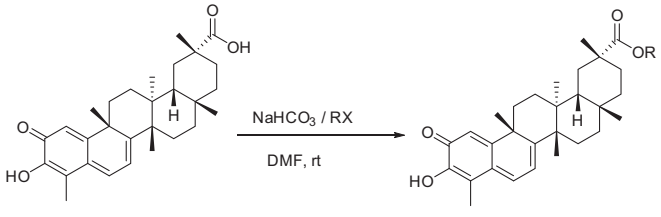
Celastrol is a natural quinone methide triterpenoid found in the extracts of *Tripterygium wilfordii* and shows anti-inflammatory, anti-proliferative, and anti-obesity activity.[1–4] In researches of celastrol on tumor cells, celastrol has been found to induce multiple biological effects, such as Hsp90 inhibition,[5,6] proteasome inhibition,[7] NF-κB pathway perturbation,[8,9] topoisomerase II inhibition,[10] and heat shock response activation.[11–13] It shows considerable potential to become an anti-tumor chemical entity.

Celastrol has been proved a special Hsp90 inhibitor, which shows cytotoxic activity. Inhibition of Hsp90 induces degradation of the misfolded clients and disrupts different pathways in development of tumors.[14,15] Previous studies showed that celastrol could disrupt the Hsp90-Cdc37 interaction and the quinone methide moiety is essential for its cytotoxic activity against tumor cell lines.[2,6,7,18] Disruption of the Hsp90-Cdc37 interaction leads to the degradation of the oncogenic kinases and may bring promising anticancer effects.[16,17] Remarkable stereospecific conjugate addition on this moiety may be the major contribution to the protein target selectivity.[19]

Some structure modifications of celastrol were reported,[20–24] but chemical modification about C-3 hydroxyl has rarely been studied before. The exact target(s) and the C-3 structure-activity relationship (SAR) of celastrol remains undefined. Meanwhile, low water solubility and obvious toxicity hindered its application.[25,26]

Because of the structure of A ring, C-3 hydroxyl forms a hydrogen bond with the neighboring carbonyl group and conjugate with the quinone methide moiety. Modification on this position may cause subtle changes on the molecule. In this study, we elaborated a series of derivatives in order to investigate the structure-activity relationship and improve the drug-like properties of celastrol. Meanwhile, oral administration was tested and found improved the compliance.

Compounds **1**–**18** were readily synthesized by nucleophilic reaction between celastrol and organic halides in DMF at room temperature, using $NaHCO_3$ as basic (Figure 1). The activity of these compounds were evaluated by MTT method with several tumor cell lines (see Table 1).



Ceastrol

Fig. 1. Synthesis of compound **1**–**18**.

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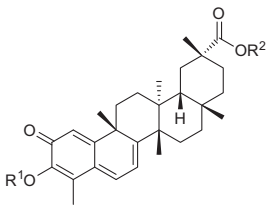
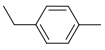
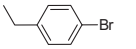
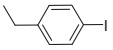
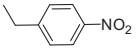
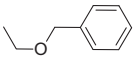
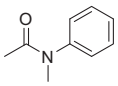
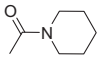
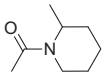
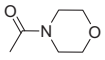
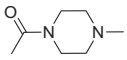
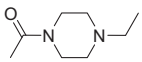
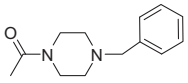
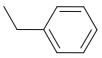
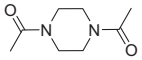
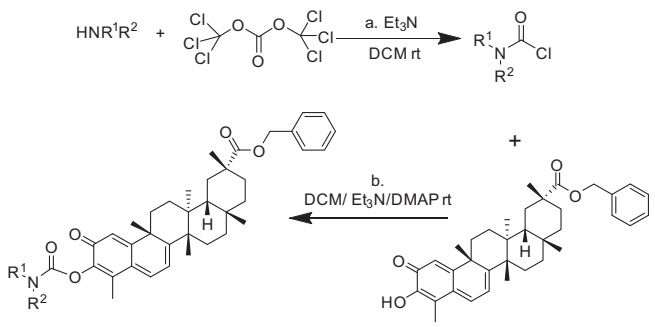
Table 1
Structure of celastrol analogues **1–26**.

Compound	R^1	R^2	Compound	R^1	R^2
			**13**	H	
Celastrol	H	H	**14**	H	
1	H	CH_3	**15**	H	
2	H	CH_2CH_3	**16**	H	
3	H	$CH_2CH_2CH_3$	**17**	H	
4	H	$CH(CH_3)_2$	**18**	H	
5	H	$CH_2CH_2CH_2CH_3$	**19**		
6	H	$CH(CH_3)CH_2CH_3$	**20**		
7	H	$CH_2CH(CH3)_2$	**21**		
8	H	$CH_2CH_2CH_2CH_2CH_3$	**22**		
9	H	$CH_2CH_2CH_2CH_2CH_2CH_3$	**23**		
10	H	$CH_2CH{=}CH_2$	**24**		
11	H	$CH_2COOCH_2CH_3$	**25**		
12	H		**26**		

Then, compound **12**, because of its better antitumor activity, was chosen to be modified in order to adjust the chemical environment of C-3 hydroxyl which may affect the electron density of the quinone methide moiety. New pharmacophores were added on the structure to alter the activity of these compounds and improve the solubility. Therefore, a series of carbamates (compounds **19–26**) of compound **12** were synthesized by one-pot reaction with BTC and chosen amine (Scheme 1). The polarity of these compounds was enhanced, which is benefit for solubility and bioavailability. The *in vitro* activity of these compounds remained as potent as celastrol, shown in Table 2.



Scheme 1. Reagents and conditions: (a) Et_3N, DCM, 0 °C to rt; (b) Et_3N, DMAP, CH_2Cl_2, 0 °C to rt, 12 h.

Total 26 compounds **1–26** were tested for their cytotoxicity against several cell lines include (A549, Bel7402, SGC7901, Hela, HepG2) by MTT assay employing celastrol as positive control. The IC_{50} values for the inhibition of proliferation are shown in Table 2.

The 29- ester derivatives **1–18** initially synthesized showed comparable cytotoxic activity with celastrol. In addition, they were easier to be synthesized and purified. These results indicated that it was suitable for improving the cytotoxicity to introduce the alkyl group at the carboxyl. This might be caused by better cell permeability. And compound **12** showed slightly better activity than the others. So, our present work focused on exploring some modifications on the hydroxyl to improve the polarity and solubility of the compounds and studying their effects on cytotoxicity. Therefore, compounds **19–26** were synthesized and tested for their cytotoxicity.

From Table 2, we found that most of our carbamates of celastrol showed potent activity as well as their precursor. However, the activity of derivatives with piperazine (compounds **23–26**) were better than that with aniline (compound **19**), Piperidine (compounds **20**, **21**) and morpholine (compound **22**). The substitution of piperazine and morpholine by piperidine and aniline weakened