| Title       | Development of Mcl-1 inhibitors for cancer therapy |
|-------------|---------------------------------------------------|
| Author(s)   | Negi, Arvind; Murphy, Paul V.                     |
| Publication Date | 2020-11-24                                      |
| Publication Information | Negi, Arvind, & Murphy, Paul V. (2021). Development of Mcl-1 inhibitors for cancer therapy. European Journal of Medicinal Chemistry, 210, 113038. doi:https://doi.org/10.1016/j.ejmech.2020.113038 |
| Publisher   | Elsevier                                          |
| Link to publisher's version | https://doi.org/10.1016/j.ejmech.2020.113038       |
| Item record | http://hdl.handle.net/10379/16438                |
| DOI         | http://dx.doi.org/10.1016/j.ejmech.2020.113038  |
Review article

Development of Mcl-1 inhibitors for cancer therapy

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A R T I C L E I N F O

Article history:
Received 4 September 2020
Received in revised form
23 October 2020
Accepted 13 November 2020
Available online 24 November 2020

A B S T R A C T

The myeloid leukemia cell differentiation protein (Mcl-1) is an anti-apoptotic protein of the B-cell lymphoma 2 (Bcl-2) family, which regulates cellular apoptosis. Mcl-1 expression plays a key role in survival of cancer cells and therefore serves as a promising target in cancer therapy. Besides, its importance as a cancer target, various peptides and small-molecule inhibitors have been successfully designed and synthesized, yet no Mcl-1 inhibitor is approved for clinical use. However, recent development on the understanding of Mcl-1’s role in key cellular processes in cancer and an upsurge of reports highlighting its association in various anticancer drug resistance supports the view that Mcl-1 is a key target in various cancers, especially hematological cancers. This review compiles structures of a variety of inhibitors of Mcl-1 reported to date. These include inhibitors based on a diverse range of heterocycles (e.g. indole, imidazole, thiophene, nicotinic acid, piperazine, triazine, thiazole, isindoline), oligomers (terphenyl, quaterpyridine), polyphenol, phenalene, anthranilic acid, anthraquinone, macrocycles, natural products, and metal-based complexes. In addition, an effort has been made to summarize the structure activity relationships, based on a variety of assays, of some important classes of Mcl-1 inhibitors, giving affinities and selectivities for Mcl-1 compared to other Bcl-2 family members. A focus has been placed on categorizing the inhibitors based on their core frameworks (scaffolds) to appeal to the chemical biologist or medicinal chemist.

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https://doi.org/10.1016/j.ejmech.2020.113038

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1. Link between apoptosis and cancer

Based on the Hanahan and Weinberg description, a cancer can be characterized by its 8 different hallmarks [1], as briefly summarized in Fig. 1. These are (a) sustaining proliferative signaling, (b) inducing angiogenesis, (c) evading immune destruction, (d) activating invasion and metastasis, (e) evading growth suppressors, (f) enabling replicate immortality, (g) reprogramming of energy metabolism, (h) resisting cell death (i.e. apoptosis). Among all these 8 hallmarks, metastasis and the evasion of apoptosis are considered as the most life-threatening and aggressive, and therefore their targeting with inhibitors could sustainably improve the longevity and standard of living for a cancer patient.

In general, apoptosis is a key function for normal cellular physiology and can be induced by two processes in a cell [1] that are known as a) the extrinsic pathway and b) the intrinsic pathway.

Evasion of apoptosis in cancer is often found to be a key attribute in resistance against various anticancer chemotherapies. The extrinsic apoptosis pathway is regulated by binding of ligands to various cell surface receptors such as the tumor necrosis factor receptors (TNFR), Fas and DR4/DR5 receptors [2]. On the other hand, the intrinsic pathway is regulated by the Bcl-2 family of proteins [3].

2. Role of the Bcl-2 family proteins in apoptosis

The intrinsic apoptosis pathway is prominently initiated by various types of exogenous and endogenous stimuli, such as DNA damage, ischemia, oxidative stress, chemotherapy, or radiotherapy which ultimately leads to activation of the proteins of the Bcl-2 family. The Bcl-2 family is divided into 2 main subgroups based on their structural homology: (a) pro-apoptotic, which are single Bcl-2 homology 3 domain proteins (also known as, “BH3-only...
Intrinsic pathway of apoptosis: The pro-apoptotic molecules cause permeabilization of the outer mitochondrial membrane, leading to efflux of cytochrome c, which binds the adaptor Apaf-1 and the initiator caspase-9 in the cytosol to form the apoptosome complex. This stimulates caspase-9, which in turn activates the effector caspases. The anti-apoptotic proteins Bcl-2 and Bcl-xl inhibit cytochrome c release, whereas Bax, Bak, and Bid, all pro-apoptotic proteins, promote its release from mitochondria. Cytochrome c and deoxyadenosine triphosphate (dATP) bind to APAF-1 to form a multimeric complex that recruits and activates pro-caspase-9, that in turn activates the caspase cascade, resulting in cell apoptosis. During this process, a number of caspase proteins (caspase-2, caspase-8, caspase-9, and caspase-10) are involved in the initiation of apoptosis. Caspase-3, caspase-6, and caspase-7 are involved in apoptosis. Caspase-3 and caspase-7 regulate the inhibition of DNA repair and start DNA degradation which also further contribute in inducing cellular apoptosis. Synthetic molecules that block the Bcl-2/Mcl-1/Bcl-xl interaction with BH3 helix of Bid, Bim, Bad, Puma, Bmf, and Noxa, leads to increase the intracellular level of BAX/BAK and resulted in their oligomerization to form a pore, through which cytochrome-c release into the cytosol and activates the intrinsic pathway of apoptosis in tumor cells.

Fig. 2: Intrinsic pathway of apoptosis: The pro-apoptotic molecules cause permeabilization of the outer mitochondrial membrane, leading to efflux of cytochrome c, which binds the adaptor Apaf-1 and the initiator caspase-9 in the cytosol to form the apoptosome complex. This stimulates caspase-9, which in turn activates the effector caspases. The anti-apoptotic proteins Bcl-2 and Bcl-xl inhibit cytochrome c release, whereas Bax, Bak, and Bid, all pro-apoptotic proteins, promote its release from mitochondria. Cytochrome c and deoxyadenosine triphosphate (dATP) bind to APAF-1 to form a multimeric complex that recruits and activates pro-caspase-9, that in turn activates the caspase cascade, resulting in cell apoptosis. During this process, a number of caspase proteins (caspase-2, caspase-8, caspase-9, and caspase-10) are involved in the initiation of apoptosis. Caspase-3, caspase-6, and caspase-7 are involved in apoptosis. Caspase-3 and caspase-7 regulate the inhibition of DNA repair and start DNA degradation which also further contribute in inducing cellular apoptosis. Synthetic molecules that block the Bcl-2/Mcl-1/Bcl-xl interaction with BH3 helix of Bid, Bim, Bad, Puma, Bmf, and Noxa, leads to increase the intracellular level of BAX/BAK and resulted in their oligomerization to form a pore, through which cytochrome-c release into the cytosol and activates the intrinsic pathway of apoptosis in tumor cells.

Fig. 3: A: Representative inhibitors of members of the anti-apoptotic Bcl-2 family. B: Surface map of co-binding of Mcl-1 protein with ε-helix peptide showing 4 pockets (P1, P2, P3, P4) of active site for corresponds to 4 hydrophobic side chain of amino acids of BH3 helix.
proteins”), such as Bid, Bim, Bad, Puma, Bmf, and Noxa; and (b) multiple BH domain containing proteins, which are further divided into two subcategories, one which includes pro-apoptotic proteins such as Bcl-2 associated X protein (Bax), Bcl-2 homologous antagonist/killer (Bak) and Bcl-2 family apoptosis regulator (Bok), while the other one includes anti-apoptotic proteins (such as Bcl-2, Bcl-xL, and Mcl-1).

When stimulated by an apoptotic signal, BH3-only proteins activate the apoptotic effector proteins Bax and Bak by two ways, either by binding directly to Bax and Bak or by binding to free state anti-apoptotic proteins (such as Bcl-2, Bcl-xL, Mcl-1) to set free Bax and Bak. Further, oligomerization of Bax and Bak forms a pore on the mitochondrial membrane (also, called “mitochondrial outer membrane permeabilization” (MOMP)), through which
cytochrome-c goes into the cytosol, leading to the formation of an apoptosome and activation of the caspase cascade and finally results in apoptosis as signaling shown in Fig. 2 [4]. On the other hand, the antiapoptotic proteins tend to block the oligomerization of Bax and Bak, which arrests cell death. Therefore, their relative ratios inside the mitochondria have a high significance in deciding the fate of cell, either to die or to live, as shown in Fig. 2.

3. Mcl-1 as a cancer target

In cancer, the relative activity is inclined more towards the antiapoptotic than apoptotic proteins, therefore, most of the antiapoptotic proteins serve as attractive targets in various cancers [5]. Various strategies were implemented to identify inhibitors of antiapoptotic protein in the last few decades, but only a few molecules have been a success clinically: \((R-(-))\)-Gossypol (AT-101) [6], GX15–070 (Obatoclax) [7], ABT-737, ABT-263 [7,8], ABT-199.

![Chemical structure, binding affinity of MIM1 and amino acid sequence of the Mcl-1 SAHBα.](image)

Fig. 7. Various derivatives of AT101 and ApoG2 and their affinities.
few compounds are also in preclinical development (see Fig. 3A). Mcl-1 is an anti-apoptotic protein and a key member of the Bcl-2 family. Various studies showed its overexpression and associated resistance towards many anticancer drugs, such as its over-expression has been reported in estrogen receptor positive human [9,10], non-small cell lung [11,12], melanoma [13,14], leukemia [15], ovarian [16] and liver cancers [17,18]. Its high expression drives the resistance against ABT-199 and its analogues and therefore limits their utility in other types of cancers [3]. Also, Mcl-1 is responsible for resistance in various other conventional chemotherapies, like cisplatin-resistant in ovarian carcinoma cells [14,19], lapatinib resistance in HCT116 cells (human colon cancer cell line) [20], resistance to prednisone in MLL-rearranged infant acute lymphoblastic leukemia [21]. However, studies show that its down-regulation or reducing its stability, improves either, the sensitivity or efficacy of other drugs in solid tumors and blood cancers.

Fig. 8. Molecular structure and affinity of Yc137 and Chai's compounds.

Fig. 9. Showing the derivative of 2-thioxo-4-thiazolidinone (from Fang's group) and thiazolidine-2,4-dione derivatives 4-4b with affinity data given against Mcl-1.
such as, it improves the chemosensitivity of gemcitabine in pancreatic carcinoma [24], enhances the anti-invasive effect of dasatinib in pancreatic adenocarcinoma [25], along with inhibition of cyclin E1 sensitizes hepatocellular carcinoma cells to regorafenib [26], assisted renieramycin T preferential cytotoxicity towards lung cancer cells [27], associated with apoptosis induced by FTY720 drug induce in oral squamous carcinoma [28], improves chemosensitivity of AML cells towards daunorubicin [29], improves efficacy of FLT3 tyrosine kinase inhibitors [30], linked with deguelin and formononetin induced tumor growth suppression in non-small cell lung cancer (NSCLC) [31,32], reverses the acquired resistance to osimertinib in EGFR-mutant lung cancer (NSCLC) [33], and triggers apoptosis in case of taxol and vincristine based chemotherapies [34]. Therefore, number of research groups have been working to develop Mcl-1 inhibitors in recent years.

4. Structure of Mcl-1 and degradation in cells

The Mcl-1 gene forms two products through alternative splicing where one is a larger form (Mcl-1 protein) whereas another form is smaller (called as Mcl-1s or Mcl-2 and works as an apoptotic protein). The larger form (i.e. Mcl-1 protein) has three BH domains, while other anti-apoptotic proteins contain four BH domains. Although it lacks one BH domain compared to other members, it...
still is the largest of all these proteins, containing 350 amino acid residues [35]. It has a different N-terminus than other anti-apoptotic proteins and also, contains PEST/pest regions (peptide sequences with proline/glutamic acid/serine/threonine, they can be ‘weak’ (pest) or ‘strong’ (rich, PEST)) [36]. It has been suggested that the presence of rich PEST regions is linked to higher rates of degradation of proteins [37] and linked to the short half-life of Mcl-1 (<1–4 h) [38]. Also, its N-terminus contains regulatory motifs that responds to stimuli in order to express the Mcl-1 protein [35] (as shown in Fig. 4). Also, it contains 4 pockets (P1, P2, P3, P4) which could occupy 4 hydrophobic side chains (H1, H2, H3, H4) of the α-helix of a BH3 peptide of a pro-apoptotic member as shown in Fig. 3B. Therefore, the BH-3 peptide is α-helical and has been used as a basis to develop small molecule ligands against Mcl-1 and these are discussed in the remainder of the review.

5. Various classes of Mcl-1 inhibitors

There are some of important reviews/perspectives which cover small heterocyclic molecules as Mcl-1 inhibitors; these also provide insights on strategies for targeting Mcl-1 protein [37], and linked to the short half-life of Mcl-1 (<1–4 h) [38]. Also, its N-terminus contains regulatory motifs that responds to stimuli in order to express the Mcl-1 protein [35] (as shown in Fig. 4). Also, it contains 4 pockets (P1, P2, P3, P4) which could occupy 4 hydrophobic side chains (H1, H2, H3, H4) of the α-helix of a BH3 peptide of a pro-apoptotic member as shown in Fig. 3B. Therefore, the BH-3 peptide is α-helical and has been used as a basis to develop small molecule ligands against Mcl-1 and these are discussed in the remainder of the review.

5.1. Polyphenols and derivatives

In 2002, Wang’s group at the University of Michigan showed that R-(−)-gossypol (AT-101) is a Bcl-2 family inhibitor. AT-101 was also found to inhibit all anti-apoptotic of Bcl-2 family except Bfl-1 [57]. The Wang group derivatized the (−)-gossypol to get a promising inhibitor of multiple anti-apoptotic Bcl-2 members TW-127 (Ki values were obtained by fluorescence polarization assay (FPA)). Here are FPA data (see Fig. 5): (−)-gossypol Bcl-xl: 0.32 ± 0.02 μM; Bcl-2: 0.48 ± 0.07 μM; Mcl-1: 0.18 ± 0.01 μM; TW-127 Bcl-xl: 0.29 ± 0.06 μM; Bcl-2: 1.11 ± 0.4 μM; Mcl-1: 0.26 ± 0.01 μM) [58]. Further, derivatization led to apogossypol (ApoG2), which showed −20-fold greater selectivity for Bcl-2/Mcl-1 as compared to Bcl-xl [59]. Later, based on AT-101, the Pellecchia
group at the Burnham Institute for Medical Research utilized structure-based design to improve the affinity against the Bcl-2 family members (Figs. 1, 2 and 6) [60]. Furthermore, they resolved the optical isomers and obtained the (R, - , R) stereoisomer for 2 (i.e. BI-97C1) and found it to be more potent [61]. Further derivatization led to formation of pan-Bcl-2 inhibitor (BI97D6) [62]; which was separated into its enantiomers such as ( )-BI97D6 [63], as shown in Fig. 6. Also, they reported antiapoptotic protein...
inhibitory activities for (−)-epigallocatechin gallate (EGCG) (FPA: Bcl-2/Bad $K_i = 490 \text{ nM}$; Bcl-xL/Bad $K_i = 335 \text{ nM}$) [64] which were similar to data later reported by Reed & coworkers [57], as shown in Fig. 5.

In 2012, the Walensky group at the Dana-Faber Cancer Institute (Harvard Medical School, Boston) identified small molecule Mcl-1 inhibitors by stapled peptide based screen. They initiated this work with a high-throughput screening (HTS) based on fluorescence polarization assay (FPA) on 71,296 compounds and attained MIM1 (IC$_{50}$ of 4.7 μM as Mcl-1 inhibitor), which on further testing showed no activity for Bcl-xL/Bid complex (IC$_{50} > 50 \text{ μM}$). It was also seen that MIM1 induced apoptosis by blocking the Mcl-1-mediated suppression of Bax.

The MIM1 structure contains a thiazolyl core substituted with methyl, cyclohexylimino, and benzenetriol groups, as shown in Fig. 7 [65]. The structure-activity relationship (SAR) studies showed the significance of positioning of the methyl and cyclic groups at the thiazolyl core and all three −OH groups on the benzenetriol moiety for Mcl-1 activity. Biophysical studies showed MIM1 occupied that part of the BH3-binding site engaged by residues ETI11RV (amino acid 211−216) of Mcl-1 SAHB0 (Fig. 7) While the cyclohexyl ring made complementary hydrophobic contacts with the region residues L213 and V216, the thiazolyl core and its methyl substituent were predicted to point directly into a deep crevice. Interestingly, the benzene-1,2,3-triol (or pyrogallol) moiety engaged in hydrophilic contacts with Asp 256 and Arg283.

### 5.2. Thiazole derivatives

Similar to polyphenols, thiazoles were also highly exploited for their Mcl-1 activities. In 2004, YC137 was reported as a potent Bcl-2 inhibitor [66]. Further testing showed that it was active against Bcl-
2, Bcl-W, Bcl-B and Mcl-1 (\( \leq 5 \) mM) and inactive against Bcl-xL and Bfl-1 (\( > 20 \) mM) [57], as shown in Fig. 8.

From previous studies [67], it was known that BH3I-1 and sanguinarine binding are in close proximity in Bcl-xL binding site, therefore Chai’s group at National University of Singapore utilized the structural features of BH3I-1 and sanguinarine in a hybrid molecule [68,69] as shown in Fig. 8.

Encouraged by inhibitory data found for WL-276, Fang’s group (at the School of Pharmacy, Shandong University, China) implemented the structure-based design to form the 2-thioxo-4-

Fig. 16. Fang’s group disclosed indole-3-carboxylic acid derivatives as Bcl-2/Mcl-1 dual inhibitors. Included are toxicity data against various cancer cell lines (N.A. = not assayed).

Fig. 17. Summary of further indole derivatives and assay data from by Fang’s group.
thiazolidinone heterocyclic core derivatives, which were pan-Bcl-2 inhibitors (3a-d) [70], as shown in Fig. 9. While the Fletcher group at the School of Pharmacy, University of Maryland, revealed thiazolidine-2,4-dione as a moderate inhibitor (4a) which was later optimized to attain the potent Mcl-1 inhibitor (4b) [71], as shown in Fig. 9.

5.3. Thiazolo[3,2-a]pyrimidinone

In 2010, Feng et al. discovered a thiazolo[3,2-a]pyrimidinone molecule (2.1.5) from a virtual database screening as a pan-Bcl-2 inhibitor [72], as shown in Fig. 10. Although they tried derivatization of the A and B-rings of 5, but this led to only diminished affinity for Mcl-1.

Later, in 2011, the Wang & Yu group performed the FPA (fluorescence polarization assay) based screening on 95 in-house synthesized compounds, where they found a potent compound (6) active against Bcl-2, Bcl-xL and Mcl-1. This compound shares similar structural features with a phosphatase inhibitor (CDC25B) [73], as shown in Fig. 11. By molecular modeling, they predicted that the phenyl amide region has π-π-interactions with Tyr195, whereas indole N-benzyl substitution was required to utilizes a binding subpocket [74]. Furthermore, they isolated single enantiomers of their most active compounds and led to the identification of more potent inhibitors of Mcl-1 in some cases [75], see in Fig. 11.

5.4. Indole derivatives

Mcl-1 inhibitors based on indole have been explored more than any other heterocyclic system. Much of the contribution is from Fesik’s group at Vanderbilt University (USA).

5.4.1. Fesik indole compounds

In 2013, Fesik’s group from Vanderbilt University utilized fragment-based methods and structure-based design to discover a series of potent and selective Mcl-1 inhibitors. Sequential optimization of the molecule in three different regions was performed: (a) fragment 1, where they selected indole over benzofuran and benzothiophene; (b) fragment-2, where they used various substituted and unsubstituted aryl groups; (c) linker region, where they used carbon backbone of 1, 2 or 3 carbon atoms [76]. After a number of
subsequent optimization steps, they finally identified a potent 2-indole carboxylic acid derivative (10a, Fig. 12), with a 16-fold selectivity for Mcl-1 over Bcl-2 and 270-fold selectivity over Bcl-xL. The co-crystal structure of 10a showed the interactions that the 2-COOH functionality of indole ring has a H-bond acceptor interaction with Arg263 of the NWGR domain of Mcl-1. The presence of the aromatic ring in fragment-2 seems essential for a π-π interaction with Ph270 (pdb id: 4HW2 & 4HW3) [76]. In another study, they transformed the indole ring into a tricyclic indole where they form a 6 membered cyclic linkage between N1/C7 of indole 2-Fig. 20. Chemical structures and binding affinities ofacenaphthylene derivatives. (The structure of 58 [S1] was reported incorrectly in early [90,91], which was corrected later [96]).

Fig. 21. Chemical structures and binding affinities of phenalene derivatives.
carboxylic acid ring (11) as shown in Fig. 12. The tricyclic indole-2-carboxylic acid (11) itself showed better affinity for Mcl-1 ($K_i = 38 \mu M$) when compared to the indole-2-carboxylic acid 9 ($K_i > 1000 \mu M$) [77].

Also, the derivatives of tricyclic indole (12–15) showed a high preferential selectivity for Mcl-1 over Bcl-xL [14 showed a 1750-fold selectivity while 15 showed ~2000-fold selectivity, see in Fig. 12] [77].

In order to guide the design of analogues that could bind to the P3 and P4 pocket of Mcl-1, they performed a fragment-based screening of 13,824 molecules while saturating the P2 pocket of Mcl-1 with compound 10a. Later, they utilized acylsulfonamide (compound 16 & 17) linkage so that it would provide them a synthetic handle for fragment linking and, also, at the same time retain the acidic group to interact with Arg263. Further, screening of various cyclic fragments with a carbon backbone linkage to sulfonamide of 18, yielded compounds 19–26, which showed submicromolar activity against Mcl-1. The crystal structure of 18a with 19 (PDB id:5FD0), shows that the substituted phenyl group pointed towards the P4 pocket and sulfonamide linker retains a H-bonding distance to the amide NH of Asn260 explaining the sudden improvement in Mcl-1 affinity. Interestingly, the NH of the pyrazole points up from the binding pocket and therefore explained why methylation did not improve Mcl-1 affinity. However, this kind of binding could be more useful if an additional group could be substituted to the NH of pyrazole to improve its drug-like characteristics or to transform the molecule into a probe for biochemical assays without affecting the affinity for Mcl-1 [78].

To improve drug-like character and retaining high on-target potency of 28, its acidic sulfonamide linker was replaced with a neutral amide linker as shown in Fig. 13. However, in this way, it lost the charge−charge interaction of its sulfonamide with Arg263, but attempts were made to compensate. Various pyridine/phenyl/ furan derivatives were used as shown in Fig. 13 (30), where they co-crystallized 30a with Mcl-1 (PDB id: 5IEZ). The binding of 30a showed that the indole amide substructure is nearly coplanar and both the NH−(indole and amide linker) could adopt a geometry to form a cyclic structure, which ultimately leads to the formation of a new scaffold (31). The compounds 32 and 33 were found to be the most potent and selective [79]. Further in-vitro investigation with 33 exhibited reduced cell activity in the presence of serum. Unlike many other acidic lipophilic compounds that bind to drug site 1 or 2, they found that 33 predominantly binds to drug site-3 of Mcl-1, which is unusual but the X-ray structure of 33 bound to site-3 provides a basis to design drugs with reduced albumin binding [80]. In another study, further derivatization of 33 leads to the picomolar range Mcl-1 inhibitor 33b [81].

### 5.4.2. (b) AbbVie compounds

Through high throughput screening (HTS) AbbVie Inc have reported an indole carboxylic acid derivative compound 34 similar to Fesik's compound 10. Initially, they formed 35, which on co-crystallization with Mcl-1, disclosed 2-COOH acid interaction with Arg263 and naphthyl functionality going deep into the cavity (packed tightly by hydrophobic side chains of Leu235, Leu 246, Met250, Phe270 (10.1021/acs.jmedchem.5b00616), and Ile 294). Later, SAR (structure activity relationship) studies showed indole N-substitution with 2 carbon tethered morpholinyl group along
with various O-tolyl substitution at C6 position on 35, improved Mcl-1 affinity to a value of even less than 10 nM. Afterward, the co-crystal structure showed that the 7-O-tolylmethyl group was oriented toward the P3 and P4 pockets of the BH3 binding groove. Further derivatization, replaces the O-tolyl with pyrazole (as in case of 36, improved the activity to the picomolar range) and extended bicyclic sulphonamide group that ultimately lead to formation of 38. Further, cellular-based assays showed 38 was effective against multiple cancer cell lines such as multiple myeloma and small cell lung cancer and is presently used as a clinical agent in various cell-based assays [83].

5.4.3. Liu & Zhou compounds

Liu & Zhou and co-workers (Shenyang Pharmaceutical University, Shenyang, China) performed a fluorescence polarization assay (FPA: Bid-BH3 peptide labeled fluorescein) on small in-house compound library, where they discovered 39 as a hit against Mcl-1 ($K_i = 7.78 \pm 1.21$ nM). This was followed by grafting various other groups to the indole residue, as indicated in Fig. 15. These optimizations led to a series of submicromolar Mcl-1 active compounds (37-43), where 37 ($K_i$ value of 0.11 $\mu M$) was the most potent and was 7-fold selective over Bcl-2, as shown in Fig. 15. Further, docking showed that these compounds bind to the pocket (P2) and interact with Arg263 [84].

5.4.4. (d) Fang group's work

Fang's group (School of Pharmacy, Shandong University, China) used structure-based design to form an inhibitor which contains three parts (a) indole ring as a heterocyclic scaffold (b) aryl sulphonamide (c) substituted biphenyl group. They synthesize compounds 44-47 as Mcl-1 inhibitor and identified the potent Mcl-1 inhibitor 45 with an $IC_{50}$ of 72 nM (10-fold selective over Bcl-xL), as shown in Fig. 16. Additionally, cell line testing against MDA-MB-231 (breast cancer cell), PC-3 (prostatic cancer cell), K562 (chronic myelogenous leukemia cells) showed moderate inhibitory activity when compared to R-(-)-gossypol, as shown in Fig. 16 [85]. Fang's group explored their scaffold further for Mcl-1 inhibitor development, where they increased the carbon linkage between sulfonamide functionality and indole core as shown in Fig. 17. This led them to identify 48-50 as dual Bcl-2/Mcl-1 inhibitors. These
compounds, especially 50, showed a better activity profile when compared to WL-276 [86], in cell line testing. Further derivatization, led to improved Mcl-1 and Bcl-2 potencies, leading to 50a-e being identified as dual inhibitors of Mcl-1/Bcl-2 as shown in Fig. 17 [87].

5.4.5. Zhang’s group work

The Zhang group (Dalian University of Technology) implemented the fragment-based design to synthesize 1-substituted-indole-2-carboxylic acids (51) as Mcl-1-selective inhibitors where their most potent compound (52) showed ~8-fold preferential selectively over Bcl-2 protein, as shown in Fig. 18. Furthermore, they tested these compounds against 4 cell lines: Mcl-1 dependent NCI-H235 (IC50 = 2.2 μM), Bcl-2 dependent HL-60 (IC50 = 63.3 μM), both Mcl-1 and Bcl-2 dependent H22 (IC50 = 9.2 μM) and MCF-7 (IC50 = 19.2 μM) [88].

5.4.6. Covalent bonded Mcl-1 inhibitor

AstraZeneca discovered a covalently bonded Mcl-1 inhibitor which they achieved by incorporating boronic acid onto the molecules and found its proposed bonding interaction with amino group of Lys 234 supported by site mutagenesis (K234A). Further, they performed time-resolved fluorescence resonance energy transfer (TR-FRET) binding assay and caspase-3/7 activation assay in MOLP-8 cells, which showed compound 53 as the most potent one, while the methylated version 56 showed a 6-fold increase in cellular potency as shown in Fig. 19 [89]. While 57 was also prepared to compare the effect of incorporation of the boronic acid residue in 53–56.

5.5. Acenaphthylene & phenalene derivatives

In 2009, Zhang’s group at Dalian University of Technology, reported a small molecule 51 (58) as a pan inhibitor with preferential selectivity (~6-fold) for Mcl-1 over Bcl-2 protein [90]. Further investigation indicated 58 can disrupt heterodimerization Bcl-2/Bax, Mcl-1/Bak and Bcl-2/Bim cancer cells. Later, SAR studies showed that 58 binds in the BH3 groove of Mcl-1 and Bcl-2, with
the thiomorpholine extended into the P2 binding pocket of Mcl-1 and Bcl-2 [91]. Also, they noted that Mcl-1 binding pocket is shorter and wider in comparison to the Bcl-2 binding pocket. Based on these differences, they further probed the difference between the P2 pocket of Mcl-1 and Bcl-2 [92] by replacing thiomorpholine with phenol derivatives, where they found more potent Mcl-1 inhibitor (p-isobutyl substitution on 59) which showed ~7-fold improvement over Bcl-2 [92].

Further in their 2nd study, they tried fragment-based design where they used fragment-1 (cynoacetamide, 60) and fragment-2 (61, 2-[2-oxo-2H-acenaphthylen-1-ylidene]-malononitrile) for further derivatization and found the compounds from fragment 1 (60) were much more potent (60a-d) [93]. The 1H-15N HSQC-NMR supported docking study revealed the binding mode of 60d. The NMR chemical shift perturbations showed a cluster of residues of R263, V253, L246, M250, F270 and K234 with 0.07 ppm, while another cluster represented by V220, R215, V216 and G219 amino acids showed chemical shift perturbation of 0.06 ppm. These residues are evenly distributed over p2, p3 and p4 pockets of Mcl-1 and finding change in their chemical shift shows that these 60d utilizes these cavities for its inhibitory potential [93]. In their third study, they derivatized the fragment-2 (61) into a series of hydroxy pyridine core-compounds (62), as shown in Fig. 20. This hydroxy-pyridine core was substituted with various thiophenol substituents (such as methyl, bromo, amino and t-amyl) but only p-isopropyl thiophenol derivative was able to show less than 1 μM activity. The isopropyl derivative (62) was further derivatized at 2-position where –OH group was replaced with amide derivatives and attained 63 as the most potent compound [94]. In their fourth study, they derivatized 58 structure at cyano and thiomorpholine functionality position and identified 64, which showed activity (IC50) at 10 nM for Mcl-1 and 2-fold less potent against Bcl-2 [95]. In this report, further experiment of site directed mutagenesis on R263A showed 20-fold decrease affinity of 64 (2.34 μM) than wild-type Mcl-1. This diminution of Mcl-1 activity could be based on anchor role of Arg263 residue to provide a H-bond network to C3, C9 substituted derivatives of 58 (like in case of 64).

In order to study phenalene derivatives as Mcl-1 inhibitors, Zhang’s group published a report where they utilized the concept that the “binding of proapoptotic proteins also differentiate the fate of the anti-apoptotic proteins: NoxaBH3 binding biases the QRN motif toward a helical conformation, leading to an enhance ubiquitination of Mcl-1, while BimBH3 binding biases the QRN motif toward a nonhelical conformation, which does not lead to ubiquitination” [48]. They worked on improvements starting from 65, which plays a role in binding to QRN motif (a Glu-Arg-Asp peptide). This led to 66, which had dual functions as it targeted the Bcl-2 homology 3 domain (BH3) and facilitates Mcl-1 ubiquitination by forming hydrogen bond with His224 to drive a QRN adopting a helical conformation [48], as shown in Fig. 21. Later in another report, the researcher...
Fig. 28. Anthraquinone and quinazolone derivatives as Mcl-1 and Bcl-2 inhibitors.

Fig. 29. Structures (UMI-59, UMI-77 & YMI-101) based on naphthol.

Fig. 30. Naphthol based inhibitors.
transformed the molecular structure 66 to 66a (submicromolar dual inhibitor Mcl-1/Bcl-2) and 66b (selective Mcl-1 inhibitor, 20 times over Bcl-2 protein) [97]. To evaluate their anticancer activity both compounds were tested on K562 cells, which prominently depends in Mcl-1 to survive), and Hela cells, which depends on both proteins Bcl-1 and Mcl-1 to survive. The results are shown in Fig. 21 [97]. $^1$H-$^{15}$N HSQC-NMR derived docking study was used to determine the binding mode of 66a and 66b. The 66b NMR titrations exhibited significant changes in chemical shift perturbations (more than 0.025 ppm) in the residues utilized by BH3 helix of proapoptotic proteins, such as Val216, Gly217, Gly219, Val220, Phe254, Arg263, Val265, Phe319. Based on their SAR, HSQC, and docking studies, they reiterated that the conserved residues Arg146/Arg263 could be utilized as “hot spot” to achieved dual function against Mcl-1/Bcl-2 proteins. However, as P1 and P2 pockets in these proteins have different characteristic and therefore pose a distinctive binding conformation, binding coefficient for structurally similar molecules, which could further be seen by these two compounds 66a and 66b, where 66a has dual function characteristic while 66b only found selective to Mcl-1 [97].

5.6. Pyrrole derivatives

5.6.1. Marinopyrrole analogues

Marinopyrrole A (maritoclax) was isolated from a marine Streptomyces species and found active against methicillin-resistant Staphylococcus aureus (MRSA) [98]. Structurally, it contains 2 pyrrole rings. Later, Doi et al. reported its preferential selective affinity for Mcl-1 compared to other members of the Bcl-2 family. They provided $^1$H-$^{15}$N HSQC-NMR supported docking data on mMcl-1, where: (a) one pyrrole moiety of maritoclix projects its chlorine atom toward the deep cleft of Mcl-1; (b) OH of one phenol residue has a H-bond with Gly308 (c) the other phenol has a H-bond with Thr247, (d) a carbonyl group shows a H-bond with Asn204 [99]. Furthermore, the Li and Qin group worked on preparing maritoclix analogues where initially they tried sulfide derivatives (67, 68, 69) and found compounds with submicromolar activity against both Mcl-1 and Bcl-xL [100], as shown in Fig. 22. Furthermore, in cellular assay, these compounds induce caspase-3 based apoptosis in breast cancer cell lines (MDA-MB-468) [100]. However, in another study, synthesized analogues 70 and 71, which are have cyclic constraints “to restrict the conformations of flexible ligand and improve mostly the preferential selectivity for a given physiological target by minimizing the entropic loss associated with the ligand adopting a preferred conformation for binding” [101]. Through 70 and 71 they achieved slight improvement in preferential selectivity to Mcl-1 over Bcl-xL [102].

Later (2015) in their third study, they incorporated various polar and non-polar substituents at the para-position in both the pyrrole rings and found better hydrophobicity tolerance than hydrophilicity (74, 75 vs 72, 73) for dual Mcl-1/Bcl-xL inhibitors [103], as shown in Fig. 22. However, further extension by a triazole ring at the para-position of both phenyl rings on pyrrole and –NH– substitution of the triazole ring, clarified a key role of lipophilicity (trends 76 → 81) in increasing potency towards Mcl-1. In order to investigate the binding mode of these marinopyrrole derivative using $^1$H-$^{15}$N HSQC-NMR with 77. A significant chemical perturbation changes were observed in several residues of Mcl-1 located...
Fig. 33. Showing 4 different series (A-D) of benzylpiperazine as Mcl-1 inhibitors, where P and Q indicating fragments used to target P and Q-site according to the authors.

Fig. 34. Pyrazolo [1,5-a]pyridine as Mcl-2 inhibitors.

Fig. 35. Isoindolines as Mcl-1 inhibitors.
in BH3 binding groove from helices α2, α3, α4, α5. Interesting, chemical perturbation in some residues in helices α1, α6 and α7 has been reported as due to allosteric effect from the binding to the BH3 binding groove, also have been documented with NMR titrations of BH3 helices and Mcl-1 [104]. Although, these molecules showed high potency against Mcl-1 as well as for Bcl-xL (as shown in Fig. 22), they did not lower Mcl-1 expression and Caspase-3 activation at as high as 10 μM μM concentration in human breast cancer cells (MDA-MB-468) [103].

5.6.2. Fang group’s pyrrole derivatives

In 2015, Fang’s group (School of Pharmacy, Shandong University) reported a tetrahydropyrrole (pyrrolidine) derivative (82) by virtual screening (Fig. 23) as a moderate Mcl-1 inhibitor (Ki = 8.4 μM) with reasonable aqueous solubility. Later, by optimization they identified 83 as a more potent Mcl-1 inhibitor and its binding mode was predicted to be similar to the α-helix of BH3-only protein (Bim) by molecular modeling; the model predicted that: (a) the acyl-sulfonyl group formed two hydrogen bonds with Arg263 and one hydrogen bond with Asn260; (b) the carbonyl group of the pyrrolidine ring interacted with Thr266 through one hydrogen bond. While compound 84, was found better to have preferential selectivity for Mcl-1 than 83 (8.2-fold greater than Bcl-2, 15.5-fold greater than Bcl-xL). Furthermore, MDA-MB-231 (breast cancer cell), PC-3 (prostatic cancer cell) and K562 (chronic myelogenous leukemia cell), were chosen to evaluate the anti-proliferative activities of 83, 84, 85 and 86 by MTT assay [105]. The interface between pro-apoptotic proteins and Mcl-1 is large and flexible therefore the Fang group further extended the aromatic amino acid region in their 2nd study reported in 2017 and synthesized 87–91 (Fig. 23) They substituted the aromatic amino acid region with various non-classical amino acids and additionally evaluated the necessity of Boc protecting group on the pyrrolidine scaffold for activity as shown in Fig. 23. The most potent compound 87 (Ki = 0.077 μM) was ~10-fold more potent than 83 and over 2-fold more potent than R-(-)-goossypol (Ki = 0.18 μM) (Fig. 13). Further molecular modelling predicted that: (a) a biphenyl group of compound 87 could mimic an interaction of the α-helical peptide of Bim; (b) an acyl-sulfonyl group could form three hydrogen bonds with Arg263 and Trp 261; (c) the Boc protecting group on the pyrrolidine ring formed one hydrogen bond with Thr266, which also explains the contribution of the Boc to binding affinities for Mcl-1. Later cell-based assays showed compound 87 had moderate activity against MDA-MB-231 and K562 cells when compared to R-(-)-goossypol while being equipotent with an IC50 of 10.2 and 7.54 μM in cytotoxicity assays with PC-3 cell line, respectively [106].

Fig. 36. Imidazolidine-2,4-dione derivative as Mcl-1 inhibitor.

Fig. 37. Terphenyl and quaterpyridines as Mcl-1 inhibitors.
5.7. Isoquinoline & quinoline derivatives

In 2008, Prakesh et al. generated a tetrahydroaminoquinoline-based library to find a small molecule modulator for protein-protein interactions. Through, in-silico and $^1$H-$^1$H HSQC-NMR studies, they filtered the molecules which showed their ability to bind to Bcl-xL and Mcl-1. The NMR study led to the identification of the tetrahydroaminoquinoline 92 as a weak inhibitor of both proteins ($K_d = 200 \mu M$ for Bcl-xL and $K_d = 300 \mu M$ for Mcl-1). Later, fragment-based design was implemented to afford the compound 93 as their most potent inhibitor of Mcl-1 and Bcl-xL ($K_d = 25$ and $70 \mu M$) [107], as shown in Fig. 24.

The first report on polyquinoline derivatives was published by the Moreau group (Clermont University) [108], where they found that polyheterocyclic compounds adopt similar conformations to the BH3 helix of Bak. Also, they used a symmetric triquinoline core, where C5 of every quinoline was substituted with a branched alkyl functionality (94). Later when they chemically modified this...
scaffold to compounds (such as 95 & 96) and tested them against a wide variety of Bcl-XL-apoptotic PPIs, they found 94 comparatively superior to the derivatives. Therefore, it was further evaluated against antiapoptotic-Bax [109], as shown in Fig. 25. In their another study, they examined the effects of substitution at C2 and C5 in both quinoline rings, giving asymmetric polyquinolines. Their most promising compounds had an isopropyl substituent at C5 position of one quinoline ring along with C2 substitution of both quinolines with either O/N-tethered heterocycles or alkyl groups (97, 98, 99, 100, 101) [110], as shown in Fig. 25. However, while 97 was found as the most potent Mcl-1 inhibitor, it was also non-selective in nature [110].

In 2013, Richard et al. published another study, they examined the effects of substitution at C2 and C5 position of one quinoline ring along with C2 substitution of both quinolines with either O/N-tethered heterocycles or alkyl groups (97, 98, 99, 100, 101) [110], as shown in Fig. 25. However, while 97 was found as the most potent Mcl-1 inhibitor, it was also non-selective in nature [110].

In 2016, Fletcher’s group disclosed a series of 3-carboxy-substituted 1,2,3,4-tetrahydroquinolines as Mcl-1 inhibitors. They used hybrid structure-based design (SBD), where they considered two molecules (111 from AbbVie Inc and Fesik’s 10b) and attained a series of compounds (e.g. ±112) including a potent inhibitor of Mcl-1 (±113) [114], as shown in Fig. 27.

5.8. Anthraquinone and quinazolone derivatives

In 2012, the Zhang group at Dalian University of Technology utilized structure-based design which was based on the concept that, “two faces of the Bim BH3 α-helix are utilized in binding with Mcl-1 and Bcl-2” [7]. However, interestingly the other known inhibitors such as ABT-737 and nonpeptide α-helix mimics, terphenyl scaffolds, only act on one face of the α-helix of Bim-BH3, and do not mimic the opposite side of another α-helix face, which is conserved in all BH3 domains [115,116]. Therefore, Zhang et al. introduced anthraquinone based inhibitors (114) which can mimic two-faces of the Bim BH3 α-helix, where one face/core of core was substituted with hydroxy groups (at C1, C2, and C3) while on other side either C6 or C7 position was substituted (with either bromo, isopropyl or thiophenols), as shown in Fig. 28. They found better Mcl-1 activities when substitutions were performed on both faces/sides than one face/side of the anthraquinones, and 115 was their most potent Mcl-1 inhibitor [117]. Based on their anthraquinone study [117], they developed quinazolones, where they found 116 to be a dual inhibitor of Bcl-2/Mcl-1, with Ki values of 179 nM and 123 nM, respectively, holding typical BH3 like two-faced binding characteristic [118].

5.9. Naphthol derivatives

In 2014, Nicolovska-Coleska and co-workers performed a high throughput screening on a library of 53,000 compounds to identify UMI-59 as a validated hit. Later they synthesized UMI-77 and UMI-101 as analogs and tested them against Bcl-2 family proteins as shown in Fig. 29. By 1H−15N HSQC-NMR-based docking, they confirmed that bioactive UMI-77 is utilizing the H2 and H3 pockets similar to the BH3 helix (which utilize its four hydrophobic residues H1, H2, H3, H4) and mimic 2 conserved hydrophobic residues from mNoxa Leu 78 and Ile 81 respectively. They also observed that the p-bromophenyl group occupies the h2 pocket and interacts through hydrophobic interactions with Met231, Met250, Val 253, Leu 267, and Phe270, which is further supported by 1H−15N HSQC 2D-NMR spectroscopy. Whereas UMI-101, lacking a carboxylic acid,
Fig. 41. Krohnke pyridines as Mcl-1 inhibitors (139 representing general structure and illustrating structure activity relationship for these molecules).

Fig. 42. Fang’s L-tyrosine derivatives as Mcl-1 inhibitor.
was found inactive. Further investigation showed that UMI-77 blocks in vitro and in vivo pancreatic cancer [119].

In 2016, Fletcher’s group used structure-based design, similar to those used for tetrahydroquinolines [114] to design naphthol derivatives. They utilized an innovative approach called, “site-identification by ligand competitive saturation (SILCS)” to quantitatively predict the relative affinities of ligands bindings to Mcl-1 and Bcl-xl, and showed could be useful for differentiating the binding behaviors of compounds to Mcl-1/Bcl-xl leading to design of Mcl-1 selective inhibitor. The initial SAR study showed that mono-N-substitution of the sulfonamide improved Mcl-1 activity (116a-h) and N,N-substitution of sulfonamide led to increase in potency for the Mcl-1 inhibitor (as N, N-, 117) [120], as shown in Fig. 30.

5.10. Salicylic, anthranilic and nicotinic acids

AbbVie pharmaceuticals implemented a NMR-based fragment screening, where they identified sulfonamide containing fragment 118, and this was altered to improve the potency for Mcl-1 as shown in Fig. 31. Later, a co-crystal structure with 120 (PDB ID: 4Q05), disclosed the mode of binding of this series of compounds: the COOH group in these ligands interacted with Arg 263, as for the

![Fig. 43. Triazine based Mcl-1 inhibitor.](image)

![Fig. 44. Zhu & Zhou Mcl-1 selective ligands.](image)

![Fig. 45. Biaryl sulphonamide based inhibitor 150 and 151 a hit obtained from virtual screening by Fang’s research group.](image)
amphipathic peptides from the pro-apoptotic proteins Bim and Noxa [121], while the naphthyl group occupies the leucine ‘hot spot’ in the Mcl-1 groove. The sulfonamide orients the bi-aryl ether along this groove [122].

In 2012, the Fletcher group reported 2,6-di-substituted nicotinates, by simplifying the α-helix mimetic (JY-1-106), as α-helix based oligoamide-foldamer potent Bcl-xL inhibitor [123]. Mechanistic interventions of JY-1-106 showed dose-dependent inhibition of the protein-protein interaction of Bak-BH3 with Bcl-xL and Mcl-1 (IC₅₀ values of 394 ± 54 nM [123] and
10.21 ± 0.83 μM [124]) respectively. However, the Mcl-1 binding affinity was found 10 times more potent than Bcl-xL [124], as shown in Fig. 32. Conversely, JY-1-106 found to induce apoptosis via intrinsic apoptosis pathway and independent of expression of Mcl-1 level [124]. Later, they simplified the JY-106 structure into a very weak Mcl-1 inhibitor (122), which on further derivatization led to a more improved and also lower molecular weight Mcl-1 inhibitor 123 as compared to JY-106, as shown in Fig. 32 [125].

5.11. Benzylpiperazine derivatives

In 2013, Ding et al. evaluated four series of benzylpiperazine derivatives as inhibitors of Mcl-1. To achieve high preferential selectivity, they compared the binding site of the BH3 domain of Bcl-xL and Mcl-1. They divided these sites in both proteins into three subsites: (i) P-site (hydrophobic site; hydrophobic, mainly consisting of Leu108, Val126 and Phe97 residues in the case of Bcl-xL, and Met231, Leu235, Val249 residues in the case of Mcl-1), (ii) Q-site (a hybridized site, which mainly consists of Arg100, Asn136 and Tyr195 residues in the case of Bcl-xL, and His224, Asn260, Phe319 residues in the case of Mcl-1); (iii) L-site (region between the P-site and the Q-site: where a linker fragment is to be placed in order to connect the chemical fragments fitting to the P-site and Q-site) [126]. They applied a fragment-based design, where they...
and D-series (m50 series showed less than 50% inhibition against these proteins at
found that it induces Bax-dependent, but not Bak-dependent
PARP cleavage, as critically required in cellular apoptosis. They also

The most potent compounds were from the A- (four series (A
e Ki values; d IC50 values.

| #  | Compound                      | Mcl-1 Ki/IC50 = μM | Bcl-xL, Ki/IC50 = μM | Ref   |
|----|-------------------------------|--------------------|----------------------|-------|
| 1  | Gymnochrome F                 | 3.3 (Mcl-1/Bak, FRET: IC50) | NR                  | [160] |
| 2  | 161                          | 2.4 ± 0.1 (Mcl-1/Bak, FRET: IC50) | NR                  | [161] |
| 3  | 162                          | 8.9 (Mcl-1/Bak, FRET: IC50) | NR                  | [161] |
| 4  | 163                          | 7.3 (Mcl-1/Bak, FRET: IC50) | NR                  | [161] |
| 5  | 164                          | 17.7 ± 3.1 (Mcl-1/Bid, FPA: IC50) | NR                  | [162] |
| 6  | 165                          | 5.8 ± 0.3 (Mcl-1/Bid, FPA: IC50) | ±3.2 ± 0.1 (Bcl-xL/Bak, FPA: IC50) | [162] |
| 7  | 166                          | 3.7 ± 2.0 (Mcl-1/Bid, FPA: IC50) | 16.3 ± 0.5 (Bcl-xL/Bak, FPA: IC50) | [162] |
| 8  | 167                          | 0.7 ± 0.1 (Mcl-1/Bid, FPA: IC50) | 1.2 ± 0.1 (Bcl-xL/Bak, FPA: IC50) | [162] |
| 9  | 168                          | 0.2 ± 0.1 (Mcl-1/Bid, FPA: IC50) | 0.3 ± 0.1 (Bcl-xL/Bak, FPA: IC50) | [162] |
| 10 | 169                          | 0.2 ± 0.1 (Mcl-1/Bid, FPA: IC50) | 0.2 ± 0.1 (Bcl-xL/Bak, FPA: IC50) | [162] |
| 11 | 170                          | 1.2 ± 0.9 (Mcl-1/Bid, FPA: IC50) | 5.7 ± 0.6 (Bcl-xL/Bak, FPA: IC50) | [162] |
| 12 | 171*                         | 14 ± 3.3 (Mcl-1/Bid, FPA: IC50) | 19.2 ± 1.6 (Bcl-xL/Bak, FPA: IC50) | [163] |
| 13 | 172                          | 13 ± 5.0 (Mcl-1/Bid, FPA: IC50) | 12.6 ± 0.2 (Bcl-xL/Bak, FPA: IC50) | [163] |
| 14 | 173                          | 5.2 ± 0.2 (Mcl-1/Bid, FPA: IC50) | >100 (Bcl-xL/Bak, FPA: IC50) | [163] |
| 15 | 174                          | 5.9 ± 0.5 (Mcl-1/Bid, FPA: IC50) | 19.4 ± 3.0 (Bcl-xL/Bak, FPA: IC50) | [163] |
| 16 | Cryptophaerolide              | 11.4 (Mcl-1/Bak, FRET: IC50) | NR                  | [159] |
| 17 | AT101                         | 1.75 (Mcl-1/Bid, FPA: IC50) | 3.03 (Bcl-xL/Bid, FPA: IC50) | [57]  |
| 18 | ApoG2                         | 0.025 (Mcl-1/Bid, FPA: IC50) | 0.66 (Bcl-xL/Bid, FPA: IC50) | [59]  |
| 19 | EGCC                         | 0.92 (Mcl-1/Bid, FPA: IC50) | 0.59 (Bcl-xL/Bid, FPA: IC50) | [57]  |
| 20 | Maritoclax                    | 8.9 ± 1.0 (Mcl-1/Bim, ELISA: IC50) | 16.4 ± 3.3 (Bcl-xL/Bim, ELISA: IC50) | [160] |
| 21 | Antimycin-A                   | 2.51 (Mcl-1/Bid, FPA: IC50) | 2.70 (Bcl-xL/Bid, FPA: IC50) | [57]  |
| 22 | Meioxygenin-A                 | 5.20 ± 1.20 (Mcl-1/Bid, FPA: IC50) | 8.3 ± 1.2 (Bcl-xL/Bak, FPA: IC50) | [157] |

*Uncharacterized cryptosphaerolide was found to have a Ki of 11.4 μM so all the isomers were listed here.

d IC50 values.

Ki values; NR not reported.

found various fragments for every site. Ultimately, they synthesized four series (A–D) of benzylpiperazine derivatives (81 compounds). The most potent compounds were from the A- (124, Ki = 0.18 μM) and D-series (125, Ki = 0.32 μM), while compounds from B and C series showed less than 50% inhibition against these proteins at 50 μM concentration, as summarized in Fig. 33.

5.12. Pyrazolo[1,5-α]pyridine

Takeda Pharmaceuticals disclosed molecules, which were Bcl-xL/Mcl-1 dual inhibitors. They used a structure-based hybridization strategy where they fused two different scaffolds. Among the series, one is selective for Bcl-xL (127) while another one was Mcl-1 selective (126) and they attained a much more potent dual inhibitor (128), compared to the parent molecule [127], as shown in Fig. 34.

5.13. Isoindolines

Zhang’s group (Dalian University of Technology) used their previously identified scaffold (129) and developed submicromolar active isoindolines 130 [128], as shown in Fig. 35.

5.14. Imidazolidine-2,4-dione

Fang’s group used structure-based design and replaced the 2-thioxo-4-thiazolidine scaffold (earlier in 3a-d) by imidazolidine-2,4-dione. Moderate activity of Mcl-1 was attained with compound 131d [129] as shown in Fig. 36.

5.15. Oligomers

5.15.1. π-Terphenyl derivative

In 2002 Hamilton’s group developed a potent Bcl-xL antagonist based on π-helix mimicry [130]. Later, they developed a pan Bcl-2 antagonist (BH3-M6), whose activity is associated with cytochrome c release from mitochondria, caspase-3 activation, and PARP cleavage, as critically required in cellular apoptosis. They also found that it induces Bax-dependent, but not Bak-dependent apoptosis [131] (Fig. 37).

5.15.2. Quaterpyridine - pyridoclax

A similar strategy based on z-helix mimicry was adopted by Gloaguen et al., in 2015 [132], where they designed and synthesized oligopyridines to target the Mcl-1 hydrophobic pocket. They identified a compound based on an oligopyridine (MR29072 or pyridoclax), Pyridoclax, which administered as a single agent, induced apoptosis in various cell lines (IGROV1, OAW42-R, SLOV3, A549 and MSTO-211 H) and ABT-737-chemoresistant ovarian cancer cells (IGROV1-R10 and SKOV3) at 25 μM (Fig. 37) [133].

5.16. Peptidomimetic and non-peptidomimetic medium-sized based on various cyclic systems

AstraZeneca synthesized a library of tripeptide from affinity-mediated screen of DNA-encoded chemical libraries and found an inhibitor 132 for Mcl-1 with a Ki = 1.49 μM. The compound contains a tripeptide with a dihydrobenzazepine incorporated. Most Mcl-1 inhibitors possess a free COOH group on their structures to interact with Arg263, but this inhibitor lacks this feature, but has a terminal primary amine. Also, SAR studies showed that any halogen atom removal diminishes the Mcl-1 activity, with most influence found from the para-halogen. Also, for Mcl-1 activity, R, S, 5 ste- reochemistry is preferred. However macrocyclization, which also leads to removal of the amine, led to improvement of the potency as shown for 133 and 134 (Fig. 38).

AstraZeneca discovered a rationally designed macrocyclic molecule (AZD5991) with high selectivity and affinity for Mcl-1. Their studies demonstrate, that AZD5991 inhibits to Mcl-1-Bak interactions which lead Bak-dependent apoptosis in myeloma and acute myeloid leukemia. SAR studies showed that incorporation of two substituents (6-CI & 1-N-Me) limits the rotation around the biaryl bond, which resulted in formation of atropisomers [135]. Also, N1-methylation pushed the COOH group orthogonal to the indole, improving H-bond acceptor interaction with Arg263 of Mcl-1, while the 6-CI atom is 3.2 Å from the peptide backbone of Ala 227, which indicates the possibility of halogen–carbonyl bond [136].
They found $^1$H NMR chemical shift for H$_2$-pyrazole (Ra)-AZD5991 was unexpectedly upfield ($\delta$ 4.75 ppm vs 2-D predicted shift $\delta$ 5.83 ppm), which indicate the strong anisotropic shielding and led to a suspicion of drift or rigid conformation by the macrocyclic structure in solution. Further extensive NMR based investigation studies showed that (Ra)-7 adopted a free ligand conformation quite like the bound conformation observed in the Mcl-1 co-crystal structure (PDB id: 6F50) [137], as shown in Fig. 39 [138].

A direct binding screen of 100,000 sp3-rich molecules using DSF (differential scanning fluorimetry), which ultimately leads to the identification of single diastereomer of a medium size lactam (macrolactam, 135) that specifically binds to Mcl-1 at 4.5 $\mu$M [139], as shown in Fig. 39.

Medium ring containing Antimycin A is a secondary metabolite produced by Streptomyces bacteria and also known for its antibiotic properties, but Reed and co-workers shows its non-selective Bcl-2 antiapoptotic protein inhibition at low $\mu$M concentration except (Bfl-1$>$ 10 $\mu$M) [157,146], as shown in Fig. 39.

Amgen Biopharmaceuticals implemented structure-based design to identify an alternative novel macrocyclic Mcl-1 inhibitor similar to AMG-176 with a focus on substituting acyl sulfonamide to form a hydrogen bond interaction with Arg263 [141]. Further derivatization and optimization leads to identify a potent Mcl-1 macromolecule inhibitor 1 featuring an $\alpha$-hydroxy phenylacetic acid phosphorochromic (1C9 = 19 nM in an OPM-2 cell viability assay) with reasonable ADME profile and in vivo efficacy in an OPM-2 multiple myeloma xenograft model as shown in Fig. 39 [142].

5.17. Miscellaneous Mcl-1 inhibitors

In 2016, Walensky's group reported a small-molecule covalent Mcl-1 inhibitor (MAIM1), which interacts at an allosteric site (with Cys 286) from the BH3-binding groove. Later studies showed that it impairs the binding capacity of Mcl-1 to BAX in vitro and in mouse cells [143]. In order to find a new scaffold for Bcl-2 inhibitor development, Yang et al., performed a virtual screening on 56,000 compounds; they selected 3-different types scaffolds (136, 137, 138) [144], as shown in Fig. 40.

The Fletcher's group disclosed Kröhnke pyridines (140-a-b & 141), as low-micromolar inhibitors of Mcl-1 wherein the 2,4,6-substituted compounds were predicted to mimic the i, i + 2 and i + 7 side chains of the BH3 $\alpha$-helix [145], as shown in Fig. 41.

Based on previous work by Fang's group (Shandong University), where an intermediate (142) was used to synthesize the thiazolodinones, thiaazolidones and pyrroldines as Bcl-2 family protein inhibitors, showed moderate activity as shown in Fig. 42 [146]. Further, they developed a series of substituted thiosynes based on molecular structure of 142. Further modification leads to the formation of 142, 143 and 144 [146]. In their another study, they further derivatize the molecular structure at R1 and R2 [147] which leads to 5 compounds (144-a-e) of reasonable submicromolar potency as shown in Fig. 42. Further evaluation against a wide variety of cell lines, which has high expression levels of Mcl-1/Bcl-2 (4 leukemia cancer cell lines: RPMI-8226, KM-3, Jurkat and Molt-4; 3 solid cancer cell lines: HepG2, PC-3, H1299) [147] showed improved anticancer activities.

The Nikolaovska-Coleska group at Michigan University performed a HTS on 102,255 compounds using two different labeled BH3 peptides derived from the Noxa and Bid pro-apoptotic proteins and identified the difuryl-triazine (145) as an Mcl-1 inhibitor [148]. The compound was further studied as part of a structure-activity relationship by substituting various groups in the place of furan rings and the benzamide. They attained 146a which was 10 times more potent compound than 145, as shown in Fig. 43. Later, in their another study, they redesign the chemical core of 145, which resulted into 146b which displayed equipotency binding to Mcl-1 and Bfl-1. Using structure-based design they improved the design and attained 146c with 15-fold potency than 146a. The 146c as dual inhibitor of Mcl-1 and Bfl-1 (Ki under 100 nM for Mcl-1 and Bfl-1 compared to Bcl-2 and Bcl-xL (Ki > 25 $\mu$M) [149]. Further, co-crystal structure of 146c binding to Mcl-1 sheds light on its molecular interaction where again an interaction with its COOH group with Arg263 acting as main anchoring point; in addition, its 2-phenylethanolnethio group interacting with Met231. The phenethylthio substituent is projected toward the p1 pocket towards hydrophobic side chain of Leu235 [149]. The target selectivity of 146c was evaluated using EJ-Myc lymphoma cell lines, which were engineered to overexpress anti-apoptotic Bcl-2 proteins and showed dose-dependent increase in cell death with significant effects at 50 $\mu$M [149].

Zhu & Zhou (School of Pharmacy, Second Military Medical University, Shanghai, China) identified a broad-spectrum inhibitor of Bcl-2 family proteins. They developed two different molecules 147 and 148 [150], forecasted their benzamide moiety binding to P1 pocket of Mcl-1. In their subsequent study, they improved the selectivity for the P1 pockets in the anti-apoptotic Bcl-2 proteins and identified the Mcl-1 selective ligand 149, which was 70-fold more potent for Mcl-1 than Bcl-2 and nearly 330-fold more potent for Mcl-1 than Bcl-xL [151], as shown in Fig. 44.

Forma Therapeutics reported nanomolar Mcl-1 inhibitor (150) using high throughput screening [152]. The co-crystal structure of 150 binding with Mcl-1 protein disclosed interactions: (a) the oxygen atom of the amide lies within H-bonding distance with His224 (3.35 Å); (b) benzyl ring found close to the P4 pocket; (c) The 2-CI and 5-CF$_3$ groups projected towards hydrophobic regions of the P2 pocket; (d) 2,5-disubstituted phenyl ring utilizes a pocket, which is absent in the BH3 bound Mcl-1 structure, demonstrating the plasticity of the P2 pocket of Mcl-1; (e) oxygen of sulfonamide found close to Arg263 (3.46 Å) (f) Sulfonamide linkage pushes the 2- chloro-5-CF$_3$ phenyl group towards the deepest part of P2 pocket while methyl thiophene ring close to the edge between the P2 and P3 sites [152] as shown in Fig. 45.

Fang research group from Shandong university used structure-based virtual screening approach combining an interaction-based pharmacophore (IBP) model and stepwise molecular docking for the identification of selective Mcl-1 inhibitors. They reported 151 as a preferential Mcl-1 inhibitor (Mcl-1 Ki = 0.53 ± 0.07 $\mu$M; Bcl-2 Ki = 15 ± 1.5 $\mu$M; Bcl-xL Ki = 50 $\mu$M) [153], as shown in Fig. 45.

The Servier Research Institute of Medicinal Chemistry reported thienopyrimidine amino acids as antiapoptotic protein inhibitor 152 [154]. Further structure-guided fine-tuning of molecules showed aromatic substituents at 5-position of thienopyrimidine core have restricted rotation due to the presence of an ortho-substituent, which assists these molecules to attain selective binding conformation with Mcl-1 protein and leads to potent inhibitor 152a. However, the other atropisomer of 152a found 25 $\mu$M which is 1000 times less active than 152a. Further, in vivo pharmacokinetic studies in mice showed that oral bioavailability 9% for 152a which indicated its limited absorption [154] as shown in Fig. 46.

5.18. Inhibitors based on natural products

Marinopyrroles were one of the most studied naturally derived Mcl-1 inhibitors and were described earlier in this review under...
the pyrrole subsection. Meiogynins is another well studied class of natural products inspired Mcl-1 inhibitors. Meiogynin A was not found to be cytotoxic, while inhibiting Mcl-1, however its derivatives showed cytotoxicity and induced apoptosis as a dual inhibitor of Bcl-xL and Mcl-1 proteins (as seen in [158, 159, & 160]). Further derivatization, by incorporating aromatic side chain in the structure did not change its dual inhibition of Bcl-xL and Mcl-1 but improved it affinity towards both targets as could be seen for compounds 153 and 154 [156,157]. Further derivatization showed the ambiguous nature of the inhibition of Bcl-2 proteins where most of the derivatives were dual inhibitors of Bcl-2 and Mcl-1 (155 to 157) [158], as shown in Fig. 47.

The Scripps Institution of Oceanography Chemical at University of California (San Francisco) investigated small molecules from marine-derived fungi as a part of NCI-supported collaborative program to identify target-specific agents for the treatment of cancer. They screened various marine microbial extracts for the ability to inhibit protein-protein interactions. They studied one of the fungal strain CNL-523 within the genus Cryptosphaeria as shown significant cytotoxicity towards HCT-116 colon carcinoma cell line. Further bioactivity guided screening leads to the novel class of terpenoids, eremophilane terpenoids. The most complex natural product Cryptosphaerolide was found an inhibitor of Mcl-1 (Mcl-1/Bak, TR-FRET, Ki = 11.4 μM).

With an in vitro cancer cell cytotoxicity of 4.5 μM (IC50) toward an HCT-116 colon. Structurally, it possesses two substructures conjugated by an ester bond: cyclic and acyclic. However, absolute stereochemistry was reported for cyclic substructure but not for the acyclic substructure. Also, they observed [159] that the cyclic structure did not show any Mcl-1 inhibition activity for the cyclic substructure of cryptosphaerolide as shown in Fig. 48.

Also, various other natural Mcl-1 inhibitors were discovered in past but most of them are either preferential selective for other Bcl-2 family members than Mcl-1 or have moderate Mcl-1 potencies. Their structures are described in Fig. 48 and corresponding Mcl-1 activities in Table 1. Biological data for other natural product derivatives mentioned above are also collected in Table 1.

### 5.19. Metal complexes based Mcl-1 inhibitors

The copper (II) complexes containing β-carboline ligands were designed and synthesize as Mcl-1 inhibitors [164], based on reasoning: a) various indole containing based molecules were identified as potent and selective Mcl-1 inhibitors as mentioned above in subsection of indoles; b) β-carbolines are also classified as potential antitumor agents [165] and C1, C3, N9-position of β-carboline skeleton were found important for their antitumor activity [166]; c) also, compels lipophilic ligand efficiency, which could improve the drug like character of the molecule [167]; d) copper complexes has comparatively lower toxicity than platinum-based drugs [168].

To improve the binding affinity to the hydrophobic pocket of Mcl-1, various hydrophobic groups were used at N9 position of β-
carboline scaffold. The anticancer and Mcl-1 binding affinity assays show a general trend where copper complexes containing β-carboline ligands were found comparatively cytotoxic/better Mcl-1 affinity then their parent β-carboline in cancer cells. It has been also found that hydrophobic substitution at N9 is key for the cytotoxicity activity and Mcl-1 affinity in the cancer cells [164]. However, these copper (II) complexes were also found Mcl-1 selective in comparison to the other antiapoptotic proteins (such as Bcl-2, Bcl-xl, Bcl-w, A1/Bfl-1s). Compound 175 (Fig. 49) was found first metal based Mcl-1 inhibitor with potent antitumor activity via Bax/Bak mediated apoptosis. Further evaluation of 175 significantly inhibition of tumor growth and improved the survival time in mouse xenograft model [164].

5.20. PROTAC strategies

Recent reports have shown the application of PROTAC (proteolysis targeting chimera) to target to Mcl-1 protein to degradation in cells. A PROTAC is bifunctional compound, consisting of two components. One component is a ligand for the Mcl-1 protein, while the other component recruits the E3 ligase for ubiquitination and subsequently degradation. Wang et al. at Dalian University of Technology have reported the development of 176 and 177 as PROTACs by using selective Mcl-1 inhibitor (analogues of compounds 65 and 66 discussed above) which have been covalently linked with the E3 ligase cereblon-binding ligand (CRBN) pomalidomide as shown in Fig. 50 [169]. The PROTACS, 176 and 177, were potent and selectively induce degradation to the Mcl-1 (DC50 = 3.0 μM) and Bcl-2 protein (DC50 = 0.7 μM). The PROTAC 177-induced Mcl-1 ubiquitination translated into more lethality in Mcl-1-dependent H23 cells compared to the potent Mcl-1/Bcl-2 inhibitor A-1210477 [169]. Furthermore, the morpholine residue of A1210477 was replaced with a piperazine, which facilitated ligation (ligated point highlighted in red color) and generating the PROTAC (178) with the thalidomide containing CRBN binding agent as shown in Fig. 50, [170]. Surface plasma resonance studies revealed a decrease in the potency of 178 (Kd = 30 nM) with respect to A-1210477 (Kd = 19 nM) [170].
6. Conclusion

Various compounds have been designed, synthesised, or isolated from natural sources, and evaluated as Mcl-1 ligands. As Mcl-1 shares similar active site structural features with other members of the Bcl-2 family, it poses a challenge to attain ligands with preferentially selectivity against it. Many reported inhibitors in this review showed the selective targeting of Mcl-1. Binding of these to the same region as that of the BH3 helix of the apoptotic proteins seems to be a requirement in order to achieve this. While some are effective inhibitors it is the case that some inhibitors lead to an increase in the level of Mcl-1 while others reduce it, resulting in different fates for cells, which cannot be predicted by binding affinity measurement alone. The various inhibitors identified contain rigid (poly)cyclic scaffolds (see Table 2 for a list of nM range inhibitors where Ki are available from FPA) substituted with a carboxylic acid, or similar biososere, to interact with Arg263 of the NWGR domain. Also, commonly the ligands have a hydrophobic hibitors where Ki are available from FPA) substituted with a carboxylic acid, or similar biososere, to interact with Arg263 of the NWGR domain. Also, commonly the ligands have a hydrophobic hibitors where Ki are available from FPA) substituted with a carboxylic acid, or similar biososere, to interact with Arg263 of the NWGR domain. Also, commonly the ligands have a hydrophobic hibitors where Ki are available from FPA) substituted with a carboxylic acid, or similar biososere, to interact with Arg263 of the NWGR domain. Also, commonly the ligands have a hydrophobic hibitors where Ki are available from FPA) substituted with a carboxylic acid, or similar biososere, to interact with Arg263 of the NWGR domain. Also, commonly the ligands have a hydrophobic hibitors where Ki are available from FPA) substituted with a carboxylic acid, or similar biososere, to interact with Arg263 of the NWGR domain. Also, commonly the ligands have a hydrophobic hibitors where Ki are available from FPA) substituted with a carboxylic acid, or similar biososere, to interact with Arg263 of the NWGR domain. Also, commonly the ligands have a hydrophobic hibitors where Ki are available from FPA) substituted with a carboxylic acid, or similar biososere, to interact with Arg263 of the NWGR domain. Also, commonly the ligands have a hydrophobic...
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