CHAPTER SEVENTEEN

Small-Molecule Antagonists of Bcl-2 Family Proteins

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1. INTRODUCTION

The Bcl-2 family of proteins is central to regulating apoptosis, or programmed cell death. Alterations to the native expression levels of Bcl-2 family proteins have been shown to correlate with cancer progression, chemotherapy and radiotherapy resistance, and overall poor clinical outcomes. Apoptosis may be initiated by two different pathways. The extrinsic pathway is initiated through activation of death receptors on the cell surface, while the intrinsic pathway is initiated by increases in mitochondrial outer membrane permeability (MOMP). Mitochondrial membrane integrity is regulated through a balance of pro- and antiapoptotic Bcl-2 family proteins. The multidomain proapoptotic proteins Bax, Bak, and Bok share sequence homology in three α-helical Bcl-2 homology domains (BH1–BH3), while the antiapoptotic proteins Bcl-2, Bcl-x_L, Mcl-1, Bcl-w, Bcl-B, and A1 (Bfl-1) share sequence homology in four domains (BH1–BH4) and similar tertiary structure. In a healthy cell, antiapoptotic proteins deactivate the

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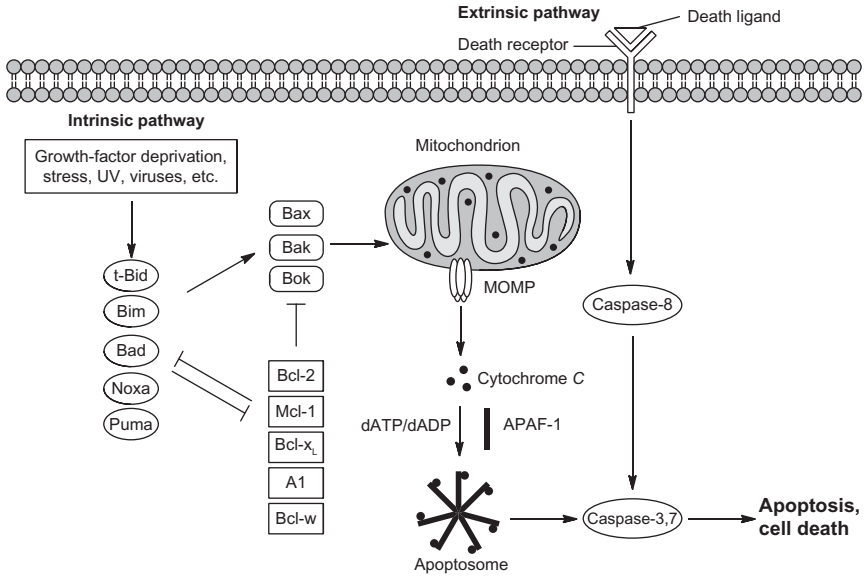


Figure 17.1 Scheme depicting intrinsic and extrinsic pathways of apoptosis.

multidomain proapoptotic proteins on the mitochondrion outer membrane (Fig. 17.1). Damaged cells receive death signals from the activated proapoptotic BH3-only proteins Bad, Bim, Puma, Bid, Bik, Noxa, Hrk, and Bmf. This initiates a cascade in which the proapoptotic proteins Bax, Bak, and Bok are activated and then homooligomerize on the outer mitochondrial membrane. This oligomerization forms pores in the mitochondrion that cause MOMP, the key step in commitment to apoptosis. Permeabilization of the mitochondrion releases cytochrome *c* into the cytosol where it complexes with APAF-1, caspase-9, and dATP/dADP to form the apoptosome that ultimately leads to caspase activation and cell death.

Although the precise mechanism of interaction between the Bcl-2 proteins is still under debate, it is widely agreed upon that the antiapoptotic proteins bind and neutralize the proapoptotic members through protein–protein interactions.[1] A hydrophobic groove displayed by the antiapoptotic Bcl-2 proteins accommodates the α-helical BH3 domain of both the proapoptotic BH3-only proteins and the multidomain proteins, creating a high-affinity interface that can potentially be disrupted with small molecules. Antagonizing the antiapoptotic Bcl-2 family proteins should result in the release of BH3-only proteins and thus induce apoptosis in the cell. Additionally, it has been shown that tumors expressing high levels of Bcl-2, Bcl-x_L, and Mcl-1 are resistant to radiation and chemotherapy, suggesting that antagonizing the antiapoptotic

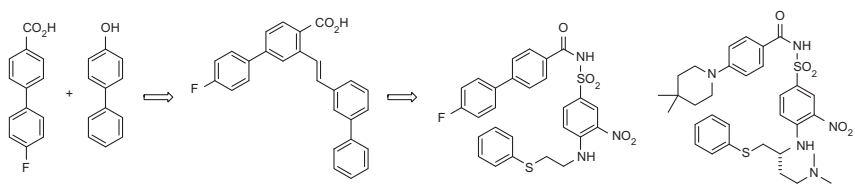
Bcl-2 proteins is a feasible and promising strategy for the treatment of cancer.[2,3] In this chapter, we present the different strategies applied to small-molecule lead generation and the progress toward the discovery of antiapoptotic Bcl-2 protein antagonists for the treatment of cancer.

2. SMALL-MOLECULE INHIBITORS OF Bcl-2 Family Proteins

2.1. Fragment-based approaches

To date, the most selective and potent inhibitors of Bcl-2 family proteins have been discovered using fragment screening followed by synthetic optimization. This flexible technique enables the discovery of leads for various targets with vastly different binding pockets. It also has advantages over other lead discovery techniques for finding inhibitors of protein–protein interactions, since these are challenging targets that do not fit the classical definition of "druggable."[4] Multiple leads and several clinical candidates have been discovered using this technique.

In 2006, a fragment screening effort was described that led to the discovery of potent Bcl-x_L inhibitors using ^{15}N HSQC NMR.[5] Initial screens identified low-affinity binders **1** ($K_d \sim 300$ μM) and **2** ($K_d \sim 6000$ μM) that were chemically linked to generate ligand **3**, a modest inhibitor of Bcl-x_L ($K_i = 1.4$ μM). Further synthetic efforts identified compound **4**, a potent inhibitor ($K_i = 0.036$ μM) of Bcl-x_L that binds to the BH3-domain. A liability of ligand **4** was low affinity in the presence of human serum (HS) ($K_i = 2.50$ μM with 1%HS). Further modifications resulted in A-385358 (**5**), an inhibitor with increased affinity in serum ($K_i = 0.36$ μM with 10%HS) as well as affinity for Bcl-2 ($K_i = 0.067$ μM). As a single agent, A-385358 showed little activity on cancer cell viability. However, it potentiated the activity of paclitaxel in A549 NSCLC cells (up to 25-fold compared to monotherapy) and significantly enhanced A549 tumor shrinkage when codosed with paclitaxel in a mouse xenograft model.[6,7]



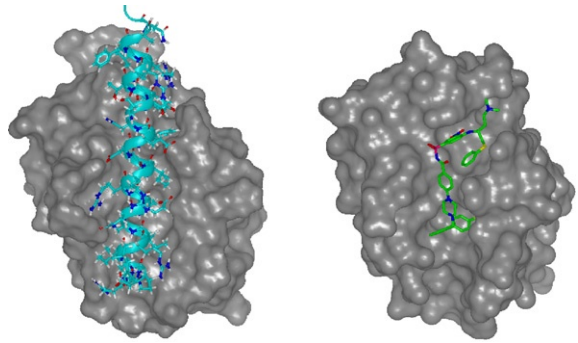
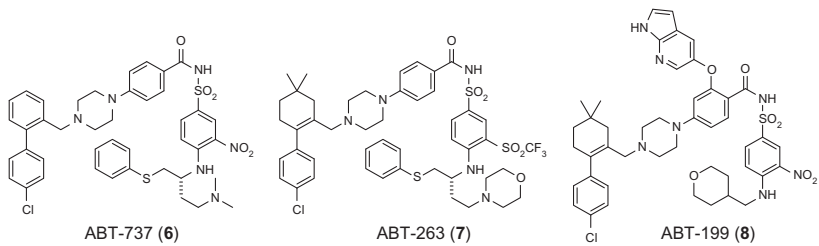


Figure 17.2 X-ray crystal structure of antiapoptotic protein Bcl-x_L bound to the Bim BH3-domain peptide (left), and the small-molecule BH3 mimetic ABT-737 (right).

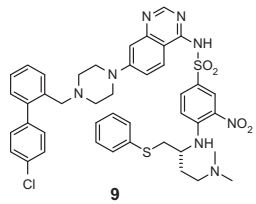
Further optimization led to the discovery of ABT-737 (**6**), an inhibitor with high affinity for Bcl-x_L, Bcl-2, and Bcl-w ($K_i < 0.001$ μM) but not Mcl-1, Bcl-B, or A1.[8] The increased affinities are rationalized by the chlorobiphenyl substituent occupying hydrophobic regions common to the three proteins.[9] ABT-737 was cocrystallized with Bcl-x_L, demonstrating that it mimics the BH3 helix and binds to the hydrophobic BH3-binding domain (Fig. 17.2). ABT-737 demonstrates efficacy as a single agent in various cancer cells lines, such as NCI-H889 SCLC cells ($IC_{50} = 20$ nmol/L). Cancer cell lines sensitive to ABT-737 generally show high expression levels of Bcl-2 and Bcl-x_L, while high levels of Mcl-1 correlate with resistance.[10] ABT-737 also potentiates the proapoptotic effects of various chemotherapeutic agents such as paclitaxel (fourfold over monotherapy in A549 cells). Additionally, in H146 SCLC tumor xenograft models, ABT-737 caused complete regression, and tumors did not regrow for 58 days after therapy cessation.[8] However, the poor oral bioavailability and solubility profile of ABT-737 hampered the progression of this molecule through clinical studies.[11]



Optimization of ABT-737 led to ABT-263 (navitoclax, **7**), an inhibitor with improved solubility and pharmacokinetic profiles.[12] ABT-263 displays high affinity for Bcl-x_L ($K_i = 0.0042$ μM) and Bcl-2 ($K_i = 0.0056$ μM) and shows oral bioavailability in various animal species. It selectively kills SCLC H146 cells as a single agent ($EC_{50} = 87$ nM with 10% HS) and has shown efficacy in other lymphoma and chronic lymphocyte leukemia lines. ABT-263 potentiates the activity of a broad range of chemotherapeutic agents over a wide range of cell types. For example, it synergizes with erlotinib in NSCLC NCI-H1650 cells with a combination index value of 0.44.[13] ABT-263 induces complete tumor regression when dosed at 100 mg/kg/day for 21 days in a SCLC H1963 xenograft model.[14] Tumor sensitivity positively correlates with Bcl-2 and Bcl-x_L expression and negatively correlates with Mcl-1 expression.[10,15] ABT-263 is currently in phase I/II clinical trials for the treatment of various solid tumors, hematologic malignancies, chronic lymphocytic leukemia, and lymphoid malignancies.[16] However, phase II results in patients with relapsed SCLC demonstrated limited efficacy (partial response in 2.6% of patients and stable disease in 23%), while dosing has been limited by Bcl-x_L-mediated thrombocytopenia.[17]

To overcome the Bcl-x_L-related platelet toxicity, ABT-199 (**8**) was designed as a selective Bcl-2 inhibitor. ABT-199 is a potent Bcl-2 inhibitor ($K_i < 0.001$ μM) but has significantly less affinity for Bcl-x_L ($K_i > 1.0$ μM). ABT-199 has a similar sensitivity profile to ABT-263 across various cells, but exhibits an approximate 10-fold increased potency in all sensitive cell lines. Importantly, ABT-199 has little effect on platelets *in vitro* compared to ABT-263, which kills platelets with an EC_{50} of 80 nM.[18] ABT-199 is currently in phase I clinical trials.[19]

Other inhibitors against the various Bcl-2 family members include quinazoline sulfonamide **9**.[20] The quinazoline sulfonamide core is a suitable isostere for the phenyl acylsulfonamide in ABT-263, and X-ray crystallographic data show that both inhibitors bind to Bcl-x_L with similar binding modes. Unlike ABT-263, quinazoline sulfonamide displays high affinity for Bcl-x_L ($IC_{50} = 0.007$ μM) and Bcl-2 ($IC_{50} = 0.0082$ μM) while displaying significantly lower affinity for Bcl-w ($IC_{50} = 0.44$ μM). These analogs currently remain in preclinical development. Selective inhibitors of Mcl-1, exemplified by indole **10**, have been disclosed although biological data have yet to be reported.[21,22]



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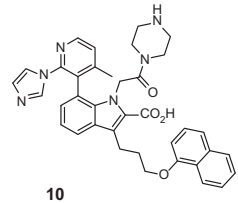
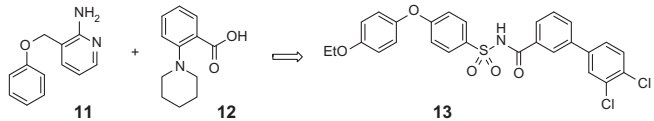


10

Finally, a fragment-based lead discovery approach reported a dual Bcl-x_L and Mcl-1 inhibitor.[23] NMR screening revealed weak-binding fragments **11** ($K_i = 690$ μM) and **12** ($K_i = 380$ μM). Fragment linking and optimization gave acylsulfonamide **13**, with affinity for both Bcl-x_L ($IC_{50} = 0.086$ μM) and Mcl-1 ($IC_{50} = 0.14$ μM).



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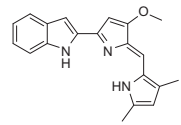
2.2. Natural product-based approaches

The discovery of several classes of cytotoxic natural products that may function by antagonizing the antiapoptotic Bcl-2 proteins has provided several small-molecule leads and multiple clinical candidates. In contrast to the potent inhibitors discovered by fragment screening, however, most of the natural product inhibitors display only modest affinity for Bcl-2 family proteins, and in several cases, additional biological activity has been reported.

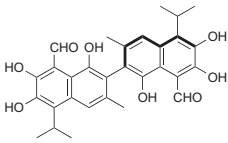
Obatoclax (GX15-070, **14**), an analog of cycloprodigiosin, inhibits all six antiapoptotic Bcl-2 proteins with IC_{50} values ranging from 1 to 7 μM. Obatoclax displays 87% growth inhibition of C33A tumors cells compared to vehicle in a mouse xenograft model and is currently in phase I/II clinical trials for solid and hematological malignancies.[24] Although obatoclax displays clinical efficacy, reducing circulating lymphocytes in 18 of 26 patients with a median reduction of 24%,[25] this efficacy may not be driven solely by antagonism of Bcl-2 proteins. In fact, obatoclax induces apoptosis in the absence of Bax and Bak and also induces an S-G2 cell cycle block, suggesting that it acts through multiple targets.[26]



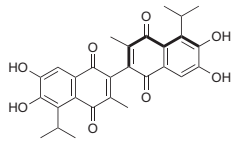
Obatoclax (GX15-070, **14**)

Gossypol is a natural polyphenol isolated from cotton seeds and roots. (±)-Gossypol (BL-193) is a mixture of atropisomers where (−)-gossypol (AT101, **15**) displays greater potency of the two isoforms. AT101

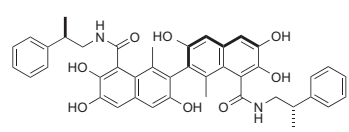
exhibits affinity for Bcl-2, Bcl-x_L, and Mcl-1 ($IC_{50} = 0.32$, 0.48, and 0.18 μM, respectively), although evidence suggests that the cytotoxicity results from additional mechanisms, such as DNA cleavage or the generation of reactive oxygen species that promote apoptosis.[27] AT101 demonstrates activity in a WSU-$DLCL_2$ mouse xenograft model, providing 51% tumor growth inhibition compared to vehicle.[28] This single-agent efficacy has prompted the therapeutic potential of AT101 to be tested in phase I/II clinical trials, but dosing has been limited by gastrointestinal toxicity.[29]



(–)-Gossypol (AT101, **15**)



Apogossypolone (ApoG2, **16**)

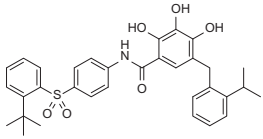


Sabutoclax (BI-97C1, **17**)

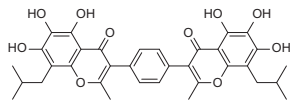
In an effort to reduce the toxicity observed with AT101 treatment, apogossypolone (ApoG2, **16**) was synthesized within a series of gossypol derivatives designed without the electrophilic aldehyde groups. ApoG2 displays increased binding affinity toward Bcl-2 ($IC_{50} = 0.040$ μM), Bcl-x_L ($IC_{50} = 0.088$ μM), Mcl-1 ($IC_{50} = 0.056$ μM), and A1 ($IC_{50} = 0.211$ μM). Further optimization culminated in the identification of BI-97C1 (sabutoclax, **17**). A pan-Bcl-2 family inhibitor, sabutoclax exhibits submicromolar affinity for Bcl-2 ($IC_{50} = 0.32$ μM), Bcl-x_L ($IC_{50} = 0.31$ μM), Mcl-1 ($IC_{50} = 0.20$ μM), and A1 ($IC_{50} = 0.62$ μM) while displaying *in vitro* cytotoxicity toward prostate cancer (PC3 cells; $EC_{50} = 0.13$ μM), lung cancer (H460 cells; $EC_{50} = 0.42$ μM), and lymphoma (BP3 cells; $EC_{50} = 0.049$ μM) cell lines. Sabutoclax has minimal effect on $Bax^{-/-}Bak^{-/-}$ cells providing evidence that its cytotoxicity is mitochondrion mediated.[30]

Efforts to design (−)-gossypol mimetics resulted in the identification of TW-37 (**18**), a pan-Bcl-2 family inhibitor.[31] TW-37 exhibits affinity for Bcl-2 ($IC_{50} = 0.29$ μM), Mcl-1 ($IC_{50} = 0.26$ μM), and Bcl-x_L ($IC_{50} = 1.11$ μM) and inhibits growth of PC3 cells with an IC_{50} of 200 nM, although its cytotoxic effects have also been attributed to other mechanisms.[32] A structure-based design effort utilizing the gossypol/Bcl-x_L structure revealed BI-33 (**19**), a modest inhibitor of Bcl-x_L ($K_i = 1.2$ μM) but a potent inhibitor of both Bcl-2 ($K_i = 0.017$ μM) and Mcl-1 ($K_i = 0.017$ μM). A related effort revealed dihydroisoquinoline TM-1206 (**20**), an inhibitor of Bcl-x_L ($K_i = 0.64$ μM), Bcl-2 ($K_i = 0.11$ μM), and

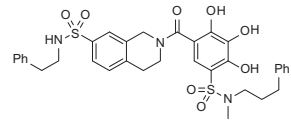
Mcl-1 ($K_i = 0.15$ μM). Both BI-33 and TM-1206 induce death in MDA-MB-231 cells with IC_{50} values of 0.11 and 0.10 μM, respectively.[33,34]



TW-37 (**18**)

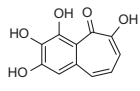


BI-33 (**19**)

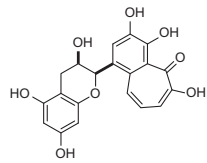


TM-1206 (**20**)

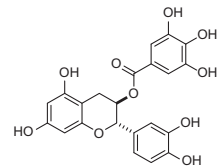
Several classes of natural polyphenols have been identified as Bcl-x_L inhibitors in screening campaigns.[35] Purpurogallin (**21**), isolated from edible oils, is a modest inhibitor of Bcl-x_L ($K_i = 2.2$ μM). Black tea component theaflavanin (**22**) has submicromolar affinities for Bcl-x_L ($K_i = 0.25$ μM) and Bcl-2 ($K_i = 0.28$ μM), and green tea component (−)-catechin-3 gallate (**23**) has similar Bcl-x_L ($K_i = 0.12$ μM) and Bcl-2 ($K_i = 0.40$ μM) affinities.[36] Both these compounds induce apoptosis in various cancer cells, but the exact mechanisms are a matter of debate.[37]



Purpurogallin (**21**)



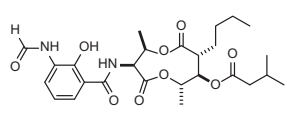
Theaflavanin (**22**)



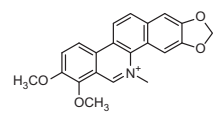
(–)-Catechin-3 gallate (**23**)

Other structurally diverse natural products with moderate affinity for Bcl-2 family proteins have been reported. Antimycin A (**24**), isolated from *Streptomyces*, was shown to selectively induce apoptosis in cells with high Bcl-x_L expression levels, although superoxide release from the mitochondria was also observed, suggesting that multiple apoptotic mechanisms may be occurring.[38] Chelerythrine (**25**), isolated from *Bocconia vulcanica*, demonstrates modest Bcl-x_L affinity ($IC_{50} = 1.5$ μM) and was shown to disrupt Bak/Bcl-x_L interactions in an immunoprecipitation assay. However, activity against Bak/Bax-deficient cell lines suggests that additional biological mechanisms may contribute to this cytotoxicity.[39] Cryptosphaerolide (**26**), isolated from *Cryptosphaeria*, is a modest binder of Mcl-1 ($IC_{50} = 11.4$ μM) and kills HCT-116 cells ($EC_{50} = 4.5$ μM) *in vitro*.[40] Marinopyrrole A (**27**), isolated from marine *Streptomycetes*, exhibits modest affinity for Mcl-1 ($IC_{50} = 10$ μM) but not for Bcl-x_L. Marinopyrrole A enhances the cytotoxicity of ABT-737 by 60-fold compared to

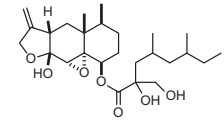
monotherapy in K562 cells.[41] Finally, tetrocacin A (**28**), isolated from *Actinomycete*, sensitizes cells overexpressing Bcl-2 and Bcl-x_L to radiation, but again evidence suggests that this activity may result from other pathways.[42]



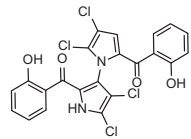
Antimycin A (**24**)



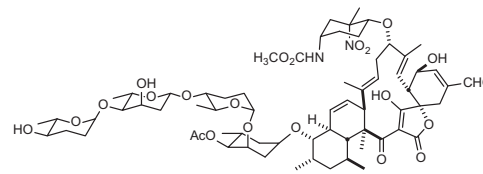
Chelerythrine (**25**)



Cryptosphaerolide (**26**)



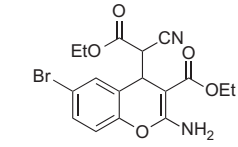
Marinopyrrole A (**27**)



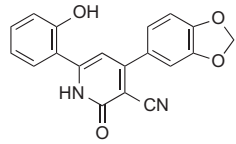
Tetrocarcin A (**28**)

2.3. *In silico* screening approaches

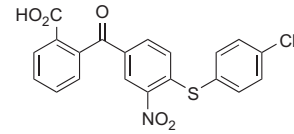
Several inhibitors with modest affinity for Bcl-2 family proteins have been discovered utilizing *in silico* screens of commercial 3D libraries. The first reported Bcl-2 inhibitor, dihydrochroman HA14-1 (**29**), was discovered through a virtual screen of the MDL/ACD-3D database and displays weak affinity for Bcl-2 ($IC_{50} = 9.0\ \mu M$) and efficacy against HL-60 cells ($EC_{90} = 50\ \mu M$). However, HA14-1 was shown to decompose to generate reactive oxygen species that may induce apoptosis.[43] A virtual screen of the Maybridge chemical library revealed BI-21C5 (**30**, $IC_{50} = 5.1\ \mu M$) and BI-21C6 (**31**, $IC_{50} = 0.5\ \mu M$) with modest affinity for Bcl-x_L.[44] BI-21C5 kills ZR-75-1 cells ($EC_{50} = 11.7\ \mu M$), while BI-21C6 is inactive. A virtual screen of the NCI-3D database led to BL-11 (**32**), with modest Bcl-x_L ($IC_{50} = 9.0\ \mu M$) and Bcl-2 ($IC_{50} = 10.4\ \mu M$) affinities.[45] However, BL-11 demonstrated similar activity in Bak/Bax-deficient cells, suggesting that activity may be off mechanism.[46] A virtual screen of the NCI-3D database revealed MNB (**33**), a Bcl-2 inhibitor ($IC_{50} = 0.7\ \mu M$) with cytotoxic activity ($EC_{65} = 5\ \mu M$) against HL-60 cells.[47] Finally, a virtual screen of the SPECS database revealed tetracycle **34**, an inhibitor with moderate Bcl-x_L ($IC_{50} = 3.4\ \mu M$), Bcl-2 ($IC_{50} = 3.1\ \mu M$), and Mcl-1 ($IC_{50} = 6.4\ \mu M$) affinities.[46] Tetracycle **34** showed dose-dependent cell killing, but reactive oxygen species were observed that may be responsible for initiating apoptosis. Compounds **29**–**34** have not been evaluated with *in vivo* tumor models.



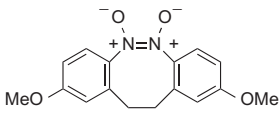
HA-14-1 (**29**)



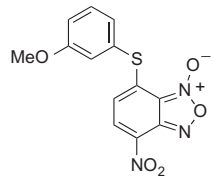
BI-21C5 (**30**)



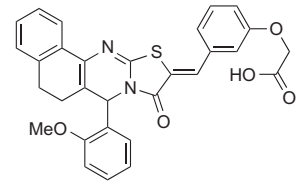
BI-21C6 (**31**)



BL-11 (**32**)



MNB (**33**)



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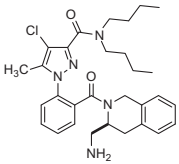
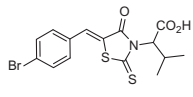
2.4. Library screening approaches

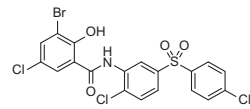
Several reports of Bcl-2 family inhibitors discovered from library high-throughput screens (HTSs) have also been published, but thus far, this approach has only led to the discovery of low-affinity inhibitors. One report describes an HTS campaign that resulted in phenylpyrazole **35**, a dual Bcl-2 ($IC_{50} = 0.16$ μM) and Bcl-x_L ($IC_{50} = 0.25$ μM) inhibitor.[48] Modest Bcl-x_L inhibitors rhodanine **36** ($K_i = 2.4$ μM) and diarylsulfone **37** ($K_i = 4.1$ μM) were discovered using an HTS of commercial compounds. However, cytotoxicity in Bak/Bax-deficient cell lines suggests that activity may be independent from Bcl-x_L binding.[49] An HTS produced compound **38** as the only hit in the screen to induce >50% inhibition of Bcl-x_L.[50] Inhibitor **38** disrupted the Bax/Bcl-x_L interaction in an immunoprecipitation assay and induced apoptosis at high concentrations ($EC_{50} \sim 0.5$ mM) in MCF7 cells.



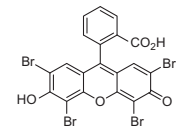
35



BH3I-1 (**36**)



BH3I-2 (**37**)

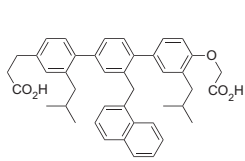


38

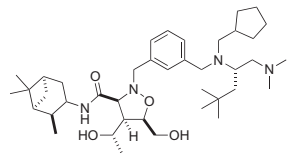
2.5. Other approaches

Efforts to mimic the α-helical BH3 domain of BH3-only proteins with a small molecule led to the discovery of terphenyl **39**, a weak inhibitor ($K_i = 114$ μM) of Bcl-x_L.[51] All attempts to improve the poor physical

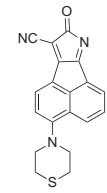
properties of this compound proved unsuccessful. A series of isooxazolidines represented by structure **40** were discovered using a diversity-oriented synthesis approach.[52] These isooxazolidines are dual Bcl-2 ($K_i < 0.001$ μM) and Bcl-x_L ($K_i < 1.0$ μM) inhibitors and demonstrate dose-dependent killing of various cell lines. An effort to design DNA intercalating agents revealed rigid chromophore S1 (**41**), a compound that reportedly lacks DNA intercalation ability but induces apoptosis in a variety of Bcl-2-sensitive cell lines. S1 was claimed to be a dual inhibitor of Bcl-2 ($IC_{50} = 0.285$ μM) and Mcl-1 ($IC_{50} = 0.035$ μM).[53] Mipralden (**42**) is a weak dual inhibitor of Bcl-2 ($K_d = 70$ μM) and Mcl-1 ($K_d = 25$ μM) that was discovered through a *de novo* structure-based approach.[54] Compounds **39**–**42** are currently in the preclinical evaluation stage.



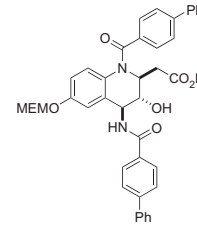
39



40



S1 (**41**)



Mipralden (**42**)

3. CONCLUSIONS

The work described in this review demonstrates that inhibition of Bcl-2 family proteins with high-affinity small molecules is possible and that Bcl-2 proteins are valid targets for clinical oncology indications. The structural diversity of chemical inhibitors suggests that a wide range of chemotypes are useful, although the physiochemical properties of these molecules may fall outside the range traditionally considered "drug-like." Development of clinical candidates with a range of selectivity profiles for the Bcl-2 family proteins will provide clarity on their role in cancer and should ultimately lead to new oncology therapies.

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