Supporting Information

Novel 3-Arylidene-indolin-2-ones as inhibitors of NAD^+-dependent histone deacetylases (sirtuins)

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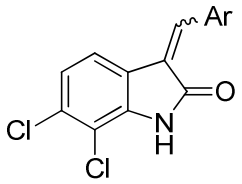
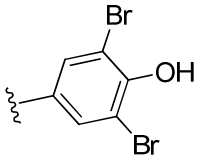
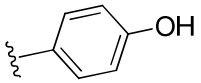
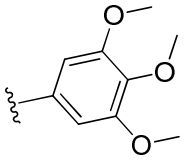
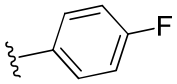
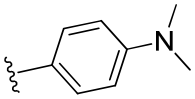
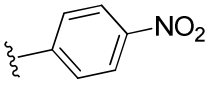
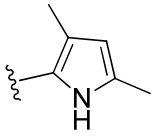
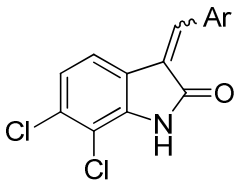
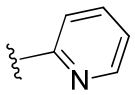
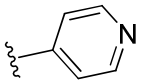
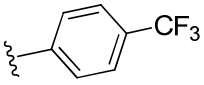
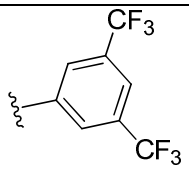
Table S1. *E/Z* ratios and obtained yields of the synthesized 3-arylidene-indolinones.



Compound	Ar	*E/Z* ratio [%][a]	Overall yield [%]
4		30:70	60
5		57:43	81
6		50:50	52
7		54:46	72
8		82:18	45
9		57:43	72
10		0:100	70



Compound	Ar	*E/Z* ratio [%][a]	Overall yield [%]
11		100:0	78
12		100:0	60
13		60:40	58
14		69:31	81

[a]Ratios were determined by ^{1}H NMR spectroscopy.

NOE-Determination of *E/Z* isomers. Since all prepared compounds may exist either as *E* or *Z* isomer, NOE experiments were performed in order to determine the configuration of the arylidene double bond. In case of the *Z*-isomer an NOE effect can be observed between the vinylic proton and proton H-4 of the oxindole core. In contrast, *E* configured compounds may only display an NOE effect between the *ortho* protons of the 3-arylidene substituents and proton H-4 (Figure S1)[1].

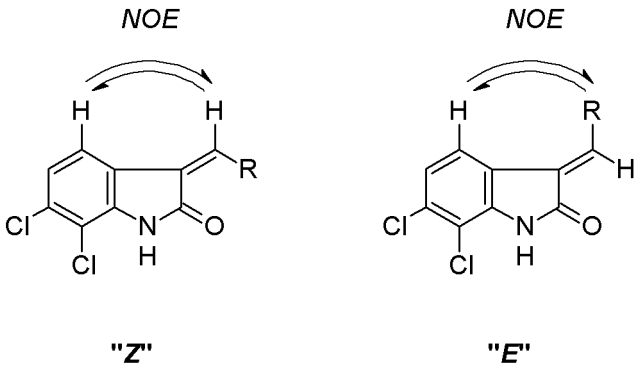
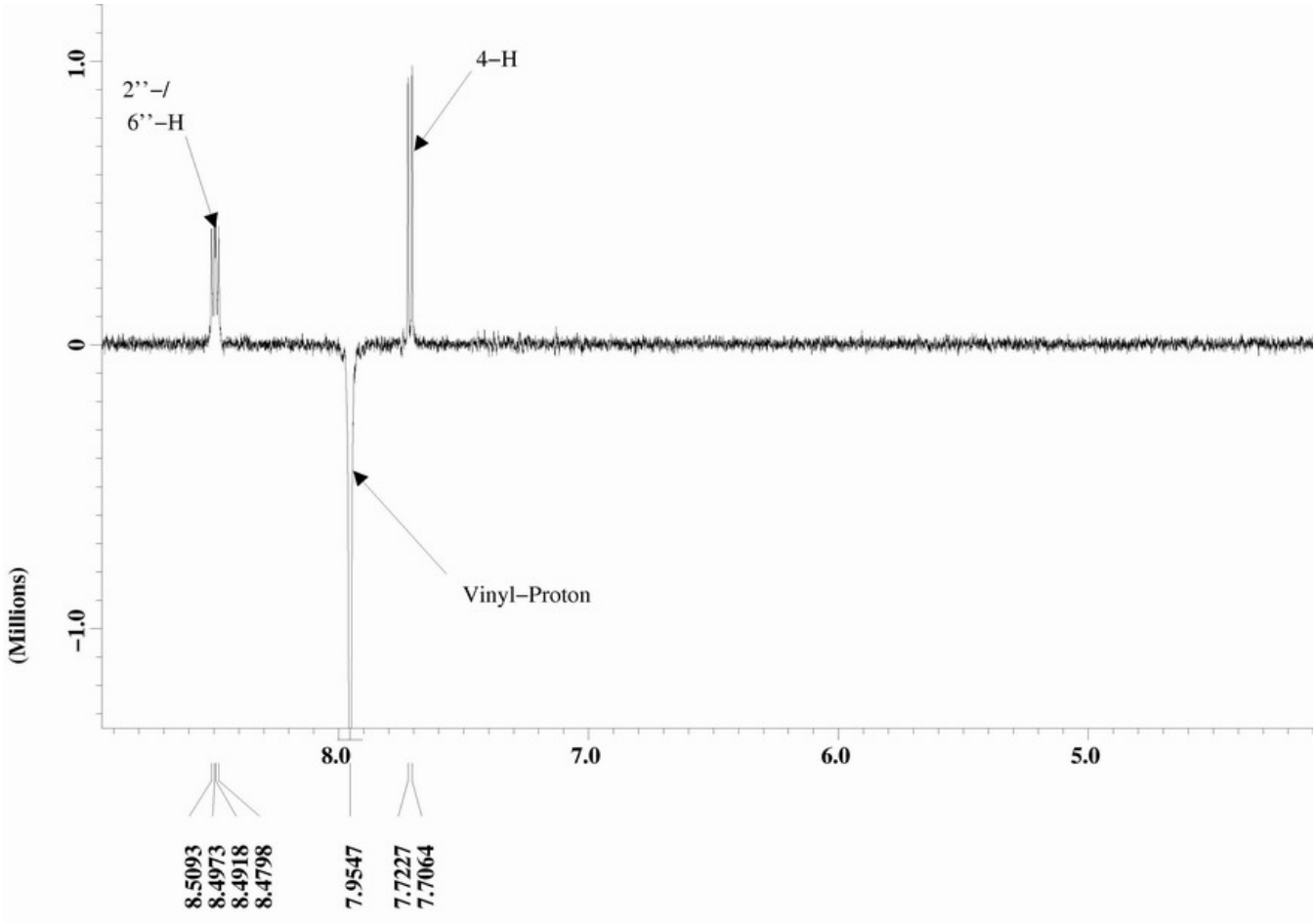


Figure S1. Expected NOE effects for *E* and *Z* isomers.

For example, irradiation at the vinylic proton of **7** induces an NOE effect with proton H-4 of the oxindole (Figure S2).



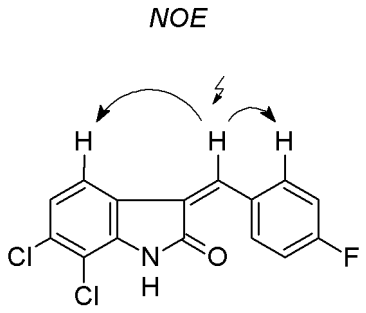
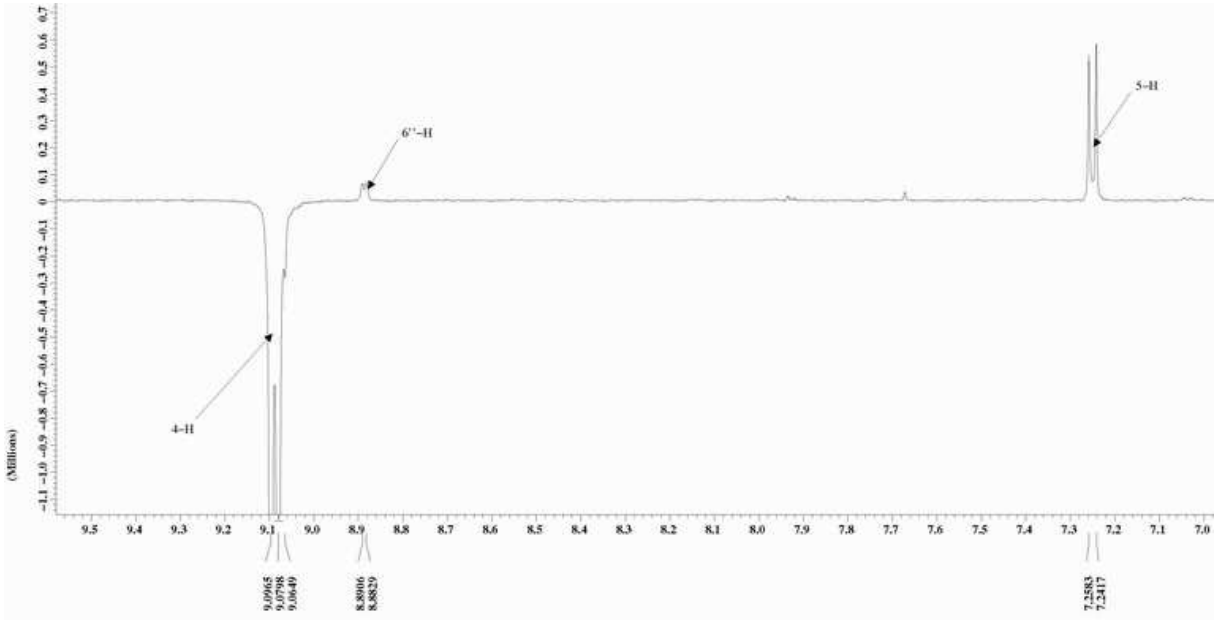


Figure S2. Observed NOE effects for the *Z*-isomer of **7** after irradiation at the vinylic proton.

On the contrary, *E* configured compounds like **11** do not exhibit an NOE between proton H-4 and the vinylic proton (Figure S3).



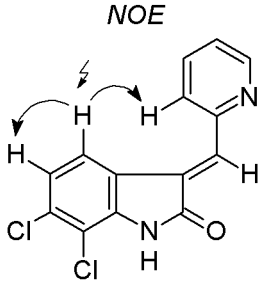


Figure S3. Observed NOE effects for **11** after irradiation at H-4 of the oxindole nucleus.

The exclusive existence of single isomers has already been examined in the literature suggesting that more or less favored intramolecular interactions can promote or stabilize the formation of either *E* or *Z* isomers[1]. For instance, only the *Z*-isomer of **10** is able to establish a hydrogen bond between the pyrrole NH and the oxindole's carbonyl (Figure S4).

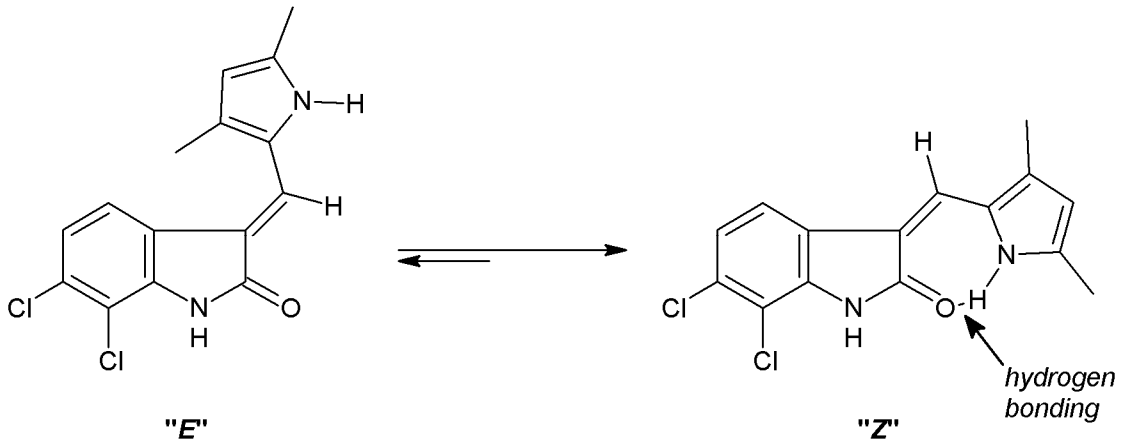


Figure S4. Rationale for the strong preference for the *Z*-isomer in the case of compound **10** due to intramolecular hydrogen bonding.

In case of the pyridine derivative **11** only the *E*-isomer prevents an unfavored electrostatic repulsion between the lone electron pairs of the pyridine nitrogen and the oxindole's carbonyl, respectively (Figure S5).

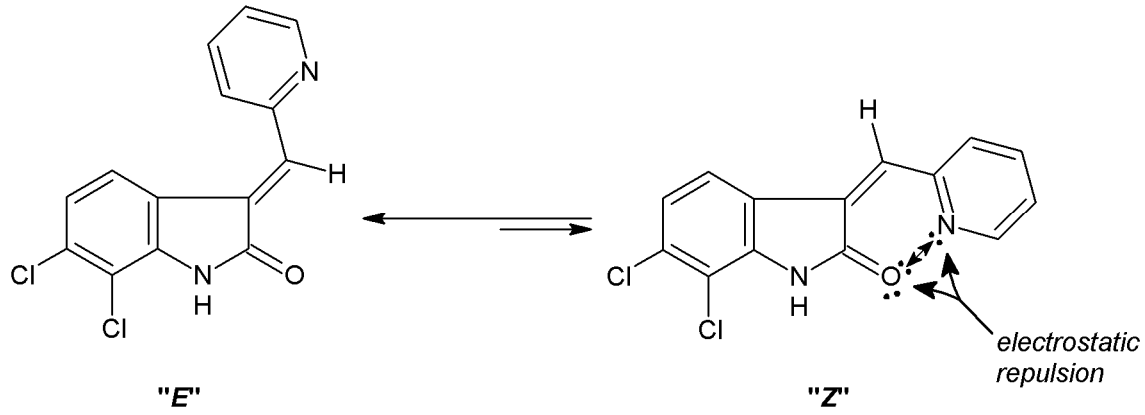


Figure S5. Favored formation of the *E* isomer in case of **11** due to electrostatic repulsion effects.

As previously described in the literature there have been reports on conversion of isomers occurring in solution for this class of compounds[2, 3]. Yet we were not able to detect any significant changes in the *E/Z* ratios either by TLC or NMR.

Biochemical assays

Recombinant proteins. Human SIRT2 and SIRT3 were expressed as N-terminally tagged His_6 fusion proteins and purified as described previously with minor modifications[4]. Human SIRT1 (N-terminally GST tagged) was prepared as described previously with minor modifications[4]. Identity and purity of the produced enzymes were verified using SDS electrophoresis. The deacetylase activity of the SIRT isoforms was dependent on NAD^+ and could be inhibited with nicotinamide.

Fluorescent deacetylase assay. All compounds were evaluated for their ability to inhibit recombinant sirtuins using a homogeneous fluorescent deacetylase assay[5]. The inhibitors were dissolved in DMSO and 3 µL or less of a concentrated stock solution (depending on the solubility of the compound) of the inhibitor in DMSO were added to a incubation mixture. The assay was carried out in 96-well plates with a reaction volume of 60 µL containing the fluorescent histone deacetylase substrate ZMAL (10.5 µM), NAD^+ (500 µM) and sirtuin. The amount of enzyme solution that was added depends on the activity of the preparation that is used and may vary from batch to batch. Usually, we adjusted to 10-30% conversion of the substrate without inhibitor. After incubation time of 4 h at 37 °C, the deacetylation reaction was stopped with a solution of trypsine buffer (60 µL) containing trypsin (1 mg × mL^{-1}) from bovine pancreas (10 00 BAEE units × mg^{-1}) and the sirtuin inhibitor nicotinamide (8 mM). The microplate was incubated with this solution for 20 min at 37 °C, fluorescence intensity was then measured in a plate reader (BMG Polarstar) with a coumarin filter (λ_{exc}=390 nm, λ_{em}= 460 nm). The amount of substrate remaining in the positive control with inhibitor versus negative control without inhibitor was employed to calculate inhibition. For all determinations at least duplicates were carried out. IC_{50} data were calculated using GraphPad Prism software.

Cell lines

MCF-7 human breast adenocarcinoma cells were grown in RPMI-1640 (PAA) supplemented with 10% fetal bovine serum (PAA) and 2 mM L-glutamine (PAA).

Hep G2 human hepatocellular carcinoma cells were grown in DMEM high glucose (PAA) supplemented with 10% fetal bovine serum (PAA), 2 mM L-glutamine (PAA), 1% Non essential amino acids (Biochrom) and 1% penicillin-streptomycin (PAA).

Western blot analysis

Cells were seeded into 6-well plates 24 h prior to the experiment at a density of 5×10^5 per well. The culture medium was renewed 1 h prior to incubation with the different compounds or DMSO vehicle only. Therefore compounds were dissolved in 20 µL DMSO, diluted in a total volume of 2 mL growth medium and added to the cells.

Following drug treatment for 6 or 16 h, cells were washed with ice cold PBS and collected with gentle lysis buffer (50 mM Tris-HCl pH 7.5, 150 mM NaCl, 0.5% IGEPAL-CA 630, 0.5 mM EDTA, Complete Protease Inhibitor Tablets (Roche)). After centrifugation of the lysed cells, protein concentrations were determined in the supernatant using the BCA Protein Assay (Pierce). About 10 µg of protein samples were electrophoresed on 15% SDS-polyacrylamide gels and transferred to an Immobilon-P transfer membrane (Millipore). Membranes were blocked with Roti-Block (Roth) and probed with anti-acetylated α-tubulin (sigma 6-11B-1) 1:2000 or anti-α-tubulin (sigma B-5-1-2) 1:2000.

Cell-based ELISA-type assay

5×10^4 cells per well were seeded into 96-well microtiter plates (clear, PAA) 24 h prior to experiments (37 °C, 5% CO_2). Drug dilutions and appropriate DMSO controls were added to the wells and incubated for 6 hours. The medium was tipped out and the cells were fixed and permeabilized with 100 µL fixing solution containing 0.25% glutaraldehyde, 4% paraformaldehyde, 0.25% Triton X-100 in phosphate buffered saline (PBS) for 30 minutes. After two washing steps with PBS, 100 µL of freshly prepared blocking solution (5% BSA in PBS) were added to each well and the plate was incubated for another 30 minutes. Following two additional washing steps with PBS, an antibody against acetylated α-tubulin (sigma 6-11B-1) diluted in PBS (1:1000) was applied. Following 2 hours of incubation time and two washing steps with Tris buffer (100 mM Tris, 150 mM NaCl, 0.1% Tween 20, pH 7.5) a secondary anti-IgG mouse antibody labelled with Eu (Perkin Elmer) diluted in Tris buffer (1:1000) was added to each well. Following another incubation step of 1 hour, the wells were washed again with Tris buffer and 100 µL of Enhancement solution (Perkin Elmer) were added to each well. After 5 minutes of incubation, fluorescence intensity was measured in a time resolved mode (340/615 nm). At the end of the procedure, the BCA Protein Assay (Pierce) was performed to normalize the fluorescent signal to the amount of protein in each well.

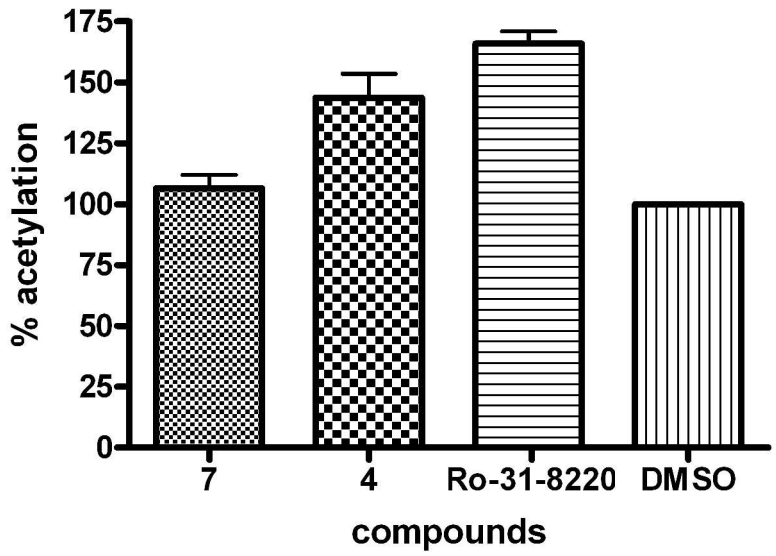


Figure S6. Cell based ELISA-type assay for tubulin hyperacetylation. Bars reflect % acetylation (100% = DMSO controls). Signal (%) for acetylation of α-tubulin. Compound **4** induces α-tubulin hyper-acetylation caused by inhibition of Sirt2. The sirtuin inhibitor Ro-31-8220 (**1**) was used as a reference compound.

Molecular Modeling and docking studies

Molecular Modeling. All calculations were performed on a Pentium IV 2.2 GHz based Linux cluster (20 CPUs). The molecular structures of the inhibitors were generated using the MOE modelling package (Chemical Computing Group). The structures were energy minimized using the MMFF94s force field and the conjugate gradient method until the default derivative convergence criterion of 0.01 kcal/(mol × Å) was met. The crystal structure of human Sirt2 (PDB code 1J8F)[6] was taken from the Protein Data Bank and prepared as described in our former docking studies[7]. Docking of the inhibitors was carried out using program GOLD 3.2 and default settings (Cambridge Crystallographic Data Centre)[8]. The binding site was defined on Ile169 with a radius of 20 Å covering the NAD^+ and acetyl-lysine substrate binding pockets. Goldscore was chosen as fitness function due to the success in our former docking studies. For each molecule 10 docking runs were performed. The resulting solutions were clustered on the basis of the heavy atom rmsd values (1 Å). The top-ranked poses for each ligand were retained and analyzed graphically within MOE 2006.08 (Chemical Computing Group)[9].

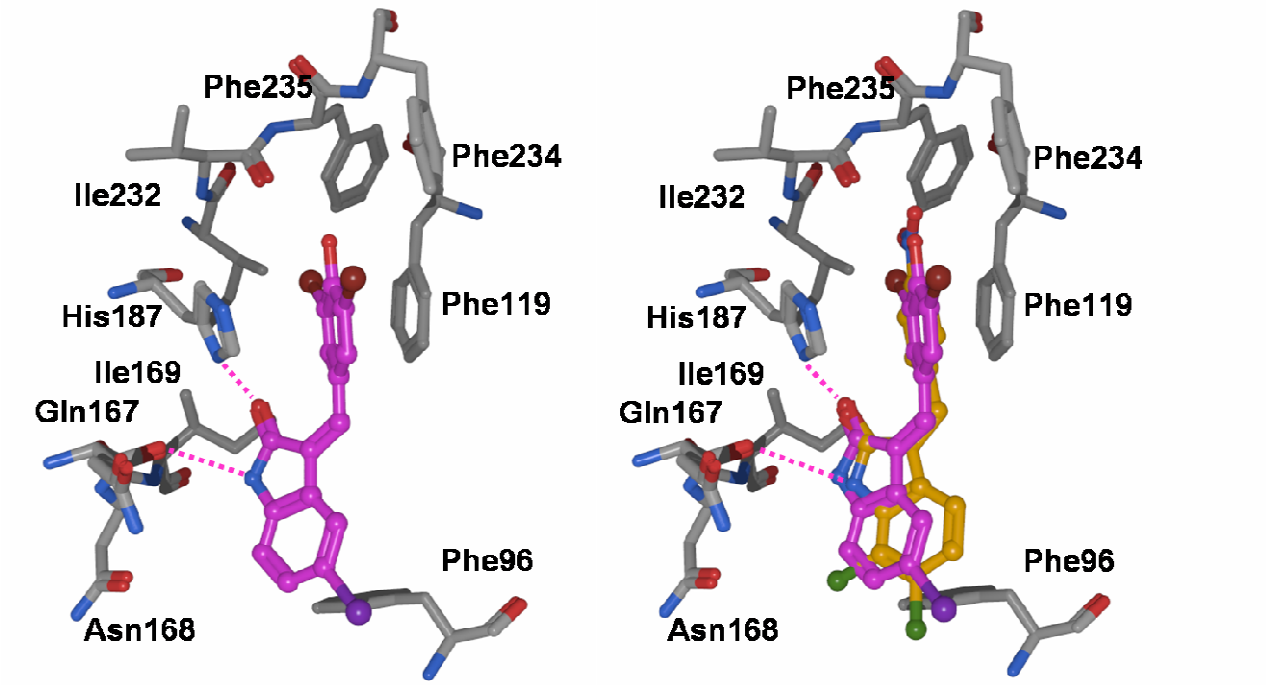


Figure S7. *Left:* Docking solution of GW5074 (**2**) (colored magenta) binding to the C pocket of human SIRT2 (PDB code: 1J8F). Only the *Z*-isomer is able to bind in such a way that allows the dibromo-phenol substituent to protrude into the hydrophobic lysine tunnel and to interact with apolar residues. The lactam group is involved in two hydrogen bonds (dashed lines) with His187 and the backbone CO of Gln167. *Right:* Comparison of the docking solution obtained for **2** (magenta) and the indolinone **9** (orange).

Procedures and experimental data for compounds 4-15 and 19:

General Information. NMR spectra were recorded using a Jeol JNMR-GSX 400, Jeol JNMR-GSX 500 (Jeol, Peabody, USA), Varian NMR-System 600 MHz or Varian NMR-System 400 MHz (Varian, Palo Alto, USA). *E/Z* ratios were determined by integration of the corresponding peaks in the ^{1}H NMR spectra, chemical shifts are given in Hertz. Mass spectra (electronic ionization, EI, 70 eV) were recorded using a Hewlett Packard 5989 A Mass Spectrometer with a 59980 B Particle Beam LC/MS-interface (Agilent Technologies, Palo Alto, USA). High resolution mass spectra were obtained using a Jeol Mstation 700 (Jeol, Peabody, USA). IR spectra were recorded as KBr discs on a Perkin Elmer FT-IR Paragon 1000 (Perkin Elmer, Waltham, USA) or Jasco FT/IR-410 (Jasco, Easton, USA). Melting points were determined with a Büchi B-540 apparatus (Büchi, Flawil, Switzerland) and are uncorrected. Elemental analyses were performed using a CHN-Elementaranalysator Rapid (Heraeus, Hanau, Germany) or Elementaranalysator Vario EL (Elementar, Hanau, Germany). Purification by flash column chromatography (FCC) was done using Silica gel 60 (Merck, Darmstadt, Germany).

All synthesis chemicals were purchased from Sigma-Aldrich, Fluka and Acros. GW5074 was purchased from Biomol International and sunitinib malate from LKT Laboratories, Inc. (St. Paul, USA).

6,7-Dichloro-3-methylthio-indolin-2-one (19). 200 mL dry dichloromethane were cooled to -78 °C under nitrogen atmosphere. 3.95 mL (30.7 mmol) ethyl (methylthio)acetate and 2.47 mL (30.7 mmol) sulfuryl chloride were added consecutively and the reaction mixture was stirred for 15 min. Subsequently a solution of 5.02 g (31 mmol) 2,3-dichloroaniline and 6.58 g (30.7 mmol) 1,8-bis(dimethylamino)naphthalene in 100 mL dry dichloromethane was added over 1 h. After stirring for 2 h at -78 °C a solution of 4.28 mL (30.7 mmol) triethylamine in 10 mL of dry dichloromethane was added and the reaction was allowed to warm up to rt. The mixture was washed with water (3 × 100 mL) and the combined aqueous layers were reextracted with 100 mL of dichloromethane. The combined organic layers were washed with brine, dried over $MgSO_4$, filtered and the solvent was evaporated. The crude brown residue was taken up in 200 mL of glacial acetic acid and stirred for 3 h under nitrogen atmosphere. The acetic acid was removed employing azeotropic rotary evaporation with dry toluene and the residue was resuspendend in 100 mL of diethyl ether, stirred for 30 min, filtered and washed with cold diethyl ether. Recrystallization from methanol gave 3.50 g (45 %) of **19** as a purple solid. Mp 181 °C; ^{1}H NMR (500 MHz, CD_2Cl_2, TMS) δ 8.43 (br. s, 1 H, N-H), 7.24 (dd, J = 8.0 Hz, 0.9 Hz, 1 H, 4-H), 7.20 (d, J = 8.0 Hz, 1 H, 5-H), 4.35 (d, J = 0.9 Hz, 1 H, 3-H), 2.04 (s, 3 H, CH_3); ^{13}C NMR (125 MHz, CD_2Cl_2, TMS) δ 176.2 (C=O), 141.1 (C-7a), 132.9 (C-6), 126.4 (C-3a), 124.5 (C-5), 124.5 (C-4), 114.2 (C-7), 47.4 (C-3), 12.5 (CH_3); MS EI *m/z* (relative intensity, %) 251 [$M^{+\bullet}$] (1), 249 [$M^{+\bullet}$] (6), 247 [$M^{+\bullet}$] (8), 200 (100); IR ν_{max} (cm^{-1}) 3396 (NH), 1709, 1610 (C=O); Anal. ($C_9H_7Cl_2NOS$) C, H, N, S.

6,7-Dichloroindolin-2-one (15). Under nitrogen, 2.94 g (9.30 mmol) 6,7-dichloro-3-methylthio-indolin-2-one (**19**) were dissolved in a mixture of 80 mL ethanol and 20 mL dichloromethane and treated with 10 g Raney nickel. The suspension was heated to reflux for 3 h and filtered through Celite after cooling. The crude product was purified using FCC (hexane/ethyl acetate 1:1) and recrystallized from ethanol to give 1.72 g (72 %) of **15** as pale brown crystals. Mp 262 °C; ^{1}H NMR (500 MHz, DMSO-d_6, TMS) δ 10.93 (br. s, 1 H, N-H), 7.17 (d, J = 8.0 Hz, 1 H, 4-H), 7.14 (d, J = 8.0 Hz, 1 H, 5-H), 3.59 (s, 2 H, 3-H); ^{13}C NMR (125 MHz, DMSO-d_6, TMS) δ 176.2 (C=O), 143.3 (C-7a), 129.9 (C-6), 126.1 (C-3a), 123.7 (C-4), 122.3 (C-5), 112.1 (C-7), 36.5 (C-3); MS EI *m/z* (relative intensity, %) 205 [$M^{+\bullet}$] (11), 203 [$M^{+\bullet}$] (62), 201 [$M^{+\bullet}$] (100), 173 (61), 166 (26), 138 (58); IR ν_{max} (cm^{-1}) 3431 (NH), 1705, 1618 (C=O); Anal. ($C_8H_5Cl_2NO$) C, H, N.

(*E/Z*)-6,7-Dichloro-3-(3,5-dibromo-4-hydroxybenzylidene)indolin-2-one (4). ^{13}C NMR (125 MHz, DMSO-d_6, TMS) δ 168.4 (C=O, *E*), 167.1 (C=O, *Z*), 153.3 (C-4'', *Z*), 152.4 (C-4'', *E*), 142.1 (C-7a, *E*), 139.3 (C-7a, *Z*), 136.5 (vinyl-C, *Z*), 136.4 (C-2''/-6'', *Z*), 135.6 (vinyl-C, *E*), 133.3 (C-2''/-6'', *E*), 131.9 (C-6, *E*), 130.4 (C-6, *Z*), 127.9 (C-1''), 126.3 (C-3, *E*), 125.2 (C-3a, *Z*), 124.3 (C-3, *Z*), 122.3 (C-5),

121.1 (C-3a, *E*), 121.0 (C-4, *E*), 118.8 (C-4, *Z*), 113.0 (C-7, *E*), 112.1 (C-7, *Z*), 111.8 (C-3''/-5'', *E*), 111.1 (C-3''/-5'', *Z*).

(*E/Z*)-6,7-Dichloro-3-(4-hydroxybenzylidene)indolin-2-one (5). 250 mg (1.24 mmol) of **15**, 181 mg (1.48 mmol) 4-hydroxybenzaldehyde and 0.01 mL piperidine were reacted as given in the general procedure. Yield: 306 mg (81%) as a yellow solid from ethanol/acetic acid (1:1). Mp 282 °C; ^{1}H NMR (500 MHz, DMSO-d_6, TMS) δ 11.19 (br. s, 2 H, N-H), 10.33 (br. s, 2 H, O-H), 8.42 (d, J = 8.8 Hz, 0.43 × 2 H, 2''-/6''-H, *Z*), 7.84 (s, 0.43 × 1 H, vinyl-H, *Z*), 7.68 (d, J = 8.1 Hz, 0.43 × 1 H, 4-H, *Z*), 7.67 (s, 0.57 × 1 H, vinyl-H, *E*), 7.65 (d, J = 8.3 Hz, 0.57 × 1 H, 4-H, *E*), 7.64 (d, J = 8.8 Hz, 0.57 × 2 H, 2''-/6''-H, *E*), 7.23 (d, J = 8.1 Hz, 0.43 × 1 H, 5-H, *Z*), 7.15 (d, J = 8.3 Hz, 0.57 × 1 H, 5-H, *E*), 6.92 (d, J = 8.8 Hz, 0.57 × 2 H, 3''-/5''-H, *E*), 6.88 (d, J = 8.8 Hz, 0.43 × 2 H, 3''-/5''-H, *Z*); ^{13}C NMR (125 MHz, DMSO-d_6, TMS) δ 169.0 (C=O, *E*), 167.2 (C=O, *Z*), 160.8 (C-4'', *Z*), 159.8 (C-4'', *E*), 141.4 (C-7a, *E*), 140.1 (vinyl-C, *Z*), 139.2 (vinyl-C, *E*), 138.7 (C-7a, *Z*), 135.3 (C-2''/-6'', *Z*), 132.2 (C-2''/-6'', *E*), 131.0 (C-6, *E*), 129.4 (C-6, *Z*), 125.9 (C-3a, *Z*), 125.2 (C-1'', *Z*), 124.3 (C-1'', *E*), 123.2 (C-3, *E*), 122.2 (C-5, *E*), 122.0 (C-5, *Z*), 121.6 (C-3a, *E*), 121.4 (C-3, *Z*), 121.0 (C-4, *E*), 118.3 (C-4, *Z*), 115.7 (C-3''/-5'', *E*), 115.3 (C-3''/-5'', *Z*), 112.7 (C-7, *E*), 111.8 (C-7, *Z*); *E/Z* ratio (%) 57:43; MS EI *m/z* (relative intensity, %) 309 [$M^{+\bullet}$] (11), 307 [$M^{+\bullet}$] (63), 305 [$M^{+\bullet}$] (100); HRMS calcd, 305.0010; found, 305.0002; IR ν_{max} (cm^{-1}) 3282 (OH), 1711, 1599 (C=O); Anal. ($C_{15}H_9Cl_2NO_2$) C, H, N.

(*E/Z*)-6,7-Dichloro-3-(3,4,5-trimethoxybenzylidene)indolin-2-one (6). 250 mg (1.24 mmol) of **15**, 291 mg (1.48 mmol) 3,4,5-trimethoxybenzaldehyde and 0.01 mL piperidine were reacted as given above. Yield: 243 mg (52%) as orange crystals from ethanol. Mp 202 °C; ^{1}H NMR (500 MHz, DMSO-d_6, TMS) δ 11.24 (br. s, 1 H, N-H), 8.01 (s, 0.50 × 2 H, 2''-/6''-H, *E*), 7.91 (s, 0.50 × 1 H, vinyl-H, *E*), 7.70 (d, J = 8.2 Hz, 0.50 × 1 H, 4-H, *Z*), 7.70 (s, 0.50 × 1 H, vinyl-H, *Z*), 7.69 (d, J = 8.2 Hz, 0.50 × 1 H, 4-H, *E*), 7.28 (d, J = 8.2 Hz, 0.50 × 1 H, 5-H, *E*), 7.20 (d, J = 8.2 Hz, 0.50 × 1 H, 5-H, *Z*), 3.86 (s, 0.5 × 6 H, 3''-/5''-O-CH_3, *E*) , 3.82 (s, 0.5 × 6 H, 3''-/5''-O-CH_3, *Z*), 3.77 (s, 0.5 × 3 H, 4''-O-CH_3, *E*), 3.76 (s, 0.5 × 3 H, 4''-O-CH_3, *Z*); ^{13}C NMR (125 MHz, DMSO-d_6, TMS) δ 168.7 (C=O, *Z*), 167.1 (C=O, *E*), 152.8 (C-3''/-5'', *Z*), 152.2 (C-3''/-5'', *E*), 141.9 (C-7a, *Z*), 140.3 (C-4'', *E*), 140.0 (vinyl-C, *E*), 139.2 (C-7a, *E*), 139.1 (C-4'', *Z*), 138.6 (vinyl-C, *Z*), 131.6 (C-6, *Z*), 130.2 (C-6, *E*), 129.0 (C-1'', *E*), 128.9 (C-1'', *Z*), 125.5 (C-3a, *E*), 125.4 (C-3, *Z*), 124.2 (C-3, *E*), 122.4 (C-5, *Z*), 122.3 (C-5, *E*), 121.7 (C-4, *Z*), 121.3 (C-3a, *Z*), 118.7 (C-4, *E*), 112.9 (C-7, *Z*), 112.0 (C-7, *E*), 110.4 (C-2''/-6'', *E*), 107.2 (C-2''/-6'', *Z*), 60.1 (4''-O-CH_3), 55.9 (3''-/5''-O-CH_3, *Z*), 55.8 (3''-/5''-O-CH_3, *E*); *E/Z* ratio (%) 50:50; MS EI *m/z* (relative intensity, %) 383 [$M^{+\bullet}$] (12), 381 [$M^{+\bullet}$] (65), 379 [$M^{+\bullet}$] (100), 364 (56), 333 (15), 159 (18); IR ν_{max} (cm^{-1}) 3444 (NH), 2827 (C-O), 1703, 1603 (C=O); Anal. ($C_{18}H_{15}Cl_2NO_4$) C, H, N.

(*E/Z*)-6,7-Dichloro-3-(4-fluorobenzylidene)indolin-2-one (7). 255 mg (1.26 mmol) of **15**, 188 mg (1.51 mmol) 4-fluorobenzaldehyde and 0.01 mL piperidine were reacted as given above. Yield: 280 mg (72%) as yellow crystals from ethanol. Mp 274 °C; ^{1}H NMR (500 MHz, DMSO-d_6, TMS) δ 11.30 (br. s, 0.46 × 1 H, N-H, *Z*), 11.27 (br. s, 0.54 × 1 H, N-H, *E*), 8.49 (dd, J = 8.8 Hz, 2.0 Hz, 0.46 × 2 H, 2''-/6''-H, *Z*), 7.95 (s, 0.46 × 1 H, vinyl-H, *Z*), 7.79 (dd, J = 8.5 Hz, 2.0 Hz, 0.54 × 2 H, 2''-/6''-H, *E*), 7.75 (s, 0.54 × 1 H, vinyl-H, *E*), 7.71 (d, J = 8.2 Hz, 0.46 × 1 H, 4-H, *Z*), 7.44 (d, J = 8.2 Hz, 0.54 × 1 H, 4-H, *E*), 7.36 (m, 4 H, 3''-/5''-H), 7.27 (d, J = 8.2 Hz, 0.46 × 1 H, 5-H, *Z*), 7.12 (d, J = 8.2 Hz, 0.54 × 1 H, 4-H, *E*); ^{13}C NMR (125 MHz, DMSO-d_6, TMS) δ 168.5 (C=O, *E*), 167.0 (C=O, *Z*), 163.3 (d, J = 250 Hz, C-4'', *Z*), 162.7 (d, J = 249 Hz, C-4'', *E*), 142.0 (C-7a, *E*), 139.5 (C-7a, *Z*), 138.1 (vinyl-C, *Z*), 137.3 (vinyl-C, *E*), 134.9 (d, J = 8.6 Hz, C-2''/-6'', *Z*), 131.9 (d, J = 8.6 Hz, C-2''/-6'', *Z*), 131.9 (C-6, *E*), 130.6 (C-6, *Z*), 130.3 (C-1''), 126.5 (C-3, *E*), 125.1 (C-3a, *Z*), 125.1 (C-3, *Z*), 122.4 (C-5, *E*), 122.3 (C-5, *Z*), 121.3 (C-4, *E*), 121.0 (C-3a, *E*), 119.1 (C-4, *Z*), 115.9 (d, J = 22.1 Hz, C-3''/-5'', *E*), 115.3 (d, J = 22.1 Hz, C-3''/-5'', *Z*), 112.9 (C-7, *E*), 112.2 (C-7, *Z*); *E/Z* ratio (%) 54:46; MS EI *m/z* (relative intensity, %) 311 [$M^{+\bullet}$] (11), 309 [$M^{+\bullet}$] (66), 307 [$M^{+\bullet}$] (100), 279 (33), 244 (15), 212 (29); IR ν_{max} (cm^{-1}) 3435 (NH), 1707, 1608 (C=O); Anal. ($C_{15}H_8Cl_2FNO$) C, H, N.

(*E/Z*)-6,7-Dichloro-3-[4-(dimethylamino)benzylidene]indolin-2-one (8). 326 mg (1.61 mmol) of **15**, 241 mg (1.61 mmol) *N,N*-dimethylaminobenzaldehyde and 0.01 mL piperidine were reacted as given above. Yield: 240 mg (45%) as orange crystals from ethanol. Mp 303 °C; ^{1}H NMR (500 MHz,

CF_3COOD) δ 8.28 (d, J = 8.5 Hz, 0.18 × 2 H, 2''-/6''-H, *Z*), 8.01 (s, 0.82 × 1 H, vinyl-H, *E*), 7.93 (d, J = 8.2 Hz, 0.82 × 2 H, 2''-/6''-H, *E*), 7.81 (s, 0.18 × 1 H, vinyl-H, *Z*), 7.77 (d, J = 8.2 Hz, 0.82 × 2 H, 3''-/5''-H, *E*), 7.67 (d, J = 8.5 Hz, 0.18 × 2 H, 3''-/5''-H, *Z*), 7.43 (d, J = 8.2 Hz, 0.18 × 1 H, 4-H, *Z*) 7.35 (d, J = 8.2 Hz, 0.82 × 1 H, 4-H, *E*), 7.25 (d, J = 8.2 Hz, 0.18 × 1 H, 5-H, *Z*), 7.09 (d, J = 8.2 Hz, 0.82 × 1 H, 5-H, *E*), 3.49 (s, 0.82 × 6 H, N-CH_3, *E*), 3.47 (s, 0.18 × 6 H, N-CH_3, *Z*); ^{13}C NMR (125 MHz, CF_3COOD) δ 174.2 (C=O, *E*), 172.1 (C=O, *Z*), 145.0 (C-4'', *E*), 144.8 (C-4'', *Z*), 141.7 (C-7a, *E*), 141.0 (vinyl-C, *E*), 140.7 (vinyl-C, *Z*), 139.9 (C-7a, *Z*), 139.4 (C-1'', *E*), 139.2 (C-1'', *Z*), 137.6 (C-6, *E*), 136.8 (C-6, *Z*), 136.1 (C-2''/-6'', *Z*), 134.0 (C-2''/-6'', *E*), 131.0 (C-3, *E*), 130.2 (C-3, *Z*), 127.0 (C-5, *E/Z*), 125.3 (C-3a, *Z*), 123.4 (C-4, *E*), 122.8 (C-3''/-5'', *E*), 121.9 (C-3a, *E*), 121.8 (C-3''/-5'', *Z*), 120.7 (C-4, *Z*), 118.5 (C-7, *E*), 117.7 (C-7, *Z*), 49.4 (N-CH_3, *E*), 49.3 (N-CH_3, *Z*); *E/Z* ratio (%) 82:18; MS EI *m/z* (relative intensity, %) 336 [$M^{+\bullet}$] (11), 334 [$M^{+\bullet}$] (70), 332 [$M^{+\bullet}$] (100), 290 (9); HRMS calcd, 332.0483; found, 332.0493; IR ν_{max} (cm^{-1}) 3440 (NH), 1685, 1568 (C=O); Anal. ($C_{17}H_{14}Cl_2N_2O$) C, H, N. N: calcd, 8.41; found, 7.81.

(*E/Z*)-6,7-Dichloro-3-(4-nitrobenzylidene)indolin-2-one (9). ^{13}C NMR (150 MHz, DMSO-d_6, TMS) δ 168.1 (C=O, *E*), 166.6 (C=O, *Z*), 147.6 (C-4'', *Z*), 147.6 (C-4'', *E*), 142.6 (C-7a, *E*), 140.7 (C-1'', *E*), 140.4 (C-7a, *Z*), 139.6 (C-1'', *E*), 136.0 (vinyl-C, *Z*), 135.4 (vinyl-C, *E*), 132.7 (C-6, *E*), 132.6 (C-3''/-5'', *Z*), 131.8 (C-6, *Z*), 130.5 (C-2''/-6'', *E*), 128.9 (C-3, *E*), 128.8 (C-3, *Z*), 124.4 (C-3a, *Z*), 123.9 (C-3''/-5'', *E*), 123.1 (C-2''/-6'', *Z*), 122.6 (C-5, *Z*), 122.6 (C-5, *E*), 122.0 (C-4, *E*), 120.6 (C-3a, *E*), 120.0 (C-4, *Z*), 113.1 (C-7, *E*), 112.4 (C-7, *Z*).

(*Z*)-6,7-Dichloro-3-[(3,5-dimethyl-1H-pyrrol-2-yl)methylene]indolin-2-one (10). 250 mg (1.24 mmol) of **15**, 183 mg (1.48 mmol) 3,5-dimethylpyrrole-2-carboxaldehyde and 0.01 mL piperidine were reacted as given above. Yield: 267 mg (70%) as an orange solid from ethanol. Mp 303 °C; ^{1}H NMR (500 MHz, DMSO-d_6, TMS) δ 13.28 (br. s, 1 H, 1''-H), 11.35 (br. s, 1 H, 1-H), 7.75 (d, J = 8.2 Hz, 1 H, 4-H), 7.67 (s, 1 H, vinyl-H), 7.21 (d, J = 8.2 Hz, 1 H, 5-H), 6.09 (s, 1 H, 4''-H), 2.35 (s, 3 H, 5''-CH_3), 2.32 (s, 3 H, 3''-CH_3); ^{13}C NMR (125 MHz, DMSO-d_6, TMS) δ 170.1 (C=O), 138.5 (C-5''), 137.2 (C-7a), 134.7 (C-2''), 127.8 (C-3''), 127.7 (C-6), 127.1 (C-3a), 126.0 (vinyl-C), 122.7 (C-5), 118.1 (C-4), 114.1 (C-4''), 112.7 (C-7), 111.4 (C-3); MS EI *m/z* (relative intensity, %) 310 [$M^{+\bullet}$] (11), 308 [$M^{+\bullet}$] (65), 306 [$M^{+\bullet}$] (100), 289 (24), 254 (45); HRMS calcd, 306.0327; found, 306.0283; IR ν_{max} (cm^{-1}) 3433 (NH), 1682, 1560 (C=O); Anal. ($C_{15}H_{12}Cl_2N_2O$) C, H, N. C: calcd, 58.65; found, 57.96.

(*E*)-6,7-Dichloro-3-(pyridin-2-ylmethylene)indolin-2-one (11). 260 mg (1.29 mmol) of **15**, 166 mg (1.55 mmol) pyridine-2-carboxaldehyde and 0.01 mL piperidine were reacted as given above. Yield: 294 mg (78%) as yellow crystals from ethanol. Mp 258 °C; ^{1}H NMR (500 MHz, DMSO-d_6, TMS) δ 11.27 (br. s, 1 H, N-H), 9.10 (d, J = 8.5 Hz, 1 H, 4-H), 8.90 (dd, J = 4.7 Hz, 1.8 Hz, 1 H, 6''-H), 7.99 (dt, J = 7.7 Hz, 1.8 Hz, 1 H, 4''-H), 7.94 (dd, J = 7.7 Hz, 1.2 Hz, 1 H, 3''-H), 7.68 (s, 1 H, vinyl-H), 7.51 (ddd, J = 7.5 Hz, 4.7 Hz, 1.2 Hz, 1 H, 5''-H), 7.26 (d, J = 8.5 Hz, 1 H, 5-H); ^{13}C NMR (125 MHz, DMSO-d_6, TMS) δ 169.2 (C=O), 152.6 (C-2''), 149.7 (C-6''), 142.7 (C-7a), 137.4 (C-4''), 135.9 (vinyl-C), 132.5 (C-6), 129.1 (C-3''), 127.9 (C-3), 127.1 (C-4), 124.6 (C-5''), 122.3 (C-5), 121.8 (C-3a), 112.3 (C-7); MS EI *m/z* (relative intensity, %) 293 [$M^{+\bullet}$] (13), 291 [$M^{+\bullet}$] (48), 289 [$M^{+\bullet}$] (67), 262 (100), 212 (57); IR ν_{max} (cm^{-1}) 3446 (NH), 1718, 1603 (C=O); Anal. ($C_{14}H_8Cl_2N_2O$) C, H, N.

(*E*)-6,7-Dichloro-3-(pyridin-4-ylmethylene)indolin-2-one (12). 365 mg (1.81 mmol) of **15**, 193 mg (1.81 mmol) pyridine-4-carboxaldehyde and 0.01 mL piperidine were reacted as given above. Yield: 315 mg (60%) as yellow crystals from ethanol. Mp 304 °C; ^{1}H NMR (500 MHz, CF_3COOD, TMS) δ 9.01 (d, J = 6.6 Hz, 2 H, 2''-/6''-H), 8.38 (d, J = 6.6 Hz, 2 H, 3''-/5''-H), 8.00 (s, 1 H, vinyl-H), 7.29 (d, J = 8.2 Hz, 1 H, 4-H), 7.18 (d, J = 8.2 Hz, 1 H, 5-H); ^{13}C NMR (125 MHz, CF_3COOD, TMS) δ 172.5 (C=O), 157.0 (C-4''), 144.2 (C-2''/-6''), 143.4 (C-7a), 140.1 (C-6), 136.1 (C-3), 133.1 (vinyl-C), 129.5 (C-3''/-5''), 127.4 (C-5), 123.9 (C-4), 120.5 (C-3a), 119.1 (C-7); MS EI *m/z* (relative intensity, %) 294 [$M^{+\bullet}$] (11), 292 [$M^{+\bullet}$] (66), 290 [$M^{+\bullet}$] (100), 262 (19), 212 (54); HRMS calcd, 290.0014; found, 290.0018; IR ν_{max} (cm^{-1}) 3421 (NH), 1735, 1600 (C=O); Anal. ($C_{14}H_8Cl_2N_2O$) C, H, N.

(*E/Z*)-6,7-Dichloro-3-[4-(trifluoromethyl)benzylidene]indolin-2-one (13). 420 mg (2.08 mmol) of **15**, 362 mg (2.08 mmol) 4-(trifluoromethyl)benzaldehyde and 0.01 mL piperidine were reacted as given

above. Yield: 431 mg (58%) as yellow crystals from ethanol. Mp 213 °C; ^{1}H NMR (500 MHz, DMSO-d_6, TMS) δ 1.33 (br. s, 1 H, N-H, *E/Z*), 8.45 (d, *J* = 8.2 Hz, 0.40 × 2 H, 2''-/6''-H, *Z*), 8.03 (s, 0.40 × 1 H, vinyl-H, *Z*), 7.91 (d, *J* = 8.2 Hz, 0.60 × 2 H, 2''-/6''-H, *E*), 7.88 (d, *J* = 8.2 Hz, 0.60 × 2 H, 3''-/5''-H, *E*), 7.84 (d, *J* = 8.2 Hz, 0.40 × 2 H, 3''-/5''-H, *Z*), 7.79 (s, 0.60 × 1 H, vinyl-H, *E*), 7.75 (d, *J* = 8.2 Hz, 0.40 × 1 H, 4-H, *Z*), 7.34 (d, *J* = 8.2 Hz, 0.60 × 1 H, 4-H, *E*), 7.29 (d, *J* = 8.2 Hz, 0.40 × 1 H, 5-H, *Z*), 7.12 (d, *J* = 8.2 Hz, 0.60 × 1 H, 5-H, *E*); ^{13}C NMR (125 MHz, DMSO-d_6, TMS) δ 168.2 (C=O, *E*), 166.7 (C=O, *Z*), 142.4 (C-7a, *E*), 140.2 (C-7a, *Z*), 138.1 (C-1'', *E*), 137.2 (C-1'', *Z*), 137.0 (vinyl-C, *Z*), 136.2 (vinyl-C, *E*), 132.5 (C-6, *E*), 132.2 (C-2''/-6'', *Z*), 131.5 (C-6, *Z*), 130.0 (C-2''/-6'', *Z*), 129.7 (q, *J* = 32 Hz, C-4'', *E*), 129.5 (q, *J* = 32 Hz, C-4'', *Z*), 128.2 (C-3, *E*), 127.9 (C-3, *Z*), 125.7 (C-3''/-5'', *E*), 125.0 (C-3''/-5'', *Z*), 124.6 (C-3a, *Z*), 123.9 (q, *J* = 272 Hz, CF_3, *E*), 123.9 (q, *J* = 272 Hz, CF_3, *Z*), 122.5 (C-5, *E/Z*), 121.7 (C-4, *E*), 120.7 (C-3a, *E*), 119.8 (C-4, *Z*), 113.1 (C-7, *E*), 112.3 (C-7, *Z*); *E/Z* ratio (%) 60:40; MS EI *m/z* (relative intensity, %) 361 [$M^{+\bullet}$] (12), 359 [$M^{+\bullet}$] (64), 357 [$M^{+\bullet}$] (100), 329 (31), 212 (32); IR ν_{max} (cm^{-1}) 3446 (NH), 1709, 1606 (C=O), 1323 (C-F); Anal. ($C_{16}H_8Cl_2F_3NO$) C, H, N.

(*E/Z*)-3-[3,5-Bis(trifluoromethyl)benzylidene]-6,7-dichloroindolin-2-one (14). 250 mg (1.24 mmol) of **15**, 358 mg (1.48 mmol) 3,5-bis(trifluoromethyl)benzaldehyde and 0.01 mL piperidine were reacted as given above. Yield: 428 mg (81%) as yellow crystals from ethanol. Mp 238 °C; ^{1}H NMR (500 MHz, DMSO-d_6, TMS) δ 11.36 (br. s, 1 H, N-H, *E/Z*), 9.04 (s, 0.69 × 2 H, 2''-/6''-H, *E*), 8.39 (s, 0.31 × 2 H, 2''-/6''-H, *Z*), 8.24 (s, 0.31 × 1 H, 4"-H, *Z*), 8.20 (s, 0.69 × 1 H, 4"-H, *E*), 8.14 (s, 0.69 × 1 H, vinyl-H, *E*), 7.84 (s, 0.31 × 1 H, vinyl-H, *Z*), 7.70 (d, *J* = 8.0 Hz, 0.69 × 1 H, 4-H, *E*), 7.33 (d, *J* = 8.0 Hz, 0.69 × 1 H, 5-H, *E*), 7.13 (d, *J* = 8.2 Hz, 0.31 × 1 H, 4-H, *Z*), 7.11 (d, *J* = 8.2 Hz, 0.31 × 1 H, 5-H, *Z*); ^{13}C NMR (125 MHz, DMSO-d_6, TMS) δ 168.0 (C=O, *Z*), 166.8 (C=O, *E*), 142.6 (C-7a, *Z*), 140.4 (C-7a, *E*), 136.7 (C-1", *Z*), 135.6 (C-1", *E*), 135.0 (vinyl-C, *E*), 134.3 (vinyl-C, *Z*), 132.8 (C-6, *Z*), 131.9 (C-6, *E*), 131.9 (C-2''/-6'', *E*), 129.9 (q, *J* = 33 Hz, C-3''/-5'', *Z*), 129.8 (C-2''/-6'', *Z*), 128.8 (q, *J* = 33 Hz, C-3''/-5'', *E*), 124.3 (C-3a, *E*), 124.3 (C-3a, *Z*), 123.4 (C-4", *E*), 123.0 (C-4", *Z*), 122.7 (C-5, *E*), 122.4 (q, *J* = 272 Hz, CF_3, *E*) 122.0 (q, *J* = 272 Hz, CF_3, *Z*) 122.1 (C-5, *Z*), 121.4 (C-3, *E*), 121.2 (C-4, *Z*), 120.6 (C-3, *Z*), 119.8 (C-4, *E*), 113.3 (C-7, *Z*), 112.5 (C-7, *E*); *E/Z* ratio (%) 69:31; MS EI *m/z* (relative intensity, %) 429 [$M^{+\bullet}$] (12), 427 [$M^{+\bullet}$] (68), 425 [$M^{+\bullet}$] (100), 397 (42), 333 (13), 212 (51); IR ν_{max} (cm^{-1}) 3433 (NH), 1697, 1612 (C=O), 1128 (C-F); Anal. ($C_{17}H_7Cl_2F_6NO$) C, H, N.

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