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Research paper

# Synthesis and biological effect of chrom-4-one derivatives as functional inhibitors of heat shock protein 27

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ABSTRACT

Heat Shock Protein 27 (HSP27) is a member of small heat shock proteins with a highly-conserved α-crystalline domain. It inhibits aggregation of damaged proteins through a complex structural systems of phosphorylation-dependent oligomerization and self-assembly. It has been demonstrated that HSP27 is involved in a variety of pathophysiological pathways with negative or positive protective activities. In this study, we synthesized six chromone analogs possessing thiiran-2-ylmethoxy or oxyran-2-ylmethoxy substituents and evaluated their biological activities against HSP27 protein. Compounds YK598-2, J4 and J2 induced significant abnormal HSP27 dimer formation in NCI-H460, a human lung cancer cell line. In synergistic anticancer activity test, the compounds effectively producing abnormal HSP27 cross-linking remarkably enhanced the antiproliferative activity of 17-AAG, a HSP90 inhibitor. Target specificity test using the HSP27-silenced cells (shHSP27) showed that compounds YK598-2, J4, and J2 significantly lost their cross-linking activity only under conditions when HSP27 was deprived of. In the evaluation of cancer cell sensitization with cisplatin, cisplatin-induced lung cancer cell growth inhibition was sensitized with statistical significance by J4 and J2 as compared to compound alone treatment. These results suggest that abnormal HSP27 dimerization can be an efficient control point for cancer cell proliferation and chromone compounds might have potential as anticancer agents that modulate abnormal HSP27 dimerization.



## 1. Introduction

Heat shock proteins (HSPs) are highly-preserved proteins that play a major role as chaperones under physiological conditions. This large family of effector components can prevent and protect cells from progressing to pathological state by functionally responding to various cellular stresses [1,2]. These molecular chaperones, including HSP27, HSP70, and HSP90, are highly elevated in cancers. In most cases, they are essentially involved in tumorigenesis. HSPs permit tumorigenic cells to accumulate, transform, and migrate from their primary sites, causing secondary tumors [3]. Thus, HSPs are considered to be strongly responsible for various malignant features of tumor cells, including uncontrollable proliferation, angiogenesis, and metastasis. HSPs can directly stabilize mutated oncogenes and promote inactivation of tumor suppressor genes, leading to cancer progression. Therefore, HSPs are of growing significance in cancer as a promising therapeutic target [4,5].

HSP27 is a member of the small heat shock proteins with a highly-conserved α-crystalline domain. Its expression is mainly regulated by transcription factors, HSF1 and HIF-1α [6]. HSP27 inhibits the aggregation of damaged proteins through a complex structural systems of phosphorylation-dependent oligomerization and self-assembly [7–10]. It has been demonstrated that HSP27 is involved in a variety of pathophysiological pathways with negative or positive protective activities [11]. Numerous studies have observed elevation of HSP27 expression in a variety of carcinomas such as ovarian, breast, and lung cancers, and most of which are closely associated with poor prognosis [12–14]. HSP27 mostly counteracts apoptotic process by interacting with key molecules associated with caspase activation [15]. HSP27 is also clinically implicated as a factor that induces chemo- and radio-resistance which limits efficacy of conventional therapies [16,17].

Although HSP27 is an attractive target, developing HSP27-targeting anticancer small molecules has long been regarded as a

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challenging task. This is due to the absence of active hot spot sites or ATP-binding domain in HSP27, unlike the HSP70 or HSP90 that essentially requires ATP binding and hydrolysis for their chaperone activity [18]. Several alternative strategies have been provoked to inhibit HSP27. However, there are only two HSP27 inhibitors currently undergoing clinical trials. One distinct approach is using short interfering RNA (siRNA) and antisense oligonucleotide (ASO) to suppress HSP27 at the mRNA level [19]. OGX-427, a second-generation ASO has been developed as a specific HSP27 inhibitor to down-regulate gene expression. Moreover, relatively less specific RP101 has been evaluated as the first small molecular inhibitor of HSP27. RP101 is a synthetic nucleoside that interacts non-covalently with Phe29 and Phe33 of HSP27 to inhibit its function. Other techniques include the utilization of HSP27 peptides that can specifically bind to HSP27 to interfere with its functional dimerization and oligomerization *in vitro* [20–22]. Despite their promising anticancer activities, there are still obstacles, including the limitations in intracellular delivery of ASO, unclarified target-specific actions of RP101, and *in vivo* instability of HSP27 peptides. Thus, developing synthetic small molecular HSP27 inhibitors that can specifically target HSP27 without any administration problems is needed to improve the clinical outcome of cancer treatment.

We have previously demonstrated that some small molecules could induce abnormal cross-linking through formation of disulfide bonds between HSP27 proteins instead of normal dimerization, resulting in functional inhibition of HSP27, thereby sensitizing tumors to conventional radiation and chemotherapies [23,24]. Among these compounds, one is zerumbone isolated from natural plant while the other is SW-15, a synthetic compound (Fig. 1). In the structural view point, zerumbone contains Michael type reaction acceptor which reacts with cysteine residue of HSP27 leading to dimerization via covalent bond formation. On the other hand, SW15 had three-atom cyclic thioepoxide ring for interacting with HSP27 for dimerization process, while oxirane analogues of SW15 were inactive to induce HSP27 dimerization. This confirms the importance of the thioepoxide ring for the inhibition of HSP27 through abnormal dimer formation.

As a continuous work to find and optimize HSP27 inhibitors through abnormal dimerization of HSP27 by cross-linking, we have additionally designed and synthesized a small number of chrome-4-one derivatives. Chromone structure is a very common core in the natural products of flavones and isoflvones which hold diverse biological functions. Chromone moiety has a benzopyrone structure in which one phenyl ring is removed from the core structure of xanthone. Our strategy is to replace the xanthone core of SW15 with the chromone core to improve physicochemical property like solubility and interaction with HSP27 to form abnormal dimerization (Fig. 2). We kept the thioepoxdie ring as an interaction spot with HSP27 and attempted to introduce an epoxide ring which possibly inactive to the dimerization process. In this work, we prepared six new chromone analogues to evaluate biological function against to HSP27. Although the number of compounds is small, we expected compounds might provide better drug-like property than SW15. The objective of this study was to identify more potent HSP27 dimerization inducers that might have potential as a novel strategy to modulate HSP27 protein.

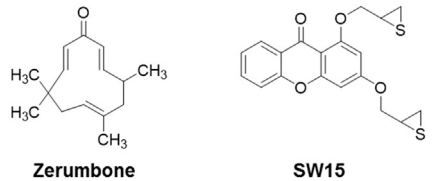


**Fig. 1.** Structures of zerumbone and SW-15 reported as HSP27 cross-linking inducers previously [23,24].

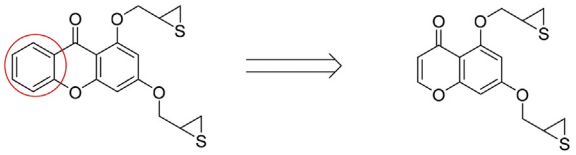

**Fig. 2.** Strategy for choosing chromone derivatives.

## 2. Chemistry

A total of eight compounds (Fig. 3) were synthesized, six of which were novel chromone analogues. Two xanthone compounds SW15 and YK594 were additionally synthesized as positive and negative controls, respectively [24]. The synthesis of target compounds was conducted with chromone as starting compounds with epichlorohydrin or epithiochlorohydrin under $K_2CO_3$ basic condition in DMF-acetone mixed solvent at 80–90 °C. Mono- and bis-(thiiran-2-ylmethoxy) substituted products were isolated from one reaction. In the $^1$H NMR spectrum, mono-(thiiran-2-ylmethoxy) substituted product showed a single peak at around 12.7 ppm, corresponding to 5-hydroxy proton of chromone ring. All the other spectral and analytical data supported the proposed structures.

## 3. Results and discussion

### 3.1. Evaluation of HSP27 cross-linking activities

Previously, we have demonstrated that compounds composed of xanthone moiety could promote abnormal dimerization of HSP27, thus inhibiting oligomerization of HSP27. In addition, the cross-linking activity of each compound differs depending on the structure of side chains [24]. To discover compounds with improved activities, we additionally synthesized chrome-4-one derivatives with different side chains in this study to determine their dimerization potentials. Five out of six compounds with chrome-4-one moiety (except YK597-1) showed apparent cross-linking activity for HSP27 in *in vitro* test using recombinant HSP27 protein along with SW15, a xanthone-derivative (Fig. 4A). YK594, a negative control, did not exhibit any cross-linking activity, similar to previous report [24]. When all compounds were used to treat NCI-H460, a human lung cancer cell line, only YK598-2, J4, and J2 induced significant formation of abnormal dimers. Chrome-4-one derivatives showed relatively higher activities than SW15 with J2 showing the most remarkable dimerization activity. By compound alone, most of them increased apoptotic proteins such as cleaved PARP and cleaved caspase 7 to a low extent. However, SW15 and YK598-2 were rather toxic (data not shown). They displayed strong apoptotic activities (Fig. 4B). Cytotoxicity and apoptotic activities of compounds SW15 and YK598-2 did not correlate with the degree of HSP27 dimerization. This implies that compounds SW15 and YK598-2 might have dimerization-independent effects on cell death.

### 3.2. Synergistic effects between chrome-4-one derivatives and HSP90 inhibitor

The significance of HSP90 in cancer development and progression has led to successful therapeutic exploitation. Outstanding efficacy of HSP90 inhibitors has been demonstrated in a variety of preclinical models. Many of these HSP90 inhibitors are now under