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Research paper

# Synthesis and biological properties of polyamine modified flavonoids as hepatocellular carcinoma inhibitors

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ABSTRACT

A series of polyamine conjugates of flavonoids with a naphthalene motif were synthesized and evaluated for their anti-hepatocellular carcinoma properties using *in vitro* and *in vivo* assays. Compound **8a** displayed favorable selectivity between hepatocellular carcinoma cells and normal hepatocyte cells, and the combination of **8a** with aspirin resulted in additive inhibition of *in vitro* tumor cell growth and migration. The **8a**-aspirin combination also inhibited H22 liver tumor growth and pulmonary metastasis and improved body weight index in animal models. Preliminary mechanistic studies indicated that **8a** increased the expression of apoptosis-related proteins such as p-p38, p-JNK, p53 and Bcl-2, an effect that was further amplified by aspirin. Therefore, a cocktail therapy of flavonoid-polyamine conjugates with aspirin has potential use as an antitumor therapy.



## 1. Introduction

Flavonoids are a vast group of polyphenolic compounds which exhibit multiple health beneficial health effects such as anti-coagulation, cancer prevention and neuroprotective properties [1]. Many naturally occurring flavonoids have drawn a great deal of attention as potential cancer therapies due to their wide safety margin [2]. Moreover, some of them are found to possess intriguing biological features, for instance, reversing multidrug resistance and reducing tumor cell migration, which are major obstacles in current cancer treatments [3,4]. Substantial studies have been conducted to identify diverse structurally-modified flavonoids with biological benefits superior to their natural counterparts [5,6]. For example, LY294002 (Fig. 1) entered clinical trials as a potential antineoplastic agent [7]. Meanwhile, significant advances have been made in the design of novel flavonoid derivatives focused on modulating multidrug resistance or inhibiting invasion and migration [8,9].

Natural polyamines play a pivotal role in eukaryotic cell growth, division, and proliferation. Both native and synthetic polyamines have been appended to diverse pharmacophores to furnish multiple biological functions, such as cancer inhibition, neuroprotection, and antiparasitic properties [10–12]. F14512 (Fig. 1), a promising polyamine-based antitumor agent, is currently evaluated in Phase I trials [13–15]. Recently, there is increasing interest in the association between polyamines and tumor migration [16]. Russo et al. found that the natural flavonoid quercetin inhibited colon cancer growth partially by decreasing the endogenous synthesis of polyamines [17]. Thus, the polyamine modified flavonoids, may possess some intriguing antitumor properties.

Hepatocellular carcinoma (HCC) accounts for the third major cause of all cancer-associated death in the world [18]. In spite of advances in detection, surgical techniques and chemotherapy, the five-year survival rate for hepatocellular carcinoma patients remains low, mainly because of a high incidence of recurrence and metastasis after tumor excision [19]. Thus, there is a need for novel drugs to treat HCC.

Thus far, there are very few reports on polyamine-based anti-metastatic drug design. We previously showed that 8-flavonemethyl nitrogen mustard was as potent as melphalan against cancerous K562 and B16 cells [20]. Here, we have investigated the potential for polyamine conjugates alone and combined with other drugs to inhibit the growth and metastasis of HCC. We

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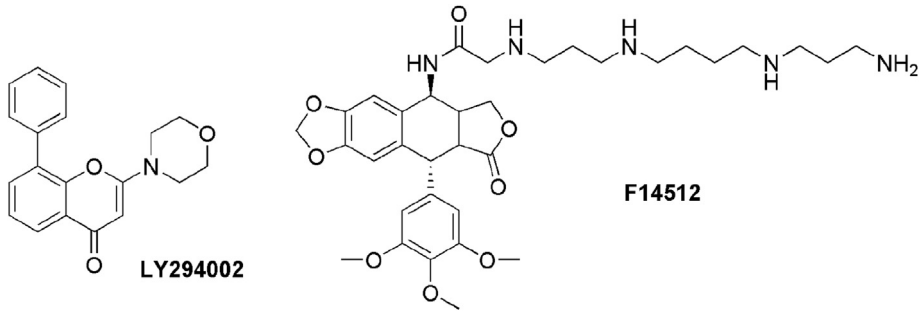



**Fig. 1.** Chemical structures of LY294002 and F14512.

used a flavonoid scaffold, composed of a naphthalene ring linked to a benzopyrano moiety, which was coupled with diverse amines or polyamines at the 8-position.

## 2. Results and discussion

### 2.1. Chemistry

For the synthesis of the 8-methyl flavonoid nucleus (**3**), we used a method previously reported by our lab [20]. In Scheme 1, the 2-acetyl-6-methylphenol (**1**), a starting material prepared from 2-methylphenol via conventional Fries rearrangement, was treated with 1-naphthaldehyde in EtOH to generate chalcone (**2**). **2** was heated in DMSO with $I_2/H_2SO_4$ as catalysts to furnish the 8-methyl flavone backbone (**3**).

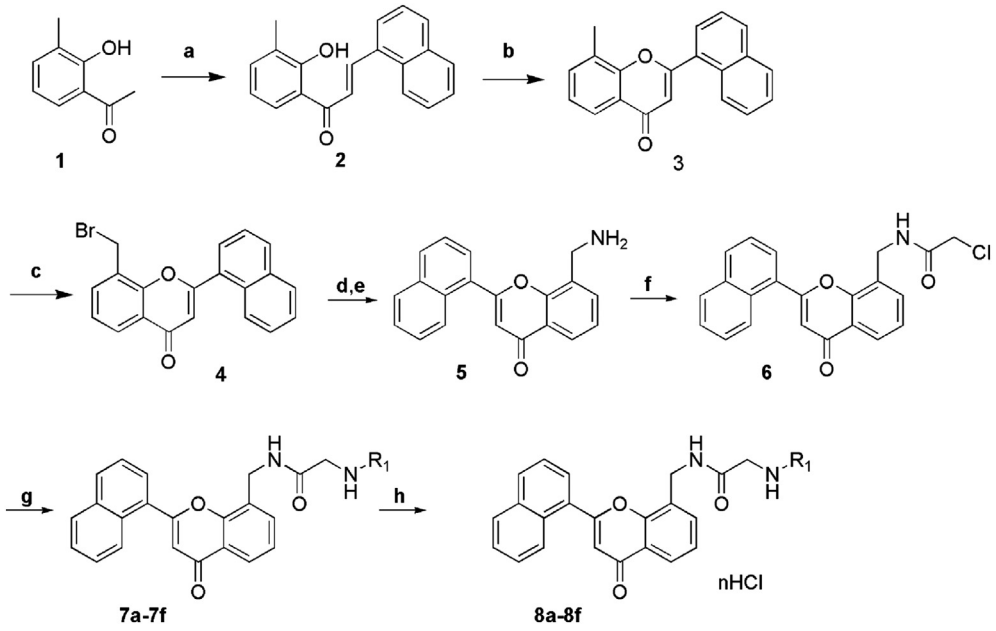
The bromination of **3** with NBS in the presence of benzoyl peroxide (BPO) afforded the intermediate **4**. [20] Intermediate **5**, prepared from **4** by the conventional Gabriel method, was reacted with chloroacetyl chloride to provide compound **6** in a nearly 100% yield. The tandem aminations of compound **6** with diverse amine/polyamine compounds **$RNH_2$** (as shown in Fig. 2), and respective Boc removal or neutralization of formed intermediates **7a-7f** with 4M HCl resulted in the target compounds **8a-8f** as hydrochloride salts [21]. The structures of all the target compounds were confirmed by $^1H$ NMR, ESI-MS and elemental analysis.

### 2.2. In vitro structure-activity relation study

On the basis of our previous findings of effective HCC growth inhibition by the polyamine conjugates [22], two HCC cell lines (HepG2, SMMC7721) were selected for the *in vitro* screen of novel flavonoid-polyamine conjugates. Polyamines and/or flavonoids were assessed for selectivity between tumor and normal cells using one normal hepatocyte cell line (QSG7701).

The inhibitory activity of target compounds against tumor cells was measured by traditional MTT (3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide) tests. Quercetin (Que) and Mitoxantrone (Mito, a clinical drug in HCC therapy), were selected as positive control drugs. The concentrations of new compounds (**8a-8f**) were 30 and 100 μM. The respective concentration of Mito was 1 and 5 μM due to its high potency. The *in vitro* structure-activity relationship of the target conjugates was obtained from the analysis of biological data as illustrated in Table 1.

Increased potency against tumor cells can be achieved by the




**Scheme 1.** Synthesis of flavonoid derivatives. Reagents and conditions: (a) 1-naphthaldehyde, KOH, EtOH, rt, 12 h; (b) $I_2$, $H_2SO_4$, DMSO, 110 °C, 5.5 h; (c) NBS, BPO, $CCl_4$, *hv*, reflux, 8 h, 55%. (d) phthalimide potassium, $CH_3COCH_3$, reflux, 8 h; (e) hydrazine hydrate, overnight, 35% for two steps; (f) chloroacetyl chloride, $K_2CO_3$, $CH_3CN$, rt, overnight, 100%; (g) $RNH_2$, $K_2CO_3$, KI, $CH_3CN$, 30 °C, overnight, 45%; (h) 4M HCl, EtOH, 0 °C, overnight, 80%.