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8-Chrysoeriol, as a potential BCL-2 inhibitor triggers apoptosis of SW1990 pancreatic cancer cells

Yiwen Zhang [a,1], Zhimei Li [b,1], Qiuxia Min [b,1], Abulizi Palida [c,1], Yiyuan Zhang [b], Ruotian Tang [b,*], Lixia Chen [a,*], Hua Li [a,b,*]

[a] Wuya College of Innovation, School of Traditional Chinese Materia Medica, Key Laboratory of Structure-Based Drug Design & Discovery, Ministry of Education, Shenyang Pharmaceutical University, Shenyang 110016, China
[b] Hubei Key Laboratory of Natural Medicinal Chemistry and Resource Evaluation, School of Pharmacy, Tongji Medical College, Huazhong University of Science and Technology, Wuhan 430030, China
[c] Department of Pharmacy, Xinjiang Medical University, Urumpi, Xinjiang 830011, China

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ABSTRACT

8-Chrysoeriol, a bioactive flavanoid, was firstly identified to bind directly to BCL-2 as BH3 mimetics by structure-based virtual ligand screening. And 3D docking model revealed the molecular basis of 8-Chrysoeriol targeting to BCL-2. The interaction between 8-Chrysoeriol and BCL-2 was further confirmed using Microscale Thermophoresis (MST) technique. Meanwhile, high expression level of antiapoptotic protein BCL-2 was detected in SW1990 pancreatic cancer cells and 8-Chrysoeriol showed obvious proapoptosis effect against SW1990 *in vitro*. Collectively, the results showed that 8-Chrysoeriol as a natural dietary product potentially targeting to BCL-2 could serve as a lead compound for SW1990 pancreatic cancer therapy.

1. Introduction

Apoptosis, a conserved process of programmed cell death, plays a key role in eliminating unneeded, abnormal or potentially cancerous cells [1,2]. Limitation or avoidance of apoptosis is a hallmark of cancer [3], and contributes to tumor progression and resistance to anticancer treatments.

BCL-2 family proteins are central apoptosis regulators, which consist of antiapoptotic protein members (*i.e.* BCL-2, BCL-XL, Mcl-1), and two proapoptotic categories: the multi-BH domain proteins (*i.e.* BAX, BAK), as well as the BH3-only proteins (*i.e.* BAD, BID, BIM, NOXA, *etc.*) [4]. BAX and BAK, existed within proteolipid pores in mitochondrial outer membrane, inducing release cytochrome *C* into the cytosol followed by the recruitment and activation of caspases to lead to cell apoptosis [5,6]. Antiapoptotic proteins favor cancer cell survival by binding to the BH3 domain of BAX and BAK to antagonize the activity of proapoptotic members. Furthermore, initiation of apoptosis is also linked to BH3-only proteins by two modes: one mode is provided by some BH3-only proteins such as BIM and BID harboring BH3 domain of BAK or BAX to induce their conformational change and activation [7], and the other mode to promote apoptosis is achieved through liberating BAK or BAX from antiapoptotic protein members, when their BH3-bingding sites were occupied by other BH3-only proteins like BAD and NOXA [8].

Currently, small-molecule inhibitors mimicing "BH3-only" proteins have been generating much interest. ABT-737 and its orally available derivative ABT-263, small-molecule inhibitors of BCL-2 family proteins, neutralize antiapoptotic function by binding to the groove on the surface of BCL-2, BCL-XL, and BCL-W [4,9-11]. Pertinently, more recent ABT-199 is developed to be specific for BCL-2 [12,13]. These mimetics have demonstrated promising anticancer efficacy in diverse cancer models, providing powerful evidence that designing BH3 mimetics is an effective strategy for cancer therapy.

Overexpression of antiapoptotic protein BCL-2 is observed in SW1990 pancreatic cancers, leading to poor response to chemotherapy, suggesting that BCL-2 as a potential therapeutic target for this kind of cancer. On the basis of protein structure of

* Corresponding authors at: Wuya College of Innovation, School of Traditional Chinese Materia Medica, Key Laboratory of Structure-Based Drug Design & Discovery, Ministry of Education, Shenyang Pharmaceutical University, Shenyang 110016, China (L. Chen and H. Li); Hubei Key Laboratory of Natural Medicinal Chemistry and Resource Evaluation, School of Pharmacy, Tongji Medical College, Huazhong University of Science and Technology, Wuhan 430030, China (R. Tang and H. Li).

E-mail addresses: 676264176@qq.com (R. Tang), syzyclx@163.com (L. Chen), li_hua@hust.edu.cn (H. Li).

[1] These authors contributed equally to this work.

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BCL-2, 8-Chrysoeriol, a natural dietary product was firstly identified as BH3 mimetic using structure-based virtual ligand screening. The compound-BCL-2 complex (3D model) revealed the molecular basis of 8-Chrysoeriol targeting to BCL-2. In addition, Microscale Thermophoresis (MST) technique confirmed the high affinity between 8-Chrysoeriol and BCL-2. And it showed obvious anticancer effect on human SW1990 pancreatic cancer cells with high level of BCL-2. Although more verified experiments should be conducted latter, the discovery of a new potential inhibitor targeting to BCL-2 is an undoubtedly important step for finding compounds derived from natural products for treatment of SW1990 pancreatic cancer with high expression level of BCL-2.

2. Results

2.1. Structure-based virtual ligand screening identified 8-Chrysoeriol binding to BCL-2

The natural products from the in-house compound library were screened by structure-based virtual ligand screening. Small molecules were docked to the BH3-binding groove of BCL-2 (PDB: 4AQ3) and the 3D docking calculation was performed with the lowest energy and the most optimal orientation of ligand. Three compounds (Fig. 1) (their ^{1}H- and ^{13}C NMR data see Supplementary Tables S1–S3) had shown significant docking mfScores and prominent capacity to interact with the critical residues in hydrophobic pocket of BCL-2 protein (Table 1).

The mfScore represented the independent score of the binding energy of compound-protein interaction. Fig. 2A and B showed the docking model of the compound **2**-BCL-2 complex. As it can be seen, the 3′-OH of ring B interacted with Arg105 through hydrogen bond. Ring A of 8-Chrysoeriol could form hydrophobic interaction (π-π stacking) with Phe71, and ring C may form hydrophobic interaction (π-π stacking) with Phe63. Ring A and ring C could interact with Met74 and Tyr67 through weaker hydrophobic interaction, respectively. In the model of compound **1**/**3**-BCL-2 complex, carbonyl of ring C interacted with Arg105 through hydrogen bond, and ring B could form weaker hydrophobic interaction with Phe63, Tyr67 and Phe71 (Fig. 3, Supplementary Figs. S1A, B).

As shown in Table 1, compound **2** (mfScore = −138.9) bound to BCL-2 with lower binding energy as compared to compound **1**/**3** (mfScore > −65). Their structural differences provide important mechanistic information for understanding the high affinity binding properties of compound **2**. One notable difference was the adjacent hydroxyls (3′-OH and 4′-OH) in ring B of compound **2**, which hinder ring B to insert into the hydrophobic pocket of BCL-2 and provide alternative spatial arrangement to allow rings A and C tightly binding in the pocket. Another marked difference was the hydrogen bond between 3′-OH in ring B and Arg105, which locked the above favorable conformation of compound **2** and further strengthened the binding. Whereas, due to the absence of 3′-OH, compound **1**/**3** cannot form hydrogen bonds in ring B to lock the favorable conformation, whereas the 4′-OH was not in the right distance to form this hydrogen bonding. Collectively, the reason for targeting ability lies in the unique structural feature of compound **2** which contains two hydroxyl groups (3′-OH and 4′-OH) in ring B. In addition, compared to compound **3**, the structure of compound **1** contains an additional methoxy group, which helps compound form more hydrophobic interaction with the hydrophobic pocket of BCL-2. Therefore, compound **1** (mfScore = −63.6) appears to show higher binding affinity than compound **3** (mfScore = −52.84).

Table 1
Binding affinity of screening hits.

No.	ICM docking mfscore[a] (kcal/mol)
1	−63.6
2	−138.9
3	−52.84

[a] Docking score/interaction potential of compounds with BCL-2 (kcal/mol).

Analysis of these three complexes and comparison with the binding modes reveal a favorable interaction in the compound **2**-BCL-2 complex, which ultimately primes for the apoptosis process. The overall binding mode of compound **2**-BCL-2 complex resembles that of the quercetin-BCL-2 complex [14]. Similarly extending to the hydrophobic cleft of BCL-2 shown in Fig. 2A and B, most importantly, the 3′-OH group of the ring B in quercetin also forms the hydrogen bonding interaction with the relevant residues, which are normally occupied by proapoptotic BAX BH3 binding site in the complex [15], suggesting a role of 3′-OH of ring B in stabilizing the binding interaction between compounds and BCL-2 during the crucial step. Therefore, the models further disclose the molecular basis of these compounds targeting to BCL-2.

2.2. Microscale thermophoresis (MST) confirmed binding affinity of compound **2** to BCL-2

Microscale thermophoresis (MST) method was further employed to validate the finding of virtual ligand screening. MST method is based on fluorescent behaviour of protein in the presence of diverse ligand concentrations during thermophoresis to achieve investigating interaction of protein-protein or protein-small molecule. In this study, dissociation constant (K_d) measured by MST was used to evaluate the affinity between compounds **1**–**3** and BCL-2, compared with the other two compounds, K_d value (36.1 ± 3.64 μM) of compound **2** and BCL-2 indicated its higher affinity to BCL-2 (Fig. 4, Table 2, Supplementary Figs. S2A, B). Results of MST measurements confirmed the prediction of molecular docking.

2.3. Overexpression of BCL-2 were detected in SW1990 cells

Two primary human pancreatic cancer cell lines including Aspc-1 and SW1990 were initially selected for examining the expression level of BCL-2 by Western blot assay. Protein content in the samples was normalized by signal intensity of the structure protein

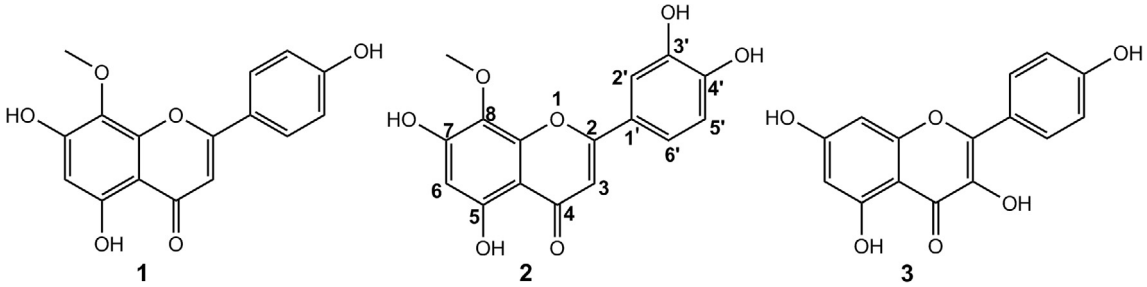

Fig. 1. Structures of 1–3.