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Chinese Journal of Natural Medicines 2016, **14**(6): 0462–0472
Chinese Journal of Natural Medicines

Synthesis and cytotoxicity evaluation of 3-amino-2-hydroxypropoxyisoflavone derivatives

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Available online 20 June, 2016

[ABSTRACT] Soy isoflavones exert a wide variety of biological activities, such as antioxidant, anti-inflammatory and anti-cancer properties. Nuclear factor erythroid 2-related factor 2 (Nrf2), a bZip transcription factor, plays a key role in soy isoflavones induced protection against oxidative stress and cancer. To obtain more effective isofavones, a series of 7,4′-bis-(3-amino-2-hydroxypropoxy), 7 or 4′-(3-amino-2-hydroxypropoxy) isoflavone derivatives have been synthesized as potential antitumor agents and Nrf2/ARE (antioxidant response element) activators. The cytotoxicity of these compounds in human cancer cell lines MDA-MB-231, HT-29, HCT116, HepG2 and 7402 was tested by MTT assay. In this study, the cytotoxicity of compound 3b exhibited highest cytotoxic activity and at the safety dose range, it also strongly up-regulated antioxidant response element (ARE)-luciferase reporter activity. In addition, compound 3b induced Nrf2 nuclear translocation and upregulated its downstream target genes NQO-1 and HO-1 at protein level. Taken together, our results suggest that compound 3b could be a potential agent for cancer themotherapy or cancer chemoprevention.

[KEY WORDS] Isoflavone; Cytotoxicity; Nrf2/ARE; Anti-cancer; Chemoprevention

[CLC Number] R284, R965 **[Document code]** A **[Article ID]** 2095-6975(2016)06-0462-11

Introduction

Isoflavones are a class of flavonoid phenolic compounds that are widely represented in nature [1], and their major producers are plants of the fabaceae [2]. Recently, isoflavones in soy products have received attention, due to their extensive biologically beneficial roles against multiple human diseases, including cancer (e.g. breast [3], colon [4], liver cancer [5]), cardiovascular disease [6], osteoporosis [7], and menopausal symptoms [8]. The major isoflavones in soybeans are daidzein and genistein, and their metabolites. Isoflavones are thought to represent a key bioactive component that has useful biological effects such as antoxidation and antiinflammation. In previous studies, daidzein was also reported to decrease the occurrence of menopausal syndromes; have positive effect in the prevention of osteoporosis; reduce the risk of the incidence of cardiovascular diseases and breast cancer [9-11]. Moreover, a positive impact of these isoflavones in the prostate gland cancer prevention was also noted [12].

Soy isoflavones have been shown to have anticancer properties, but the underlying mechanisms are not clear. It was thought that the anticancer effects of these compounds vary significantly among various isoflavones and different cell types under investigation. For example, the inhibition of cancer cell by isoflavones may involve interference with the estrogenic effect that blind to estrogen receptor similar to estrogen [13-14], effects on cell cycle [15], signaling via the epidermal growth factor receptor kinase [16], transforming

[Received on] 12-Jun.-2015

[Research funding] This work was supported by the National Natural Science Foundation of China (No. 81372268), Program of State Key Laboratory of Natural Medicines, China Pharmaceutical University (No. SKLNMZZCX201405), the Natural Science Foundation for Distinguished Young Scholars of Jiangsu Province (No. BK20130026), the Fundamental Research Funds for the Central Universities (No. 2016ZZD003) and the Program for Jiangsu Province Innovative Research Team.

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These authors have no conflict of interest to declare.

growth factor β or antioxidant activity. Notably, soy isoflavones have been linked to decreased risk of other diseases including osteoporosis, fat [13] or dry mouth [17], due in part to their possible antioxidant activities. Cancer is related to break balance of reactive oxygen species (ROS) in body, and the excess production of ROS occurs during normal physiological processes by both no-enzymatic and enzymatic sources, causes continuous damage to lipids, proteins, and nucleic acids [18]. Nuclear factor-erythroid 2-related factor 2 (Nrf2) is an important sensor of oxidative and electrophilic stress, ensuring the maintenance of redox homeostasis by regulating transcriptional activation of phase II defense and antioxidant genes [19]. In response to ROS, Nrf2 undergoes translocation to the nucleus to bind the antioxidant response element (ARE) of antioxidant genes and facilitate transcription. For example, heme oxygenase-1 (HO-1), NAD (P) H quinine oxidoreductase 1 (NQO1) are increased when Nrf2/ARE signaling pathway was activated. Nrf2 was reported to suppress carcinogenesis especially in the early stages. This topic has been extensively reviewed and several anti-carcinogenic activity of chemopreventive drugs has been reported to be abolished in Nrf2 KO mice [20-22]. Furthermore, several Nrf2 inducers with chemopreventive activity have been studied in mouse cancer models, such as skin, lung, colon and breast [23-25].

Almost all currently known ARE inducers (or activators) are indirect inhibitors of Keap1–Nrf2 interaction and they are believed to form covalent adducts with the sulfhydryl groups of cysteines in Keap1 (Fig. 1A). Electrophilicity is a common property of most of the known ARE inducers. It is well known that the biological effect of electrophiles being therapeutic to toxic depends on its hardness (“hard” and “soft”) that define the rate and selectivity of interactions with nucleophiles. Specifically, soft electrophiles will react predominantly with soft nucleophiles such as the sulfhydryl groups of cysteine, whereas hard nucleophiles target the amino and hydroxyl groups on nucleic acids and thus induce carcinogenicity and genotoxicity.

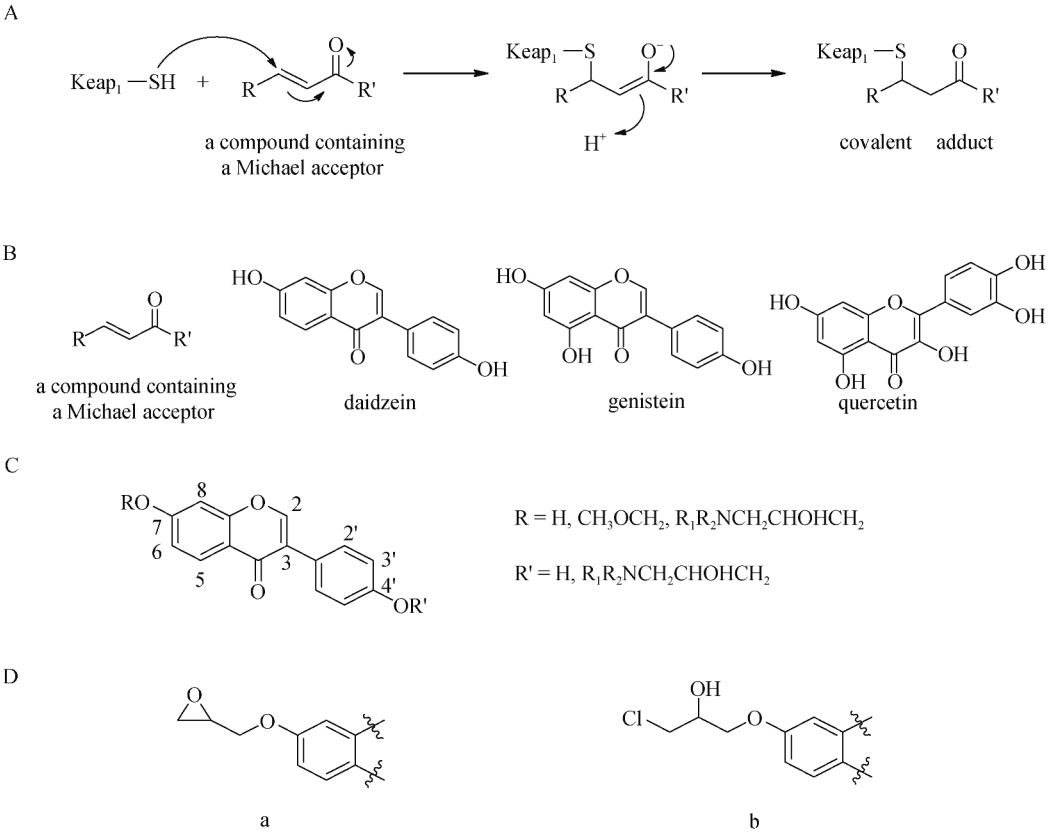

Fig. 1 (A) The proposed reaction mechanism of Michael addition reactions between Michael acceptors and cysteine sulfhydryl groups in Keap1. (B)Flavonoids that that contain electrophilic Michael acceptors; (C) Chemical structure of target compounds. (D) a: epoxy compound, b: open loop

A number of flavonoids have been reported to have ARE gene-induction properties, including natural and synthetic flavonoids such as natural isoflavone daidzein, genistein and a natural flavonoid quercetin (Fig. 1B). Structurally, they are derivatives of 2-phenylchromen-4-one, which contain α, β-unsaturated ketone (a Michael acceptor). Michael acceptors (olefins or acetylenes conjugated with electron-withdrawing carbonyl groups) are a prominent class of indirect inhibitors of Keap1–Nrf2 interaction. As shown in Fig. 1A, Michael acceptors can react with the critical cysteine thiolate groups in