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Research paper

The lead optimization of the polyamine conjugate of flavonoid with a naphthalene motif: Synthesis and biological evaluation

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ABSTRACT

Polyamine conjugated flavonoid with a naphthalene moiety (**ZYY14**) displayed excellent therapeutic activity against hepatocellular carcinoma. In this study, three different series of novel flavonoid-polyamine conjugates were designed and screened against tumor cell lines. The structure-activity relationship study demonstrated the importance of the naphthalene moiety (as the B-ring), the basic side chains in the A-ring, and the methoxy group linked to the C-ring. The optimized compound **9b** displayed better antitumor potency *in vitro* and *in vivo* than the lead compound **ZYY14**. Fluorescent assays revealed that **9b** could enter cancer cells via polyamine transporter (PAT) and locate in mitochondria and endoplasmic reticulum. Compound **9b** and **ZYY14** demonstrated similar apoptotic mechanism in the cytotoxicity studies and stimulated the expression of apoptosis-related proteins, such as p-p38, p-JNK, p53 and Bax. In addition, **9b** can initiate autophagy which inhibited the occurrence of apoptosis. Thus, **9b** can be used as a valuable lead for the future development of antitumor agents.

1. Introduction

Flavonoids, a well-known class of polyphenolic substances widely distributed in fruits and vegetables, have attracted much attention in the field of drug discovery due to their intriguing pharmacological properties embracing antioxidant, antitumor, neuroprotective and anti-inflammatory activities [1–3]. Chromone (4H-benzopyran-4-one) is a key scaffold for synthesizing various flavonoid derivatives [4,5]. Many commercial drugs or lead compounds were invented by modifying the structure of chromone-containing flavonoids, for example: NU7441 (a DNA-PK inhibitor with a fused dibenzothiophene moiety linked to A ring) [6,7] and pranlukast (an anti-asthmatic drug with a tetrazole group as the B ring and a side group at 8-position) [8] (Fig. 1).

Putrescine, spermidine and spermine are important eukaryotic polyamines, responsible for cellular survival, growth, division and proliferation [9,10]. Cancer cells are unable to biosynthesize enough polyamines to sustain their rapid growth rate, they consequently rely on more exogenous native polyamines (or polyamine derivatives), imported by the polyamine transporter (PAT) which is actually hyperactive in cancer cells [11]. Thus, polyamine analogues and antitumor agents conjugated with polyamines may reach tumor tissues more specially.

Many scientists have confirmed that conjugation of polyamine moieties with a parent drug may endow some intriguing biological functions [12–17], our group has also focused on the polyamine-based antitumor agents for many years, mainly composed of polyamines with various drugs and/or pharmacophores, for example: flavones (with a chromone scaffold and phenyl group as the B-ring) may serve as antitumor [18] and anti-cholinesterase agents [19]. In addition, the polyamine modified flavonoids with a naphthalene moiety as the B-ring may inhibit the progression of hepatocellular carcinoma (HCC) (ZYY14, compound **8a** in Ref. [20]; WYX63, compound **6c** in Ref. [21]) (Fig. 2). WYX63 was able to enter tumor cells via PAT and exhibited significant cell selectivity between HepG2 and QSG-7701 (normal hepatocyte) cells. In

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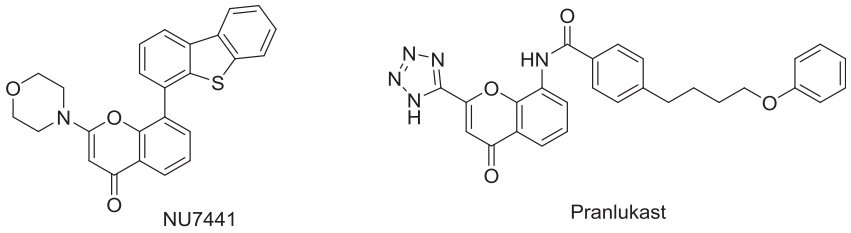

Fig. 1. Chemical structures of NU7441 and Pranlukast.

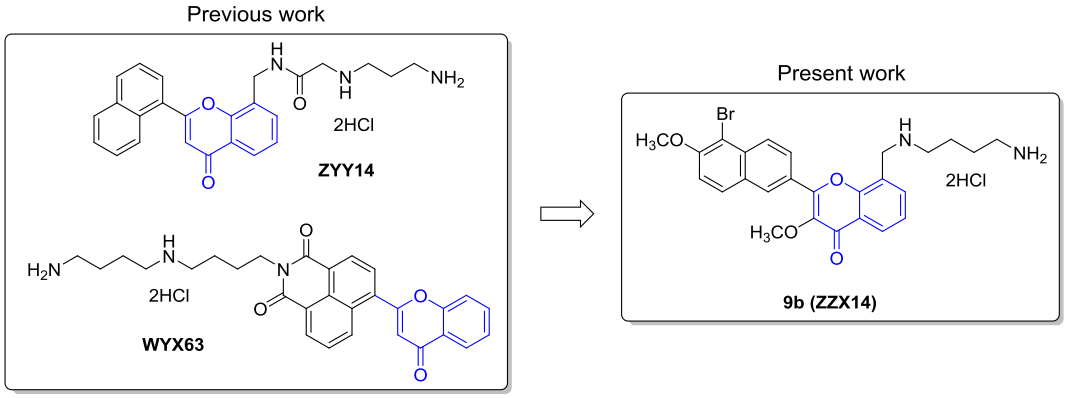

Fig. 2. Previous work and present target compound **9b** (**ZZX14**).

addition, the presence of naphthalimide moiety guaranteed WYX63 as a fluorescent probe which could utilized in cell location. While ZYY14 did not exhibit expected fluorescent property, and it did not show potent activity as a single agent, therefore, in present work we optimized the structure of **ZYY14** and synthesized three series of polyamine-flavonoid conjugates with B ring and side chain modifications. We hypothesized that these conjugates could serve as dual functional agents with anti-cancer ability and subcellular localization. Moreover, the anti-HCC activities and apoptotic mechanisms of the unexpected compound **9b** (**ZZX14**) were evaluated.

2. Results and discussion

2.1. Chemistry

The lead optimization strategy was mainly focused on (i) the B ring, (ii) the position of basic side chains, and (iii) substituents located on B and C rings. The 3-methoxy-8-methyl flavonoid motifs (**3a** and **3b**) were synthesized by a modified Algar-Flynn-Oymada reaction [22], where the starting compound 2-acetyl-6-methylphenol (**1**) reacted with two aromatic aldehydes (Fig. 3) by

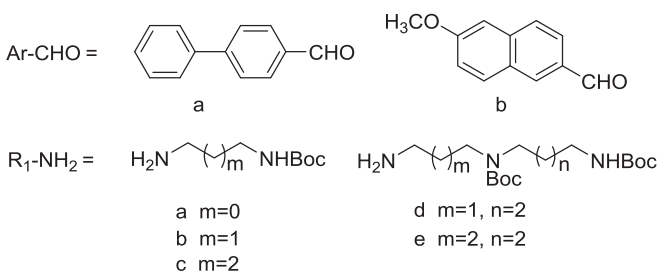

Fig. 3. Building blocks for the designed library.

a Claisen-Schmidt-type reaction to generate relevant chalcones (Scheme 1). The chalcones were oxidized and cyclized (using H_2O_2/I_2/KOH as catalysts) to obtain the 3-hydroxy-8-methyl flavone backbone (**2a**, **2b**), and the hydroxy group was methylated by $(CH_3)_2SO_4$ to get **3a** and **3b**. Their ^{1}HNMR data illustrated that the Ar groups of **3a**/**3b** have little impact on the methyl (δ2.60/2.61) and methoxy group (δ 3.96/3.95) on chromone moiety, respectively.

Bromination of the methyl group of **3a** with N-bromosuccinimide (NBS) and benzoylperoxide (BPO) yielded **4** [13]. Tandem aminations of compound **4** with diverse Boc-protected amines/polyamines R_1NH_2 (Fig. 3), and successive Boc removal from the intermediates **5a-5e** with 4 M HCl yielded the desired compounds **6a-6e** (R_2, Table 1) as hydrochloride salts (Scheme 2).

Bromination of **3b** with NBS and BPO afforded an unexpected intermediate **7** with an extra bromine located on naphthalene moiety. The ^{1}H NMR with one missed aromatic hydrogen signal of **7** (Supporting Information) indicates the occurrence of aromatic bromination in addition to the formation of conventional bromomethyl group. In addition, the ^{1}H NMR signal of methoxy group on chromone moiety has a slight change from δ 3.95 to 3.94 while the signal of methoxy group on naphthalene motif moves reversely from δ 4.05 to 4.08, indicating that the aromatic bromination occurred in the naphthalene motif. To assign the position of bromine, a single crystal of **7** was obtained. As shown in Fig. 4, the bromine is located at the α-site, adjacent to the methoxy group of the naphthalene moiety. Subsequent amination of the compound **7** with Boc-protected amine/polyamines R_1NH_2 (Fig. 3, synthesized according to our previous procedure [23,24]), and successive Boc removal from the intermediates **8a-8c** with 4 M HCl afforded the desired compounds **9a-9c** (R_2, Table 1) as hydrochloride salts (Scheme 3).

Synthesis of the flavonoids with a basic side chain at position-3 is shown in Scheme 4. The 3-methylflavonoid nucleus (**11**) was constructed with 2-propionylphenol (**10**) as the starting compound following a previously reported procedure. The subsequent