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Original article

Halogenated flavanones as potential apoptosis-inducing agents: Synthesis and biological activity evaluation

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ABSTRACT

A series of halogenated flavanones were synthesized from 2-hydroxychalcones and tested for their cytotoxicity against a panel of human cancer cell lines. Among the synthesized compounds, 3′,7-dichloroflavanone (**2d**) showed the highest activity against MCF-7, LNCaP, PC3, Hep-G2, KB and SK-N-MC cells. However, 3′,6-dichloroflavanone (**2g**) with IC_{50} value of 2.9 ± 0.9 μM was the most potent compound against MDA-MB-231 cells, being approximately 12 times more active than etoposide as reference drug. According to the flow-cytometric analysis, compound **2g** can induce apoptosis by 66.19 and 21.37% in PC3 and MDA-MB-231 cells, respectively. The results of acridine orange/ethidium bromide staining and TUNEL assay suggested that the cytotoxic activity of this compound in PC3 and MDA-MB-231 cells occurs via apoptosis.

1. Introduction

Despite significant investment in the field of cancer chemotherapy, limited improvement in patient survival has been achieved in many countries. Cancer is the second leading cause of death worldwide after heart diseases [1,2]. Natural or semi-synthetic compounds may be used to prevent or treat the development of invasive cancers [2]. Flavonoids are naturally occurring polyphenolic compounds that have been reported to possess anticancer or anti-carcinogenic/antimutagenic activities [3]. These compounds possess a common phenylbenzopyrone structure (C6–C3–C6), consisting of two aromatic rings linked by three carbons that are usually in an oxygenated central pyran ring. They are categorized according to the saturation level and opening of the central pyran ring, mainly into flavones, isoflavones, flavonols, flavanonols, flavanols and flavanones (Fig. 1) [1,4,5]. Several beneficial biological effects of flavonoids including antioxidant, antitumor, and anti-inflammation properties have been ascertained in several previous studies [6–8]. Flavonoids have been identified to inhibit proliferation in many kinds of cultured human cancer cell lines, whereas less or no toxic to human normal cells [9–11]. The significant anticancer properties of flavonoids may be via induction of apoptosis [5].

Flavanones have been a potential source in the search for new lead compounds in the field of cancer chemotherapy. Usman et al. [12] had reported cytotoxic properties of flavanones isolated from the tree barks of *Cryptocarya costata*. A study of eight flavanones on colorectal carcinoma cells indicated that 2′-OH flavanone showed the most potent cytotoxic effect on these cancer cells, and cell death induced by 2′-OH flavanone was via the occurrence of DNA ladders, apoptotic bodies, and hypodiploid cells, all characteristics of apoptosis [13]. In 2007, Hsiao et al. described that flavanone and 2′-OH flavanone inhibited cell growth of A549, LLC, AGS, SK-Hepl and HA22T cancer cells, while other flavanones (4′-OH flavanone, 6-OH flavanone, naringin and naringenin) showed little or no inhibition [14]. The results of another study explored that synthetic flavanone derivatives have strong anti-proliferative effects on human breast cancer cells by way of p53-mediated apoptosis and the induction of cell cycle arrest at the G1 phase [15]. Also, Choi et al. reported that 4′,7-dimethoxyflavanone, exhibits potent anti-cancer activity and induces cell cycle arrest and apoptosis in human breast cancer MCF-7 cells [16].

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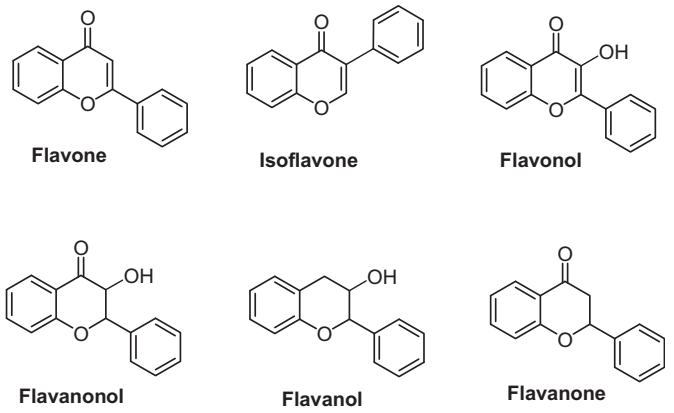


Fig. 1. The main structural categories of flavonoids.

It is now well documented that most cytotoxic anticancer agents induce apoptosis. Therefore, an attractive method for cancer chemoprevention or chemotherapy is new therapeutic agents that induce tumor cells death [17–19]. Because no systemic inflammatory responses have been observed in cells induced to undergo apoptosis, several studies have suggested that the induction of cell death via apoptosis reserves a physiological advantage in the cancer treatment [20,21].

In continuation of our previous work on the synthesis of potential cytotoxic agents and apoptosis inducers [22–25], and based on the diverse biological activities of flavanone derivatives, in this paper we have synthesized and evaluated cytotoxicity and apoptosis-inducing activity of a series of halogenated flavanones **2** in order to develop novel anticancer agents. These compounds are small molecules which consist of chromanone ring and aryl ring attached to the 2 position. Since halogens like chlorine, are very useful to modulate the electronic and steric characteristics of drugs and may also influence the hydrophilic–hydrophobic balance of the molecules, thus chlorine substitution on the chromanone ring and on the C-2 attached phenyl ring was used for structural modification and modulation of basic pharmacophore of flavanones.

2. Chemistry

A general synthesis of the flavanone derivatives **2a**–**k** is shown in Scheme 1. The condensation of 2-hydroxyacetophenone derivatives with the corresponding aldehyde in a basic media afforded hydroxychalcones **1a**–**k**. The compounds **1a**–**k** were cyclized in refluxing ethanol in the presence of sodium acetate to give flavanone derivatives **2a**–**k**. The structural characterization of flavanone compounds **2a**–**k** is based on their ^{1}H NMR and IR spectral data. For example, their ^{1}H NMR spectra showed typical chemical shifts and coupling pattern of the H-2, H-3ax and H-3eq protons of chroman ring in flavanone structures (Fig. 2). In the ^{1}H NMR spectra of flavanones (Table 1), the H-2 signal appears at approximately at 5.24–5.88 ppm as doublet of doublet. The resonances of the diastereotopic H-3ax and H-3eq protons occurred approximately at 3.03–3.20 and 2.80–2.90 ppm as two doublets of doublets.

The H-3ax and H-3eq of chroman ring are coupled with a constant of 16.4–17.6 Hz related to the geminal coupling. The value of the coupling constant between H-2 and H-3ax is too large ($J_{2,3ax}$ = 12.4–13.6 Hz) which can only arise from a *trans*-diaxial coupling, thus H-2 is axial and the 2-aryl group has equatorial orientation. Furthermore, the value of $J_{2,3eq}$ which is in the range of 2.8–3.2 Hz confirms the conformation of the flavanone structure (Fig. 2). FT-IR spectra of flavanone derivatives also revealed a very strong band about 1642–1697 cm^{-1} related to C=O stretching.

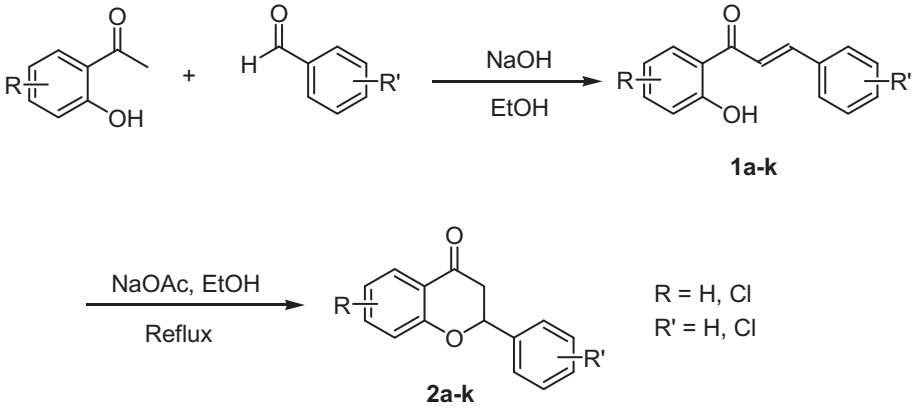
3. Biology

3.1. In vitro cytotoxicity assay

The in vitro cytotoxic activity of the test compounds **2a**–**k** against eight human cancer cell lines include MCF-7, MDA-MB-231 (human breast cancer), LNCaP, PC3 (human prostate cancer), Hep-G2 (human liver carcinoma), KB (human nasopharyngeal epidermoid carcinoma), SK-N-MC (human neuroblastoma) and K-562 (human erythroleukemic) was assessed using MTT (3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyl tetrazolium bromide) colorimetric assay [26]. The results of cytotoxic assay in comparison with etoposide were listed in Table 2.

3.2. Acridine orange/ethidium bromide staining method

Apoptosis was determined morphologically after staining PC3 and MDA-MB-231 cells with acridine orange/ethidium bromide using fluorescence microscopy according to the previously described method [27]. Acridine orange penetrates into living and dead cells, emitting green fluorescence as a result of intercalation in double-stranded DNA. Ethidium bromide emits red fluorescence after intercalation in DNA of cells with an altered cell membrane. Ethidium bromide staining due to loss of membrane integrity identifies the population of late stage of apoptotic cells and necrotic cells. Analysis of the acridine orange/ethidium bromide staining of the synthetic compounds **2d** and **2g** are shown in Fig. 3.



Scheme 1. Synthesis of flavanone derivatives.