Antagonists Against Anti-Apoptotic Bcl-2 Family Proteins for Cancer Treatment

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Abstract: Apoptosis is a programmed cell death process, critical for normal cellular development and tissue homeostasis. B-cell lymphocyte/leukemia 2 (Bcl-2) family proteins are important regulators of apoptosis. Numerous studies have demonstrated that over-expressing anti-apoptotic Bcl-2 proteins is one mechanism for cancer cells to acquire resistance against cancer chemotherapies, suggesting antagonizing these proteins would be a potential approach to overcoming such drug resistance. This review briefly discusses the principle and the recent advances in the development of such antagonists, with some highlights about several promising antagonists.

Keywords: Bcl-2 antagonists, drug resistance, apoptosis, cancer.

INTRODUCTION

Apoptosis is a highly regulated and energy-dependent process of cellular suicide that can be triggered by a multitude of internal and external stimuli [1]. Apoptosis plays an important role in many developmental processes as well as maintenance of tissue homeostasis. Indeed, impaired apoptosis is a hallmark of various cancers [2]. As many cancer therapies eliminate tumor cells through induction of apoptosis directly or indirectly, impairment in apoptosis also leads to drug resistance to cancer therapies [3].

REGULATION OF LIFE AND DEATH BY THE BCL-2 FAMILY PROTEINS

One mechanism for cancers to acquire resistance to apoptosis is through the over-expression of anti-apoptotic Bcl-2 family proteins, a subfamily of Bcl-2 family proteins. Bcl-2 family proteins comprise of over 20 members, which are the crucial regulators of apoptosis [4]. The Bcl-2 family proteins can be functionally classified into two broad subfamilies. The first subfamily comprise of family members that protect a cell from apoptosis, named as anti-apoptotic Bcl-2 proteins (Bcl-2, Bcl-X_L, Bcl-w, Mcl-1, A-1, and Bcl-B) [5, 6]. The other subfamily consists of family members that promote apoptosis, named pro-apoptotic Bcl-2 proteins. These two subfamilies antagonize each other and the balance between them determines the cell's fate to undergo apoptosis or to survive. Most of the anti-apoptotic members contain four conserved motifs named as Bcl-2 homology (BH) domains (BH1 – BH4) except Mcl-1 contains only three conserved domains (BH1 – BH3) and lacks the BH4 domain [7] (Fig. **1**). The pro-apoptotic Bcl-2 proteins can be further classified into two subsets. Some members (Bax, Bak, and Bok) have three BH domains (BH1, BH2, and BH3) while other members only have BH3 domain, named as BH3-domain only pro-apoptotic Bcl-2 proteins (Bik, Bid, Bad, Bim, Bmf, Hrk, Noxa, and Puma) [8]. Structural studies have revealed that the BH1, BH2, and BH3 domains in the anti-apoptotic proteins are folded to form an elongated hydrophobic cleft. This cleft serves as the binding site to interact with the pro-apoptotic members through their BH3 domain, forming heterodimer [9, 10]. Heterodimerization of a pro-apoptotic Bcl-2 family member and an anti-apoptotic Bcl-2 family member is one proposed mechanism for these two subfamilies to antagonize each other. The anti-apoptotic Bcl-2 family proteins engage pro-apoptotic Bcl-2 proteins and keep them in check, thereby preventing apoptosis. The pro-apoptotic Bcl-2 family proteins, when in excess or activated (not bound by anti-apoptotic ones), can permeabilize the mitochondrial membrane, resulting in the leakage of cytochrome c and other molecules involved in caspase activation, which leads to apoptosis [11, 12].

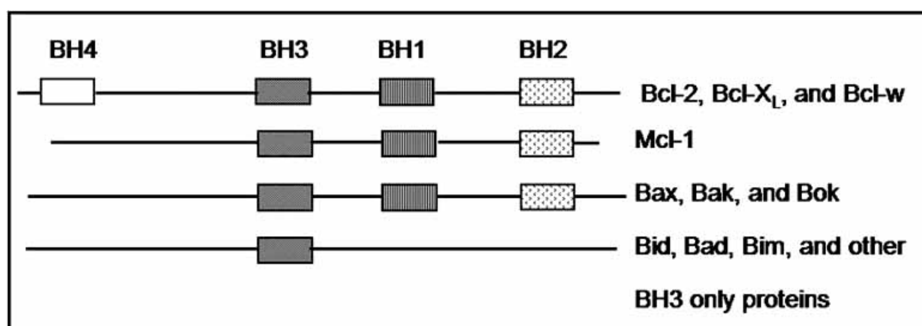

Fig. (1). Schematic representation of structural domains of the Bcl-2 family proteins.

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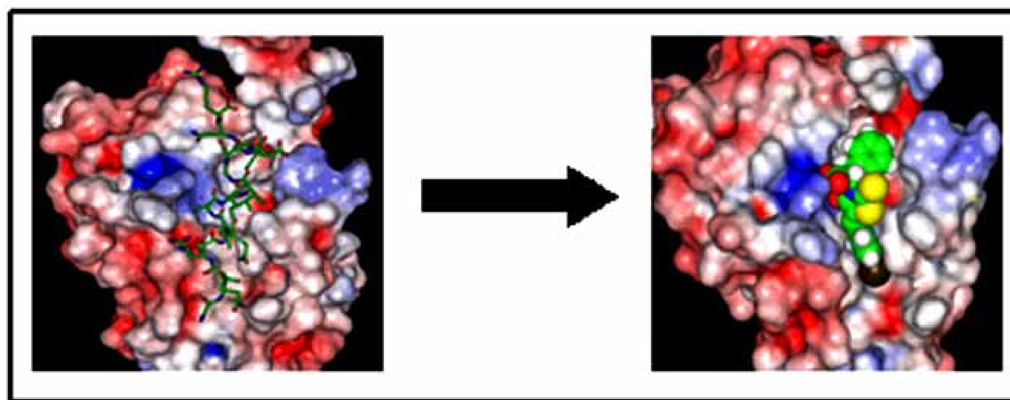

Fig. (2). Basis for the design of small molecule antagonists against the anti-apoptotic Bcl-2 proteins.

LOSS OF APOPTOSIS IN CANCERS

Increasing literature evidence suggests that stress stimuli imposed by most anticancer agents on a cell would result in activation of one or more pro-apoptotic Bcl-2 proteins as signal transducers to activate apoptosis [13, 14]. Overexpression of the anti-apoptotic Bcl-2 family proteins prevent such signal transduction, therefore, is one source of chemoresistance [15, 16]. For instance, over-expression of Bcl-2 has been shown to be the underlying cause for resistance to a wide range of cancer therapies, such as cisplatin [17], glucocorticoids [18], doxorubicin [19], staurosporine [20], docetaxel [21], thapsigargin [22], 5-flurouracil [23], rituximab [24], TNF and Fas ligand [25], and imatinib [26]. Likewise overexpression of Bcl-X_L has been associated with resistance to apoptosis induced by methotrexate [27], 5-flurouracil [23, 27] Fas ligand [28], staurosporine [29], and tumor necrosis factor (TNF) ligand [25]. Mcl-1 has been demonstrated to prevent apoptosis induced by ZD1839, an epidermal growth factor receptor (EGFR) kinase inhibitor, and ionizing radiation [30].

ANTAGONIZING ANTI-APOPTOTIC BCL-2 PROTEINS AS A NEW APPROACH COUNTERACTING DRUG RESISTANCE

The data above suggest that nullifying the protective effects of anti-apoptotic Bcl-2 proteins may restore sensitivity of cancers towards radio/chemotherapies. The over-expressed anti-apoptotic Bcl-2 proteins can be negated by the following three strategies: i) indirect regulation of gene expression, ii) downregulation of protein expression, and iii) direct antagonism of the anti-apoptotic proteins.

Indirect Regulation of Gene Expression

Several agents reduce the transcription of the genes encoding anti-apoptotic Bcl-2 proteins. These include small molecule modulators of steroid or retinoid family nuclear receptors, such as tamoxifen, which suppresses estrogen receptor-dependent transcription of *BCL-2* in breast cancers [31], and 9-cis-retinoic acid, which suppress the transcription of *BCL-2* and *BCL-XL* in myeloid leukemias [32]. Inhibitors of histone deacetylase (HDACs), such as trichostatin A and sodium butyrate, have also been demonstrated to reduce transcription of anti-apoptotic *BCL2*-family genes [33].

Downregulation of Protein Expression

Several antisense oligonucleotides for Bcl-2, Bcl-X_L, and Mcl-1 have shown encouraging results in preclinical evaluation for antagonizing these proteins [34], one of which, Oblimersan sodium (G3139, a Bcl-2 antisense oligonucleotide), has advanced into clinical trials. Oblimersan sodium is an 18-mer phosphorothioate antisense oligonucleotide that has completed phase-3 clinical trials for refractory chronic lymphocytic leukemia (CLL), acute myeloid leukemia (AML), and myeloma [35]. This agent showed promise in AML but failed to show any effect in myeloma. A few trials are underway to explore its effectiveness as a chemosensitizer with current anticancer therapies like cyclophosphamide, dacarbazine, carboplatin, and etoposide [36, 37]. The study with dacarbazine demonstrated improved response time but failed to prolong survival [37]. A potential reason for this could be the simultaneous overexpression of multiple anti-apoptotic members to induce drug resistance, which leads to the development of multiple/dual antisense oligonucleotides [38, 39].

Direct Antagonism of Anti-Apoptotic Proteins

As the BH3 domains of pro-apoptotic proteins are known to bind the surface cleft on the anti-apoptotic proteins for heterodimer formation, BH3 mimetic agents may bind and antagonize the anti-apoptotic members to overcome drug resistance induced by the over-expression of the anti-apoptotic Bcl-2 family proteins (Fig. **2**). Peptides containing the BH3 domains of several Bcl-2 family members such as Bax, Bak, Bik, Bid, and Bad have been demonstrated to bind to the anti-apoptotic Bcl-2 family members, such as Bcl-X_L [40]. The cell permeable forms of these BH3 domain peptides also reveal the capability of triggering intracellular apoptosis [41]. One such example is CPM-1285, which binds to Bcl-2 with an IC_{50} of 130 nM competing against a fluorescence-tag labeled Bak BH3 peptide. In HL-60 cells, CPM-1285 induced caspase-3 activation and triggered deoxyribonucleic acid (DNA) fragmentation and poly ADP ribose polymerase (PARP) cleavage, characteristics of apoptosis [42]. The limitations of peptide as drugs has been alleviated by developing either α-helix constrained BH3 peptides or unnatural amino acid-based peptides [43-45]. Peptidomimetic approaches using a terphenyl scaffold as an α-helix mimic

afforded an antagonist of Bcl-X_L with a Ki of 114 nM [46]. Such a scaffold may bind to Bcl-X_L through hydrophobic interactions with L130, W137, R139, I140, F146, E193, Y195, S203 of Bcl-X_L [46].

ORGANIC SMALL MOLECULES AS ANTAGONISTS OF ANTI-APOPTOTIC BCL-2 PROTEINS

Another approach to directly antagonize the anti-apoptotic Bcl-2 proteins is to develop small molecules mimicking the BH3 domain peptide. Over the past eight years, quite a few small molecules have been reported to interact with the anti-apoptotic Bcl-2 proteins and function as antagonists against these proteins (Fig. **3**, Table **1**). These small molecules are identified from natural products, by library screening, or via molecular design.

Antagonists from Natural Products

Natural products from several sources have been demonstrated to antagonize anti-apoptotic Bcl-2 proteins. These include an antibiotic produced by an Actinomycete – tetrocarcin A, originally found to be active against Gram-positive bacteria [47]. Tetrocarcin A was demonstrated to inhibit the anti-apoptotic functions of Bcl-2 or Bcl-X_L transiently transfected in HeLa cells, leading to cell death via loss of mitochondrial membrane potential and release of cytochrome c [48]. It remains to be determined whether tetrocarcin directly interacts with anti-apoptotic Bcl-2 family proteins. Another antibiotic antimycin A, active against a variety of fungi and yeasts and an inhibitor of mitochondrial respiration, was also demonstrated to antagonize anti-apoptotic Bcl-2 proteins [49], with their binding interactions characterized via several biophysical methods [50]. A group of polyphenolic compounds with Bcl-X_L binding activity have also been identified, including purpurogallin – an antioxidant and tyrosine kinase inhibitor from Quercus sp. nutgall and gossypol – a male contraceptive from cottonseed extracts [51]. Several polyphenolic compounds from tea, including epigallocatechin gallate, catechin gallate, and their epimers, and theaflavin and theaflavanin, also revealed varied binding interactions with Bcl-2 and Bcl-X_L via NMR and fluorescence polarization based assays [52]. Another natural product identified as an inhibitor of Bcl-X_L was chelerythrine, a benzophenanthridine alkaloid known as a protein kinase C inhibitor. A fluorescence polarization assay-based screen of a library of 107,423 natural products for inhibition of Bak binding to Bcl-X_L led to the identification of chelerythrine as an inhibitor with IC_{50} of 1.5 μM, which effectively eliminates SH-SY5Y cells overexpressing Bcl-X_L [53].

Antagonists Obtained by Library Screening

One of the earliest reported antagonists against the anti-apoptotic Bcl-2 proteins was ethyl-2-amino-6-bromo-4-(1-cyano-2-ethoxy-2-oxoethyl)-4*H*-chromene-3-carboxylate (HA 14-1), identified through a virtual screening. HA 14-1 was subsequently shown to interact with Bcl-2 and induce apoptosis [54]. As detailed later, HA 14-1 represents one of the most widely studied antagonist against the anti-apoptotic Bcl-2 proteins. Following the identification of HA 14-1 was the report by Degterev *et al.* who screened a library of 16,320 compounds from Chembridge Corp. using fluorescence polarization competition assay, followed by NMR studies of the identified lead candidates. They identified two series of small molecule antagonists against Bcl-X_L, the benzyl substituted thiazolidines (BH3I-1s) and the benzamide analogs (BH3I-2) [55]. Shortly after, Enyedy *et al.* applied a virtual screening technique to screen the National Cancer Institute (NCI) 3D open database (206,876 compounds) and identified 35 potential binders, which were subsequently evaluated for binding interactions with Bcl-2 using a fluorescence polarization assay. These efforts resulted in the identification of NSC 365400, which demonstrated binding affinities (Ki) of 10 μM and 7 μM for Bcl-2 and Bcl-X_L respectively [56].

Antagonists Obtained *via* Molecular Design

In this category, one of the earliest described compounds was YC137, obtained by molecular design starting from NSC 365400 [57]. YC137 was demonstrated to selectively induce toxicity in cancer cells overexpressing Bcl-2 while sparing normal blood cells. Abbott laboratories used a combination of NMR-based screening and structure based design to identify an antagonist (compound **31**) with nanomolar potency in antagonizing Bcl-X_L [58]. Structurally, the 4-flurophenyl group occupies a hydrophobic pocket formed by Y101, L108, V126, F146. The phenyl ring of F97 of Bcl-X_L stacks against the S-phenyl ring of compound **31**. This S-phenyl ring also forms an intermolecular stack with the nitrophenyl ring, while the nitrophenyl ring stacks with Y194 of Bcl-X_L. This extensive π-stacking arrangement probably accounts for the higher binding affinity of this compound [58]. However this candidate non-selectively binds to plasma protein, which greatly decreased its *in vivo* potency. Further structure based optimization led to the discovery of ABT-737 [59a]. ABT-737 is an antagonist with nanomolar potency against Bcl-2 and Bcl-X_L and has demonstrated efficacy in numerous pre-clinical studies as discussed in detail in the following section. ABT-737 was designed from Compound **31** and similar interactions were retained. The fluorophenyl was replaced by a substituted piperazine. An additional lipophilic chlorobiphenyl group was added to the piperazine to access a deep hydrophobic pocket on Bcl-X_L [59a]. Crystal structure of ABT-737 complexed with Bcl-X_L reveals that acylsulphonamide of ABT-737 forms a long hydrogen bond (3.1 Å) to the backbone amide of G138 of Bcl-X_L. The other hydrogen bond present in the complex is between E96 and the 2-dimethylaminoethyl group of ABT-737 [59b].

Reed and coworkers used molecular modeling, NMR-based structural analysis, fluorescence polarization, and cell viability assays to develop a gossypol-based antagonist targeting Bcl-X_L [60]. Because of the potential toxicity that may be induced by the two reactive aldehyde moiety on gossypol, the authors developed an aldehyde-free analog – apogossypol, which exhibited a Ki of 2.3 μM for Bcl-X_L compared to 0.3 μM by gossypol. Wang and colleagues have developed several classes of antagonists using a molecular modeling based rational design approach [61-63]. Based on docking studies, (-) -gossypol formed a hydrogen bonding network with residues R146 and N143 in Bcl-2 *via* the aldehyde group and the hydroxyl group on the right naphthalene

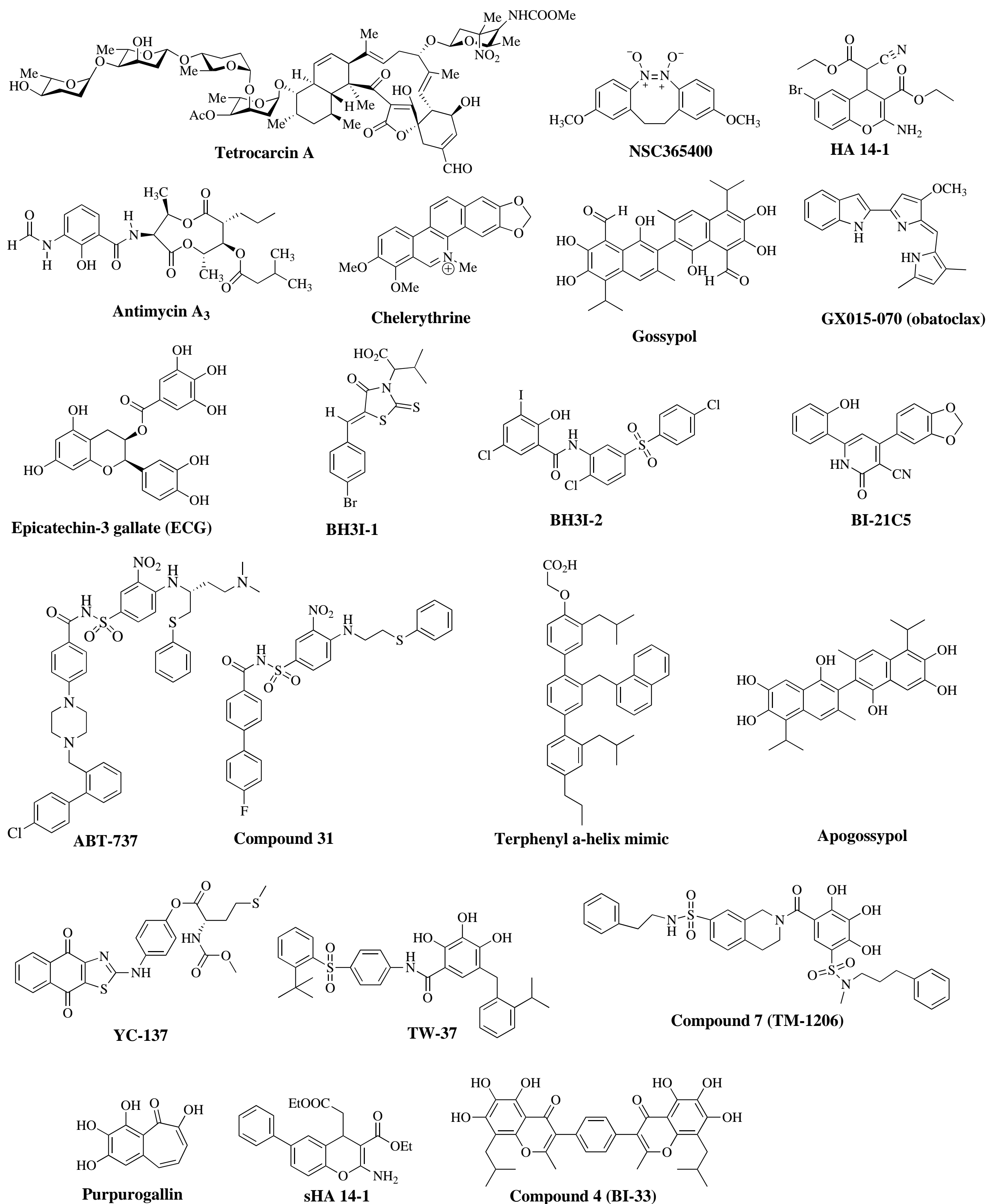

Fig. (3). Representative small molecule antagonists against anti-apoptotic Bcl-2 proteins.

ring. This mimicked the hydrogen bonding network formed by D99 and N102 in Bim and R146 and N143 in Bcl-2. The isopropyl group on the naphthalene ring occupied the hydrophobic pocket on Bcl-2 mimicking the F101 in Bim. The left half of (-)-gossypol interacts with Bcl-2 via hydrophobic contacts mimicking I97 in Bim. This model suggests that the

Table 1. Binding Interactions of Small Molecule Antagonists with Anti-Apoptotic Bcl-2 Proteins

Compound	Assay	Binding Interaction			Competing Peptide	Protein	Reference
		K_i	IC_{50}	K_d			
Antimycin A3	ITC	--	--	0.82 µM	--	Bcl-2Δ22	[50]
Chelerythrine	FP	--	1.5 µM	--	Fl-GQVGRQLAIIGD-DINR[b]	GST-Bcl-X_L ΔC19	[53]
Gossypol	FP	--	0.5 µM	--	NLWAAQRYGRELR-RMSD-K(FITC)FVD	Bcl-X_L[a]	[51]
		0.17 µM	--	--	DMRPEIWIAQELRR-IGDEFNAYYARR	His-Bcl-2 isoform 2	[60]
		0.28 µM	--	--		Mcl-1 fragment 171-327	
ECG	FP	120 nM	--	--	NLWAAQRYGRELR-RMSD-K(FITC)FVD	Bcl-X_L[a]	[52]
	FP	400 nM	--	--		Bcl-2[a]	
Purpurogallin	FP	--	2.2 µM	--	NLWAAQRYGRELR-RMSD-K(FITC)FVD	Bcl-X_L[a]	[51]
Apogossypol	FP	2.3 µM	--	--	NLWAAQRYGRELR-RMSD-K(FITC)FVD	Bcl-X_L[a]	[60]
Terphenyl α-helix mimic	FP	--	--	114 nM	Fl-GQVGRQLAIIGD-DINR-$CONH_2$[b]	GST-Bcl-X_L	[46]
YC137	FP	1.3 µM	--	--	Fl-QEDIIRNIARHLA-QVGDSMDR[b]	His-Bcl-2	[57]
		>100 µM	--	--		His-Bcl-X_L	
NSC365400	FP	--	10.4 µM	--	Fl-GQVGRQLAIIGDDINR[b]	GST-Bcl-2	[56]
BH3I1	FP	2.4 µM	--	--	Og-KGGGQVGRLAIIGDDINR[c]	GST-Bcl-X_L	[55]
	NMR	7.8 µM	--	--	KGGGQVGRLAIIGDDINR	His-Bcl-X_L	
BH3I2	FP	3.3 µM	--	--	Og-KGGGQVGRLAIIGDDINR[c]	GST-Bcl-X_L	[55]
HA 14-1	FP	9 µM	--	--	Fl-GQVGRLAIIGDDINR[b]	GST-Bcl-2	[54]
Compound 31	FP	36 µM	--	--	NLWAAQRYGRELRRMSD-K(FITC)FVD	Bcl-X_L Δ45-84 ΔTM	[58]
ABT-737	FP	<1 nM	--	--	NLWAAQRYGRELRRMSD-K(FITC)FVD	Bcl-2[a]	[59a]
		<1 nM	--	--		Bcl-X_L	
		<1 nM	--	--		Bcl-w[a]	
TW-37	FP	0.29 µM	--	--	QEDIIRNIARHLAQVGDSMDR	His-Bcl-2 isoform2	[61]
	ELISA	--	0.7 µM	--	Biotin-ADMRPEIWIAQELRR-IGDEFNAYYARP-$CONH_2$		
	FP	1.11 µM	--	--	FAM Bak BH3[d]	Bcl-X_L Δ45-84 Δ212-233	
	FP	0.26 µM	--	--	FAM Bid BH3[d]	Mcl-1 171-327	
TM-1206	FP	0.11 µM	--	--	QEDIIRNIARHLAQVGDSMDR	His-Bcl-2 isoform2	[63]
	ELISA	--	0.33 µM	--	Biotin-ADMRPEIWIAQELRR-IGDEFNAYYARP-$CONH_2$		
	FP	0.64 µM	--	--	FAM Bak BH3[d]	Bcl-X_L Δ45-84 Δ212-233	
	FP	0.15 µM	--	--	FAM Bid BH3[d]	Mcl-1 171-327	
	ELISA	--	39 nM	--	Biotin-ADMRPEIWIAQELRR-IGDEFNAYYARP-$CONH_2$		
BI-33	FP	17 nM	--	--	DMRPEIWIAQELRR-IGDEFNAYYARP	His-Bcl-2 isoform2	[62]
		18 nM	--	--	FAM Bim BH3[d]	Mcl-1 171-327	
		1.2 µM	--	--	FAM Bid BH3[d]	Bcl-X_L Δ45-84 Δ212-233	
BI-21C5	DELFIA	--	5.1 µM	--	Biotin-$(CH_2)_6$-GGG-QVGRLAIIGDDINR	Bcl-X_L ΔTM	[64]

[a] Protein construct details not reported, [b] Fl - Fluorescein, [c] Og - Oregon green, [d] FAM - 6-carboxyfluorescein.

two halves of (-)-gossypol interact differently with Bcl-2 [61]. Wang *et al.* designed a class of antagonists that structurally mimic the interaction between (-)-gossypol and Bcl-2. Specifically, the hydrogen bonding due to the three phenolic hydroxyl groups and lipophilic interaction by the isopropyl moiety of (-)-gossypol were retained. The most potent member of this series was TW-37 with Ki values of 0.29 μM, 1.1 μM, and 0.26 μM for Bcl-2, Bcl-X_L, and Bcl-w respectively [61]. For TW-37, a simple phenyl ring tethered to the polyphenolic ring via an amide bond mimicked the hydrophobic interaction between the right half of (-)-gossypol and Bcl-2. A substituted benzyl group was used to maximize the binding interaction with the pocket occupied by isopropyl group in (-)-gossypol. A phenyl ring tethered via a sulfone linker was utilized to occupy the hydrophobic pocket occupied by L94 in Bim [61]. In 2007, the same group obtained a second series of pyrogallol-based antagonists using structure-based design, the most effective of which, TM-1206, disrupted the binding of Bim peptide to Bcl-2, Bcl-X_L, and Mcl-1 with Ki values of 110, 639, and 150 nM respectively [63]. Structurally, two of the hydroxyl groups on the phenyl ring form hydrogen bonds with R146 and N143 in Bcl-2, mimicking residue D99 of Bim. The isopropyl group occupied the pocket occupied by F101 of Bim. The core tetrahydroisoquinolone ring retains the hydrogen bonding interactions with improved hydrophobic interactions. The phenylethyl group occupied a hydrophobic pocket in Bcl-2 occupied by L94 of Bim. The *N*-methyl-*N*-(3-phenylpropyl) sulfamoyl group was a large, polar group attached to core tetrahydroisoquinoline ring to occupy the deep pocket on Bcl-2 occupied by F101 of Bim. The sulfonyl group also formed a hydrogen bond with Y108 [63]. Recently, the same group employed the structure-based design strategy to obtain a series of flavonoid analogs as mimics of Bim BH3 peptide. The most active compound, BI-33, demonstrated binding affinities (Ki) of 17 and 18 nM for Bcl-2 and Mcl-1 respectively [62]. The trihydroxy flavonoid ring mimicked the interactions between F101, I97 and D99 in Bim and Bcl-2. This part of the molecule also formed extensive hydrogen bonding network with R146 and N143 of Bcl-2. An additional hydrogen bond was seen for the second flavonoid moiety and V133 on Bcl-2. The isobutyl and methyl groups mimicked the I90 and L94 residues of the Bim peptide. The phenyl group provided an optimal linker to orient the flavonoid moieties so as to mimic I90, L94, I97, and F101 in Bim peptide [62].

Pellecchia et al. recently reported structure-based discovery of a new class of Bcl-X_L antagonists using virtual docking, DELFIA (dissociation enhanced lanthanide fluoroimmuno assay), ^{13}C-filtered 1D NMR experiments. The most potent candidate, BI-2IC, analogs displaced BH3 peptide from Bcl-X_L with IC_{50} in the low micro molar range [64].

SELECTED SMALL MOLECULE ANTAGONISTS OF SPECIAL BIOLOGICAL INTEREST

Obatoclax (GX15-070)

Obatoclax is an antagonist developed by Gemin-X biosciences through structure-activity relationship (SAR) studies on prodiginines (tripyrrolic compounds) of bacterial origin. It has a broad spectrum against anti-apoptotic Bcl-2 proteins, antagonizing Mcl-1, Bcl-2, Bcl-X_L, and Bcl-w. Obatoclax has also been shown to synergize other anti-cancer therapies such as cisplatin - a DNA alkylator; bortezomib - a protease inhibitor, and Lapatinib - a reversible inhibitor of EGFR-Her-2/neu kinase [65-67]. This compound is currently in Phase I and II clinical trials in both single-agent and dual-agent formats for different types of cancer [68].

Gossypol and Apogossypol

Gossypol is a polyphenolic compound derived from cottonseed extracts, exhibiting cytotoxic effect in a host of human cancer cell lines including prostate [69], colon [70], and head and neck squamous cell carcinoma [71]. In cells lacking Bax and Bak, which are largely resistant to apoptosis, gossypol readily induces apoptosis compared to the vehicle control [72]. An enantiomer of gossypol, (-)-gossypol induced apoptosis via the mitochondrial pathway and was able to overcome the resistance conferred by the overexpression of Bcl-2 or Bcl-X_L in Jurkat T leukemia cells [73]. In another study, (-)-gossypol was able to reverse cisplatin resistance in head and neck squamous cell carcinoma associated with Bcl-X_L overexpression [74]. Despite these promising activities, its therapeutic usefulness could be compromised by the two reactive aldehyde groups, potentially causing side effects. To circumvent this problem, Reed and coworkers, removed the two reactive aldehydes from gossypol to obtain apogossypol, which results in moderate loss of potency in antagonizing anti-apoptotic Bcl-2 proteins and cytotoxicity [60]. In a transgenic mouse model with B cells overexpressing Bcl-2 protein, apogossypol showed a much better toxicity and efficacy profile over gossypol [75]. Hepatoxicity and gastrointestinal toxicity, the major adverse effects observed for gossypol, were absent in apogossypol treated mice.

HA 14-1 and sHA 14-1

HA 14-1 is one of the first small-molecule antagonists against anti-apoptotic Bcl-2 family proteins, identified by Huang *et al.* in 2000 via a virtual screen. It was subsequently demonstrated to selectively induce apoptosis in malignant cells that overexpress Bcl-2 [26, 54, 76]. More interesting is its ability to sensitize different type of cancer cells to a wide range of cancer therapies [66, 77-84]. Two studies also reported *in vivo* tumor suppressing activity for HA 14-1 [85, 86]. To improve the potency of HA 14-1, we performed SAR studies on HA 14-1 and obtained an analog with submicromolar *in vitro* cytotoxicity [87]. Despite its interesting activity, HA 14-1 is not stable with a half-life of only 15 minutes in cell culture medium. Concomitant generation of reactive oxygen species (ROS) was also observed during this decomposition process. Furthermore, the decomposition-generated ROS is the major contributor to its cytotoxicity since N-acetyl-L-cysteine (a ROS scavenger) greatly reduced the cytotoxicity, caspase-3/-7 activation, and DNA fragmentation by HA 14-1 [88]. These decomposition studies led to the development of stable analogs. Unlike HA 14-1, the stable analog (sHA 14-1) did not generate ROS. Overexpression of Bcl-2 or Bcl-X_L failed to induce resistance to sHA 14-1. Moreover, sHA 14-1 was able to synergize the activity of Fas ligand and dexamethasone in human leukemia cells [89].

ABT-737 and ABT-263

ABT-737 is a nanomolar antagonist developed by Abbott laboratory researchers using a combination of NMR-based screening and structure based design [58, 59a]. ABT-737 is one of the first small-molecule antagonists with demonstrated *in vivo* efficacy, inducing complete regression in small-cell lung carcinoma (SCLC) tumor xenografts in mice. Moreover, ABT-737 also synergized the activity of a number of clinically used agents such as vincristine, L-asparaginase, dexamethasone, carboplatin, and melphalan both *in vitro* and *in vivo* [90-92]. The therapeutic promise of ABT-737 as a chemopotentiator has been recently reviewed elsewhere [93]. Despite its ability to inhibit Bcl-2, Bcl-X_L, and, Bcl-w with subnanomolar potency, ABT-737 lacked antagonism against Mcl-1 [59a]. This has been attributed as a cause of resistance of some SCLC and solid tumors to ABT-737 [94]. Down-regulation of Mcl-1, therefore, potentiates the cytotoxicity of ABT-737 [95, 96]. Recently, the crystal structure of ABT-737 complex with Bcl-X_L was solved, providing insight for its selectivity among the anti-apoptotic Bcl-2 proteins. It has been suggested that a different fold of the α-3 helix of Bcl-X_L compared to Mcl-1 results in the p2 pocket of Mcl-1 to differ from that of Bcl-X_L, which accounts for the lack of activity of ABT-737 against Mcl-1 [59b]. Another problem with ABT-737 was its lack of oral bioavailability. Subsequent SAR investigations on ABT-737 resulted in the identification of ABT-263, an orally active antagonist. ABT-263 holds great promise as it showed complete regression in SCLC tumor xenografts models upon oral dosing [97] .

SUMMARY

Overexpressing anti-apoptotic Bcl-2 family proteins is a frequently used mechanism by cancer cells to evade apoptosis and to develop drug resistance in cancer therapy. One approach to tackle this is to reduce Bcl-2 protein expression. An alternate approach is to use antagonists against the anti-apoptotic proteins. Further, the availability of the three dimensional structure of most of the anti-apoptotic proteins has greatly facilitated the rational design approach. Some of these antagonists have shown promise as single therapy agents at various stages of pre-clinical and clinical studies. Another interesting feature of these agents is their ability to synergize a variety of cancer therapies, underscoring their therapeutic potential as sensitizers. In light of increasing evidence of involvement of anti-apoptotic Bcl-2 proteins in drug resistance to cancer therapy; the advances in the discovery of antagonists against these proteins exhibit considerable potential in the fight against cancer.

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