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Structural Re-Engineering of the α -Helix Mimetic JY-1-106 into Small-Molecules: Disruption of the Mcl-1–Bak-BH3 Protein–Protein Interaction with 2,6-Di-Substituted Nicotinates

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Abstract

The disruption of aberrant protein–protein interactions (PPI) with synthetic agents remains a challenging goal in contemporary medicinal chemistry but some progress has been made. One such dysregulated PPI is that between the anti-apoptotic Bcl-2 proteins, including Mcl-1, and the α -helical BH3 domains of its pro-apoptotic counterparts, such as Bak. Herein, we describe the discovery of small-molecule inhibitors of the Mcl-1 oncoprotein based on a novel chemotype. Particularly, re-engineering of our α -helix mimetic JY-1-106 into 2,6-di-substituted nicotinates afforded inhibitors of comparable potencies but with significantly reduced molecular weights. The most potent inhibitor **1r** ($K_i = 2.90 \mu\text{M}$) likely binds in the p2 pocket of Mcl-1 and engages R263 in a salt bridge through its carboxylic acid, as supported by 2D ^1H – ^{15}N HSQC NMR data. Significantly, inhibitors were easily accessed in just four steps, which will facilitate optimization efforts.

Abstract

Keywords

Mcl-1; protein–protein interaction; cancer; nicotinic acid; JY-1-106

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Introduction

Apoptosis, or programmed cell death, ensures normal tissue homeostasis, and its dysregulation can lead to several human pathologies, including cancer.^[1,2] Whilst the extrinsic apoptosis pathway is initiated through the activation of cell surface receptors, the intrinsic apoptosis pathway occurs at the mitochondrial outer membrane, and is governed by the binding interactions between pro- and anti-apoptotic Bcl-2 family proteins.^[3] The anti-apoptotic proteins, which include Bcl-2, Bcl-xL, Bcl-w, Bcl-A1 and Mcl-1, bind to the α -helical Bcl-2 homology-3 (BH3) domains of pro-apoptotic proteins, which include Bax, Bak and Bim, effectively neutralizing them and inhibiting apoptosis.^[4,5] In many cancers, the anti-apoptotic Bcl-2 proteins are upregulated and in this way the cancer cells can evade apoptosis.^[2] Accordingly, synthetic strategies to inhibit the Bcl-2 proteins has been adopted as a new avenue for cancer therapy.^[6]

The *Mcl-1* gene is frequently overexpressed in human cancers,^[7] including lung,^[8] breast,^[9] pancreatic,^[10] cervical cancers^[11] as well as leukaemia.^[12] Importantly, genetic mouse models have shown that Mcl-1 is involved in tumour initiation and development.^[13] Thus, for these reasons, Mcl-1 has emerged as an attractive target for anti-cancer therapy. Indeed, in the last few years, several groups, including ours, have developed inhibitors of Mcl-1,^[14,15] which span a variety of scaffolds such as indoles,^[16-18] 5-phenylsalicylates, 1-hydroxynaphthalenes,^[19] 1-hydroxy-2-naphthoates^[20] and tetrahydroquinolines,^[21] all of which share a carboxylic acid to bind R263. In addition, the neutral pyrogallol MIM1^[22] and an 8-hydroxyquinoline derivative^[23] have also been described as Mcl-1 inhibitors, and it is possible these neutral agents still engage R263 through hydrogen bond(s). However, no Mcl-1 inhibitor has advanced to the clinic. Finally, it is especially noteworthy that Leverson and colleagues recently reported that their highly potent and selective Mcl-1 inhibitor A-1210477 demonstrates ontarget cellular effects, thus establishing for the first time that Mcl-1 is a viable cell target for the development of novel anti-cancer therapies.^[24]

In addition to conventional small-molecule strategies, Mcl-1 inhibitors have been fashioned through the structural mimicry of BH3 α -helical domains.^[25-31] However, such “ α -helix mimetics” targeting the Bcl-2 proteins have not advanced to the clinic, not for this nor any other targeted PPIs,^[32-34] and this may be due to their high molecular weights (MWs), which contravene Lipinski’s rule as they are typically in excess of 500, and/or a lack of specificity. α -Helix mimetics typically reproduce functionality on only one face of the α -helix, specifically the *i*, *i*+3/4 and *i*+7 positions. These are often rather hydrophobic molecules and their inhibitory activities might stem from the hydrophobic effect, at least in part. We believe that the recognition properties of an α -helix mimetic can be enhanced by mimicking additional faces of the α -helix.^[28,31,35] On the other hand, a successful α -helix mimetic might be used as the inspiration from which a more traditional small-molecule could be realized. Along these lines, we herein describe the structural re-engineering of our α -helix mimetic JY-1-106^[26,27] into 2,6-di-substituted nicotinates that are equipotent at inhibiting Mcl-1 but bear significantly reduced MWs, and are selective for Mcl-1 over Bcl-xL.

Design

BH3 α -helices recognize Mcl-1 through binding four hydrophobic sub-pockets p1-p4 via conserved hydrophobic residues at the i , $i+3/4$ and $i+7$ and $i+11$ positions along one face of the α -helix. Additionally, a conserved aspartate on the opposing face of the α -helix binds R263. As an example, the co-crystal structure of Mcl-1–Bim-BH3 is given in Figure 1A, and the key residues of the Bim-BH3 α -helix are shown more clearly in Figure 1B. We recently reported on the discovery of the BH3 α -helix mimetic JY-1-106 (Figure 1C), which is a dual Bcl-x_L/Mcl-1 inhibitor (Bcl-x_L: $K_i = 179 \pm 24$ nM; Mcl-1: $K_i = 1.79 \pm 0.15$ μ M).^[26,27] The isopropyl groups of JY-1-106 are believed to target the p2, p3 and p4 pockets of Mcl-1, whilst the role of the carboxylic acid group was to enhance compound solubility rather than target any residue in particular.

We hypothesized that the central picolinamide subunit of JY-1-106 could serve as a scaffold to generate synthetically-accessible, small-molecule inhibitors of Mcl-1. We postulated that retaining but modifying the substitution at both positions flanking the pyridine nitrogen of the picolinamide fragment might still permit binding to both the p2 and p3 pockets, whilst relocation of the carboxylic acid *meta* to the nitrogen atom might also allow the formation of a salt bridge with R263. One such simple molecule, **1a**, is shown in Figure 2. Computational modeling of **1a** with Mcl-1 (after ligand extraction, Mcl-1 coordinates from PDB ID: 4HW2) was conducted with GOLD, and a low energy docked solution is given in Figure 3. The small-molecule–Mcl-1 crystal structure 4HW2 was selected for docking studies because our synthetic ligand is now more akin to a traditional small-molecule rather than an α -helix or an α -helix mimetic. The phenyl ring snugly fits into the p2 pocket (partially obscured) and the isobutyl group is pointed towards the p3 pocket, docking nicely into a new pocket bounded by A227 and M231 not found in crystal structures of Mcl-1 with BH3 ligands.^[16] A weak salt bridge (3.9 Å) is detected between the carboxylic acid and R263, although given the plasticity^[16] of the hydrophobic groove of Mcl-1, as revealed by multiple crystal structures (e.g. PDB IDs: 4HW2, 4HW3, 4HW4, 3WIX), we envisage stronger interactions between the acid and R263 may be possible. With this modeling data in hand, we then prepared a library of analogs of **1a** in which we modified the alkoxy (R¹O) and aryloxy (R²O) groups.

Accordingly, 2,6-dichloronicotinic acid (**2**) was regioselectively alkoxylated *ortho* to the acid with various R¹OH alcohols to afford **3** using chemistry recently developed in our laboratory.^[36] Incidentally, the ability to selectively control the displacement of the two chlorines in 2,6-dichloronicotinic acid directly facilitated the development of a new α -helix mimetic.^[37] Subsequent esterification of **3** was accomplished with thionyl chloride and methanol to yield methyl esters **4**. A second S_NAr reaction was effected with phenols R²OH to deliver compounds **5**, or thiophenols R²SH to yield compounds **6**, which were finally saponified to furnish the target molecules **1a–1r** and **7a–7d**, respectively, in just four steps.

Results

In order to quantify the abilities of compounds to inhibit Mcl-1, a fluorescence polarization competition (FPC) assay was employed in which the compounds competed with an FITC-

labeled Bak-BH3 peptide for binding to Mcl-1¹⁷²⁻³²⁷. IC₅₀ data resulting from the analysis were converted to K_i values using the Nikolovska-Coleska equation.^[38] For further details, see the Experimental Section. The simplest compound **1a** exhibited the weakest binding to Mcl-1 with a K_i of 260 μ M. However, the introduction of halogens into the phenyl R² group resulted in improved inhibition, and the effect was cumulative, which can be seen by comparing the data for **1c** and **1e** with that for **1f**. Indeed, **1f** (K_i = 6.69 μ M) was almost 40-fold more potent than parent compound **1a**. Naphthyl R² derivatives also proved better inhibitors of Mcl-1 over **1a** with the 1-naphthyl derivative **1b** superior to its regioisomer **1g**. Whilst substitution off the phenyl R² moiety with hydrophobic groups always enhanced the inhibitory activity over the parent compound **1a**, *para*-substitution led to the greatest increases in inhibition. For example, compare the data for 2-chlorophenyl (**1c**) with 4-chlorophenyl (**1e**) derivatives, and 3,5-dimethylphenyl (**1k**) with 4-methylphenyl (**1h**) derivatives. In every case, replacement of the R² ether oxygen (X group) with a sulfur atom (compounds **7a–7d**) resulted in an improvement in inhibitory activity of between 2.5-fold and 12-fold. **7c** was the most potent of the thioether series with a K_i of 3.69 μ M. Finally, we examined the effect of varying the R¹ group. As the size of the hydrophobic R¹ group was increased, the resulting inhibitor generally became more potent. In fact, **1r** was the most potent compound of the entire series: K_i = 2.90 μ M.

Evidence for the direct binding of **1r** to Mcl-1 was provided by heteronuclear NMR studies. 2D ¹H–¹⁵N heteronuclear single quantum coherence (HSQC) spectra of ¹⁵N-Mcl-1 were collected in the absence and presence of **1r**. An overlay of the two spectra (Figure 4: black = Mcl-1 alone; red = Mcl-1 + **1r**) revealed significant chemical shift changes, particularly for R263 and those residues located around the p2 pocket; those 0.3 ppm have been mapped onto the Mcl-1 crystal structure 4HW2 and shown in red in Figure 5.

Discussion

Overall, larger and more hydrophobic R¹ and R² groups afforded greater inhibition of Mcl-1. This finding may be due to more efficient interactions with the hydrophobic p2 pocket, which is supported by the HSQC NMR data. The inhibitory activity was enhanced further still by replacing the R² phenyl ether oxygen with a sulfur atom, which can be rationalized by the greater hydrophobicity of sulfur over oxygen coupled with the idea that the R² group is directed into the hydrophobic p2 pocket. In addition, the carboxylic acid of the inhibitors was critical to activity since the methyl ester derivative of **1r** exhibited no effect on the Mcl-1–Bak-BH3 PPI (data not shown), which is consistent with the design rationale wherein the carboxylic acid was proposed to form a salt bridge with R263.

Many of the inhibitors described in the present work exhibited similar K_i values to the α -helix mimetic JY-1-106 (K_i = 1.79 μ M), yet they have much lower MWs. Indeed, the two most potent compounds **7c** and **1r** are about two-thirds the mass of JY-1-106, bringing their MWs within the threshold proposed by Lipinski (MW < 500). Furthermore, their lipophilicities are also significantly reduced. For example, cLogP and cLogD (pH 7.4) for JY-1-106 are 7.37 and 4.32, respectively, whilst the corresponding values for **1r** are 6.11 and 2.98, respectively. Finally, we evaluated the selectivity profile of **1r** for Bcl-2 family members. As shown in Figure 6, the IC₅₀ values for **1r** against Mcl-1 and Bcl-x_L were 11.35

$\pm 4.85 \mu\text{M}$ and $31.53 \pm 6.19 \mu\text{M}$, respectively, which correspond to K_i values of $2.87 \pm 1.24 \mu\text{M}$ and $2.04 \pm 0.40 \mu\text{M}$, respectively. It is noteworthy that the 10-fold Bcl-x_L-selectivity of JY-1-106 has been almost completely eroded upon its transformation into small-molecule nicotines; future work will focus on the acquisition of crystal structures of **1r** with Mcl-1 and Bcl-x_L to assist in further enhancement in affinity and selectivity for Mcl-1.

Conclusions

In summary, using our previously reported α -helix mimetic JY-1-106 as a starting point, we have developed a new family of readily accessible Mcl-1 selective inhibitors based on a 2,6-di-substituted nicotinic acid core. HSQC NMR data supported the hypothesis that the R¹ group and/or the R² group probe(s) into the p2 pocket, whilst the carboxylic acid likely engages R263 through a salt bridge, although the exact binding mode remains unknown at this time. It is noteworthy that our most potent compounds are about as potent as the α -helix mimetic JY-1-106 yet exhibit significantly reduced MWs and cLogDs. Additional structure-activity studies are currently underway, which, as well as enhancing compound affinity, will include further investigation into a better understanding of the specific binding mode and how this can be exploited towards achieving selectivity for specific Bcl-2 family members of anti-apoptotic proteins.

Experimental Section

GOLD Molecular Docking

Compound **1a** was first MM2 energy minimized in ChemDraw3D. The 4HW2 pdb file was uploaded into GOLD, all the appropriate hydrogens were added, waters were removed and then the ligand was extracted. The binding site was defined as 10 Å about the side chain sulfur of Met231; no further constraints were used.

General Procedure A First S_NAr

Alcohol R¹OH (5 eq.) was added to a solution of anhydrous THF (0.10 M). The reaction was then cooled to 0 °C then NaH (5 eq.) was slowly added to the solution. The reaction mixture was stirred for 30 min. before 2,6-dichloronicotinic acid (1 eq.) was added portionwise, and then the mixture was heated overnight at 60 °C in an oil bath. The next day, the reaction was quenched with brine (1 mL), then concentrated to *ca* 10 mL. The reaction mixture was partitioned between CH₂Cl₂ and 0.1 M HCl. The organic layer was collected, and the aqueous layer was extracted with CH₂Cl₂ twice further. The organic layers were combined, dried with Na₂SO₄, concentrated to yield the crude *ortho*-substituted nicotinic acid.

General Procedure B: Esterification

The *ortho*-substituted nicotinic acid (1 eq.) from *General Procedure A* was dissolved in MeOH (0.10 M), Thionyl chloride (3 eq.) was slowly added to the reaction mixture 0 °C, and then the mixture was refluxed for 3 h. The reaction mixture was concentrated to dryness. The crude material was adsorbed onto silica gel, then purified by flash column chromatography (eluent: Hex/EtOAc, 4:1).

General Procedure C: Second S_NAr

The methyl ester (1 eq.) from *General Procedure B* was dissolved in DMF (0.10 M). The appropriate phenol (R²OH) or thiophenol (R²SH) (4 eq.) was added to the reaction followed by K₂CO₃ (3 eq.). The reaction was heated at 100 °C for 72 h. The reaction was partitioned between EtOAc and H₂O. The organic layer was isolated and washed repeatedly (5x) with H₂O. The organic layer was then collected, dried with Na₂SO₄ and concentrated under vacuum. The crude material was adsorbed onto silica gel, then purified by flash column chromatography (eluent: Hex/EtOAc, 4:1).

General Procedure D: Saponification

The *o,p*-disubstituted nicotinate (1 eq.) from *General Procedure C* was dissolved in a 3:1:1 solution of THF/MeOH/H₂O (0.10 M). LiOH•H₂O (4 eq.) was added, and the reaction mixture was stirred at RT for 3 h. If necessary, the reaction volume was reduced to *ca* 10 mL, then partitioned between ether and H₂O. The ethereal layer was discarded, the aqueous layer was acidified with 0.1 M HCl, then extracted with CH₂Cl₂. The organic layer was collected, the aqueous layer was extracted once more with CH₂Cl₂, then the organic extractions were combined, dried with Na₂SO₄, filtered and concentrated to produce the *o,p*-disubstituted nicotinic acid.

Fluorescence polarization experiments

Fluorescence polarization experiments were conducted using a BMG PHERAstar FS multimode microplate reader equipped with two PMTs for simultaneous measurements of the perpendicular and parallel fluorescence emission. For the competition assay, inhibitors were titrated into a solution of Mcl-1¹⁷²⁻³²⁷ (or Bcl-x_L²⁻²¹²) and the fluorescently-labeled Bak-BH3 peptide FITC-Ahx-GQVGRQLAIIGDDINR-CONH₂ (hereafter “FITC-Bak”), where FITC = fluorescein isocyanate; Ahx = 6-aminohexanoyl linker. Regression analysis was carried out using Origin (OriginLab, Northampton, MA) to fit the data to the Hill equation to determine the initial binding affinity (*K*_d) and the IC₅₀ in the FPC assay. *K*_ds for the FITC-Bak peptide to Mcl-1 and Bcl-x_L were 33.8 ± 0.50 nM and 6.67 ± 0.05 nM, respectively. For the fluorescence polarization competition titrations, an equation derived by Nikolovska-Coleska *et al.* was used to calculate the *K*_i values from the IC₅₀ data.^[38] All experiments were run in three biological replicates, each in triplicate.

NMR Spectroscopy

2D HSQC (heteronuclear single quantum coherence) NMR spectra were collected at 25 °C with a Bruker AVANCE 800 NMR spectrometer (800.27 MHz for protons) equipped with pulsed-field gradients, four frequency channels, and triple resonance, z-axis gradient cryogenic probes. A one-second relaxation delay was used, and quadrature detection in the indirect dimensions was obtained with states-TPPI phase cycling; initial delays in the indirect dimensions were set to give zero- and first-order phase corrections of 90° and -180°, respectively. Data were processed using the processing program nmrPipe on Linux workstations. All proton chemical shifts are reported with respect to the H₂O or HDO signal, taken to be 4.658 ppm relative to external TSP (0.0 ppm) at 37 °C. The ¹⁵N chemical shifts were indirectly referenced using the zero-point frequency at 37 °C of 0.10132905

for ^1H - ^{15}N , as previously described. Uniformly ^{15}N -labeled Mcl-1 was used to collect two-dimensional ^1H - ^{15}N -fast HSQC spectra of Mcl-1 with and without compound to detect changes in the backbone ^{15}N and ^1H resonances of Mcl-1 due to the direct interaction with compound **1r**, which itself was initially dissolved in 100% d_6 -DMSO. The NMR samples contained 61.9 μM ^{15}N -labeled Mcl-1, 20 mM HEPES, pH 6.8, 36.4 mM NaCl, 0.20 mM NaN₃, 2.2 mM DTT, 4.2% DMSO, 20% D₂O. Concentrated **1r** was added in excess to a final protein:ligand ratio of 1:2 (i.e. 123.8 μM). NMR datasets were acquired with 200 indirect points and 32 scans at 299K on a Bruker Avance 800 MHz spectrometer equipped with a z-gradient cryogenic probe.

Supplementary Material

Refer to Web version on PubMed Central for supplementary material.

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References

- [1]. Hanahan D, Weinberg RA. Cell. 2000; 100:57–70. [PubMed: 10647931]
- [2]. Adams JM, Cory S. Oncogene. 2007; 26:1324–1337. [PubMed: 17322918]
- [3]. Kroemer G, Galluzzi L, Brenner C. Physiol. Rev. 2007; 87:99–163. [PubMed: 17237344]
- [4]. Cory S, Adams JM. Nat. Rev. Cancer. 2002; 2:647–656. [PubMed: 12209154]
- [5]. Youle RJ, Strasser A. Nat. Rev. Mol. Cell Biol. 2008; 9:47–59. [PubMed: 18097445]
- [6]. Billard C. Mol. Cancer Ther. 2013; 12:1691–1700. [PubMed: 23974697]
- [7]. Beroukhi R, Mermel CH, Porter D, Wei G, Raychaudhuri S, Donovan J, Barretina J, Boehm JS, Dobson J, Urashima M, et al. Nature. 2010; 463:899–905. [PubMed: 20164920]
- [8]. Song L, Coppola D, Livingston S, Cress WD, Haura EB. Cancer Biology & Therapy. 2004; 4:267–276. [PubMed: 15753661]
- [9]. Ding Q, He X, Xia W, Hsu J-M, Chen C-T, Li L-Y, Lee D-F, Yang J-Y, Xie X, Liu J-C, et al. Cancer Res. 2007; 67:4564–4571. [PubMed: 17495324]
- [10]. Miyamoto Y, Hosotani R, Wada M, Lee JU, Koshiba T, Fujimoto K, Tsuji S, Nakajima S, Doi R, Kato M, et al. Oncology. 1999; 56:73–82. [PubMed: 9885381]
- [11]. Zhang T, Zhao C, Luo L, Zhao H, Cheng J, Xu F. Med. Oncol. 2012; 29:1985–1991. [PubMed: 21674276]
- [12]. Andersen MH, Becker JC, Straten P. Thor. Leukemia. 2005; 19:484–485. [PubMed: 15618955]
- [13]. Glaser SP, Lee EF, Trounson E, Bouillet P, Wei A, Fairlie WD, Izon DJ, Zuber J, Rappaport AR, Herold MJ, et al. Genes Dev. 2012; 26:120–125. [PubMed: 22279045]
- [14]. Belmar J, Fesik SW. Pharmacology & Therapeutics. 2015; 145:76–84. [PubMed: 25172548]
- [15]. Chen L, Lanning ME, Fletcher S. Austin J Anal Pharm Chem. 2015; 1:1015.
- [16]. Friberg A, Vigil D, Zhao B, Daniels RN, Burke JP, Garcia-Barrantes PM, Camper D, Chauder BA, Lee T, Olejniczak ET, et al. J. Med. Chem. 2013; 56:15–30. [PubMed: 23244564]
- [17]. Burke JP, Bian Z, Shaw S, Zhao B, Goodwin CM, Belmar J, Browning CF, Vigil D, Friberg A, Camper DV, et al. J. Med. Chem. 2015; 58:3794–3805. [PubMed: 25844895]
- [18]. Bruncko M, Wang L, Sheppard GS, Phillips DC, Tahir SK, Xue J, Erickson S, Fidanze S, Fry E, Hasvold L, et al. J. Med. Chem. 2015; 58:2180–2194. [PubMed: 25679114]
- [19]. Abulwerdi FA, Liao C, Mady AS, Gavin J, Shen C, Cierpicki T, Stuckey JA, Showalter HDH, Nikolovska-Coleska Z. J. Med. Chem. 2014; 57:4111–4133. [PubMed: 24749893]

- [20]. Lanning ME, Fletcher S, et al. *J. Med. Chem.* 2015 submitted.
- [21]. Chen L, Fletcher S, et al. *Org. Biomol. Chem.* 2015 submitted.
- [22]. Cohen NA, Stewart ML, Gavathiotis E, Tepper JL, Bruekner SR, Koss B, Opferman JT, Walensky LD. *Chem. Biol.* 2012; 19:1175–1186. [PubMed: 22999885]
- [23]. Richard DJ, Lena R, Bannister T, Blake N, Pierceall WE, Carlson NE, Keller C, Eberhart, Koenig M, He Y, Minond D, Mishra J, Cameron M, Spicer T, Hodder P, Cardone MH. *Bioorg. Med. Chem.* 2013; 21:6642–6649. [PubMed: 23993674]
- [24]. Levenson JD, Zhang H, Chen J, Tahir SK, Phillips DC, Xue J, Nimmer P, Jin S, Smith M, Xiao Y, et al. *Cell Death Dis.* 2015; 6:e1590. [PubMed: 25590800]
- [25]. Kazi A, Sun J, Doi K, Sung S-S, Takahashi Y, Yin H, Rodriguez JM, Becerril J, Berndt N, Hamilton AD, et al. *J. Biol. Chem.* 2011; 286:9382–9392. [PubMed: 21148306]
- [26]. Yap JL, Cao X, Vanommeslaeghe K, Jung K-Y, Peddaboina C, Wilder PT, Nan A, MacKerell AD, Smythe WR, Fletcher S. *Org. Biomol. Chem.* 2012; 10:2928–2933. [PubMed: 22395339]
- [27]. Cao X, Yap JL, Newell-Rogers MK, Peddaboina C, Jiang W, Papaconstantinou HT, Jupiter D, Rai A, Jung K-Y, Tubin RP, et al. *Mol. Cancer.* 2013; 12:42. [PubMed: 23680104]
- [28]. Jung K-Y, Vanommeslaeghe K, Lanning ME, Yap JL, Gordon C, Wilder PT, MacKerell AD, Fletcher S. *Org. Lett.* 2013; 15:3234–3237. [PubMed: 23763721]
- [29]. Moon H, Lee WS, Oh M, Lee H, Lee JH, Im W, Lim H-S. *ACS Comb. Sci.* 2014; 16:695–701. [PubMed: 25336412]
- [30]. Barnard A, Long K, Martin HL, Miles JA, Edwards TA, Tomlinson DC, Macdonald A, Wilson AJ. *Angew. Chem. Int. Ed.* 2015; 54:2960–2965.
- [31]. Lanning ME, Wilder PT, Bailey H, Drennen B, Cavalier M, Chen L, Yap JL, Raje M, Fletcher S. *Org. Biomol. Chem.* 2015; 13:8642–8646. [PubMed: 26204921]
- [32]. Azzarito V, Long K, Murphy NS, Wilson AJ. *Nature Chemistry.* 2013; 5:161–173.
- [33]. Lanning M, Fletcher S. *Future Med. Chem.* 2013; 5:2157–2174. [PubMed: 24261892]
- [34]. Moon H, Lim H-S. *Curr. Opin. Chem. Biol.* 2015; 24:38–47. [PubMed: 25461722]
- [35]. Lanning ME, Fletcher S. *Biology.* 2015; 4:540–555. [PubMed: 26404384]
- [36]. Yap JL, Hom K, Fletcher S. *Tetrahedron Lett.* 2011; 52:4172–4176.
- [37]. Drennen B, MacKerell AD Jr. Fletcher S. *Tetrahedron Lett.* 2015 In press.
- [38]. Nikolovska-Coleska Z, Wang R, Fang X, Pan H, Tomita Y, Li P, Roller PP, Krajewski K, Saito NG, Stuckey JA, et al. *Anal. Biochem.* 2004; 332:261–273. [PubMed: 15325294]

Figure 1.

A. Structure of Mcl-1–Bim-BH3 (PDB ID: 4HW4). Mcl-1 coloured by atom type: carbon = grey; blue = nitrogen; red = oxygen; yellow = sulfur. Bim-BH3 α-helix coloured in green. Key residues and binding pockets on Mcl-1 shown in black. **B.** Key residues of the Bim-BH3 α-helix. **C.** The α-helix mimetic JY-1-106.

Figure 2. Reduction of the α -helix mimetic JY-1-106 into the small-molecule **1a**. Labels in half-bubbles refer to Mcl-1 residues and sub-pockets.

Figure 3.
Low energy GOLD docking solution of **1a** bound to Mcl-1 (coordinates from PDB ID: 4HW2).

Figure 4. ^1H - ^{15}N HSQC spectra overlay of apo-Mcl-1 (black) and **1r**-bound Mcl-1 (red).

Figure 5.
 ^1H - ^{15}N Mcl-1 Chemical shift perturbations ≥ 0.3 ppm in the presence of **1r** mapped onto the Mcl-1 crystal structure 4HW2 are shown in red.

Figure 6. Inhibition of Mcl-1 and Bcl-x_L by compound 1r, as determined by a FPC assay with FITC-Bak. Errors represent standard deviations of three biological replicates performed in triplicate.

Scheme 1.

(a) R^1OH , NaH, THF, 60 °C, 16 h; (b) SOCl_2 , MeOH, 60 °C, 3 h; (c) R^2OH or R^2SH , K_2CO_3 , DMF, 100 °C, 72 h; (d) $\text{LiOH}\cdot\text{H}_2\text{O}$, THF–MeOH– H_2O , 3:1:1, RT, 3 h.

Table 1

Mcl-1 structure–activity relationships of 2,6-di-substituted nicotinates. IC₅₀ data from a fluorescence polarization competition assay with Mcl-1¹⁷²⁻³⁷¹ and FITC-labeled Bak were converted to K_i values using the Nikolovska-Coleska equation.³⁸

Compound Number	R ¹	R ²	X	K_i (μM)
1a			O	261 ± 33
1b			O	40.0 ± 4.2
1c			O	72.8 ± 4.6
1d			O	55.6 ± 4.9

Compound Number	R ¹	R ²	X	K _i (μM)
1e			O	39.8 ± 2.1
1f			O	6.69 ± 0.71
1g			O	59.6 ± 3.4
1h			O	87.8 ± 18.4
1i			O	47.0 ± 10.6
1j			O	12.7 ± 1.3
1k			O	123 ± 16

Compound Number	R ¹	R ²	X	K _i (μM)
1l			O	11.1 ± 2.3
7a			S	48.1 ± 7.1
7b			S	7.11 ± 0.78
7c			S	3.69 ± 0.17
7d			S	50.3 ± 3.7
1m			O	25.4 ± 1.4
1n			O	26.9 ± 8.6

Compound Number	R ¹	R ²	X	K _i (μM)
1o			O	20.0 ± 1.1
1p			O	4.58 ± 1.20
1q			O	4.62 ± 0.77
1r			O	2.90 ± 1.24

^aData represent an average of at least two biological replicates and errors represent standard deviations.