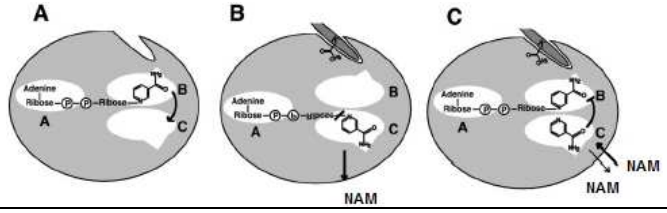
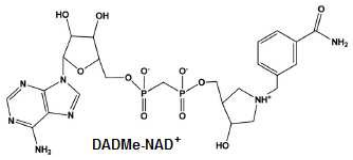
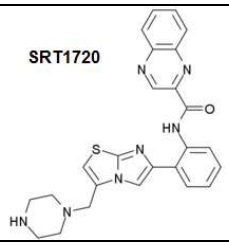
Summary of Sirtuin Kinetic Parameters

<table>
<tr><th>Sirtuin</th><th>Remarks</th><th>References</th></tr>
<tr><td>ySir2
hSIRT1</td><td>M15, sirtinol, splitomicin, nicotinic acid, TSA, and NAM were tested. IC50 (NAM) <50 uM. Model for noncompetitive inhibition of ySir2 and hSIRT1 by NAM.(HDAC fluorescent activity assay kit)
 
</td><td>1.Sinclair group, J Biol. Chem. 2002</td></tr>
<tr><td>ySir2</td><td><table><tr><th></th><th>[IsoNAM] = 0mM</th><th>[IsoNAM] = 60mM</th><th>[IsoNAM] = 100mM</th></tr><tr><td>Ki(NAM),uM</td><td>115±15</td><td>210±17</td><td>295±25</td></tr><tr><td>Vmax</td><td>1.15±0.04</td><td>1.11±0.06</td><td>1.10±0.06</td></tr><tr><td>Km (NAM), uM</td><td>110±10</td><td>212±15</td><td>370±40</td></tr></table></td><td>2. Schramm group, Mol. Cell 2005</td></tr>
<tr><td>Sir2Tm</td><td><table><tr><th></th><th>kcat, s^{-1}</th><th>km, uM</th><th>kcat/km, $s^{-1}mM^{-1}$</th><th>IC50(NAM), mM</th><th>IC50(Nicotinic acid), mM</th></tr><tr><td>NAD^+</td><td>0.17±0.006</td><td>53±11</td><td>3.2±0.8</td><td>1.0±0.2</td><td>>100</td></tr></table></td><td rowspan="2">3. Wolberger group, Mol. Cell 2005</td></tr>
<tr><td>Sir2Tm (D101N)</td><td><table><tr><th></th><th>kcat, s^{-1}</th><th>km, uM</th><th>kcat/km, $s^{-1}mM^{-1}$</th><th>IC50(NAM), mM</th><th>IC50(Nicotinic acid), mM</th></tr><tr><td>NAD^+</td><td>(1.8±0.1)X10^{-3}</td><td>1.17±0.18</td><td>(1.5±0.84)X10^{-3}</td><td>9.0±2.0</td><td>11.3±3.3</td></tr><tr><td>NAAD</td><td>(1.1±0.1)X10^{-3}</td><td>617± 43</td><td>(1.8±0.3)X10^{-3}</td><td>14.6±3.4</td><td>6.2±2.0</td></tr></table>Fluor de Lys-SIRT1 assay for deacety-lation activity; TLC detection of base exchange activities.</td></tr>
<tr><td>yHst2</td><td><table><tr><th></th><th>kcat, min^{-1}</th><th>km, uM</th></tr><tr><td>NAD^+</td><td rowspan="2">1.01±0.23</td><td>10.2±0.3</td></tr><tr><td>Ac-poly-L-lysine</td><td>0.92±0.07</td></tr></table>Mutations of G32A, S36A, R45A, N116A, H135A, K178A, V228A, P230A, N248A, D263A, H338A were tested.Gel-based assay with [^{14}C]acetyl-poly-L-lysine substrate.</td><td>4. Lewis group, J Biol. Chem. 2006</td></tr>
<tr><td>yHst2</td><td><table><tr><th>By NAM</th><th>Km(NAD^+), uM</th><th>Kii, uM</th><th>Kis, uM</th></tr><tr><td>yHst2</td><td>16.1</td><td>170±28</td><td>140±3</td></tr><tr><td>I117V</td><td>25.5</td><td>64±40</td><td>65±1.1</td></tr><tr><td>I117F</td><td>25.8</td><td>1000±30</td><td>720±40</td></tr><tr><td>D118N</td><td>453.5</td><td>180±50</td><td>320±30</td></tr></table>Fluor de Lys fluorescent activity assay kit.</td><td>5. Marmorstein group, Mol. Cell 2007</td></tr>
<tr><td>Sir2Tm</td><td><table><tr><th>Km, uM (NAD^+)</th><th>Ki, uM (DADMe-NAD^+)</th><th>IC50, uM (DADMe-NAD^+)</th></tr><tr><td>125</td><td>360</td><td>720</td></tr></table>Fluorescence-based assay
 
</td><td rowspan="2">6.Wolberger group, Structure 2008</td></tr>
<tr><td>Sir2Tm (F33A)</td><td><table><tr><th></th><th>Kcat(NAD^+), s^{-1}</th><th>IC50(NAM), uM</th></tr><tr><td>Sir2Tm</td><td>5.9</td><td>480</td></tr><tr><td>Sir2Tm(F33A)</td><td>0.3</td><td>0.1</td></tr></table>Fluorescence-based assay and HPLC.</td></tr>
<tr><td>mSIRT3 (54-334)</td><td><table><tr><th>Substrate</th><th>Km, uM</th><th>Ki, uM (SRT1720)</th><th>Ki, uM (NAM)</th></tr><tr><td>NAD^+</td><td>280</td><td>0.34 (uncompetitive)</td><td>2.84 (competitive)</td></tr><tr><td>AceCS2</td><td>2.44</td><td>0.56 (competitive)</td><td>4.62 (competitive)</td></tr></table>Mass Spectrometry.
 
</td><td>7. Jin-Sirtris, Protein Sci. 2009</td></tr>
<tr><td>hSIRT1</td><td><table><tr><th>Km (NAD^+), uM</th><th>Km (p53), uM</th></tr><tr><td>94±5</td><td>4.5±0.17</td></tr></table>HPLC for kinetic study; NMR, ISC(isothermal titration calorimetry), SPR(surface plasmon resonance) for binding study</td><td>8.Pacholec-Pfizer, J Biol. Chem. 2010</td></tr>
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<table>
<tr>
<td>hSIRT1</td>
<td>
<table>
<tr><th>Substrate</th><th>Kcat, s^{-1}</th><th>Km(NAD^+), uM</th><th>Kcat/Km, s^{-1} mM^{-1}</th></tr>
<tr><td>EEK^{B}GQSTSSHSK^{Ac}JSTEGK^{T}EE</td><td>0.036</td><td>8.3</td><td>4.3</td></tr>
<tr><td>EEK^{B}GQSTSSHSK^{Ac}JSTEGKEE</td><td>0.038</td><td>33</td><td>1.2</td></tr>
<tr><td>EEKGQSTSSHSK^{Ac}JSTEGKEE</td><td>0.044</td><td>40</td><td>1.1</td></tr>
<tr><td>SKKGQSRHKK^{Ac}LJKTEGP</td><td>0.36</td><td>8.5</td><td>42</td></tr>
<tr><td>RHKK^{Ac}-AMC</td><td>0.091</td><td>110</td><td>0.8</td></tr>
<tr><td>RHKK^{Ac}</td><td>0.14</td><td>23</td><td>6</td></tr>
<tr><td>RHKK^{Ac}A</td><td>0.15</td><td>13</td><td>12</td></tr>
<tr><td>RHKK^{Ac}F</td><td>0.16</td><td>7.1</td><td>23</td></tr>
<tr><td>RHKK^{Ac}W</td><td>0.19</td><td>4</td><td>48</td></tr>
</table>
ITC for binding study, and MS for kinetic study.
</td>
<td>9. Dai-Sirtris, J Biol. Chem. 2010</td>
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<td>Sirtuins</td>
<td>
<table>
<tr><th>Sirtuin</th><th>Acetylated substrate</th><th>IC50, uM</th></tr>
<tr><td>Sir2Af2</td><td>NBK(Ac)AAAOMe</td><td>131±26.0</td></tr>
<tr><td>ySir2</td><td>H3mer</td><td>19.5±5.8</td></tr>
<tr><td>hSIRT1</td><td>p53mer</td><td>6.5±1.0</td></tr>
<tr><td>hSIRT2</td><td>p53mer</td><td>13.1±0.54</td></tr>
<tr><td>hSIRT3</td><td>H3mer</td><td>272±80.3</td></tr>
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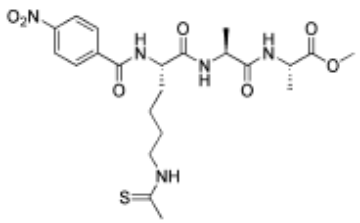
By
 
HPLC method
</td>
<td>10. Sauve group, Organic & Biomolecular Chem. 2011</td>
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<td>hSIRT3</td>
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<table>
<tr><td>100uM</td><th>hSIRT1 activity</th><th>hSIRT2 activity</th><th>hSIRT3 activity</th></tr>
<tr><td>Control</td><td>100±5</td><td>100±2</td><td>100±7</td></tr>
<tr><td>Suramin</td><td>18±1</td><td>20±1</td><td>NA</td></tr>
<tr><td>NAM</td><td>NA</td><td>71±3</td><td>20±2</td></tr>
<tr><td>Compound 2</td><td>88±6</td><td>92±2</td><td>16±2</td></tr>
<tr><td>Compound 6</td><td>105±5</td><td>110±1</td><td>36±1</td></tr>
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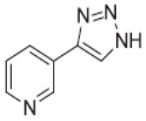
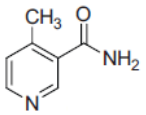
Compound 2  Compound 6 
SIRT1/SIRT2 fluorimertic drug discoverykit.
</td>
<td>11. Genazzani group, Euro J Med. Chem. 2012</td>
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<td>Sirtuins</td>
<td>
<table>
<tr><th></th><th>NAM</th><th>AGK2</th><th>Ex527</th><th>Suramin</th></tr>
<tr><td>hSIRT1</td><td>~50 uM</td><td></td><td>98 nM</td><td>297 nM</td></tr>
<tr><td>hSIRT2</td><td>~50 uM</td><td>3.5 uM</td><td></td><td>1.15 uM</td></tr>
<tr><td>hSIRT5</td><td></td><td></td><td></td><td>22 uM</td></tr>
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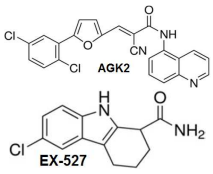
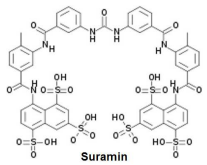

AGK2

EX-527
 
Suramin
</td>
<td>12.Altucci, Frontiers in Pharmacology 2012</td>
</tr>
<tr>
<td>hSIRT1</td>
<td>
<table>
<tr><th>Km, uM</th><td>82.6±14.6</td></tr>
<tr><th>Vmax, uM min^{-1}</th><td>6.8±0.5</td></tr>
<tr><th>Kcat, s^{-1}</th><td>0.18±0.01</td></tr>
<tr><th>Kcat/Km(x10^3), $M^{-1}s^{-1}$</th><td>2.1±0.4</td></tr>
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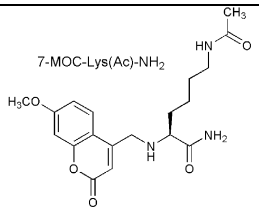
Capillary electrophoresis –based assay
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<td>13. Scriba group, Electrophoresis 2012</td>
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References

1. Bitterman KJ, Anderson RM, Cohen HY, Latorre-Esteves M, and Sinclair DA. Inhibition of silencing and accelerated aging by nicotinamide, a putative negative regulator of yeast Sir2and human SIRT1. J Biol. Chem. 277 (2002) 45099-45107.
2. Sauve AA, Moir RD, Schramm VL, and Willis IM. Chemical activation of Sir2-dependent silencing by relief of nicotinamide inhibition. Mol. Cell 17 (2005) 595-601.

3. Avalos JL, Bever KM, and Wolberger C. Mechanism of sirtuin inhibition by nicotinamide: altering the NAD+ cosubstrate specificity of a Sir2 enzyme. Mol. Cell 17 (2005) 855-868.
4. Khan AN, Lewis PN. Use of substrate analogs and mutagenesis to study substrate binding and catalysis in the Sir2 family of NAD-dependent protein deacetylases. J Biol. Chem. 281 (2006) 11702-11711.
5. Sanders BD, Zhao K, Slama JT, and Marmorstein M. Structural basis for nicotinamide inhibition and base exchange in Sir2 enzymes. Mol. Cell 25 (2007) 463-472.
6. Hawse WF, Hoff KG, Fatkins DG, Daines A, Zubkova OV, Schramm VL, Zheng W, and Wolberger C. Structural insights into intermediate steps in the Sir2 deacetylation reaction. Structure 16 (2008) 1368-1377.
7. Jin L, Galonek H, et al. Biochemical characterization, localization, and tissue distribution of the longer form of mouse SIRT3. Protein Sci. 18 (2009) 514-525.
8. Pacholec M, Bleasdale JE, et al. SRT1720, SRT2183, SRT1460, and Resveratrol are not direct activators of SIRT1. J Biol. Chem. 285 (2010) 8340-8351.
9. Dai H, Kustigian L, et al. SIRT1 activation by small molecules. Kinetic and biophysical evidence for direct interactionof enzyme and activator. J Biol. Chem. 285 (2010) 32695-32703.
10. Cen YN, Falco JN, Xu P, Youn DY, and Sauve AA. Mechanism-based affinity capture of sirtuins. ORGANIC & BIOMOLECULAR CHEMISTRY 9 (2011) 987-993.
11. Galli U, Mesenzani O, Coppo C, sorba G, Canonico PL, Tron GC, and Genazzani. Identification of a sirtuin 3 inhibitor that displays selectivity over sirtuin1 and 2. European J Med. Chem. 55(2012) 58-66.
12. Carafa V, Nebbioso A, and Altucci L. Sirtuins and disease: the road ahead. Frontiers in Pharmacology 3(2012)1-6.
13. Abromeit H, Kannan S, Sippl W, Scriba GKE. A new nonpeptide substrate of human sirtuin in a capillary electrophoresis-based assay. Investigation of the binding mode by docking experiments. Electrophoresis 33 (2012) 1652-1659.