

Chromo-pharmacophores:

Photochromic diarylmaleimide inhibitors for sirtuins

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Supporting Information

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Synthesis

General preliminary remark: Starting materials were purchased from commercial suppliers and used without any further purification. The syntheses of compounds **2**¹, **5**² and **13**¹ were described elsewhere. Solvents were used in p.a. quality and dried according to common procedures, if necessary. Dry nitrogen was used as inert gas atmosphere. Thin-layer chromatography (TLC) for reaction monitoring was performed with alumina plates coated with Merck silica gel 60 F₂₅₄ (layer thickness: 0.2 mm) and analyzed under UV-light (254 nm). Flash column chromatography was performed with Sigma Aldrich MN silica gel 60M (0.040-0.063 mm, 230-400 mesh) as stationary phase on a Biotage Isolera One automated flash purification system with UV-Vis detector. Melting points were determined using a Büchi Melting point B-545 or a Stanford Research Systems OptiMelt MPA 100 and are uncorrected. NMR spectra were recorded using a Bruker Avance 300 (¹H: 300 MHz, ¹³C: 75 MHz, T = 295 K) or a Bruker Avance 400 (¹H: 400 MHz, ¹³C: 101 MHz, T = 300 K) or a Varian 400 (¹H: 400 MHz, ¹³C: 101 MHz, T = 295 K) instrument. The spectra are referenced against the NMR solvent and are reported as follows: ¹H: chemical shift δ (ppm), multiplicity (s = singlet, d = doublet, t = triplet, m = multiplet, m_c = symmetrical multiplet, b = broad), integration, coupling constant (*J* in Hz). ¹³C: chemical shift δ (ppm), abbreviations: (+) = primary/tertiary, (–) = secondary, (q) = quaternary carbon. The assignment resulted from DEPT, APT, HMBC and HSQC experiments. Mass spectra were measured with a Finnigan MAT 95, Finnigan MAT SSQ 710 A, ThermoQuest Finnigan TSQ 7000 or an Agilent Q-TOF 6540 UHD instrument. Standard hand-held lamps (Herolab, 312 nm, 6 W) were used for visualizing TLC and to perform the ring closure reactions. The ring opening reactions were performed using a LED (2.5 W, 530 nm emission maximum). Absorption spectra were recorded on a Varian Cary 50 Bio UV/Vis spectrophotometer or an Agilent 8453 UV-visible Spectroscopy System or an Perkin Elmer Lambda 25 UV/Vis Spectrometer. IR spectra were measured using a BioTools Chiral IR_2X. Photostationary states and purity of the final compounds were measured on a Agilent 1290 Series HPLC: Column: Phenomenex Luna C18, 3 μ m, 150 x 2.00 mm, 100 Å; flow: 0.3 mL/min; solvent A: H₂O [0.059 Gew% TFA], solvent B: MeCN; gradient: 0-35 min: A/B 40/60, 35-40 min: A/B 5/95.

Synthesis and characterization of new compounds:

3-(2-Methyl-5-phenylthiophen-3-yl)-4-(2-methylbenzo[b]thiophen-3-yl)-1H-pyrrole-2,5-dione

(3): Maleimide **15** (90 mg, 0.2 mmol), K₃PO₄ (0.2 g, 1.0 mmol), phenylboronic acid (44 mg,

0.4 mmol), XPhos (11 mg, 0.024 mmol) and $\text{Pd}_2(\text{dba})_3$ (11 mg, 0.012 mmol) were stirred in 1-BuOH (2 mL) at 100 °C overnight. The reaction mixture was filtered and 1 M HCl (3 mL) was added. The aqueous phase was extracted with EtOAc (2 x 3 mL). The combined organic phases were washed with brine (10 mL), dried over MgSO_4 and filtered. The solvent was evaporated and the crude product was purified by automated flash column chromatography (heptane/EtOAc: 5/1). Compound **3** (40 mg, 40%) was obtained as red solid with traces of **15**. mp 124 °C. ^1H -NMR (400 MHz, CDCl_3): δ = 1.77 (s, 3H), 2.28 (s, 3H), 7.20-7.32 (m, 4H), 7.37-7.45 (m, 5H), 7.49-7.53 (m, 2H), 7.89 (d, 1H, J = 8.0 Hz), 11.33 (s, 1H). ^{13}C NMR (101 MHz, CDCl_3): δ = 14.7 (+), 15.3 (+), 122.5 (+), 122.9 (q), 123.1 (+), 124.7 (+), 124.8 (+), 125.2 (+), 125.4 (+), 128.2 (+), 128.5 (q), 129.7 (+), 133.4 (q), 133.6 (q), 137.1 (q), 137.8 (q), 138.3 (q), 140.0 (q), 140.9 (q), 142.0 (q), 171.4 (q), 171.8 (q). IR [cm^{-1}]: ν = 2935, 1717, 1653, 1434, 1026. HR-MS (ESI, $[\text{M}+\text{H}]^+$): calcd. for $\text{C}_{24}\text{H}_{18}\text{NO}_2\text{S}_2$: 418.0764; found 418.0762.

Methyl-2-(5-chloro-2-methylthiophen-3-yl)acetate (6): Acetate **5** (0.87 g, 5.00 mmol) was added to a suspension of TTN (2.30 g, 6.00 mmol) in MeOH (21 mL) and HClO_4 (2.5 mL) at RT. After stirring overnight the mixture was concentrated under vacuum and diluted with water (3 mL). The aqueous phase was extracted with CHCl_3 (3 x 3 mL), dried over MgSO_4 and filtered. The solvent was evaporated and purification of the crude product by flash column chromatography (PE/EtOAc: 5/1) yielded compound **6** (0.85 g, 83%) as yellow liquid. ^1H -NMR (400 MHz, CDCl_3): δ = 2.31 (s, 3H), 3.46 (s, 2H), 3.69 (s, 3H), 6.72 (s, 1H). ^{13}C -NMR (101 MHz, CDCl_3): δ = 13.0 (+), 33.7 (−), 52.1 (+), 125.4 (q), 127.9 (+), 128.8 (q), 134.3 (q), 171.0 (q). IR [cm^{-1}]: ν = 3493, 2147, 1663, 1435, 1406, 1001. HR-MS (APCI, $[\text{M}+\text{H}]^+$): calcd. for $\text{C}_8\text{H}_{10}\text{ClO}_2\text{S}$: 205.0084; found 205.0085.

Methyl 2-(2-methyl-5-phenylthiophen-3-yl)acetate (7): Acetate **6** (170 mg, 0.83 mmol), K_3PO_4 (264 mg, 1.25 mmol), phenylboronic acid (152 mg, 1.25 mmol), XPhos (40 mg, 0.083 mmol) and $\text{Pd}_2(\text{dba})_3$ (38 mg, 0.042 mmol) were stirred in 1,4-dioxane (1.5 mL) at 100 °C overnight. After cooling to RT the reaction mixture was diluted with EtOAc (5 mL) and the organic phase was washed with water (2 x 5 mL). The organic phase was dried over MgSO_4 , filtered and the solvent was removed under reduced pressure. The crude product was purified by automated flash column chromatography (PE/EtOAc: 10/1). The acetate **7** (105 mg, 52%) was obtained as yellow oil. ^1H -NMR (300 MHz, CDCl_3): δ = 2.42 (s, 3H), 3.56 (s, 2H), 3.71 (s, 3H), 7.12 (s, 1H), 7.20-7.29

(m, 1H), 7.31-7.38 (m, 2H), 7.51-7.56 (m, 2H). ^{13}C -NMR (75 MHz, CDCl_3): δ = 13.3 (+), 34.1 (-), 52.1 (+), 125.1 (+), 125.4 (+), 127.1 (+), 128.8 (+), 130.2 (q), 134.3 (q), 135.3 (q), 140.0 (q), 171.5 (q). HR-MS (EI, $[\text{M}^{+\bullet}]$): calcd. for $\text{C}_{14}\text{H}_{14}\text{O}_2\text{S}$: 246.0715; found 246.0716.

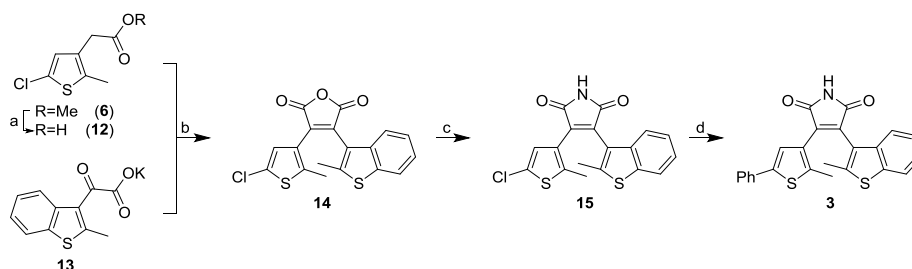
Methyl-2-(5-chloro-2-methylthiophen-3-yl)-2-oxoacetate (8): Methyl chlorooxoacetate (760 μL , 8.29 mmol) and **4** (1.00 g, 7.54 mmol) were added to a suspension of AlCl_3 (4.02 g, 30.2 mmol) in CH_2Cl_2 (40 mL) at 0 $^\circ\text{C}$. The reaction was stirred for 2 h at 0 $^\circ\text{C}$ and then quenched by a mixture of water/ice. The aqueous phase was extracted with CH_2Cl_2 (2 x 40 mL) and the combined organic phases were washed with a saturated solution of NaHCO_3 (70 mL) and brine (70 mL). The organic phase was dried over MgSO_4 , filtered and the solvent was evaporated. The crude product was purified by automated flash column chromatography (heptane/EtOAc: 10/1) and yielded **8** (1.20 g, 73%) as light yellow solid. mp 33 $^\circ\text{C}$. ^1H -NMR (400 MHz, CDCl_3): δ = 2.70 (s, 3H), 3.93 (s, 3H), 7.36 (s, 1H). ^{13}C -NMR (101 MHz, CDCl_3): δ = 16.1 (+), 53.0 (+), 126.0 (q), 127.9 (+), 130.7 (q), 153.0 (q), 163.4 (q), 179.0 (q). IR [cm^{-1}]: ν = 2957, 1738, 1678, 1522, 1460, 1292, 1198, 1132. HR-MS (EI, $[\text{M}^{+\bullet}]$): calcd. for $\text{C}_8\text{H}_7\text{ClO}_3\text{S}$: 217.9804; found 217.9808.

Methyl 2-(2-methyl-5-phenylthiophen-3-yl)-2-oxoacetate (9): Acetate **8** (500 mg, 2.29 mmol), K_3PO_4 (729 mg, 3.44 mmol), phenylboronic acid (418 mg, 3.44 mmol), XPhos (109 mg, 0.23 mmol) and $\text{Pd}_2(\text{dba})_3$ (104 mg, 0.12 mol) were stirred in 1,4-dioxane (1.5 mL) at 100 $^\circ\text{C}$ overnight. After cooling to RT the reaction mixture was diluted with EtOAc (5 mL) and the organic phase was washed with water (2 x 5 mL). The organic phase was dried over MgSO_4 , filtered and the solvent was removed under reduced pressure. The crude product was purified by automated flash column chromatography (PE/EtOAc: 0% $\rightarrow$ 15% EtOAc). The oxoacetate **9** (377 mg, 63%) was thus obtained as brown oil. ^1H -NMR (400 MHz, CDCl_3): δ = 2.79 (s, 3H), 3.96 (s, 3H), 7.30-7.34 (m, 1H), 7.35-7.43 (m, 2H), 7.52-7.57 (m, 2H), 7.67 (s, 1H). ^{13}C NMR (101 MHz, CDCl_3): δ = 16.3 (+), 52.8 (+), 124.6 (+), 125.8 (+), 127.9 (+), 128.1 (+), 128.6 (+), 129.0 (+), 130.5 (q), 132.3 (q), 133.1 (q), 140.5 (q), 153.8 (q), 164.1 (q). HR-MS (APCI, $[\text{M}+\text{H}]^+$): calcd. for $\text{C}_{14}\text{H}_{13}\text{O}_3\text{S}$: 261.0583; found 261.0580.

2-(2-Methyl-5-phenylthiophen-3-yl)-2-oxoacetamide (10): To a solution of the oxoacetate **9** (150 mg, 0.61 mmol) in THF (1.5 mL) was added a NH_4OH solution (25% in H_2O) (214 mg,

6.11 mmol) at 0 °C. The reaction was stirred overnight at RT and then diluted with water (3.0 mL). The aqueous phase was extracted with EtOAc (3 x 5 mL). The combined organic phases were dried over MgSO₄, filtered and the solvent was removed under reduced pressure. Amide **10** (120 mg, 80%) was obtained as colorless solid and used without further purification. mp 136 °C. ¹H-NMR (300 MHz, DMSO-d₆): δ = 2.72 (s, 3H), 7.32-7.38 (m, 1H), 7.40-7.50 (m, 2H), 7.57-7.67 (m, 2H), 7.80 (s, 1H), 7.92 (s, 1H), 8.26 (s, 1H). ¹³C-NMR (75 MHz, DMSO-d₆): δ = 15.4 (+), 125.0 (+), 125.2 (+), 128.0 (+), 129.2 (+), 132.4 (q), 133.0 (q), 138.9 (q), 151.0 (q), 167.0 (q), 185.5 (q). HR-MS (ESI, [M+H]⁺): calcd. for C₁₃H₁₂NO₂S: 246.0583; found 246.0587.

3,4-Bis(2-methyl-5-phenylthiophen-3-yl)-1H-pyrrole-2,5-dione (11): KOtBu (1 M in THF) (490 μL, 0.49 mmol) was added to a solution of amide **10** (107 mg, 0.41 mmol) in THF (2.0 mL) at 0 °C. After 90 min stirring at 0 °C ester **7** (100 mg, 0.41 mmol) was added at 0 °C and stirred overnight at RT. The reaction was quenched with 1 M HCl (5 mL) and diluted with EtOAc (5 mL). The organic phase was washed with water (3 x 5 mL) and brine (10 mL). The organic phase was dried over MgSO₄, filtered and the solvent was removed under reduced pressure. Purification by automated flash column chromatography (CH₂Cl₂/MeOH: 0% → 10% MeOH) yielded **11** (30 mg, 17%) as dark purple solid. mp 176 °C. ¹H-NMR (300 MHz, DMSO-d₆): δ = 1.99 (s, 6H), 7.28-7.34 (m, 2H), 7.39-7.45 (m, 6H), 7.54-7.59 (m, 4H), 11.28 (s, 1H). ¹³C-NMR (75 MHz, DMSO-d₆): δ = 14.2 (+), 124.7 (+), 124.9 (+), 127.6 (+), 128.1 (q), 129.1 (+), 132.9 (q), 133.6 (q), 139.7 (q), 139.9 (q), 171.3 (q). HR-MS (ESI, [M+NH₄]⁺): calcd. for C₂₆H₂₃N₂O₂S₂: 459.1195; found 459.1199.



Scheme S1: Synthetic strategy for the preparation of **3**: a) NaOH, EtOH, RT, overnight (86%); b) Ac₂O, 110 °C, 3 h, (60%); c) HMDS, MeOH, DMF, overnight, (73%); d) Pd₂(dba)₃, XPhos, phenyl boronic acid, K₃PO₄, 1,4-dioxane, 100 °C, overnight, (40%).

2-(5-Chloro-2-methylthiophen-3-yl)acetic acid (12): NaOH (0.21 g, 5.13 mmol) was added to a solution of **6** (0.70 g, 3.42 mmol) in EtOH (9 mL) at RT and stirred overnight. The solution was

concentrated under vacuum and water (9 mL) was added. The aqueous phase was washed with Et₂O (2 x 10 mL) and finally acidified to pH 1 with conc. HCl. The aqueous phase was extracted with Et₂O (2 x 20 mL). The combined organic phases were dried over MgSO₄, filtered and the solvent was removed under reduced pressure. The acid **12** (0.56 g, 86%) was obtained as light brown solid and used without further purification. mp 84 °C. ¹H-NMR (400 MHz, DMSO-d₆): δ = 2.26 (s, 3H), 3.46 (s, 2H), 6.86 (s, 1H), 12.33 (bs, 1H). ¹³C-NMR (101 MHz, DMSO-d₆): δ = 13.1 (+), 33.7 (–), 123.2 (q), 129.5 (+), 131.2 (q), 134.5 (q), 172.2 (q). IR [cm^{–1}]: ν = 2935, 1717, 1201, 1072, 995. HR-MS (ESI, [M–H][–]): calcd. for C₇H₆ClO₂S: 188.9783; found 188.9786.

3-(5-Chloro-2-methylthiophen-3-yl)-4-(2-methylbenzo[b]thiophen-3-yl)furan-2,5-dione (14): A mixture of the acid **12** (0.31 g, 1.64 mmol), the potassium salt **13** (0.42 g, 1.64 mmol) and acetic anhydride (4 mL) was heated to 110 °C for 3 h. The reaction was cooled to RT and quenched by adding water (5 mL). The aqueous phase was extracted with EtOAc (3 x 5 mL) and the combined organic phases were washed with brine (8 mL). The organic phase was dried over MgSO₄, filtered and the solvent was removed under reduced pressure. The crude product was purified by automated flash column chromatography (heptane/EtOAc: 5/1) to yield the maleic anhydride **14** (0.37 g, 60%) as orange foam. ¹H-NMR (400 MHz, DMSO-d₆): δ = 1.70 (s, 3H), 2.31 (s, 3H), 7.05 (s, 1H), 7.29 (m_c, 2H), 7.50 (m_c, 1H), 7.91 (m_c, 1H). ¹³C-NMR (101 MHz, DMSO-d₆): δ = 14.5 (+), 15.3 (+), 121.3 (q), 122.7 (+), 123.1 (+), 124.9 (+), 125.0 (+), 125.5 (q), 125.9 (q), 127.7 (+), 135.0 (q), 137.6 (q), 137.9 (q), 138.1 (q), 142.3 (q), 144.1 (q), 164.9 (q), 165.2 (q). IR [cm^{–1}]: ν = 3393, 2939, 2818, 1764, 1439, 1406, 1003. HR-MS (ESI, [M+H]⁺): calcd. for C₁₈H₁₂ClO₃S₂: 374.9911; found 374.9910.

3-(5-Chloro-2-methylthiophen-3-yl)-4-(2-methylbenzo[b]thiophen-3-yl)-1H-pyrrole-2,5-dione (15): HMDS (0.86 g, 5.33 mmol) was added to a solution of **14** (0.20 g, 0.53 mmol) in MeOH (110 μL, 2.66 mmol) and DMF (2 mL) at RT and stirred overnight. The reaction was quenched by adding 0.1 M HCl (4 mL) and the aqueous phase was extracted with EtOAc (3 x 5 mL). The combined organic phases were washed with brine (15 mL), dried over MgSO₄, filtered and the solvent was removed under reduced pressure. The maleimide **15** (0.15 g, 73%) was obtained as orange-yellow solid. mp 228 °C. ¹H-NMR (400 MHz, DMSO-d₆): δ = 1.68 (s, 3H), 2.28 (s, 3H), 7.04 (s, 1H), 7.27 (m_c, 2H), 7.40 (m_c, 1H), 7.89 (m_c, 1H), 11.33 (s, 1H). ¹³C-NMR (101 MHz,

DMSO- d_6): δ = 14.4 (+), 15.3 (+), 122.5 (+), 122.7 (q), 123.0 (+), 124.6 (q), 124.7 (+), 124.8 (+), 127.1 (q), 128.4 (+), 134.0 (q), 136.2 (q), 137.9 (q), 138.2 (q), 140.3 (q), 142.2 (q), 171.2 (q), 171.6 (q). IR [cm^{-1}]: ν = 3369, 2939, 2899, 1437, 1406, 1003. HR-MS (ESI, $[\text{M}-\text{H}]^-$): calcd. for $\text{C}_{18}\text{H}_{11}\text{ClNO}_2\text{S}_2$: 372.9955; found 372.9953.

Photochromism:

Photochemical syntheses of the closed and open photoisomers:

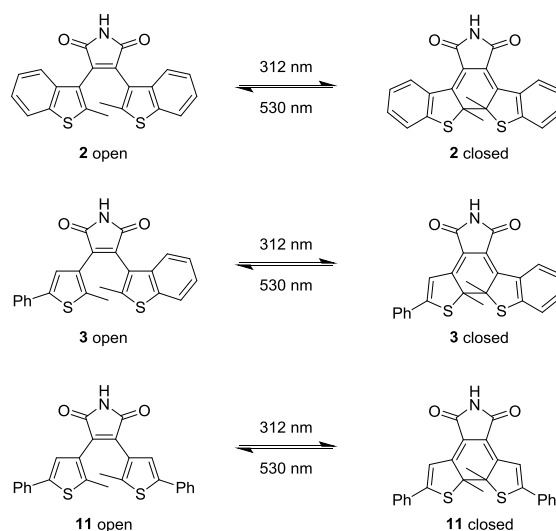


Figure S1: Photochemical ring closure and ring opening reactions of compounds **2**, **3** and **11**.

The reversible interconversion of compounds **2**, **3** and **11** (10 μM in DMSO) was performed by irradiating the solutions with a lamp (λ = 312 nm) to obtain the closed photoisomers. The spectral changes were complete after irradiation for 14 s (compound **2**), 38 s (compound **3**) and 60 s (compound **11**). The open photoisomers were reobtained with a LED (530 nm) (Figure S1). The spectral changes were complete after 15 min, respectively. Figure 3 shows exemplarily the changes in the UV/Vis absorption spectrum of compound **11**.

Photostationary states: The photostationary states were analyzed by HPLC measurements and contained 62% (compound **2**), 87% (compound **3**) and 94% (compound **11**) of the closed photoisomers. The ratios were determined at the isosbestic points, respectively. The respective HPLC chromatograms of the open and closed photoisomers of **11** are shown in Figure S2.

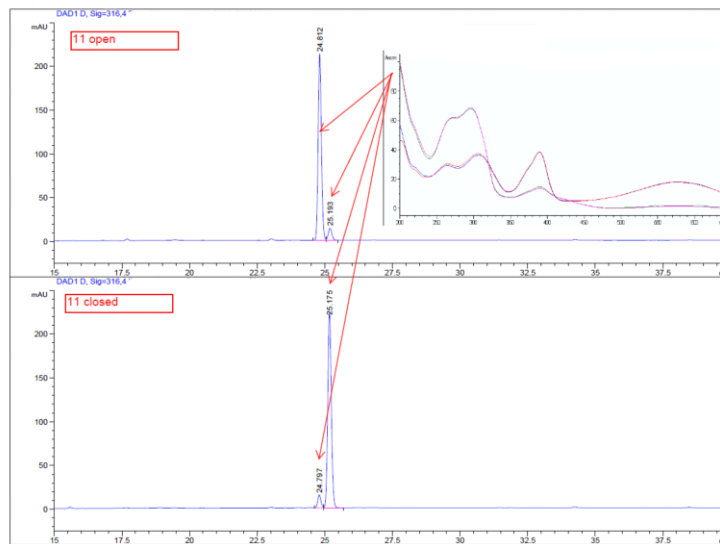


Figure S2: Representative HPLC chromatograms for the determination of the photostationary state of compound **11**. Ratios were determined at 316 nm. The upper chromatogram shows the open photoisomer of **11**, the lower chromatogram shows the closed photoisomer of **11** after irradiation with $\lambda = 312$ nm for 60 s. The absorption spectra of both photoisomers of **11** are shown in the right panel.

Cyclic performance: Photochemical cycling studies were performed to show the fatigue resistance of compounds **2**, **3** and **11**. Therefore solutions of compounds **2**, **3** and **11** (10 μ M in DMSO) were alternately irradiated with $\lambda = 312$ nm for 60 s and with $\lambda = 530$ nm for 15 min. The absorption change at 495 nm (compound **2**), 527 nm (compound **3**) and 580 nm (compound **11**) was measured. A representative cycle performance is shown for compound **11** in Figure S3.

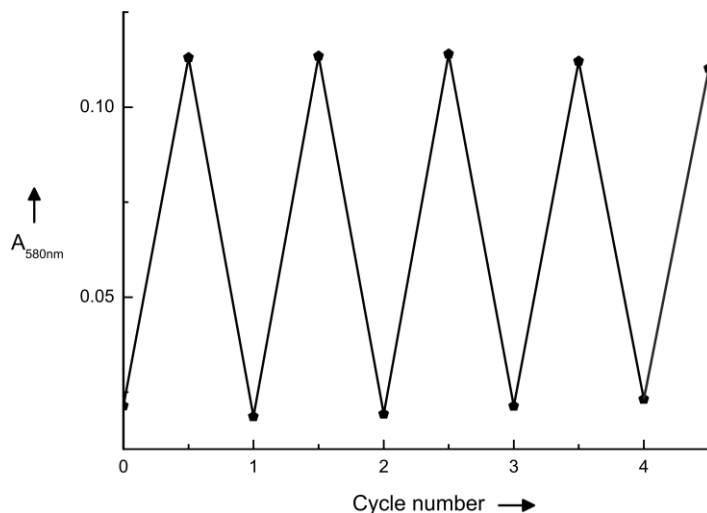


Figure S3: Representative cycle performance of compound **11** (10 μ M in DMSO). The repetitive switching cycles were performed using $\lambda = 312$ nm for ring closing (60 s) and $\lambda = 530$ nm for ring opening (15 min) of **11**.

Biological test results:

Recombinant proteins: Human Sirt1₁₃₃₋₇₄₇ was expressed as a GST-tagged enzyme and purified as described previously.³ Human Sirt2₂₂₅₋₃₈₉ was expressed N-terminally tagged with His₆⁴ with minor modifications.³ The gene sequence coding for human Sirt3₁₁₈₋₃₉₅ was cloned in a modified pET15b vector that contained His₁₀-Tag instead of a His₆-Tag and a cleavage site for TEV protease instead of one for thrombin. Sirt3₁₁₈₋₃₉₅ was expressed in E. coli strain BL21(DE3) Codonplus RIPL cells overnight at 18 °C. Overexpression was induced with IPTG, (0.1 mM) at an OD₆₀₀ of 0.6. Cells were harvested, resuspended in lysis buffer (50 mM HEPES, 500 mM NaCl, 5% (v/v) glycerol, 5 mM beta-mercaptoethanol, pH 7.5) and lysed using a microfluidizer (Microfluidics). After removal of cell debris, the supernatant was applied to a HisTrapFF 5 mL column (GE Healthcare), washed intensively and eluted using an imidazole gradient (20 mM-300 mM). The eluted protein was concentrated and further purified using a Superdex S75 26/60 gel filtration column (25 mM HEPES, 200 mM NaCl, 5% (v/v) glycerol, 5 mM beta-mercaptoethanol, pH 7.5). Sirt3-containing fractions were collected and concentrated. Identity and purity of the produced enzymes was verified using SDS-PAGE.⁵ Protein concentration was determined by the Bradford assay.⁶ Deacetylase activity of Sirtuin isoforms was NAD⁺-dependent and could be inhibited by nicotinamide.

Homogenous fluorescent assay: The inhibitory effect towards human Sirt1, Sirt2 and Sirt3 was determined by a histone deacetylase assay, previously established by Jung *et. al.*⁷ Therefore human Sirt1₁₃₃₋₇₄₇, Sirt2₂₂₅₋₃₈₉ or human Sirt3₁₁₈₋₃₉₅ were mixed with assay buffer (50 mM Tris, 137 mM NaCl, 2.7 mM KCl, pH 8.0), NAD⁺ (final assay concentration 500 µM), the substrate Z-(Ac)Lys-AMC, also termed ZMAL (final assay concentration 10,5 µM), the inhibitor dissolved in DMSO at various concentrations or DMSO as a control (final DMSO concentration 5% (v/v)). Total substrate conversion of controls was driven to about 15% - 30% to assure initial state conditions. The assay was carried out in 96-well plates with a reaction volume of 60 µL per well. All determinations were performed as triplicates. After an incubation of 4 h at 37 °C and 140 rpm, deacetylation was stopped by addition of 60 µL of a solution containing trypsin and nicotinamide (50 mM Tris, 100 mM NaCl, 6.7% (v/v) DMSO, trypsin 5.5 U/µL, 8 mM nicotinamide, pH 8.0). The microplate was further incubated for 20 min at 37 °C and 140 rpm. Finally, fluorescence intensity was measured in a microplate reader (BMG Polarstar, λ_{ex} 390nm,

λ_{em} 460nm). Rates of inhibition were calculated by using the controls, containing no inhibitor, as a reference. Graphpad Prism software (La Jolla, USA) was employed to determine IC₅₀ values.

Kinetic measurements: For the kinetic measurements the closed photoisomer of **11** was incubated with the enzyme under assay conditions (with an assay concentration of 10 μ M) in a 96-well plate, wrapped with aluminum foil. The plate was covered with a black foil and step by step the foil was removed and the single rows were irradiated with a LED (2.5 W, 530 nm emission maximum) for 0, 2.5, 5, 7.5, 10, 20 and 30 min. After irradiation the cofactor NAD⁺ and ZMAL were added and the assay was performed under the usual conditions. All determinations were performed as triplicates.

To measure the photochemical conversion of the closed photoisomer to the open isomer of **11**, the sample (10 μ M in buffer) was irradiated in a quartz cuvette with a LED (2.5 W, 530 nm emission maximum) for 0, 2.5, 5, 7.5, 10, 20 and 30 min and the absorption changes were monitored on a UV/Vis spectrometer. The ratio of the open to the closed photoisomer of **11** was determined according to the following equation:

$$\frac{c_{open}}{c_{closed}} = \frac{A_{closed} - A}{A - A_{open}}$$

Equation S1: Determination of the ratio between the closed and the open photoisomer of **11**. A_{closed} = absorption of the complete closed photoisomer of **11**; A_{open} = absorption of the complete open photoisomer of **11**; A = absorption measured after irradiation with a LED (2.5 W, 530 nm emission maximum).

Analysis of tubulin acetylation: HeLa cells (DSMZ accession no. 057) were grown in RPMI1640 medium (Pan Biotech) containing 10% (vol/vol) fetal bovine serum (PAN Biotech), 1% (vol/vol) penicillin (PAA), 1% (vol/vol) streptomycin (PAA), 1% L-glutamine (PAA) at 37 °C in a 5% (vol/vol) CO₂ atmosphere. Cells were seeded in 24-well plates (greiner bio-one) in a volume of 400 μ L/well and incubated overnight to a confluency of 30 – 40%. Compounds were dissolved in DMSO (concentration 200fold of final test concentration). Inhibitor stock solution in DMSO or DMSO alone (negative control) were first diluted 1:20 with sterile PBS and 40 μ L were added to the cells (max. DMSO concentration 0.5% (v/v)). After 4 h incubation time a washing step with pre-warmed PBS and removal of the final wash solution was performed. 100 μ L RIPA buffer (50 mM TrisHCl, pH 7.4, 150 mM NaCl, 1% Triton X-100 (vol/vol), 0.5% (wt/vol) sodium

deoxycholate, 0,1% (vol/vol) SDS, 1 mM EDTA, 1 x protease inhibitor cocktail (complete mini, Roche)) was added to each well. The plates were incubated at 4 °C in a refrigerator for 5 min and scraped. Extracts were centrifuged at 8000 x g at 4 °C for 10 min to pellet the cell debris. Protein concentrations were determined using the BCATM Protein Assay Kit (Pierce). About 2.4 µg of protein samples were electrophoresed on 12,5% SDS-polyacrylamide gels and transferred to a PVDF membrane using Trans-Blot Turbo Transfer Pack (BIO-RAD). Membranes were blocked with 5% milk powder TBST 0,1% for 1 h and probed with anti-acetyl- α -tubulin (1:1000, Sigma-Aldrich, T6793) and anti GAPDH (1:1000, Sigma-Aldrich; G9545) as a loading control. **Ro31-8220 (1)** was used as a positive control.⁸

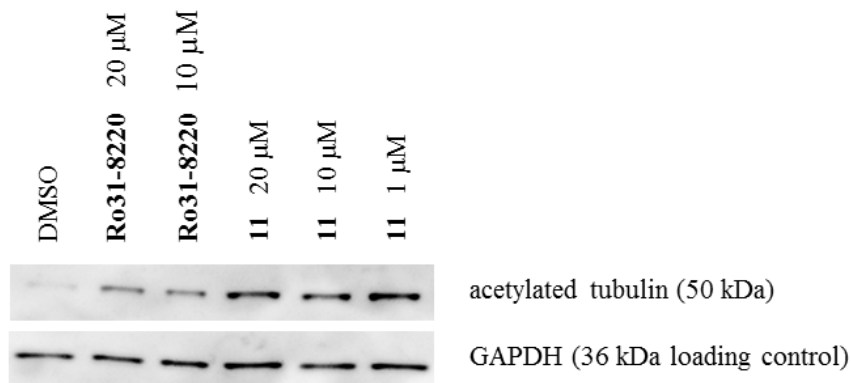


Figure S4: Western blot analysis for the validation of Sirt2 inhibition in cell culture for compound **11** (20, 10 and 1 µM) and as a positive control **Ro31-8220 (1)** (20 and 10 µM), using an antibody to the acetylated form of tubulin.

Molecular modeling:

General preliminary remark: Crystal structures of hSirt1, hSirt2 and hSirt3 were taken from the Protein Data Bank (PDB).^{9, 10} The following structures were analyzed in the current work: open conformation of hSirt1 (4IG9.pdb), closed conformation of hSirt1 (4KXQ.pdb), apo (open conformation) hSirt2 (3ZGO.pdb), hSirt2 complexed with ADP-Ribose (3ZGV.pdb), hSirt2 inhibited with a cyclic peptide inhibitor (4L3O.pdb) and apo (open conformation) hSirt3 (3GLS.pdb). The apo structures represent the open conformation of sirtuins whereas the substrate-complexed or inhibited conformations show a more closed substrate binding site. All protein structures were first prepared by using the Structure Preparation module in MOE 2012.10.¹¹ Hydrogen atoms were added, for titratable amino acids the protonation state was calculated using the Protonate 3D module in MOE. All protein structures were energy minimized

using the AMBER99 force field¹² using a tethering force constant of $(3/2) \text{ kT} / \sigma^2$ ($\sigma = 0.5 \text{ \AA}$) for all atoms during the minimization. Atom types and AM1-BCC atomic charges were generated for the ligand using the Antechamber module. Docking studies were performed using the GOLD 5.2 program.¹³ Bound water molecules at the binding pocket of the sirtuins were considered for docking taking the toggle water mode within GOLD. Goldscore was taken as scoring function to choose the docking pose. The potential binding site for the docking study was defined by taking into account all amino acids found within a 15 Å radius of Val179 in hSirt2 or the corresponding residue in hSirt1 (Val412) and hSirt3 (Val292). The best ranked pose from each docking run was included in the analysis and viewed graphically together with the hSirt2 structure using program MOE. Rescoring of the dockings poses was carried out using the scoring function within GOLD (Goldscore, Chemscore, ASP, PLP Score) and rescoring using the MM-GBSA method implemented in MOE 2012.10. For the MM-GBSA calculation a single protein-inhibitor complex was selected and minimized taking the protocol described above and treating the ligand fully flexible. The applied docking protocol was able to correctly reproduce the location and conformation of the bound ADPR (3ZGV.pdb) and cyclic peptide (4L3O.pdb) in hSirt2.

Supplementary Figures and Tables:

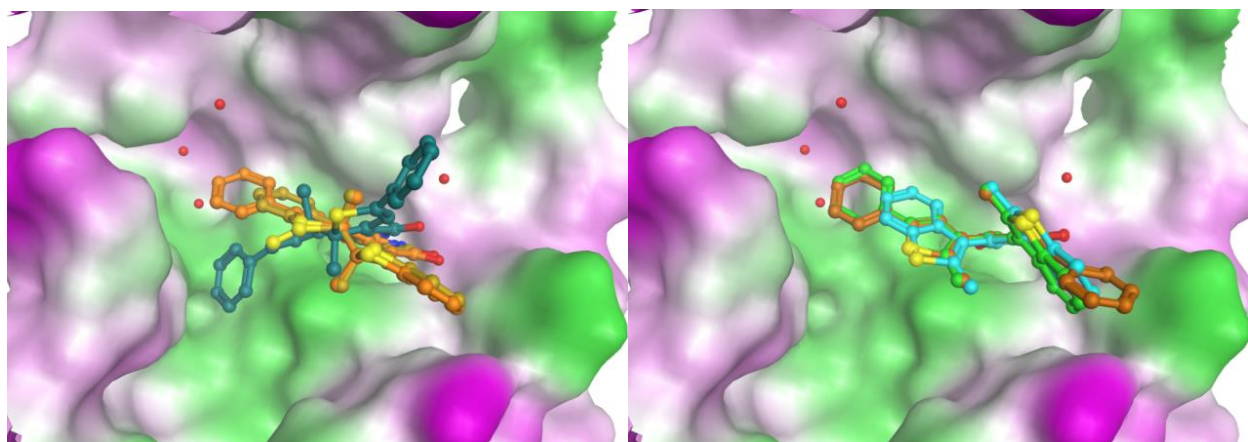


Figure S5: left) Predicted binding pose for the closed photoisomers **2** (yellow), **3** (orange) and **11** (dark green) within the hSirt2 binding pocket; right) Binding mode for the open photoisomers **2** (cyan), **3** (green) and **11** (orange). Bound water molecules within the hSirt2 binding pocket are shown as red balls. The molecular surface is colored according to the hydrophobicity (hydrophobic = green, hydrophilic = magenta).

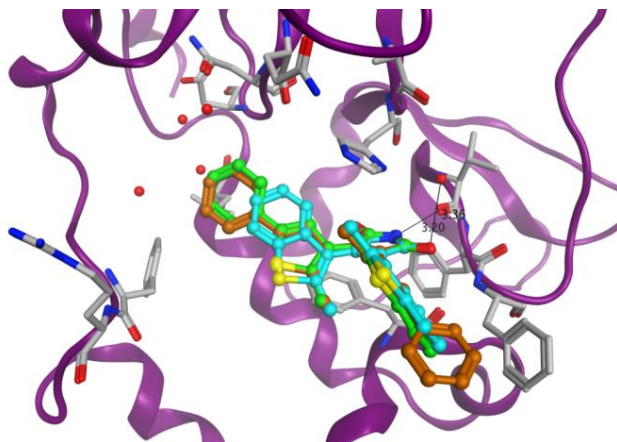


Figure S6: Interaction of open photoisomer **2** (cyan), **3** (green) and **11** (orange) at the hSirt2 (violet ribbon) substrate binding site. Only amino acids close to the inhibitors are shown. Bound water molecules are shown as red balls. The hydrogen bond to the backbone of Val233 is shown as black line (distance given in Å).

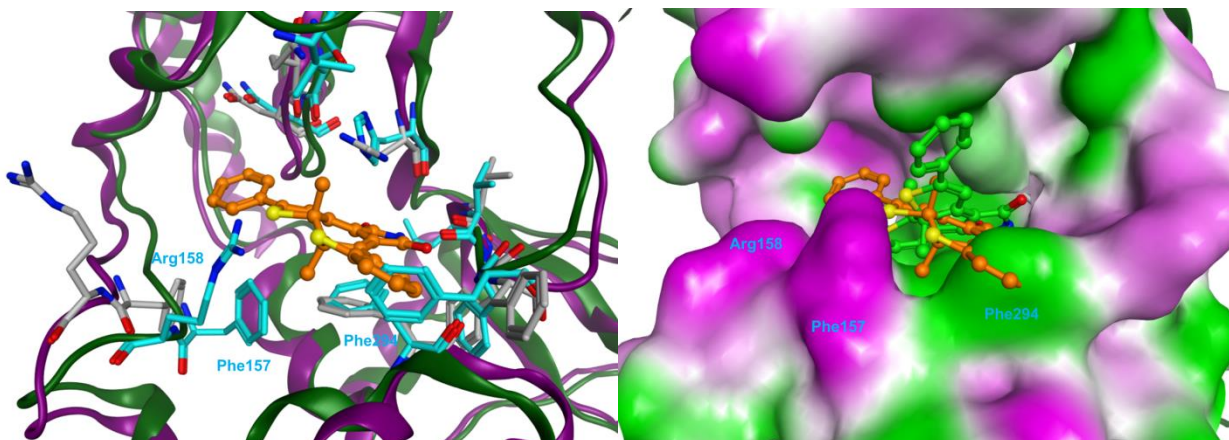


Figure S7: left) Comparison of hSirt2 (violet ribbon, grey amino acid residues) with docked closed photoisomer **3** (orange) and hSirt3 (dark green ribbon, cyan amino acid residues). In the hSirt3 structure (open conformation), Phe157, Arg158 and Phe294 hinder the interaction of the diarylmales; right) The molecular surface of the hSirt3 binding pocket is displayed and color coded according to the hydrophobic potential. A steric clash between the inhibitors (conformations taken from the hSirt2 complexes) and Phe157, Arg158 and Phe294 is observed.

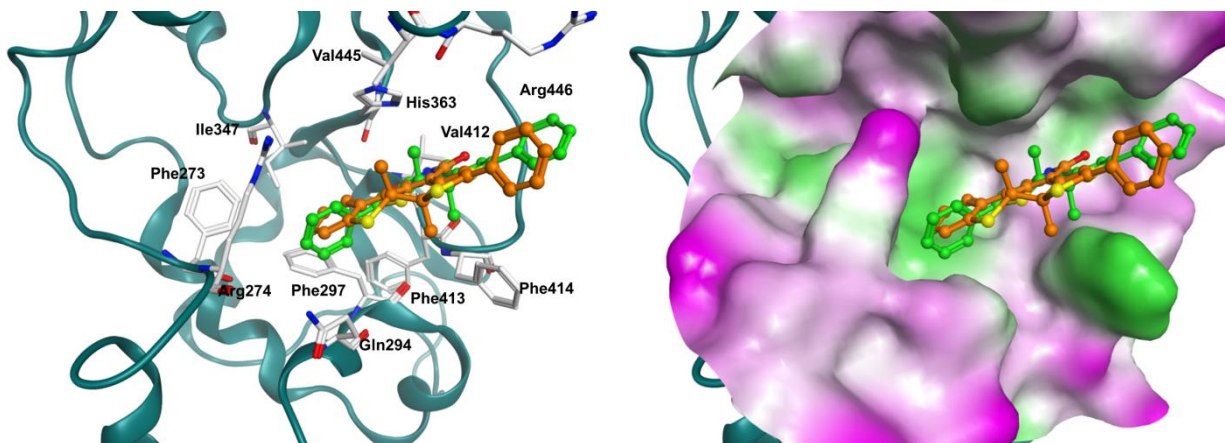


Figure S8: left) Predicted binding pose for the closed photoisomers **3** (orange) and **11** (green) within the hSirt1 binding pocket. Due to the different conformation of the flexible loops no hydrogen bond between the maleimide and Val412 is observed; right) The molecular surface of the hSirt1 binding pocket is displayed and color coded according to the hydrophobic potential. Arg274 is restricting the interaction of the maleimides in the deeper part of the binding pocket.

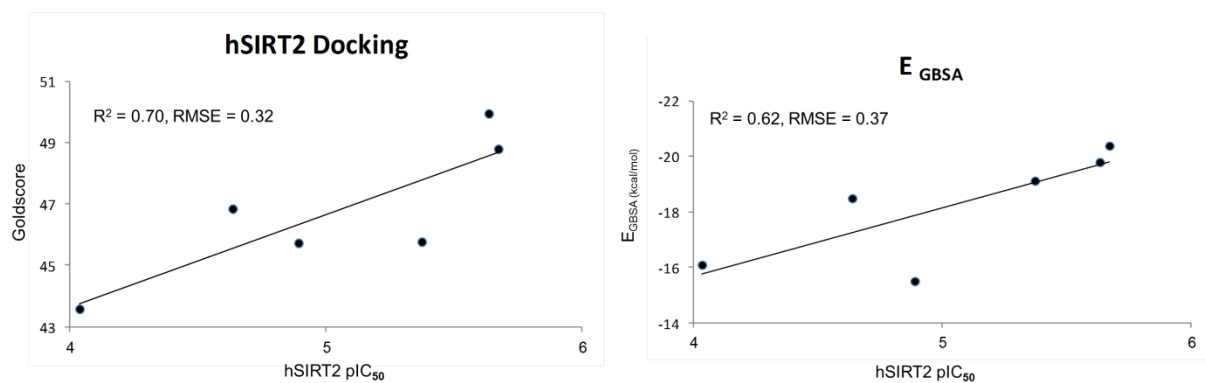


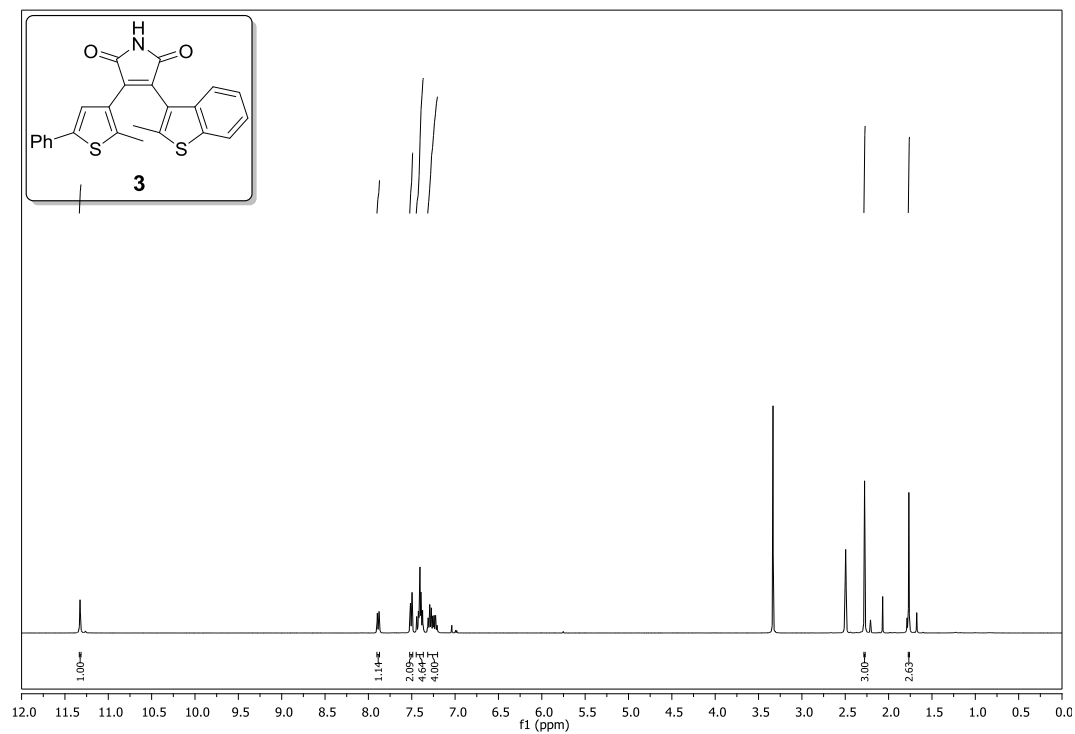
Figure S9: left) Correlation between experimental pIC_{50} values and Goldscores for the six diarylmaleimide derivatives **2**, **3** and **11**. A correlation coefficient of $r^2 = 0.70$ and a RMSE value of 0.32 was obtained; right) Correlation between experimental pIC_{50} values and E_{GBSA} scores for the six diarylmaleimides derivatives **2**, **3** and **11**. A correlation coefficient of $r^2 = 0.62$ and a RMSE value of 0.37 was obtained.

Table S1: In vitro data (IC_{50} hSirt2) and calculated scoring values.

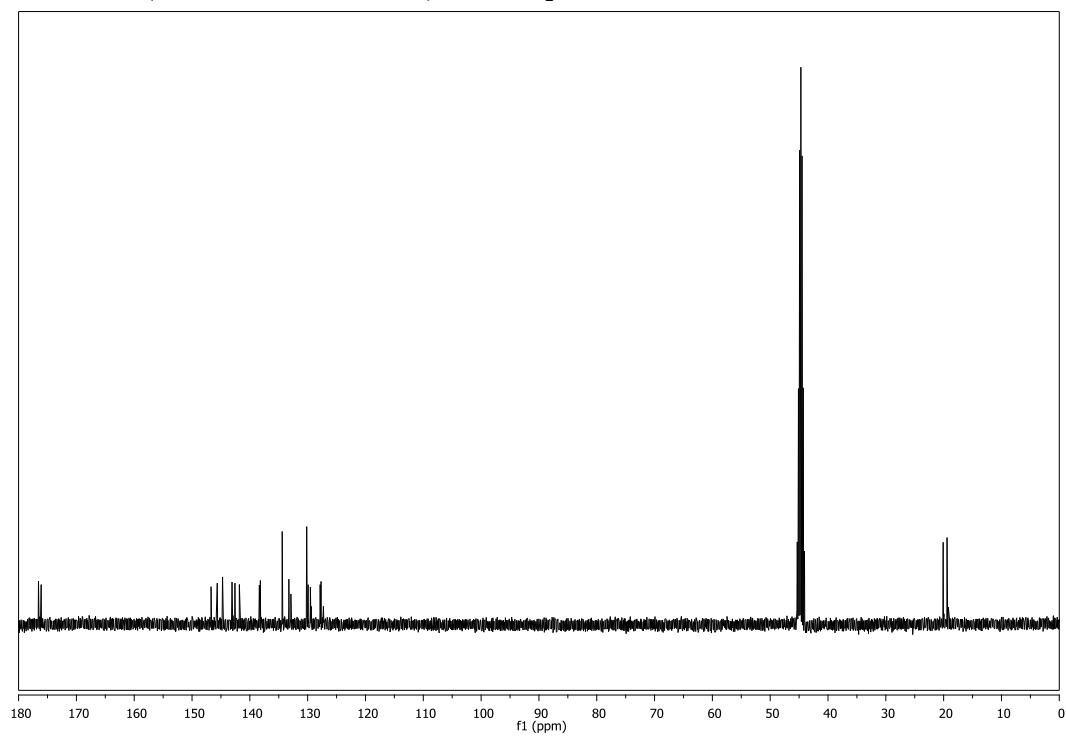
Entry	Compound	hSirt2 IC_{50}	hSirt2 pIC_{50}	Goldscore	E (GBSA)
1	3 closed	2.13	5.67	48.81	-20.41
2	3 open	2.32	5.63	49.95	-19.79
3	2 closed	23.06	4.64	46.83	-18.52
4	2 open	12.86	4.89	45.73	-15.51
5	11 closed	92.25	4.04	43.58	-16.09
6	11 open	4.23	5.37	45.76	-19.12

Supplementary diarylmaleimide NMR spectra

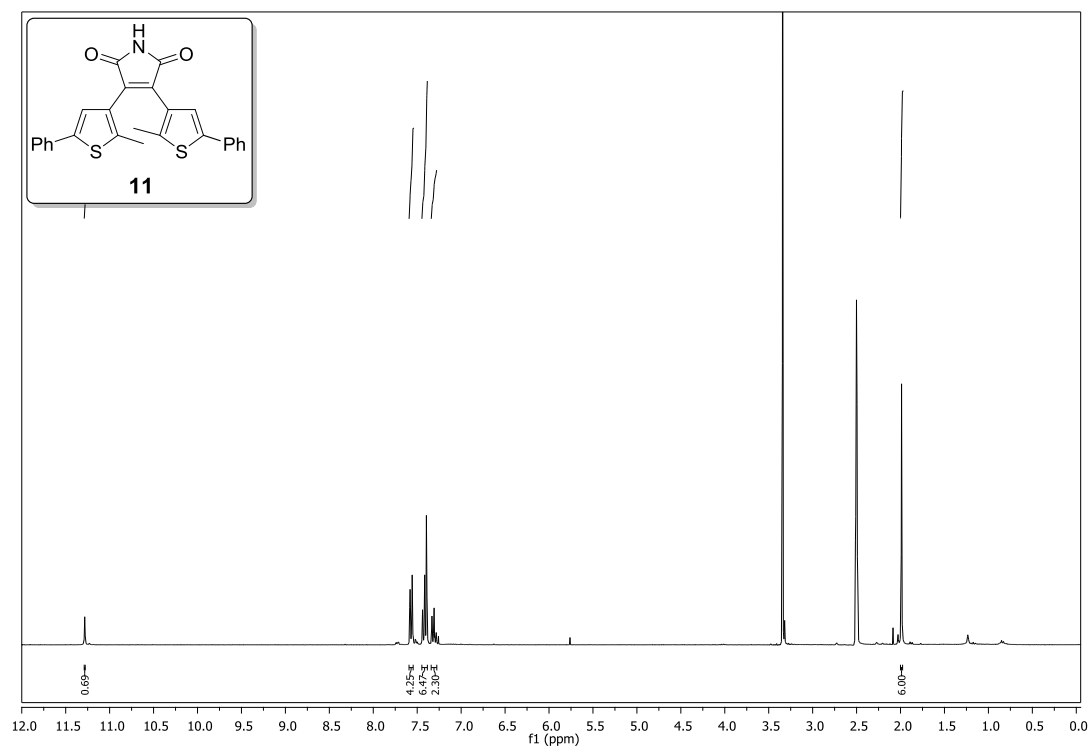
^1H -NMR (400 MHz, DMSO- d_6) for compound **3**:



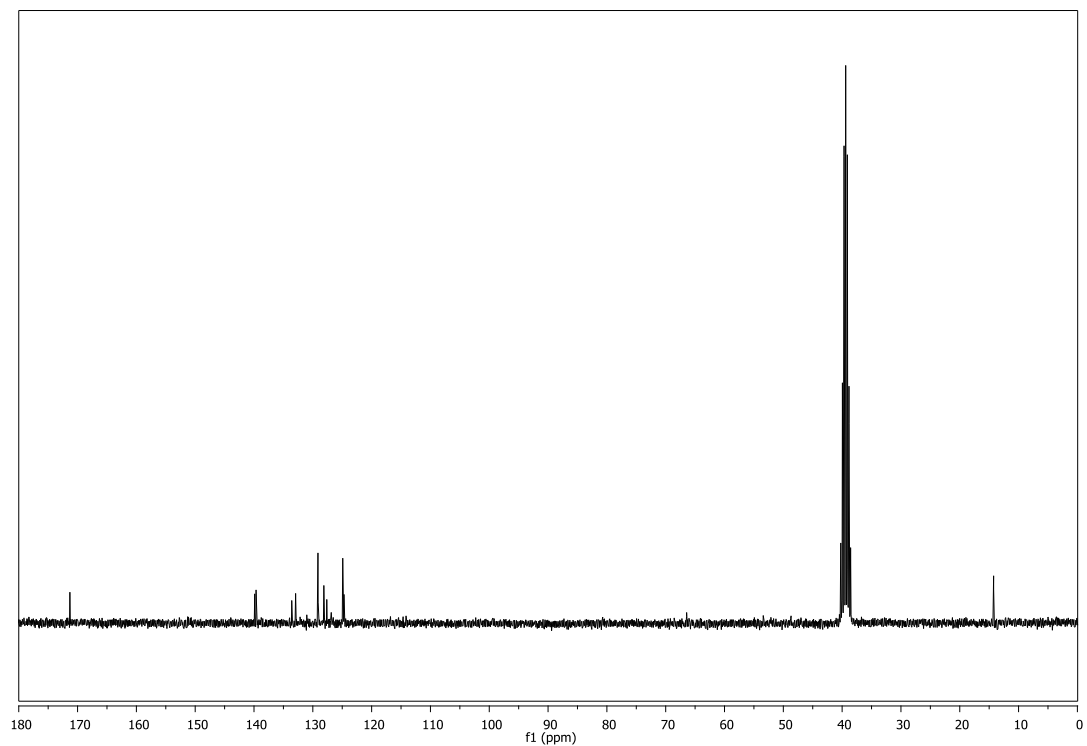
^{13}C -NMR (101 MHz, DMSO- d_6) for compound **3**:



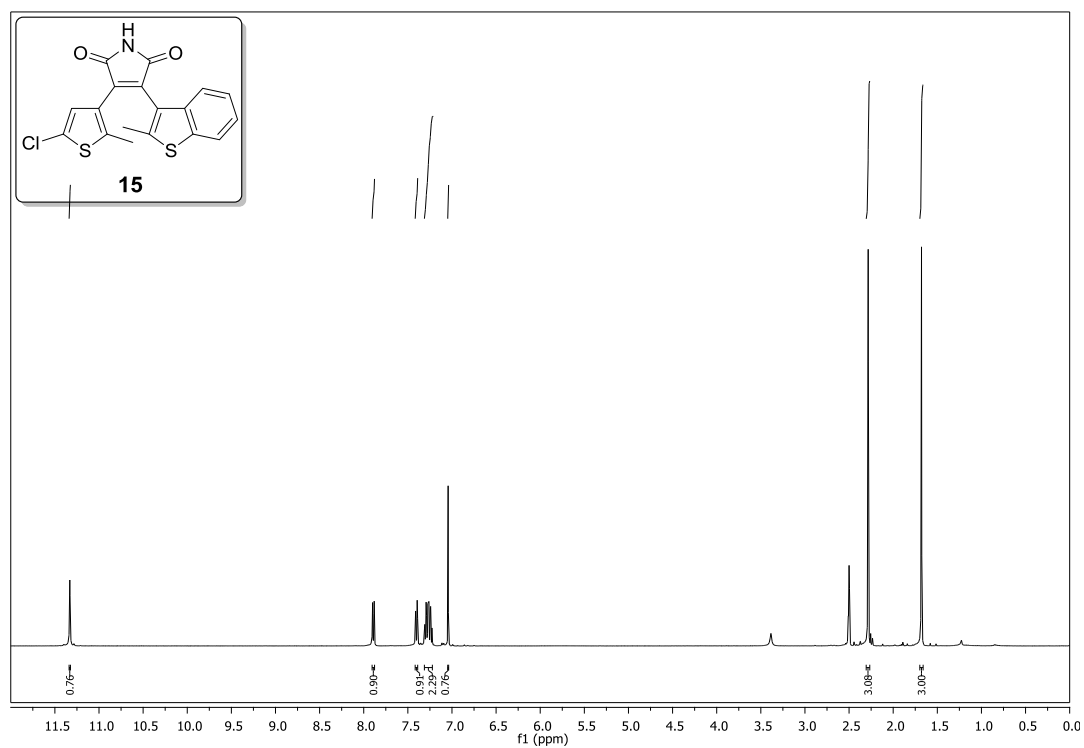
^1H -NMR (300 MHz, DMSO- d_6) for compound **11**:



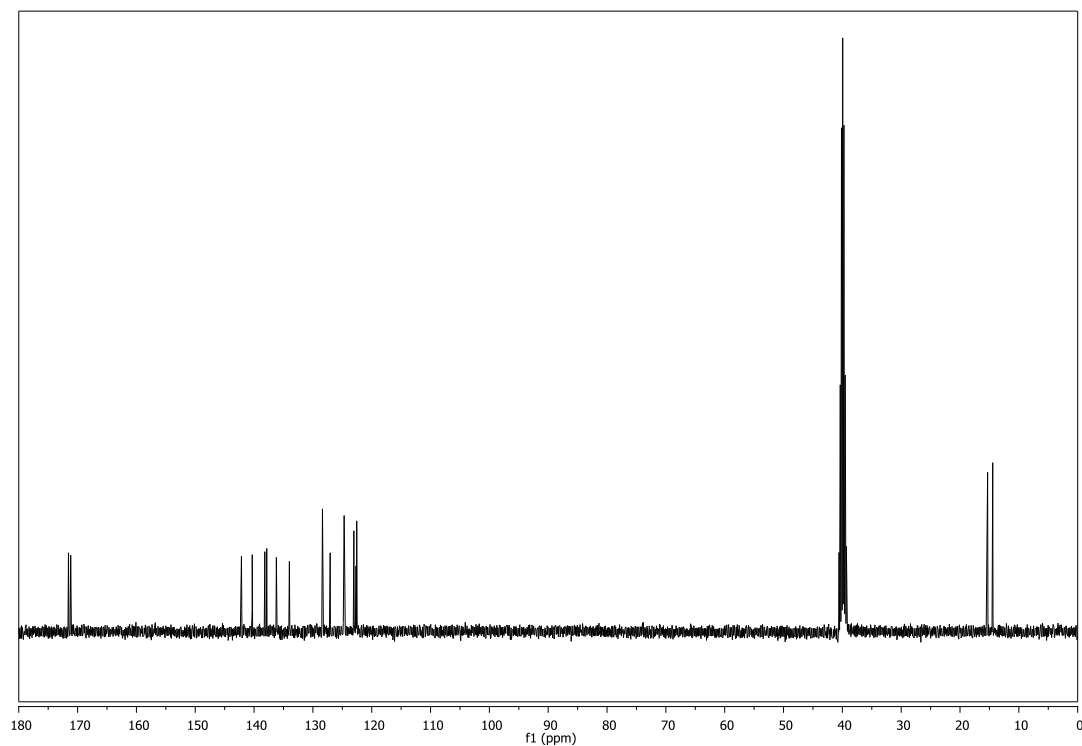
^{13}C -NMR (75 MHz, DMSO- d_6) for compound **11**:



¹H-NMR (400 MHz, DMSO-d₆) for compound **15**:



¹³C-NMR (101 MHz, DMSO-d₆) for compound **15**:



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