The Allosteric Sirtuin 2 Modulator FLS-359 Inhibits Viral Replication

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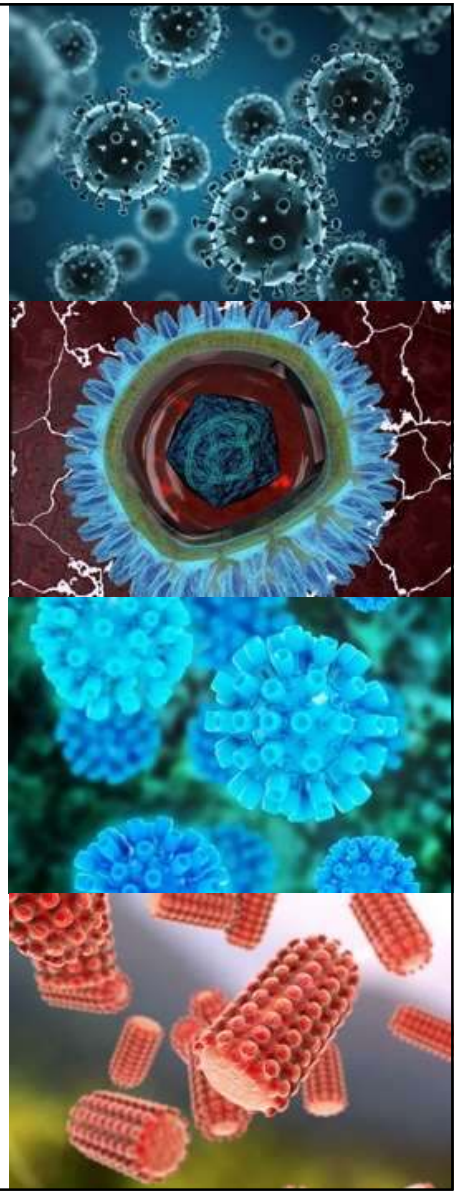
Invited Speaker

Denver ACS Meeting August 19, 2024

Session: Approaches to the Modulation of Enzyme Activation and/or Substrate Recognition



Agenda

- Introduction & identification of sirtuins (SIRTs) as viral restriction factors
- SIRT2 Enzyme mechanism and overview of SIRT2 structure
- FLS-359 broad spectrum antiviral activity and SIRT2-mediated cellular effects
- Mechanism of FLS-359 SIRT2 enzyme activity modulation
- Comparison structures of FLS-359 & literature SIRT2 modulators
- Summary

Human cytomegalovirus (HCMV)

- b-herpesvirus: 200 ORFs and 28 miRNAs
- Ubiquitous: 1% of newborns; 10% 6-month-olds; 60-99% of adults
- Infection in healthy individuals generally asymptomatic
  - Acute phase (weeks) - many cell types & organs
  - Persistent phase (years) - salivary, mammary & kidney epithelial cells
  - Latent phase (lifetime) - $CD34^+$ bone marrow cells
- Congenital infection: hearing loss, profound CNS damage
- Infection can present in immunosuppressed: AIDS & transplant
  - Provides an opportunity to develop a therapy for an unmet medical need
- Standards of care – direct acting antivirals: ganciclovir, letermovir, maribavir
  - DNA polymerase (pUL54) inhibitor, terminase (pUL56) inhibitor and kinase (pUL97) inhibitor

Direct acting antivirals (DAAs) suffer from therapeutic shortcomings

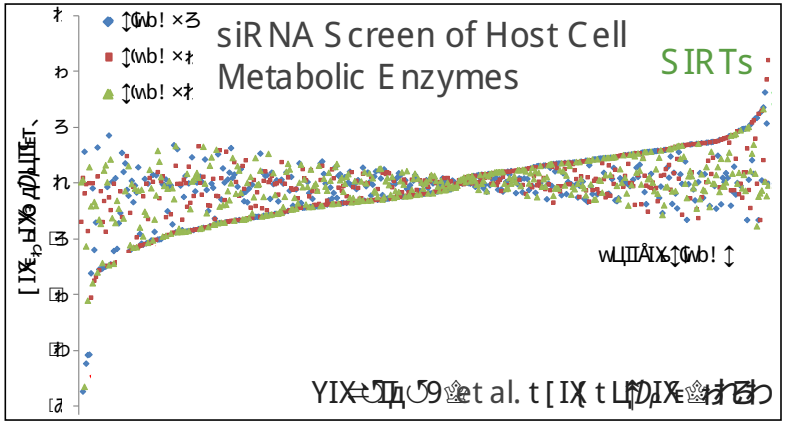
- Targeting specific a viral protein can lead to drug-resistance
- One drug generally specific for one virus
- Novel emergent viruses will likely not be affected by marketed DAAs

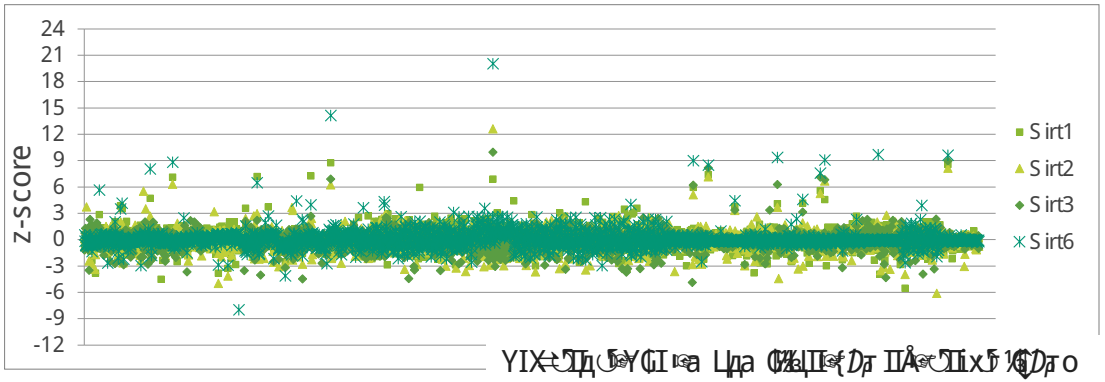
- Host targeted antivirals can likely address these issues

Sirtuins (SIRTs) ID ̆d as affecting HCMV growth & SIRT2 modulator ID ̆d inhibiting HCMV growth

Tom Shenk lab Princeton:

- HCMV virus infection reprograms host-cell metabolism
- Genetic screen identified SIRT1-7 as viral restriction factors
- A 13 K small molecule screen ID ̆d 85 SIRT modulators; 61/85 cpds active vs. HCMV in secondary screen





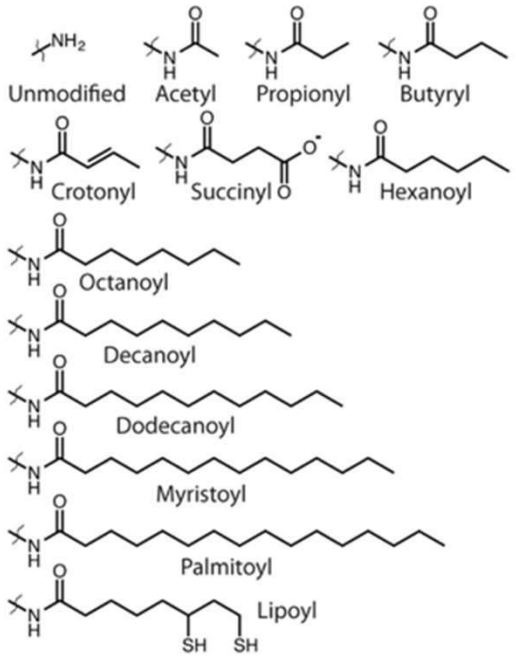
Host Target: Human Sirtuin 2 Protein

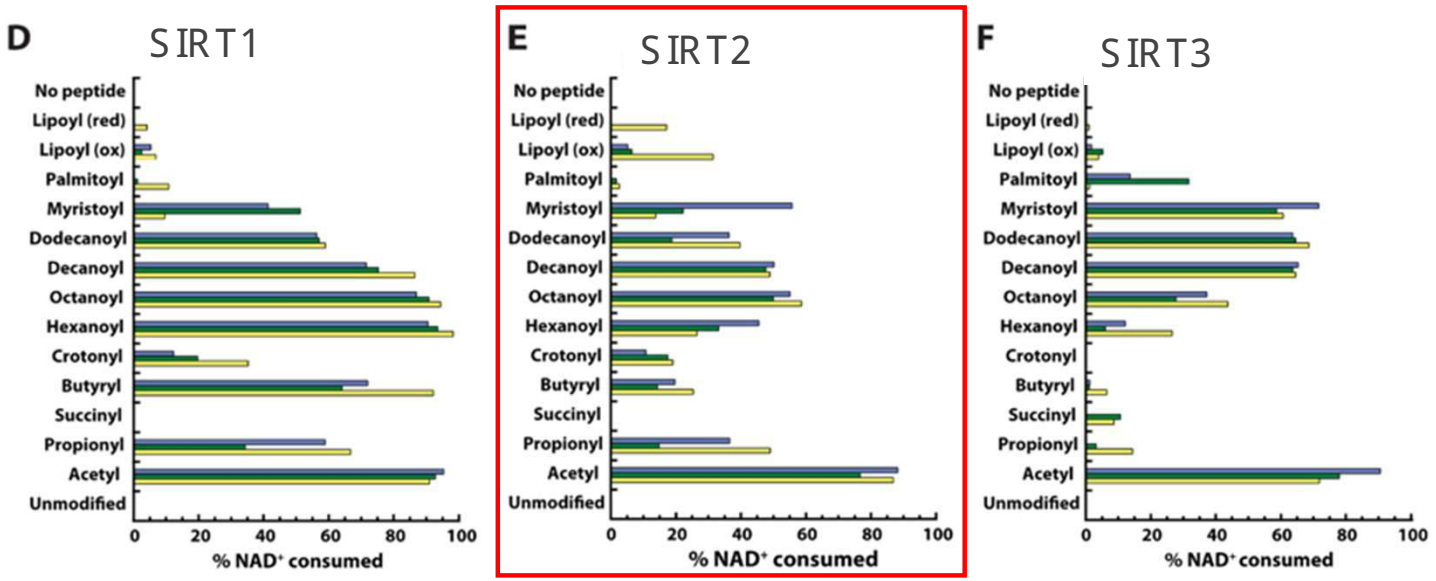
- Sirtuins (SIRTs) are NAD^+-dependent Class 3 lysine de-acylases (KDACs) that regulate transcription, genome stability, cellular signaling, and energy metabolism in response to the metabolic status of the cell
- Sirtuin family of 7 proteins vary in sub-cellular location and function to remove a large variety of protein acyl-lysine modifications
- Viral infection disrupts the metabolic status of the cell and depends on sirtuin regulated functions to replicate productively
- SIRT2 modulators (nicotinamide, sirtinol, cambinol, AGK2) can engage cellular reprogramming to inhibit cytomegalovirus[1,2], hepatitis A virus[3], hepatitis B virus[4,5], Listeria[6,7], Salmonella[8], Tuberculosis[9]
- SIRT2 KO mouse is healthy and less susceptible to infection[10]
- Evrys SIRT2 targeted drugs are allosteric enzyme activity modulators

[1,2]Mao 2016, Koyuncu 2014, [3]Kanda 2015, [4,5]Piracha 2018, Yu 2018, [6,7]Eskandarian 2013, Pereira 2018, [8]Gogoi 2018, [9]Bhaskar 2020, [10]Ciarlo 2017

SIRT1, 2 & 3 are closely related and deacylate various lysine acyl groups

GlnThrAlaArg-Lys(Acyl)- SerThrGlyGly





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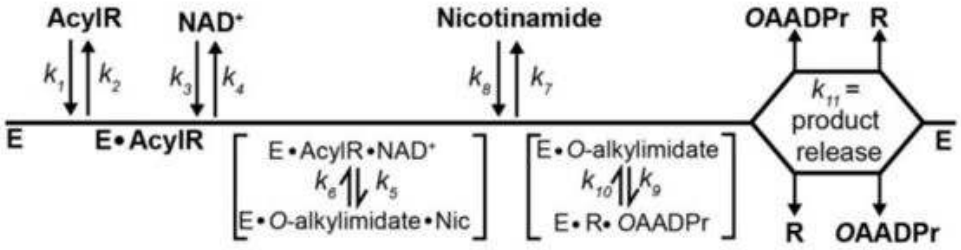
evrys bio

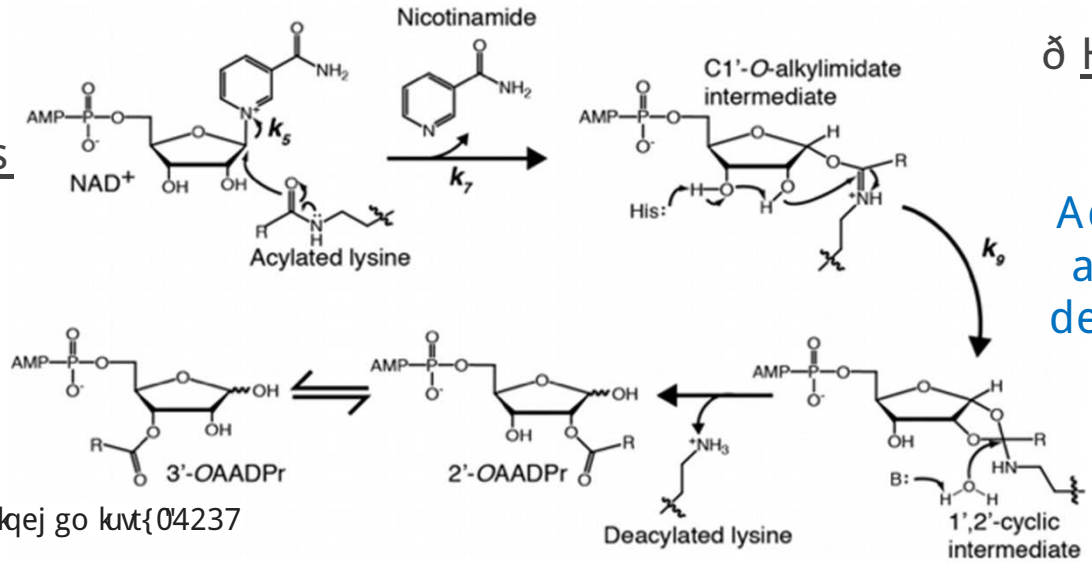
SIRT2 enzyme reaction is complex with multiple substrates, products, transition states, and rate constants

ð Rxn Mechanism:
2 substrates
Ordered binding
Acyl-peptide, then NAD^+



ð3 Products:
Nicotinamide
O-Acyl-ADP-ribose
de-Acyl-peptide

ð Chemical Intermediates



ð HTMS assay, detecting:
Ac-ADPR
De-acyl-peptide
Advantage: Can use any acyl chain length since de-acyl-peptide the same

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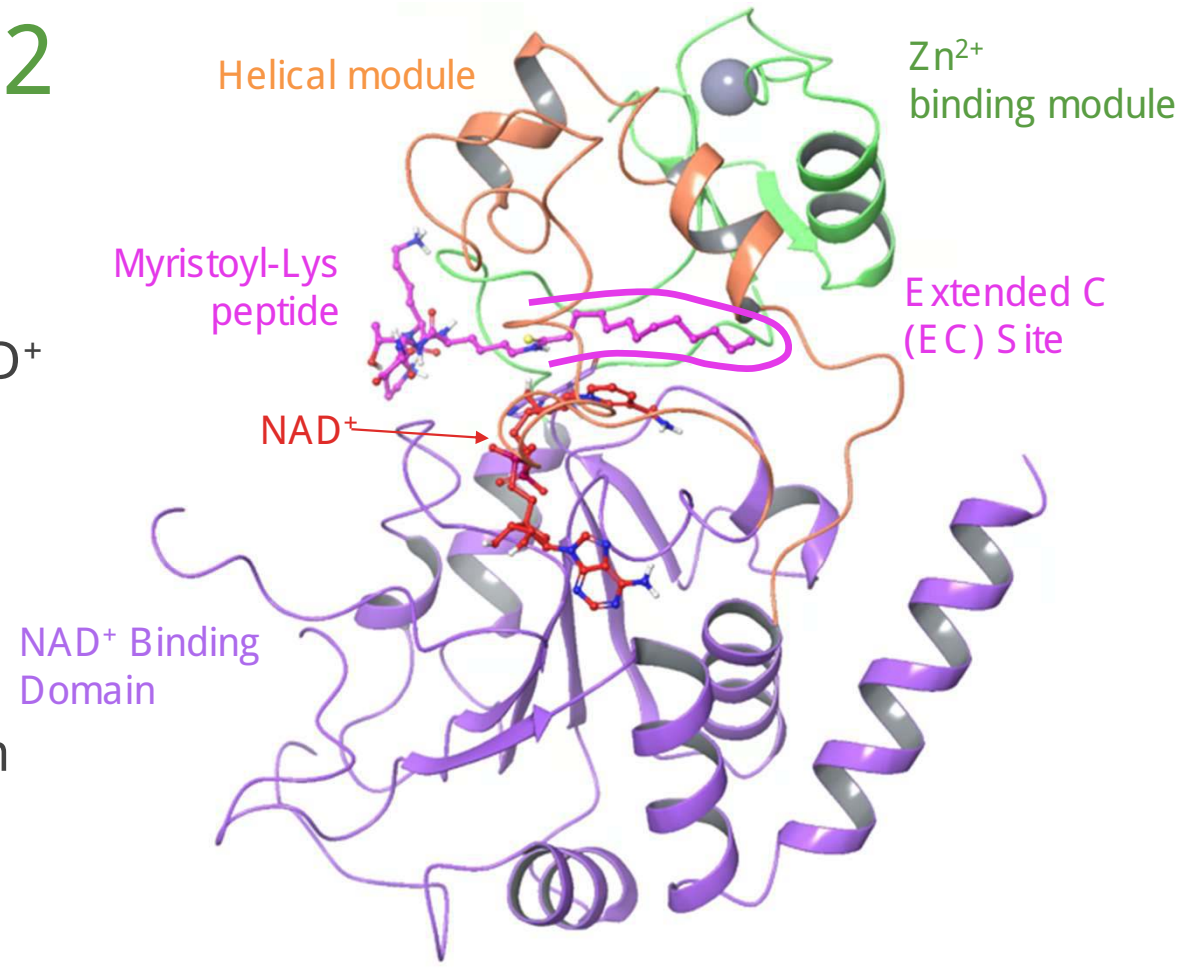
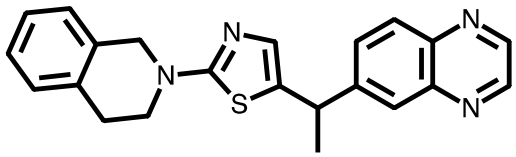
Structure of SIRT2

- SIRT2 X-ray bound to a myristoylated peptide and NAD^+ (PDB 4X3P)
- The myristoyl binding site (EC site) is induced by the binding of the peptide (or small molecules) and is not present in the apo-protein



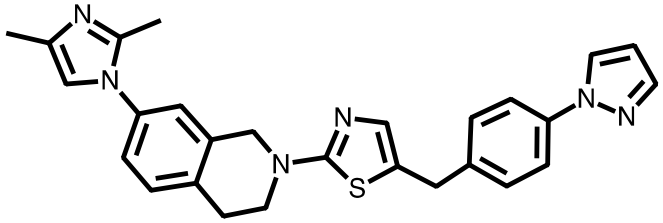
Hit to Lead

- A 13 K small molecule screen at Princeton ID'd FH003 as an antiviral SIRT2 modulator



Princeton: FH003
HCMV IC_{50} = 8.7 ı M
SIRT2 EC_{50} = 4.1 ı M

Evrys
Med Chem



Evrys: FLS-359
HCMV IC_{50} = 0.5 ı M
SIRT2 EC_{50} = 3.0 ı M

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FLS-359: Host targeted SIRT2 modulator results in broad-spectrum antiviral efficacy in vitro

Virus/ Host Cell	Virus Family	FLS-359 IC_{50} (µM)	Host Cell CC_{50} (µM)	[1]SI CC_{50}/IC_{50}	[2]SOC/C IC_{50} (µM)	SOC/ Comparator (C)
[3]SARS-CoV-2/Human Calu3	β-Coronavirus	0.3	15.8	52.7	0.4	Remdesivir (C)
[3]Zika/Human HFF	Flavivirus	0.4	41.6	104.0	2.8	Amodiaquine (C)
[4]HCMV/Human MRC5	β-Herpesvirus	0.5	>15.8	>40	2.7	Ganciclovir (SOC)
[5]Influenza A/Human dNHBE	Orthomyxovirus	[7]1.2	>100	>83.3	[7]0.7	Ribavirin (C)
[4]HCoV-OC43/MRC5	β-Coronavirus	1.7	>50	>30.1	0.1	Remdesivir (C)
[3]Junin/Human HFF	Arenavirus	3.2	>25	>7.8	0.2	RIID E-1 (C)
[6]Hepatitis B/Human PHH	Hepadnavirus	4.8	>10	>2.1	0.03	Tenofovir (SOC)
Epstein-Barr/Human Akata	g-Herpesvirus	3.8	>100	>26.3	43	Ganciclovir (C)
RSV/Human MRC5	Orthopneumovirus	6.7	>12.5	>1.9	16.1	Ribavirin (SOC)

Results are shown for representative assays. [1]SI = selectivity index, [2]SOC = standard of care or C = comparator compound, [3]HFF = human foreskin fibroblasts, [5]dNHBE = differentiated normal human bronchial epithelial cells, PHH = primary human hepatocytes, [7]IC_{90}.

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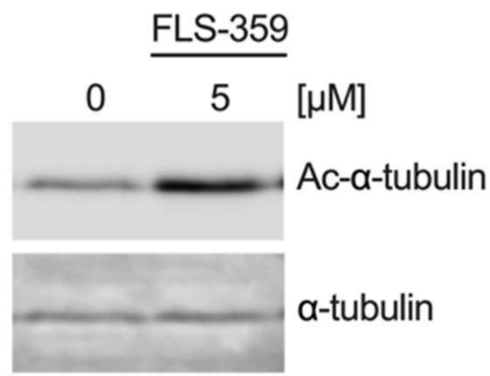
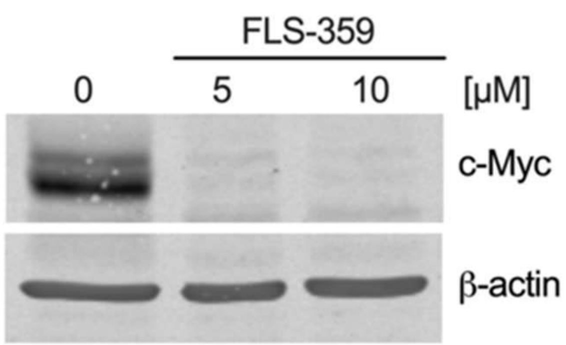
FLS-359 affects known cellular targets of SIRT2

From HepG2 cells

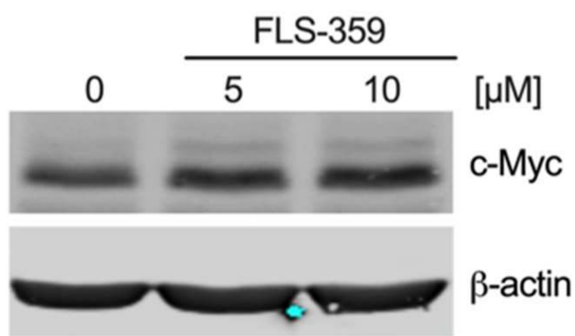


HepG2 cells were treated with FLS-359 plus 1 ≈M trichostatin A for 24 h and levels of acetylated a-tubulin (Ac-a-tubulin) were determined by Western blot. Total a-tubulin was monitored as a control

From MDA-MB-231 cells



From MRC-5 cells



MDA-MB-231 cells or MRC-5 fibroblasts were treated with drug for 72h and c-Myc was monitored by Western blot with b-actin as a control.

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evrys bio

Evrys FLS-359 SIRT2 modulator is not a classic competitive inhibitor

- SIRT2 Acetyl-peptide hydrolysis rate is reduced
  - Effect not altered when raising [NAD]
  - Effect marginally altered raising [Ac-peptide]
  - Cpds not fully competitive with substrates
- SIRT2 activity is not reduced to 0
  - Compounds are SIRT2 modulators
- Evrys compounds are selective for SIRT2

Drug	SIRT1 de-Acetyl EC_{50}	SIRT2 de-Acetyl EC_{50}	SIRT3 de-Acetyl EC_{50}
FLS-359	>100 µM	3 µM	>100 µM

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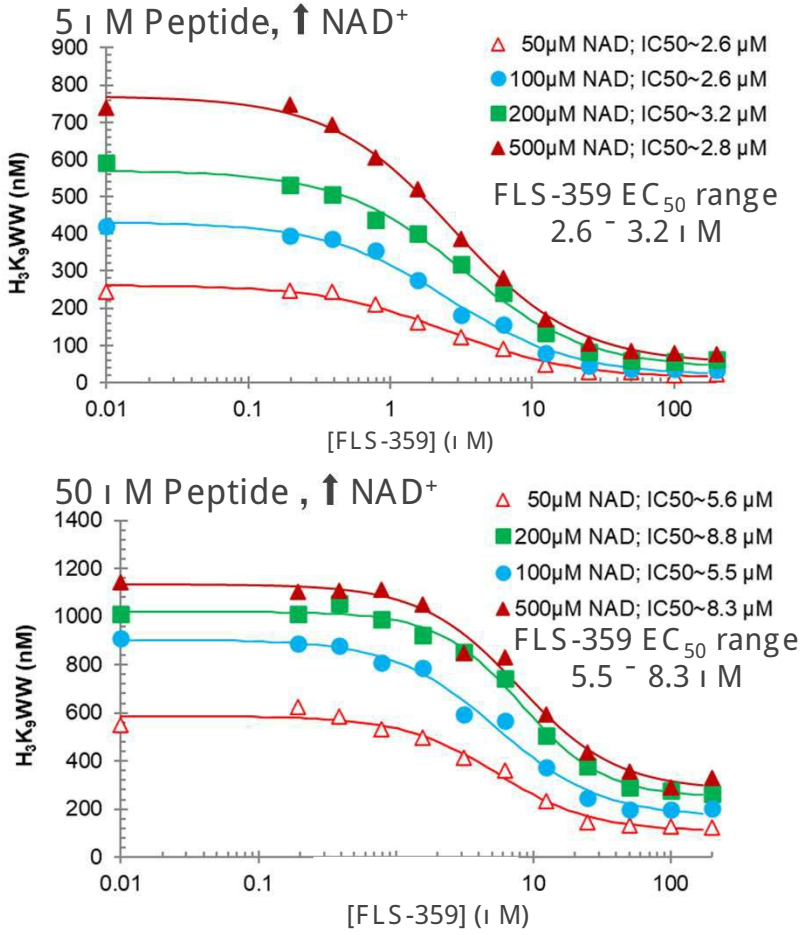
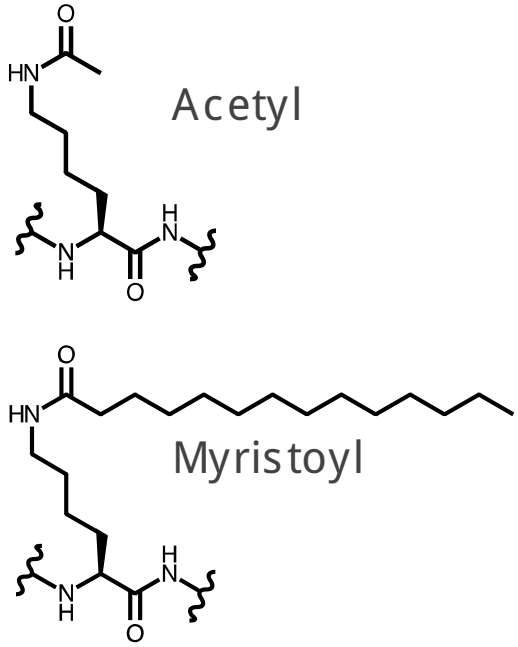
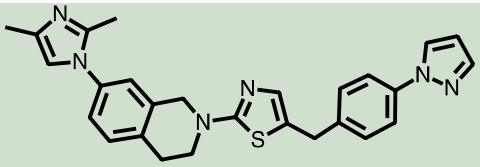
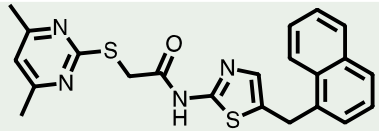
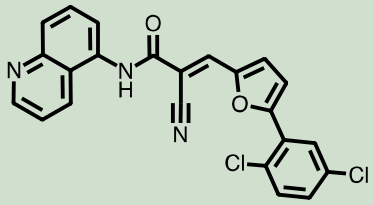


FLS-359 is a selective peptide substrate modulator



Cpd	Structure	Deacetylase IC_{50} (μM)	Demyristoylase IC_{50} (μM)
FLS-359		3.0	>100
SirReal2		0.2	>100
AGK2		0.5	>100

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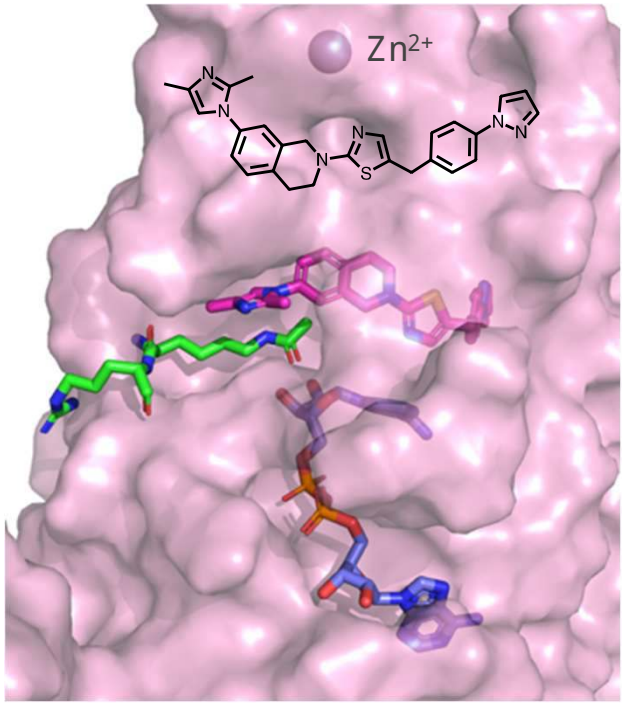
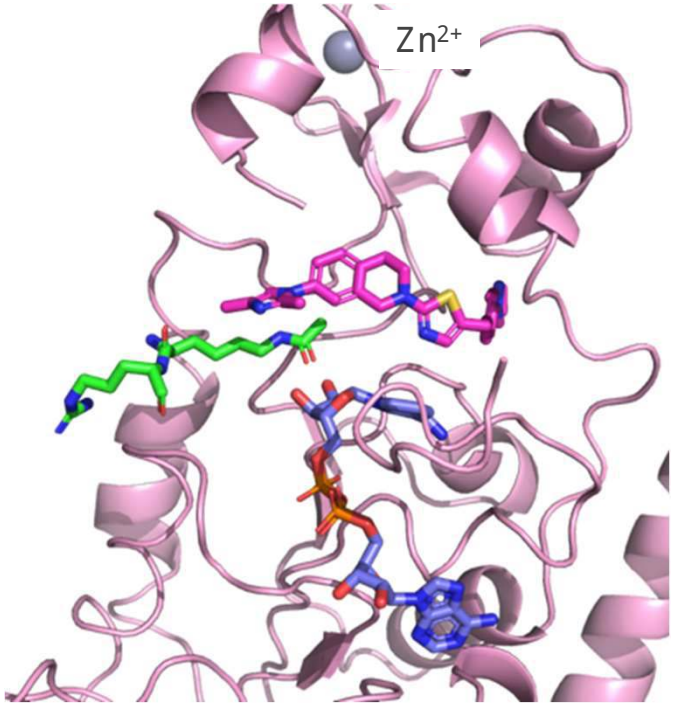
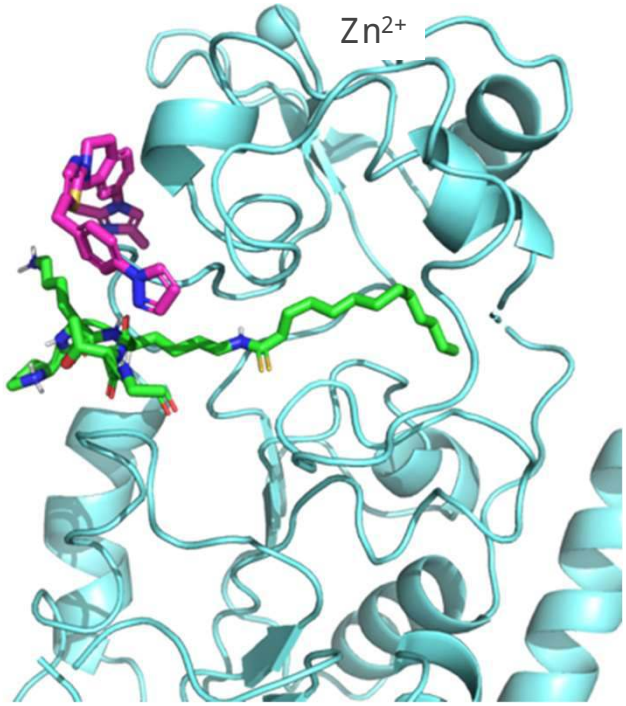
SIRT2 can accommodate FLS-359, NAD^+, and Acetyl- but not myristoylated-substrate



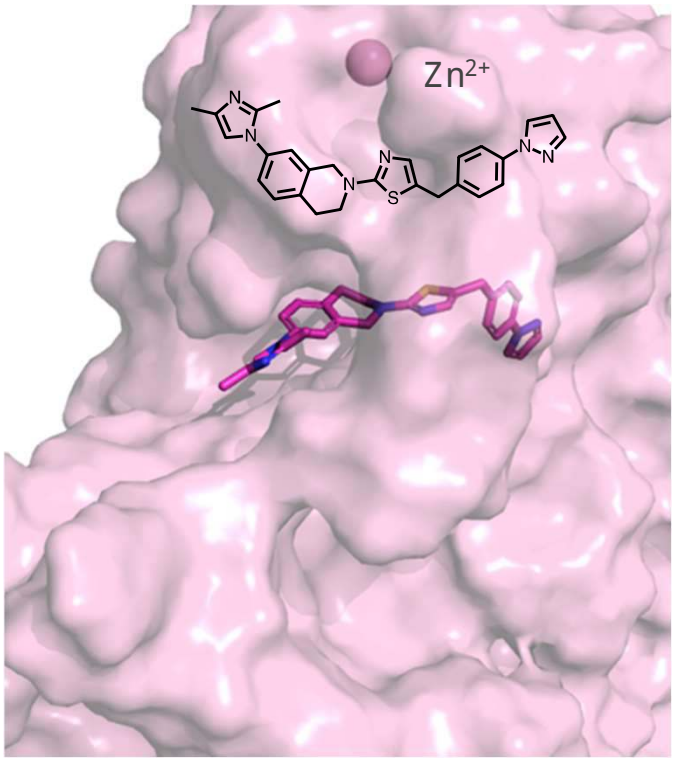


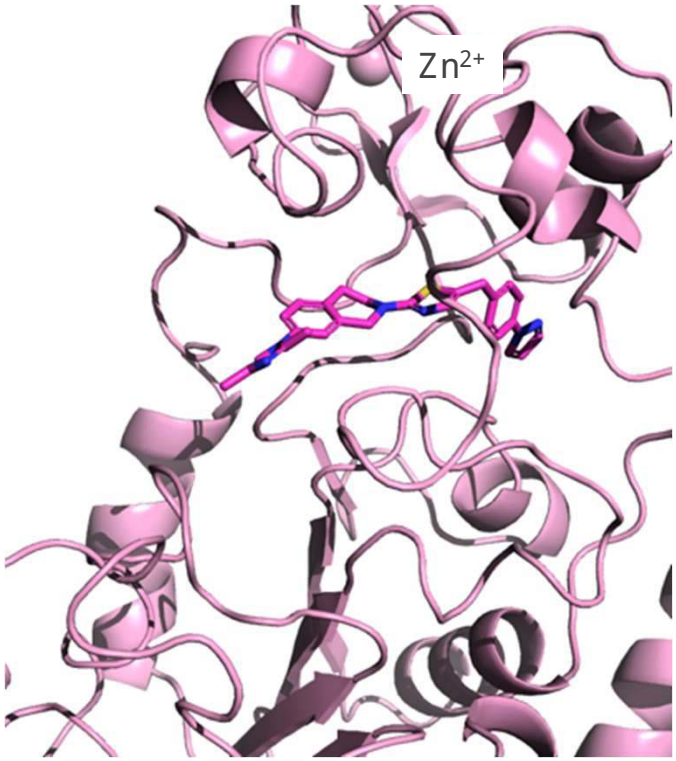


FLS-359 docked to SIRT2 with acetylated lysine PDB 4RMH. Binding of FLS-359 (magenta sticks) does not prevent binding of acetyl lysine substrate (green sticks) or NAD^+ (purple sticks, from PDB 4RMG). Left panel: SIRT2 with surface. Center panel: SIRT2 with ribbon structure only. w[illegible] et al. W[illegible]

Myristoylated peptide (green sticks, PDB 4Y6O) competes for binding with FLS-359 (magenta sticks) preventing compound binding to SIRT2.

FLS-359 – SIRT2 co-crystal PDB 7T1D



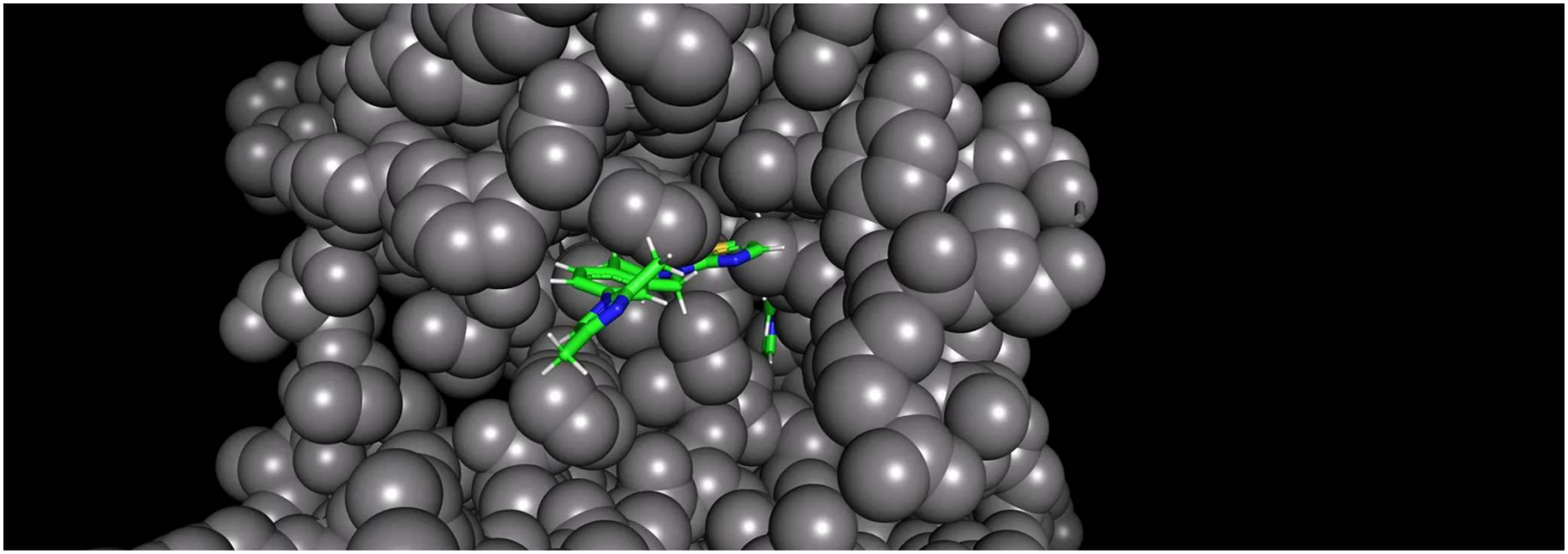


FLS-359 SIRT2 cocrystal shows compound filling EC site.
Roche KL, et al. J Clin Invest. 2023

FLS-359 SIRT2 cocrystal 7T1D (pink) overlayed with myristoylated peptide 4Y6O (cyan).

EC site is induced by FLS-359 binding to SIRT2

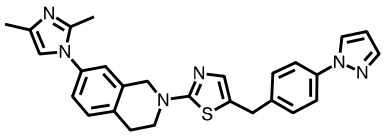
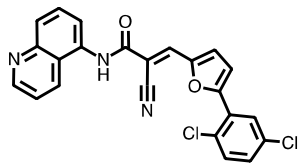
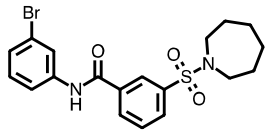
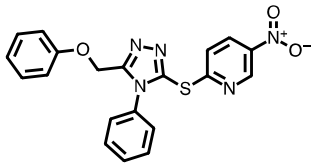
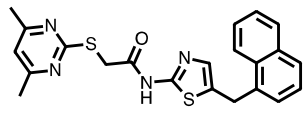
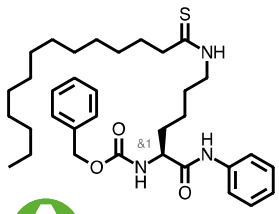
- Start: Apo-SIRT2 – X-ray; End: FLS-359-bound SIRT2 X-ray, conformation change



Published SIRT2 modulators reduce HCMV yield in vitro

Compound	HCMV IC_{50} (μM)	MRC-5 CC_{50} (μM)	SI: CC_{50}/IC_{50}	SIRT2 Deacetyl IC_{50} (μM)
FLS-359	0.5	>15.8	>40	3.0
AGK2	3.4	>100	>29.4	3.5
AK-7	8.2	>25	>3	15.5
MIND4	14.4	19.8	1.4	3.5
SirReal2	14.9	>20	>1.3	0.2
TM	26.0	46.3	1.8	0.03

FLS-359 AGK2 AK7 MIND4 SirReal2 TM

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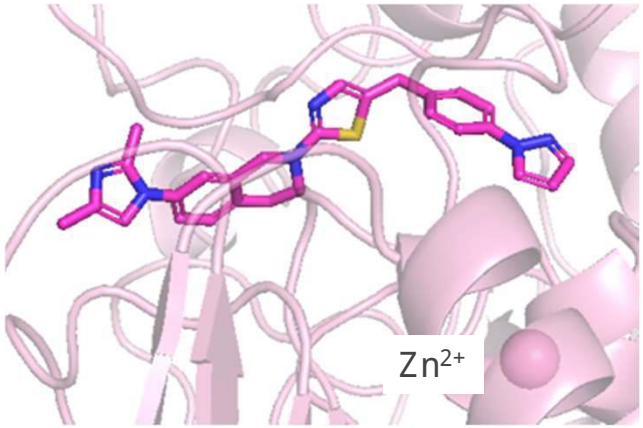
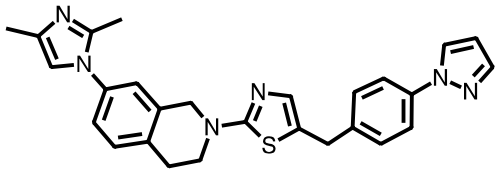
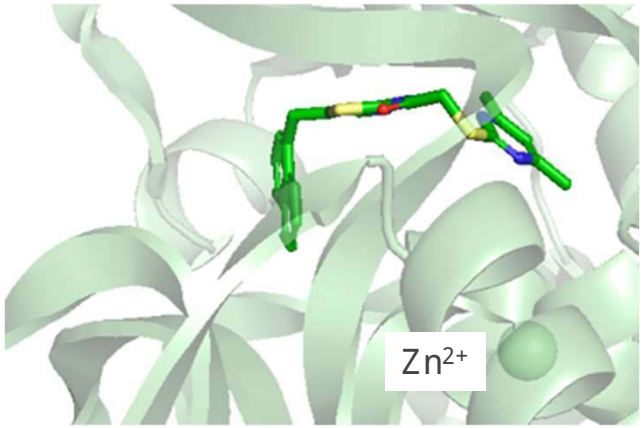
Small molecule SIRT2 modulators have different SIRT binding conformations

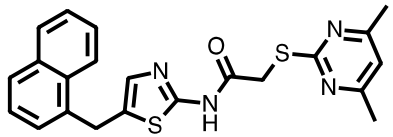


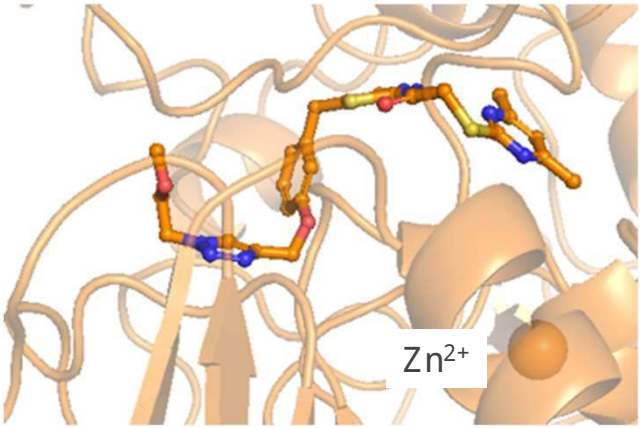
FLS-359 7T1D



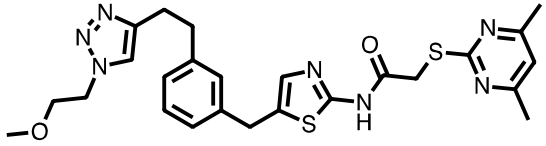


SirReal2 4RMG

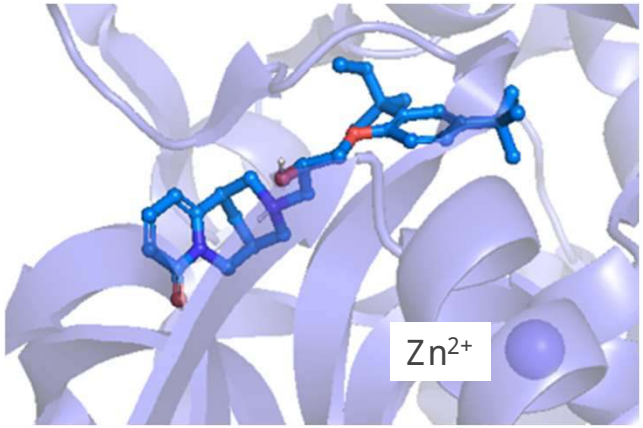




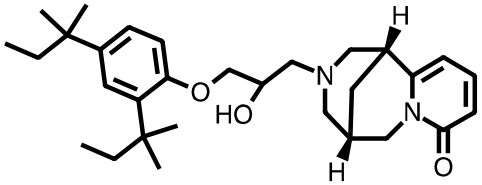
SirReal1.2 8OWZ

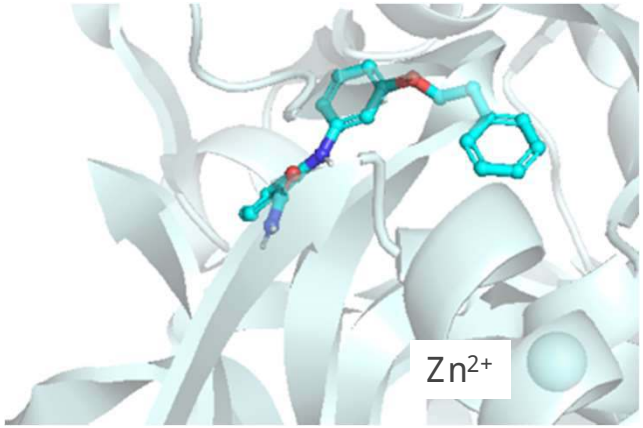


Small molecule SIRT2 modulators have different SIRT binding conformations

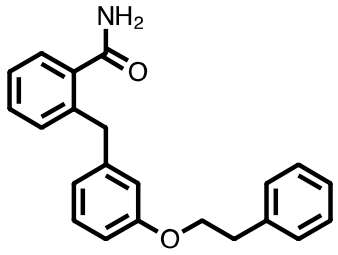


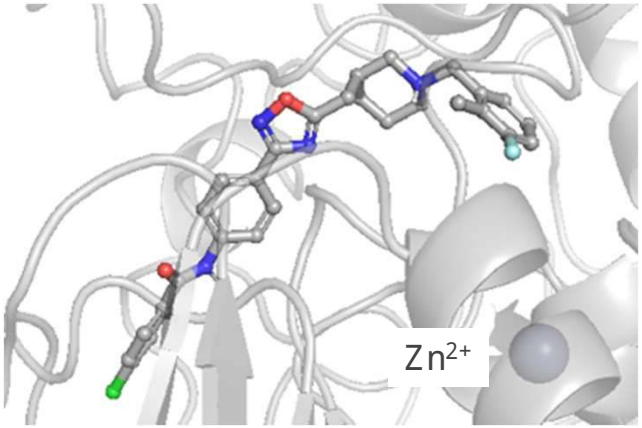
NPD11033 5Y0Z



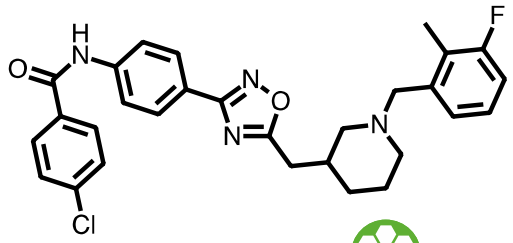


Benzamide 6 5Y5N





Oxadiazole 16 8PY3



Summary

- An unbiased siRNA genetic screen in HCMV infected fibroblasts identified the enzyme class Sirtuins as viral restriction factors
- A small molecule SIRT enzyme screen found 85 SIRT modulators with 65 having anti-HCMV activity
- SIRT2 modulation affects multiple human pathogens
- SIRT2 can deacylate lysines with chain lengths ranging from C2 acetyl to C14 myristoyl
- The mechanism of SIRT2-mediated deacylation is multistep

Summary

- A variety of SIRT2 modulators affect HCMV replication
- Evrys lead optimization resulted in FLS-359, which allosterically modulates SIRT2
- Host-targeted FLS-359 has broad-spectrum antiviral activity
- The EC pocket is absent in apo-SIRT2 and is induced by substrate and small molecule binding
- The EC pocket is flexible and can accommodate a variety of small molecule binders that adopt very different conformations in their interactions with SIRT2

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