Bioorganic & Medicinal Chemistry 20 (2012) 4194–4200

Contents lists available at SciVerse ScienceDirect

Bioorganic & Medicinal Chemistry

journal homepage: www.elsevier.com/locate/bmc

Structure-based design of rhodanine-based acylsulfonamide derivatives as antagonists of the anti-apoptotic Bcl-2 protein

Huan-qiu Li [a,†], Jing Yang [a,†], Shuhua Ma [b], Chunhua Qiao [a,*]

[a] College of Pharmaceutical Science, Soochow University, PR China
[b] Department of Chemistry, Towson University, 8000 York Road, Towson, MD 21252, USA

ARTICLE INFO

Article history:
Received 25 April 2012
Revised 31 May 2012
Accepted 31 May 2012
Available online 7 June 2012

Keywords:
Bcl-2
Apoptosis
Antagonist
Structure-based design
Acylsulfonamide

ABSTRACT

A series of novel rhodanine-based acylsulfonamide derivatives were designed, synthesized, and evaluated as small-molecule inhibitors of anti-apoptotic Bcl-2 protein. These compounds exhibit potent antiproliferative activity in three human tumor cell lines (Hep G2, PC-3 and B16-F10). Among them, the most potent compounds **10** and **11** bind to Bcl-2 with a K_i of 20 and 25 nM, respectively. Docking studies demonstrated that these two compounds orient similarly at the binding site of Bcl-2, and the calculated binding affinities (Glide XP score) of compound **10** is more negative than that of compound **11**. The binding interactions of compounds with high binding affinity to Bcl-2 protein were analyzed.

1. Introduction

Apoptosis is an evolutionarily conserved process that regulates normal development and tissue homeostasis by eliminating unwanted and damaged cells. Inhibition of apoptosis is implicated in virtually every known human malignancy.[1,2]

Proteins of the Bcl-2 (B-cell lymphocyte/leukemia-2) family are the key inhibitors of apoptosis in the mitochondria-mediated pathway and comprise both pro- and anti-apoptotic proteins.[3–5] Several homologues, as defined by sequence similarity to some or all of the four Bcl-2 homology (BH) domains in Bcl-2, are found in humans and function as either blockers or inducers of apoptosis. The most structurally disparate of the Bcl-2 family members are those that contain a single BH domain. As molecular sentinels, in response to cellular damage, the proapoptotic BH3-only proteins (e.g., Bad, Bim, Noxa) are activated to propagate death signaling. Antiapoptotic Bcl-2 proteins are over-expressed in many human cancers. Bcl-2 is over-expressed in 80% of B-cell lymphomas, 30–60% of prostate cancers, 90% of colorectal adenocarcinomas, and a wide variety of other cancers; and Bcl-xL is over-expressed in breast and lung cancers.[6–8] Therefore, the antiapoptotic Bcl-2 family protein represents an attractive target for the development of a novel therapy for the treatment of many forms of cancer.

Intense interest in small-molecule BH3 mimetics is reflected in numerous recent publications (Fig. 1). The acylsulfonamide series of inhibitors from Abbott Laboratories, exemplified by **A** (ABT-737), was reported to inhibit Bcl-xL, Bcl-2, and Bcl-w with an IC_{50} less than 1.0 nM, greater than 3 orders of magnitude more potently than previously described small-molecule inhibitors.[9] **A** related compound, **B** (ABT-263), was subsequently prepared and demonstrated oral availability across multiple species, and has now entered phase II clinical trials.[10] A hallmark of this class of drugs is the presence of an acylsulfonamide core. This moiety represents a potential metabolic liability and indeed has been used in prodrug approaches.[11] In recent years, the structure-based approach has become very powerful for the discovery of novel lead compounds and for lead optimization, and new rhodanine-based derivatives as potent Bcl-2 inhibitors are reflected in recent publications (Fig. 1).[12,13] The reported anti-apoptotic/prosurvival pan-Bcl-2 inhibitors are some biarylrhodanine and pyridylrhodanine derivatives. Thus, we postulated that a rhodanine-based sulfonamide may serve such a purpose while being topographically appropriate to maintain high affinity binding to Bcl-2 family proteins (Fig. 1). Herein, we report the synthesis of a series of rhodanine-based acylsulfonamide derivatives, their in vitro antitumor activity, and their binding affinities to Bcl-2 protein.

2. Results and discussions

2.1. Chemistry

The synthesis of the rhodanine-based acylsulfonamide derivatives is outlined in Scheme 1. The intermediates **2a–2l** and **3a–3l** were prepared by the Knoevenagel reaction of various substitute

* Corresponding author. Address: 199 Ren Ai Road, Suzhou 215123, China. Fax: +86 512 65882089.
E-mail address: qiaochunhua@suda.edu.cn (C. Qiao).
† These authors contributed equally to this work.

0968-0896/$ - see front matter © 2012 Elsevier Ltd. All rights reserved.
http://dx.doi.org/10.1016/j.bmc.2012.05.079

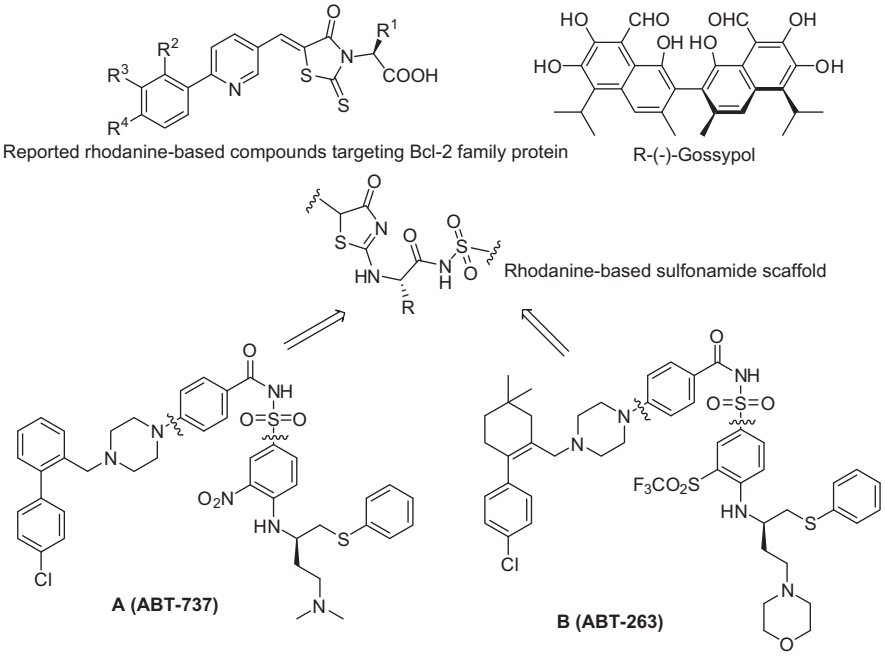


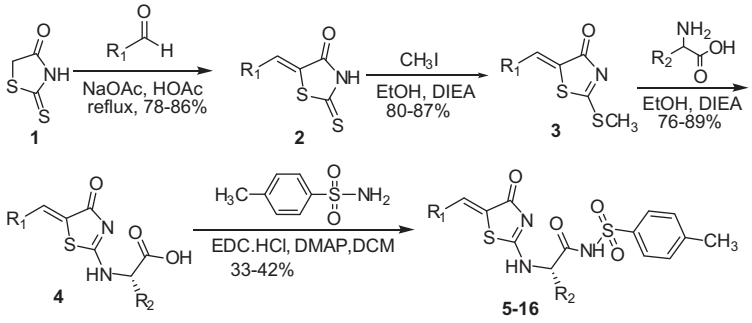
Figure 1. Structures of known BH3 mimetics with pro-apoptotic activity, acylsulfonamides **A** and **B**, R-(−)-Gossypol and the proposed rhodanine-based sulfonamide scaffold.

aldehydes with rhodanine, and methylated by CH_3I. Then, the rhodanine derivatives **4a–4l** were synthesized from various L-amino acids with **3a–3l** and diisopropylethyl amine (DIEA). Finally, rhodanine derivatives **4a–4l**, 4-methylbenzenesulfonamide, dimethylamino pyridine (DMAP) and 1-(3-dimethylaminopropyl)-3-ethylcarbodiimide hydrochloride (EDC) were dissolved in CH_2Cl_2 at room temperature to give the desired rhodanine-based acylsulfonamide derivatives **5–16.** All compounds were purified by silica gel column and identified by elemental H and C NMR and HRMS.

2.2. Antiproliferative assay

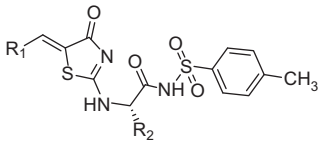
Although Bcl-2 was identified as an oncogene initially in follicular lymphoma, its expression has been identified in a wide array of cancers, including melanoma, myeloma, small cell lung cancer, prostate and acute leukemias.[14] Thus, all the synthesized rhodanine-based acylsulfonamide derivatives **5–16** were evaluated for their antiproliferative activity against three human tumor cell lines Hep G2 (human liver cancer cell), B16-F10 (melanoma cancer cell) and PC-3 (prostate cancer cell) by applying the MTT colorimetric assay. The results were summarized in Table 1. (*R*-)-Gossypol (Fig. 1), reported to bind to and antagonize the anti-apoptotic Bcl-2 family protein,[15] was also screened under identical conditions for comparison. As expected, these rhodanine-based acylsulfonamide derivatives exhibited remarkable antiproliferative activity comparable to the positive control (*R*-)-Gossypol.

Generally, the results in Table 1 showed that those applied to B16-F10 cells would perform better than that in Hep G2 and PC-3 cells. The substituents on the phenyl ring at R^1 position didn't input substantial effects on the antiproliferative capability of the compounds. Compound **9, 11, 12** exhibited single digit IC_{50}value, which is slightly more potent compared to other compounds, suggesting substitute size or position on the phenyl ring would not exert significant effect on the compound anti-proliferative activity. Accordingly, compound **6**, **9**, **11**, **12** displayed moderate activity, indicating relatively bulky isobutyl or isopropyl group at R_2 position would slightly favor the compound potency. Nonetheless, an aromatic benzyl group at R_2 would not significantly tamper the compound inhibitory activity compared to the aliphatic groups. Taken together, the designed and synthesized rhodanine-based acylsulfonamide derivatives did demonstrate fairly potent antiproliferative activity against all three tested cancer cell lines, although the structural-activity relationship study couldn't reveal any defined structural feature at R_1 and R_2 position. Therefore, more structurally diversified derivatives would be synthesized and evaluated to provide clues on the antagonist–protein interaction. Moreover, future study on the sulfonamide moiety variation may afford remarkable cell anti-proliferative candidates.



Scheme 1. Synthesis of the desired rhodanine-based acylsulfonamide derivatives **5–16**.

Table 1
Structure of 4,6-substituted-(diaphenylamino) quinazolines and the anti-proliferative effect from MTT assay



Compound	R_1	R_2	IC_{50}(μg/mL)		
			Hep G2	PC-3	B16-F10
5	4-MeO-Ph	$(CH_3)_2CH$	12.79 ± 0.72	20.27 ± 3.31	11.62 ± 0.83
6	2-F-Ph	$(CH_3)_2CHCH_2$	16.43 ± 1.33	15.31 ± 1.22	11.29 ± 0.39
7	2-F-Ph	$PhCH_2$	18.73 ± 1.35	22.07 ± 1.98	11.90 ± 0.58
8	3-Cl-Ph	$(CH_3)_2CHCH_2$	16.51 ± 1.10	16.75 ± 1.66	13.37 ± 1.30
9	2-Cl-Ph	$(CH_3)_2CHCH_2$	13.35 ± 1.01	15.61 ± 2.03	8.52 ± 1.11
10	2-Cl-Ph	$PhCH_2$	21.01 ± 1.21	16.01 ± 1.79	12.20 ± 0.93
11	2-Cl-Ph	$(CH_3)_2CH$	21.25 ± 1.41	11.87 ± 1.37	8.80 ± 0.49
12	Ph	$(CH_3)_2CH$	18.23 ± 1.22	11.90±1.22	7.46 ± 0.52
13	2-MeO-Ph	$PhCH_2$	23.88 ± 2.31	12.84 ± 0.88	11.84±1.01
14	Ph	$(CH_3)_2CHCH_2$	13.43 ± 0.93	16.64 ± 1.13	14.03 ± 1.27
15	2-MeO-Ph	$PhCH_2$	16.58 ± 1.39	16.37 ± 1.23	12.54 ± 1.32
16	2-MeO-Ph	$(CH_3)_2CH$	20.17 ± 1.36	15.04 ± 0.73	13.49 ± 1.59
(R)-Gossypol	—	—	10.16 ± 0.66	6.71 ± 0.37	5.56 ± 0.31

Table 2
The binding affinities of representative rhodanine-based acylsulfonamide derivatives to Bcl-2 protein

	Compound					
	5	10	11	12	15	(*R*)-Gossypol
K_i ± SD (nM)	671.5 ± 27	20 ± 1.3	25 ± 2.4	3010 ± 72	310 ± 1.3	334 ± 17

2.3. Binding assay to Bcl-2 protein

The Bcl-2 binding affinity of these rhodanine-based acylsulfonamide derivatives and positive control (*R*-)-Gossypol was examined and the results are summarized in Table 2. Except for compound **5**, **10**, **11**, 12, **15**, the binding affinity of all other compounds are greater than 1.0 mM, thus the exact values were not listed in the table. We have used a sensitive and quantitative in vitro fluorescence polarization (FP) based binding assay.[16] The basic principle behind this assay is competition: a fluorescent peptide tracer (Flu-Bak-BH3) and the nonfluorescent small molecule inhibitor competes for binding to the target protein Bcl-2. Using this method, a binding affinity of 300 nM (reported value 270 nM)[17] IC_{50} was obtained for Bak-BH3 peptide binding to the Bcl-2, and the binding affinity of (*R*)-gossypol was determined to be 334 ± 17 nM (reported value 320 nM),[18] both values are consistent with that reported in the literature. As shown in Table 2, compounds **10** and **11** are the most tight binding compounds in the FP assay, with binding affinities of 20 and 25 nM, respectively. This result basically correlates well with that from the cell based assay. Another compound **15**, with the 4-phenyl phenyl at R^1 position and the benzyl at R^2 position, demonstrated comparable binding affinity to the positive control (*R*-)-Gossypol. This biological assay indicated that rhodanine-based acylsulfonamide derivatives **10** and **11** are potential small-molecule Bcl-2 inhibitors as anticancer agents.

2.4. Molecular modeling

The binding affinity of these rhodanine-based acylsulfonamide derivatives to Bcl-2 was also examined through docking studies. All docking calculations were performed using the Glide (Version 5.7, Schrödinger, LLC, New York, NY, 2011) module of a computational program Schrödinger. To validate the performance of Glide docking and scoring, compounds **5**, **10**, **11**, **12** and **15** were docked to the binding site of Bcl-2 using the Glide extra precision (XP) mode.[19] Fig. 2 shows the correlation between the computed Glide XP score and the measured K_i values for the above mentioned 5 inhibitors of Bcl-2.

The Bcl-2—compound **10** complex structure obtained from docking calculations is shown in Figure 3 (a). The acyl-phenyl-sulfonamide group occupies the similar Bcl-2 location as indicated in the NMR structure, in which the benzene ring of the phenyl-sulfonamide group formed π–π interaction with the side chain of Tyr199,[20] And the $PhCH_2$-group at R_2 site of compound **10** occupied the binding site of –SPh group of inhibitor 43B in the NMR structure (PDB ID:2O21);

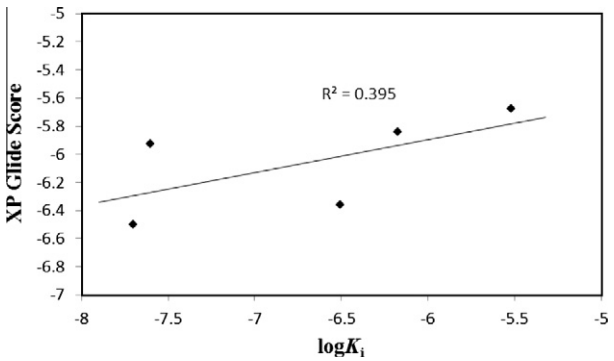


Figure 2. Correlation between the computed Glide XP score and the measured $\log K_i$ values.

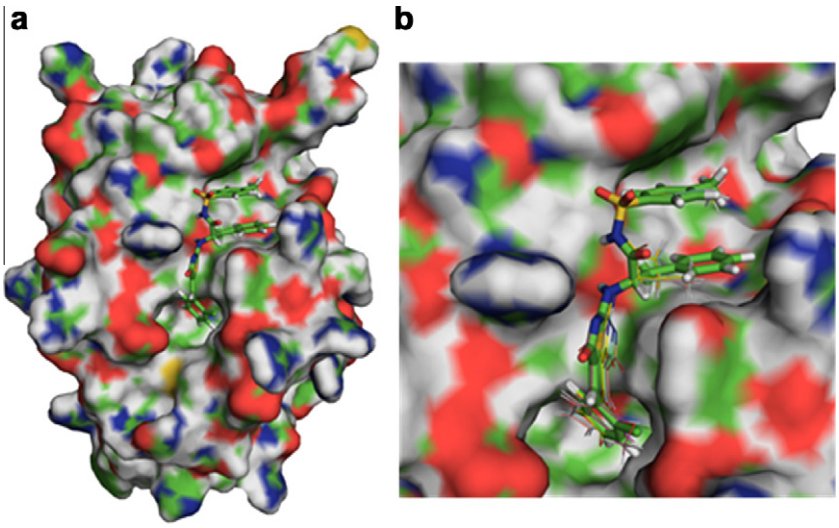


Figure 3. Molecular surface of the complex structure of Bcl-2 and compound **10** (docked structure from Glide); (b) Zoom-in image of compounds **5**, **10**, **11**, **12**, and **15** binding to Bcl-2 protein.

the 2-Cl-Ph-group at R_1 site of compound **10** falls into the hydrophobic site that the benzothiazole group occupied in Bcl-2 and 43B complex structure. Compounds **5**, **11**, **12** and **15** bind to Bcl-2 protein in a similar pattern as compound **10**.

3. Conclusions

In summary, a series of rhodanine-based acylsulfonamide derivatives were designed, synthesized, and evaluated as anti-apoptotic Bcl-2 protein antagonist. Two of these compounds exhibit protein binding affinity comparable to positive control (*R*-)-Gossypol. The cell based MTT assay demonstrated that some compounds displayed fairly potent cytotoxicity. The docking experiment indicated that all potent compounds bind tightly to Bcl-2 protein.

4. Experimental section

4.1. General chemistry

All chemicals (reagent grade) used were purchased from Sigma–Aldrich (USA) and Sinopharm Chemical Reagent Co. Ltd. (China). 1H NMR spectra were measured on Varian Unity Inova 300/400 MHz NMR Spectrometer at 25 °C and referenced to TMS. Chemical shifts are reported in ppm (δ) using the residual solvent line as internal standard. Splitting patterns are designed as s, singlet; d, doublet; t, triplet; m, multiplet. HRMS spectra were acquired on Bruker Esquire Liquid Chromatography-Ion Trap Mass Spectrometer. Analytical thin-layer chromatography (TLC) was performed on the glass-backed silica gel sheets (silica gel 60 Å GF254). All compounds were detected using UV light (254 or 365 nm). Analytical HPLC was conducted on SHIMADZU LC-20AD. Prior to biological evaluation, all compounds were determined to be >95% pure using appropriate analytical methods (MeOH/H_2O 80% v/v, MeOH/H_2O 75% v/v, MeOH/H_2O 60% v/v) based on the peak area percentage.

4.2. General procedure for the preparation of compounds 2a–2l (2j)

Rhodanine **1** (0.73 g, 5.48 mmol), biphenyl-4-carbaldehyde (1.00 g, 5.48 mmol) and sodium acetate (1.57 g, 19.18 mmol) were suspended in acetic acid (50 mL) and the mixture was refluxed for 10 h. The resulting mixture was cooled to room temperature, and then 150 mL water was added. The yellow precipitate that formed was filtered, washed with ethyl ether, and dried to give **2j** (1.32 g, 4.43 mmol) (Yield: 80.9%).^{1}H NMR (300 MHz, DMSO-d_6) δ 7.38–7.58 (m, 3H), 7.64–7.80 (m, 5H), 7.82–7.92 (m, 2H).

4.3. General procedure for the preparation of compounds 3a–3l (3j)

Compound **2j** (1.00 g, 3.37 mmol), DIEA (0.65 g, 5.05 mmol) were suspended in anhydrous ethanol (50 mL), then CH_3I (0.72 g, 5.05 mmol) was slowly added within ten minutes, the mixture was stirred at room temperature for 2 h. Then 150 mL water was added, the yellow precipitate was filtered, and dried to give **3j** (0.85 g, 2.64 mmol) (Yield: 81.1%). ^{1}H NMR (300 MHz, DMSO-d_6) δ 2.85 (s, 3H), 7.38–7.56 (m, 3H), 7.72–7.81 (m, 4H), 7.84–7.92 (m, 3H).

4.4. General procedure for the preparation of compounds 4a–4l (4j)

Compound **3j** (0.60 g, 1.93 mmol), DIEA (0.25 g, 1.93 mmol) and L-phenylalanine (0.32 g, 1.93 mmol) were suspended in ethanol (25 mL) and heated at reflux at 70–80 °C for 7 h. The reaction mixture was cooled to room temperature and concentrated under reduced pressure. The resulting solid was extracted with ethyl acetate for column chromatography. Column chromatography was performed using silica gel (200–300 mesh), eluted with ethyl acetate and petroleum ether (1:1, v/v) to give **4j** (0.66 g, 1.42 mmol) (Yield: 80.2%).^{1}H NMR (400 MHz, DMSO-d_6) δ 3.07 (dd, J = 9.2 Hz, 13.6 Hz, 1H), 3.26 (dd, J = 4.4 Hz, 13.6 Hz, 1H), 4.83–4.94 (m, 1H), 7.18–7.34 (m, 5H), 7.38–7.42 (m, 1H), 7.46–7.52 (m, 2H), 7.61–7.67 (m, 3H), 7.72–7.75 (m, 2H), 7.80–7.86 (m, 2H).

4.5. General procedure for the preparation of compounds 5–16

Compound **4j** (0.60 g, 1.40 mmol), p-TSA (0.36 g, 2.10 mmol), EDC·HCl (0.40 g, 2.10 mmol), DMAP (0.14 g, 1.12 mmol) were suspended in CH_2Cl_2 (25 mL) and stirred at room temperature for 24 h. The mixture was cooled to 5 °C, and acidified to pH 1.0 with addition of HCl aqueous solution (10%), followed by extraction with CH_2Cl_2/MeOH (9:1, 3 × 100 mL). The combined organic layers

were washed with H_2O and brine, dried over Na_2SO_4, and concentrated in vacuo. The residue was subjected to silica gel chromatography or crystallization if necessary to afford the compounds **5–16.** Yield: 33–42%.

4.5.1. (*Z*)-2-(5-(4-Methoxybenzylidene)-4-oxo-4,5-dihydrothiazol-2-ylamino)-3-methyl-*N*-tosylbutanamide 5

^{1}H NMR (400 MHz, DMSO-d_6) δ 0.80 (d, J = 6.8 Hz, 3H), 0.83 (d, J = 6.7 Hz, 3H), 2.05–2.13 (m, 1H), 2.37 (s, 3H), 3.78–3.86 (m, 4H), 7.08 (d, J = 8.7 Hz, 2H), 7.40 (d, J = 8.2 Hz, 2H), 7.51 (d, J = 8.7 Hz, 2H), 7.57 (s, 1H), 7.82 (d, J = 8.2 Hz, 2H), 9.69 (d, J = 7.8 Hz, 1H); ^{13}C NMR (75 MHz, DMSO-d_6) δ 17.6, 18.7, 21.1, 30.5, 55.4, 63.0, 114.8 (2C), 125.6, 126.3, 127.5 (2C), 129.5 (2C), 129.9, 131.3 (2C), 136.2, 144.4, 160.4, 169.3, 174.3, 179.5. ESI-MS: Calcd for $C_{23}H_{25}N_3O_5S_2$ $[M+H]^+$ 488.1308, Found 488.1317 (error 1.8 ppm).

4.5.2. (*Z*)-2-(5-(2-Fluorobenzylidene)-4-oxo-4,5-dihydrothiazol-2-ylamino)-4-methyl-*N*-tosylpentanamide 6

^{1}H NMR (400 MHz, DMSO-d_6) δ 0.85 (d, J = 6.1 Hz, 3H), 0.89 (d, J = 6.1 Hz, 3H), 1.48–1.60 (m, 3H), 2.37 (s, 3H), 4.67–4.74 (m, 1H), 7.34–7.43 (m, 4H), 7.48–7.55 (m, 2H), 7.66 (s, 1H), 7.81 (d, J = 8.3 Hz, 2H), 9.96 (d, J = 7.9 Hz, 1H); ^{13}C NMR (75 MHz, DMSO-d_6) δ 21.1 (2C), 22.8, 24.5, 56.7, 116.2 (d, J = 21.3 Hz), 120.9 (d, J = 6.7 Hz), 121.9 (d, J = 12.0 Hz), 125.4, 127.5 (2C), 128.5, 129.6 (2C), 131.1, 132.0 (d, J = 8.4 Hz), 136.2, 144.4, 158.8, 162.2, 170.2, 173.8, 178.9. ESI-MS: Cacld for $C_{23}H_{24}FN_3O_4S_2$ $[M+H]^+$: 490.1265, Found 490.1274 (error 1.8 ppm).

4.5.3. (*Z*)-2-(5-(2-Fluorobenzylidene)-4-oxo-4,5-dihydrothiazol-2-ylamino)-3-phenyl-*N*-tosylpropanamide 7

^{1}H NMR (300 MHz, DMSO-d_6) δ 2.40 (s, 3H), 2.82 (dd, J = 9.9, 13.5 Hz, 1H), 3.13 (dd, J = 3.6, 13.8 Hz, 1H), 4.82–4.99 (m, 1H), 7.16–7.24 (m, 5H), 7.33–7.44 (m, 4H), 7.46–7.54 (m, 2H), 7.61 (s, 1H), 7.82 (d, J = 8.1 Hz, 2H), 10.08 (d, J = 7.4 Hz, 1H); ^{13}C NMR (75 MHz, DMSO-d_6) δ 21.1 (2C), 22.8, 24.5, 56.7, 116.2 (d, J = 21.3 Hz), 120.9 (d, J = 6.7 Hz), 121.9 (d, J = 12.0 Hz), 125.4, 127.5 (2C), 128.5, 129.6 (2C), 131.1, 132.0 (d, J = 8.4 Hz), 136.2, 144.4, 158.8, 162.2, 170.2. ESI-MS: Cacld for $C_{23}H_{24}FN_3O_4S_2$ $[M+H]^+$: 490.1265, Found 490.1274 (error 1.8 ppm).

4.5.4. (*Z*)-2-(5-(3-Chlorobenzylidene)-4-oxo-4,5-dihydrothiazol-2-ylamino)-4-methyl-*N*-tosylpentanamide 8

^{1}H NMR (400 MHz, DMSO-d_6) δ 0.85 (d, J = 6.1 Hz, 3H), 0.89 (d, J = 6.2 Hz, 3H), 1.49–1.66 (m, 3H), 2.37 (s, 3H), 4.65–4.79 (m, 1H), 7.38–7.46 (m, 2H), 7.49–7.58 (m, 3H), 7.60–7.67 (m, 2H), 7.79–7.90 (m, 2H), 9.91 (d, J = 7.0 Hz, 1H); ^{13}C NMR (100 MHz, DMSO-d_6) δ 17.6, 18.8, 21.2, 30.7, 63.3, 127.7 (2C), 127.8, 128.4, 129.2, 129.5, 129.7 (2C), 130.4, 131.2, 134.0, 136.2, 136.3, 144.6, 169.4, 174.4, 179.3. ESI-MS: Cacld for $C_{22}H_{22}ClN_3O_4S_2$ $[M+H]^+$: 492.0813, Found 492.0820 (error 1.4 ppm).

4.5.5. (*Z*)-2-(5-(2-Chlorobenzylidene)-4-oxo-4,5-dihydrothiazol-2-ylamino)-4-methyl-*N*-tosylpentanamide 9

^{1}H NMR (400 MHz, DMSO-d_6) δ 0.86 (d, J = 5.8 Hz, 3H), 0.89 (d, J = 5.8 Hz, 3H), 1.47–1.63 (m, 3H), 2.38 (s, 3H), 4.67–4.78 (m, 1H), 7.39–7.61 (m, 6H), 7.75–7.84 (m, 3H), 9.97 (d, J = 7.5 Hz, 1H); ^{13}C NMR (75 MHz, DMSO-d_6) δ 21.1 (2C), 22.7, 24.4, 40.1, 56.7, 125.2, 127.4 (2C), 127.9, 128.5, 129.5 (2C), 130.1, 131.1, 132.0, 132.1, 134.1, 136.2, 144.3, 170.1, 173.9, 178.6. ESI-MS: Cacld for $C_{23}H_{24}ClN_3O_4S_2$ $[M+H]^+$: 506.0970, Found 506.0955 (error 2.9 ppm).

4.5.6. (*Z*)-2-(5-(2-Chlorobenzylidene)-4-oxo-4,5-dihydrothiazol-2-ylamino)-3-phenyl-*N*-tosylpropanamide 10

^{1}H NMR (300 MHz, DMSO-d_6) δ 2.40 (s, 3H), 2.82 (dd, J = 9.9, 13.8 Hz, 1H), 3.13 (dd, J = 4.5, 14.4 Hz, 1H), 4.89–4.99 (m, 1H), 7.15–7.27 (m, 5H), 7.40–7.62 (m, 6H), 7.75 (s, 1H), 7.82 (d, J = 8.4 Hz, 2H), 10.08 (d, J = 7.5 Hz, 1H); ^{13}C NMR (100 MHz, DMSO-d_6) δ 21.2, 36.7, 59.5, 125.3, 127.0, 127.6 (2C), 128.1, 128.4 (2C), 128.7, 129.2 (2C), 129.7 (2C), 130.3, 131.3, 132.0, 132.1, 134.2, 135.8, 136.1, 144.6, 169.1, 174.1, 178.7. ESI-MS: Cacld for $C_{26}H_{22}ClN_3O_4S_2$ $[M+H]^+$: 540.0813, Found 540.0824 (error 2.0 ppm).

4.5.7. (*Z*)-2-(5-(2-Chlorobenzylidene)-4-oxo-4,5-dihydrothiazol-2-ylamino)-3-methyl-*N*-tosylbutanamide 11

^{1}H NMR (400 MHz, DMSO-d_6) δ 0.78 (d, J = 6.8 Hz, 3H), 0.82 (d, J = 6.8 Hz, 3H), 2.04–2.13 (m, 1H), 2.37 (s, 3H), 4.57–4.63 (m, 1H), 7.39–7.62 (m, 6H), 7.78 (s, 1H), 7.82 (d, J = 8.3 Hz, 2H), 9.93 (d, J = 7.9 Hz, 1H); ^{13}C NMR (100 MHz, DMSO-d_6) δ 17.6, 18.7, 21.1, 30.6, 63.1, 125.1, 127.6 (2C), 128.1, 128.6, 129.6 (2C), 130.2, 131.2, 132.1, 132.2, 134.2, 136.1, 144.5, 169.2, 174.5, 178.8. ESI-MS: Cacld for $C_{22}H_{22}ClN_3O_4S_2$ $[M+H]^+$: 492.0813, Found 492.0819 (error 1.2 ppm).

4.5.8. (*Z*)-2-(5-Benzylidene-4-oxo-4,5-dihydrothiazol-2-ylamino)-3-methyl-*N*-tosylbutanamide 12

^{1}H NMR (400 MHz, DMSO-d_6) δ 0.80 (d, J = 6.5 Hz, 3H), 0.83 (d, J = 6.7 Hz, 3H), 2.05–2.13 (m, 1H), 2.37 (s, 3H), 4.58–4.68 (m, 1H), 7.34–7.45 (m, 3H), 7.49–7.58 (m, 4H), 7.61 (s, 1H), 7.82 (d, J = 7.2 Hz, 2H), 9.79 (d, J = 7.1 Hz, 1H) ; ^{13}C NMR (75 MHz, DMSO-d_6) δ 17.7, 18.8, 21.2, 30.6, 63.1, 125.7, 127.6, 128.5, 129.3 (2C), 129.6 (2C), 129.8, 130.0, 134.0, 136.2, 141.5, 141.9, 144.5, 169.4, 174.5, 179.4. ESI-MS: Cacld for $C_{22}H_{23}N_3O_4S_2$ $[M+H]^+$: 458.1203, Found 458.1190 (error 2.8 ppm).

4.5.9. (*Z*)-2-(5-Benzylidene-4-oxo-4,5-dihydrothiazol-2-ylamino)-4-methyl-*N*-tosylpentanamide 13

^{1}H NMR (400 MHz, DMSO-d_6) δ 0.85 (d, J = 6.1 Hz, 3H), 0.88 (d, J = 6.1 Hz, 3H), 1.45–1.65 (m, 3H), 2.37 (s, 3H), 4.64–4.77 (m, 1H), 7.38–7.45 (m, 3H), 7.49–7.57 (m, 4H), 7.63 (s, 1H), 7.77–7.82 (m, 2H) , 9.93 (d, J = 7.3 Hz, 1H); ^{13}CNMR (75 MHz, DMSO-d_6) δ 21.2 (2C), 23.0, 24.6, 29.2, 56.8, 127.6 (2C), 128.4, 129.4 (2C), 129.6 (2C), 129.7 (2C), 130.0, 130.2, 134.0, 136.4, 144.5, 170.5, 174.1, 179.5. ESI-MS: Cacld for $C_{23}H_{25}N_3O_4S_2$ $[M+H]^+$: 472.1359, Found 472.1359 (error 0 ppm).

4.5.10. (*Z*)-2-(5-(Biphenyl-4-ylmethylene)-4-oxo-4,5-dihydrothiazol-2-ylamino)-3-phenyl-*N*-tosylpropanamide 14

^{1}H NMR (400 MHz, DMSO-d_6) δ 2.40 (s, 3H), 2.84 (dd, J = 10.0 Hz, 14.0 Hz, 1H), 3.14 (dd, J = 4.4 Hz, 14.0 Hz, 1H), 4.89–4.97 (m, 1H), 7.17–7.26 (m, 5H), 7.39–7.43 (m, 3H), 7.46–7.52 (m, 2H), 7.62–7.66 (m, 3H), 7.72–7.76 (m, 2H), 7.80–7.85 (m, 4H) , 9.94 (d, J = 10.1 Hz, 1H); ^{13}C NMR (100 MHz, DMSO-d_6) δ 21.3, 36.8, 59.5, 126.9 (2C), 127.1, 127.5(2C), 127.7(2C), 128.2, 128.3, 128.5(2C), 129.2(3C), 129.3(2C), 129.8(2C), 130.3(2C), 133.0, 136.0, 136.2, 139.1, 141.3, 144.6, 169.3, 174.1, 179.5. ESI-MS: Cacld for $C_{32}H_{27}N_3O_4S_2$ $[M+H]^+$: 582.1521, Found 582.1525 (error 0.6 ppm).

4.5.11. (*Z*)-2-(5-(2-Methoxybenzylidene)-4-oxo-4,5-dihydrothiazol-2-ylamino)-3-phenyl-*N*-tosylpropanamide 15

^{1}H NMR (300 MHz, DMSO-d_6) δ 2.40 (s, 3H), 2.80 (dd, J = 9.6, 13.5 Hz, 1H), 3.12 (dd, J = 4.2, 13.5 Hz, 1H), 3.86 (s, 3H), 4.86–4.96 (m, 1H), 7.04–7.12 (m, 2H), 7.18–7.26 (m, 5H), 7.35–7.43 (m, 4H), 7.78–7.85 (m, 3H), 9.92 (d, J = 7.8 Hz, 1H); ^{13}C NMR (75 MHz, DMSO-d_6) δ 21.1, 36.8, 55.7, 59.5, 111.7, 120.8, 122.5, 124.5, 126.8, 127.5 (2C), 128.0, 128.3 (2C), 128.4, 129.2 (2C), 129.5 (2C), 131.5, 136.1, 136.7, 144.0, 157.8, 169.5, 174.1, 179.3. ESI-MS: Cacld for $C_{27}H_{25}N_3O_5S_2$ $[M+H]^+$: 536.1308, Found 536.1310 (error 0.3 ppm).

4.5.12. (*Z*)-2-(5-(2-Methoxybenzylidene)-4-oxo-4,5-dihydrothiazol-2-ylamino)-3-methyl-*N*-tosylbutanamide 16

^{1}H NMR (300 MHz, DMSO-d_6) δ 0.78–0.84 (m, 6H), 2.02–2.11 (m, 1H), 2.38 (s, 3H), 3.88 (s, 3H), 4.59 (t, J = 6.9 Hz, 1H), 7.06–7.14 (m, 2H), 7.39–7.42 (m, 4H), 7.81–7.84 (m, 3H), 9.72 (d, J = 7.7 Hz, 1H); ^{13}C NMR (100 MHz, DMSO-d_6) δ 17.7, 18.8, 21.2, 30.7, 55.8, 63.1, 111.8, 121.0, 122.6, 124.7, 127.7 (2C), 128.1, 128.6, 129.7 (2C), 131.7, 136.3, 144.6, 158.0, 169.6, 174.8, 179.6. ESI-MS: Cacld for $C_{23}H_{25}N_3O_5S_2$ [M+H]$^+$: 488.1308, Found 488.1306 (error 0.4 ppm).

4.6. Cell proliferation assay

The antiproliferative activities of chalcone thiosemicarbazide derivatives were determined using a standard (MTT)-based colorimetric assay (Sigma). Briefly, cell lines were seeded at a density of 7×10^3 cells/well in 96-well microtiter plates (Costar). After 24 h, exponentially growing cells were exposed to the indicated compounds at final concentrations ranging from 0.1 to 40 mg/mL. After 48 h, cell survival was determined by the addition of an MTT solution (20 μL of 5 mg/mL MTT in PBS). After 6 h, 100 mL of 10% SDS in 0.01 N HCl was added, and the plates were incubated at 37 °C for a further 4 h; optical absorbance was measured at 570 nm on an LX300 Epson Diagnostic microplate reader. Survival ratios are expressed in percentages with respect to untreated cells. IC_{50} values were determined from replicates of 6 wells from at least two independent experiments.

4.7. Protein expression and purification

The isoform 2 construct of the human Bcl-2 with an N-terminal 6xHis tag was used.[21] Bcl-2 protein was produced in *E. coli* BL21 (DE3) cells. Cells were grown at 37 °C in 2xYT containing antibiotics to an OD600 of 0.6. Protein expression was induced by 0.4 mM IPTG at 20 °C for 20 h. Cells were lysed in 50 mM Tris pH 8.0 buffer containing 500 mM NaCl, 0.1%bME and 40 μL of Leupectin/Aprotin. The protein was purified from the soluble fraction using Ni-NTA resin, following the manufacturer's instructions. The protein was further purified on a Superdex75 column (Amersham Biosciences) in 25 mM Tris pH 8.0 buffer containing 150 mM NaCl and 2 mM DTT.

4.8. Fluorescence polarization-based binding assay for Bcl-2 protein

A 21-residue Bid BH3 peptide (residues 79–99:QEDIIRNIARHLAQVGDSMDR) derived from its BH3 domain, was synthesized and labeled at the N-terminus with 6-carboxyfluorescein succinimidyl ester (FAM) as the fluorescence tag (FAM-Bid). Saturation experiments determined that FAM-Bid binds to Bcl-2 protein with a K_d value of 7.1 nM.

For competitive binding experiments, Bcl-2 protein (40 nM) and FAM-Bid peptide (2.5 nM) were preincubated in the assay buffer (100 mM potassium phosphate, pH 7.5; 100 μg/mL bovine gamma globulin; 0.02% sodium azide, purchased from Invitrogen, Life Technologies). 5 μL of a solution in DMSO of the tested compound was added to the Bcl-2/FAM-Bid solution in Dynex 96-well, black, round-bottom plates (Fisher Scientific) to produce a final volume of 125 μL. For each experiment, a control containing Bcl-2 and Flu-Bid peptide (equivalent to 0% inhibition), and another control containing only FAM-Bid, were included on each assay plate. After 4 hours incubation, the polarization values in milipolarization units (mP) were measured at an excitation wavelength at 485 nm and an emission wavelength at 530 nm using the Ultra plate reader (Tecan U.S. Inc., Research Triangle Park, NC). The K_i value for each inhibitor was calculated using the equation developed for FP-based assays. [22]

4.9. Molecular modeling procedure

All docking calculations were performed using the Glide module of a computational program Schrödinger. The NMR-derived structure of Bcl-2 complexed with an acyl-sulfonamide based inhibitor 3-nitro-*N*-{4-[2-(2-phenylethyl)-1,3-benzothiazol-5-yl]benzoyl}-4-{[2-(phenylsulfanyl)ethyl]amino}benzenesulfonamide (43B in PDB code: 2O21) was used as the receptor for this docking experiment. The hydrogen atoms from the original NMR structure were removed and then the complex structure was processed with the Protein Preparation Wizard of Schrödinger. In this step, hydrogen atoms were added to the protein and ligand, the protein–ligand complex was then refined by Impact minimization.

All the inhibitor structures used in the docking experiment were modeled with Schrödinger and refined by its LigPrep module. The docking calculations were performed using the Glide extra precision (XP) mode. Default input parameters were employed in docking calculations and a core of acyl-phenyl-sulfonamide group from the prepared receptor structure was used as a reference. To compensate for the rigid protein structure, Glide provides the options to scale the van der Waals radii of nonpolar atoms of both the protein and ligand. In this work, the default scaling factors were used, that is, the van der Waals radii for nonpolar atoms were scaled by a factor of 0.8, and those of protein atoms were not scaled.

Acknowledgment

This work was financially supported by grants from Chinese National Science & Technology Major Project on "Key New Drug Creation and Manufacturing Program" (2009ZX09103-129) to Professor Chunhua Qiao at Soochow University. Foundation from the Priority Academic Program Development of Jiangsu Higher Education Institutions is also acknowledged; The docking study work was supported by Towson University start-up funds to Professor Ma.

References and notes

1. McDonald, E. N.; El-Deiry, W. S. *Death Recept. Cancer Ther.* **2005**, 1–41.
2. Amundson, S. A.; Myers, T. G.; Scudiero, D.; Kitada, S.; Reed, J. C.; Fornace, A. J. *Cancer Res.* **2000**, *60*, 6101–6110.
3. Adams, J. M.; Cory, S. *Curr. Opin. Immunol.* **2007**, *19*, 488–496.
4. Youle, R. J.; Strasser, A. *Nat. Rev. Mol. Cell Biol.* **2008**, *9*, 47–59.
5. van Delft, M. F.; Huang, D. C. *Cell Res.* **2006**, *16*, 203–213.
6. Adams, J. M.; Cory, S. *Oncogene* **2007**, *26*, 1324–1337.
7. Lessene, G.; Czabotar, P.; Colman, P. M. *Nat. Rev. Drug Discovery* **2008**, *7*, 989–1000.
8. Cory, S.; Adams, J. M. *Cancer Cell* **2005**, *8*, 5–6.
9. Oltersdorf, T.; Elmore, S. W.; Shoemaker, A. R.; Armstrong, R. C.; Augeri, D. J.; Belli, B. A.; Bruncko, M.; Deckwerth, T. L.; Dinges, J.; Hajduk, P. J.; Joseph, M. K.; Kitada, S.; Korsmeyer, S. J.; Kunzer, A. R.; Letai, A.; Li, C.; Mitten, M. J.; Nettesheim, D. G.; Ng, S.; Nimmer, P. M.; O'Connor, J. M.; Oleksijew, A.; Petros, A. M.; Reed, J. C.; Shen, W.; Tahir, S. K.; Thompson, C. B.; Tomaselli, K. J.; Wang, B.; Wendt, M. D.; Zhang, H.; Fesik, S. W.; Rosenberg, S. H. *Nature* **2005**, *435*, 677–681.
10. Park, C.-M.; Bruncko, M.; Adickes, J.; Bauch, J.; Ding, H.; Kunzer, A.; Marsh, K. C.; Nimmer, P.; Shoemaker, A. R.; Song, X.; Tahir, S. K.; Tse, C.; Wang, X.; Wendt, M. D.; Yang, X.; Zhang, H.; Fesik, S. W.; Rosenberg, S. H.; Elmore, S. W. *J. Med. Chem.* **2008**, *51*, 6902–6915.
11. Huang, S.; Connolly, P. J.; Lin, R.; Emanuel, S.; Middleton, S. A. *Bioorg. Med. Chem. Lett.* **2006**, *16*, 3639–3641.
12. Bernardo, P. H.; Sivaraman, T.; Wan, K. F.; Xu, J.; Krishnamoorthy, J.; Song, C. M.; Tian, L.; Chin, J. S.; Lim, D. S.; Mok, H. Y.; Yu, V. C.; Tong, J. C.; Chai, C. L. *Pure. Appl. Chem.* **2011**, *83*, 723–731.
13. Chai, C. C. L.; Bernardo, P. H., Xu, J. WO 2010/024783 A1. US Patent.
14. Zeitlin, B. D.; Zeitlin, I. J.; Nor, J. E. *J. Clin. Oncol.* **2008**, *26*, 4180–4188.
15. Kitada, S.; Leone, M.; Sareth, S.; Zhai, D.; Reed, J. C.; Pellecchia, M. *J. Med. Chem.* **2003**, *46*, 4259–4264.
16. Tang, G.; Yang, C.-Y.; Nikolovska-Coleska, Z.; Guo, J.; Qiu, S.; Wang, R.; Gao, W.; Wang, G. P.; Stuckey, J.; Krajewski, K.; Jiang, S.; Roller, P. P.; Wang, S. M. *J. Med. Chem.* **2007**, *50*, 1723–1726.
17. Enyedy, I. J.; Ling, Y.; Nacro, K.; Tomita, Y.; Wu, X.; Cao, Y.; Guo, R.; Li, B.; Zhu, X.; Huang, Y.; Long, Y.-Q.; Roller, P. P.; Yang, D.; Wang, S. *J. Med. Chem.* **2001**, *44*, 4313–4324.

18. Wang, G.; Nikolovske-Coleska, Z.; Yang, C.-Y.; Wang, R.; Tang, G.; Guo, J.; Shangary, S.; Qiu, S.; Gao, W.; Yang, D.; Meagher, J.; Stuckey, J.; Krajewski, K.; Jiang, S.; Roller, P. P.; Abaan, H. O.; Tomita, Y.; Wang, S. *J. Med. Chem.* **2006**, *49*, 6139–6142.
19. Friesner, R. A.; Murphy, R. B.; Repasky, M. P.; Frye, L. L.; Greenwood, J. R.; Halgren, T. A.; Sanschagrin, P. C.; Mainz, D. T. Extra Precision Glide: Docking and Scoring Incorporating a Model of Hydrophobic Enclosure for Protein-Ligand Complexes *J. Med. Chem.* **2006**, *49*, 6177–6196.
20. Bruncko, M.; Oost, T. K.; Belli, B. A.; Ding, H.; Joseph, M. K.; Kunzer, A.; Martineau, D.; McClellan, W. J.; Mitten, M.; Ng, S.-C.; Nimmer, P. M.; Oltersdorf, T.; Park, C.-M.; Petros, A. M.; Shoemaker, A. R.; Song, X.; Wang, X.; Wendt, M. D.; Zhang, H.; Fesik, S. W.; Rosenberg, S. H.; Elmore, S. W. *J. Med. Chem.* **2007**, *50*, 641–662.
21. Petros, A. M.; Medek, A.; Nettesheim, D. G.; Kim, D. H.; Yoon, H. S.; Swift, K.; Matayoshi, E. D.; Oltersdorf, T.; Fesik, S. W. *Proc. Natl. Acad. Sci. USA* **2001**, *98*, 3012–3017.
22. Nikolovska-Coleska, Z.; Wang, R.; Fang, X.; Pan, H.; Tomita, Y.; Li, P.; Roller, P. P.; Krajewski, K.; Saito, N.; Stuckey, J.; Wang, S. *Anal Biochem.* **2004**, *332*, 261–273.