# Design, synthesis and biological evaluation of C(6)-indole celastrol derivatives as potential antitumor agents

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# Design, synthesis and biological evaluation of C(6)-indole celastrol derivatives as potential antitumor agents†

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Celastrol is a natural triterpenoid which possesses diverse pharmacological activities including potent antitumor activity. Previously, we reported a C(6)-modified celastrol derivative NST001A which possesses cytotoxic activity against Colon 205 cells with a 60 nM $IC_{50}$ value. To further explore the structure activity relationships, a new class of C(6)-indole substituted celastrol derivatives were designed and synthesized. Biological evaluation of these compounds includes their cytotoxic activity against human hepatocellular carcinoma Bel7402 and human glioblastoma cell line H4. Among all these semisynthetic analogues, compound **4f** and **4h** displayed excellent *in vitro* antiproliferative activities against Bel7402 cancer cells ($IC_{50}$ = 0.02 μM and 0.01 μM, respectively).

## Introduction

Triterpenoids are members of a larger family of structurally related compounds known as cyclosqualenoids that are widely distributed in nature.[1] Celastrol (Fig. 1) is a quinone methide triterpenoid extracted from the root bark of the traditional Chinese medicine "Thunder of God Vine" (*Tripterygium wilfordii* Hook F.). It was reported that celastrol has diverse biological activities, such as antitumor,[2–4] antioxidant,[5–7] anti-inflammatory,[5,6,8–10] antifungal,[11,12] immunomodulatory,[13] protective and antiangiogenic effects.[13–15] Celastrol has promising clinical potential as both a therapeutic and chemopreventive agent for various cancers, including gliomas, hepatocellular carcinomas, prostate cancer, renal cell carcinoma, head and neck carcinoma, non-small cell lung carcinoma.[16,17] The antitumor activity of celastrol may result from targeting multiple signaling pathways such as NF-Kb,[18] Notch,[19] HSP90,[20] Cdc37, p23, IKKβ, proteasome, Bcr-Abl, and p-Akt,[3] which suggests its potential use as an antitumor agent and has attracted many oncologists into the exploration of novel celastrol derivatives.

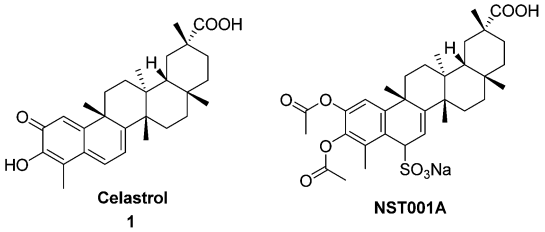


Fig. 1 Structure of celastrol and NST001A.

Some celastrol analogues like pristimerin and dihydrocelastrol are reported to be inducers of the heat shock response and cytoprotective agents of lethal stress in Hela cells and SH-SY5Y neuronal cells.[21] Current structure modifications of celastrol mainly focus on its 20-carboxylic acid group. Klaić[22] and Sun[23] disclosed that amidation and esterification of the carboxylic acid group of celastrol may influence its bioactivity and the amide linkage is more appropriated for tethering celastrol to a bioprobe. It was found that the carboxyl of celastrol was not required for its apoptotic activity, and the quinone methide moiety is not responsible for its chemical chaperone activity, but is crucial for its cytotoxic activity in cancer cells. In the studies conducted by Westerheide,[21] dihydrocelastrol is active as a heat shock promoter. However, it is inactive in the cellular assays.[24]

Previously, we disclosed a series of C(6)-sulfonated celastrol derivatives as potent antitumor agents.[25] Compared with the parent compound celastrol, NST001A displayed good cytotoxicity against Colon 205 cell line and can potently inhibit the growth of Colo 205 xenografts in nude mice with a better safety profile. This study indicated that the C(6)-position of celastrol was suitable for modification.

Indole has a wide range of biological activities and many antitumor agents such as sunitinib,[26] SU5416,[27] SU6668,[28] vinblastine,[29] vincristine[30] (Fig. 2) share the indole moiety.[31] In

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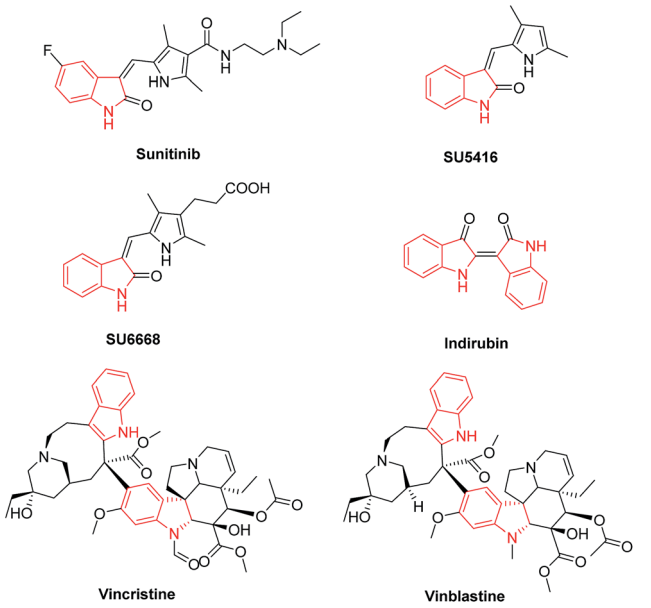


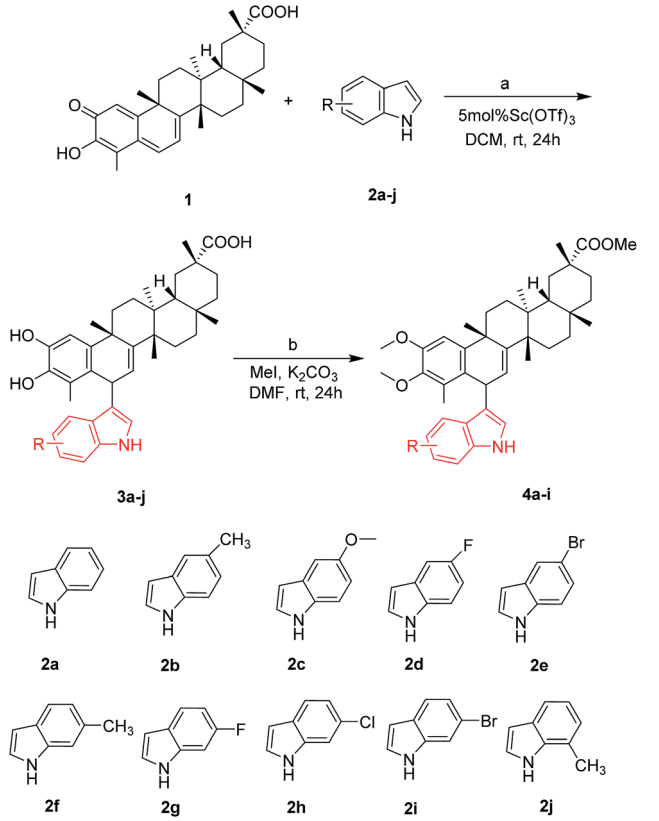

Fig. 2 Chemical structures of indole derivatives.

order to further explore the SAR of C6 position, we designed and synthesized a new series of C(6)-indole substituted celastrol analogues. Biological evaluation of these compounds includes their antiproliferative activity against human hepatocellular carcinoma Bel7402 and human glioblastoma cell line H4.

## Results and discussion

### Chemistry

The general route used for the synthesis of the celastrol derivatives is depicted in Scheme 1. Novel C(6)-indole substituted celastrol analogues **3a–3j** were synthesized by Friedel–Crafts reaction in good yields (43–88%).[32,33] Subsequently, the phenolic hydroxyl group along with the carboxylic acid group were methylated in the presence of potassium carbonate and methyl iodide, and afforded celastrol analogues **4a–4g** and **4i**. Compound **4h** was the only *N*-substituted indole derivative under the same condition.



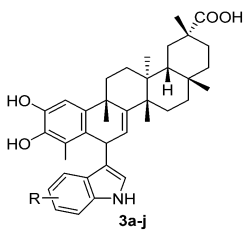

Scheme 1 Synthesis of C(6)-indole analogues of celastrol. Reagents and conditions: (a) scandium(III) triflate, DCM and (b) MeI, $K_2CO_3$, DMF.

### Biological assay

***In vitro* cell growth inhibition.** All synthesized celastrol analogues, along with celastrol as positive control were tested for their cytotoxic activity against human hepatocellular carcinoma Bel7402 and human glioblastoma cell line H4.

As shown in Table 1, celastrol exhibited potent anti-tumor effect with $IC_{50}$ values in the low micromolar range. Compared with the positive control compound, C(6)-indole substituted celastrol **3a** showed poor cytotoxicity. When methyl group was introduced to 7-position of indole, the antiproliferative activity against Bel7402 was increased (**3j** *vs.* **3a**). The 6-substituted indole analogues showed better cytotoxicity than 5-substituted ones (**3f** *vs.* **3b**, **3g** *vs.* **3d** and **3i** *vs.* **3e**). In this series, compound **3f** and **3i** displayed the best *in vitro* anti-cancer activity ($IC_{50}$ = 4.52–8.85 μM), which is comparable with celastrol.

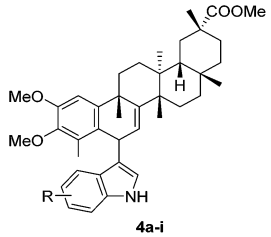
Table 1 *In vitro* cell growth inhibition[a]




| Compounds | R | $IC_{50}$ (μM) H4 | $IC_{50}$ (μM) Bel7402 |
|---|---|---|---|
| Celastrol | — | 2.09 | 1.55 |
| **3a** | H | 56.22 | >100 |
| **3b** | 5-Me | 67.00 | 55.24 |
| **3c** | 5-OMe | 24.39 | 45.42 |
| **3d** | 5 F | 31.75 | 34.30 |
| **3e** | 5-Br | 69.76 | 18.91 |
| **3f** | 6-Me | 6.66 | 5.52 |
| **3g** | 6-F | 60.90 | 11.88 |
| **3h** | 6-Cl | 7.83 | 6.53 |
| **3i** | 6-Br | 8.85 | 4.52 |
| **3j** | 7-Me | 80.48 | 34.19 |

[a] Each value was reproduced in three experiments.

Table 2 *In vitro* cell growth inhibition[a]




4a-i

| Compounds | R | $IC_{50}$ (μM) H4 | $IC_{50}$ (μM) Bel7402 |
|---|---|---|---|
| Celastrol | — | 2.09 | 1.55 |
| **4a** | H | 5.87 | 20.45 |
| **4b** | 5-Me | >100 | >100 |
| **4c** | 5-OMe | 4.59 | 0.51 |
| **4d** | 5-F | 4.66 | 1.58 |
| **4e** | 5-Br | 5.44 | 1.65 |
| **4f** | 6-Me | 7.88 | 0.02 |
| **4g** | 6-F | 14.56 | 23.91 |
| **4h** | 6-Cl, *N*-Me | 2.03 | 0.01 |
| **4i** | 6-Br | >100 | >100 |

[a] Each value was reproduced in three experiments.

Overall, another series of celastrol derivatives showed better anticancer activity (Table 2). Compared with the positive control compound celastrol, C(6)-indole substituted analogue **4a** showed moderate cytotoxicity. The cytotoxicity was maintained when 5-substituted indole was introduced into the C6 position of celastrol except compound **4b**. On the other hand, **6**-substituted indole derivatives showed entirely different antiproliferative activity. Compound **4f** showed excellent *in vitro* anticancer activity on Bel7402 cell line while 6-bromo substituted analogue **4i** lost its cytotoxicity. Interestingly, the only *N*-substituted indole derivative **4h** displayed the best antiproliferative activity in this series ($IC_{50}$ = 2.03 μM and 0.01 μM, respectively).

## Conclusions

In summary, a new series of semisynthetic C(6)-indole substituted celastrol derivatives were designed and synthesized. Part of these analogues showed similar *in vitro* anticancer activity to the parent compound celastrol. Notably, compound **4f** and **4h** showed excellent antiproliferative activity on Bel7402 cancer cell line. To the best of our knowledge, this work is the only example of celastrol derivatives bearing a C–C bond at the C-6 position with potent *in vitro* anti-cancer activity. The formation of C–C bond at this position results in the inactivation of Michael acceptor, which suggests the Michael acceptor is not necessary for its anti-tumor activity. The present encouraging results give a promising future optimization of the series.

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