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Research paper

Novel celastrol derivatives with improved selectivity and enhanced antitumour activity: Design, synthesis and biological evaluation

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ABSTRACT

Celastrol is one of the most active antitumour compounds among the natural triterpenoids. It has been reported to be highly active against a wide variety of tumours and to affect multiple cellular pathways. A series of new celastrol derivatives, including compounds bearing a urea group, have been synthesised and analysed for their biological activity against human cancer cell lines. Several compounds presented a stronger growth inhibition effect than celastrol on the cell lines studied. Among them, compound **24** was the most promising derivative, as it exhibited both a remarkable antiproliferative activity and an improved selectivity in tumour versus non-tumour cells. The anticancer molecular mechanism of compound **24** in the human ovary cancer cell line SKOV-3 was further studied and the results showed that compound **24** induced apoptosis through the activation of the extrinsic death receptor pathway. Interestingly, the results revealed that compound **24** might be able to decrease the levels of dysfunctional p53. The assays also suggested that compound **24** is an Hsp90 inhibitor, and that the Akt/mTOR pathway might be involved in the downstream regulation that leads to its antiproliferative activity. Moreover, a synergistic anticancer effect was evidenced when SKOV-3 cells were simultaneously treated with compound **24** and cisplatin. Taken together, these results suggest that compound **24** may be a promising lead for the development of new cancer therapies.

1. Introduction

The rational drug design for the discovery of new potent anticancer agents with minimal side effects is a major goal of modern medicinal chemistry [1]. The development of new chemotherapy options is often aimed at finding new compounds for combination therapies, due to the drug resistance and considerable side effects usually related with monotherapy in cancer [2].

Natural products are a unique source for the development of novel effective cytotoxic agents [3–6]. Celastrol **1** is one of the most active antitumour compounds among the natural triterpenoids [7]. It is a chemical substance isolated from the root bark of the Chinese medicinal plant *Tripterygium wilfordii Hook F.*, which belongs to the Celastraceae family, an important source of bioactive secondary metabolites [8].

Structurally, celastrol **1** is a triterpenoid quinone methide that, as implied in its name, bears a structure that is analogous to a quinone with one of the carbonyl oxygens replaced by a methylene group [9]. Moreover, it has a hydroxyl group *ortho* to the quinone carbonyl group and extended conjugation at the exocyclic methylene group. The stability of the chemical structure is further influenced by the steric and conformational features of the 5-ring triterpene [10,11]. The A and B rings of celastrol **1** make the molecule polarised and, thus, extremely reactive. It incorporates a Michael acceptor in which the C-6 position is highly prone to nucleophilic addition [12,13]. Therefore, celastrol **1** has the ability to form covalent Michael adducts by reacting with the nucleophilic thiol groups of the cysteine residues of biomolecules, such as DNA and proteins [14]. This seems to be the chief mechanism responsible for the wide range of biological activities associated with

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celastrol **1** [8,9,14,15]. Among these pharmacological activities, its potent antitumour effect has been the most widely investigated [13,16–19].

Celastrol **1** has been reported to be highly active against a wide variety of human tumour cell lines [16,20–24]. Its potential mechanisms of action have also been studied, and it has been revealed that celastrol **1** can regulate the survival [25], proliferation [26], invasion [27], angiogenesis [28] and metastasis [29] of tumour cells via several pathways. Despite the huge potential of celastrol **1** as an anticancer agent, it presents some important limitations for clinical application, such as systemic toxicity, poor aqueous solubility and low bioavailability [30,31]. For this reason, in recent years, an effort has been made to develop and optimise new celastrol **1** derivatives [13,32,33].

Urea-containing derivatives of some chemical products have been recently synthesised, resulting in enhanced compounds with versatile properties that helped to improve their pharmacological and pharmacokinetic profile [34–36]. In this article, we report a rational approach to the synthesis and characterization of several new urea-containing derivatives of celastrol **1**, resulting in antitumour compounds that are even more effective and less toxic. The most active compound, compound **24**, was selected for additional studies aimed at gaining insight into the mechanism of action via which this derivative causes a decrease in the viability of cancer cells.

2. Results and discussion

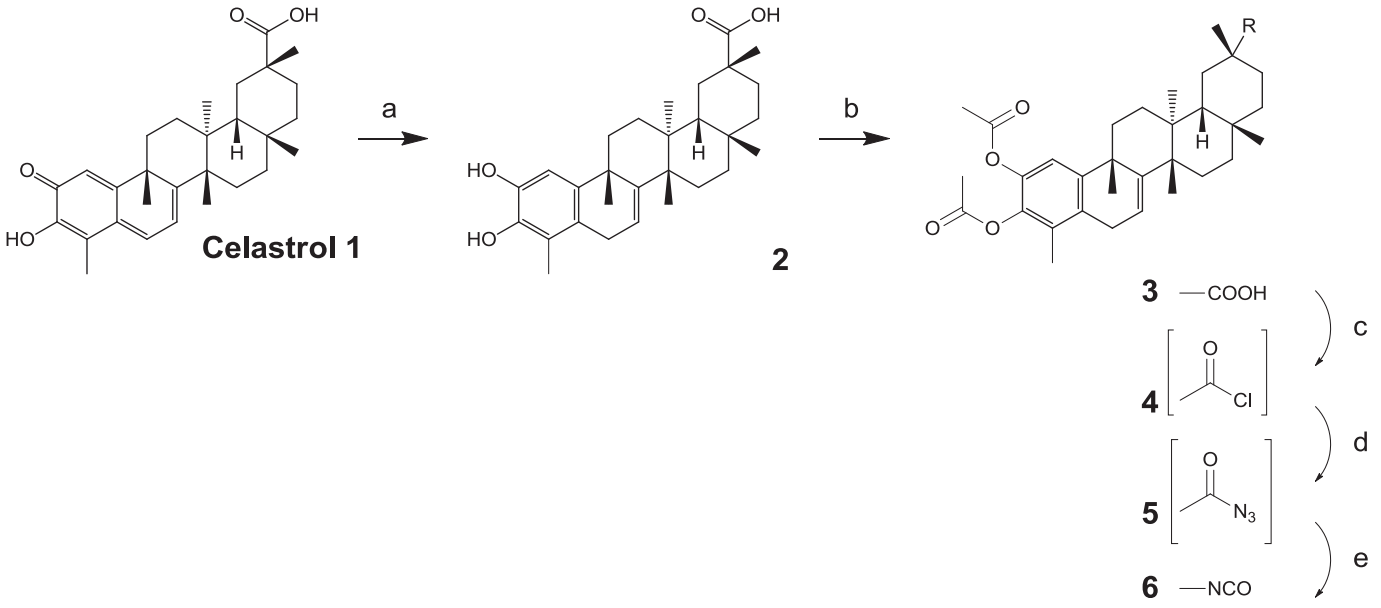
2.1. Chemistry

The synthetic routes used here for the novel celastrol 1 derivatives are outlined in Schemes 1–3. The structures and high purity of all compounds were corroborated by melting point (mp) determination, infrared spectroscopy (IR), nuclear magnetic resonance spectroscopy (^{1}H NMR and ^{13}C NMR), mass spectrometry (MS) and elemental analysis.

Celastrol **1** contains a hydroxyquinone methide moiety which can be easily reduced with sodium borohydride to the dihydro derivative **2** (Scheme 1) [37]. This derivative is readily converted back to its parent celastrol **1** by aerial oxidation [38]. Interestingly, the progress of these reactions can be monitored visually by following changes in the colour of the reaction mixture. The disappearance of the orange–red colour that is characteristic of celastrol **1** is linked to the loss of the ortho-quinonoid structure [39]. Phenolic hydroxyl groups are highly reactive; in order to avoid the formation of multiple derivatives, the protection of these groups was carried out by preparing the respective diacetate **3** using acetic anhydride (Scheme 1) [12,38,40,41].

Compound **3** was treated with oxalyl chloride in dichloromethane to give directly the acid chloride of dihydrocelastrol diacetate **4**, which was further converted to the acid azide **5** using sodium azide in aqueous acetone; Curtius rearrangement in toluene after 2 h of reflux gave isocyanate **6** (Scheme 1) [35,42], a central intermediate to obtain urea-type compounds. The introduction of an isocyanate function in compound **6** was confirmed by the specific IR observation at 2253 cm^{-1}, in combination with the observation of a signal for the quaternary carbon attached to the nitrogen at 120.77 ppm in the ^{13}C NMR spectrum.

To verify the importance of the quinone methide moiety for the anticancer activity in the urea derivatives of celastrol, some ureas 6-oxo derivatives (Scheme 2) were also synthesised. We adapted a previously described procedure [43] for the allylic oxidation of compound **3** using *tert*-butyl hydroperoxide in the presence of sodium chlorite in aqueous acetonitrile at room temperature, to give 6-oxo celastrol diacetate **7** in good yield (Scheme 2). Successful allylic oxidation was confirmed by the observation of an IR band at 1715 cm^{-1}, corresponding to the C=O stretching vibration and of a ^{13}C NMR signal for the α,β-unsaturated carbonyl C-6 at 187.13 ppm. The treatment of compound **7** with ammonium acetate catalysed efficiently the deprotection of the aromatic acetates in aqueous methanol at room temperature, to yield the corresponding diphenol **8** (Scheme 2) [44]. This spectroscopic evidence was consistent with the data reported in the literature, thus confirming that the structure of compound **8** corresponds to the natural triterpenoid Wilforol A [45]. Compound **7** was converted to the corresponding isocyanate **9** in a manner similar to that described previously. For the synthesis of urea **11** and **12a**, intermediate isocyanate **9** was treated with ammonium acetate and methylamine, respectively.



Scheme 1. *Reagents and conditions:* a) $NaBH_4$, MeOH, R.T., 10 min; b) $(CH_3CO)_2O$, DMAP, THF, R.T., N_2, 4 h; c) $(COCl)_2$, CH_2Cl_2, R.T., N_2, 4 h; d) NaN_3, H_2O, acetone, 0 °C, 1 h; e) in toluene, reflux, 2 h.