Novel synthetic luteolin analogue-caused sensitization of tumor necrosis factor-α-induced apoptosis in human tumor cells†

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Received 12th August 2008, Accepted 24th September 2008
First published as an Advance Article on the web 9th October 2008
DOI: 10.1039/b813904k

Studies on the sensitization, by novel alkynyl luteolin analogues, of TNF-α-induced apoptosis in HeLa and HepG2 cells revealed that LA-12 showed better sensitizing effects on TNF-α-induced cell death than luteolin, suggesting great potential for alkynyl luteolin analogues in cancer therapy.

Luteolin is an important member of the flavonoid family, and has been shown to exhibit anti-mutagenic,[1] anti-inflammatory,[2,3] and antioxidant[4] activities. Recently, luteolin has attracted much attention as a potential anti-cancer and anti-proliferative agent. The potential of luteolin in cancer therapy was demonstrated by its inhibition of DNA topoisomerase I and II.[5,6] Ko *et al.* also showed that luteolin was effective at the inhibition of proliferation and induction of apoptosis in human myeloid leukemia cells.[7] Very recently, the potential uses of luteolin as an anti-tumor agent in prostate, colon, lung and mammary cancers were studied.[8–11]

Tumor necrosis factor (TNF) is a proinflammatory cytokine with a wide spectrum of functions in many biological processes, including cell growth, death and development, oncogenesis, immunity, and inflammatory and stress responses.[12] Recently, we reported that luteolin could significantly sensitize TNF-α-induced apoptotic cell death in a number of human cancer cell lines,[13] which demonstrated a novel anti-cancer effect of luteolin and supported its potential application in cancer therapy. Herein, we wish to report the discovery of a novel luteolin analogue that possesses enhanced sensitizing effects on TNF-α-induced apoptosis in human tumor cells.

Structure–activity relationship (SAR) studies on luteolin have been very limited. More extensive studies on luteolin structural analogues would be highly desirable due to its biological importance. In our design of novel luteolin analogues (Fig. 1), we decided to keep the core structural scaffold of luteolin (rings A, B and C) intact to maintain the key biological activities of luteolin. The two hydroxy groups at the C-5 and C-7 positions were also kept as they are generally important in flavonoid activities.[14]

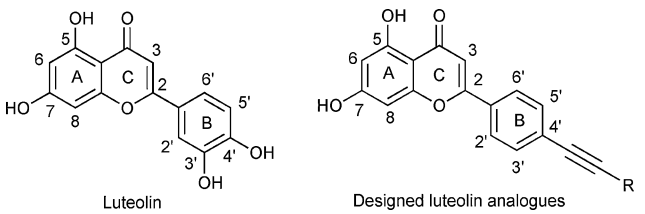

Fig. 1 Structures of luteolin and its analogues.

The hydroxy groups at C-3′ and C-4′ are deemed to be less important,[14] and we intended to delete these groups and install a series of alkynyl groups at the C-4′ position. Alkynes are useful building blocks for unsaturated molecular scaffolds because of their rigid structures and conjugating π systems. Alkynes are surprisingly common in natural products that have been isolated from plants and marine organisms, and they are also common motifs in drugs *e.g.* enediyne antibiotics and contraceptive pills. Furthermore, the unsaturated, high-energy carbon–carbon triple bond makes alkyne an attractive functional group for further derivation by many synthetic transformations.[15] We anticipated that the above described modifications to the luteolin could result in novel analogues with interesting activity profiles.

The synthesis of luteolin analogues is outlined in Scheme 1. 2′,4′,6′-Trihydroxyacetophenone was protected as its methoxymethyl ether (**1**). 4-Iodobenzaldehyde, which was readily prepared from 4-iodobenzoic acid, then underwent aldol condensation with ketone **1** to afford chalcone **2**. The cyclization of **2** proceeded efficiently in the presence of 2,3-dichloro-5,6-dicyano-1,4-benzoquinone (DDQ) to yield the key intermediate 4′-iodoflavone (**3**), which was then subjected to Sonogashira couplings with various terminal alkynes, followed by deprotection, to generate a series of desired luteolin analogues (**LA-1** to **LA-16**). The Sonogashira coupling under conventional thermal heating conditions was very sluggish, and carrying out the reactions under microwave irradiation[16] greatly shortened the reaction time, and provided much cleaner product.

The luteolin analogues that we intended to synthesize are illustrated in Fig. 2. Alkynes are readily available, making structural variations straightforward. In addition, we also prepared a number of aromatic alkynes (alkyne moieties in **LA-2**, **LA-4**, **LA-6**, **LA-9** and **LA-11**) following the literature procedure.[17] Various aliphatic alkynes which contained long alkyl chain, cycloalkyl, hydroxyalkyl or ether structures were incorporated into the synthetic luteolins. To examine the effects of aromatic alkynyl moieties in the luteolin analogue structures, neutral, electron-deficient, electron-rich and heteroaromatic (4-pyridine, imidazole) alkynes were used in the Sonogashira coupling reactions.

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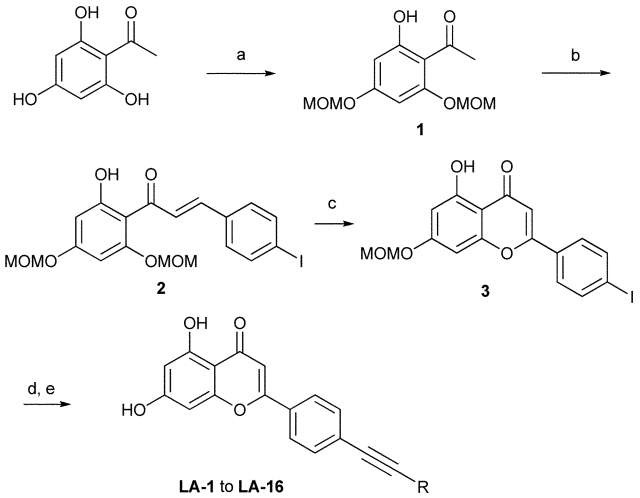
† Electronic supplementary information (ESI) available: Experimental procedures and characterization of the novel luteolin analogues; details of the cytotoxicity studies and sensitization experiments; ^{1}H NMR spectra of the products. See DOI: 10.1039/b813904k



Scheme 1 Synthesis of luteolin analogues. *Reagents and conditions*: (a) DIPEA, MOMCl, CH_2Cl_2, 0 °C to room temperature, 4 h; (b) NaOH, 1,4-dioxane, 4-iodobenzaldehyde, room temperature, 20 h; (c) DDQ, 1,4-dioxane, reflux, 36 h; (d) $Pd(PPh_3)_4$, CuI, THF, triethylamine, microwave irradiation, 10 min; (e) HCl, THF or MeOH, reflux.

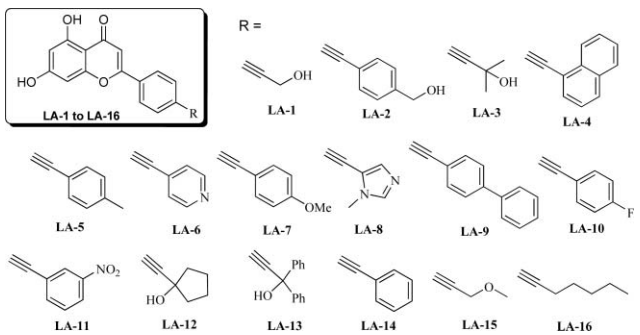


Fig. 2 Structures of synthetic luteolin analogues.

Having synthesized all the luteolin analogues, we first performed cytotoxicity studies with all the synthetic compounds using the MTT assay.[18] Among the 16 compounds tested, **LA-4**, **LA-11**, **LA-13**, **LA-14** and **LA-15** had similar cytotoxicity to luteolin, and compounds **LA-12** and **LA-16** appeared to be more cytotoxic than luteolin.

Luteolin has been shown to sensitize TNF-α-induced apoptosis in cancer cells.[13] We next tested whether some of the synthetic analogues of luteolin were able to sensitize TNF-α-induced cell death in both HeLa and HepG2 cells (Fig. 3). HeLa cells were treated with selected analogues together with TNF-α for 24 hours. The results showed that **LA-12** significantly promoted TNF-α-induced cell death. Similar results were also observed in HepG2 cells. Moreover, we compared the sensitization effect of **LA-12** with that of luteolin. As shown in Fig. 4, at the same concentration, **LA-12** was more effective at sensitizing TNF-α-induced cell death than luteolin, based on cell viability determined by MTT assay. Consistent results were also obtained based on the morphological changes of the cells (Fig. 5).

In conclusion, we prepared a number of novel luteolin analogues containing various alkynyl groups. The cytotoxicities of the

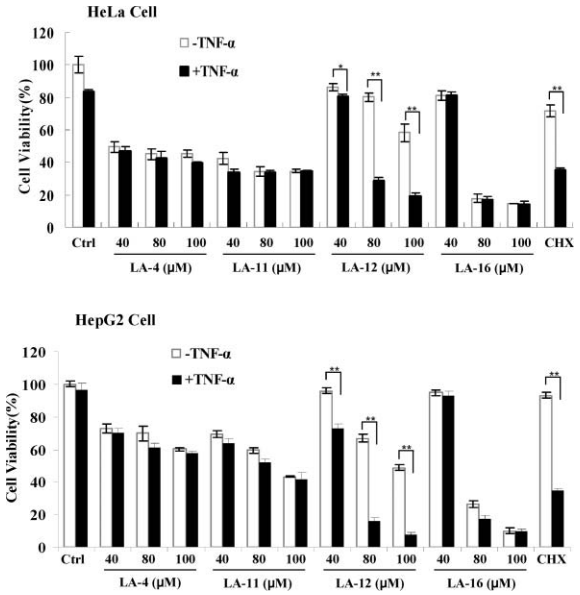


Fig. 3 Luteolin analogue-sensitized TNF-α-induced apoptosis.

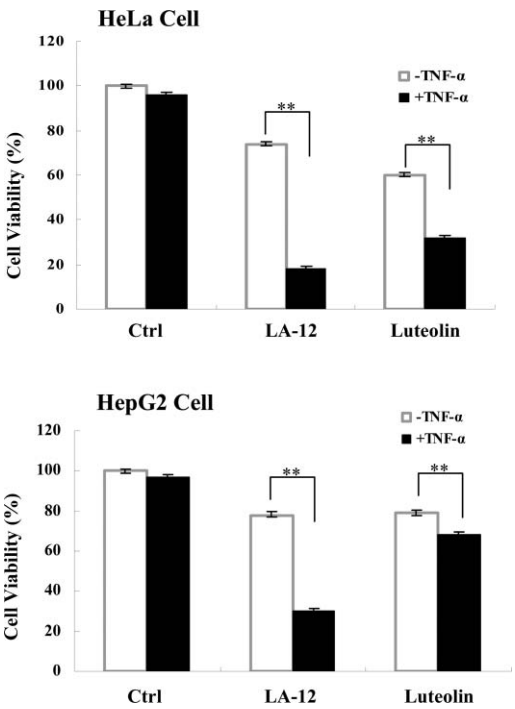


Fig. 4 A comparison of **LA-12**- and luteolin-sensitized TNF-α-induced cell death.

synthetic luteolin analogues were evaluated, and some of the potent synthetic compounds were tested for their sensitizing effects on TNF-α-induced apoptosis in HeLa and HepG2 cells. It was discovered that the analogue **LA-12** displayed better sensitizing effects on TNF-α-induced cell death than luteolin. We are currently preparing more luteolin analogues possessing similar structural motifs to those of **LA-12**, and thoroughly investigating the sensitization effects of luteolin analogues in cancer therapy.

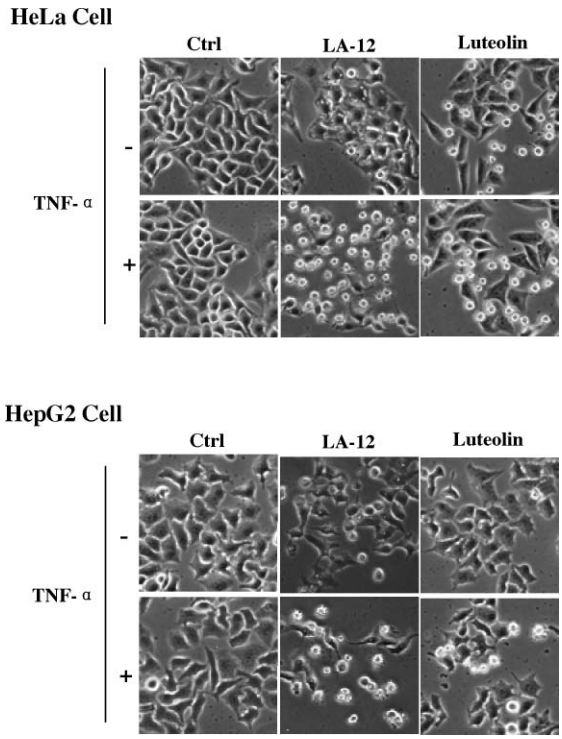


Fig. 5 Morphological changes confirm that **LA-12** is more effective than luteolin at sensitizing TNF-α-induced cell death.

Acknowledgements

We thank the National University of Singapore and the Agency for Science, Technology and Research (A*STAR) of Singapore for generous financial support.

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