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Synthesis and biological evaluation of farnesylthiosalicylamides as potential anti-tumor agents

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ABSTRACT

Fourteen hybrids of farnesylthiosalicylic acid (FTS) with various diamines were synthesized and biologically evaluated. It was found that FTS-monoamide molecules (**10a**–**g**) displayed strong anti-proliferative activity against seven human cancer cell lines, superior to FTS and FTS-bisamide compounds (**11a**–**g**). The mono-amide **10f** was the most active, with IC_{50}s of 3.78–7.63 μM against all tested cancer cells, even more potent than sorafenib (9.12–22.9 μM). In addition, **10f** induced SMMC-7721 cell apoptosis, down-regulated the expression of Bcl-2 and up-regulated Bax and caspase-3. Furthermore, **10f** had the improved aqueous solubility relative to FTS. Finally, treatment with **10f** dose-dependently inhibited the Ras-related signaling pathways in SMMC-7721 cells. Collectively, **10f** could be a promising candidate for the intervention of human cancers.

1. Introduction

The Ras proteins are low molecular-weight GTP-binding proteins, which function as biological switches playing a key role in mediation of signal transduction between G-protein-coupled receptors and downstream events, such as mitogen-activated protein kinase (MAPK) and Akt.[1,2] Mutations or excessive activation of Ras proteins are found in approximately 30% of human cancers.[3–5] It was reported that the inhibition of excessive activated Ras proteins may revert malignant cells to a nonmalignant phenotype and cause tumor regression both in vitro and in vivo.[6] As a result, the Ras protein has become an attractive therapeutic target for intervention of a number of cancers.

Farnesylthiosalicylic acid (FTS, Fig. 1), structurally mimicking the carboxyl-terminal farnesylcysteine moiety of Ras proteins, can recognize the anchorage and dislodge the active Ras protein from the cell membrane, thereby blocking the initiation of downstream signaling events, inhibiting tumor cell proliferation and promoting the tumor cell apoptosis.[7–9] Although FTS was well preclinically studied for the treatment of a wide range of malignancies, including lung, breast, and pancreatic cancers,[10–13] it has not got approval for clinical trials mainly due to its limited therapeutic efficacy.[14] Therefore, development of new FTS-based Ras inhibitors with more potent anti-tumor activity should be of clinical significance.

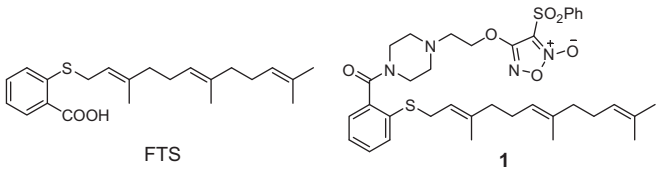

Figure 1. The chemical structures of FTS and furoxan-based nitric oxide derivative of FTS (compound **1**).

Previously, a series of furoxan-based nitric oxide donating FTS derivatives with anti-hepatocellular carcinoma activity was reported by our group.[15] It has been observed that the amide derivatives exhibit much stronger inhibitory effects than the corresponding esters. The most active compound **1** is an amide bearing a piperazine moiety (Fig. 1). It is known that a diamine moiety exists in various pharmaceutical compounds[16] and is often used as a structural block in rational drug design because of its improved water-solubility and enhanced bioavailability and metabolic stability in vivo.[17] It was therefore interesting to determine whether conjugating such diamine moiety with the carboxylic acid group of FTS would provide the derivatives that possess enhanced anticancer activity as well as the desired water-solubility. As part of this ongoing program, we now describe the synthesis of

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mono- (**10a–g**) and bis-amides (**11a–g**) of FTS, and evaluation as potential anti-cancer agents.

2. Results and discussion

2.1. Chemistry

FTS was previously prepared by direct conjugation of thiosalicylic acid with farnesyl bromide in the presence of guanidine carbonate,[18] but some disadvantages limit the utility of this approach. Firstly, hydrogen-bonding effect between the carboxyl with the sulfhydryl of thiosalicylic acid could attenuate the reactivity of sulfhydryl with farnesyl bromide. Secondly, the carboxyl of thiosalicylic acid could also react with farnesyl bromide, producing some by-products, reducing the yields and leading to purification problem. Herein, FTS was obtained using a protection strategy. The synthetic routes of **10a–g** and **11a–g** including FTS are depicted in Scheme 1. Triethylphosphonoacetate (TEPA) was treated with (*E*)-geranyl acetone (**2**) in the presence of NaH to form (*E*,*E*)-ethyl farnesylate (**3**) as previously reported.[19] Compound **3** was reduced by $LiAlH_4$ giving (*E*,*E*)-farnesol (**4**), which was then converted to (*E*,*E*)-farnesyl bromide (**5**) by treatment with PBr_3. In addition, the carboxyl group of thiosalicylic acid (**6**) was protected to form methyl thiosalicylate (**7**). Coupling of **5** with **7** furnished methyl (*E*,*E*)-farnesylthiosalicylicate (FTM, **8**), followed by hydrolysis in aqueous solution of NaOH to provide free carboxylic acid (FTS, **9**). Subsequently, **9** was treated with oxalylchloride to produce (*E*,*E*)-farnesylthiosalicyl chloride, which was treated without further purification with several diamines including cyclic piperazine in the presence of triethylamine, giving target compounds **10a–g** and **11a–g**. The target compounds **10a–g** and **11a–g** were purified by column chromatography, and their structures were characterized by IR, ^1H NMR and MS.

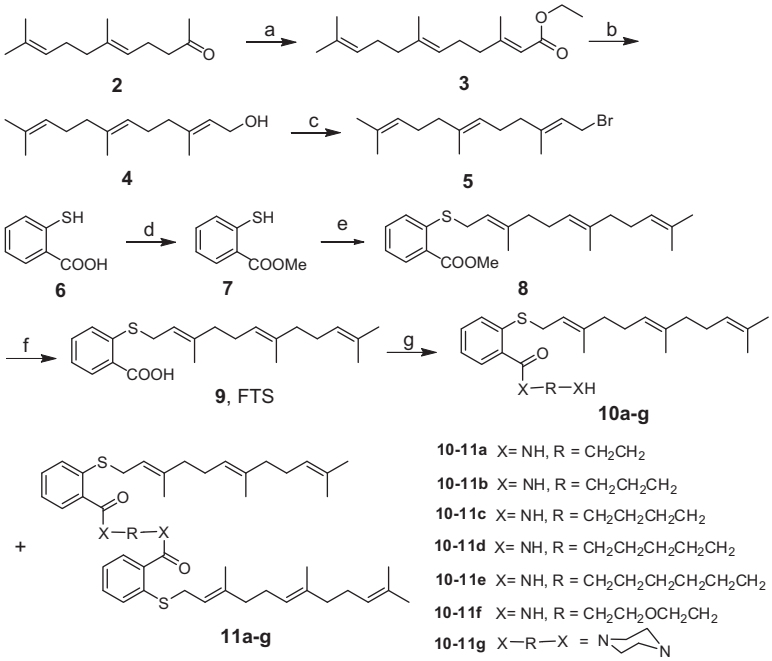
2.2. Biological evaluation

The cell growth inhibitory activity of target compounds **10a–g** and **11a–g** against seven human cancer cell lines SGC7901 (human gastric cancer cells), SMMC-7721 (human hepatocellular carcinoma cells), EJ (human bladder carcinoma cells), SKOV-3 (human ovarian cancer cells), MCF-7 (human breast cancer cells), H460 (human lung cancer cells), Panc-1 (human pancreatic carcinoma cells) were evaluated by MTT assays *in vitro* using FTS and sorafenib, a well-known Ras-related signal inhibitor, as positive controls. The IC_{50} values were summarized in Table 1. As can be seen, monoamides **10a–g** displayed dramatically improved antiproliferative effects compared to FTS. Among them, compounds **10c–f** (IC_{50} = 3.78–15.0 μM) showed slightly stronger antiproliferative effects than sorafenib (IC_{50} = 9.12–22.9 μM). Notably, **10f** exhibited most potent inhibitory activity with IC_{50} value range of 3.78–7.63 μM against all tested cancer cells. However, compounds **11a–f**, the bis-amide derivatives of FTS showed very week inhibitory activity.

Given that FTS is a known Ras inhibitor and could block Ras-related signaling events, inducing tumor cell apoptosis, the most active compound **10f** was selected to determine its effect on SMMC-7721 cells apoptosis. The SMMC-7721 cells were incubated with vehicle alone or with different concentrations of **10f** (3, 6 or 12 μmol/L), or FTS (12 μmol/L) for 48 h, and the percentages of the apoptotic SMMC-7721cells were determined by FITC-Annexin V/PI staining and flow cytometry. As shown in Figure 2, the percentage of annexin V + apoptotic SMMC-7721 cells gradually increased for those cells exposed to increasing concentrations of **10f** (25.9% for 3 μmol/L; 48.2% for 6 μmol/L; and 84.5% for 12 μmol/L), demonstrating that incubation with **10f** induced SMMC-7721cell apoptosis in a dose-dependent manner. In contrast, incubations performed using FTS (12 μmol/L) only induced apoptosis of 19.4% SMMC-7721cells.

Next, a Western blotting analysis was conducted to check the expression levels of Bcl-2, Bax and caspase-3 proteins. It was observed that incubation of **10f** significantly increased levels of pro-apoptotic Bax and caspase 3, but reduced levels of anti-apoptotic Bcl-2 (Fig. 3) in a dose-dependent manner.

Finally, in order to get insight to the mechanisms underlying the activity of these farnesylthiosalicylamide derivatives, we examined the inhibitory effects of the active compound **10f** on the Ras-re-



Scheme 1. Reaction reagents and conditions: (a) TEPA, NaH, ether, 0 °C to rt, 12 h, 81%; (b) $LiAlH_4$, $AlCl_3$, ether, 0 °C to rt, 4 h, 88%; (c) PBr_3, pyridine, *n*-hexane, ether, 0 °C to rt, 4 h, 76%; (d) $SOCl_2$, MeOH, 0 °C-reflux, 8 h, 85%; (e) **5**, K_2CO_3, CH_3CN, 50 °C, 6 h; (f) 1 N NaOH, MeOH, 60 °C, 10 h, 82%; (g) oxalylchloride, diamines or piperazine, TEA, CH_2Cl_2, 0 °C to rt, 2 h.