Published in final edited form as:

Curr Opin Chem Biol. 2017 August ; 39: 133–142. doi:10.1016/j.cbpa.2017.06.014.

Progress in targeting the BCL-2 family of proteins

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Abstract

The network of protein-protein interactions among the BCL-2 protein family plays a critical role in regulating cellular commitment to mitochondrial apoptosis. Anti-apoptotic BCL-2 proteins are considered promising targets for drug discovery and exciting clinical progress has stimulated intense investigations in the broader family. Here, we discuss recent developments in small molecules targeting anti-apoptotic proteins and alternative approaches to targeting BCL-2 family interactions. These studies advance our understanding of the role of BCL-2 family proteins in physiology and disease, providing unique tools for dissecting these functions. The BCL-2 family of proteins is a prime example of targeting protein-protein interactions and further chemical biology approaches will increase opportunities for novel targeted therapies in cancer, autoimmune and aging-associated diseases.

Graphical abstract

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Conflict Of Interest Statement

Nothing declared.

Introduction

Our understanding that regulation of cell survival and death through the mitochondrial apoptotic pathway is important in development, homeostasis, and many human diseases has led to intensive investigation of the BCL-2 family of proteins over the last two decades [1]. Protein-protein interactions of the BCL-2 family members dictate cell survival and death decisions in the mitochondrial pathway. Their deregulation can lead to imbalance of homeostasis contributing to the development of a variety of diseases [2]. Mitochondrial apoptosis is driven by the activity of the conserved BCL-2 homology domain 3 (BH3) of pro-apoptotic BCL-2 members [3,4]. Pro-apoptotic BH3-only proteins such as BIM, BID, PUMA and NOXA use their BH3 domain to inhibit anti-apoptotic BCL-2 proteins such as BCL-2, BCL-X_L and MCL-1 and activate pro-apoptotic BCL-2 proteins BAX and BAK [2,5,6]. When BAX and BAK are directly activated by BH3-only proteins or released from inhibited anti-apoptotics, they use their BH3 domain to oligomerize and assemble mitochondrial pores that induce mitochondrial outer membrane permeabilization, a key event that leads to apoptosis (Figure 1) [7,8].

Anti-apoptotic BCL-2 proteins possess a hydrophobic groove, comprising conserved BCL-2 homology domain 1 (BH1) and 2 (BH2) that binds and sequesters the BH3 domains of pro-apoptotic members [9]. Structural elucidation of anti-apoptotics bound to a range of BH3 domains has led to the development of a large number of small molecules targeting the hydrophobic groove of anti-apoptotic BCL-2, BCL-X_L and MCL-1 [10,11]. These small molecules, called BH3 mimetics, bind to one or more of P1-P4 sub-pockets in the BH3 groove of anti-apoptotic proteins, releasing pro-apoptotic BH3-only proteins that can activate BAX and BAK and lead to apoptosis. Here, we discuss the progress with the development of selective BH3 mimetics and the emerging approaches associated with targeting non-canonical pockets and pro-apoptotic BCL-2 proteins (Table 1). Figure 2 shows select small molecules and probes targeting the BCL-2 proteins that will be discussed in this review.

BH3 mimetics of anti-apoptotic BCL-2 proteins

Several BH3 mimetics, either small molecules or peptidomimetics have been identified and previously reviewed [11–13]. The first generation of BH3 mimetics had limited selectivity for a specific anti-apoptotic BCL-2 protein and most of them were also found to promote cell death independently of BAX/BAK proteins. Compounds as AT-101 and Obatoclax progressed to clinical evaluation, although significant toxicities associated with off-target effects halted their development further [14,15].

The discovery of ABT-737 and its orally available analogue ABT-263 (navitoclax), demonstrated a more potent and selective profile for BAX/BAK dependent apoptosis [16,17]. ABT-737 was discovered at Abbot Laboratories using a fragment-based drug design approach by NMR and binds P1-P4 sub-pockets of BCL-X_L, BCL-2 and BCL-W with subnanomolar affinity ($K_i < 1\text{nM}$). Navitoclax showed efficacy in clinical trials for hematological malignancies and small cell lung cancer, however, its effectiveness is limited by dose dependent BCL-X_L-mediated thrombocytopenia due to platelets apoptosis [18,19].

Dual BCL-2/BCL-X_L inhibitors

Based on the success of ABT-737/ABT-263, several dual BCL-2/BCL-X_L inhibitors were developed using their arylsulfonamide scaffold. Wang and colleagues generated BM-1197, a dual BCL-2/BCL-X_L inhibitor with subnanomolar affinity for both BCL-2/BCL-X_L and >1,000-fold selectivity over MCL-1 [20]. BM-1197 exerted potent antitumor activity in small cell lung cancer cells and complete and long-term tumor regression in xenograft models after intravenous administration at daily or weekly schedule. BM-1197 caused reversible platelet reduction in mice at highly efficacious doses. Servier also generated a nanomolar affinity dual BCL-2/BCL-X_L inhibitor, S44563, which exerted promising inhibitory efficacy in patient-derived uveal melanoma cell lines and xenografts and enhanced sensitivity of small cell lung cancer to radiation [21,22].

A dual inhibitor, BCL-32, with nanomolar affinity for BCL-2 ($K_i = 3.3$ nM) and BCL-X_L ($K_i = 8.5$ nM) was reported by AstraZeneca [23]. BCL-32 showed potent inhibitory efficacy *in vivo* allowing intermittent dosing schedule and full platelet recovery between doses. Another subnanomolar inhibitor of both BCL-2 and BCL-X_L, AZD4320, was recently reported as a clinical candidate by AstraZeneca [24]. AZD4320 exhibited potent antitumor activity in BCL-2/BCL-X_L-driven cell lines and RS4;11 ALL xenograft model. Reversible reduction of platelets counts was detected after 72 hours.

BCL-2 inhibitors

Structure-based design based on the ABT-263 co-crystal structure with BCL-2, enabled tailoring the interaction with the P4 sub-pocket of BCL-2, leading to the first potent and selective BH3 mimetic ABT-199 (Figure 3a) [25]. ABT-199 (venetoclax) has subnanomolar affinity ($K_i = 0.01$ nM) for BCL-2 protein inducing potent apoptosis in BCL-2-dependent patient-derived cells lines and xenografts from a variety of leukemia and lymphoma malignancies without triggering thrombocytopenia. Venetoclax became the first BH3 mimetic to be FDA-approved in April 2016, for use in patients with chronic lymphocytic leukemia (CLL) with the 17p deletion [26]. Venetoclax is currently being evaluated in multiple clinical trials as a monotherapy and combination therapy for non-Hodgkin lymphomas, acute myeloid leukemia, multiple myeloma and breast cancer.

Following the paradigm of ABT-199 for high selectivity against one anti-apoptotic protein, monoselective BH3 mimetics for each BCL-2, BCL-X_L and MCL-1 have been recently generated. Servier and Vernalis collaboration yielded a novel class of BCL-2 selective compounds based on tetrahydroisoquinoline amide substituted pyrazoles [27]. S55746 derived from this class has entered phase I trials as single agent in patients with CLL and Non-Hodgkin lymphoma and in combination with the P13K δ inhibitor idelalisib in follicular lymphoma and mantle cell lymphoma [28,29].

BCL-X_L inhibitors

Lessene *et al.* utilized an alpha screen competition assay to disrupt BIM BH3 interaction with BCL-X_L and not MCL-1 to identify a chemical scaffold with selectivity for Bcl-X_L [30]. Structure-based guided medicinal chemistry focused on specific interactions with the P2 and P4 sub-pockets led to WEHI-539, an inhibitor with subnanomolar affinity for BCL-

X_L (IC₅₀ = 1.1 nM) and >400-fold selectivity against other anti-apoptotics. WEHI-539 promoted robust apoptosis induction in BCL-X_L dependent small cell lung cancer cells, however, its poor physicochemical properties limits its activity *in vivo*.

Abbvie colleagues combined NMR fragment screening and structure-based design to optimize WEHI-539 leading to A-1155463 and A-1331852 [31,32]. A-1331852 is the most potent and selective orally available BCL-X_L inhibitor (K_i = < 0.010 nM) reported with 10–50 fold improved cellular activity than A-1155463 and ABT-263 (Figure 3b). A-1331852 induced tumor regression in xenograft models of solid tumors including ovarian cancer, breast cancer and NSCLC. Moreover, A-1331852 showed improved efficacy when tested in combination with docetaxel with no apparent toxicity [31].

MCL-1 inhibitors

The structure of MCL-1 suggested that its BH3 groove is particularly shallow in P1, P2 and P4 sub-pockets compared to BCL-2 and BCL-X_L [33]. Interestingly, alanine-scanning mutagenesis suggested that BH3 binding to the P4 sub-pocket is not as important as in BCL-2 and BCL-X_L [34]. Earlier reports demonstrated high affinity for MCL-1 and selectivity over BCL-2 and BCL-X_L with BIM BH3-based peptides, stapled peptides based on the MCL-1 BH3 domain and the natural product maritoclax [35–37].

Nikolovska-Coleska and colleagues identified UMI-77 through HTS and structure-based design, with moderate binding affinity (K_i = 490 nM) and selectivity for MCL-1 [38]. UMI-77 induces apoptosis by disrupting MCL-1/BAX and MCL-1/BAK complexes. In an *in vivo* pancreatic xenograft model, intravenous dosing of UMI-77 in two cycles provided evidence of tumor growth inhibition.

Abbvie and independently the Fesik lab adopted a strategy to grow P2 bound hits from fragment-based NMR screens and generate compounds based on the 2-carboxy indole core. These approaches led to larger P2-P4 binders such as A-1210477 by Abbvie [39] and compounds 4 and 5 by the Fesik lab [40], which exhibit subnanomolar affinity (K_i = 0.045 nM and K_i = 0.05 nM respectively) and selectivity >1,000-fold over BCL-2 and BCL-X_L. These compounds induced single-agent mitochondrial apoptosis in MCL-1 dependent cell lines of hematologic cancers.

Servier recently reported the development of S6384 [41], with subnanomolar (K_i = 0.15 nM) binding to MCL-1 and selectivity of ~10,000 fold over BCL-2 and BCL-X_L (Figure 3c). S63845 has a highly potent and broad pro-apoptotic killing activity in a variety of MCL-1-addicted hematopoietic malignancies as well as select breast and NSCLC cell lines. S63845 was highly effective *in vivo* in xenograft models of AML, lymphoma and multiple myeloma. Moreover, when it was combined with MEK1/2 inhibitor, trametenib, HER-2 inhibitor, lapatinib, B-Raf inhibitor, PLX-4032 and EGFR inhibitor, tarceva, it exhibited effective synergistic cytotoxicity in solid tumors [41].

AMG176 developed by Amgen is the first MCL-1 inhibitor to enter phase 1 trials for the treatment of relapsed and refractory multiple myeloma. Data disclosed recently identify AMG176 as a macrocyclic inhibitor that binds with subnanomolar affinity (K_i = 0.13 nM)

around the P2 sub-pocket forming a hydrogen bond with Arg 263 of MCL-1 [42]. AMG176 induced potent apoptosis in MCL-1-addicted cell lines derived from a variety of hematologic malignancies exhibiting on-mechanism activity. AMG176 demonstrated robust efficacy in a multiple myeloma xenograft using a discontinuous dosing schedule of once or twice weekly.

AZD5991 is another rationally designed macrocyclic MCL-1 inhibitor with sub-nanomolar affinity for MCL-1 ($K_i = 0.13$ nM) recently disclosed by AstraZeneca [43]. AZD5991 demonstrated potent and rapid apoptosis induction in MCL-1-addicted cell lines and achieves complete tumor growth inhibition *in vivo* in multiple myeloma xenografts from a single intravenous dose.

BFL-1 inhibitors

Although studies have demonstrated BFL-1 upregulation as a chemoresistance factor in subsets of leukemia, lymphoma and melanoma, targeting studies have been limited. Recent efforts have been conducted towards the development of peptide-based inhibitors taking advantage of the high affinity and selective interaction of NOXA BH3 helix with BFL-1 and the reactive cysteines at each interaction partner that can form a covalent bond. Walensky and coworkers introduced to stapled peptides after NOXA and BIM BH3 domains an acrylamide moiety that reacts with BFL-1 Cys55 to generate an irreversible and highly selective BFL-1 inhibitor that enhanced apoptosis in BFL-1 driven melanoma cells [44]. Pellecchia and coworkers took a similar approach and replaced the Cys25 of NOXA BH3 with a mild Michael acceptor (Dap-2-chloroacetamide). A nanomolar ($IC_{50} = 9$ nM) BFL-1 peptide inhibitor was generated with 2–5-fold selectivity over other anti-apoptotics and cellular activity in BFL-1 expressing melanoma and lymphoma cells [45].

Non-canonical targeting of anti-apoptotic BCL-2 proteins

Besides targeting the P1-P4 sub-pockets of the BH3 groove, additional mechanisms of BCL-2 family protein interactions have been investigated and small molecule or protein-based probes have been identified to bind non-canonical surfaces.

Berger *et al.* used a 3-helix bundle protein, BINDI that utilizes a BH3-like central helix with two flanking helices for additional contacts [46]. Computational mutagenesis and docking was used to develop a series of mutants capable of selectively inhibiting each of the six anti-apoptotic BCL-2 proteins. The success of this technique is attributed to the exploitation of residues outside of the canonical BH3 groove unique to each anti-apoptotic protein and formed by $\alpha 3$ – $\alpha 5$ region (Figure 3e). This increase in contact surface area is translated into increased affinity and specificity. When these designed proteins were expressed in cells, they inhibited their corresponding BCL-2 protein providing unique cellular probes.

BCL-2

Using a computational docking screen to target the BH4 domain (aa 6–31) of BCL-2 protein that is essential for its anti-apoptotic function, BDA-366 was identified to bind selectively to BCL-2 and antagonize it. BDA-366 results in a BCL-2 conformational change that exposes its BH3 domain resulting in pro-apoptotic activity [47]. BDA-366 was shown to suppress lung cancer growth in lung cancer cell lines and xenografts alone and in synergy with an

mTOR inhibitor. Although the mechanism for this structural transition of BCL-2 is elusive, similar conclusions were previously reported for NuBCP-9, a short Nur77-derived peptide, which bound to the loop region between the BCL-2 BH3 and BH4 domains with $K_d \sim 200$ nM [48]. NuBCP-9 was able to induce exposure of the BCL-2 BH3 domain, which in turn suppressed anti-apoptotic activity of BCL-X_L facilitating BAX-dependent apoptosis and inhibition of tumor growth *in vitro* and *in vivo* [48].

MCL-1

Song *et al.* utilized NMR, limited proteolysis, and *in vitro* ubiquitination assays to demonstrate that subtle conformational changes in loop 2 (between $\alpha 2$ – $\alpha 3$) within the BH3 binding groove of MCL-1 could influence ubiquitination and MCL-1 turnover [49]. A new MCL-1 inhibitor was identified with modest affinity, compound 5, capable of influencing the conformation of loop 2 and subsequently promoting MCL-1 degradation. In cellular assays compound 5 was able to both dissociate proteins bound to MCL-1 and lower MCL-1 levels allowing it to outperform higher affinity small molecules in the killing of MCL-1-addicted cell lines. This conformational regulation also explained the action of MCL-1 inhibitors including Maritoclax which induces the same loop 2 conformation and enhances MCL-1 turnover, while A-1210477, which fails to alter loop 2, leads to increase in MCL-1 levels in cells potentially antagonizing its efficacy [49].

Targeted covalent inhibitors combine a small molecule with specificity for a target with a covalent “warhead” which covalently binds to the target and dramatically increases the binding energy. Lee *et al.* identified a small molecule to covalently bind to MCL-1 Cys286, which is both unique to MCL-1 and distant from the canonical binding groove [50]. The compound induced conformational changes and the allosteric inhibition of BH3-domain interaction with MCL-1. Another study by Akçay *et al.* modified a small molecule inhibitor of MCL-1 to include a strategically placed boronic acid group, which reversibly reacts with Lys234 [51]. Covalent binding to this lysine, unique to MCL-1, allowed specific increase in binding to MCL-1 over other BCL-2 family members. The resulting compound showed encouraging results in MCL-1-dependent cell lines.

Targeting of pro-apoptotic BAX and BAK

Several recent studies have significantly advanced our understanding of the different activation and regulation mechanisms of pro-apoptotic BAX and BAK. Elucidation of binding surfaces and different conformations in solution and on the membrane provides the means for selectively targeting these proteins to modulate their function.

BAX

Structural analysis of a stapled BIM BH3 helix, BIM SAHB, bound to monomeric BAX uncovered an activation site (trigger site) at the N-terminal surface of BAX [52]. This activation site regulates the trigger mechanism for conformational activation of cytosolic BAX, leading to the release of the C-terminal transmembrane $\alpha 9$ helix, exposure of the hydrophobic $\alpha 2$ helix (BH3 domain) and ultimately to mitochondrial BAX oligomer formation [53,54]. BAX without the transmembrane $\alpha 9$ helix additionally interacts with

BID and BIM BH3 peptides at its canonical groove suggesting an additional activation mechanism for loosely attached mitochondrial BAX [55,56]. Recently, an autoinhibited dimer crystal structure of full-length BAX revealed an additional mechanism of BAX regulation that is mediated by the interaction of $\alpha 9$ helix from one BAX molecule with the trigger site of the second BAX molecule [57].

Gavathiotis *et al.* performed a computational screen and a competitive binding assay for binding small molecules to the trigger site and identified BAX activator molecule, BAM7 [58]. BAM7 directly and selectively over anti-apoptotic proteins and BAK engages the BAX trigger site promoting functional oligomerization of BAX and BAX-dependent apoptosis (Figure 3d). Thus, direct small molecule activation of BAX suggested a new paradigm for pharmacologic induction of apoptosis.

Uchime *et al.* identified synthetic antibodies through a phage display screen that bind with low nanomolar affinity to BAX [59]. A representative Fab 3G11 found to bind an extensive surface of BAX that includes the trigger site (helices $\alpha 1/\alpha 6$) and the closed $\alpha 1$ - $\alpha 2$ loop (Figure 3g). 3G11 blocked BAX mitochondrial translocation and BAX-mediated cytochrome *c* release consistent with a mechanism of BAX conformational suppression through extensive contacts with BAX, which prevent opening of the $\alpha 1$ - $\alpha 2$ loop that is essential for BAX activation [53,57].

Moreover, Barclay *et al.* identified a stapled peptide based on the BCL-2 BH4 domain to bind with nanomolar affinity to BAX and suppress BAX conformational activation [60]. Using NMR and paramagnetically-labeled peptides, the BCL-2 BH4 was localized on a discrete binding site formed by residues of $\alpha 1$, $\alpha 1$ - $\alpha 2$ loop, and $\alpha 2$ - $\alpha 3$ and $\alpha 5$ - $\alpha 6$ hairpins on the BAX surface (Figure 3f). Residues of this binding site between the BAX trigger site and the canonical groove presumably mediate conformational BAX activation.

BAK

Structural and biochemical studies determined activating interactions of BAK by native and stapled peptides of BID BH3 through its BH3-binding groove ($\alpha 3$ - $\alpha 5$) [61–63]. These studies led to the conclusion that BAK is activated through interaction with the canonical groove and opened the opportunity for pharmacological activation of BAK. However, small molecules targeting this canonical BH3-groove are still elusive. Interestingly, Kluck and colleagues identified an antibody (7D10), which bound to the $\alpha 1$ - $\alpha 2$ loop of BAK, opposite to the canonical BH3-groove and promoted BAK conformational activation and oligomerization leading to MOMP [64]. The physiological relevance of this novel activation mechanism has not yet been established, although a movement of this loop on BAX to open position was determined to be essential upon BH3 trigger site activation [53]. The development of small-molecules that target the trigger site of BAX provides hope that this novel site of BAK can also be exploited pharmacologically.

Conclusions and perspectives

Significant progress has been made in targeting the BCL-2 family of proteins. The FDA-approval of ABT-199 has fueled with excitement further efforts that BH3 mimetics could

have a promising future to treat cancers. The paradigm for potent and selective targeting of individual anti-apoptotic proteins to promote BAX- and BAK- dependent apoptosis has been followed with new compounds targeting both BCL-2/BCL-X_L, or individual BCL-2, BCL-X_L, MCL-1 and BFL-1. It remains to be seen whether these new selective BCL-2 inhibitors or their derivatives can achieve improved efficacies as monotherapies or can be combined for increased efficacy with manageable undesired effects.

Several novel approaches to target BCL-2 proteins, beyond the canonical BH3 groove binding, have been identified in recent years including allosteric or covalent small molecules, peptides and antibodies. These recent findings suggest that there are several ways to modulate the surfaces and function of these proteins. Such approaches offer an alternative path to enhanced potency and selectivity that may stimulate more efficient drug discovery. The advances in our understanding of the mechanisms of activation of BAX and BAK have already generated valuable tools and targeting strategies. The possibility of developing activators and inhibitors of apoptosis through pro-apoptotic proteins would have promising application in cancer, autoimmune and aging-associated diseases. Without doubt, this is an exciting time for the application of chemical biology and drug discovery against BCL-2 family proteins.

Acknowledgments

This review aims to highlight select recent studies within the allocated space and we apologize for any omissions. The authors would like to acknowledge the support from National Institute of Health (R01CA178394), the American Heart Association, the Sidney Kimmel Foundation for Cancer Research, the Gabrielle's Angels Foundation for Cancer Research, the Alexandrine and Alexander L. Sinsheimer Foundation, the Pershing Square Sohn Cancer Research Alliance, the Melanoma Research Alliance and the Irma T. Hirsch Trust.

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Highlights

- Selective BH3 mimetics for interrogating the BCL-2 protein family interactions and their development for cancer therapy
- Non-canonical interactions of anti-apoptotic BCL-2 proteins offer alternative targeting from BH3 mimetics
- Advances in understanding pro-apoptotic BCL-2 protein interactions leads to novel targeting opportunities

Figure 1. The mitochondrial pathway of apoptosis

The intrinsic or mitochondrial apoptotic pathway is characterized by the mitochondrial outer membrane permeabilization (MOMP). Upon variety of stress stimuli, pro-apoptotic BH3-only proteins inhibit anti-apoptotic BCL-2 proteins and activate pro-apoptotic BAX and BAK. Activated BAX and BAK oligomerize and form pores to the mitochondrial outer membrane triggering MOMP. MOMP induces release of mitochondrial intermembrane space proteins such as cytochrome *c* and second mitochondria-derived activator of caspases (SMAC) into the cytosol. While SMAC boosts apoptosis by blocking caspase inhibitor X-

linked inhibitor of apoptosis protein (XIAP), cytochrome *c* promotes apoptosis by activating the caspase cascade. Cytochrome *c* interacts with the apoptotic protease activating factor 1 (APAF1), leading to the activation of caspase-9 and the apoptosome assembly. Activated caspase-9 activates caspase-3 and caspase-7, leading to apoptosis. Anti-apoptotic BCL-2 inhibitors promote apoptosis by releasing sequestered BH3-only proteins and BAX and BAK from anti-apoptotic BCL-2 proteins. In contrast, BAX and BAK activators can bind directly to these pro-apoptotic proteins and activate them to promote cell death.

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Figure 2.
Small molecules targeting the BCL-2 family of proteins

Figure 3. Representative structures of BCL-2 family proteins in complex with modulators
 Small molecule inhibitors of anti-apoptotic members bind to the 4 sub-pockets (P1-P4) of the canonical groove, (a) BCL-2 in complex with an analogue of ABT-199* (PDB:4MAN), (b) BCL-X_L in complex with A-1155463 (PDB:4QVX) and (c) MCL-1 in complex with S63845 (PDB:5LOF). (d) BAM7 binds to a hydrophobic N-terminal trigger site of BAX (Modified PDB:2K7W), surface properties illustrated as hydrophobic:yellow, hydrophilic:green, acidic:red, basic:blue. (e) BINDI helical bundles utilize both canonical groove and the surrounding surface to increase specificity and potency. MCL-1 in complex

with a MCL-1 specific BINDI (PDB:5JSB). (f) A stapled peptide derived from the BH4 domain of BCL-2 binds to a surface of BAX distinct from both the canonical and trigger site of BAX [56]. (g) The antibody 3G11 binds BAX through interaction with an extended N-terminal trigger site inhibiting BAX conformational activation [55].

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Table 1

Characteristics of inhibitors and activators of the BCL-2 family of proteins through canonical and non-canonical interactions^a.

Name	Target(s)	In vivo antitumor effects	Clinical Stage	References
AT-101 (Gossypol)	BCL-2, BCL-X _L , MCL-1	Yes	Phase II ^b	14
Obatoclox	BCL-2, BCL-X _L , MCL-1, BCL-W, BFL-1	Yes	Phase II ^b	15
ABT-737	BCL-X _L , BCL-2, BCL-W	Yes		16
ABT-263 (Navitoclax)	BCL-X _L , BCL-2, BCL-W	Yes	Phase II ^b	17
BM-1197	BCL-2, BCL-X _L	Yes		20
S44563	BCL-2, BCL-X _L	Yes		21,22
BCL-32	BCL-2, BCL-X _L	Yes		23
AZD4320	BCL-2, BCL-X _L	Yes		24
ABT-199 (Venetoclax)	BCL-2	Yes	FDA-Approved	25,26
S55746 (BCL201)	BCL-2	Yes	Phase I	27–29
WEHI-539	BCL-X _L	No		30
A-1155463	BCL-X _L	No		31
A-1331852	BCL-X _L	Yes		32
<i>MCL-1-SAHB_D</i>	MCL-1	No		35
Bim ₂ A	MCL-1	No		36
Maritoclax	MCL-1	Yes		37
UMI-77	MCL-1	Yes		38
Compounds 4/5	MCL-1	No		40
A-1210477	MCL-1	No		39
S63845	MCL-1	Yes		41
AMG176	MCL-1	Yes	Phase I	42
AZD5991	MCL-1	Yes		43
<i>BH3-SAHB_{A-3}</i>	BFL-1	No		44
<i>hNOXA 130G4</i>	BFL-1	No		45
<i>BINDI</i>	BCL-2, BCL-X _L , MCL-1, BCL-W, BFL-1, BCL-B	No		46
BDA-366	BCL-2	Yes		47
<i>NuBCP-9</i>	BCL-2	Yes		48
Compound 5	MCL-1	No		49
MAIM1	MCL-1	No		50
Compound 11	MCL-1	No		51
<i>BIM-SAHB</i>	BAX	Yes		52–54
BAM7	BAX	No		58
<i>3G11</i>	BAX	No		59
<i>BCL2-BH4 SAHB</i>	BAX	No		60
<i>BID-SAHB</i>	BAK	No		61–62
<i>7D10</i>	BAK	No		64

^aCanonical binders in light grey background, non-canonical/covalent binders in dark grey background, peptide/protein in italics.

^b completed clinical trials

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