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Original article

Synthesis and biological evaluation of novel farnesylthiosalicylic acid/salicylic acid hybrids as potential anti-tumor agents

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ABSTRACT

A series of FTS/salicylic acid hybrids was designed and synthesized and their *in vitro* antitumor activities were evaluated. It was found that the anti-proliferation activities of hybrids were better than that of FTS. Compound **10a** displayed the strongest antitumor activities with IC_{50} values of 5.72–9.76 μmol/L and selectively inhibited tumor cell proliferation. In addition, **10a** induced tumor cell apoptosis in a dose-dependent manner by up-regulating the expression of Bax and caspase-3 and down-regulating Bcl-2. Our findings suggest that these novel hybrids may hold a great promise as therapeutic agents for the intervention of human cancers.

1. Introduction

Malignant neoplasm is severe life threatening. Ras proteins encoding by *ras* genes serve as molecular switches tightly regulating intracellular signal transduction pathways controlling cell proliferation, differentiation, and cell apoptosis in normal cells [1–3]. However, oncogenic mutations in *ras* genes result in development of the biological process disorders, especially the occurrence of tumors in humans [4–6]. Therefore, Ras proteins and Ras related signaling are considered as promising targets in anticancer drug discovery. Farnesylthiosalicylic acid (FTS), a potent competitive Ras inhibitor, has been reported to display chemo-preventive activities in clinical trials [7–11]; however, it displays a limited therapeutic effect [12,13]. Our previous studies have developed a number of promising FTS derivatives that displayed significant cytotoxicity against cancer cells [14–17]. Among these FTS derivatives, FTS-diamines evidently improved the antitumor activities of FTS, but failed to be selective to tumor cells [14]. It would be great significant to searching for more potent and safer inhibitors targeting Ras proteins and Ras-related signaling pathway.

Acetylsalicylic acid (aspirin), a well known nonsteroidal anti-inflammatory agent, has been revealed to inhibit cyclooxygenase (COX) activity and exhibit the extraordinary potent for the treatment of cancer [18–21]. Epidemiological studies suggested that the regular intake of aspirin was associated with a reduction in the incidence of malignancies, including colorectal, gastrointestinal, and lung cancer [21,22]. What's more, reports demonstrated that acetylsalicylic acid and its metabolite salicylic acid (SA) could selectively induce apoptosis in several colorectal carcinoma cell lines [23–25]. Thus, acetylsalicylic acid or SA would be an excellent antitumor active fragment for the development of novel anticancer agents.

Given these, novel series of FTS/SA hybrids were designed by introducing salicylic acid fragment into parent molecule FTS with linkers of different length of diamines. We hypothesized that these new hybrids would exert inhibitory activity to tumor cells in a synergistic effect, leading to tumor cell apoptosis. Herein, we reported ten novel FTS/SA hybrids and the *in vitro* biological evaluation of their antitumor activity, selective cytotoxicity and apoptosis-inducing effects.

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2. Experimental

2.1. Chemistry

General: Infrared (IR) spectra were recorded on a Nicolet Impact 410 instrument (KBr pellet). ^{1}H NMR spectra were recorded with a Bruker Avance 300 MHz spectrometer at 300 K with TMS as an internal standard. MS spectra were recorded on a Mariner Mass Spectrum (ESI). Element analysis was performed on an Eager 300 instrument. All compounds were routinely checked by TLC and ^{1}H NMR. TLCs and preparative thin-layer chromatography were performed on silica gel GF254, and the chromatograms were conducted on silica gel (200–300 mesh, Merck) and visualized under UV light at 254 and 365 nm. All solvents were reagent grade and, when necessary, were purified and dried by standards methods. Solutions after reactions and extractions were concentrated using a rotary evaporator operating at a reduced pressure of *ca.* 20 Torr. Organic solutions were dried over anhydrous sodium sulfate. Compounds **2** and **3** were commercially available.

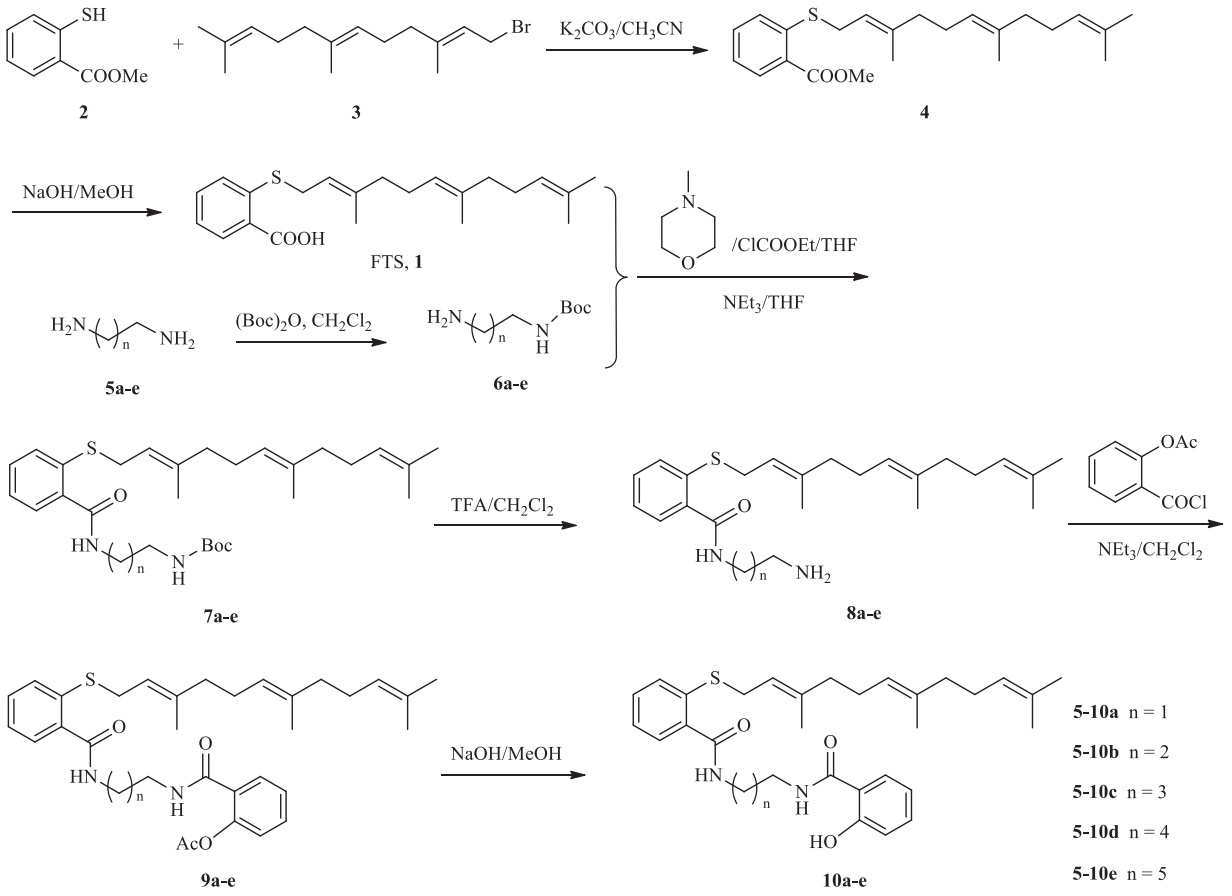
The synthetic route of **9a–e** and **10a–e** was outlined in Scheme 1.

(*E*,*E*)–Bromofarnesyl **3** was reacted with methyl thiosalicylate **2** in the presence of K_2CO_3 and KI to obtain methyl (*E*,*E*)-farnesylthiosalicylicate **4**, which was followed hydrolyzed with NaOH solution to gain parent compound **1** (FTS). In addition, different diamines **5a–e** was treated with $(Boc)_2O$ to generate one side *N*-protection of **6a–e**. Then FTS was respectively reacted with **6a–e** to form **7a–e** in the presence of ethyl chloroformate and *N*-methylmorpholine. Compounds **9a-e** were deprotected by treating with trifluoroacetic acid (TFA), then treated with *O*-acetylsalicylryl chloride prepared from the acid with $SOCl_2$ to yield target compound **9a–e**. Finally, compounds **9a–e** was hydrolyzed with NaOH to gain **10a–e**. The detailed experimental procedures and data of selected compounds were shown in Ref. [26].

2.2. Biological evaluation

MTT assay: Human hepatocellular carcinoma cells (SMMC-7721 and HepG2), human bladder carcinoma cells (EJ), human gastric cancer cells (SGC7901), human lung cancer cells (H460), human breast cancer cells (MCF-7) and human hepatocellular normal cells (LO2) at 10^4 cells per well were cultured in 10% FBS DMEM in 96-well flat-bottom microplates overnight. The cells were incubated in triplicate with, or without, different concentrations of each tested compound for 48 h. During the last 4 h incubation, 30 μL of tetrazolium dye (MTT) solution (5 mg/mL) was added to each well. The resulting MTT-formazan crystals were dissolved in 150 μL DMSO, and absorbance was measured spectrophotometrically at 570 nm using an ELISA plate reader. The inhibition induced by each tested compound at the indicated concentrations was expressed as a percentage. The concentration required for 50% inhibition (IC_{50}) was calculated using the software (GraphPadPrism Version 4.03).

Flow cytometry assay of cell apoptosis: SMMC-7721 cells were cultured overnight and incubated in triplicate with the tested compound **10a** (3.0, 6.0 and 12 μmol/L) or vehicle for 48 h. The cells were harvested and stained with FITC-Annexin V and PI (BioVision) at room temperature for 15 min. The percentage of apoptotic cells was determined by flow cytometry (Beckman Coulter) analysis.



Scheme 1. The synthetic route of **9a–e** and **10a–e**.