Discovery of Potent Myeloid Cell Leukemia 1 (Mcl-1) Inhibitors Using Fragment-Based Methods and Structure-Based Design

Advanced Computational Methods in Drug Discovery: AI & Physics Based Simulations

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Introduction

Myeloid cell leukemia-1 (MCL-1) is an anti-apoptotic protein whose inhibition has been suggested as a promising target in various types of cancers (Osman et al., 2021). Overexpression of MCL-1 has been shown in a variety of cancer, including leukemia (Craig, 2002; Kang et al., 2008), breast cancer (Campbell et al., 2018) and pancreatic cancer (Friberg et al., 2013). In line with this observation, overexpression of MCL-1 has also been associated with poor prognosis and resistance against various conventional anticancer drugs such as paclitaxel, vincristine, and gemcitabine (Friberg et al., 2013). Interestingly, MCL-1 has also been suggested as a potential target in neurodegenerative and autoimmune diseases where apoptosis seems to play a role, such as Alzheimer's Disease (Cen et al., 2020) or Crohn's Disease (Nijhuis et al., 2017).

MCL-1 belongs to a large group of proteins called B cell lymphoma-2 (BCL-2) which are involved in controlling of apoptosis (through intrinsic pathway) via interacting with other BCL-2 family. The MCL1 protein is encoded by the MCL1 gene. MCL1 has two isoforms that are created through alternative splicing. Isoform 1 enhances cell survival by inhibiting apoptosis and isoform 2 promotes cell death by promoting apoptosis (Xiang, Yang and Bai, 2018). Despite sharing various sequence and functional similarity, MCL-1 expression is regulated differently than that of other BCL-2 members (Craig, 2002). This leads to MCL-1 having a very short half-life compared to other BCL-2 members (Akgul, 2009). Thus, MCL-1 inhibition makes MCL-1-dependent cells more susceptible to apoptosis, making it an attractive target for cancer treatment. On top of its antiapoptotic activity, MCL1 is also the only member of BCL-2 family that interacts with Proliferating Cell Nuclear Antigen (PCNA), which disrupts S-phase of cell, inhibits cell cycle progression (Fujise et al., 2000), thus further contributes to its role as anti-cancer target.

MCL-1 is primarily localized to the outer mitochondrial membrane, embedding itself via a hydrophobic tail. Under normal condition, MCL-1 promotes cell survival by suppressing cytochrome C release from mitochondria and inhibits mitochondrial outer membrane permeabilization (Wang et al., 2021). MCL-1 achieves this via heterodimerization to other proapoptotic effector of the BCL-2 family. An important binding site of MCL-1 that allows it to serve this function is the conserved hydrophobic groove that binds to BH3-only proteins (Xiang, Yang and Bai, 2018). Under normal ("survival") condition, MCL-1 sequester proapoptotic protein BAX and BAK, through binding with BH3 protein (tBid, PUMA, BIM), or directly, respectively, which reinforces its anti-apoptotic activity (Akgul, 2009; Xiang, Yang and Bai, 2018). However, when apoptotic signals are received, MCL-1 binds to NOXA instead, which lead to

Bak oligomerization and cytochrome c release form mitochondria. MCL-1's interaction with other members of the BCL2 family is illustrated in figure 1 below.

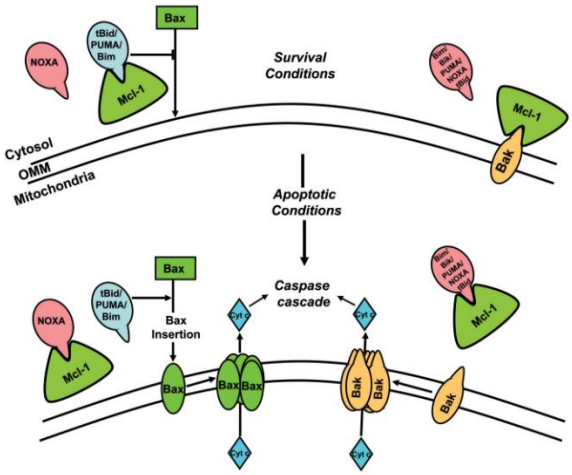


Figure 1: MCL-1 interaction with other BCL-2 protein under both survival (normal) condition and apoptotic condition. OMM: Outer Mitochondrial Domain. Taken from Akgul (2009).

Our standard reference ligand, 19G is synthesized following a NMR-based fragment screening and NOE-guided molecular modelling experiment done by Friberg et al. (2015). Original screening of compound library using SOFAST 1H-15N HMQC spectra of MCL-1 identified two classes of compound that binds to two different regions of MCL-1. Class I inhibitors are generally 6,5-fused heterocyclic carboxylic acids, meanwhile class II inhibitors are generally hydrophobic aromatic system tethered by a linker to a polar functional group. NOE-guided molecular docking then show that four-atom-long linker yield the most potent MCL-1 inhibition, as shown by the affinity constant and ligand efficiency (LE) value (Fig 2).

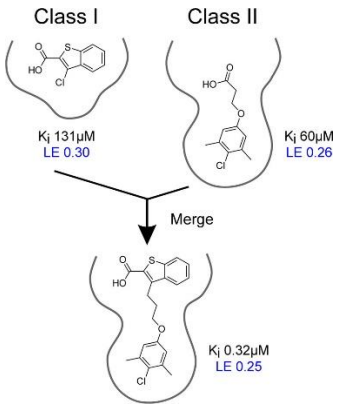
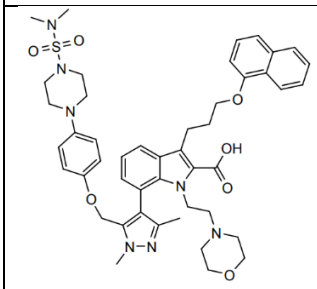
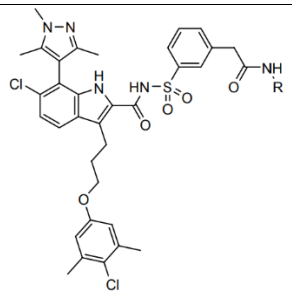
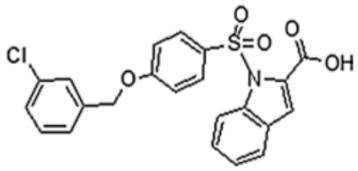
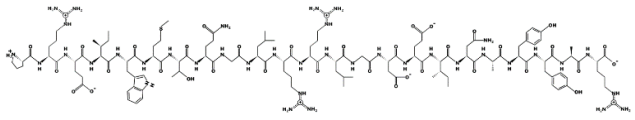
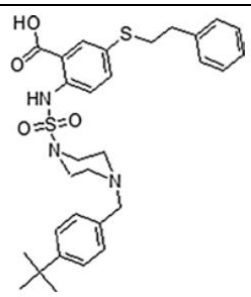


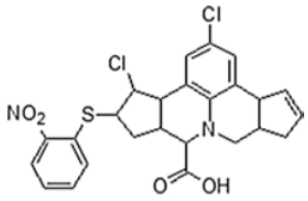
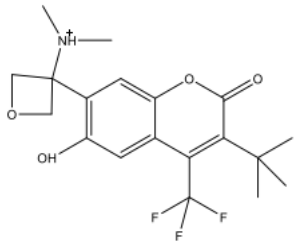
Figure 2: Synthesis of ligand 19G as done by Friberg *et al*. (2015). Ki: Inhibitory rate constant. LE: Ligand Efficiency score.

Therapeutic strategy to inhibit MCL-1 also occurs upstream or downstream of protein expression (e.g., by downregulating MCL-1 expression, or deubiquitinase inhibitors to induce MCL-1 degradation). However, for the purpose of this report, we will focus on inhibitors that specifically target the hydrophobic binding site of MCL-1. To the best of our knowledge, there are still yet a single drug that

has been established as a specific, potent inhibitor of MCL1. Some therapeutic targets out there are general inhibitors for BCL-2 family, with anti MCL-1 activity, such as Sabutoclax (Xiang, Yang and Bai, 2018). However, there are several MCL-1 specific inhibitors currently in various stages of clinical trials. Most of these inhibitors share structural similarity or backbone with various BH3 protein, but most notably, Bim and Noxa. Table 1 below showcases several MCL-1 inhibitors of in the literature that are highlighted due to their method of discovery, or as the most potent ones by their bioactivity.

Table 1: Several MCL-1 inhibitors curated from literature. Figures are taken and modified from Xiang, Yang and Bai (2018), Wang *et al.* (2021), and Osman *et al.* (2021)

Structure of Inhibitors	Bioactivity	Notes
	Ki: 0.454 nM IC50: 26.2 nM	Tested against non-small-cell lung cancer cell lines. Has also been tested in other studies using various other cell lines
	NA	Discovered using fragment-based drug design and structure-based drug design approaches
	IC50: 2.2 µM	NOXA-like compound. Discovered though fragment-based approach
	Kd:1.9±1.0 nM, EC50: 2–18 µM	BH3 domain of BIM. Discovered through screening of the yeast surface display library of the BIM-BH3
	Ki : 100 nM	Discovered through high throughput screening & virtual screening. Tested against lymphoma cell lines

	Ki : 0.53±0.07 µM	Discovered through structure based virtual screening. Tested against haematological and solid cancer cell lines
	IC50: 0.0809 nM	Coumarin derivative discovered through QSAR

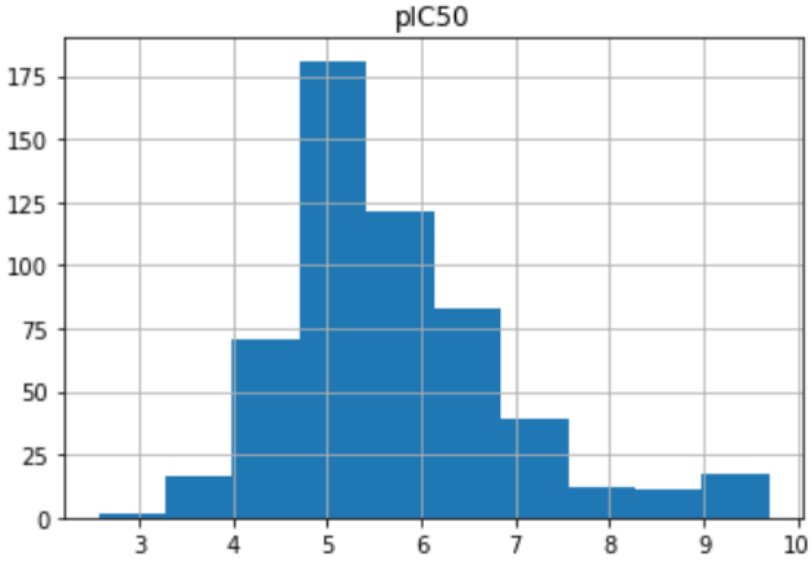
In this report, we attempted to identify novel 19G inhibitors through fragment-based methods and structure-based design. We will assess and discuss how these ligands perform as well as their potential therapeutic potential.

Simulation Results

Data Acquisition (talktorial 1)

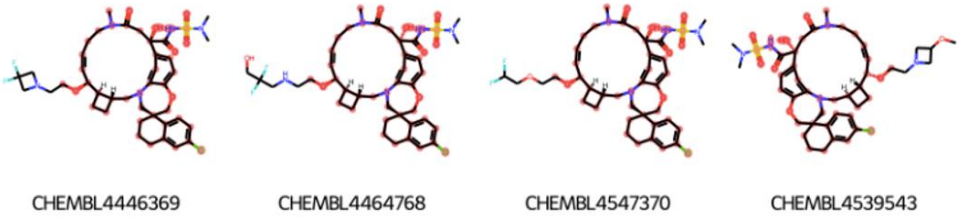
Mcl-1 uniprot_id is "Q07820”and there are 1825 compounds for Mcl-1 in ChEMBL data set.

In talktorial 1 and 2, we filter these compounds with "standard_unit == nM”, 'Assay Type'= 'B', 'Standard Units' = 'nM', "Standard Relation" = "'='" and in the final there are 553 compounds eligible.



Compound data: pIC50 value distribution

The pIC50 values are distributed quite normal but with a bit of a ‘tail’ end where compounds are that have a high pIC50. Usually, cyclic compounds have high pIC50 values. Using PubChem to find the IC50 values found for these compounds, the following results were presented:



CHEMBL4446369: 0.0003uM -> pIC50 8.5 M

CHEMBL4464768: 0.0003uM -> pIC50 8.5 M

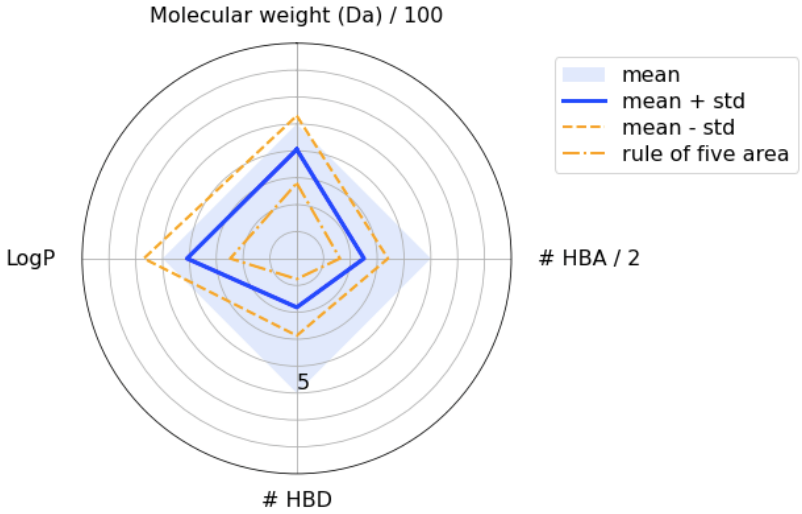
CHEMBL4547370: 0.0006uM -> pIC50 8.2 M

CHEMBL4539543: 0.0003uM -> pIC50 8.5 M

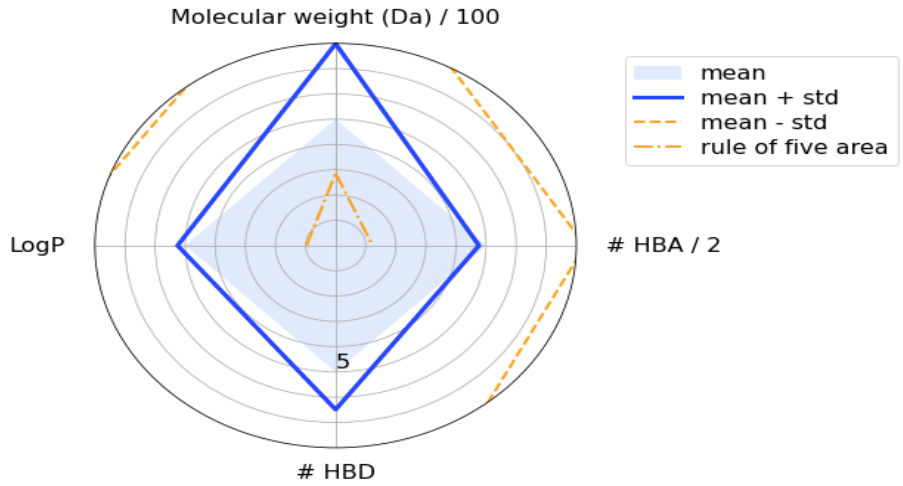
The IC50 values can be converted into pIC50 values according to the formula pIC50 = -log10(IC50). The calculated pIC50 values that are all between 8 and 9 M are good values and indicate that these compounds are potent inhibitors. These cyclic molecules are also indeed forming the 'tail' part of the normally distributed pIC50.

Molecular Filtration using ADME criteria (talktorial 2)

In talktorial 2, we filter data set by Lipinski's rule of five and it shows the number of compounds not compliant with the Ro5 is 137.



the radarplot for the dataset of compounds that fulfill the Ro5

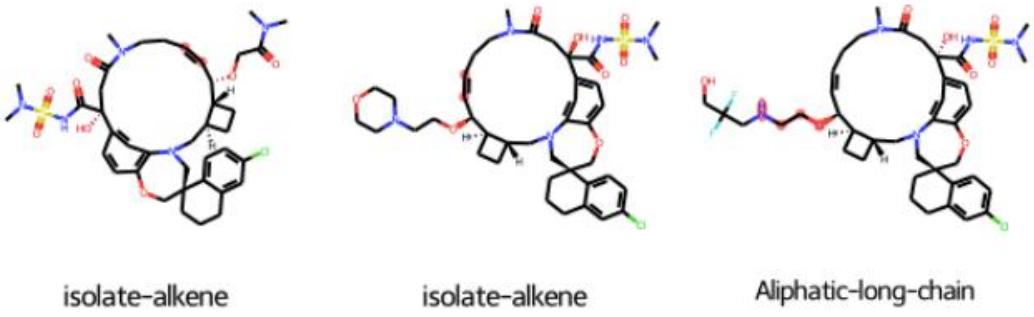


the radarplot for the dataset of compounds that violate the Ro5

Molecular Filtration by Unwanted Structures (talktorial 3)

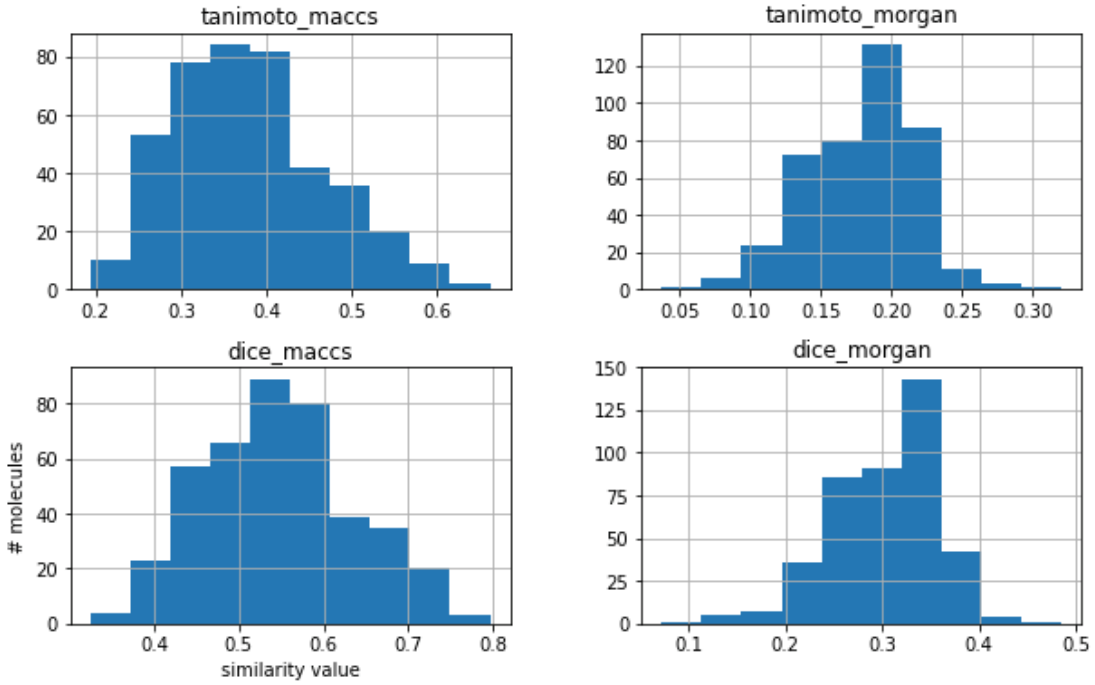
In talktorial 3, we filter out unwanted structure by PAINS and also highlight substructures.

For unwanted structures, we just take PAINS as example, because after searching literature, we found that there is no external list and therefore we can't get structure matches manually.

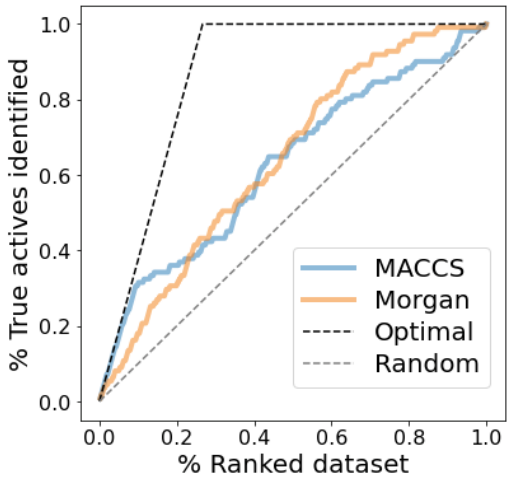


Ligand-based Screening: Compound Similarity (talktorial 4)

First we calculate MACCS/Morgan fingerprints and Tanimoto/Dice similarity for data set.



The distribution of Tanimoto similarity of MACCS fingerprints

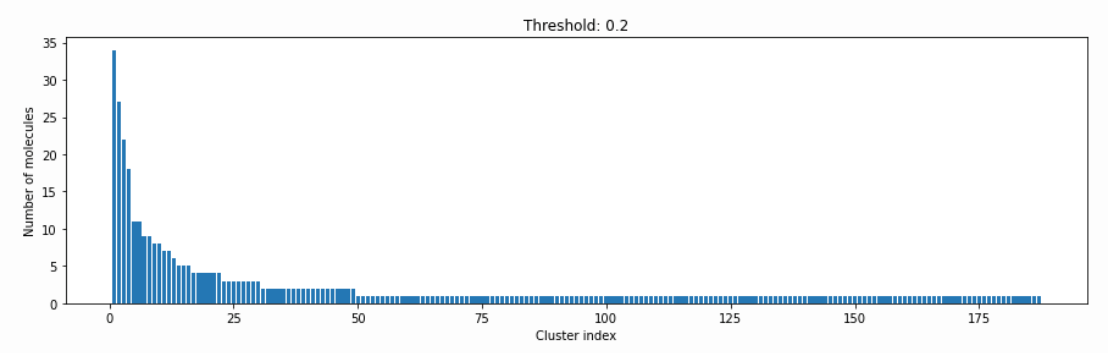


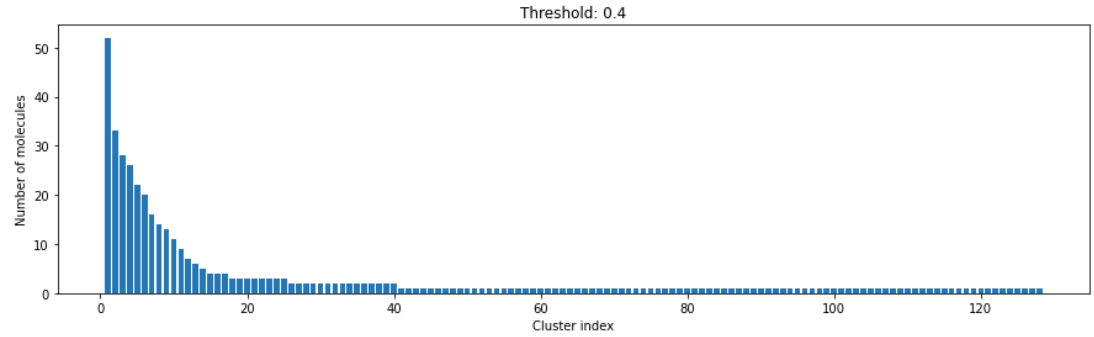
Data set ranked by similarity to query compound (Query: AZD5991)

Second we split data set into active & inactive compounds, the value of PIC_{50} cutoff is 5.92 from literature : https://www.sciencedirect.com/science/article/pii/S2352914821002392

Compound clustering with the Butina algorithm (talktorial 5)

For picking a reasonable cut-off to visualize the size of the clusters, we tried values from 0.0 to 1.0 by 0.2. When comparing threshold 0.2 with 0.4, threshold 0.4 has not many singletons and the cluster sizes don't have an extreme but smooth distribution, that's better than threshold 0.2. So we choose threshold 0.4 as visualization.



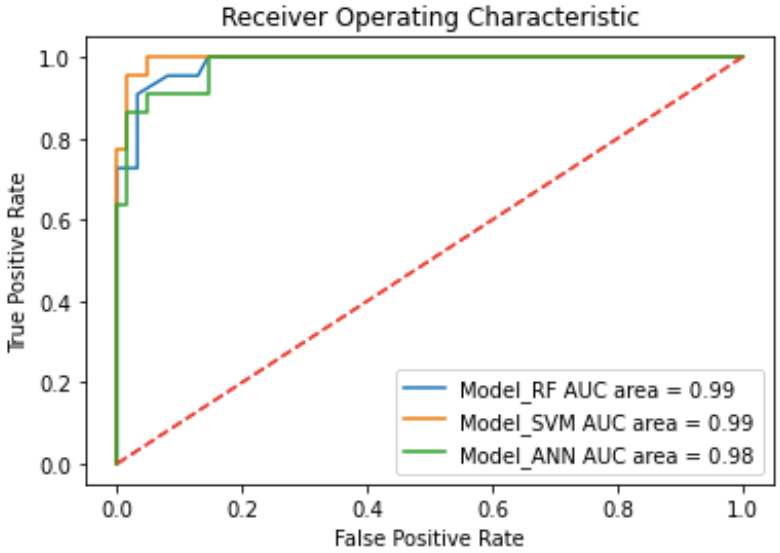


Maximum Common Substructures (talktorial 6)

We are trying to show MCS for largest cluster using FMCS algorithm.

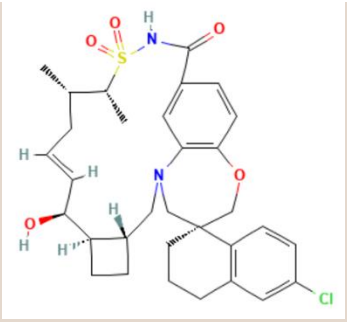
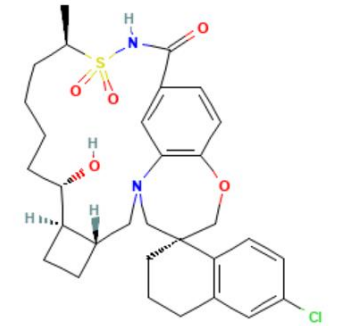
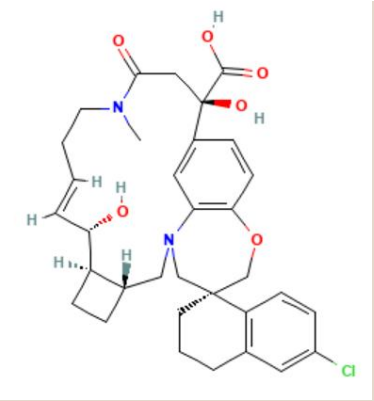
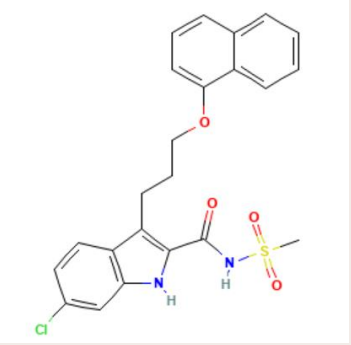
Ligand-based screening: Machine learning (talktorial 7)

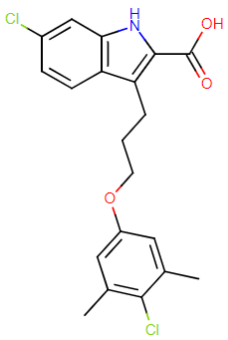
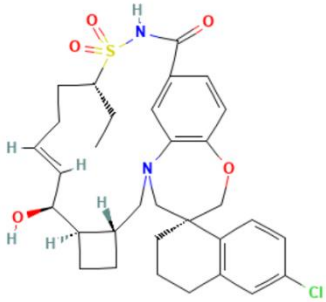
We use random forests, support vector machines & artificial neural networks to trainML classifiers.



Our result shows that the training part and also test part fit well.

Table 2: Structure of 6 novel ligand identified by out machine learning algorithm, as well as their affinity value and pChEMBL value as identified by docking vina

Ligand no	Structure	Chemical ID	Affinity (kcal/mol)	pChEMBL
0		Q51	-11.974	8.78
1		N0M	-10.692	7.84
2		Q4V	-10.683	7.84
3		5WL	-8.751	6.42

4			19H	-8.591	6.30
5			N0J	-10.518	7.72

Discussion

In this report, we have run several algorithms to visualize the binding pocket of MCL-1 and how our standard ligand binds to it. We have also succeeded in retrieving information about possible bioactive compound from a large chemical space that could bind to MCL-1. We have identified 6 of such compounds, perform molecular docking using autovina, and assess their on-target binding affinity to MCL-1. Out of these compounds, we note that ligands with better binding affinity and higher pChEMBL values are all cyclic.

In literature, cyclic peptides have been indeed suggested to give a more comprehensive understanding of a protein's substrate specificity (Kuhn et al., 2016), as well as being more stable, more cost efficient, less immunoreactive, while being easier to modify to aid immobilization and detection (Heurich et al., 2013). While physiological relevancies of cyclic peptide in eukaryotic system is not really known, cyclic macropeptides are naturally synthesized in several species of prokaryotes (Pelay-Gimeno et al., 2015), mostly serving defensive functions (Fjell et al., 2012; Oliva et al., 2018). These peptides are natural inhibitors of various protein target, often with antimicrobial activity (Oliva et al., 2018). Hence, it is not illogical to assume that these peptides could have structural scaffold that inspires design of potent MCL-1 inhibitors.

Our observation also seems to be supported by our ChEMBL data mining for compounds with high pIC50 values – all of which are cyclic. Cyclic peptides tend to have a more rigid structure that stabilizes interactions within itself, and around it. Instead of fitting itself into the binding site, the amino acid residues around it "wrap" themselves around the ligand, causing it to form a more stable conformation. This leads to a better affinity value, as shown by the pIC50 which are superior to 8. While we are unable to confirm the structure or other compounds with pIC50 values of 9-10, we are confident to hypothesise that these compounds are also likely to be cyclical in nature.

However, we do question the translatability of our prediction to real life clinical trials. Not only that cyclic peptides are often toxic to eukaryotic cells (Bondaryk et al., 2017), cyclic peptides are notoriously difficult to synthesise in laboratory settings. There are several in vitro expression systems that synthesises cyclic peptides using non-canonical amino acids. One of them uses *E. coli* ribozymes, coupled with puromycin-mediated mRNA display system which aids screening (Passioura and Suga, 2017; Katoh and Suga, 2020). However, there are not many literatures that elaborate on eukaryotic expression system for cyclic peptides. One of them uses yeast as expression system, but the synthesized peptide is still linear, and cyclized using linkers (Bacon et al., 2020). Moreover, the cyclic peptides that we have identified also contain cross-linking to the backbone, which adds to the difficulty of the synthesis.

In conclusion, we have found several promising inhibitors for MCL-1 by screening compound library with relatively good affinity on the binding site. However, its synthesisability and real-life application remains a topic to be explored.

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