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Design, synthesis and biological evaluation of 3-aryl-rhodanine benzoic acids as anti-apoptotic protein Bcl-2 inhibitors

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ABSTRACT

A new class of 3-aryl-rhodanine benzoic acid derivatives were designed, synthesized, and evaluated for their inhibition activities against anti-apoptotic Bcl-2 proteins. The potent compounds **33** and **41** bound to Bcl-2 with submicromolar K_i values and had selectivities to Bcl-2/Mcl-1 over Bcl-xL. In addition, they exhibited obvious antiproliferative activities in three human tumor cell lines (MDA-MB-231, K562 and PC-3).

Apoptosis is a highly conserved process which is triggered by cellular stimuli of internal or external. Evasion of apoptosis is a hallmark of human cancer and contributes to the resistance to conventional therapies.[1] As a result, targeting key apoptosis regulators is promising therapeutic strategy for malignancies treatment.

The Bcl-2 (B-cell lymphocyte/leukemia-2) family proteins are key regulators of apoptosis and composed of anti-apoptotic proteins, including Bcl-2, Bcl-xL, Mcl-1, Bfl-1/A1, Bcl-B and Bcl-w, and pro-apoptotic proteins, such as BAK, BAX, BID, BIM and BAD.[2,3] Studies show that the anti-apoptotic Bcl-2 proteins are over-expressed in multiple types of human cancers such as B-cell lymphomas,[4] prostate cancers[5] and breast carcinomas.[6] And they are contributors of cancer initiation, progression, and resistance to current anti-tumor treatments.[7] As a consequence, anti-apoptotic Bcl-2 proteins have become attractive targets for the treatment of various human cancers and different series of small-molecule inhibitors have been reported.[8–13] Navitoclax (ABT-263, **1**), a dual inhibitor of Bcl-2 and Bcl-xL,[14–17] and Obatoclax (GX15-070, **2**), a pan Bcl-2 family inhibitor,[18–21] were currently in phase I/II clinical trials (Fig. 1).

Rhodanine (2-thioxo-4-thiazolidinone) is a very common active fragment in antitumor agents,[22–24] and some rhodanine-based derivatives have been developed as potent Bcl-2 family inhibitors.[25–30] The first of them, BH3I-1 (**3**), bound to the BH3 binding site of Bcl-2 proteins and induce apoptosis.[25] After that, WL-276 (**4**) was developed and the preliminary biological activity assay showed that it could suppress tumor growth.[30] And further SAR studies and optimization of WL-276 has been done by our group.[31] Recently, we found a new rhodanine compound **5** with

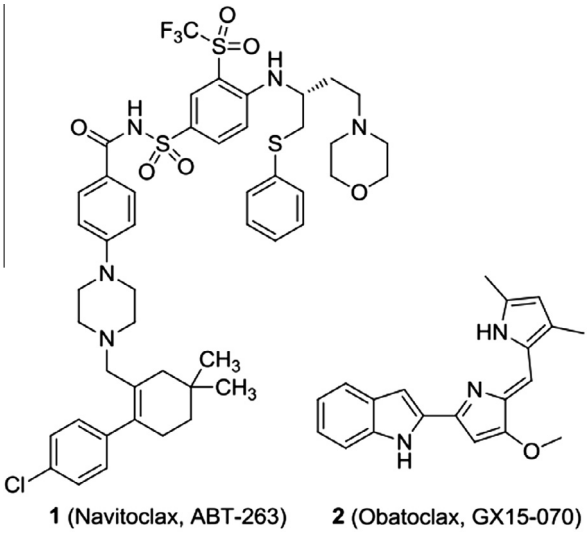

Figure 1. Representative small-molecule inhibitors of anti-apoptotic Bcl-2 proteins.

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3-aryl-rhodanine benzoic acid structure showed 18% inhibition against Bcl-2 protein at 100 μM, which indicated that the 3-aryl-5-ylidene-rhodanine benzoic acid derivatives might possess potential Bcl-2 inhibition. In our on-going studies, further structural modifications were focused on the 3-position of rhodanine with more complicated aryl or benzyl group and another aromatic ring (–Ar–X–) was introduced to 5-position of rhodanine based on previous studies (Fig. 2). Herein, we report the synthesis of a series of 3-aryl-rhodanine benzoic acid derivatives, their inhibition activities against Bcl-2 proteins and their in vitro antiproliferative activities.

As shown in Scheme 1, the intermediates substituted **8a**–**8e** were prepared through substitution[32] and/or coupling[33] reaction. And the *N*-arylsubstituted rhodanines were prepared through two different methods. While **13a**–**13c** and **13e**–**13o** were following the same literature,[34] 4-nitrophenyl rhodanine (**13d**) was synthesized from 4-nitroaniline (**12d**) and bis(carboxymethyl) trithiocarbonate[35] (Scheme 2).

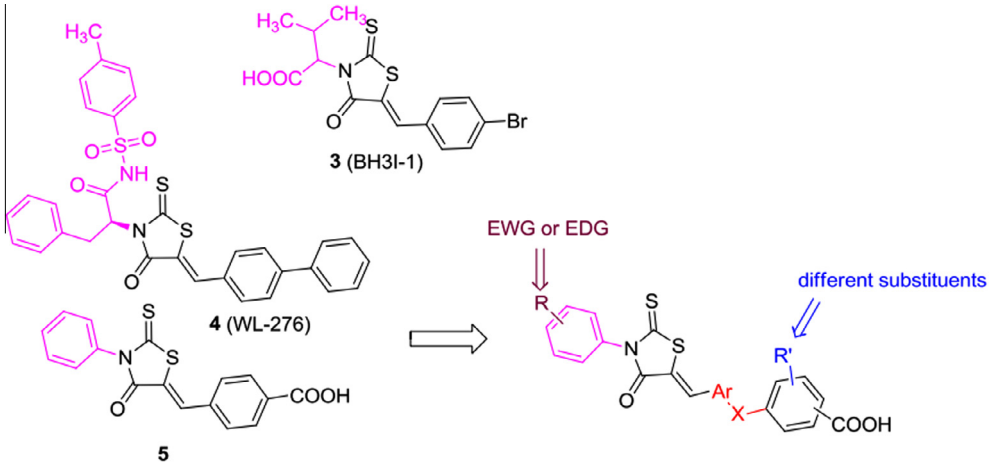
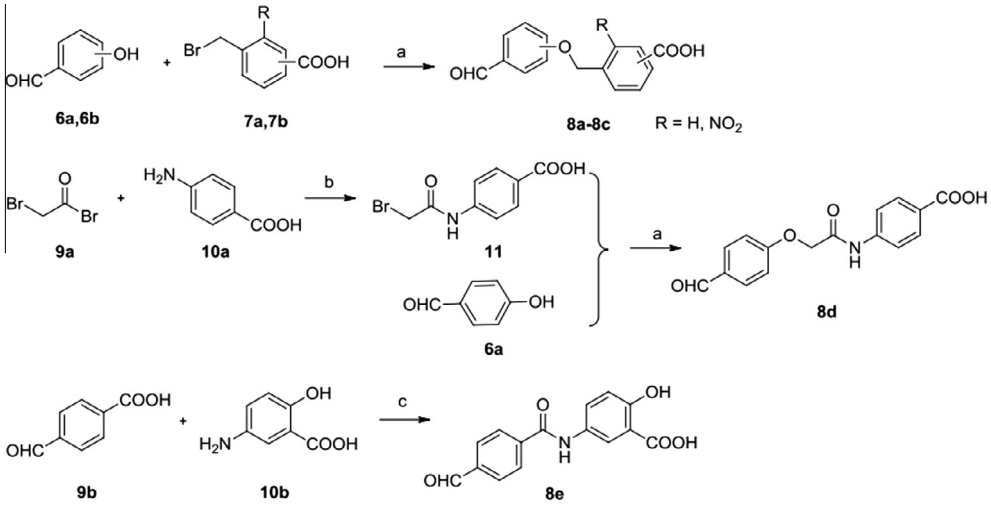
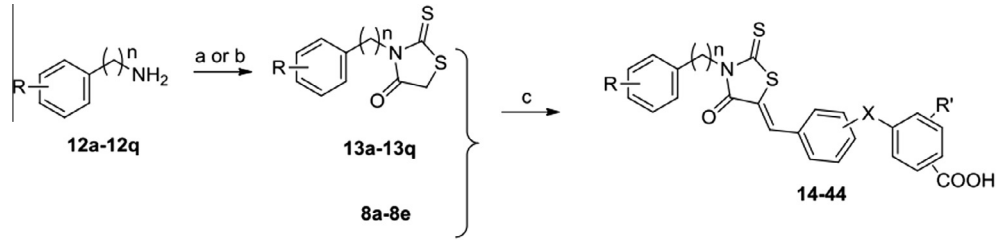


Figure 2. Representative rhodanine-based Bcl-2 inhibitors and design of our 3-aryl-rhodanine benzoic acid derivatives.



Scheme 1. Synthesis of intermediates **8a**–**8e**. Reagents and conditions: (a) K_2CO_3, acetone, reflux; (b) $NaHCO_3$, THF, 0 °C; (c) oxalyl chloride, $NaHCO_3$, THF, 0 °C.



Scheme 2. Synthesis of target compounds **14**–**44**. Reagents and conditions: (a) (i) CS_2, Et_3N, ethanol; (ii) $ClCH_2COONa$, H_2O; (iii) 6 M HCl, 85 °C; (b) $SC(SCH_2COOH)_2$, H_2O, reflux; (c) NH_4Ac, HAc, reflux.

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After all the intermediates had been synthesized, the target compounds **14**–**44** were afforded through the aldol reaction of **13** with **8** in the presence of acetic acid and ammonium acetate.[36]

Using fluorescence polarization assays (FPAs), affinities of the target compounds for Bcl-2 were tabulated as K_i values in Table 1. According to the data in Table 1, many target compounds showed better affinities for Bcl-2 protein than the previous compound **5**. And some exhibited similar activities compared with WL-276. Also, both the substituents on aromatic ring A (R) or C (R′, –COOH) and the linker X between aromatic ring B and C had important effects on the binding affinity for Bcl-2. For example, when R was hydrogen, compounds **14**–**18** displayed poor affinities ($K_i \geqslant 9$ μM). And most of the substitutions on 3- or 4-position of the phenyl A did not improve the activities except 4-nitro, 4-bromo and 4-iodo (**27**, **41** and **42**). While 4-nitro and 4-iodo groups minor increased the affinities of **27** (K_i = 3.1 μM) and **42** (K_i = 4.3 μM), 4-bromo substituted **41** showed higher affinities with the K_i value of 0.61 μM. The results indicated that halogen substitution on 4-position of phenyl A exhibited significant effect on binding affinities. On the other hand, the substituents on *ortho*-position of phenyl A significantly impacted the activities as well. For example, *o*-methyl and *o*-methoxy substituted compounds (**19**, **21**, **22**, **33**, **34**) showed much better activities than unsubstituted **14**–**18**. The results indicated that electron donating groups (EDGs) on *ortho*-position of phenyl A were benefit for the interaction of inhibitors and the Bcl-2 protein. Moreover, the activity of **43** also confirmed this hypothesis. Its poor affinity for Bcl-2 was likely due to the electron-withdrawing fluorine substituents. Besides, the fact that target compound **44** displayed poor affinity for Bcl-2 suggested addition of one methylene between phenyl A and rhodanine did not improve the compounds' activities.

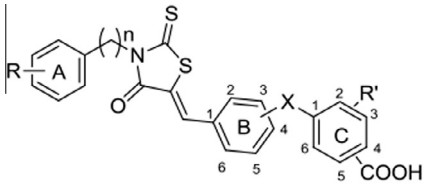
And for the substituents on aromatic ring C, 2-nitro and 5-carboxy disubstitution was better than 4-carboxyl monosubstitution,

Table 2
The binding affinities of representative target compounds to three Bcl-2 proteins

Compd	K_i[a] (μM)		
	Bcl-xL	Bcl-2	Mcl-1
19	>10	3.1 ± 0.8	3.2 ± 0.5
21	>10	2.0 ± 0.2	3.9 ± 1.7
22	>10	1.6 ± 0.2	3.7 ± 1.2
33	3.9 ± 0.9	0.90 ± 0.10	0.72 ± 0.07
41	>10	0.61 ± 0.06	0.70 ± 0.11
42	>10	4.3 ± 0.7	1.6 ± 0.4
WL-276	0.57 ± 0.24	0.50 ± 0.04	0.29 ± 0.04
Gossypol	0.64	0.44	0.31

[a] Each value was reproduced in three independent assays and expressed with standard deviations.

Table 1
The structure and in vitro Bcl-2 inhibitory activity of **14**–**44**



Compd	R	*n*	X	R′	Position of –COOH	K_i[a] (μM)
5						>10
14	H	0	4-OCH_2–	—	4	>10
15	H	0	3-OCH_2–	—	4	>10
16	H	0	4-OCH_2CONH–	—	4	>10
17	H	0	4-OCH_2–	2-NO_2	5	9.0 ± 4.4
18	H	0	4-CONH–	4-OH	3	>10
19	2-CH_3	0	3-OCH_2–	—	4	3.1 ± 0.8
20	2-CH_3	0	4-OCH_2CONH–	—	4	>10
21	2-OCH_3	0	4-OCH_2–	—	4	2.0 ± 0.2
22	2-OCH_3	0	3-OCH_2–	—	4	1.6 ± 0.2
23	2-OCH_3	0	4-OCH_2CONH–	—	4	10 ± 2.5
24	4-NO_2	0	4-OCH_2–	—	4	>10
25	4-NO_2	0	3-OCH_2–	—	4	>10
26	4-NO_2	0	4-OCH_2CONH–	—	4	>10
27	4-NO_2	0	4-OCH_2–	2-NO_2	5	3.1 ± 0.9
28	4-NO_2	0	4-CONH–	4-OH	3	>10
29	3-CH_3	0	4-OCH_2–	—	4	>10
30	3-CH_3	0	4-OCH_2CONH–	—	4	>10
31	4-CH_3	0	4-OCH_2–	—	4	>10
32	4-CH_3	0	4-OCH_2CONH–	—	4	>10
33	2,6-$(CH_3)_2$	0	4-OCH_2–	—	4	0.90 ± 0.10
34	2,6-$(CH_3)_2$	0	4-OCH_2CONH–	—	4	7.6 ± 1.5
35	4-OCH_3	0	4-OCH_2–	—	4	>10
36	3-NO_2	0	4-OCH_2–	—	4	9.6 ± 2.2
37	4-$COCH_3$	0	4-OCH_2–	—	4	>10
38	3-CF_3	0	4-OCH_2–	—	4	>10
39	4-F	0	4-OCH_2–	—	4	>10
40	4-Cl	0	4-OCH_2–	—	4	>10
41	4-Br	0	4-OCH_2–	—	4	0.61 ± 0.06
42	4-I	0	4-OCH_2–	—	4	4.3 ± 0.7
43	2,6-F_2	0	4-OCH_2–	—	4	>10
44	4-F	1	4-OCH_2–	—	4	>10
WL-276						0.50 ± 0.04

[a] Each value was reproduced in three independent assays and expressed with standard deviations.

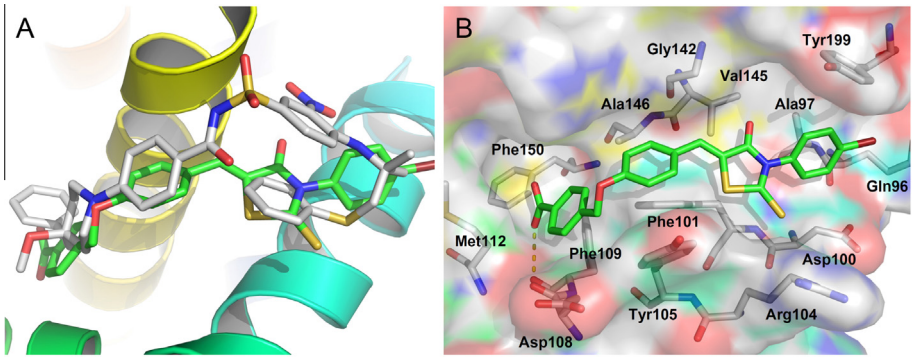


Figure 3. (A) Docked compound **41** with Bcl-2 (PDB code 2O2F). (B) The docking mode of compound **41** in the active site of Bcl-2 protein.

as both the two target compounds **17** and **27** showed more potent activities than 4-carboxyl substituted **14** and **24**.

In addition, the linker X between aromatic ring B and C also affected the affinity activities. When R′ was the same substituent, the compounds **21** and **22** had good appetency with K_i value in the low micromolar range in the case of the phenoxyl group was connected with alkylbenzoic acid. Nevertheless, **23** showed poor affinity activity (K_i = 10 μM) owing to the acetamidobenzoic acid linked. Also, **19**–**20** and **33**–**34** had the same trends. This result suggested that X was important to binding affinities as well.

We also explored if these rhodanine-based benzoic acid compounds could inhibit other anti-apoptotic Bcl-2 proteins. Six compounds **19**, **21**, **22**, **33**, **41** and **42**, which showed potent binding affinities for Bcl-2 with K_i of low micromolar, were chosen to evaluate their activities against Mcl-1 and Bcl-xL (Table 2). The results showed that all the compounds exhibited good affinities for Bcl-2 and Mcl-1. But they had poor inhibitory activity against Bcl-xL. It suggested that the rhodanine-based benzoic acid derivatives might have Bcl-2/Mcl-1 selectivities.

To better studying the interactions of these rhodanine-based benzoic acid derivatives to Bcl-2 protein, the most potent compound **41** were chosen to dock with Bcl-2 protein in the active site using AutoDock 4.2,[37] which was a widely used docking program with exceptional accuracy (Fig. 3). The results suggested that compound **41** could reasonably bind to the active pocket of Bcl-2 protein. And the benzoic acid group could form one hydrogen bond with Phe109.

To further examine the activities of rhodanine-based benzoic acids at the cellular level, the potent target compounds **33** and **41** were selected to test their antiproliferative activities taking WL-276 and Gossypol as control. Three cancer cells, MDA-MB-231 (human breast cancer cell), K562 (human myelogenous leukemia cell) and PC-3 (human prostate cancer cell), were evaluated using MTT (3-[4,5-dimethyl-2-thiazolyl]-2,5-diphenyl-2*H*-tetrazoliumbromide) assay. According to the data in Table 3, both **33** and **41** displayed obvious antiproliferative activities, which were better or similar compared with WL-276 against all the tested cancer cells. And it appeared that all the target compounds showed better growth inhibition activities toward solid tumor cell (MDA-MB-231 and PC-3) than blood cancer (K562). Of them, **41** had slightly higher inhibitory potency than **33** against the tested tumor cells.

Table 3
Antiproliferative activities of representative compounds

Compd	IC_{50}[a] (μM)		
	MDA-MB-231	K562	PC-3
33	25.6 ± 1.8	46.6 ± 3.2	25.8 ± 3.4
41	19.8 ± 1.1	43.8 ± 1.5	18.9 ± 1.9
WL-276	>30	32.8 + 1.4	>30
Gossypol	4.80 ± 1.00	7.31 ± 0.34	5.61 ± 0.18

[a] Inhibitory data are means of no fewer than two independent determinations and expressed with standard deviations.

In conclusion, we had designed and synthesized a series of rhodanine-based benzoic acid derivatives as Bcl-2 inhibitors. The strong affinities of compounds **19**, **21**, **22**, **33** and **41** suggested that the *ortho*-methylphenyl, *ortho*-methoxyphenyl and *para*-bromophenyl substituents on 3-position of rhodanine and a suitable linking group between aromatic rings B and C were benefit for potency. Also, they had much better activities than the initial compound **5**. In addition, all the tested compounds showed Bcl-2/Mcl-1 selectivities over Bcl-xL, and the most potent **33** and **41** displayed obvious antiproliferative activities. These results indicated that rhodanine-based benzoic acid derivatives could be used as lead compounds to develop novel Bcl-2 inhibitors.

Acknowledgments

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36. Procedure for the synthesis of representative (*Z*)-4-((4-((3-(4-bromophenyl)-4-oxo-2-thioxothiazolidin-5-ylidene)methyl)phenoxy)methyl)benzoic acid (**41**): 3-(4-bromophenyl)rhodanine (**13m**, 0.14 g, 0.5 mmol) and 4-((4-formylphenoxy)methyl)benzoic acid (**8a**, 0.13 g, 0.5 mmol) were dissolved in acetic acid (3 ml) under reflux condition. Ammonium acetate (0.15 g, 2 mmol) was then added. The mixture was stirred 0.5 h, and precipitation occurred. The precipitate was filtered off, washed with acetic acid and absolute ethanol to afford the product. Yellow solid: mp 263–265 °C; yield 90%. ^{1}H NMR (300 MHz, DMSO-d_6) δ 12.99 (s, 1H), 7.98 (d, J = 8.4 Hz, 2H), 7.82 (s, 1H), 7.78 (d, J = 8.7 Hz, 2H), 7.68 (d, J = 8.7 Hz, 2H), 7.59 (d, J = 8.4 Hz, 2H), 7.41 (d, J = 8.7 Hz, 2H), 7.24 (d, J = 8.7 Hz, 2H), 5.32 (s, 2H). ^{13}C NMR (100 MHz, DMSO-d_6) δ 191.8, 167.0, 165.4, 160.9, 141.3, 135.6, 133.4, 130.4, 129.5, 127.5, 125.5, 118.2, 116.1, 113.0, 112.9, 112.8, 112.7, 69.0. HRMS (AP-ESI) m/z calcd for $C_{24}H_{16}BrNO_4S_2$ $[M+H]^+$ 525.9777, found 525.9836.
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