Structure Guided Design for Improved Anticancer Compounds

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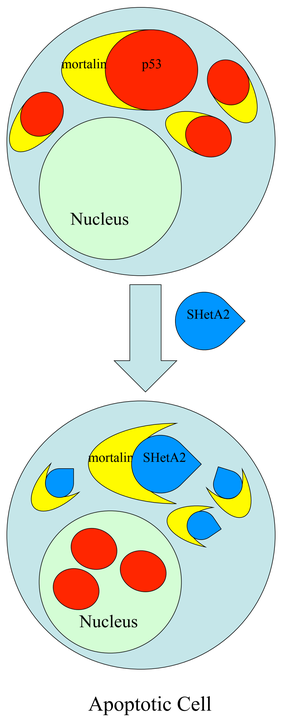
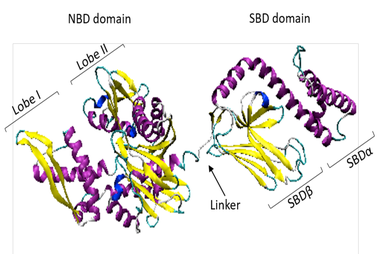
Introduction

Few other public health issues can match the tax on society that cancer has placed. Contributing to this are inadequate treatments with low efficiency and large number of negative side effects. Therefore, it is necessary to develop a highly efficient low toxic cure for cancer. Cancer drug candidate SHetA2, which was developed by scientists at OSU and OUHSC, has shown promising results: it is capable of inhibiting the growth of all cancer cell lines in the National Cancer Institute without toxic side effects in animal tests[1]. Phase 0 clinical tests are currently being conducted at OUHSC for ovarian cancer prevention. The application of SHetA2 as a cure, however, is limited by its low efficiency, only killing about 80% of cancer cells and leaving the rest to continue cancerous growth. Therefore, the creation of SHetA2 analogs[2] with increased efficiency that retain low toxicity to healthy tissues would be a major advantage in combating cancer.

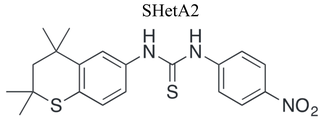
Anticancer Mechanism



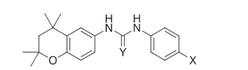
1. Mortalin sequesters the apoptotic regulator protein p53 in the cytoplasm preventing apoptosis.
2. The SHetA2 molecule binds to mortalin and prevents formation of the complex. The displaced p53 protein is free to migrate to the nucleus and induce apoptosis[3].



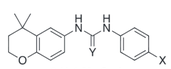
SHetA2



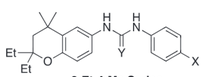
Analogs


2-Me-4-Me Series

2a (X = NO_2, Y = S) **3a** (X = NO_2, Y = O)
2b (X = CO_2Et, Y = S) **3c** (X = CF_3, Y = O)
2c (X = CF_3, Y = S) **3d** (X = CN, Y = O)
2e (X = SO_2NH_2, Y = S) **3f** (X = OCF_3, Y = O)

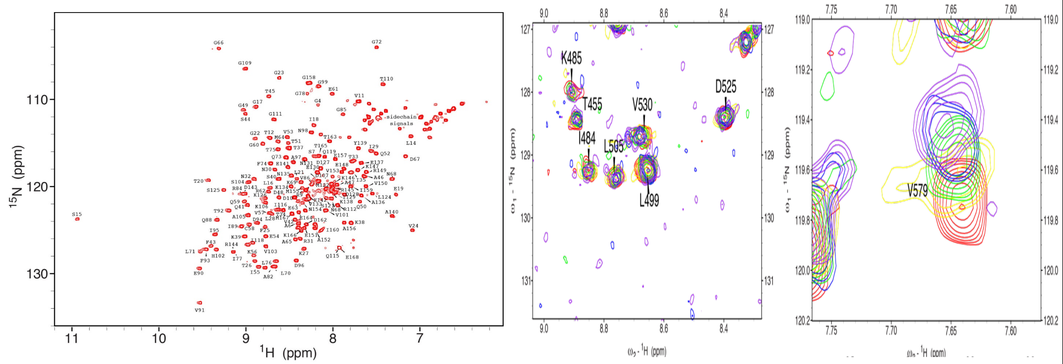

2-H-4-Me Series

4a (X = NO_2, Y = S) **5a** (X = NO_2, Y = O)
4b (X = CF_3, Y = S) **5b** (X = CF_3, Y = O)
4c (X = OCF_3, Y = S) **5c** (X = OCF_3, Y = O)

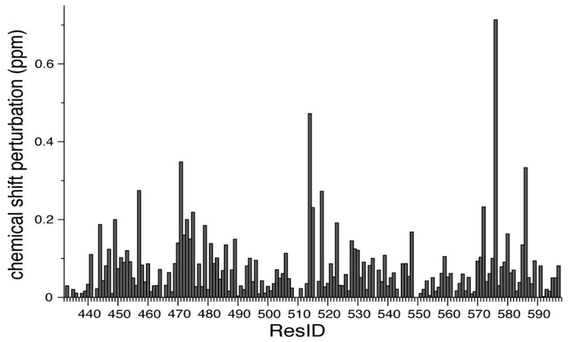

2-Et-4-Me Series

6a (X = NO_2, Y = S)
6b (X = NO_2, Y = O)
6c (X = CF_3, Y = O)

NMR Spectroscopy

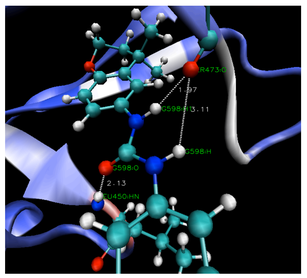


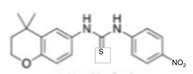
HSQC spectra and shifted peaks. Subjecting analogs to NMR study allows identification of the amino acids that are critically involved with binding. In the HSQC spectra, peaks correspond to each amino acid residue of mortalin. Peak shift observed following titration with SHetA2 indicates perturbation of that amino acid.



Molecular Modeling

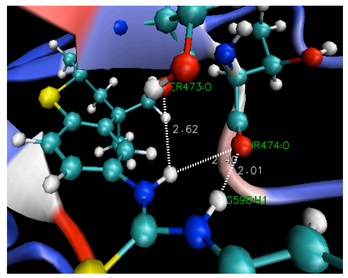
Analog 5A

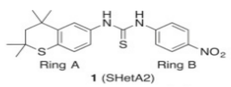




- -148.6 kJ/mol

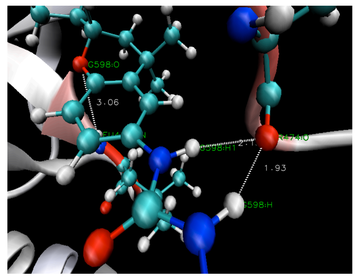
SHetA2

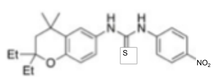




- -167.9 kJ/mol

Analog 6B

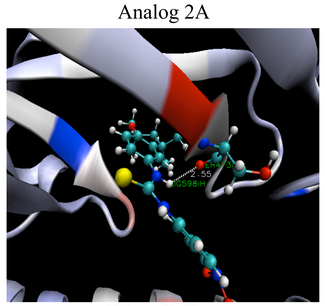


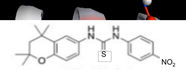


- -192.3 kJ/mol

Comparison of SHetA2 and analogs at lowest binding energy during 50 ns GROMACS simulation. Hydrogen bonding is indicated with dotted lines, amino acids with large contributions are shaded red. Above: Effect of increased hydrophobicity in ring A due to added geminal diethyl groups (6B). Right: Comparison of thiourea (2A) and urea linkers (3A). Data for hydrogen bonding is summarized in the table.

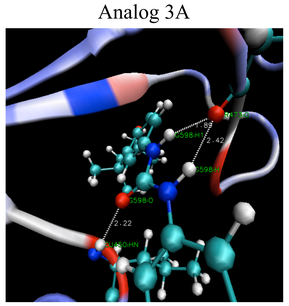
Analog 2A

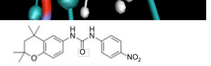




- -161.5 kJ/mol

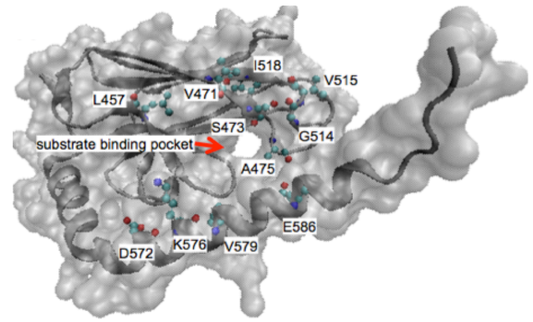
Analog 3A





- -140.4 kJ/mol

Results



Location of the residues that exhibit the most shifted peaks during SHEtA2 binding.

Acceptor/Donor (Lowest BE)	2a (-161.5 kJ/mol)	3a (-140.4 kJ/ mol)	5a (-148.6 kJ/ mol)	6b (-192.3 kJ/ mol)	SHetA2 (-167.9 kJ/ mol)
S473_CO_Lig_NH	62.46%	52.97%	8.44%	0.367%	51%
S473_CO_Lig_N1H1	0.00%	82.88%	37.12%	0.00%	2.14%
T474_CO_Lig_NH	0.00%	0.095%	0.00%	81.83%	30.06%
T474_CO_Lig_N1H	0.00%	0.00%	0.00%	94.33%	92.76%
L450_HN_Lig_O	0.00%	97.95%	65.47%	0.00%	0.00%
L450_HN_Lig_O (ring A)	0.00%	0.00%	0.00%	1.31%	0.00%

Summary of hydrogen bonding during 50 ns simulation. Percentage indicates the amount of time during simulation that hydrogen bond was formed. Note the improved performance of with increased hydrophobicity (6B), and the urea linker (3A). This data is in agreement with the biological information.

Implication

The data presented here will help guide the development of the next generation of SHetA2 derived analogs to further its potential as a cancer treatment.

Acknowledgements and References

This work was generously supported by the Lew Wentz Foundation (GR), OSU President's Fellows Faculty Research and Stephenson Cancer Research Center/ Oklahoma Tobacco Settlement Endowment Trust (TSET) .

[1] Nammalwar, B., et al., *SHetA2 – A Mini Review of a Promising Anticancer Drug.* JSM Chemistry, 2013. **1**: p. 1005.
[2] Watts, F.M. Jr., et al., *Activity of oxygen-versus sulfur-containing analogs of the Flex-Het anticancer agent SHetA2.* European Journal of Medicinal Chemistry, 2018. **5**: p. 720-732.
[3] Benbrook, D.M., et al., *SHetA2 interference with mortalin binding to p66shc and p53 identified using drug-conjugated magnetic microspheres.* Investigational New Drugs, 2014. **32**: p. 412-423.