Development of a NanoBRET assay to validate dual inhibitors of Sirt2-mediated lysine deacetylation and defatty-acylation that block prostate cancer cell migration

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Supplementary Data

Figure S1: Docking of compound 4 and SirReal2. ................................................................. 3

Figure S2. Results of MTS assays......................................................................................... 4

Figure S3. Time-dependent effect on cell viability in HGC-27 cells. ....................................... 4

Figure S4. c-Myc protein levels after treatment with Sirt2 inhibitor (10 µM) or DMSO for 48 h. 5

Figure S5. Proteasomal degradation of c-Myc in PC-3M-luc cells induced by Sirt2 inhibitors..... 5

Figure S6. Correlation between inhibition of migration and reduction of c-Myc protein levels..... 5

Figure S7. Immunostaining of acetylation levels of α-tubulin. ............................................... 6

Figure S8. Cellular permeability studies using the NanoBRET assay. ..................................... 7

Figure S9. Determination of $K_i$ for 12 based on Cheng-Prusoff using the NanoBRET assay. 7

Table S1 .................................................................................................................................. 8

Table S2. Results of MTS assay.............................................................................................. 9

Table S3. Inhibition of colony formation.............................................................................. 10

Table S4: Results from in vitro fluorescence polarization binding assay............................... 10

Scheme S1. Synthesis of SirReal-based Sirt2 inhibitors. ...................................................... 11

Compound syntheses ............................................................................................................ 12

NMR spectra......................................................................................................................... 23

Western Blot images ............................................................................................................ 24

References.............................................................................................................................. 28
**Figure S1: Docking of compound 4 and SirReal2.** Docking pose of 4 (R)-isomer colored orange, (S)-isomer colored magenta) in comparison with the crystal structure of Sirt2 bound with SirReal2 (colored cyan, PDB ID 4RMH). In the case of compound 4, the methoxy substituted naphthyl ring no longer fits well in the binding pocket due to steric effects and is unable to occupy the position of the naphthyl ring of SirReal2.
Figure S2. Results of MTS assays. Concentration-dependent cell viability curves of HGC-27, PC-3M-luc, HEK293T, HL-60 and MCF-7 cells are displayed for different Sirt2 inhibitors.

Figure S3. Time-dependent effect on cell viability in HGC-27 cells. Comparison of cell viability of HGC-27 for different Sirt2 inhibitors (10 µM) after 3 (dark grey) and 5 days (bright grey) of treatment. Values are presented as mean ± SD.
Figure S4. c-Myc protein levels after treatment with Sirt2 inhibitor (10 µM) or DMSO for 48 h.

Figure S5. Proteasomal degradation of c-Myc in PC-3M-luc cells induced by Sirt2 inhibitors. Immunoblot for c-Myc protein levels after treatment with Sirt2 inhibitor (10 µM) or DMSO in the presence or absence of the proteasome inhibitor MG-132.

Figure S6. Correlation between inhibition of migration and reduction of c-Myc protein levels. Plot correlating inhibition of migration and reduction of c-Myc levels at 10 µM of Sirt2 inhibitor in PC-3M-luc cells.
Figure S7. Immunostaining of acetylation levels of α-tubulin. Acetylation levels of α-tubulin (red) in the presence or absence of Sirt2 inhibitor (20 µM) in PC-3M-luc cells. Nuclei were DAPI-stained (blue).
Figure S8. Cellular permeability studies using the NanoBRET assay. A) NanoBRET assay curves displaying the relative affinity of the tracer (13) in permeabilized (digitonin-treated, 50 ng/µL) and non-permeabilized cells. B) NanoBRET assay curves displaying the relative affinity of 2 (left) and 12 (right) in permeabilized respectively untreated cells.

Figure S9. Determination of $K_i$ for 12 based on Cheng-Prusoff using the NanoBRET assay. A) Determination of apparent IC$_{50}$ values by fitting individual curves with the sigmoidal dose-response curve. B) Replots of apparent IC$_{50}$ values as a function of tracer concentration.
Table S1: Measured thermal shift assay $\Delta T_m$ values, pIC$_{50}$ values for Sirt2 inhibition and the calculated MM-GBSA interaction energies (PDB ID 5DYS) for the studied inhibitors.

| Name | $\Delta T_m$ ($^\circ$C) | pIC$_{50}$ | E GBSA (kcal/mol) |
|------|----------------|---------|------------------|
| 1    | 3.75           | 6.36    | -58.71           |
| 2    | 5.69           | 6.92    | -84.07           |
| (S)-3| 3.01*          | 6.59*   | -49.36           |
| (R)-3|                |         | -46.40           |
| (S)-4| 2.70*          | 4.34*   | -41.10           |
| (R)-4|                |         | -44.70           |
| 5    | 4.28           | 6.34    | -72.77           |
| (S)-6| 2.55*          | 5.22*   | -55.35           |
| (R)-6|                |         | -52.72           |
| 7    | 1.40           | 3.21    | ---              |
| 8    | 4.26           | 6.80    | -82.47           |
| 9    | 5.35           | 6.77    | -86.53           |
| 10   | 4.51           | 6.82    | -90.34           |
| 11   | 4.17           | 6.96    | -84.35           |
| 12   | 6.63           | 6.92    | -98.22           |

* Measured for the racemate.
**Table S2. Results of MTS assay.** Results of MTS assay for different Sirt2 inhibitors (10 µM) in HGC-27, PC-3M-luc, HEK293T, HL-60 and MCF-7 cells. Values represent % inhibition of cell viability as mean ± SD. Experiments were performed at least twice as triplicates. n.i. = no inhibition (% inhibition < 10%).

| Compound | HGC-27       | PC-3M-luc    | HEK293T      | HL-60       | MCF-7       |
|----------|--------------|--------------|--------------|-------------|-------------|
| SirReal2 | 27.2 ± 10.1  | 12.1 ± 1.1   | 18.8 ± 1.6   | n.i.        | 16.2 ± 3.5  |
| 5        | 44.2 ± 21.9  | 21.2 ± 13.8  | n.i.         | 24.3 ± 6.9  | 11.0 ± 5.0  |
| 6        | 11.6 ± 6.6   | n.i.         | n.i.         | n.i.        | n.i.        |
| 7        | 29.0 ± 7.0   | n.i.         | 11.6 ± 1.6   | n.i.        | n.i.        |
| 2        | 6.8 ± 4.4    | 27.4 ± 1.9   | 20.8 ± 8.3   | 9.9 ± 2.8   | 10.5 ± 1.7  |
| 8        | 40.6 ± 4.5   | 22.0 ± 1.4   | 18.4 ± 2.9   | 16.6 ± 4.7  | n.i.        |
| 9        | 32.7 ± 10.8  | 27.0 ± 0.2   | 55.1 ± 8.0   | 7.7 ± 2.0   | 10.8 ± 3.1  |
| 10       | 53.1 ± 10.7  | 33.8 ± 2.1   | 70.9 ± 1.7   | 4.6 ± 1.4   | n.i.        |
| 11       | 13.5 ± 2.9   | 29.2 ± 3.4   | 32.1 ± 7.6   | 4.6 ± 3.6   | n.i.        |
| 12       | 60.3 ± 6.6   | 30.9 ± 2.2   | 63.0 ± 6.5   | 13.4 ± 9.0  | n.i.        |
Table S3. Inhibition of colony formation. Results of colony formation assays (CFU) in PC-3M-luc cells after treatment with 25, 10 and 1 µM of selected Sirt2 inhibitors. Results are presented as mean ± SD.

| Compound | Concentration | Inhibition (%) | Compound | Concentration | Inhibition (%) |
|----------|---------------|----------------|----------|---------------|----------------|
| SirReal2 | 25 µM         | 4.3 ± 0.4      | 10       | 25 µM         | 50.1 ± 4.8     |
|          | 10 µM         | 13.8 ± 8.7     | 10       | 10 µM         | 30.4 ± 13.2    |
|          | 1 µM          | 27.4 ± 8.7     | 10       | 1 µM          | 10.8 ± 4.3     |
| 2        | 25 µM         | 44.0 ± 7.8     | 11       | 25 µM         | 42.4 ± 0.8     |
|          | 10 µM         | 15.1 ± 13.0    | 10       | 10 µM         | 29.8 ± 6.3     |
|          | 1 µM          | 13.5 ± 10.8    | 10       | 1 µM          | 17.0 ± 9.9     |
| 9        | 25 µM         | 32.9 ± 5.4     | 12       | 25 µM         | 94.2 ± 2.6     |
|          | 10 µM         | 28.0 ± 7.6     | 10       | 10 µM         | 8.7 ± 7.8      |
|          | 1 µM          | 4.8 ± 3.1      | 10       | 1 µM          | 7.2 ± 7.4      |

Table S4: Results from in vitro fluorescence polarization binding assay. IC₅₀ [µM] values (mean ± SD) are presented for different Sirt2 inhibitors. n.i. = no inhibition (% inhibition < 10% @ 10 µM)

| Compound | SirReal2 | 2   | 8    | 9    | 10   |
|----------|----------|-----|------|------|------|
| IC₅₀ [µM]| 0.37 ± 0.10 | 0.35 ± 0.13 | 0.76 ± 0.16 | 0.16 ± 0.04 | 0.15 ± 0.03 |

| Compound | 11 | 12 |
|----------|----|----|
| IC₅₀ [µM]| 0.22 ± 0.04 | 0.07 ± 0.03 |
Scheme S1. Synthesis of SirReal-based Sirt2 inhibitors. Reagents and conditions: a) propargyl bromide, NaOH, acetonitrile, 20 °C, overnight, 54% yield; b) NaNO₂, HCl, water, 0 °C, 20 min; acrolein, CuCl₂·2 H₂O, acetone, 20 °C, 3 h; thiourea, ethanol, reflux, 2 h, 16% yield; c) chloroacetyl chloride, N,N-diisopropylethylamine, acetonitrile, 20 °C, 2 h, 99% yield; d) 4,6-dimethyl-2-methylsulfanylpyrimidine, Na₂CO₃, KI, DMSO, 20 °C, 2 h, 76% yield; e) benzyl/aryl azide, sodium ascorbate, CuSO₄, TBTA, water/tBuOH/DMF (1:1:1), 20 °C, 2 h; 50-59% yield f) 1-azido-2-methoxyethane, sodium ascorbate, CuSO₄, TBTA, water/tBuOH/DMF (1:1:1), 20 °C, 2 h, 75% yield; g) chloroacetyl chloride, acetonitrile, N,N-diisopropylethylamine, 20 °C, 2 h, 88% yield; h) for 5: octanoyl chloride, DIPEA, acetonitrile, 20 °C, 3.5 h, 81% yield; for 6: Ibuprofen, EDC*HCl, DMAP, DCM, 20 °C, overnight, 97% yield; i) mercaptopyrimidine derivative, DMSO, Na₂CO₃, KI, 20 °C, 2 h, 37% yield.
Compound syntheses

The syntheses of 15, 16, 20 and 21 were performed according to previously published procedures.¹

5-(3-((1-{2-methoxyethyl}-1H-1,2,3-triazol-4-yl)methoxy)benzyl)thiazol-2-amine (17)

16 (8.19*10⁻⁴ mol, 200 mg) and 1-azido-2-methoxyethane (8.19*10⁻⁴ mol, 165.4 µl, 82.8 mg, 1 equiv.) were dissolved in tertbutanol/water (1:1, 2.2 mL) and a solution of TBTA (8.19*10⁻⁵ mol, 43.46 mg, 0.1 equiv.) in DMF (8.2 mL) was added. A copper sulfate solution (0.1 M, 812 µL, 0.1 equiv.) and subsequently a solution of sodium ascorbate (0.2 M, 1.6 mL, 0.2 equiv.) were added and the mixture was stirred at room temperature for 2 h. After quenching the reaction with water (4 mL) all solvents were evaporated and the residue was purified by automated flash chromatography (gradient of DCM/methanol) to get 213 mg (6.20*10⁻⁴ mol, 75 %) of 17 as a white solid. R_f: 0.62 (DCM/MeOH 95:5); ¹H-NMR (DMSO-D₆, 400 MHz, δ[ppm]): 8.17 (s, 1 H, triazole-H); 7.21-7.25 (m, 1 H, phenyl-H-5); 6.80-6.91 (m, 3 H, phenyl-H-2,4,6); 6.72 (bs, 3 H, amine-H₂ and aminothiazole-H); 5.11 (s, 2 H, triazole-CH₂-O-Ar); 4.54 (t, 2 H, ³J = 5.08, H₃-C-O-CH₂-CH₂-triazole); 3.87 (s, 2 H, Ar-CH₂-aminothiazole); 3.74 (t, 2 H, ³J = 5.08, H₃-C-O-CH₂-CH₂-triazole); 3.24 (s, 3 H, H₃-C-O-CH₂-CH₂-triazole); impurities: 3.35 (H₂O), 5.77 (DCM); ¹³C-NMR (DMSO-D₆, 100 MHz, δ[ppm]): 158.6 (phenyl C3, aminothiazole C2); 143.0 (triazole C4); 142.6 (phenyl C1); 136.0 (aminothiazole C4); 129.9 (phenyl C5); 125.3 (triazole C5); 121.2 (phenyl C6); 115.2 (phenyl C2); 112.7 (phenyl C4); 70.5 (H₃-C-O-CH₂-CH₂-triazole); 61.4 (triazole-CH₂-O-Ar); 58.4 (H₃-C-O-CH₂-CH₂-triazole); 49.7 (H₃-C-O-CH₂-CH₂-triazole); 32.9 (Ar-CH₂-aminothiazole); LRMS m/z (ESI⁺): [M+H]⁺ calculated for C₁₆H₂₀N₅O₂S⁺: 346.13, found: 346.2; HPLC (C18): retention time 13.24 min, 96.1 %.
2-chloro-N-(5-3-[(1-(2-methoxyethyl)-1H-1,2,3-triazol-4-yl)methoxy]benzyl)thiazol-2-yl)acetamide (18)

The amine 17 (95 mg, 0.3 mmol) was dissolved in acetonitrile (5 mL) and N,N-diisopropyl amine (72 µL, 53 mg, 0.4 mmol, 1.5 equiv.) was added. At 0 °C chloroacetyl chloride (33 µL, 47 mg, 0.4 mmol, 1.5 equiv.) was slowly added whilst stirring. The mixture was allowed to warm up to room temperature and stirred for 2 h. Volatiles were evaporated to give a red-brown oil. Addition of water (5 mL) led to precipitation of a light brown solid. The precipitate was washed with hydrochloric acid (1 M) and water to yield 112 mg (0.27 mmol, 88%) of a beige solid after drying. 

Rf: 0.69 (DCM/MeOH 95:5); \(^1^H\)-NMR (CDCl\(_3\), 400 MHz, \(\delta[ppm]\)): 7.74 (s, 1 H, triazole-H); 7.24 (t, 1 H, \(^3^J = 8.00\) Hz, phenyl H-5); 7.17 (s, 1 H, aminothiazole-H); 6.90-6.84 (m, 3 H, phenyl H-2,4,6); 5.19 (s, 2 H, triazole-CH\(_2\)-O-Ar); 4.54 (t, 2 H, \(^3^J = 4.98\) Hz, H\(_3\)C-O-CH\(_2\)-CH\(_2\)-triazole); 4.24 (s, 2 H, CO-CH\(_2\)-Cl); 4.06 (s, 2 H, Ar-CH\(_3\)-aminothiazole); 3.76 (t, 2 H, \(^3^J = 5.07\) Hz, H\(_3\)C-O-CH\(_2\)-CH\(_2\)-triazole); 3.25 (s, 3 H, H\(_3\)C-O-CH\(_2\)-CH\(_2\)-triazole).

\(^1^C\)-NMR (DMSO-D\(_6\), 100 MHz, \(\delta[ppm]\)): 172.4 ((C=O)-CH\(_2\)-Cl); 158.5 (aminothiazole C2); 157.5 (phenyl C3); 143.8 (triazole C4); 140.5 (phenyl C1); 133.2 (aminothiazole C4); 129.8 (phenyl C5); 123.8 (triazole C5); 121.3 (phenyl C6); 115.1 (phenyl C2); 113.0 (phenyl C4); 70.6 (H\(_3\)C-O-CH\(_2\)-CH\(_2\)-triazole); 61.9 (triazole-CH\(_2\)-O-Ar); 59.0 (H\(_3\)C-O-CH\(_2\)-CH\(_2\)-triazole); 50.3 (H\(_3\)C-O-CH\(_2\)-CH\(_2\)-triazole); 41.9 ((C=O)-CH\(_2\)-Cl); 32.8 (Ar-CH\(_2\)-aminothiazole). LRMS m/z (ESI\(^+\)): [M+H]\(^+\) calculated for C\(_{18}\)H\(_{21}\)ClN\(_5\)O\(_3\)S\(_2\): 422.10 and 424.1 in a 3:1 ratio, found: 422.3 and 424.3 in a 3:1 ratio.

4,6-diphenylpyrimidine-2-thiol (23)

To a solution of sodium tertbutyloxide (480 mg, 5 mmol, 1 equiv.) in ethanol (17 mL) thiourea (381 mg, 5 mmol) and 1,3-Diaryl-2-propen-1-one (1041 mg, 5 mmol, 1 equiv.) were added
successively. After refluxing for 1 h the solvent was evaporated, and the residue was dissolved in water (25 mL) and the mixture was acidified with acetic acid (1.7 mL). The resulting precipitate was filtered off and washed with ethanol. The crude product was dissolved in a solution of tert-butyloxide in ethanol (3 M, 100 mL) and refluxed for 2 h to get the 283 mg (1.07 mmol, 21%) of 23 as yellow solid. \( R_f: 0.54 \) (CH/EE 9:1); \(^1^H\)-NMR (CDCl\(_3\), 400 MHz, \( \delta [ppm] \)): 8.08-8.06 (m, 4 H, 2x phenyl H2,6); 7.60-7.48 (m, 6 H, 2x phenyl H3,4,5); 7.15 (s, 1 H, pyrimidine H5). LRMS m/z (ESI\(^+\)): [M+H]\(^+\) calculated for C\(_{16}\)H\(_{13}\)N\(_2\)S\(^+\): 265.08, found: 265.3.

2-((4,6-diphenylpyrimidin-2-yl)thio)-N-(5-((1-(2-methoxyethyl)-1H-1,2,3-triazol-4-yl)methoxy)benzyl)thiazol-2-yl)acetamide (7)

23 (12.1 mg, 0.05 mM, 1 equiv.) was dissolved in 183 µL DMSO. Na\(_2\)CO\(_3\) (11.0 mg, 0.09 mM, 2 equiv.) and KI (7.6 mg, 0.05 mM, 1 equiv.) were added. The mixture was stirred for 15 min at room temperature before the alkyl chloride 18 (20 mg, 0.05 mM, 1 equiv.) was added to the reaction mixture. After stirring for 2 h 914 µL water were added. The aqueous layer was extracted with ethyl acetate (3×20 mL) and the combined organic layers were dried over Na\(_2\)SO\(_4\) and solvents were evaporated. The crude products were purified by automated flash column chromatography (CH/EE gradient), to get 11 mg (17 µmol, 37%) of 7 respectively. \( R_f: 0.36 \) (DCM/MeOH 95:5); \(^1^H\)-NMR (CDCl\(_3\), 400 MHz, \( \delta [ppm] \)): 10.70 (s, 1 H, aminothiazole-NH-(C=O)); 8.12-9.09 (m, 4 H, pyrimidine-(phenyl H2,6)\(_2\)); 7.85 (s, 1 H, pyrimidine H5); 7.71 (s, 1 H, triazole H); 7.53-7.48 (m, 6 H, pyrimidine-(phenyl H3,4,5)\(_2\)); 7.20 (t, 1 H, \(^3^J = 8.02\) Hz, phenyl H5); 7.06 (s, 1 H, aminothiazole H); 6.88-6.80 (m, 3 H, phenyl H2,4,6); 5.16 (s, 2 H, O-CH\(_2\)-triazole); 4.52 (t, 2 H, \(^3^J = 5.09\) Hz, triazole-CH\(_2\)-CH\(_2\)-O-CH\(_3\)); 4.11 (s, 2 H, phenyl-CH\(_2\)-aminothiazole); 4.00 (s, 2 H, (C=O)-CH\(_2\)-S-pyrimidine); 3.75 (t, 2 H, \(^3^J = 5.08\) Hz, triazole-CH\(_2\)-CH\(_2\)-O-CH\(_3\)); 3.33 (s, 3 H, triazole-CH\(_2\)-CH\(_2\)-O-CH\(_3\)). \(^{13}C\)-NMR (CDCl\(_3\), 100 MHz, \( \delta [ppm] \)): 170.0 (pyrimidine C2); 166.8 ((C=O)-CH\(_2\)-S-pyrimidine); 165.6 (pyrimidine C4,6); 158.5 (phenyl C1); 143.9 (triazole C4); 140.9 (phenyl C3); 136.1 (pyrimidine-(phenyl C4)\(_2\)); 134.1 (aminothiazole C2); 131.4 (aminothiazole C3); 129.7...
(phenyl C5); 129.0 (pyrimidine-(phenyl C3,5)2); 127.3 (pyrimidine-(phenyl C2,6)2); 123.8 (triazole C5); 121.3 (phenyl C4); 115.1 (phenyl C2); 112.8 (phenyl C6); 109.4 (pyrimidine C5); 70.7 (triazole-CH2-CH2-O-CH3); 61.9 (O-CH2-triazole); 59.0 (triazole-CH2-CH2-O-CH3); 50.3 (triazole-CH2-CH2-O-CH3); 34.9 ((C=O)-CH2-S-pyrimidine); 32.9 (phenyl-CH2-aminothiazole). HRMS m/z [ESI⁺]: [M+ACN+H]⁺ calculated for C37H36N2O3S2⁺: 691.23, found: 691.26. HPLC (C18): retention time 24.77 min, 95.8%.

**N-(5-(3-((1-(2-methoxyethyl)-1H-1,2,3-triazol-4-yl)methoxy)benzyl)thiazol-2-yl)octanamide (5)**

To a cooled solution of the amine 17 (20 mg, 0.06 mmol, 1 equiv.) in acetonitrile (2 mL) 
N,N-diisopropylethylamine (14.7 µL, 11.0 mg, 0.09 mmol, 1.5 equiv.) and octanoyl chloride (14.8 µL, 14.1 mg, 0.09 mmol, 1.5 equiv.) were added whilst stirring. After 3.5 h at 30 °C the solvent was evaporated, and water was added to the residue. The aqueous phase was washed with ethyl acetate (3 x 20 mL). Subsequently, the combined organic phase was washed with brine, dried with MgSO4 and the solvent was evaporated. The product was purified via flash chromatography (DCM/MeOH 95/5). Afterwards, the product was dissolved in a sodium hydroxide solution (2 M) and the alkaline solution was again extracted with ethyl acetate (3 x 20 mL) to remove traces of the actanoic acid, giving 22.1 mg (0.05 mmol, 81 %) of 5. 

Rf: 0.57 (DCM/MeOH 95:5); ¹H-NMR (DMSO-D₆, 400.13 MHz, δ [ppm]): 11.91 (br s, 1 H, amide-H); 8.18 (s, 1 H, triazole-H); 7.23-7.24 (m, 1 H, phenyl-H-5); 7.23 (s, 1 H aminothiazole-H); 6.84-6.93 (m, 3 H, phenyl-H-2,4,6); 5.11 (s, 2 H, triazole-CH2-O-Ar); 4.54 (dd, 2 H, 3J = 5.03, 5.33 Hz, H3C-O-CH2-CH2-triazole); 4.04 (s, 2 H, Ar-CH2-aminothiazole); 3.73 (dd, 2 H, 3J = 5.36, 5.07 Hz, H3C-O-CH2-CH2-triazole); 3.24 (s, 3 H, H3C-O-CH2-CH2-triazole); 2.37 (t, 2 H, J = 7.33, CO-CH2-CH2-C4H8-CH3); 1.55 (m, 2 H, CO-CH2-CH2-C4H8-CH3); 1.25 (s, 8 H, CO-CH2-CH2-C4H8-CH3); 0.85 (t, 3 H, J = 6.71, CO-CH2-CH2-C4H8-CH3); impurities: 3.34 (H2O) ¹³C-NMR (DMSO-D₆, 100.61 MHz, δ [ppm]): 171.46 q (octanoyl-CH); 158.61 (phenyl C-3); 157.34 (aminothiazole C-2); 142.92 (triazole C-4); 142.36 (phenyl C-1); 134.94 (aminothiazole C-4); 131.15 (aminothiazole C-5); 130.05 (phenyl C-5); 125.31 (triazole C-5); 121.31 (phenyl C-4); 115.36 (phenyl C-2); 112.81 (phenyl C-6); 70.53 (H3C-O-CH2-
CH₂-triazole); 61.38 (triazole-CH₂-O-Ar); 58.34 (H₃C-O-CH₂-CH₂-triazole); 49.65 (H₃C-O-CH₂-CH₂-triazole); 35.20 (octanoyl-C₂); 32.32 (Ar-CH₂-aminothiazole); 31.51 (octanoyl-C₂); 28.88 (octanoyl-C₂); 28.78 (octanoyl-C₂); 25.14 (octanoyl-C₂); 22.47 (octanoyl-C₂); 14.35 (octanoyl-C₂); the carbon atoms of the octanoyl residue were assigned according to Paukstelis et al.¹³⁶ LMRS m/z (ESI⁺): [M+Na]⁺ calculated for C₂₄H₃₃N₅NaO₃S⁺: 494.21, found: 494.2, [M+Li]⁺ calculated for C₂₄H₃₅LiN₅O₃S⁺: 478.23, found: 478.2; HPLC (C18): retention time 23.60 min, 96.08%.

2-(4-isobutylphenyl)-N-(5-((3-(1-(2-methoxyethyl)-1H-1,2,3-triazol-4-yl)methoxy)benzyl)thiazol-2-yl)propenamide (6)

![Image of compound 6]

A mixture of ibuprofen (29.8 mg, 0.15 mmol, 2.5 equiv.), EDCI (27.9 mg, 0.15 mmol, 2.5 equiv.) and DMAP (14.2 mg, 0.12 mmol, 2 equiv.) in DCM (3 mL) was stirred for 30 min, before 17 (20 mg, 0.06 mmol, 1 equiv.), dissolved in DCM (2 mL), was added. The reaction mixture was stirred overnight and then quenched with water. The aqueous phase was extracted with DCM (3 x 10 mL) and the combined organic phases were washed with brine, dried with MgSO₄ and evaporated. The residue was purified via flash chromatography (DCM/MeOH 95/5) to give 29.9 mg (0.06 mmol, 97 %) of 6. Rf: 0.76 (DCM/MeOH 95:5); ¹H-NMR (DMSO-D₆, 400.13 MHz, δ [ppm]): 12.13 (br s, 1 H, amide-H); 8.16 (s, 1 H, triazole-H); 7.23-7.25 (m, 4 H, aminothiazole-H, phenyl-H-5, ibuprofen-Ar-H2,6); 7.09-7.11 (m, 2 H, ibuprofen-Ar-H3,5); 6.82-6.91 (m, 3 H, phenyl-H-2,4,6); 5.11 (s, 2 H, triazole-CH₂-O-Ar); 4.53 (dd, 2 H, 3J = 5.01, 5.39 Hz, H₃C-O-CH₂-CH₂-triazole); 4.03 (s, 2 H, Ar-CH₂-aminothiazole); 3.90 (q, 1 H, 3J = 6.94 Hz, ibuprofen-Ar-CH(CH₃)-CO) 3.72 (dd, 2 H, 3J = 5.34, 5.01 Hz, H₂C-O-CH₂-CH₂-triazole); 3.23 (s, 3 H, H₃C-O-CH₂-CH₂-triazole); 2.39 (d, 2 H, 3J = 7.30 Hz, ibuprofen-Ar-CH₂-CH(CH₃)-CO); 1.79 (m, 1 H, ibuprofen-Ar-CH₂-CH(CH₃)-CO); 1.38 (d, 3 H, 3J = 6.88 Hz, ibuprofen-Ar-CH₂-CH(CH₃)-CO); 0.84 (d, 6 H, 3J = 6.66, ibuprofen-Ar-CH₂-CH(CH₃)-CO); impurities: 3.35 (H₂O) ¹³C-NMR (DMSO-D₆, 100.61 MHz, δ [ppm]): 172.57 (ibuprofen- CO); 158.59 (phenyl C-3); 157.31 (aminothiazole C-2); 142.92 (triazole C-4); 142.30 (phenyl C-1); 140.22 (ibuprofen-Ar-C-4); 138.62 (ibuprofen-Ar-C-1); 135.05 (aminothiazole C-4); 131.48 (aminothiazole C-5); 130.06 (phenyl C-5); 129.44 (2 C, ibuprofen-Ar-C-2,6); 127.46 (2 C, ibuprofen-Ar-C-3,5);
125.30 (triazole C-5); 121.29 (phenyl C-4); 115.32 (phenyl C-2); 112.85 (phenyl C-6); 70.52 (H3C-O-CH2-CH2-triazole); 61.38 (triazole-CH2-O-Ar); 58.33 (H3C-O-CH2-CH2-triazole); 49.64 (H3C-O-CH2-CH2-triazole); 44.62 (ibuprofen-Ar-CH(CH3)-CO); 44.58 (ibuprofen-Ar-CH2-CH(CH3)2); 32.31 (Ar-CH2-aminothiazole); 30.01 (ibuprofen-Ar-CH2-CH(CH3)2); 22.59 (2 C, ibuprofen-Ar-CH2-CH(CH3)2); 18.63 (ibuprofen-Ar-CH(CH3)-CO); the carbon atoms of the ibuprofen residue were assigned according to Marathias et al.\textsuperscript{13}\textsuperscript{7} LMRS m/z (ESI\textsuperscript{+}): [M+Na]\textsuperscript{+} calculated for C\textsubscript{29}H\textsubscript{35}N\textsubscript{5}NaO\textsubscript{3}S\textsuperscript{+}: 556.22, found: 556.2, [M+Li]\textsuperscript{+} calculated for C\textsubscript{29}H\textsubscript{35}LiN\textsubscript{5}O\textsubscript{3}S\textsuperscript{+}: 540.25, found: 540.2; HPLC (C18): retention time 25.33 min, 96.90%.

2-((4,6-dimethylpyrimidin-2-yl)thio)-N-((5-((1-(2-methylbenzyl)-1H-1,2,3-triazol-4-yl)methoxy)benzyl)thiazol-2-yl)acetamide (8)

![Chemical Structure](image)

21 (0.08 mmol, 34 mg) and 1-azidomethyl-2-methylbenzole (0.08 mmol, 160 µl, 0.5 M in TBME, 1 equiv.) were dissolved in tertbutanol/water (1:1, 1.4 mL) and a solution of TBTA (8*10\textsuperscript{3} mmol, 4.00 mg, 0.1 equiv.) in DMF (0.8 mL) was added. A copper sulfate solution (0.1 mmol, 80 µL, 0.1 equiv.) and subsequently a solution of sodium ascorbate (0.2 mmol, 160 µL, 0.2 equiv.) were added and the mixture was stirred at room temperature for 2 h. After quenching the reaction with water (2 mL) all solvents were evaporated and the residue was purified by automated flash chromatography (gradient of DCM/methanol) to get a 19.5 mg (0.03 mmol, 43%) of 8 as a white solid. \textsuperscript{1}H-NMR (acetone-D\textsubscript{6}, 400.16 MHz, δ [ppm]): 11.51 (s, 1H, amide-H); 7.93 (s, 1H, triazole H-5); 7.30-7.14 (m, 6H, benzyl H-3,4,5,6 & aminothiazole H-4 & Phenyl H-5); 6.99 (s, 1H, pyrimidine H-5); 6.94 (s, 1H, phenyl H-2); 6.89 (d, \textsuperscript{3}J = 7.5 Hz, 2H, phenyl H-4,6); 5.65 (s, 2H, Ar-CH\textsubscript{2}-N); 5.15 (s, 2H, triazole-CH\textsubscript{2}-O-Ar); 4.08 (s, 4H, S-CH\textsubscript{2}-CO-N & Ar-CH\textsubscript{2}-aminothiazole); 2.41 (s, 6H, 2x CH\textsubscript{3}-pyrimidine); 2.35 (s, 3H, CH\textsubscript{3}-benzyl). \textsuperscript{13}C-NMR (acetone-D\textsubscript{6}, 100.61 MHz, δ [ppm]): 169.6 (pyrimidine C-2); 167.7 (pyrimidine C-4,6); 166.9 (Ar-NH-CO-CH\textsubscript{2}-S-Ar); 158.7 (phenyl C-3); 143.6 (triazole C-1); 141.9 (Phenyl C-1); 136.6 (benzyl C-4); 134.9 (aminothiazole C-4); 134.0 (benzyl C-1); 130.5 (phenyl C-5); 128.9 (benzyl C-6); 123.6 (triazole C-5); 120.9 (phenyl C-6); 116.3
(pyrimidine C-5); 115.0 (phenyl C-2); 112.7 (phenyl C-4); 61.4 (triazole-CH₂-O-Ar); 51.3 (Ar-CH₂-triazole), 34.0 (CO-CH₂-S); 32.3 (Ar-CH₂-aminothiazole); 22.7 (2x CH₃-pyrimidine); 18.15 (CH₃-benzyl). LMRS m/z (ESI⁺): [M+Na]⁺ calculated for C₂₉H₂₉N₇NaO₂S₂⁺: 594.17, found: 594.2; HPLC (C18): retention time 22.8 min, 98.7%.

2-((4,6-dimethylpyrimidin-2-yl)thio)-N-(5-3-((1-(4-methylbenzyl)-1H-1,2,3-triazol-4-yl)methoxy)benzyl)thiazol-2-yl)acetamide (9)

21 (0.08 mmol, 34 mg) and 1-azidomethyl-4-methylbenzene (0.08 mmol, 192 µl, 0.5 M in TBME, 1.2 equiv.) were dissolved in tertbutanol/water (1:1, 1.4 mL) and a solution of TBTA (8×10⁻³ mmol, 4.00 mg, 0.1 equiv.) in DMF (0.8 mL) was added. A copper sulfate solution (0.1 M, 80 µL, 0.1 equiv.) and subsequently a solution of sodium ascorbate (0.2 M, 160 µL, 0.2 equiv.) were added and the mixture was stirred at room temperature for 2 h. After quenching the reaction with water (2 mL) all solvents were evaporated and the residue was purified by automated flash chromatography (gradient of DCM/methanol) to get 27.0 mg (0.05 mmol, 59%) of 9 as a white solid. \(^1\)H-NMR (DMSO-D₆, 400.16 MHz, δ [ppm]): 12.23 (s, 1 H, amide-H); 8.22 (s, 1 H, triazole H-5); 7.26 (1 H, aminothiazole H-4); 7.24-7.15 (m, 5 H, benzyl H-2,3,5,6 and Phenyl H-5); 6.93 (s, 1 H, pyrimidine H-5); 6.91-6.80 (m, 3 H, phenyl H-2,4,6); 5.54 (s, 2 H, Ar-CH₂-N); 5.09 (s, 2 H, triazole-CH₂-O-Ar); 4.09 (s, 2 H, S-CH₂-CO-NO); 4.03 (s, 2 H, Ar-CH₂-aminothiazole); 2.28 (s, 9H, 2x CH₃-pyrimidine and CH₃-benzyl). \(^13\)C-NMR (DMSO-D₆, 100.61 MHz, δ [ppm]): 169.3 (pyrimidine C-2); 167.4 (pyrimidine C-4,6); 167.2 (Ar-NH-CO-CH₂-S-Ar); 158.6 (phenyl C-3); 157.3 (aminothiazole C-2); 143.3 (triazole C-1); 142.3 (phenyl C-1); 137.9 (benzyl C-4); 135.2 (aminothiazole C-4); 133.4 (benzyl C-1); 131.5 (aminothiazole C-5); 130.0 (phenyl C-5); 129.7 (benzyl C3,5); 128.4 (benzyl C-2,6); 124.9 (triazole C-5); 121.3 (phenyl C-6); 116.5 (pyrimidine C-5); 115.3 (phenyl C-2); 112.9 (phenyl C-4); 61.4 (triazole-CH₂-O-Ar); 53.0 (Ar-CH₂-triazole), 34.5 (CO-CH₂-S); 32.3 (Ar-CH₂-aminothiazole); 23.7 (2x CH₃-pyrimidine); 21.1 (CH₃-benzyl). LMRS m/z (ESI⁺): [M+Na]⁺ calculated for C₂₉H₂₉N₇NaO₂S₂⁺: 594.17, found: 594.2; HPLC (C18): retention time 22.9 min, 96.4%.
2-((4,6-dimethylpyrimidin-2-yl)thio)-N-{5-((3-((1-(4-cyanobenzyl)-1H-1,2,3-triazol-4-yl)methoxy)benzyl)thiazol-2-yl)acetamide (10)

[Chemical structure image]

21 (0.08 mmol, 34 mg) and 1-azidomethyl-2-methylbenzol (0.08 mol, 160 µl, 0.5 M in TBME, 1 equiv.) were dissolved in tertbutanol/water (1:1, 1.4 mL) and a solution of TBTA (8*10⁻³ mmol, 4.00 mg, 0.1 equiv.) in DMF (0.8 mL) was added. A copper sulfate solution (0.1 M, 80 µL, 0.1 equiv.) and subsequently a solution of sodium ascorbate (0.2 M, 160 µL, 0.2 equiv.) were added and the mixture was stirred at room temperature for 2 h. After quenching the reaction with water (2 mL) all solvents were evaporated and the residue was purified by automated flash chromatography (gradient of DCM/methanol) to get 23.2 mg (0.04 mmol, 50%) of 10 as a white solid.

¹H-NMR (DMSO-D₆, 400.16 MHz, δ [ppm]): 12.23 (s, 1 H, amide-H); 8.32 (s, 1 H, triazole H-5); 7.89-7.84 (m, 2 H, benzyl H-3,5); 7.45 (d, J = 8.5 Hz, 2 H, benzyl H-2,6); 7.26 (1 H, aminothiazole H-4); 7.25-7.20 (m, 1 H, phenyl H-5); 6.94 (s, 1 H, pyrimidine H-5); 6.92-6.82 (m, 3 H, phenyl H-2,4,6); 5.73 (s, 2 H, Ar-CH₂-N); 5.12 (s, 2 H, triazole-CH₂-O-Ar); 4.09 (s, 2 H, S-CH₂-CO-N); 4.03 (s, 2 H, Ar-CH₂-aminothiazole); 2.28 (s, 6H, 2x CH₃-pyrimidine). ¹³C-NMR (DMSO-D₆, 100.61 MHz, δ [ppm]): 169.3 (pyrimidine C-2); 167.4 (pyrimidine C-4,6); 167.3 (Ar-NH-CO-CH₂-S-Ar); 158.5 (phenyl C-3); 157.3 (aminothiazole C-2); 143.6 (triazole C-1); 142.3 (phenyl C-1); 141.9 (benzyl C-1); 135.2 (aminothiazole C-4); 133.2 (benzyl C-2,6); 131.5 (aminothiazole C-5); 130.1 (phenyl C-5); 129.1 (benzyl C3,5); 125.4 (triazole C-5); 121.4 (phenyl C-2); 119.0 (C≡N); 116.5 (pyrimidine C-5); 115.4 (phenyl C-2); 112.9 (phenyl C-4); 112.9 (triazole-CH₂-O-Ar); 52.6 (Ar-CH₂-triazole), 34.5 (CO-CH₂-S); 32.2 (Ar-CH₂-aminothiazole); 23.7 (2x CH₃-pyrimidine). LMRS m/z (ESI⁺): [M+Na]⁺ calculated for C₂⁹H₂₆N₈NaO₂S₂⁺: 605.15, found: 605.2; HPLC (C18): retention time 21.2 min, 99.9%.
2-((4,6-dimethylpyrimidin-2-yl)thio)-N-5-(3-((1-(4-chlorophenyl)-1H,1,2,3-triazol-4-yl)methoxy)benzyl)-thiazol-2-yl)-acetamide (11)

![Chemical Structure](image)

21 (0.08 mmol, 34 mg) and 1-azido-4-chlorobenzene (0.10 mmol, 160 µl, 0.5 M in TBME, 1 equiv.) were dissolved in tertbutanol/water (1:1, 1.4 mL) and a solution of TBTA (8*10⁻³ mmol, 4.00 mg, 0.1 equiv.) in DMF (0.8 mL) was added. A copper sulfate solution (0.1 M, 80 µL, 0.1 equiv.) and subsequently a solution of sodium ascorbate (0.2 M, 160 µL, 0.2 equiv.) were added and the mixture was stirred at room temperature for 2 h. After quenching the reaction with water (2 mL) all solvents were evaporated and the residue was purified by automated flash chromatography (gradient of DCM/methanol) to get 18.4 mg (0.03 mmol, 40%) of 11 as a white solid. ¹H-NMR (DMSO-D₆, 400.16 MHz, δ [ppm]): 12.23 (s, 1 H, Amide-H); 8.97 (s, 1 H, triazole H-5); 7.99-7.92 (m, 2 H, benzyl H-2,6); 7.73-7.63 (m, 2 H, benzyl H-3,5); 7.29-7.22 (m, 2 H, phenyl H5 and aminothiazole H-4); 6.99-6.86 (m, 4 H, phenyl H-2,4,6 and pyrimidine H-5); 5.21 (s, 2 H, triazole-CH₂-O-Ar); 4.08 (s, 2 H, S-CH₂-CO-N); 4.06 (s, 2 H, Ar-CH₂-aminothiazole); 2.28 (s, 6 H, 2x CH₃-pyrimidine). ¹³C-NMR (DMSO-D₆, 100.61 MHz, δ [ppm]): 169.3 (pyrimidine C-2); 167.4 (pyrimidine C-4,6); 167.2 (Ar-NH-CO-CH₂-S-Ar); 158.6 (phenyl C-3); 157.3 (aminothiazole C-2); 144.5 (triazole C-1); 142.4 (phenyl C-1); 135.8 (benzyl C-1); 135.2 (aminothiazole C-4); 133.4 (benzyl C-4); 131.4 (aminothiazole C-5); 130.3 (benzyl C-3,5); 130.1 (phenyl C-5); 123.3 (triazole C-5); 122.3 (benzyl C-2,6); 121.5 (phenyl C-6); 116.5 (pyrimidine C-5); 115.4 (phenyl C-2); 112.9 (phenyl C-4); 61.3 (triazole-CH₂-O-Ar); 34.5 (CO-CH₂-S); 32.3 (Ar-CH₂-aminothiazole); 23.7 (2x CH₃-pyrimidine). LMRS m/z (ESI⁺): [M+Na]⁺ calculated for C₄₂H₂₄N₇NaO₂S₂Cl⁺: 600.10, found: 600.1; HPLC (C18): retention time 24.1 min, 98.3%.
4-(2-(4-(azidomethyl)phenoxy)ethyl)morpholine (23)

(4-(2-morpholinoethoxy)phenyl)methanol\(^2\) (1.26 mmol, 300 mg, 1 equiv.), sodium azide (1.52 mmol, 99 mg, 1.2 equiv.) and triphenylphosphane (1.39 mmol, 365 mg, 1.1 equiv.) were dissolved in a mixture of CCl\(_4\)/DMF (1/4, 10 mL). The mixture was heated to 90 °C and refluxed for 3 h under N\(_2\)-atmosphere. After cooling down, diethylether (10 mL) was added and the organic phase was washed with water (10 mL). The ether was evaporated, and the remaining mixture cooled to 0 °C and filtered to remove crystallized triphenylphosphanoxide. The organic phase was dried with MgSO\(_4\) and the solvents were evaporated. The product was purified via flash chromatography (DCM/MeOH; 1-10 % MeOH gradient) to give 102 mg (0.389 mmol, 31%) of 23 as a yellow oil. \(R_f\): 0.52 (DCM/MeOH 95/5). \(^1\)H-NMR (DMSO-D\(_6\), 400.16 MHz, \(\delta\) [ppm]): 7.33-7.25 (m, 2 H, Aryl-\(H\)\(_2,6\)); 7.00-6.93 (m, 2 H, Aryl-\(H\)\(_3,5\)), 4.35 (s, 2 H, Aryl-\(CH_2\)-\(N_3\)); 4.08 (t, \(J=5.8\) Hz, 2 H, O-\(CH_2\)-\(CH_2\)); 3.61-3.52 (m, 4 H, morpholine-\(H\)\(_2,6\)); 2.68 (t, \(J=5.8\) Hz, 2 H, N-\(CH_2\)-\(CH_2\)), 2.43 (m, 4 H, morpholine-\(H\)\(_3,5\)). \(^13\)C-NMR (DMSO-D\(_6\), 100.61 MHz, \(\delta\) [ppm]): 158.40 (Aryl-\(C_1\)); 132.04 (Aryl-C 4); 130.08 (2 C, Aryl-C 2,6); 114.63 (2 C, Aryl-C 3,5); 66.18 (2 C, morpholine-C 2,6); 65.31 (O-\(CH_2\)-\(CH_2\)); 57.01 (N-\(CH_2\)-\(CH_2\)); 53.64 (2 C, morpholine-C 3,5); 53.18 (N_3-\(CH_2\)-Aryl).

2-((4,6-dimethylpyrimidin-2-yl)thio)-N-{(3-{(1-(4-(2-morpholinoethoxy)benzyl)-1H-1,2,3-triazol-4-yl)methoxy}benzyl)thiazol-2-yl)acetamide (12)

21 (0.38 mmol, 162 mg, 1 equiv.) and 23 (0.38 mol, 100 mg, 1 equiv.) were dissolved in tertbutanol/water (1:1, 10 mL). A copper sulfate solution (0.1 M, 382 µL, 0.1 equiv.) and subsequently a solution of sodium ascorbate (0.2 M, 763 µL, 0.2 equiv.) were added and the mixture was stirred at room temperature for 2 h. After quenching the reaction with water (2 mL) all solvents were evaporated and the residue was purified by automated flash chromatography
(gradient of DCM/methanol) to get 105 mg (0.15 mmol, 40 %) of 12 as a white solid. Yield: 40%,

Rf: 0.68 (DCM/MeOH 95:1), 1H-NMR (DMSO-D6, 400.16 MHz, δ [ppm]): 12.22 (s, 1 H, amide-H);
8.20 (s, 1 H, triazole H-5); 7.27 (m, 2 H, benzyl H2,6); 7.25 (1 H, aminothiazole H-4); 7.23-7.18 (m, 1 H,
phenyl H-5); 6.95-6.90 (m, 3 H, pyrimidine H-5 and benzyl H-3,5); 6.90-6.85 (m, 2 H, phenyl H-2,6);
6.82 (d, 3J = 7.6 Hz, 1 H, phenyl H-4); 5.49 (s, 2 H, Ar-CH2-N); 5.07 (s, 2 H, triazole-CH2-O-Ar); 4.08
(s, 2 H, S-CH2-CO-N); 4.05 (t, 3J = 5.8 Hz, 2 H, O-CH2-CH2-morpholin); 4.02 (2, 2 H, Ar-CH2-
aminothiazole); 3.61-3.50 (m, 4 H, morpholine H-2,6); 2.66 (t, 3J = 5.8 Hz, 2 H, O-CH2-CH2-
morpholine); 2.48-2.40 (m, 4 H, morpholine H-3,5); 2.27 (s, 6H, 2x CH3-pyrimidine). 13C-NMR
(DMSO-D6, 100.61 MHz, δ [ppm]): 168.9 (pyrimidine C-2); 167.0 (pyrimidine C-4,6); 166.9 (Ar-NH-
CO-CH2-S-Ar); 158.4 (benzyl C1); 158.2 (phenyl C-3); 156.9 (aminothiazole C-2); 142.9 (triazole C-
1); 141.9 (phenyl C-1); 134.8 (aminothiazole C-4); 131.0 (aminothiazole C-5); 129.6 (benzyl C3,5
and phenyl C-5); 128.0 (benzyl C-4); 124.3 (triazole C-5); 120.9 (phenyl C-2); 116.1 (pyrimidine C-
5); 114.9 (phenyl C-2); 112.5 (phenyl C-4); 66.2 (morpholine C-2,6); 65.3 (O-CH2-CH2-morpholine);
61.0 (triazole-CH2-O-Ar); 57.0 (O-CH2-CH2-morpholine); 53.6 (morpholine C-3,5); 52.4 (Ar-CH2-
triazole), 34.1 (CO-CH2-S); 31.9 (Ar-CH2-aminothiazole); 23.3 (2x CH3-pyrimidine). LMRS m/z (ESI\+)
: [M+Na]+ calculated for C34H38N8NaO4S2+: 709.23, found: 709.2, [M+H]+ calculated for
C34H39N8O4S2+: 687.25, found: 687.3; HPLC (C18): retention time 16.7 min, 99.5%.
NMR spectra

Exemplary $^1$H NMR spectra (top) and $^{13}$C NMR spectra (bottom) of compound 12
Western Blot images

Effects on c-Myc level by different Sirt2 inhibitors:

Concentration-dependent effect on c-Myc levels:
**Time-dependent effect on c-Myc levels:**

|        | DMSO | SirReal2 | 2   | 12  |
|--------|------|----------|-----|-----|
| Time   | 6    | 12       | 24  | 24  |

**Western Blot Analysis**

- **c-Myc**
- **GAPDH**

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DMSO  | SirReal2 | 2 | 12 |
------|----------|---|----|
10    | 1        | 0.1| 10 | 1 | 0.1| 10 | 1 | 0.1|

**Western Blot Analysis**

- **c-Myc**
- **GAPDH**
Proteasomal degradation of c-Myc:
Detection of KRas4a fatty acylation levels:

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