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Celecoxib and Bcl-2: emerging possibilities for anticancer drug design

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Abstract

Celecoxib is a multifaceted drug with promising anticancer properties. A number of studies have been conducted that implicate the compound in modulating the expression of Bcl-2 family members and mitochondria-mediated apoptosis. The growing data surrounding the role of celecoxib in the regulation of the mitochondrial death pathway provides a platform for ongoing debate. Studies that describe celecoxib's properties as a BH3 mimic or as a direct inhibitor of Bcl-2 are not available. The motivations for this review are: to provide the basis for the development of novel compounds that modulate Bcl-2 expression using celecoxib as a structural starting point and to encourage additional biological studies (such as binding and enzymatic assays) that would provide information regarding celecoxib's role as a Bcl-2 antagonist. The current review summarizes work that identifies the role of celecoxib in blocking the activity of Bcl-2.

Bcl-2 family of proteins

Bcl-2 protein (the use of Bcl-2 throughout this review will refer to the founding member unless otherwise indicated) is derived from the second gene identified at the translocation breakpoint in B-cell lymphoma by Tsujimoto *et al.* [1,2]. It is the founding member of the Bcl-2 family of proteins that boast more than 25 pro- and anti-apoptotic constituents (Table 1). The prosurvival members are further classified based on the presence of four highly conserved Bcl-2 homology regions, BH1–4. The pro-apoptotic members are structurally similar to the anti-apoptotic proteins and are characterized as multidomain members, typically having BH1–3, and single-domain members, which have only the BH3 motif. With the redefining of the BH4 by Kvansakul *et al.*, several pro-apoptotic members are believed to contain this domain [3].

The role of Bcl-2 in cancer cell survival

The integral role of the Bcl-2 family members in regulating cell survival has been emphasized in a number of studies. For instance, the osteoblast obtained from mice void of the anti-apoptotic protein Bcl-2 reflected an impeded growth in comparison to that of normal mice [4]. Likewise, tumors in which Bcl-2-like protein 4 and Bcl-2 killer 1 (Bax and Bak) were silenced were resistant to apoptosis resulting in mice with growth abnormalities, such

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as interdigital webs [5]. Studies involving several prostate cancer models (androgen dependent and independent), reported a reduction in the size of tumors when the levels of Bcl-2 prosurvival members were reduced [6]. Kumar and others showed that pancreatic intraepithelial neoplasm, the precursor to pancreatic cancer, is characterized by the overexpression of these members, among other molecular changes [7,8]. In chronic lymphocytic leukemia (CLL), an incurable neoplastic cancer, cell survival is also attributed to the overexpression of Bcl-2 and its anti-apoptotic members [9–11]. These findings highlight the significance of the Bcl-2 family in promoting cell survival and cancer progression. Additional characterization of the oncogenic activity of the family is attributed to its interactions at the mitochondria [12]. In the presence of the Bcl-2 prosurvival proteins, cytochrome c is not released from the mitochondria and the cell remains viable. When the oncogenes are downregulated or deactivated (neutralized), Bax-like proteins can be activated. Arguments have been presented for both the indirect activation of Bax-like proteins, as a result of their displacement from their prosurvival counterpart, and for the direct activation of cytosolic Bax-like proteins [13]. As a result of either process, the integrity of the mitochondrial membrane is not maintained triggering the release of cytochrome c, the activation of downstream caspases and the onset of cell death (Figure 1).

On the molecular level, the apoptotic process can be characterized by the interaction of Bcl-2 anti-apoptotic proteins with its pro-apoptotic members. The proteins are composed primarily of hydrophobic α -helices [14]. BH1 and BH2 domains of the anti-apoptotic members align to form a hydrophobic groove capable of accommodating the BH3 region of the pro-apoptotic members. The BH3 and BH4 domains are connected by an unstructured loop; the site of key residues (namely Ser70 in Bcl-2, Ser62 in Bcl-xL and Ser64 in Mcl-1) whose phosphorylation impacts the prosurvival and pro-apoptotic conformation of the proteins [14,15]. In their prosurvival form, Bcl-2, Bcl-xL and Mcl-1 are sequestered to the surface of the mitochondria via their carboxyl-terminal hydrophobic domain [14]. The active form is found complexed with the BH3 domain of its pro-apoptotic member (Bax BH3; Figure 2). As seen in the model, Bax BH3 sits in the hydrophobic groove on the surface of Bcl-2, Figure 2a. In the groove, Bax donates a hydrogen bond to Arg107, Arg140, Gly145 and Leu137, and accepts hydrogen bonds from Asp148. The Bax protein has dual hydrogen-bonding interactions with Arg146 and Arg110, and shares hydrophobic interactions with Phe112 and Phe153. In the grooves of Bcl-xL (Figure 2b), the Bax BH3 peptide forms dual hydrogen bonds with Arg139, and also has hydrogen-bonding interactions with Tyr101, Gln111 and Gln125. For Mcl-1 (Figure 2c), the Bax BH3 peptide hydrogen bonds with Asp256 and Arg263, and has hydrophobic interactions with His224. To initiate apoptosis, it is believed that a BH3-only protein interacts with Bcl-2 or its other anti-apoptotic counterparts to liberate the Bax-like proteins [14,16]. The free Bax-like protein then forms oligomers that facilitate the release of cytochrome c, activates downstream caspases and signals the onset of apoptosis [17]. This mechanism has been explored to understand apoptotic events in cancer cells and has been exploited in the development of chemotherapeutics.

Bcl-2 inhibitors

Antisense therapy—Early therapeutic strategies to reduce the anti-apoptotic activity of Bcl-2 have included the development of antisense oligonucleotides (ASOs). Antisense-based drugs reduce the levels of Bcl-2 by interfering with mRNA expression. Among these were Genasense® (oblimersen, 5'TCTCCCAGCGTGCGCCAT-3'), a late-stage pro-apoptotic drug that binds to Bcl-2 mRNA and prevents tumor cell viability [18]. Despite its promise towards the treatment of a number of tumors in preclinical studies, Genasense showed limited benefits in clinical trials. The success of the molecule is complicated by its unfavorable off-target activities. This was also observed in the development of the antisense

SPC2996, a 16-mer oligonucleotide. SPC2996 diminished Bcl-2 expression in several cancer cell lines. However, the drug produced less than promising results in clinical studies involving patients diagnosed with CLL [19]. ASOs such as Custirsén (OGx-11, 5'-CAGCAGCAGAGTCTTCATCAT-3') and ISIS 20408 (5'-TTGGCTTTGTGTCTTGGCG-3') demonstrated clinically significant responses in non-small-cell lung cancer patients when administered in concert with gemcitabine–cisplatin [20].

Antibiotics—Like ASOs, antibiotics have been identified that demonstrate favorable activity in various cell lines with enhanced results observed when combined with other therapeutic agents [21]. Such molecules have the ability to directly bind to the anti-apoptotic members to perturb the progression of tumorigenesis. Natural and synthetic antibiotics have been identified that impair mitochondrial function and antagonize Bcl-2 activity (Figure 3). Antimycin A3 (**1**) demonstrated low micromolar affinity for Bcl-2 ($IC_{50} = 2.0 \mu M$) [22]. Tetrocarcin-A (**2**) diminished growth in immortal human cervical cancer cells (HeLa cells) that overexpressed the oncogene, although it is often reported that the molecule functions independently of Bcl-2 to reduce **mitochondrial membrane potential** [23].

Peptidomimetics—Improved therapeutic success has been observed for BH3 **peptidomimetics** over that of the ASOs. The BH3 peptides are amphiphilic α -helices that resemble the 3D structure and function of the BH3 domain. The peptidomimetics are developed by coupling a component of the so called 'minimal death domain' (typically consisting of the BH3 residues of Bax, Bid or Bad proteins) with structural components that enhance binding to anti-apoptotic members and increase death signals (box 1) [24]. To increase cell permeability, antennapedia (Ant) or polyarginine (R8) transduction domains were added to the structure. The interaction of these peptides with the hydrophobic domain of Bcl-2 family proteins was found to be necessary for the molecules to achieve successful therapeutic outcomes. Such interactions have led to the induction of cell death in neuroblastoma and neck squamous carcinoma. In addition, the ability of BH3 peptidomimetic to promote apoptosis is believed to be dependent on the expression of caspase-9 [25]. There is an emerging generation of peptidomimetics called 'stapled BH3 peptides'. In one class of stapled peptides, the structure of the peptides contains the BID–BH3 motif with an alkenyl linker forming a semi-rigid loop across three to six of the BID residues [26]. The novel chemotherapeutics were found to neutralize the Bcl-2 protein and to be effective against T-cell leukemia in mouse models. Efforts in this area are ongoing and have involved Bid, Bim, Bad, Bax and Mcl-1 stapled compounds [27]. The efforts provide insight into the BH3 domain interactions that are necessary to reduce the prosurvival activity of Bcl-2.

Small-molecule inhibitors of Bcl-2—Beyond peptidomimetics, antibodies and antisense therapies, approaches to block the activity of Bcl-2 have involved the development of small, orally available molecular inhibitors. Small-molecule inhibitors represent a very promising area in the discovery of cytotoxic agents. The known Bcl-2 inhibitors reflect a range of templates including polyphenols and terphenyls to sulfonamides and azo compounds (Table 2) [7,28]. Several of the molecules have been designed using rational approaches aided by a molecular understanding of the Bcl-2 protein interactions. Others were derived from natural sources. While there is some debate regarding whether these agents act through direct or indirect binding to Bcl-2, several molecules have entered clinical and preclinical trials. In many cases, the efficacy of the inhibitors is enhanced when administered in combination with other chemotherapeutic agents [7,29]. This highlights a viable option for overcoming the therapeutic resistance of other existing drugs.

Some small-molecule inhibitors of Bcl-2 family members are known to have other biological targets that produce unfavorable pharmaceutical outcomes, thereby diminishing their utility. In clinical trials, some inhibitors failed to give the desired apoptotic benefit and there are noted occurrences of drug resistance for many of the molecules [7,30]. Furthermore, many of the inhibitors are innately promiscuous with multiple prosurvival members [29,31]. This makes it difficult to identify the specific interaction that characterizes their anticancer properties and complicate the process of identifying structures with improved therapeutic outcomes. Ongoing efforts to develop novel inhibitors must consider this promiscuity and should involve *in silico* methods based on the structural understanding of both the proteins and known ligands.

Celecoxib as a modulator of Bcl-2 activity in cancer cells

There is growing enthusiasm regarding derivatives of the nonsteroidal anti-inflammatory drug (NSAID) **celecoxib** (Figure 4 [14], Celebrex®) and its potential use as a Bcl-2 antagonist. Initially characterized for its cyclooxygenase-2 (COX-2) activity, the compound has been approved by the US FDA in the treatment of a number of malignancies, including breast, colon and urinary cancers. Adding to its utility, the compound was shown to inhibit growth in human endometrial, prostate and gastric carcinomas [32–34]. Not only has the compound been implicated in the inhibition of proliferation, but it has also been proven effective at preventing the development of cancer in patients with key risk factors (e.g., individuals with a previous history of smoking, colorectal polyps or high prostate-specific antigen levels) [35,36].

Despite the concern that the clinically relevant dose (400–800 mg daily) required to produce notable anticancer effects increases the potential for cardiovascular and cerebral vascular incidence, the activity of celecoxib has been exploited in the design of pyrazole-based molecules that target a number of biological mechanisms (Figure 5) [37–39]. The mechanism for the anticancer activity of celecoxib and its derivatives has been described independently and dependently of its COX-2 activity [40,41]. The promiscuity (or polypharmacology) of celecoxib and its derivatives has great benefits in that the various mechanisms produce favorable biological cially among pathways involving Bcl-2 family members. While the intentional development of molecules that hit multiple targets is an evolving idea, such pharmacological properties complicate the process of isolating an exact mechanism by which celecoxib prevents cancer development and survival. The pharmacology of celecoxib as it relates to mitochondrial membrane stability is representative of this promiscuity. There have been reports that this activity is dependent on the ability of celecoxib to modulate the levels of Bcl-2 family members [42,43]. At the same time, celecoxib has also demonstrated the ability to induce membrane instability independent of these proteins [44]. In the latter case, this most likely occurs as the molecule can penetrate the membrane causing an increase in intracellular calcium ions. The resulting hyperpolarization allows cytochrome c to be dephosphorylated and liberated from the mitochondria. The sequence of events is a hallmark of apoptosis triggered by celecoxib.

With respect to mitochondrial pathways mediated by Bcl-2 family members, the involvement of celecoxib in this pathway circles back to the COX-2 enzyme. The enzyme catalyzes the synthesis of prostaglandins, which triggers inflammation and apoptosis [45,46]. The apoptotic pathway can be driven by the destabilization of the mitochondrial membrane as a result of the dephosphorylation of Bad and other single-domain members [47]. In the presence of an NSAID such as celecoxib, prostaglandin synthesis is blocked, Bad is activated and cell death is initiated. Work surrounding the role of celecoxib in the onset of apoptosis continues to grow offering mechanisms of action with varying dependence on the concentrations of Bcl-2 and its family members. Sugimoto *et al.*

hypothesized that celecoxib would enhance adenovirus type 5 gene E1A and Bcl-2-mediated apoptosis in a COX-2-dependent pathway [48]. The results of the study showed a suppression in breast (MDA-MB-231 and MDA-MB-435) cancer cells lines that overexpressed COX-2, but did not impact the level of Bcl-2 in these cells. Additional research in this area links celecoxib to the altered concentration of Bcl-2-related protein resulting in impaired cell viability. As identified in studies of rhabdomyosarcoma, the most common soft-tissue sarcoma, celecoxib reduced the expression of Bcl-2 and other effectors of apoptosis [49]. It also impacted cell migration and clonogenic colony formation. In Jurkat T lymphoma cells, celecoxib-induced apoptosis could not be overcome by the increased presence of Bcl-2 and the presence of celecoxib did not impact Bcl-xL in these cells [50]. It appears that in these cells celecoxib-induced death signals were due to its modulation of Mcl-1 and the upregulation of Bak. Furthermore, when Bak was not present, Jurkat T lymphoma cells failed to respond to treatment by celecoxib and maintained viability [42,43]. Likewise, treatment with celecoxib was associated with the activation of Bax, decreased expression of Mcl-1, loss of the mitochondrial membrane potential and increased expression of caspase-9-dependent apoptosis in human T-cell leukemia virus type I. In a follow-up study, it was observed that the apoptotic effects were independent of Bcl-2 [51]. The study further supported that celecoxib-induced apoptosis was reliant on the presence of Mcl-1 and Noxa. It was believed that celecoxib facilitated the neutralization of Mcl-1 and Bcl-xL through the upregulation of Bax.

Despite the findings by Rudner *et al.*, evidence for celecoxib's activity against Bcl-2 was observed in hepatocellular carcinoma and gastric cancers. A reduction in cell growth and Bcl-2 expression was observed in a multidrug-resistant (MDR) form of human hepatocellular carcinoma [52]. In the MDR cells, the levels of both Bcl-xL and Bcl-2 were attenuated in the presence of 10 μ M of celecoxib [53]. This effect, however, was diminished at higher concentrations of celecoxib. These findings showed that celecoxib restored the release of cytochrome c from the mitochondria. The deactivation of anti-apoptotic Bcl-2 members has been attributed to celecoxib's ability to overcome cell survival in gastric cancers. Celecoxib has also demonstrated the ability to impact traits essential for cancer cell persistence. As observed for colon cancer cells (HT29), celecoxib induced an increase of both Bax and Bid and reduction of Bcl-2, which led to the disruption of cell adhesion [54]. In osteosarcoma cells (MG-63), celecoxib downregulated Bcl-2 in a process independent of cyclooxygenase [55]. In addition, there has been evidence that the presence of celecoxib leads to an increase in the Bax/Bcl-2 ratio in amygdala following myocardial infarction and in liver cells [56,57]. In human gastric carcinoma cells, apoptosis was facilitated by the upregulation of PUMA in the presence of celecoxib [58]. When the cells were transfected with siRNA specific for PUMA, the carcinoma gained protection against treatment with celecoxib. In light of the information showing celecoxib's ability to alter the concentration of Bcl-2 family member, data regarding the exact mechanism, by which celecoxib elevates Bax/Bcl-2 ratio, would greatly benefit current efforts. Further, many of the afore mentioned studies described celecoxib's ability to alter Bax/ Bcl-2 levels or increase the concentration of Bax without impacting Bcl-2. Contrary to this, work conducted by Chen *et al.* found that celecoxib decreased the Bcl-2 concentration in glioma cells, but Bax was not impacted [59]. Conceivably, celecoxib could modulate and potentially interact with Bcl-2. However, additional information is needed to support such activity.

Co-therapies involving celecoxib

The protective nature of Bcl-2 in the development of most cancers is linked to the therapeutic resistance of several anticancer drugs including gemcitabine, daunorubicin and cisplatin [60–62]. An improved efficacy was noted for the drugs when given in combination with other small molecules that are able to reduce the levels of Bcl-2 in the cell. Therefore,

combination therapy presents a viable option for reducing drug resistance in cancer cells. For instance, the coupling of celecoxib with established chemotherapeutics has led to improved apoptotic outcomes. In the presence of compounds such as matrine and norcantharidin (naturally derived compounds with known antiproliferative and apoptotic activity), celecoxib was able to increase the Bax/Bcl-2 ratio in cancer cells [63,64]. Similar outcomes were observed for celecoxib when combined with baicalein in a human epidermoid carcinoma cell line and with irinotecan and valproic acid (VPA) in neuroblastomas [65–67]. Adding to the benefits of combination therapy, berbamine and celecoxib impair the survival pathways in estrogen receptor-negative and -positive breast cancer cell lines. The synergy of the two drugs reduced the migration and invasiveness of the cells [68]. Moreover, the combination of MG132 and celecoxib decreased cell survival in human liver tumors by repressing *Bcl-2* gene promoters [69]. In colon and breast cancer cells, therapeutic strategies utilizing celecoxib in concert with ABT 737 (or MK886) and theaflavin, respectively, showed greater death benefits in comparison to those utilizing either compound alone [70–72]. It was found that the enhanced outcomes are achieved due to the ability of ABT 737 to deactivate the anti-apoptotic Bcl-2 family members and could also be attributed to the ability of celecoxib to neutralize Mcl-1 [50]. Celecoxib induced a dose-dependent antiproliferative effect in osteo sarcoma cells, an effect that was enhanced by the presence of the co-agent cisplatin.

Structural relevance of celecoxib towards Bcl-2 activity

Coxibs—Coxibs represent a generation of NSAIDs that stimulated much interest in the early 1990s. Along with celecoxib, the group includes rofecoxib (Vioxx®, **15**) and valdecoxib (Bextra®, **16**) (Figure 4). Despite the success of the molecules as NSAIDs, the FDA removed the molecules from the market due to unfavorable pharmacological side effects. The inclusion of the molecules in this discussion aids in the understanding the role of the central ring in the biological function.

Each of the coxibs displayed antiproliferative properties and the ability to modulate apoptosis. Valdecoxib was able to reduce the expression of Bcl-2 without altering COX-2 activity, while rofecoxib produced a COX-2-mediated increase of cytosolic Bad. Both mechanisms lead to apoptosis and reduction in tumor progression. The molecules are more potent COX-2 inhibitors than celecoxib. However, celecoxib displayed a superior potency in inducing apoptosis [41,73]. While celecoxib's mechanism of action as an anti-inflammatory agent is not solely responsible for its activity as an anticancer agent, the various routes by which the molecule induces apoptosis adds to its appeal as a chemotherapeutic [74]. As such, there is an overlap in the structure–activity requirements for celecoxib in its various mechanisms of action, including those for COX-2 binding and the induction of cytotoxicity in tumors [44,75]. Similar to the structure–activity relationship found in the proposed pharmacophore for COX-2, the benzenesulfonamide moiety is a source of both negative- and positive-electrostatic attraction in pharmacophore for PDK-1 (a target for the induction of apoptosis with implications in the Bcl-2-mediated pathway), producing hydrogen bonds with Ser160 and Ala162 in the ATP-binding site of the protein [76]. The electrostatic potential of this portion of the molecule was enhanced when the benzenesulfonamide group was replaced with an acetamide group, which will be discussed later in this review [44,76]. Likewise, the central pyrazole ring supports hydrophobic interactions in the binding site of PDK-1. The hydrophobicity of the central ring system was found to be key in inducing apoptosis in human prostate cancer cells (PC-3) [41]. In comparison with rofecoxib (**15**), which possesses a more polar central lactone ring, celecoxib had a larger apoptotic effect on malignant cells [41,73].

Other cyclooxygenase inhibitors—Other cyclooxygenase compounds to consider are NS-398 (**17**) and nimesulide (**18**) (Figure 6). Structurally, the compounds lack the tricyclic motif of celecoxib, significantly decreasing its size and lipophilicity. Nevertheless, both compounds contain a substituted benzenesulfonamide that appears to be sufficient for the compound to maintain celecoxib-like activity in both anti-inflammatory and apoptotic targets.

NS-398: Iwase *et al.* examined the capability of the selective COX-2 inhibitor, NS-398, on prostaglandin E₂ (PGE₂) release and Fas-mediated apoptosis in activated neutrophils [77]. The study revealed that NS-398 not only suppressed PGE₂ release (at 1 μ M), but also enhanced Fas-mediated apoptosis of cytokine-activated neutrophils (GM-CSF, IL-8 and IL-1 β) at higher concentrations (100 μ M). It appears that the pro-apoptotic activity of NS-398 is independent of COX-2 activity. These results are consistent with the fact that the COX-2 inhibitors celecoxib and NS-398 induce apoptosis in COX-2-negative cancer cells [78–80]. In subsequent studies, NS-398 increased radiosensitivity in esophageal cancer cells and decreased cell viability in colon and in various lung (NSCLC, SC, AC and BAC) cancer cell lines [81]. Furthermore, the compound produces apoptotic cell death in pancreatic cancer as a co-therapeutic agent with rosiglitazone [46]. The apoptotic and antiproliferative activity of the NS-398 has been attributed to its ability to increase the activation of caspase-3 [82]. In combination with EGCG (chemopreventative agent derived from green tea), NS-398 was able to modulate the activation of caspase-9 and -6 and to increase the Bax/Bcl-2 ratio in prostate cancer cells [83].

Nimesulide: Nimesulide is a selective COX-2 inhibitor. Unlike celecoxib, nimesulide's ability to act as an antiproliferative and apoptotic agent is largely related to its ability to inhibit PGE₂ in the COX-2-mediated pathway [84]. Similar to celecoxib, it is able to impact a wide range of cancers and its activity may be dependent on the presence of a specific carcinogen or a co-agent. In mice with *Helicobacter pylori*-associated gastric cancers, there was a significant decrease in the levels of Bcl-2 in the presence of nimesulide [84]. Combinatorial chemistry has facilitated the development of a library of nimesulide-derived molecules with promising antiproliferative properties [85].

Celecoxib analogs—Typically, celecoxib analogs (or celecoxib-related compounds) are tricyclic systems with a central heteroaromatic ring attached to adjacent phenyl groups (Figure 7). At least one of the phenyl rings bears a polar/hydrogen-bonding substituent. Potentially, the aromatic component contributes to the activity by inducing π , steric and lipophilic interactions in the binding site of a biological target. This component is most significant and could potentially be modified to enhance hydrophobic interactions in the BH3 groove of Bcl-2 and other anti-apoptotic targets. Studies involving compounds (**20,21**) developed at Ohio State University (OSU), USA, demonstrated that increasing the size of the substituent is sufficient to remove the COX-1 and -2 activities and enhance the anticancer activity. Such celecoxib analogs with high anticancer activity and negligible COX-1 and -2 activities are referred to as **noncoxib** derivatives. Another area of hydrophobic contact and a component key to apoptosis is the trifluoromethyl group. Overall, celecoxib analogs are characterized by their size to lipophilicity ratio, rigidity and spatial arrangement of the substituted phenyl groups. The molecules in Figure 7 illustrate the potential for developing celecoxib-based molecules with enhanced apoptotic activity and highlight the potential for such compounds to have implication in the regulation Bcl-2 expression.

SC-58125 (**19**) is a fluorinated precursor of celecoxib. The molecule has been shown to modulate the levels of Bcl-2 in human colon cancer cells [86]. However, the inhibition was

overcome by an increased expression of PGE₂. The molecule has no impact on Bcl-xL and Bax. Studies recently showed that the molecule was more effective than celecoxib at reducing growth in human bladder cancer, but was unable to impact Bax/Bcl-2 expression [87]. However, earlier studies showed the molecule was poorly metabolized, a fate attributed to the presence of the fluoro group. The metabolic issue was overcome by replacing the fluoro group with a methyl group to produce the widely studied celecoxib molecule. The compound was useful for establishing the necessity of the toluene substituent in the pyrazole derivatives.

OSU compounds—In an effort to better understand the antiproliferative activity of celecoxib, several noncoxib celecoxib analogs were generated at OSU [44]. The first of these compounds were evaluated against premalignant and malignant human oral epithelial cells. Bearing various biphenyl and fused aromatic ring systems, the initial analogs demonstrated the utility of increasing the size of the celecoxib template. The molecules were found to inhibit cell growth in micromolar concentrations through the disruption of mitochondrial membrane potential. Of the analogs analyzed, the trichlorobiphenyl-substituted compound (**20**) was the most effective at inhibiting cell growth in both cell lines. In addition, the sulfonamide analogs were slightly more potent than their carboxylamide counterparts.

Subsequent work by the group led to the discovery of OSU03012 (**21**) and OSU03013 (**22**). OSU03012 is an orally bioavailable therapeutic agent that has potent *in vitro* activity against primary CLL cells [88]. Unlike celecoxib, the cytotoxicity of OSU03012 was not surmounted by the overexpression of Bcl-2 and was mediated through caspases-independent pathways. As previously described herein, celecoxib can activate the caspase-9 apoptotic pathway; consequently, celecoxib-induced apoptosis can be overcome by a caspase-9 inhibitor. Further delineating the biological activity of the two pyrazole-based molecules, Johnson *et al.* reported that celecoxib-induced apoptosis was prevented by Bcl-2 overexpression, while OSU03012 induced apoptosis independent of Bcl-2 concentration in the 697 lymphoblastic cell line [88].

The structural differences between celecoxib and OSU03012 have proven to have a significant impact on the activity of the molecules. Replacing the *N*-toluene and sulfonamide groups of celecoxib with an *N*-anthracene and acetamide groups, respectively, in OSU03012 enhanced the antiproliferative and apoptotic activity of the molecule over that of celecoxib. Similar outcomes were observed for more than 40 celecoxib-based OSU derivatives [89]. Of these, it was found that the *N*-anthracene derivatives, with varying polar replacements for the acetamide group (OSU03013), displayed slightly higher antiproliferative effects than their acetamide counterpart. Since these initial findings, the molecule has been shown to potentially act through pathways involving regulation of the mitochondrial membrane permeability and has been shown to be effective against a number of cancers, including non-small cell lung cancer [90]. In addition, micromolar concentrations of both OSU03012 and OSU03013 were found to inhibit growth in ovarian, renal melanoma, prostate and breast cancers [90]. By far, the OSU compounds are the most-studied noncoxib derivatives of celecoxib.

2,5-dimethyl celecoxib—With the addition of a second methyl group, dimethyl celecoxib (DMC) (**23**) is a noncoxib derivative that is slightly more lipophilic than celecoxib. The modified compound is able to mimic the activity of celecoxib in most tumor cells. In glioblastoma cells implanted in nude mice, DMC performed better than celecoxib towards the reduction of tumor volume [91]. However, in recent studies DMC and celecoxib were able to induce the activation of caspase-9 and apoptosis through a mitochondrial-mediated pathway in gastric cancers [92]. The combination prevents the formation of the

Bax–Bcl-2 complex leading to the permeability of the mitochondrial membrane. In studies that fail to implicate Bcl-2 in the apoptotic activity of this and other celecoxib analogs, there is evidence that the compound disrupts the mitochondrial membrane potential. Studies by Zhu *et al.* showed that replacing the sulfonamide with a carboxylamide (**24**) did little to improve the cytotoxic effects of the compounds, although it improved the water solubility [93]. The molecule was, however, twice as effective as celecoxib in inhibiting growth in oral epithelial premalignant and malignant cells. The compound was not able to activate caspase-3, -8 or -9.

Selenocoxib-1—Selenium-containing compounds have received considerable attention in light of their vast nutritional and biological benefits. For instance, selenoproteins and novel organoselenium compounds have noted antioxidant and anti-tumor properties. Adding to the growing number of selenium-based compounds, Desai and colleagues developed a selenium-based celecoxib derivative (**25**) [94]. The molecule inhibits growth in human metastatic prostate cancer cells. Although the trifluoromethyl group has been replaced with a slightly more lipophilic group, the size of the molecule is relatively similar to celecoxib. The structure was able to reduce the expression of Bcl-2 and enhance the pro-apoptotic ability of the compound comparable to that of celecoxib.

TT101 & TT201—In addition to cancer-related interest, the apoptotic properties of celecoxib have been exploited to address rheumatoid arthritis. Uncontrolled growth in synovial fibroblasts has been attributed to the progression of the disease which makes modulating apoptosis a feasible therapeutic option. To this end, the celecoxib analogs TT101 (**26**) and TT201 (**27**) were developed. TT101, in which an ethyl amine group was added to the sulfonamide substituent, was able to produce the desired apoptotic effect with a fivefold greater potency than celecoxib [95]. The molecule did not impact Bcl-2 expression but was able to induce BID-mediated apoptosis. The data illustrate the potential for incorporating an alkylamine into the molecule and for modifying the polar group of the molecule to gain enhanced apoptotic results. Modifications of celecoxib to give TT201, however, produced less than promising apoptotic results in the synovial fibroblasts. This is believed to further support the need for the nonpolar tolyl on the celecoxib compound.

FR122047—FR122047 (**28**) is a selective COX-1 inhibitor. The compound's ability to suppress cell growth in MCF-7 breast cancer cells was recently highlighted in work by Jeong *et al.* [96]. Apoptosis in the presence of this compound was initiated by an increase in the Bax/Bcl-2 ratio and a release of cytochrome c. The work also indicates that the molecule prevents the cleavage of the effector caspase-7 and PARP in the caspase-3-deficient cells. It appears that in the caspase-3-deficient MCF-7 cells, the activity of the compound is dependent on its impact on caspase-8 activity. Structurally, the compound contains a central thiazole ring that is flanked by two *para*-methoxy phenyl groups. The tricyclic motif of the compound resembles the spatial arrangement of celecoxib. The third substituent is a piperazine-substituted carboxyl group. Due to its activity, the structural elements of the compound should be considered in the design of future anticancer agents.

Alternative celecoxib-like structures—In light of the established molecular understanding of celecoxib activity, the structure-activity relationship of small tricyclic molecules with lipophilic and aromatic character comparable to celecoxib provides further insight into biological significance of similar templates (Figure 8). In particular, they define the potential to interchange five-membered aryl rings, using bioisosteres and isosteres, while maintaining targeted or enhanced biological outcomes. Triazole rings, as in compound (**29**), are known to activate caspase-3 and downregulate Bcl-2 in Jurkat cells [97]. The derivative of the South African Bushwillow tree extract, Combretastatin A-4, contains a thiazole ring,

compound **30**. After 48 h, HeLa cell treated with the compound showed a significantly increased Bax/Bcl-2 ratio with a resulting reduction in growth. In the same cell line, there was a dose-dependent upregulation of caspase-3 in the presence of the molecule. Both the triazole- and the thiazole-based molecules contain trimethoxy-substituted benzene groups, which could be incorporated into the celecoxib template to produce a biologically interesting derivative. Nutlin-3a (**31**) is a tetracyclic molecule that is capable of downregulating anti-apoptotic (namely Bcl-2 and Bcl-xL) and upregulating pro-apoptotic (namely Bax and Puma) members of the Bcl-2 family [98]. The molecule illustrates how removing rigidity and destroying the aromaticity of the central ring could lead to a molecule with improved biological outcomes. The molecule also contains an alkoxy substituted benzene group, the utility of which should be further explored. The anti-cancer activity (primarily in breast and prostate cancer cells) of the molecules **32** and **33** has been reported [99,100]. However, their activity in the Bcl-2 family-mediated pathway has not been reported. Nevertheless, the molecules provide additional insight into structural modification that maintain or improve the anticancer activity of celecoxib-like molecules.

Future perspective

The information summarized herein demonstrates that celecoxib is able to reduce cell proliferation and induce apoptosis by modulating elements of the Bcl-2 pathway. The activity of the COX-2 inhibitor in this pathway has consequences in a number of cancers. Both direct and indirect inhibition of mitochondrial membrane stability has been implicated in the apoptotic nature of celecoxib. Further, there are encouraging results regarding the use of celecoxib in combination therapy to overcome drug resistance and increase radiosensitivity in some cancers. Despite the studies providing data from protein expression and membrane potential assays, there is no binding affinity data available to definitively support how (or if) celecoxib disrupts protein–protein interaction within the Bcl-2 family. This is a major challenge. In the future, enzymatic and binding affinity assays that quantify the degree to which celecoxib or its derivatives interacts directly with Bcl-2 will greatly aid progress in this area.

Structural considerations for the biological activity of celecoxib can be leveraged from what is currently known about its ability to bind to known targets (namely PDK-1 and COX-2) with high affinity. The binding interactions of celecoxib have been attributed to three structural components:

- An *N*-aryl group with an electronic component to facilitate hydrogen bonding;
- A region of hydrophobicity;
- A substituent with aromatic character.

For the OSU analogs and other celecoxib derivatives, the most favorable apoptotic inhibition was observed for nonpolar aryl substituents. This suggests that increasing the hydrophobic or pi character may lead to an improved therapeutic outcome.

To better understand the potential of celecoxib to interact with the Bcl-2 proteins, docking models were created to compare potential binding interactions. Protein structural data exist showing Bcl-2 in complex with an acylsulfonamide derivative (not shown) and Bcl-xL in complex with ABT-737 (PDB codes: 202F and 2YXJ). The ligands are known to disrupt protein–protein interactions within the Bcl-2 family and fit well in the hydrophobic groove of the proteins between α -helices 3 and 4. The piperazine ring of ABT-737 resides in a portion of the protein directed away from the groove. The ligand is anchored in the pocket through hydrogen bonds formed with Gly142 (in Bcl-2) and Gly138 (in Bcl-xL). A flexible alignment of celecoxib and ABT-737 was generated to model the interactions that may lead

to their affinity for the protein (Figure 9). The superimposed conformation of each molecule was optimized in the binding site. The model assumes that celecoxib would displace Bak and reside in a binding pocket typical of known Bcl-2 inhibitors. In complex with Bcl-2, the sulfonamide group of celecoxib acts as a hydrogen bond acceptor from Gly142 (Figure 9b). Additional interactions include hydrophobic contact with Gly142 and Trp 141. Overall, the molecule fits tightly within this pocket as evident by the contact map. The binding interactions are similar to what was observed for ABT-737 in the Bcl-2 hydrophobic groove (Figure 9a). In Bcl-xL, it is shown that both molecules are able to form hydrogen bonds with Gly138 (Figure 10). Unlike ABT-737, celecoxib does not produce hydrophobic interactions within the protein's binding pocket (Figure 10b). The models propose the relative orientation of the molecules when bound to Bcl-2 and Bcl-xL. The information aids in understanding the potential molecular alterations that could lead to enhanced binding and to the design of design molecules with increased affinity for the proteins.

Currently, there are no studies describing the use of celecoxib as a template to develop BH3 mimics. The proposed model in Figures 9 & 10 could be used as a pharmacophore to facilitate such efforts. A database of known celecoxib analogs could be screened against the pharmacophore to identify a molecular starting point for novel designs. A more beneficial approach would involve using the model as 'fuzzy pharmacophore' to dictate where the structure can be enlarged or modified. In a fuzzy pharmacophore, the entire binding site is considered so as to take advantage of near or potential interactions that could occur between the ligand and protein to facilitate binding. In addition, modification can be aided by fragment-based approaches utilizing the known 3D structure of the protein and of its high-affinity ligands. Fragment-based design is an excellent approach for enhancing the ability of a structural template to fit a desired molecular target. Cheminformatics tools that feature lead optimization (lead design and virtual screening) can be utilized to aid such efforts. The approach can be separated into three methods:

Scaffold replacement;

Ligand growing;

Fragment linking.

For scaffold replacement, the core of a selected lead molecule (in this case celecoxib) is removed. Various software (including Recore, ClassPharmer, BROOD, Caveat, LigBuilder, CombiGlide, Pro_Ligand and MOE) can be utilized to search for suitable replacements for the core resulting in the generation of a database of new molecules. As it relates to this review, the central ring of celecoxib could be replaced (Figure 11). Alternatively, the central core can be maintained and substituents or groups can be altered in a process broadly known as ligand growing. A selected lead molecule (in this case, celecoxib) can be docked into a binding site allowing areas of potential structure modification to be identified. As illustrated in Figure 12, one of celecoxib's aryl groups can be replaced or simply enlarged to create a new compound. With fragment linking, disconnected structural fragments that produce high affinity and low energy binding in a target are maintained. Most often, such structural fragments are identified through x-ray crystal and NMR data. The fragments can originate from the same molecule or from different active compounds. Again, using computational tools (CoLibri with FlexNova, SPROUT, DLD, LUDI and some previously mentioned), various scaffolds of appropriate size and shape can be screened to connect the fragments. The various structures presented in this review can be used to guide fragment selection and placement in the pharmacophore. For example, structural components from celecoxib and ABT-737 can be utilized as illustrated in Figure 13. One advantage of the available chem-informatic tools is the ability to analyze the appropriateness of the resulting compounds *in silico*.

From a biochemical standpoint, polypharmacology should be fully exploited. Identifying a molecule that is capable of engaging both protein partners is an emerging approach in the developing inhibitors that disrupt protein–protein interactions. This approach can be exploited to simultaneously neutralize multiple Bcl-2 anti-apoptotic proteins. In the pathway, BH3 only peptides are able to displace Bax-like proteins from the binding site of the prosurvival member. This action has been identified in a number of apoptotic cells. To enhance the deactivation of anti-apoptotic Bcl-2 members, a bifunctional molecule could be utilized. Such a molecule would serve the dual purpose of displacing the Bax-like members while engaging and deactivating two anti-apoptotic proteins simultaneously. Although the native complex involving Bcl-2 and its BH3 only members would not form, the presence of the bifunctional molecule would lead to the same biological outcome (displacement and activation of Bax and release of cytochrome c). A strategy for producing a bifunctional small molecule would be to link two molecules having the desired pharmacology. A molecule that has high affinity for targeted pro-apoptotic members and another with high affinity for Bcl-2 could be connected using an inert flexible linker to create a novel compound.

The production of an anticancer agent that directly or indirectly modulates Bcl-2 utilizing celecoxib as a molecular template might be a daunting task. However, based on the multifaceted pharmacological actions of celecoxib and utilizing the strategies described here, there is a high probability for accomplishing this task and generating promising therapeutic agents.

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Key Terms

Bcl-2	Protein derived from B-cell lymphoma. There are more than 25 Bcl-2 related proteins organized into anti- and pro-apoptotic members.
Mitochondrial membrane potential	The electronic state that governs the permeability of the mitochondrial membrane. Healthy membrane potential = 80–140 mV.
Peptidomimetics	Helical structures that mimic the binding of target proteins and often contain an amino acid sequence similar to the target.
Celecoxib	Tricyclic molecule that has demonstrated selective activity for COX-2 over COX-1 and activity in various cancers.
Noncoxibs	Compounds structurally similar to celecoxib that possess no cyclooxygenase activity.

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Executive summary

Bcl-2 family of proteins

The Bcl-2 family of proteins consists of more than 25 pro- and anti-apoptotic members.

The role of Bcl-2 in cancer cell survival

A number of studies have been summarized, which emphasize the integral role of the Bcl-2 family members in promoting cell survival and cancer progression.

Bcl-2 inhibitors

Molecules that are currently known to inhibit Bcl-2 anti-apoptotic family members represent a range of structural classes and are often promiscuous with multiple members. They act by direct interaction with the anti-apoptotic members and by modulating the expression of pro- and anti-apoptotic members.

Celecoxib as a modulator of Bcl-2 activity in cancer cells

Celecoxib is a selective inhibitor of cyclooxygenase-2, which exhibits an amazing range of biological activities dependent and independent of cyclooxygenase-2 activity. It affects apoptotic signaling in several different systems at multiple levels, including elements of the Bcl-2 family-mediated pathway.

Co-therapies involving celecoxib

The protective nature of Bcl-2 in the development of most cancers is linked to the therapeutic resistance of several anticancer drugs including gemcitabine, daunorubicin, and cisplatin. In addition, a greater death benefit was shown for celecoxib in combination therapy with Baicalein, irinotecan, MG132, and ABT 737.

Structural relevance of celecoxib towards Bcl-2 activity

Structural considerations, focusing mainly on small tricyclic molecules with lipophilic and aromatic character comparable to celecoxib for the biological activity of celecoxib, have been summarized based on what is currently known about the ability of the molecule to bind to known targets and impact the survival of various tumors.

Future perspective

Consistent with pharmacophore of celecoxib, future analogs should incorporate tricyclic moieties with lipophilic and aromatic character comparable to that of celecoxib. Such compounds could be used to provide further insight into biological significance of the structures in the Bcl-2 family-mediated pathway. Future studies should probe the direct binding and inhibitor effects of the molecules.

Box 1**Box 1**

Example antennapedia and polyarginine antisense.

Ant[†]-Bax[‡] RQIKIWFQNRRMKWKK[‡]STKKLSECLKRIGDELDSNM[‡]
 Ant[†]-BaxE[‡] RQIKIWFQNRRMKWKK[‡]STKKLSECEKRIGDELDSNM[‡]
 Ant[†]-Bak[‡] RQIKIWFQNRRMKWKK[‡]MGQVGRQLAIIGDDINRRY[‡]
 Ant[†]-Bak[‡] RQIKIWFQNRRMKWKK[‡]MNLWAAQRYGRELRRMSDEFVD[‡]
 R8[§]-Bax[‡] RRRRRRRRGC[§]STKKLSECLKRIGDELDSNM[‡]
 R8[§]-BaxE[‡] RRRRRRRRGC[§]STKKLSECEKRIGDELDSNM[‡]
 R8[§]-Bak[‡] RRRRRRRRGC[§]MGQVGRQLAIIGDDINRRY[‡]
 R8[§]-Bak[‡] RRRRRRRRGC[§]MNLWAAQRYGRELRRMSDEFVD[‡]

[†]The antennapedia group.

[‡]BH3 peptide.

[§]Polyarginine.

Ant: Antennapedia; R8: Polyarginine.

Figure 1. Intrinsic cell death pathway that occurs as a result of Bax-like proteins mediated damage to the mitochondrial membrane.

Figure 2. The BH3 domain of Bax is represented as a green ball and stick formula

The residues of the anti-apoptotic protein that interacts with the Bax BH3 domain are in gray stick formula and the α -helices of the protein are shown as a red ribbon structure. The transparent surface highlights the hydrophobic binding groove showing variations in the electrostatic potential from positive (blue) to negative (red). The model was created using MOE and default parameters. The protein was protonated in 3D and original confirmation of the complex as defined in the PDB file were maintained. (A) Bcl-2 in complex with Bax BH3, PDB: 2XA0. (B) Bcl-xL with Bax BH3, PDB: 3PL7. (C) Mcl-1 in complex with Bax BH3, PDB: 3PK1.

Figure 3.
Anti-Bcl-2 antibiotics.

Figure 4.
Coxibs.

Figure 5. Polypharmacology of celecoxib influencing cancer cell survival

Upregulated or increased levels of targets = ↑; downregulated or decreased levels of targets = ↓.

Figure 6.
Noncoxib cyclooxygenase inhibitors.

Figure 7.
Celecoxib analogs.

Figure 8.
Celecoxib-like molecules.

Figure 9. Bcl-2 in complex with celecoxib and ABT-737 (PDB: 2O2F)

The interactions can be interpreted using the legend provided. For the interaction maps, the fit of the molecule inside the groove is highlighted by the gray dotted line. The portions of the molecules that reside close to this dotted gray line indicate a tight fit. **(A)** The proposed binding mode as a ligand-interaction map illustrating the residues of Bcl-2 that interact with the ABT-737. **(B)** The proposed binding mode as a ligand-interaction map illustrating the residues of Bcl-2 that interact with the celecoxib. **(C)** The general fit of the overlaid structure in the hydrophobic groove of Bcl-2. The 3D structure of Bcl-2 (red ribbon structure) in complex with an overlay of celecoxib (orange ball and stick formula) and ABT-737 (aqua ball and stick formula). The transparent surface highlights the hydrophobic binding groove showing electrostatic variations from positive (blue) to negative (red). The model was created using MOE and default parameters. The protein was protonated in 3D and original orientation of the complex as defined in the PDB file were maintained. Celecoxib and ABT-737 was docked with respect to the original acylsulfonamide derivative (not shown) of the PDB file and the best conformation obtained was energy minimized within the binding site.

Figure 10. Bcl-xL in complex with celecoxib and ABT-737 (PDB: 2YXJ)

The interactions can be interpreted using the legend provided. For the interaction maps, the fit of the molecule inside the groove is highlighted by the gray dotted line. The portions of the molecules that reside close to this dotted gray line indicate a tight fit. **(A)** The proposed binding mode as a ligand-interaction map illustrating the residues of Bcl-xL that interact with the ABT-737. **(B)** The proposed binding mode as a ligand-interaction map illustrating the residues of Bcl-xL that interact with the celecoxib. **(C)** The general fit of the overlaid structure in the hydrophobic groove of Bcl-xL. The 3D structure of Bcl-xL (red ribbon structure) in complex with an overlay of celecoxib (orange ball and stick formula) and ABT-737 (aqua ball and stick formula). The transparent surface highlights the hydrophobic binding groove showing electrostatic variations from positive (blue) to negative (red). The model was created using MOE and default parameters. The protein was protonated in 3D and original orientation of the complex as defined in the PDB file where maintained. The original conformation as found in the PDB file was maintained for ABT-737. Celecoxib was docked with respect to the ABT-737 and the best conformation obtained was energy minimized within the binding site.

Figure 11. Scaffold replace

(A) The central or core element of a molecule can be removed and replaced with a suitable structural unit, bioisosteres or isostere. (B) The central or core element of celecoxib (docked in the binding site of Bcl-2, gray bond line) is highlighted in green. Conceivably, it can be replaced with a cyclic structure of similar flexibility and electronic character.

Figure 12. Ligand growing

(A) Groups can be replaced on the lead molecule to generate new structures. (B) One of the aryl groups of celecoxib has been removed (indicated by the green arrow and pink dot). The chemical make-up of the Bcl-2 protein (gray bond line) in this area will guide the replacement of this group.

Figure 13. Fragment linking

(A) Active structural fragments from various sources can be linked using a novel central core. (B) A portion of celecoxib (orange) and ABT-737 (aqua) have been retained. The fragments can be linked using suitable structural units dictated by the structure of the Bcl-2 site (gray bond line).

Table 1

A list of the most noted Bcl-2 family members.

Role	Abbreviation	Synonyms	Full names
Anti-apoptotic members	Bcl-2		B-cell lymphoma 2
	Bcl-xL	Bcl-2L, Bcl-2L1, Bcl-X, Bcl-XS	B-cell lymphoma extra large; Bcl-2-like 1 protein
	Mcl-1		Myeloid cell leukemia
	Bcl-2A1	A1, ACC-1, ACC-2, Bcl-2L5, BFL1, GRS	Bcl-2-related protein
	Bcl2L2	Bcl-W, HBPA1, KIAA0271	Bcl-2-like protein 2; Hemopoietic specific early response protein
Pro-apoptotic members and multidomain (also known as the Bax-like members)	Bcl-2L10	Bcl-B, Boo, DIVA	Bcl2-like 10 (apoptosis facilitator)
	Bax	Bcl-2L4	Bcl-2-like protein 4; Bcl-2-like associated X protein
	Bak		Bcl-2 antagonist/killer 1
	Bok	Bcl-2L9, BokL, MGC4631, MTD	Bcl-2-related ovarian killer; Matador
	Bad	BBC2, Bcl-2L8	Bcl-2 antagonist of cell death
Pro-apoptotic members and single domain	Bid		Bcl-2-interacting domain death agonist
	Bim	Bcl-2L11, Bod, BIML, BIMEL	Bcl-2-like 11 (apoptosis Facilitator)
	Bik		Bcl-2-interacting killer
	Puma	BBC3, JFY1	p53 upregulated modulator of apoptosis; Bcl-2-binding component 3
	Bmf	FLJ00065	Bcl-2-modifying factor

Table 2

Structure of known inhibitors of Bcl-2, Bcl-xL and Mcl-1.

Agent	Target proteins	Pre-clinical and clinical trials ^{§,§}
Gossypol 3	Bcl-2, Bcl-xL, Bcl-w and Mcl-1	Sponsor: National Cancer Institute (USA) Condition: brain and CNS tumors (Phase I) Sponsor: Ascenta Therapeutics Condition: prostate and small-cell lung, Phase I/II; non-small cell lung, lymphoma (Phase II) Sponsor: Mayo Clinic Condition: lung (Phase II)
(-)-epigallocatechin gallate 4	Bcl-2 and Bcl-xL	Sponsor: University of Arizona [¶] (USA) Condition: prostate, Phase I; leukemia (Phase II) Sponsor: MD Anderson Cancer Center [‡] (USA) Condition: breast (Phase I)
Theaflavin 5	Bcl-2 and Bcl-xL
6	Bcl-xL	Sponsor: Harvard University (USA) Preclinical
BH3I-1 7	Bcl-xL	Sponsor: Harvard University (USA) Preclinical
Obatoclax mesylate 8	Bcl-2, Bcl-xL, Bcl-w and Mcl-1	Sponsor: Gemin X and Cephalon Condition: lung, leukemia, lymphoma, unspecified solid tumors (Phase I/II); myelofibrosis (Phase II); hematological malignancies (Phase I)

Agent	Target proteins	Pre-clinical and clinical trials ^{§,§}
Chelerythrine 9	Bcl-2	Sponsor: Ricerca Biosciences Preclinical
NSC 365398 10
HA14-1 11	Bcl-2
12	Bcl-2, Bcl-xL	Sponsor: Novartis Pharmaceuticals Condition: multiple myeloma (Phase III) Sponsor: medac GmbH Condition: leukemia (Phase I/II) Sponsor: Centre Francois Baclesse Condition: ovarian (Phase II)
13	Bcl-xL	Sponsor: Harvard University Preclinical

[§]The condition gives a general description of the cancers or cancer-related diseases involved in the study and is not an exhaustive list.

[§]The phase of the trial is listed in parenthesis following the related condition.

[§]In collaboration with the US National Cancer Institute.