

ABSTRACT

Human cancers have multiple genetic and epigenetic alterations leading to deregulation of variety of cellular processes and the dysfunction of the programmed cell death (apoptosis) machinery is recognized as one of the cancer hallmarks. Myeloid cell leukemia-1 (Mcl-1) is a potent anti-apoptotic protein, member of the anti-apoptotic Bcl-2 family. Amplification of the gene encoding the Mcl-1 protein is a common genetic aberration in human cancer. Overexpression of Mcl-1 is associated with high tumor grade, resistance to chemotherapy and poor prognosis in many types of cancers. Thus Mcl-1 is emerging as a critical survival factor in a broad range of human cancers and represents an attractive molecular target for development of a new class of cancer therapy.

Applying an integrated screening strategy through combining high throughput and virtual screenings, multiple hit compounds with structural diversity were validated as Mcl-1 inhibitors using biochemical and biophysical methods. Based on the confirmed hit molecule with 5,6-difuran-2-yl-1,2,4-triazine core structure, we designed and synthesized a novel series of Mcl-1 inhibitors. Analyzing established structure activity relationship (SAR) together with computational docking predicted binding pose supported by HSQC NMR studies, we have *de novo* designed and optimized a class of small-molecule inhibitors of Mcl-1 using a 2,4,5 substituted benzoic acid as a scaffold. Several co-crystal structures of this class of inhibitors in complex with Mcl-1 have provided a basis for their further optimization which ultimately led to the discovery of potent and selective ligands that bind to the BH3 hydrophobic groove of the Mcl-1 protein. Mechanistic studies performed in genetically engineered cell lines revealed that our inhibitors have on-target activity and induce Bax/Bak dependent apoptosis, selectively antagonize Mcl-1 function and disrupt the interactions with pro-apoptotic Bcl-2 family proteins, concomitant with loss of mitochondrial membrane potential and subsequent cytochrome c release to the cytosol, leading to activation of the caspase cascade and apoptosis.

Using BH3 profiling, a unique functional method to measure the dependence to the anti-apoptotic proteins in live cancer cells, we identified heterogeneous dependency on Bcl-2 family members in hematologic malignancies (multiple myeloma and leukemia), as well as in solid

human cancers (head and neck squamous cell carcinoma and pancreatic cancer cell lines). The mitochondrial response to NOXA and MS1 BH3 peptides which selectively bind Mcl-1 protein, predicted the *in vitro* sensitivity to Mcl-1 inhibitors of several cell lines found to be Mcl-1 dependent, including H929 a multiple myeloma cell line. 483LM, one of the most potent developed Mcl-1 inhibitors, inhibited the cell growth and induced mechanism-based apoptotic cell death in H929 cell line. Intraperitoneal treatment of H929 cancer xenograft model with 483LM led to significant dose-dependent tumor regression. Collectively, our data demonstrate that the new class of selective Mcl-1 inhibitors has promising *in vitro* and *in vivo* efficacy warranting further development toward clinical use in the treatment of multiple myeloma and other human cancers.