Design, Synthesis and Evaluation of a Novel Bcl-2/Mcl-1 Inhibitor

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Abstract. Bcl-2 and Mcl-1 are crucial regulators of apoptosis, therefore dual inhibitors of both proteins could serve as promising new anticancer drugs. Structure-based drug design was utilized to develop a novel, 1-oxo-1H-phenalene-2,3-dicarbonitrile (OPD)-based small molecule inhibitor (S2) of Bcl-2/Mcl-1, which was synthesized readily by nucleophilic substitution reaction and click reaction. S2 exhibited potent binding capability to both Bcl-2 (Ki=0.60±0.20) and Mcl-1 (Ki=0.87±0.25 μM). Furthermore, S2 exhibited potent lethality on Hela cells.

Keywords: Apoptosis, Bcl-2/Mcl-1 protein, Inhibitor, Structure-based drug design.

1. Introduction

Apoptosis, or programmed cell death, is a major source of cell attrition, and the evasion of apoptosis is a hallmark of cancer [1-2]. The cell's decision to undergo apoptosis is regulated by a balance of pro-apoptotic and anti-apoptotic proteins that respond to various extracellular and intracellular stress factors [3]. Bcl-2 and Mcl-1 proteins, known as the two arms of the anti-apoptotic Bcl-2 family proteins, have been identified as key regulators of apoptosis and effective therapeutic targets of cancer [4].

During the past decade, much more efforts has been made to the discovery of inhibitors targeting Bcl-2 family [5-6]. Recently, ABT199, the most promising Bcl-2 inhibitor, was approved by FDA and it was used to the therapy of chronic lymphocytic leukemia (CLL) [7]. However, the cancer killing ability of ABT-199 is limited. It only has effects on tumors which were depended on Bcl-2 to survive, and it will meet with drug-resistance in cancer cells overexpressing Mcl-1 protein [8]. Thus, Consequently, targeting Bcl-2 and Mcl-1 proteins represents an attractive strategy for cancer drug discovery.

Previously, we have reported a Bcl-2/Mcl-1 dual inhibitor, 4-thiomorpholinyl-2, 3-dicyanophenanone (S1), which occupied the p2 pocket in the BH3 groove of both Bcl-2 and Mcl-1. Based on S1, we developed a novel Bcl-2/Mcl-1 dual inhibitor, which was capable of occupying p2 and p3 [9]. S2 exhibited potent binding capability to both Bcl-2 (Ki=0.60±0.20) and Mcl-1 (Ki=0.87±0.25 μM), with Ki values in the sub-micromolar range. Furthermore, S2 exhibited lethality and showed the same order of affinity as (-)-Gossypol and S2 on Hela cells.

2. Experimental

2.1. Material and Methods

All solvents and reagents involving in experiments were purchased from commercial suppliers and directly used without any further purification. The solution of S2 was dissolved in DMSO at a concentration of 10 mM as the stock solution. 1H NMR spectra were recorded on a Bruker 500 spectrometer. Mass spectrometric data were achieved with HP1100LC/MSD MS and an LC/Q-TOF-MS instruments.

2.2. Fluorescence Polarization (FP) Assay

For the competitive binding assay for the Mcl-1 and Bcl-2 protein, FAM-Bid peptide (10 nM), Mcl 1 protein (55 nM), and Bcl-2 protein (140 nM) were preincubated in the assay buffer (100 mM potassium phosphate, pH 7.5; 100 μg/mL bovine gamma globulin; 0.02% sodium azide). Next, serial dilutions of compounds were added. After a 30 min incubation, the polarization values were measured using the Spectra Max M5 Detection System in a black 96-well plate. Saturation experiments determined that FAM-Bid binds to the Mcl-1 and Bcl-2 proteins with Kd values of 1.9 and 8 nM, respectively. The Ki value for each inhibitor was calculated using the equation: $Ki = [I]_{50}/([L]_{50}/Kd+[P]_0/Kd+1)$ where $[I]_{50}$ denotes the concentration of the free inhibitor at 50% inhibition, $[L]_{50}$ is the concentration of the free labeled ligand at 50% inhibition, $[P]_0$ is the concentration of the free protein at 0% inhibition, and Kd is the dissociation constant of the protein ligand complex.

2.3. Cell lines

Hela Cells were cultured in DMEM/high glucose supplemented with 10% fetal bovine serum (Gibco Company, USA) and streptomycin was used to culture the cells at 37°C and 5% CO_2 [10].

2.4. Annexin-V Staining Assay

Annexin-V apoptosis detection kit (Roche, Indianapolis, IN) was used to detect apoptosis and the procedure was conducted according to the manufacturer's instructions. For specific experimental details refer to our previous reports [10].

2.5. Synthesis

OPD (1.0 eq.) was dissolved in 25 mL acetonitrile, and then propargylamine (2.0 eq.) was added to the reaction system and stirred at room temperature for 3 h. After the full reaction of OPD, the solvent was removed by steam. The crude product was separated by silica gel column with dichloromethane: petroleum ether (v/v, 80:1) as eluent, and OPDA was obtained. Yield: 45 %. ^{1}H NMR (500 MHz, Chloroform-d) δ 9.01 (d, J = 8.1 Hz, 1H), 8.68 (d, J = 7.3 Hz, 1H), 8.25 (s, 1H), 8.08 (d, J = 9.1 Hz, 1H), 7.98 (t, J = 7.9 Hz, 1H), 7.19 (d, J = 9.0 Hz, 1H), 3.80 (t, J = 6.4 Hz, 2H), 3.12 (t, J = 6.4 Hz, 1H). MS $[M+H]^+$ calculated 284.1, found 284.1.

Compound OPDA (0.283 g, 1mmol) and (azidomethyl)benzene (1.5 eq.) were dissolved in tetrahydrofuran, and a mixed aqueous solution of NaVc (0.2eq.) and CuSO4 (0.1 eq.) was added to the solution at room temperature. After the full reaction of OPDA, the solvent was removed by steam. The crude product was separated by silica gel column with dichloromethane: petroleum ether (v/v, 50:1) as eluent, and the active molecule S2 was obtained. Yield: 76 %. ^{1}H NMR (500 MHz, DMSO-d_6) δ 8.97 (d, J = 8.1 Hz, 1H), 8.64 (d, J = 7.7 Hz, 1H), 8.24 (s, 1H), 8.04 (d, J = 8.9 Hz, 1H), 7.94 (t, J = 7.8 Hz, 1H), 7.35 (q, J = 7.8 Hz, 3H), 7.29 (d, J = 7.5 Hz, 2H), 7.17 (d, J = 9.0 Hz, 1H), 5.58 (s, 2H), 4.89 (s, 2H). MS $[M-H]^+$ calculated 415.1, found 415.1.

3. Results and Discussion

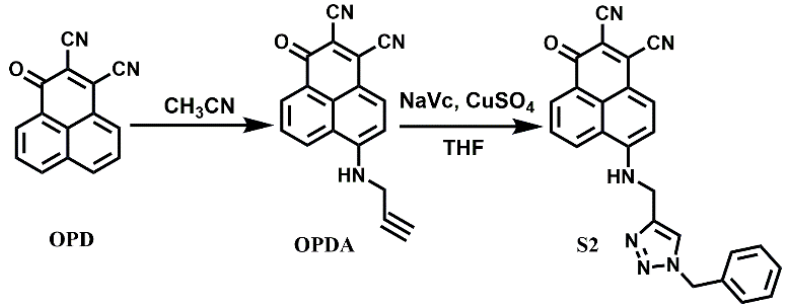

Figure 1. Synthesis of S2.

Previously, we optimized a series of N-substituted piperazine groups based on a Bcl-2/Mcl-1 dual inhibitor scaffold S1 and the substituting groups occupy the P2 pocket to increase affinity with the target protein. In this study, we introduced a new Bcl-2/Mcl-1 inhibitor S2, which was achieved by substituting the 6-thiomorpholine with a triazole group for P2 occupation. Compound S2 was synthesized readily by nucleophilic substitution reaction and click reaction (Figure 1). The structure of compound S2 was confirmed by ^{1}H NMR and MS.

Table 1. Binding affinities (by FPAs) of compounds to Mcl-1/Bcl-2.

Compound		FPAs [Ki ± SD (μM)] [b]	
		Bcl-2	Mcl-1
(-)-Gossypol [a]		0.45 ± 0.10	0.20 ± 0.05
S2		0.60 ± 0.20	0.87 ± 0.25

[a] (-)-Gossypol was obtained from Selleck, China.
[b] Values are the mean ± standard deviation of three independent experiments.

Subsequently, the binding affinity was evaluated through fluorescence polarization assay (FPA), using Bcl-2/Mcl-1 dual inhibitor (-)-Gossypol as control. As depicted in Table 1, S2 demonstrated a potent binding capability to both Bcl-2 (Ki=0.60 ± 0.20 μM) and Mcl-1 (Ki=0.87 ± 0.25 μM). Molecular docking simulation further elucidated that the triazole group occupied the upper part of the P2 pocket, and the benzyl group reached deeper into the P2 pocket like the thiomorpholine group of S1 (Figure 2) [11].

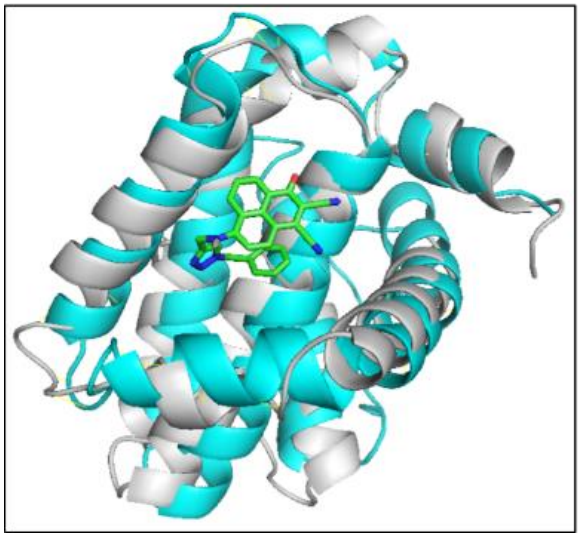

Figure 2. Predicted binding models of S2 (green) in complex with Bcl-2/Mcl-1 (PDB ID: 2XA0 for Bcl-2 and 2NLA for Mcl-1).

Next, the cytotoxic activity of S2 was assessed on Hela cells, which rely on antiapoptotic Bcl-2 family proteins for survival. Cells were incubated with a gradient concentration of S2 for 72 hr, following which annexin-V staining was carried out to ascertain the apoptosis rate (Figure 3). Commercial Bcl-2/Mcl-1 dual inhibitors (-)-Gossypol was used as a positive reference. S2 potently induced apoptosis, showing a potent lethality in Hela cells.

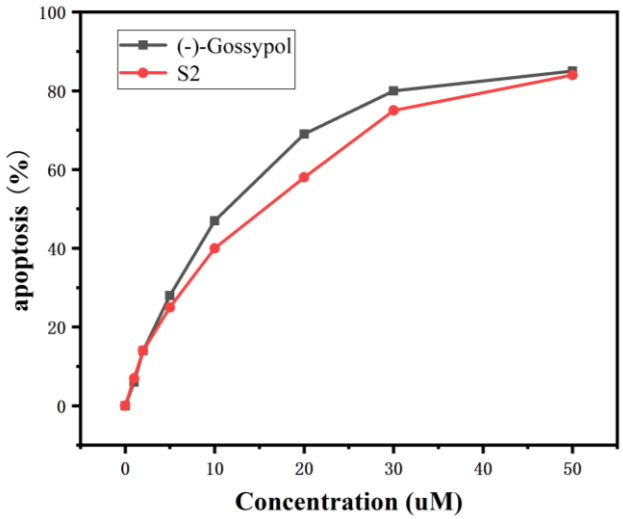

Figure 3. Cell apoptosis assays of Hela cells upon adding S2 and (-)-Gossypol as determined by annexin-V staining

4. Conclusion

In summary, a novel Bcl-2/Mcl-1 inhibitor (S2) was designed through a structure-based scaffold-hopping approach from the previously identified Bcl-2/Mcl-1 dual inhibitor S1. Compound S2 was synthesized facilely by nucleophilic substitution reaction and click reaction, and exhibited potent binding capability to both Bcl-2 (Ki=0.60 ± 0.20 μM) and Mcl-1 (Ki=0.87 ± 0.25 μM). Moreover, S2 displayed potent lethality effect on Hela cells.

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