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Small molecule Mcl-1 inhibitors for the treatment of cancer

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ABSTRACT

The Bcl-2 family of proteins serves as primary regulators of apoptosis. Myeloid cell leukemia 1 (Mcl-1), a pro-survival member of the Bcl-2 family of proteins, is overexpressed and the Mcl-1 gene is amplified in many tumor types. Moreover, the overexpression of Mcl-1 is the cause of resistance to several chemotherapeutic agents. Thus, Mcl-1 is a promising cancer target. This review highlights the current progress on the discovery of small molecule Mcl-1 inhibitors.

Contents

1. Introduction

Apoptosis is a natural process for eliminating unwanted or damaged cells that represent a threat to the health of an organism. This process is highly regulated, and the B-cell lymphoma-2 (Bcl-2) family of proteins serves as the main regulators. Indeed, dysregulation and evasion of apoptosis is one of the hallmarks of cancer (Hanahan & Weinberg, 2000, 2011).

Members of the Bcl-2 family proteins share conserved sequences in regions known as Bcl-2 homology (BH) domains (BH1–BH4) (Korsmeyer, 1999; Pang et al., 2012). Members within the same family can have opposite effects. The anti-apoptotic or pro-survival proteins, including Bcl-2, Bcl-X_L, Bcl-w, Bfl-1/A1 and Mcl-1 (Adams & Cory, 2007), keep cells alive; whereas, the pro-apoptotic proteins (e.g., Bim, tBid, Bad, Puma, Noxa, Bak, and Bax) (Youle & Strasser, 2008) promote cell death. The relative levels of the anti- and pro-apoptotic proteins govern whether a cell will live or die. Recently, much has been learned about how the Bcl-2 proteins regulate apoptosis (Burlacu, 2003; van Delft & Huang, 2006; Volkmann et al., 2014). Upon triggering a death signal, a subset of the pro-apoptotic proteins with homology only in the BH3 region cause Bak and Bax to homo-oligomerize and

Abbreviations: Bad, Bcl-2-associated death promoter; Bak, Bcl-2 homologous antagonist/killer; Bax, Bcl-2 associated X; Bcl-2, B-cell lymphoma-2; BH, Bcl-2 homology; BH3, Bcl-2 homology domain 3; Bcl-X_L, B-cell lymphoma-extra large; Bim, Bcl-2 interacting mediator; Bfl-1/A1, Bcl-2-related protein A1; Mcl-1, myeloid cell leukemia-1; Noxa, phorbol-12-myristate-13-acetate-induced protein 1; Puma, p53 upregulated modulator of apoptosis; SAHB, stapled alpha-helix of Bcl-2 domains; SAR, structure activity relationships; SPR, surface plasmon resonance; tBid, truncated BH3-interacting domain death agonist.

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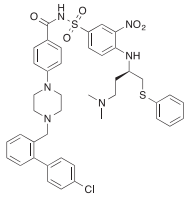
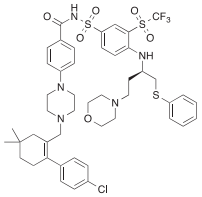
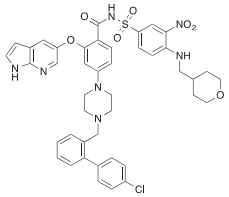
form pores in the mitochondrial membrane leading to cytochrome c release into the cytosol. This activates the caspase cascade and causes cell death. The anti-apoptotic proteins block cell death by binding and sequestering, with varying specificity the BH3-only proteins (Chen et al., 2005). The binding specificity and affinity exhibited by the anti-apoptotic proteins for the pro-apoptotic proteins is defined by hydrophobic and electrostatic interactions between the BH3 region of the pro-apoptotic proteins and the binding groove formed by the BH1, BH2 and BH3 regions of the anti-apoptotic proteins (Sattler et al., 1997; Dutta et al., 2010; Moldoveanu et al., 2014). Of the BH3-only proteins, Bim and Puma are the least selective, binding to all five anti-apoptotic proteins. Bad binds strongly to Bcl-2, Bcl-X_L and Bcl-w; whereas, Noxa binds exclusively to Mcl-1 and Bfl-1/A1. These observations suggest that apoptosis is regulated by the interactions between particular subsets of these proteins and that apoptosis can be initiated by the inhibition of the pro-survival members of the Bcl-2 family proteins. Indeed, this has been demonstrated by the BH3-mimetics ABT-737 (**1**) (Oltersdorf et al., 2005) and its orally available derivative ABT-263 (**2**; navtioclax) (Tse et al., 2008) which bind to Bcl-2, Bcl-X_L and Bcl-w. As expected, ABT-737 and ABT-263 induce apoptosis in tumor cells that are dependent on Bcl-2 and Bcl-X_L. More recently, a selective Bcl-2 inhibitor was discovered (ABT-199) that also demonstrates the utility of inhibitors of the Bcl-2 family (Souers et al., 2013). Indeed, Navitoclax and ABT-199 have shown efficacy in several clinical trials in patients with lymphoid malignancies that are believed to be Bcl-2 dependent (Tse et al., 2008; Roberts et al., 2012; Choo et al., 2014). However, there are some cancers that cannot be treated by these compounds alone. Several studies have shown that upregulation of Mcl-1 is a key factor in the development of resistance to ABT-737 and ABT-263 in several tumor types (Konopleva et al., 2006; van Delft et al., 2006; Tahir et al., 2007).


ABT-737 (**1**)


ABT-263 (**2**)

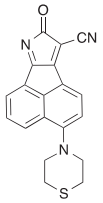

ABT-199 (**3**)

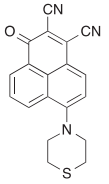
Mcl-1 has a number of functions and features that make it unique among the anti-apoptotic Bcl-2 family members. Mcl-1 is essential for early embryogenesis (Rinkenberger et al., 2000) as well as the development and maintenance of lymphocytes (Opferman et al., 2003; Dzhagalov et al., 2008), neurons (Arbour et al., 2008), synovial fibroblasts (Liu et al., 2005) and hematopoietic stem cells (Opferman et al., 2005). Mcl-1 is also unique in that it has a very short half-life of <1–4 h, depending on cellular conditions (Yang-Yen, 2006), and multiple pathways tightly regulate Mcl-1 transcription, translation, and degradation (Thomas et al., 2010). Structurally, Mcl-1's N-terminus also differs from that of the other anti-apoptotic Bcl-2 proteins in that it contains two PEST (proline/glutamic acid/serine/threonine-containing) regions (Germain & Duronio, 2007). Indeed, the N-terminal region may serve as a regulatory domain for Mcl-1's rate of turnover, localization, and phosphorylation, and may thus provide a mechanism to rapidly fine-tune the expression of Mcl-1 in response to environmental and cellular input (Thomas et al., 2010).

There is a lot of evidence to suggest that Mcl-1 is an important cancer target. For example, Mcl-1 amplification is one of the most common genetic aberrations observed in human cancer (Beroukhim et al., 2010; Wei et al., 2012), including lung (Song et al., 2005), breast (Ding et al., 2007), prostate (Krajewska et al., 1996), pancreatic (Miyamoto et al., 1999), ovarian and cervical cancers (Brotin et al., 2010), as well as melanoma (Boisvert-Adamo et al., 2009) and leukemia (Derenne et al., 2002; Andersen et al., 2005; Kang et al., 2008). Furthermore, Mcl-1 overexpression induces resistance against the aforementioned Bcl-2-inhibitors, as well as a number of widely used anticancer therapies including paclitaxel (Wertz et al., 2011), vincristine (Wertz et al., 2011) and gemcitabine (Wei et al., 2008). Moreover, RNA-mediated knockdown of Mcl-1 has shown tumor growth inhibition and cell death in Mcl-1 overexpressing lung, colon, ovarian and lymphoma cells (Moulding et al., 2000; Thallinger et al., 2003; Konopleva et al., 2006; Qin et al., 2006; Akgul, 2008; Chetoui et al., 2008; Boisvert-Adamo et al., 2009; Hauck et al., 2009; Keuling et al., 2009; Chen et al., 2010; Lucas et al., 2012). Silencing of Mcl-1 also restores sensitivity in chemoresistant cells (Taniai et al., 2004; Lin et al., 2007; Meng et al., 2007). Given these data, Mcl-1 represents a very promising cancer target. An Mcl-1 inhibitor would be expected to be useful as a single agent against cancers that depend on Mcl-1 for survival and in combination with other drugs where Mcl-1 overexpression is the major resistance factor.

This review focuses on the current state of Mcl-1 inhibitors. Although peptide-based inhibitors have been described, including stapled alpha-helix of Bcl-2 domains (SAHB) (Stewart et al., 2010; Muppidi et al., 2012), alpha-/beta-peptide foldamers (Smith et al., 2013) and reverse BH3 (rBH3) peptides (Placzek et al., 2011), we focus this review on small molecule inhibitors that have been reported to function as BH3-mimetics. As proposed by Lessene and co-workers true BH3-mimetics should exhibit Bak/Bax-dependent biological activity and high-affinity binding to at least one Bcl-2 family pro-survival protein, specifically Mcl-1 in the case of this review (Lessene et al., 2008). Therefore, Obatoclax (GX15-070) (Nguyen et al., 2007), a putative pan-inhibitor that binds to all Bcl-2 family pro-survival proteins with low affinity (Nguyen et al., 2007; Tse et al., 2008) and kills wild-type and Bak/Bax-deficient cells with equal potency (Vogler et al., 2009), as well as other chemical entities that exert their biological activities through possible off-target or unknown mechanisms of action are not included in this review (Billard, 2013). In addition, this review does not cover molecular entities disclosed exclusively within patents. This information has been reviewed in detail elsewhere (Bajwa et al., 2012).

2. S1


S1 (**4**)


Revised S1 (**5**)

Efforts aimed at designing novel DNA intercalating agents led to the discovery of S1 (**4**), a rigid, planar chromophore, which exhibited anti-tumor activity yet surprisingly lacked the ability to intercalate into DNA (Zhang et al., 2007). The structure of S1 was originally reported as possessing an 8-oxo-8*H*-acenaphtho[1,2-*b*]pyrrole-9-carbonitrile (**4**) backbone. However, the structure was later revised by Song and co-workers to a 1-oxo-1*H*-phenalene-2,3-dicarbonitrile (**5**) (Song et al., 2013a). S1 has been touted as a pan-Bcl-2 family inhibitor as it has been reported to bind to Mcl-1 (K_d = 58 nM, Bid-BH3, FPA) and Bcl-2 (K_d = 310 nM, Bid-BH3, FPA), disrupt Bax/Bcl-2 and Bak/Mcl-1 complexes in a dose- and time-dependent manner, and induce Bax/Bak-dependent apoptosis (Zhang et al., 2010). However, Eastman and co-workers suggest that S1 does not function as a pan-Bcl-2 inhibitor in cells but rather it upregulates the BH3-only protein Noxa, which inhibits Mcl-1 and leads to its degradation and an increase in cellular

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