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Review Article

Apoptosis in cancer: from pathogenesis to discovery of advanced selective Bcl-2 family Inhibitors

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ABSTRACT

Cancer is a genetic disease characterized by two features: unregulated cell growth and tissue invasion (metastasis). It can be viewed as the result of a succession of genetic changes during which a normal cell is transformed into a malignant one. Evasion of cell death, apoptosis, is one of the essential changes in a cell that cause this malignant transformation. Hence, reduced apoptosis or its resistance plays a vital role in carcinogenesis. The Bcl-2 family of proteins regulates the mitochondrial apoptotic pathway. Disease states arise upon deregulation of the Bcl-2 family of proteins, where cell death is either promoted or evaded; one of the most common tactic cancer cells utilize to promote survival is anti-apoptotic protein overexpression. Specifically, Bcl-2 overexpression has been shown to be a major chemoresistance factor in a number of human cancers, and for this reason, Bcl-2 targeting is a pharmacologic priority in the quest to reactivate cell death for therapeutic benefit in cancer.

Keywords: *Apoptosis; Bcl-2; Extrinsic pathway; Intrinsic pathway; Caspase; BH3.*

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1. INTRODUCTION

Cancer is a genetic disease characterized by two main features: unregulated cell growth and tissue invasion/metastasis. The malignant phenotype requires mutations in several genes that regulate cell proliferation, motility, survival, DNA repair, invasion, and angiogenesis. The normal cell has protective mechanisms that can repair any damage occurs during DNA synthesis or in response to environmental mutagens which are usually abnormal in cancer cells. Too much damage to a normal cell activates a suicide pathway to prevent the damage of the organ. Such a pathway is usually altered in cancer cells, leading to the survival of the damaged cells that normally die. The novel phenotypic characteristics include those that facilitate invasion and metastasis **[1]**.

2. Apoptosis

The term apoptosis comes from Greek words and refers to the falling of leaves from trees in autumn. It is used to describe the situation in which a cell actively pursues a course toward death upon receiving certain stimuli **[2]**. Being a crucial process, apoptosis is important in both physiological and pathological conditions (**Table 1**) **[3, 4]**.

Table 2: Conditions involving apoptosis

Physiological Conditions
Programmed cell destruction in embryonic development for the purpose of sculpting of tissue
Physiologic involution such as shedding of the endometrium, regression of the lactating breast
Normal destruction of cells accompanied by replacement proliferation as in the gut epithelium
lnvolution of the thymus in early age
Pathological Conditions
Anticancer drug induced cell death in tumors
Progressive cell death and depletion of CD4+ cells in AIDs
Some forms of virus-induced cell death, such as hepatitis B or C
Cell death due to injurious agents like radiation, hypoxia, and mild thermal injury
Cell death in degenerative diseases such as Alzheimer's disease
Cell death that occurs in heart diseases such as myocardial infarction

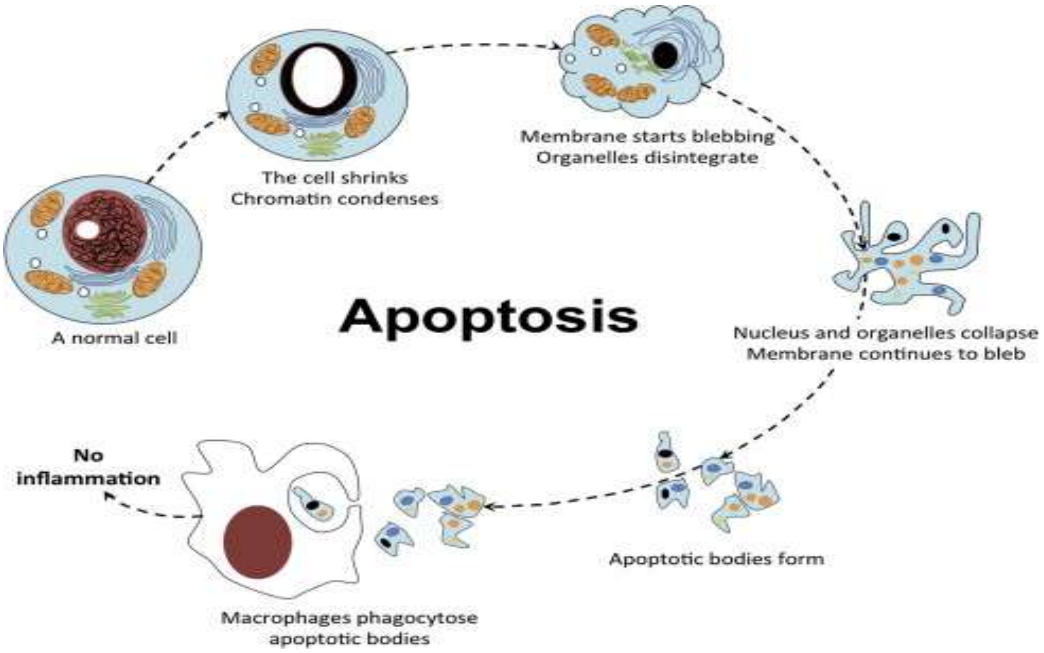


Fig. 1: Stages of apoptotic cell death **[5]**

The different stages of apoptotic cell death start by cellular shrinkage and chromatin condensation, concomitant with the formation of membrane blebs. Organelles, nucleus fragment, and the blebs begin formation of apoptotic bodies, which are eventually engulfed by macrophages or neighboring cells by endocytosis/phagocytosis. The lack of release of cellular components to the extracellular results in absence of inflammation **(Fig. 1) [5]**.

2.1. Biochemical changes in apoptosis

In apoptosis, three types of biochemical changes are observed 1) activation of caspases, 2) breakdown of DNA and protein, and 3) membrane changes and recognition by phagocytic cells **[6]**. Early in apoptosis, there is an expression of phosphatidylserine (PS) in the outer layers of the cell membrane, which has been flipped out from the inner layers. This allows early recognition of dead cells by macrophages, resulting in phagocytosis without the release of proinflammatory cellular components **[7]**. This is followed by a characteristic breakdown of DNA into large 50 to 300 kilobase pieces **[8]**. Later, there is internucleosomal cleavage of DNA into oligonucleosome in multiples of 180 to 200 base pairs by endonucleases. Another specific feature of apoptosis is the activation of a group of enzymes belonging to the cysteine protease family named caspases. The "c" of "caspase" refers to a cysteine protease, while the "aspase" refers to the enzyme's unique property to cleave after aspartic acid residues **[8]**. Activated caspases cleave many vital cellular proteins and break up the nuclear scaffold and cytoskeleton. They also activate DNAase, which further degrade nuclear DNA **[9]**.

2.2. Mechanisms of apoptosis

Understanding the mechanisms of apoptosis is crucial and helps in the understanding of the pathogenesis of conditions because of disordered apoptosis. This, in turn, may help in the development of drugs that target certain apoptotic pathways. Caspases are central to the mechanism of apoptosis as they are both the initiators and executioners. There are three pathways by which caspases can be activated. The two initiation pathways are the intrinsic and extrinsic pathways of apoptosis **(Fig. 2) [10]**. Both pathways lead to a common pathway or the execution phase of apoptosis.

2.1.1. The extrinsic death receptor pathway

Begins when death ligands bind to a death receptor. These death receptors have an intracellular death domain that recruits adaptor proteins such as TNF receptor-associated death domain (TRADD) and Fas-associated death domain (FADD), as well as cysteine proteases like caspase 8 **[11]**. Binding of the death ligand to the death receptor results in the formation of a binding site for an adaptor protein and the whole ligand-receptor-adaptor protein complex is known as the death-inducing signaling complex (DISC). DISC then initiates the assembly and activation of pro-caspase 8. The activated form of the enzyme, caspase 8 is an initiator caspase, which initiates apoptosis by cleaving other executioner caspases **[12]**.

2.1.2. The intrinsic mitochondrial pathway

As its name implies, it is initiated inside the cell and triggered by internal stimuli such as irreparable of cytosolic Ca^{2+} and severe oxidative stress **[12]**. As a result, increased mitochondrial permeability and the release of pro-apoptotic molecules such as cytochrome-c into the cytoplasm take place **[13]**. Other apoptotic factors that are released from the mitochondrial intermembrane space into the cytoplasm include apoptosis-inducing factor (AIF), a second mitochondria-derived activator of caspase (Smac), direct IAP Binding protein with low pI

(DIABLO) and Omi/high-temperature requirement protein A (HtrA2). The cytoplasmic release of cytochrome-c activates caspase 3 via the formation of a complex known as apoptosome, which is made up of cytochrome-c, Apaf-1, and caspase 9 **[14]**. On the other hand, Smac/DIABLO or Omi/HtrA2 promotes caspase activation by binding to inhibitor of apoptosis proteins (IAPs) which subsequently leads to disruption in the interaction of IAPs with caspase-3 or -9 **[15]**. This pathway is closely regulated by a group of proteins belonging to the BCl-2 family, named after the BCl-2 gene originally noticed at the chromosomal breakpoint of the translocation of chromosome 18 to 14 in follicular non-Hodgkin lymphoma [16]. There are two main groups of the BCl-2 proteins, namely:

The pro-apoptotic proteins (e.g.Bax, Bak, Bad, BCl-Xs, Bid, Bik, Bim, and Hrk) and
The anti-apoptotic proteins (e.g. BCl-2, BCl-XI, BCl-W, Bfl-1 and Mcl-1).

While the anti-apoptotic proteins regulate apoptosis by blocking the mitochondrial release of cytochrome-c, the pro-apoptotic proteins act by promoting such release. It is not the absolute quantity but rather the balance between the pro- and anti-apoptotic proteins that determine whether apoptosis would be initiated or not **[17]**.

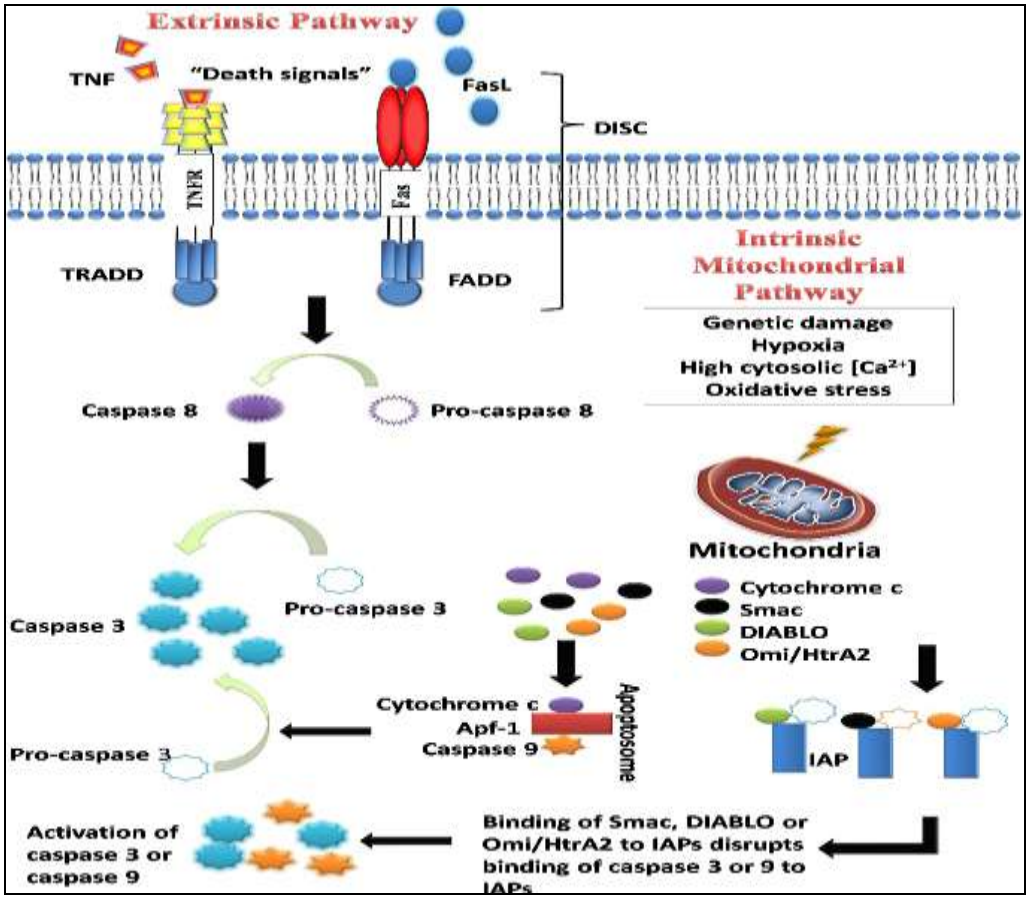


Fig. 2: The intrinsic and extrinsic pathways of apoptosis **[10]**

2.1.3. The common pathway

The execution phase of apoptosis involves the activation of a series of caspases. The upstream caspase for the intrinsic pathway is caspase 9 while that of the extrinsic pathway is caspase 8. The intrinsic and extrinsic pathways converge to caspase 3. Caspase 3 then cleaves the inhibitor of the caspase-activated deoxyribonuclease, which results in nuclear apoptosis. In addition, downstream caspases induce cleavage of protein kinases, cytoskeletal proteins, DNA repair proteins, and inhibitory subunits of endonucleases family. They also have an effect on the cytoskeleton, cell, and signaling pathways, which together contribute to the typical morphological changes in apoptosis **[18]**.

3. Apoptosis and carcinogenesis

Cancer can be viewed as the result of a succession of genetic changes during which a normal cell is transformed into a malignant one while evasion of cell death is one of the essential changes in a cell that cause this malignant transformation **[19]**. Hence, reduced apoptosis or its resistance plays a vital role in carcinogenesis. There are many ways a malignant cell can acquire reduction in apoptosis or apoptosis resistance **(Fig. 3) [20]**.

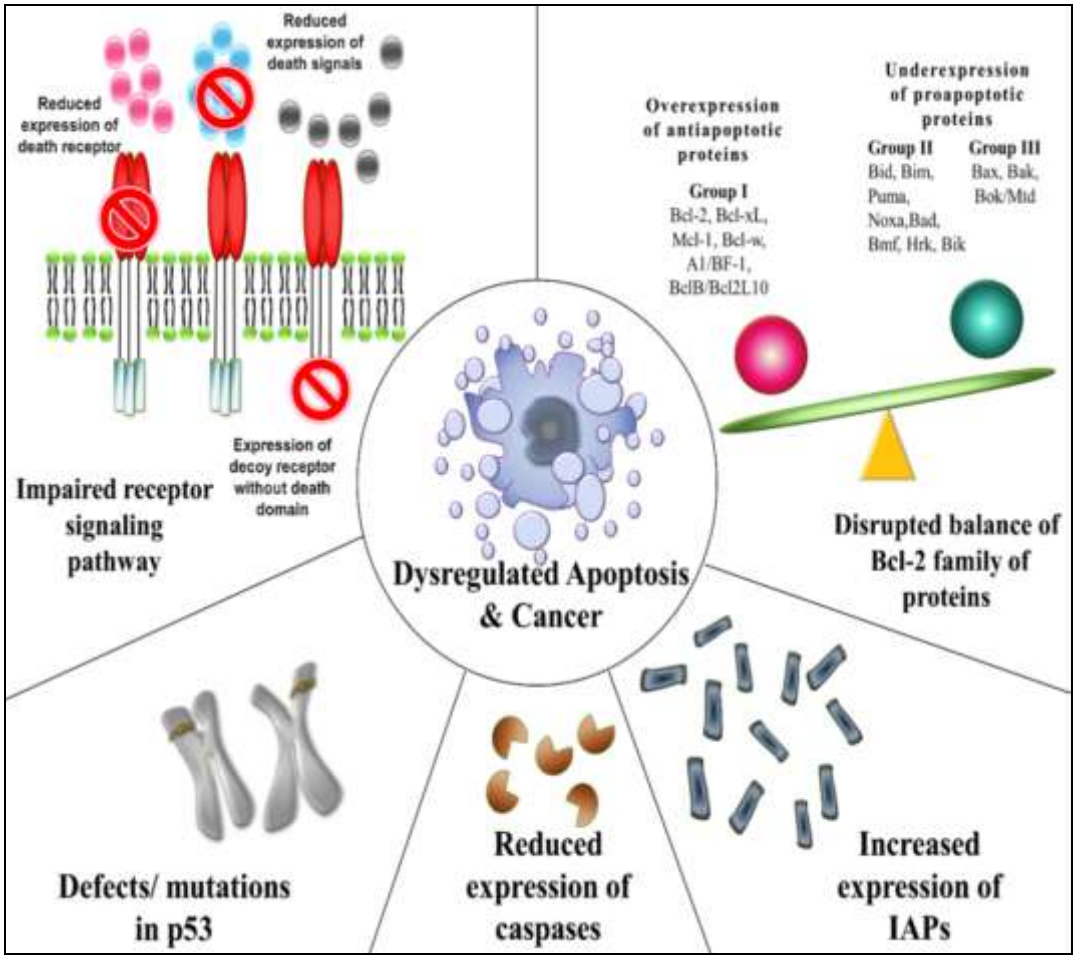


Fig. 3: Mechanisms contributing to evasion of apoptosis and carcinogenesis **[20]**

3.1. Impaired death receptor signaling

As mentioned before, death receptors and ligands of the death receptors are key regulators of the extrinsic pathway. These receptors possess a death domain and when triggered by a death signal, a number of molecules are attracted to the death domain, resulting in the activation of a signaling cascade. However, death ligands can also bind to decoy death receptors that do not possess a death domain, hence they fail to form signaling complexes and initiate the signaling cascade. Several abnormalities in the death signaling pathways that can lead to evasion of the extrinsic pathway of apoptosis have been observed. Such abnormalities include downregulation of the receptor or impairment of receptor function regardless of the mechanism or type of defects, as well as a reduced level in the death signals, all of which contribute to impaired signaling and hence a reduction of apoptosis **[21]**.

3.2. Defects/Mutations in the p53 protein

The p53 protein also called tumor protein 53 (or TP 53), is one of the best-known tumor suppressor proteins encoded by the tumor suppressor gene TP53 located at the short arm of chromosome 17. It is named after its molecular weight, i.e., 53 kDa **[22]**. Initially, it was found to be slightly oncogenic. It was later discovered that the oncogenic property was due to a p53 mutation, or what was later called a gain of oncogenic function **[23]**. It is not only involved in the induction of apoptosis but also it is a key player in cell cycle regulation, development, differentiation, gene amplification, DNA recombination, chromosomal segregation and cellular senescence **[24]** and is called the guardian of the genome **[25]**.

3.3. Reduced caspases activity

The caspases can be broadly classified into two groups: 1) those related to caspase 1 (e.g. caspase-1, -4, -5, -13, and -14) and are mainly involved in cytokine processing during inflammatory processes and 2) those that play a central role in apoptosis (e.g. caspase 2, 3, 6, 7, 8, 9 and 10). The second group can be further classified into:

1) Initiator caspases (e.g. caspase 2, 8, 9 and 10) which are primarily responsible for the initiation of the apoptotic pathway and

2) Effector caspases (caspase 3, 6 and 7) which are responsible in the actual cleavage of cellular components during apoptosis **[26] (Fig. 4).**

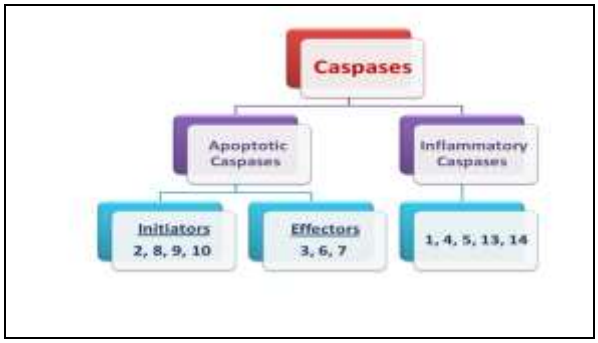


Fig. 4: Classification of caspases

Caspases remain one of the important players in the initiation and execution of apoptosis. It is, therefore, reasonable to believe that low levels of caspases or impairment in caspase function may lead to a decrease in apoptosis and carcinogenesis.

3.4. Increased expression of IAPs (inhibitor of apoptosis proteins)

The inhibitor of apoptosis proteins a group of structurally and functionally similar proteins that regulate apoptosis, cytokinesis and signa transduction. They are characterized by the presence of a baculovirus IAP repeat (BIR) protein domain **[27]**. IAPs are endogenous inhibitors of caspases and that inhibit caspase activity by binding their conserved BIR domains to the active sites of caspases, thus promoting

degradation of active caspases or keeping the caspases away from their substrates **[28]**. Thus, overexpression of the IAPs is considered one of the mechanisms by which cancer cells can evade apoptosis.

3.5. The disrupted balance of pro-apoptotic and anti-apoptotic proteins

Many proteins exert pro- or anti-apoptotic activity in the cell. It is not the absolute quantity but rather the ratio of these pro-and anti-apoptotic proteins that play a crucia role in the regulation of cell death. Moreover, over- or under-expression of certain genes, hence the corresponding regulatory proteins have been found to contribute to carcinogenesis by reducing apoptosis in cancer cells.

3.5.1. The BCl-2 families of proteins

The Bcl-2 family of proteins is divided into proapoptotic and antiapoptotic proteins that play a pivota role in the regulation of apoptosis, especially via the intrinsic pathway as they reside upstream of irreversible cellular damage and act mainly at the mitochondria level. Bcl-2 was the first protein of this family to be identified and the BCl-2 gene, which derives its name from B cell lymphoma 2, encodes it the second member of a range of proteins found in human B cell lymphomas (14; 18) chromosomal translocation **[29]**. All the BCl-2 members are located on the outer mitochondria membrane. They are dimers are responsible for membrane permeability either in the form of an ion channe or through the creation of pores **[30]**. Based of their function and the BCl-2 homology (BH) domains, the BCl-2 family members are further divided into three groups **[31]** 1) the first group is the anti-apoptotic proteins that contain all four BH domains and they protect the cell from apoptotic stimuli. 2) The second group is made up of the BH-3 only proteins, so named because in comparison to the other members, they are restricted to the BH3 domain. In times of cellular stresses such as DNA damage, growth factor deprivation, and endoplasmic reticulum stress, the BH3-only proteins, which are initiators of apoptosis, are activated. Therefore, they are pro-apoptotic. 3) Members of the third group contain all four BH domains and they are pro-apoptotic **(Fig. 5) [32]**.

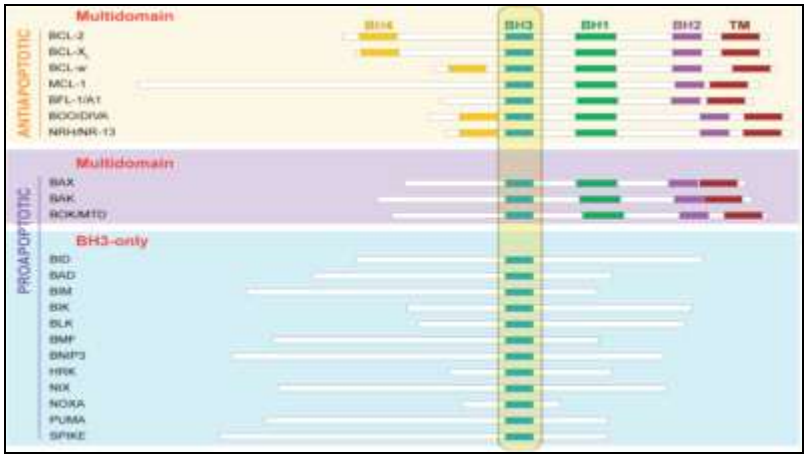


Fig. 5: Members of Bcl-2 family of proteins **[32]**

3.5.2. Role of BCl-2 family in apoptosis

Both the anti-apoptotic and pro-apoptotic functions of BCl-2 family members are regulated through their BH domains. Furthermore, the BH1- BH3 domains of anti-apoptotic proteins form a hydrophobic binding pocket that binds the α-helix of the BH3-only pro-apoptotic proteins **[33]**.

In norma cells: Pro-apoptotic BH3-only proteins use their BH3 domain to inhibit anti-apoptotic BCl-2 proteins such as BCl-2, BCl-xl, and MCl-1 and activate pro-apoptotic BCl-2 proteins BAX and BAK **[34]**. When BH3-only proteins directly activate BAX and BAK, they use their BH3 domain to oligomerize and assemble mitochondria pores that induce mitochondria outer membrane permeabilization (MOMP) **[35]**. This MOMP induces the release of mitochondria 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 **[36]**.

In cancer cells: There is a disruption in the balance between anti-apoptotic and a pro-apoptotic member of the BCl-2 family. This can be due to an overexpression of one or more anti-apoptotic proteins or an underexpression of one or more pro-apoptotic proteins or a combination of both. This imbalance results in sequestering the pro-apoptotic proteins by the anti-apoptotic ones, BAX, and BAK inactivation leading to dysregulated apoptosis **(Fig. 6) [37]**.

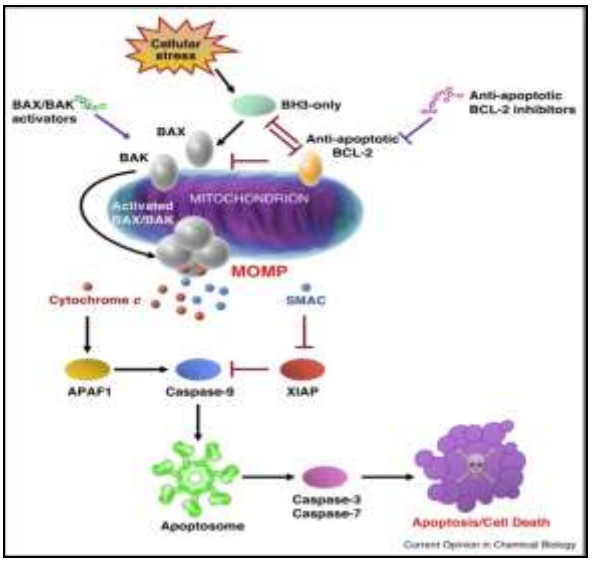


Fig. 6. Role of Bcl-2 family in apoptosis **[37]**

4. Targeting the BCl-2 family of proteins in cancer treatment

Like a double-edged sword, every defector abnormality along the apoptotic pathways may also be an interesting target of cancer treatment. Drugs or treatment strategies that can restore the apoptotic signaling pathways towards normality have the potentia to eliminate cancer cells, which depend on these defects to stay alive. Many recent and important discoveries have opened new doors into potentia new classes of anticancer drugs.

Therapeutic agents used in targeting the BCl-2 family of proteins can be divided into 1) molecules that affect gene or protein expression and 2) sma molecules called BH3 mimetics, so named because they mimic the binding of the BH3-only proteins to the hydrophobic groove of anti-apoptotic proteins of the BCl-2 family. Early attempts focused on interfering with BCl-2 gene expression, thereby reducing the level of BCl-2 protein synthesized. Oblimersen (Genasense®, Genta®), an antisense oligonucleotide that bind to Bcl2 mRNA, thus interfering with its translation, was the first agent to enter clinica trials. It was considered to have significant

potentia during early development **[38]** but ultimately proved in PhaseIII studies to have insufficient clinica efficacy **[39]**.

4.1. BH3 mimetics of anti-apoptotic BCl-2 proteins

The main strategy against BCl-2 has been the development of BH3 mimetics **(Fig. 7).** They can promote apoptosis by releasing sequestered BH3-only proteins, BAX and BAK from anti-apoptotic BCl-2 proteins **[40]**.

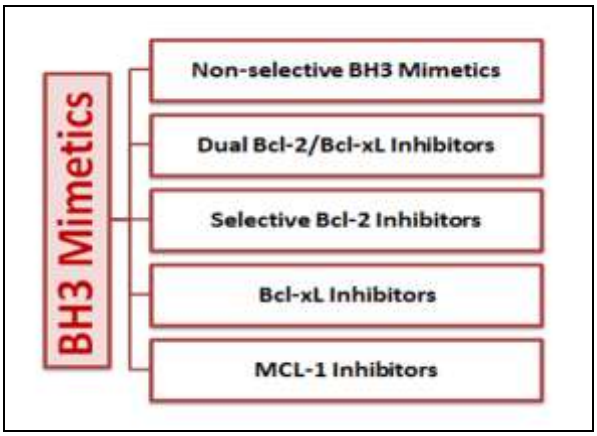
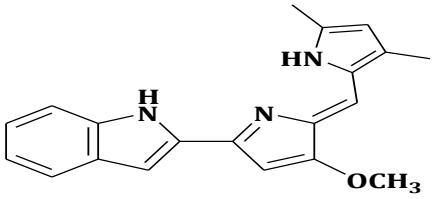


Fig. 7. Classes of BH3 mimetics

4.1.1. Non-selective BH3 mimetics

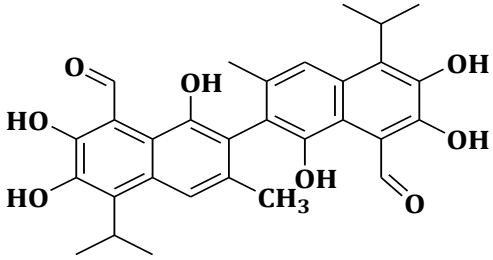
The first generation of BH3 mimetics has limited selectivity for a specific anti-apoptotic BCl-2 protein. Unfortunately, Significant toxicities associated with off-target effects halt their further development **[41]**.

Obatoclax (GX15-070) (1) binds to BCl-2, BCl-XI, BCl-W, and Mcl1. However, the response rates in clinica trials have been low and its development has ceased **[42]**.



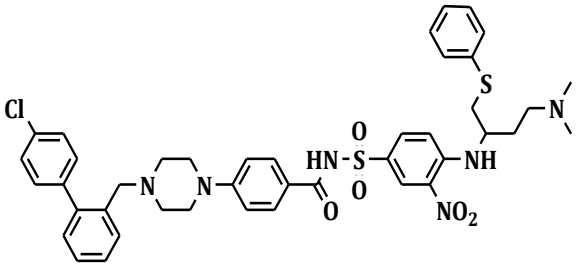
Obtoclax (GX15-070)(1)

The natura product **Gossypol**, a polyphenolic aldehyde isolated from the cotton plant, and its synthetic derivative **AT-101 (2)** bind to BCl-2, BCl-XI, and Mcl1 **[43]**. As with obatoclax, these agents have demonstrated minima activity in clinica trials **[44]**.



AT-101(2)

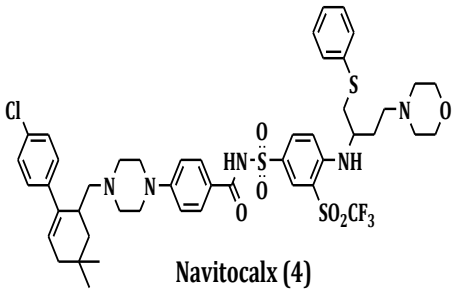
A breakthrough in the development of BCl-2 inhibitors occurred when Abbot Laboratories discovered **ABT-737 (3)** using fragment-based drug design approach by NMR **[45]**. It inhibits BCl-2, BCl-XI, and BCl-W with high affinity (Ki ≤ 1nM). It was shown to exhibit cytotoxicity in lymphoma, small cell lung carcinoma cell line, primary patient-derived cells, and regression of established tumors in anima models with a high percentage of cures **[46]**.



ABT-737 (3)

Navitoclax (ABT-263) (4) is a second generation, structurally related molecule that is orally available and has more favorable pharmacokinetics. It has an ora bioavailability of 20–50% and a half-life of 8.9 hours, making it suitable for once-daily dosing. Its specificity mirrors that of ABT-737, with a Ki of ≤1nM against Bcl-2, Bcl-XI, and Bcl-W, and a Ki of 550 nM against Mcl-1. The dose limiting toxicity

of navitoclax was a dose-dependent reduction in platelet count (thrombocytopenia) **[47]**.

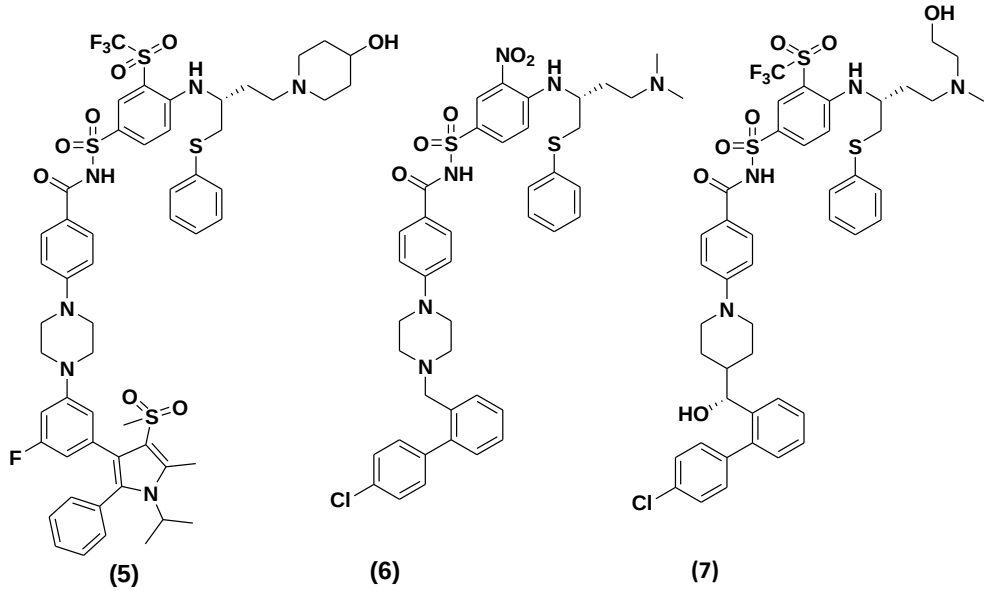


Navitocalx (4)

Since the disclosures of ABT-737 **(3)** and navitoclax **(4),** severa inhibitors of BCl-2 and BCl-XI that contain the acylsulfonamide pharmacophore or its isosteres have been reported. All those inhibitors are sti at the preclinica level.

4.1.2. Duane BCl-2/Bcl-XI inhibitors

Based on the success of ABT-737 **(3)** and navitoclax **(4)**, several dual BCl-2/Bcl-XI inhibitors were developed using their arylsulfonamide scaffold. **BM-1197 (5) [50]**, **S44564 (6) [51]** and **AZD4320 (7) [52]** are examples of dual inhibitors with subnanomolar affinity. They exert potent antitumor activity in small cell lung cancer cells. They also cause reversible platelet reduction in mice at highly efficacious doses.

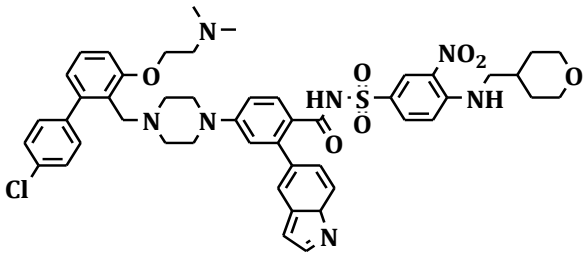


(5) (6) (7)

4.1.3. Selective Bcl-2 inhibitors

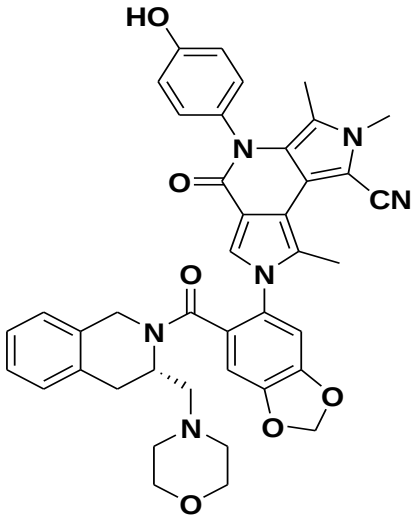
Structure-based drug design based on Navitocalx **(4)** co-crystall structure with BCl-2 protein enabled tailoring new BH3 mimetics selective to BCl-2 protein only. Examples are shown in **table 2**.

Venetoclax (ABT-199) (8) has demonstrated the most promising clinical results of all the putative agents targeting BCl-2 to date. It is the result of reverse engineering of navitoclax to increase BCl-2 selectivity to avoid thrombocytopenia. Accordingly, venetoclax has subnanomolar affinity for BCl-2 (Ki<0.01nM), but significantly weaker binding to BCl-XI (Ki = 48nM), BCl-W (Ki = 245nM), and Mcl-1 (Ki > 444nM) **[53]**. Venetoclax has adequate oral bioavailability and an estimated half-life of 26 hours **[54]**.



Venetoclax (8)

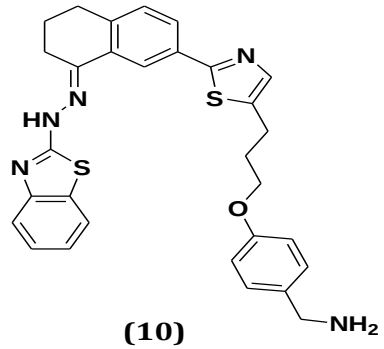
S55746 (9) is the second BCl-2-selective inhibitor (Ki = 1.3 nM) to enter clinical development. It is an orally compound is also known as BCl201 [55].



S55746 (9)

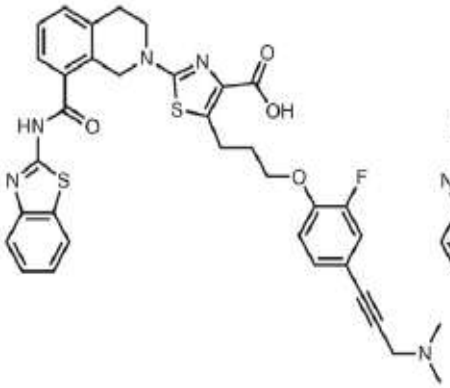
4.1.4. BCl-XI inhibitors

Structure-based guided medicina chemistry focused on specific interactions with the P2 and P4 pockets to identify a chemica scaffold with selectivity for BCl-XI. The first BCl-XI is **WEHl-539 (10),** an inhibitor with subnanomolar affinity for BCl-XI (IC_{50} = 1.1 nM). It promoted robust apoptosis induction in BCl-XI dependent lung cancer cells; however, its poor physicochemica properties limit its activity in vivo **[56]**.



(10)

Optimization of compound **(10)** using a combination between NMR fragment screening and structure-based design led to the discovery of **A-1155463 (11) [57]** and **A-1331852 (12) [58]**. Compound **(12)** is the most potent and selective orally available BCl-XI inhibitor (Ki = <0.010 nM) reported with 10–50 fold improved cellular activity than **(11)** and **(4)**.



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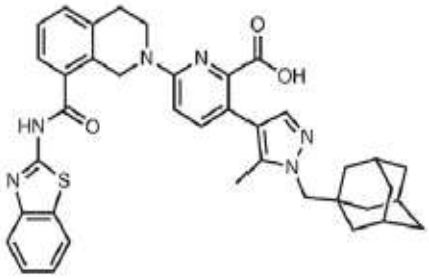
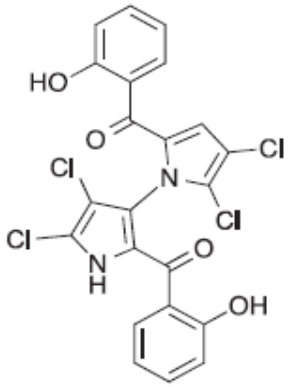


(12)

4.1.5. MCl-1 inhibitors

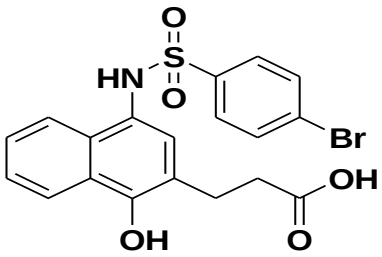
The structure of MCl-1 suggested that its BH3 groove is particularly shallow in P1, P2, and P4 pockets compared to BCl-2 and BCl-XI **[59]**. Interestingly, it is suggested that BH3 binding to the P4 pocket is not as important as in BCl-2 and BCl-XI **[60]**.

The natural product, **Maritoclax (13),** is the first known MCl-1 inhibitor (IC_{50}=10.1 μM) **[61]**.



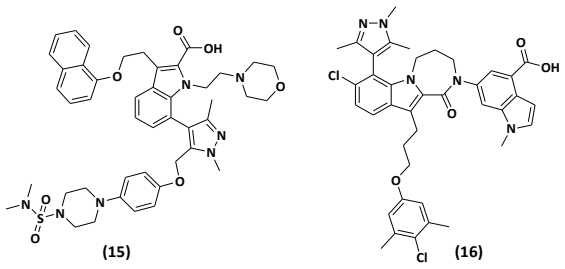
(13)

UMl-77 (14) was then identified through HTS and structure-based design **(14)** has moderate binding affinity (Ki = 490 nM) and selectivity for MCl-1 **[62]**. It induces apoptosis by disrupting MCl-1/ BAX and MCl-1/BAK complexes.



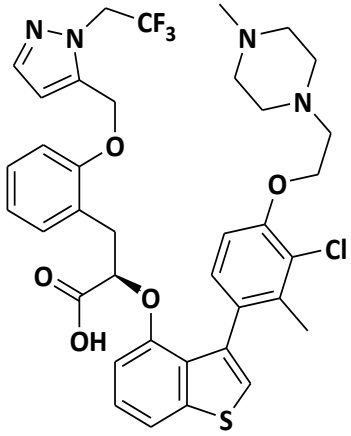
(14)

Another strategy was adopted to grow P2 bound hits from fragment-based NMR screens based on the 2-carboxy indole core. This approach led to larger P2-P4 binders such as **A-1210477 (15) [63]** and compound **(16) [64]**, which exhibit subnanomolar affinity (Ki = 0.045 nM and Ki = 0.05 nM respectively).



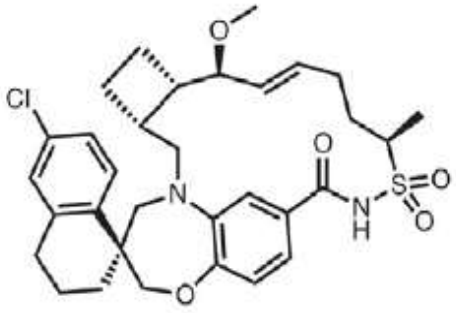
(15) **(16)**

S6384 (17) is recently reported with subnanomolar affinity to MCl-1 (Ki = 0.15 nM) and selectivity of 10000 fold over Bcl-2 and Bcl-xl **[65]**.



(17)

AMG176 (18) is the first MCl-1 inhibitor to enter phase 1 trials for the treatment of relapsed and refractory multiple myeloma with subnanomolar affinity (Ki = 0.13 nM) **[66]**.

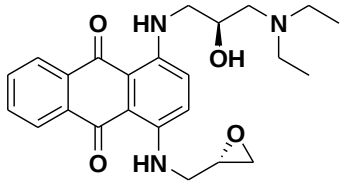
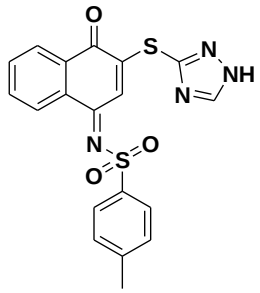
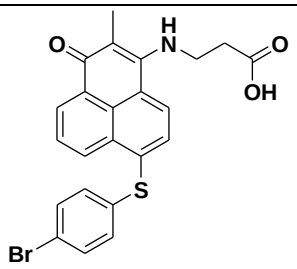
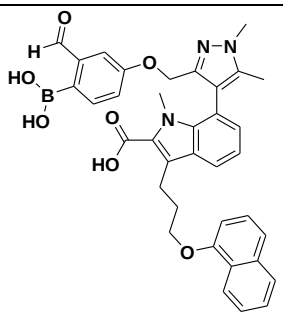


(18)

Table 2. Some Bcl-2 family protein inhibitors, their uses, and toxicities **[48, 49]**

Name	Type of Tumor	Clinical Stage	Key Toxicities
Oblimersen Sodium (Genasense®)	Melanoma, multiple myeloma, chronic lymphocytic leukemia, Non-small cell lung cancer, hormone-refractory prostate cancer	Phase III	Fatigue, lFTs elevation
Obatoclax (1)	leukemia, lymphoma, unspecified childhood solid tumor	Phase I	Neurological including somnolence, dizziness, euphoric mood, and gait disturbance
Gossypol & AT-101 (2)	Metastatic breast cancer and Advanced hematological cancers	Phase I\II	Fatigue, nausea, lymphopenia, diarrhea, hypophosphatemia, neutropenia and intestinal obstruction
ABT-737 (3)	Solid tumor, Chronic lymphocytic leukemia (CII)	Phase II	Thrombocytopenia, nausea, fatigue, elevated AlT and bronchitis
ABT-263 (Navitoclax®) (4)	Solid tumor, Chronic lymphocytic leukemia (CII)	Phase I\II	Thrombocytopenia, nausea, fatigue, elevated AlT and bronchitis

Table 3. Non-canonical modulators of anti-apoptotic Bcl-2 proteins

Name	Structure	Target
BDA-366 (19) [68]		Bcl-2 BH4 antagonist
MAlM1 (20) [69]		MCl-1 allosteric inhibitor
Compound 5 (21) [70]		MCl-1 inhibitor/ degradation inducer
Compound 11 (22) [71]		MCl-1 covalent inhibitor

4.2. Non-canonicall targeting of anti-apoptotic BCl-2 proteins

Besides targeting the BH3 groove, additional mechanisms of BCl-2 family protein interactions have been investigated and molecule or protein-based probes have been identified to bind noncanonica surfaces. The success of this technique is attributed to the exploitation of

residues outside of the canonica BH3 groove unique to each anti-apoptotic protein and formed by α3–α5 regions. When these designed proteins were expressed in cells, they inhibited their corresponding BCl-2 protein providing unique cellular probes **[67]**. Examples are shown in **table 3**.

5. Conclusion

In summary, the development of BCl-2 inhibitors as potential anti-cancer agents represents a triumph of the rational scientific investigation. While initial attempts at BCl-2 family inhibition did not achieve the desired specificity and efficacy, rational drug design eventually yielded a viable approach with BH3 mimetics. The task now turns to discover new candidates with better activity and least toxicity. While many unanswered questions yet remain, the future of BCl-2 inhibition in cancer treatment is bright.

Conflict of interest

The authors declare no conflict of interest.

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