SUPPORTING INFORMATION

New geldanamycin derivatives with anti Hsp properties by mutasynthesis

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1. General Methods

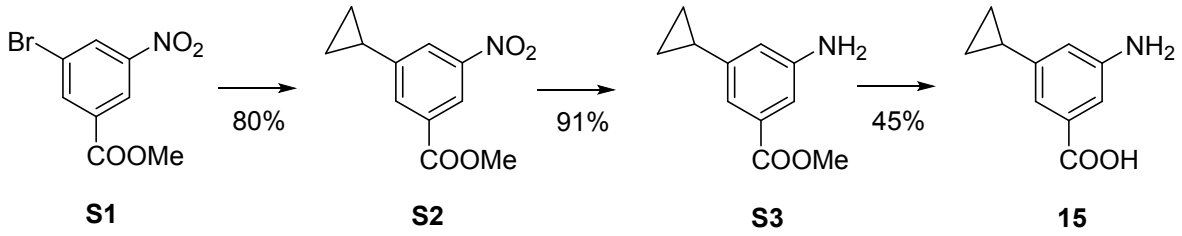
^{1}H NMR spectra were recorded at 400 MHz with a Bruker AVS-400 or at 500 MHz with a Bruker DRX-500. ^{13}C NMR spectra were recorded at 100 MHz with a Bruker AVS-400 and at 125 MHz with a Bruker DRX-500. Multiplicities are described using the following abbreviations: s = singlet, d = doublet, t = triplet, q = quartet, m = multiplet, b = broad. Chemical shift values of ^{1}H and ^{13}C NMR spectra are commonly reported as values in ppm relative to residual solvent signal as internal standard. The multiplicities refer to the resonances in the off-resonance decoupled spectra and were elucidated using the distortionless enhancement by polarization transfer (DEPT) spectral editing technique, with secondary pulses at 90° and 135°. Multiplicities are reported using the following abbreviations: s = singlet (due to quaternary carbon), d = doublet (methine), t = triplet (methylene), q = quartet (methyl). Mass spectra (EI) were obtained at 70 eV with a type VG Autospec spectrometer (Micromass), with a type LCT (ESI) (Micromass) or with a type Q-TOF (Micromass) spectrometer in combination with a Waters Aquity Ultraperformance LC system. Analytical thin-layer chromatography was performed using precoated silica gel 60 F_{254} plates (Merck, Darmstadt), and the spots were visualized with UV light at 254 nm or by staining with H_2SO_4/4-methoxybenzaldehyde in ethanol. Flash column chromatography was performed on Merck silica gel 60 (230-400 mesh). Isolation of geldanamycin derivatives was commonly achieved by preparative high performance liquid chromatography (Merck Hitachi, pump L-7150, interface D-7000, diode array detector L-7450).

As most of the isolated derivatives rather resemble reblastatin and not geldanamycin (because of the absence of the C-21 hydroxyl group and the quinone moiety) we choose to name these metabolites reblastatins.

2. Preparation of 3-aminobenzoic acid derivatives

The preparation of the benzoic acids were achieved according to literature procedures: **14** and **17** (ref. [S1]). Aminobenzoic acids **10-13** and **21**, **26-31**, **S1**, **S4**, **S7**, **S13**, **S17**, **S20**, **S25** and **S30** are commercially available.

3-Amino-5-cyclopropylbenzoic acid (15)



S1 **S2** **S3** **15**

Methyl 3-bromo-5-nitrobenzoate **S1** (1.00 g, 3.86 mmol, 1.0 eq.) was dissolved in toluene (20 mL) followed by addition of $Pd(PPh_3)_4$ (0.22 g, 0.19 mmol, 0.05 eq.), cyclopropylboronic acid (0.50 g, 5.79 mmol, 1.5 eq.) and K_2CO_3 (1.76 g, 12.8 mmol, 3.3 eq.). The reaction mixture was heated by microwave irradiation at 150 °C for 1 h. Then, the reaction was cooled to room temperature, followed by addition of a mixture of ethyl acetate and water (1:1, 40 mL). The phases were separated, the aqueous phase was extracted twice with ethyl acetate and the combined, organic extracts were washed with brine, dried over magnesium sulfate and evaporated. The crude product was purified by flash column chromatography (petroleum ether/ethyl acetate 10:1) afforded methyl 3-cyclopropyl-5-nitrobenzoate **S2** (0.68 g, 3.07 mmol, 80 %) as a crystalline, colorless solid.

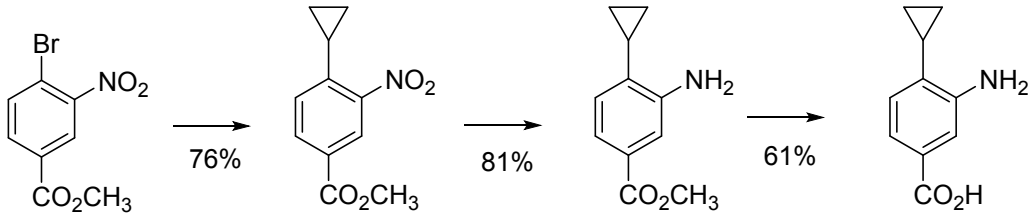
Methyl 3-cyclopropyl-5-nitrobenzoate **S2** (1.14 g, 5.15 mmol, 1.0 eq.) was dissolved in ethyl acetate (200 mL), followed by addition of tin(II) chloride dihydrate (5.80 g, 25.7 mmol, 5.0 eq.). The mixture was heated under refluxing conditions for 24 h. After cooling, the solution was hydrolyzed by addition of crushed ice and neutralization was achieved by addition of saturated hydrogen bicarbonate solution and the phases were separated. The aqueous phase was extracted with ethyl acetate and the combined organic extracts were dried over magnesium sulfate and evaporated. Purification by flash column chromatography (petroleum ether/ethyl acetate= 5:1) afforded compound **S3** (0.89 g, 4.66 mmol, 91 %) as a crystalline, yellow solid.

Methyl 3-amino-5-cyclopropylbenzoate **S3** (0.80 g, 4.19 mmol, 1.0 eq.) was dissolved in methanol (150 mL) and an aqueous 1M LiOH-solution (20.9 mL, 20.9 mmol, 5.0 eq.) was added. The reaction mixture was stirred at room temperature for 15 h, and then hydrolyzed by addition of crushed ice. The solution was acidified (pH= 3) using HCl (1 M) and extracted three times with ethyl acetate. The combined, organic phases were dried over magnesium sulfate and evaporated to dryness to yield 3-amino-5-cyclopropylbenzoic acid **15** (0.33 g, 1.86 mmol, 45 %) as a crystalline, yellow solid.

^{1}H NMR (400 MHz, MeOD, CD_2HOD= 3.31 ppm) δ 7.15-7.14 (m, 1H, H-Ar), 7.07-7.06 (s, 1H, H-Ar), 6.67-6.65 (m, 1H, H-Ar), 1.90-1.78 (m, 1H, H-cyclopropyl), 0.98-0.89 (m, 1H, H-cyclopropyl), 0.98-0.89 (m, 1H, H-cyclopropyl), 0.70-0.62 (m, 1H, H-cyclopropyl), 0.70-0.62 (m, 1H, H-cyclopropyl) ppm; **^{13}C NMR** (100 MHz, MeOD-d_4, MeOD-d_4= 49.0 ppm): δ 170.6 (s, COOH), 149.0 (s, C-Ar), 146.6 (s, C-Ar), 132.5 (s, C-Ar), 118.1 (d, C-Ar), 117.7 (d, C-Ar), 114.7 (d, C-Ar), 16.0 (d, C-cyclopropyl), 9.5 (t,

C-cyclopropyl), 9.5 (t, C-cyclopropyl) ppm; **HRMS** (ESI) *m/z* for $C_{10}H_{10}NO_2$ [M-H]$^-$: cacl.: 176.0712, found: 176.0708; **m.p.** = 159 °C.

3-Amino-4-cyclopropylbenzoic acid (16)



S4 **S5** **S6** **16**

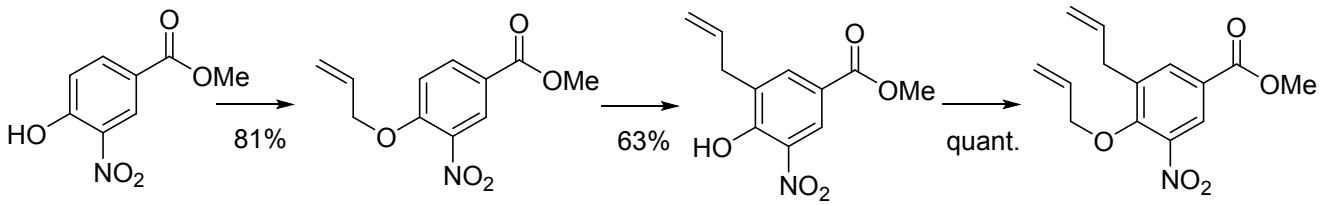
Methyl 4-bromo-3-nitrobenzoate **S4** (0.50 g, 1.93 mmol, 1.0 eq.) was dissolved in toluene (30 mL) followed by addition of $Pd(PPh_3)_4$ (0.11 g, 0.10 mmol, 0.05 eq.), cyclopropylboronic acid (0.25 g, 2.90 mmol, 1.5 eq.) and K_2CO_3 (0.88 g, 6.37 mmol, 3.3 eq.). The reaction mixture was heated at 95 °C overnight. Then, the reaction was cooled to room temperature, followed by addition of a mixture of ethyl acetate and water (1:1, 40 mL). The phases were separated, the aqueous phase was extracted twice with ethyl acetate and the combined organic extracts were washed with brine, dried over magnesium sulfate and evaporated. The crude product was purified by flash column chromatography (petroleum ether/ethyl acetate 10:1) afforded methyl 4-cyclopropyl-3-nitrobenzoate **S5** (0.32 g, 1.46 mmol, 76 %) as a crystalline, colorless solid.

Methyl 4-cyclopropyl-3-nitrobenzoate **S5** (0.32 g, 1.45 mmol, 1.0 eq.) was dissolved in ethyl acetate (60 mL), followed by addition of tin(II) chloride dihydrate (1.60 g, 7.25 mmol, 5.0 eq.). The mixture was heated under refluxing conditions for 24 h. After cooling, the solution was hydrolyzed by addition of crushed ice and neutralization was achieved by addition of saturated hydrogen bicarbonate solution and the phases were separated. The aqueous phase was extracted with ethyl acetate and the combined organic extracts were dried over magnesium sulfate and evaporated. Purification by flash column chromatography (petroleum ether/ethyl acetate= 10:1) afforded compound **S6** (0.22 mg, 1.18 mmol, 81 %) as a crystalline, yellow solid.

Methyl 3-amino-4-cyclopropylbenzoate **S6** (0.20 g, 1.05 mmol, 1.0 eq.) was dissolved in methanol (40 mL) and an aqueous 1 M LiOH-solution (10.5 mL, 10.5 mmol, 10 eq.) was added. The reaction mixture was stirred at 50 °C until the transformation was completed (as judged by TLC) and then hydrolyzed by addition of crushed ice. The solution was acidified (pH = 2) using HCl (1 M) and extracted with ethyl acetate (3x). The combined, organic layers were dried over magnesium sulfate and evaporated to dryness to yield 3-amino-4-cyclopropylbenzoic acid **16** (112 mg, 0.63 mmol, 61 %) as a crystalline, colorless solid.

^{1}H NMR (400 MHz, DMSO-d_6, DMSO-d_5 = 2.50 ppm): δ 7.23 (d, *J* = 1.5 Hz, 1H, H-Ar), 7.06 (dd, *J* = 1.5, 7.9 Hz, 1H, H-Ar), 6.85 (d, *J* = 7.9 Hz, 1H, H-Ar), 5.23 (br.s., 2H, NH_2), 1.72 (m, 1H, H-cyclopropyl), 0.89 (m, 2H, H-cyclopropyl), 0.53 (m, 2H, H-cyclopropyl) ppm; **^{13}C NMR** (100 MHz, DMSO-d_6, DMSO-d_6 = 39.52 ppm): δ 167.8 (s, Ar*C*OOH), 147.6 (s, C-Ar), 130.7 (s, C-Ar), 128.8 (s, C-Ar), 125.9 (d, C-Ar), 117.0 (d, C-Ar), 114.3 (d, C-Ar), 11.0 (d, C-cyclopropyl), 6.3 (t, C-cyclopropyl) ppm; **HRMS** [ESI] *m/z* for $C_{10}H_{11}NO_2$ $[M]^+$: cacl.: 177.0790 found: 177.0791; **m.p.** = 124.5 - 126 °C.

Aminobenzofuran-5-carboxylic acid (28)



S7 **S8** **S9** **S10**

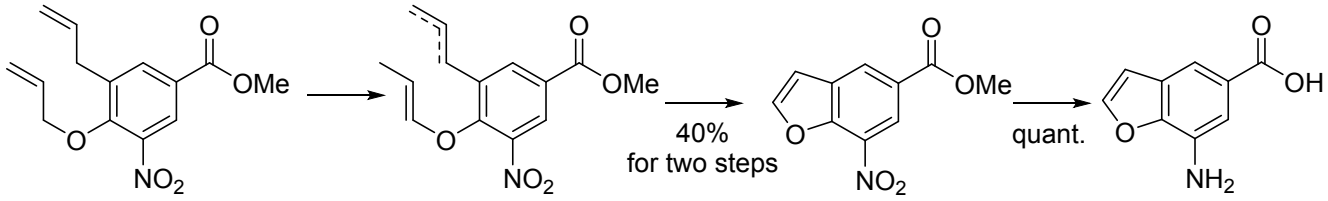
Methyl 4-hydroxy-3-nitrobenzoate **S7** (10.0 g, 50.7 mmol, 1.0 eq.) was dissolved in acetone (250 mL) followed by addition of potassium carbonate (14.0 g, 101.4 mmol, 2.0 eq.) and allyl bromide (13.2 ml, 152.1 mmol, 3.0 eq.). The reaction mixture was heated under refluxing conditions for 24 hours, and then cooled to room temperature. Then, the solution was filtered over Celite™ and concentrated under reduced pressure. The residue was taken up in ethyl acetate and water. The phases were separated, the aqueous phase was extracted twice with ethyl acetate and the combined, organic extracts were dried over magnesium sulfate and evaporated to yield methyl 4-allyloxy-3-nitrobenzoate **S8** (9.69 g, 40.9 mmol, 81 %) as a crystalline, yellow solid.

The ester **S8** (1.50 g, 6.32 mmol, 1.0 eq.) was dissolved in toluene (10 mL), treated with SiC (0.80 g) and heated under microwave irradiation at 200 °C for 2 h. After cooling to room temperature the solution was filtered over Celite™ and evaporated. Purification by flash column chromatography (petroleum ether/ethyl acetate= 30:1) afforded compound **S9** (0.95 g, 3.98 mmol, 63 %) as a crystalline, yellow solid.

The alcohol **S9** (0.60 g, 2.53 mmol, 1.0 eq.) was dissolved in acetone (20 mL), and treated with K_2CO_3 (0.70 g, 5.06 mmol, 2.0 eq.) and allyl bromide (0.7 mL, 7.59 mmol, 3.0 eq.). The reaction mixture was heated under refluxing condition for 24 h. After cooling to room temperature the solution was filtered over Celite™, washed with acetone and econcentrated under reduced pressur. The residue was taken up in water and extracted with ethyl acetate. The combined, organic extracts were dried over magnesium sulfate and removed in vacuo to yield ether **S10** (0.70 g, 2.53 mmol, quant.) as a yellow oil.

^{1}H NMR (400 MHz, $CDCl_3$, $CHCl_3$ = 7.26 ppm): δ 8.34 (d, 1H, *J* = 2 Hz, Ar-H), 8.10 (d, 1H, *J* = 2 Hz, Ar-H), 6.04 (ddt, 1H, *J* = 17.0, 10.4, 5.9 Hz, H-allyl), 5.95 (ddt, 1H, *J* = 17.2, 9.9, 6.5 Hz, H-allyl), 5.39 (dd, 1H, *J* = 17.0, 1.2 Hz, H-allyl), 5.31 (dd, 1H, *J* = 10.4, 1.2 Hz, H-allyl), 5.18 (dd, 1H, *J* = 9.9, 1.4 Hz,

H-allyl), 5.12 (dd, 1H, *J* = 17.2, 1.4 Hz, H-allyl), 4.53 (d, 2H, *J* = 5.9 Hz, CH_2), 3.51 (d, 2H, *J* = 6.5 Hz, CH_2), 3.87 (s, 3H, $COOCH_3$) ppm; **^{13}C NMR** (100 MHz, $CDCl_3$, $CDCl_3$= 77.0 ppm): δ 165.0 (s, *C*$OOCH_3$), 153.8 (s, C-Ar), 144.2 (s, C-Ar), 137.3 (d, C-allyl), 135.7 (d, C-Ar), 135.2 (d, C-allyl), 132.3 (d, C-Ar), 126.0 (s, C-Ar), 125.1 (d, C-Ar), 119.6 (t, C-allyl), 117.8 (t, C-allyl), 76.4 (t, CH_2), 52.8 (q, $COOCH_3$), 34.0 (t, CH_2) ppm; **HRMS** (ESI) *m/z* for $C_{14}H_{15}NO_5$ $[M]^+$: calc.: 277.0950, found: 277.0948.



S10 **S11** **S12** **19**

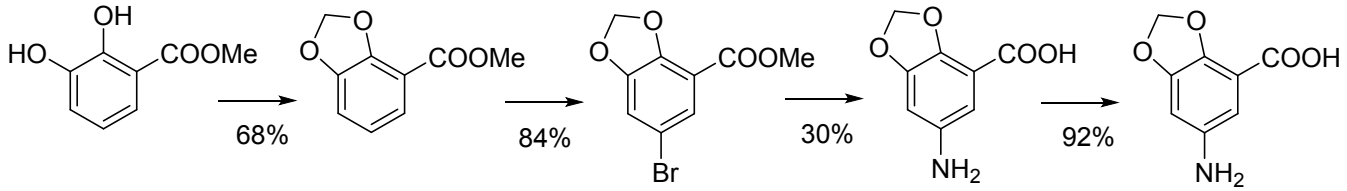
Methyl 3-allyl-4-allyloxy-5-nitrobenzoate **S10** (380 mg, 1.38 mmol, 1.0 eq.) was dissolved in toluene (5 mL), and treated with $[RuClH(CO)(PPh_3)_3]$ (180 mg, 0.19 mmol, 14 mol%). The reaction mixture was heated at 90 °C for 72 h. After cooling to room temperature the solution was filtered through a pad of silica gel and concentrated under reduced pressure. The resulting crude product **S11** (270 mg) was directly used in the next step.

The ester **S11** (0.5 g, 1.81 mmol, 1.0 eq.) was dissolved in toluene (5 mL), and treated with Grubbs-Hoveyda catalyst (40 mg, 0.06 mmol, 34 mol%). The reaction mixture was heated at 90 °C for 5.5 h. After cooling to room temperature the solution was filtered over a pad of silica gel, washed with ethyl acetate and concentrated under reduced pressure. Purification by flash column chromatography (petroleum ether/ethyl acetate= 20:1) afforded compound **S12** (0.14 g, 0.73 mmol, 40 %) as a crystalline, beige solid.

Ester **S12** (20.0 mg, 0.09 mmol, 1.0 eq.) was dissolved in methanol (5 mL) and an aqueous 1 M LiOH-solution (0.45 mL, 0.45 mmol, 5.0 eq.) was added. The reactions mixture was stirred at room temperature for 4 h, and then hydrolyzed by addition of HCl (2 M). After evaporation of the solvent the residue was taken up in water and extracted with ethyl acetate. The combined, organic extracts were dried over magnesium sulfate and concentrated under reduced pressure to yield the carboxylic acid (18.5 mg, 0.09 mmol, quant.) as a crystalline, colorless solid. The acid (23.0 mg, 0.11 mmol, 1.0 eq.) was dissolved in ethyl acetate (3 mL), followed by addition of tin(II) chloride dihydrate (100 mg, 0.44 mmol, 4.0 eq.). The reaction mixture was heated under refluxing conditions for 4 h. After cooling, the solution was hydrolyzed by addition of crushed ice and neutralization was achieved by addition of a saturated hydrogen bicarbonate solution and the phases were separated. The aqueous phase was extracted with ethyl acetate and the combined, organic extracts were dried over magnesium sulfate and evaporated to yield the title compound **19** (19.5 mg, 0.11 mmol, quant.) as a crystalline, beige solid.

^{1}H NMR (400 MHz, DMSO-d_6, DMSO-d5 = 2.50 ppm): δ 7.99 (d, 1H, *J* = 2.4 Hz, H-Ar), 7.50 (d, 1H, *J* = 1.7 Hz, H-Ar), 7.22 (d, 1H, *J* = 1.7 Hz, H-Ar), 6.94 (d, 1H, *J* = 2.4 Hz, H-Ar) ppm; **^{13}C NMR** (100 MHz, DMSO-d6, DMSO-d6 = 39.52 ppm): δ 168.1 (s, COOH), 146.1 (s, C-Ar), 145.6 (d, C-Ar), 133.5 (s, C-Ar), 127.4 (s, C-Ar), 126.5 (s, C-Ar), 110.9 (d, C-Ar), 109.7 (d, C-Ar), 107.7 (d, C-Ar) ppm; **HRMS** [ESI] *m/z* for $C_9H_6NO_3$ [M-H]+: calc. 176.0348 found 176.0347; **m.p**.: 161-167 °C (decomposition).

6-Aminobenzo[d][1,3]dioxole-4-carboxylic acid (20)



S13 **S14** **S15** **S16** **20**

Methyl 2,3-dihydroxybenzoate **S13** (5.0 g, 29.94 mmol, 1.0 eq.) was dissolved in DMF (80 mL) followed by addition of potassium fluoride (8.64 g, 148.7 mmol, 5.0 eq.). After 30 min diiodomethane (2.87 mL, 35.7 mmol, 1.2 eq.) was added and then the reaction mixture was heated at 110 °C for 30 h. Then, the solution was cooled to room temperature, filtered over Celite^{TM} and diluted with a mixture of diethyl ether and water. The phases were separated, the aqueous phase was extracted twice with diethyl ether and the combined, organic extracts were washed with NaOH-solution (1 M), dried over magnesium sulfate and evaporated under reduced pressure to yield methyl benzo[d][1,3]dioxole-4-carboxylate **S14** (3.62 g, 20.06 mmol, 68 %) as a crystalline, colorless solid.

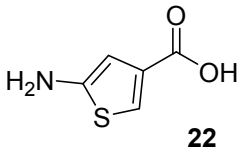
Ester **S14** (0.30 g, 1.67 mmol, 1.0 eq.) was suspended in acetic acid (8 mL), followed by addition of sodium acetate (0.31 g, 4.18 mmol, 2.5 eq.) and bromine (0.1 mL, 1.67 mmol, 1.0 eq). The reaction mixture was stirred at room temperature for 12 h. After removal of the solvent, the residue was taken up in water and extracted with diethyl ether. The combined, organic extracts were dried over magnesium sulfate and evaporated. Purification by flash column chromatography (petroleum ether/ethyl acetate= 20:1) afforded methyl 6-bromobenzo[d][1,3]dioxole-4-carboxylate **S15** (0.36 g, 1.4 mmol, 84 %) as a crystalline, colorless solid.

Aryl bromide **S15** (0.40 g, 1.36 mmol, 1.0 eq.), Cs_2CO_3 (0.88 g, 2.71 mmol, 2.0 eq.), CuI (0.03 g, 0.14 mmol, 0.1 eq.) and 2,4-pentadione (56.0 µL, 0.54 mmol, 0.4 eq.) were dissolved in DMF (3 mL) followed by addition of ammonia (28 %, 400 µL). The reaction mixture was heated at 70 °C for 12 h. After cooling to room temperature the solution was filtered over Celite^{TM}, washed with ethyl acetate and concentrated under reduced pressure. The crude product was purified by flash column chromatography (petroleum ether/ethyl acetate= 5:1) afforded amine **S16** (0.08 g, 0.41 mmol, 30 %) as a crystalline, beige solid.

Ester **S16** (50.0 mg, 0.26 mmol, 1.0 eq.) was dissolved in methanol (5 mL) and an aqueous 1 M LiOH-solution (0.13 mL, 1.13 mmol, 5.0 eq.) was added. The reaction mixture was stirred at room temperature for 4 h, and then hydrolyzed by addition of crushed ice. The solution was acidified (pH= 5) with HCl (2 M) and extracted with ethyl acetate (3x). The combined, organic layers were dried over magnesium sulfate and concentrated to dryness to yield 6-aminobenzo[1,3]dioxol-4-carboxyl acid **20** (44.0 mg, 0.24 mmol, 92 %) as a crystalline, beige solid.

^{1}H NMR (400 MHz, MeOD-d_4, D_2HCOD = 3.31 ppm) δ 7.06 (s, 1H, H-Ar), 6.80 (s, 1H, H-Ar), 6.08 (s, 2H, CH_2) ppm; **^{13}C NMR** (100 MHz, MeOD-d_4, MeOD-d_4 = 49.0 ppm): δ 166.3 (s, COOH), 151.4 (s, C-Ar), 147.0 (s, C-Ar), 133.5 (s, C-Ar), 113.7 (s, C-Ar), 105.9 (d, C-Ar), 105.8 (d, C-Ar), 104.0 (t, CH_2) ppm; **HRMS** (ESI) *m/z* for $C_8H_8NO_4$ $[M+H]^+$: cacl.: 182.0453, found: 182.0457; **m.p.** = 175 °C.

5-Aminothiophene-3-carboxylic acid (22)

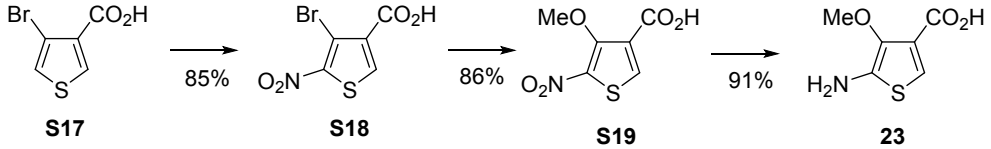


22

5-Nitrothiophene-3-carboxylic acid (100 mg, 0.58 mmol, 1.2 eq.) was dissolved in ethyl acetate (25 mL), treated with Pd/C (12.3 mg, 0.12 mmol, 20 mol %) and triethylsilane (1.12 mL, 7.2 mmol, 15 eq.). The suspension was stirred at room temperature for 24 h. The catalyst was removed by filtration through Celite™ and the filtrate was concentrated *in vacuo*. The crude product was purified by flash column chromatography (petroleum ether/ethyl acetate= 2:1) afforded 5-aminothiophene-3-carboxylic acid **22** (44.3 mg, 0.31 mmol, 53 %) as a crystalline, brown solid.

^{1}H NMR (400 MHz, DMSO-d_6, DMSO-d_5 = 2.50 ppm): δ 7.12 (m, 1H, H-Ar), 6.15 (m, 1H, H-Ar), 5.65 (s, 2H, NH_2) ppm; **^{13}C NMR** (400 MHz, DMSO-d_6, DMSO-d_6 = 39.25 ppm): δ 163.8 (s, *C*O_2H), 154.2 (s, Ar-*C*-CO_2H), 132.7 (s, Ar-*C*-NH_2), 116.9 (d, Ar-C), 103.3 (d, Ar-C) ppm; **HRMS** [ESI] *m/z* for $C_5H_6NO_2S$ $[M+H]^+$: calc.: 144.0119, found: 144.0117.

5-Amino-4-methoxythiophene-3-carboxylic acid (23)



S17 **S18** **S19** **23**

A suspension of 4-bromothiophene-3-carboxylic acid (**S17**) (0.50 g, 2.41 mmol, 1.0 eq.) in conc. H_2SO_4 (4 mL) was added dropwise at –20 °C into a solution of conc. HNO_3 (0.11 mL, 2.65 mmol, 1.10 eq.) in conc. H_2SO_4 (0.5 mL) and stirred for 3.5 h between –20 °C and –10 °C. H_2O (20 mL) was cautiously

added and the resulting precipitate was filtered and washed with water. The solid was dried in vacuo to yield the nitrothiophene **S18** (0.52 g, 2.06 mmol, 85%) as a yellow solid [S2].

^{1}H-NMR (200 MHz, DMSO-d_6): δ (ppm) = 8.68 (s, 1 H, 2-H); **^{13}C-NMR** (100 MHz, DMSO-d_6): δ (ppm) = 161.5 (CO), 147.7 (C-5), 139.2 (C-2), 133.0 (C-3), 113.3 (C-4); **mp**: 239–241 °C (decomposition).

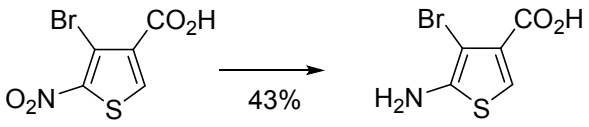
A suspension of thiophene derivative **S18** (0.25 g, 0.94 mmol, 1.0 eq.), copper(I)bromide (20.0 mg, 0.14 mmol, 0.15 eq.) and sodium iodide (21.0 mg, 0.14 mmol, 0.15 eq.) in MeOH (5 mL) was treated with sodium methanolate (0.15 g, 2.83 mmol, 3.0 eq.) under a nitrogen atmosphere. The mixture was stirred for 16 h at 75 °C. After cooling to room temperature H_2O (10 mL) was added and the pH was adjusted to 1 by addition of conc. HCl. EtOAc (10 mL) was added, the organic phase was separated and the aqueous phase was extratced with EtOAc (2 × 10 mL). The combined organic phases were dried $MgSO_4$, filtered and the solvent was removed under reduced pressure. The thiophene derivative **S19** (165 mg, 0.81 mmol, 86%) was obtained as a yellow solid.

^{1}H-NMR (200 MHz, MeOH-d_4): δ (ppm) = 8.39 (s, 1 H), 4.06 (s, 3 H); **^{13}C-NMR** (100 MHz, MeOH-d_4): δ (ppm) = 163.1 (CO), 157.1 (C-4), 140.1 (C-5), 137.6 (C-2), 128.6 (C-3), 63.5 (OMe).

A suspension of thiophene derivative **S19** (70.0 mg, 0.35 mmol, 1.0 eq.), palladium on charcoal (10%, 7 mg) and ammoniumformiate (98.0 mg, 1.55 mmol, 4.50 eq.) in MeOH (3 mL) was treated under a nitrogen atmosphere for 19 at rt. The suspension was passed through a pad silica and the filtrate was concentrated under reduced pressure. The title compound **23** (65.6 mg, 0.38 mmol, 91%) was obtained as a colorless solid.

^{1}H-NMR (200 MHz, MeOH-d_4): δ (ppm) = 7.95 (s, 1 H), 4.08 (s, 3 H); **^{13}C-NMR** (100 MHz, MeOH-d_4): δ (ppm) = 169.4 (CO), 156.8 (C-4), 139.9 (C-5), 136.5 (C-3) 133.0 (C-2), 62.8 (OMe).

5-Amino-4-methoxythiophene-3-carboxylic acid (24)

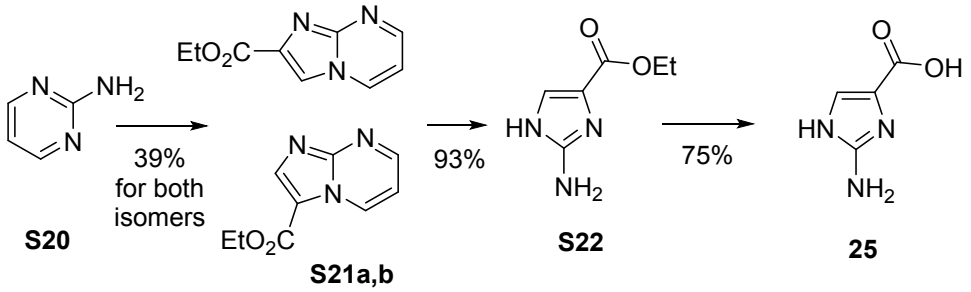


S18 **24**

A suspension of 4-bromo-5-nitrothiophene-3-carboxylic acid (**S18**) (0.10 g, 0.40 mmol, 1.0 eq.), Pd/C (5 mg) and ammoniumformiate (11 mg, 0.18 mmol, 4.50 eq.) in MeOH (3 mL) were shaken at rt for 17 hunder an argon atmosphere. The mixture was passed through a pad of silica and the filtrate was concentrated under reduced pressure. The resulting residue was suspended with a small amount of water and filtered. The resulting product **24** (37 mg, 0.17 mmol, 43%) was a colorless solid.

^{1}H-NMR (200 MHz, MeOH-d_4): δ (ppm) = 8.23 (s, 1 H, 2-H); **^{13}C-NMR** (100 MHz, MeOH-d_4): δ (ppm) = 166.1 (CO), 140.9 (C-5), 134.7 (C-2), 126.8 (C-3), 113.4 (C-4).

2-Amino-1*H*-imidazole-4-carboxylic acid (25)



A solution of 2-aminopyrimidine **S20** (3.80 g, 40.0 mmol, 1.0 eq.) and ethyl-bromopyruvate (90%, 5.58 mL, 40.0 mmol, 1.0 eq.) in EtOH (60 mL) was stirred for 18 h at 75 °C. After cooling to room temperature the solvent was removed under reduced pressure. The residue was dissolved in CH_2Cl_2 (40 mL) and a saturated bicarbonate solution (20 mL) was added. The organic phase was separated and the aqueous phase was extracted with CH_2Cl_2 (3 × 30 mL). The combined organic phases were dried ($MgSO_4$), filtered, the solvent was removed under reducced pressure. The residue was purified by column chromatography (silica gel; EtOAc) and recrystalisation of the crude product yielded **S21a** (1.53 g, 7.98 mmol, 20%) and **S21b** (1.49 g, 7.79 mmol, 19%) each of which are yellowish crystalline [S3].

S21a: **^{1}H-NMR** (200 MHz, $CDCl_3$): δ (ppm) = 8.64 (dd, J = 4.1, 2.1 Hz, 1 H, 7-H), 8.57 (dd, J = 6.9, 2.1 Hz, 1 H, 5-H), 8.16 (s, 1 H, 3-H), 6.96 (dd, J = 6.9, 4.1 Hz, 1 H, 6-H), 4.44 (q, J = 7.1 Hz, 2 H, OEt), 1.41 (t, J = 7.1 Hz, 3 H, OEt).

S21b: **^{1}H-NMR** (200 MHz, $CDCl_3$): δ (ppm) = 9.55 (dd, J = 6.9, 2.1 Hz, 1 H, 5-H), 8.71 (dd, J = 4.2, 2.1 Hz, 1 H, 7-H), 8.44 (s, 1 H, 2-H), 7.11 (dd, J = 6.9, 4.2 Hz, 1 H, 6-H), 4.41 (q, J = 7.1 Hz, 2 H, OEt), 1.41 (t, J = 7.1 Hz, 3 H, OEt).

A solution of **S21** (0.46 g, 2.39 mmol, 1.0 eq.) and hydrazine-monohydrate (0.13 mL, 2.63 mmol, 1.10 eq.) in ethanol (15 mL) was stirred for 16.5 h at 75 °C. After cooling to room temperature the solvent was removed under reduced pressure to yield a solid material which was washed with Et_2O (3 × 5 mL). The yellow solid **S22** (0.34 g, 2.22 mmol, 93%) was dried under vacuum [S3].

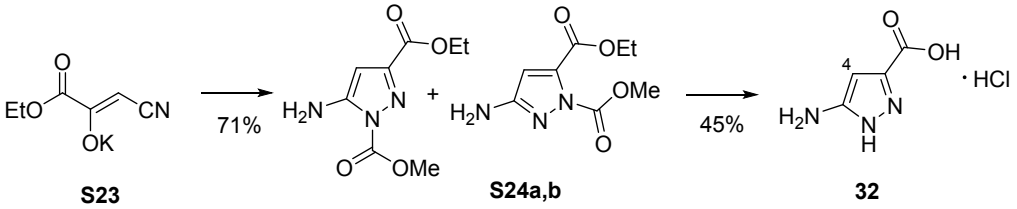
^{1}H-NMR (200 MHz, MeOH-d_4): δ (ppm) = 7.25 (s, 1 H, 5-H), 4.25 (q, J = 7.1 Hz, 2 H, OEt), 1.32 (t, J = 7.1 Hz, 3 H, OEt); **mp**: 179–180 °C.

A solution of **S22** (1.82 g, 11.7 mmol, 1.0 eq.) in THF (30 mL) was treated with NaOH (2.34 g, 58.5 mmol, 5.0 eq.) in H_2O (10 mL) and stirred for 17 h at room temperature. Then, the reaction mixture was neutralised by addition of a 2 M solution of HCl. The resulting precipitate was filtered off, dried in

vacuo and recrystalised in semiconcentrated HCl. The title compound **25** (1.43 g, 8.74 mmol, 75%) was obtained as a brownish solid.

^{1}H-NMR (400 MHz, DMSO-d_6): δ (ppm) = 7.68 (s_{br}, 2 H, NH_2), 7.58 (s, 1 H, 5-H); **^{13}C-NMR** (100 MHz, DMSO-d_6): δ (ppm) = 159.7 (CO), 148.4 (C-2), 120.0 (C-4), 118.9 (C-5); **$C_4H_5N_3O_2$ + H^+ (ESI)** HRMS: calc.: 128.0460, found: 128.0458; **mp**: 188 °C (decomposition).

5-Amino-1*H*-pyrazole-3-carboxylic acid (32)



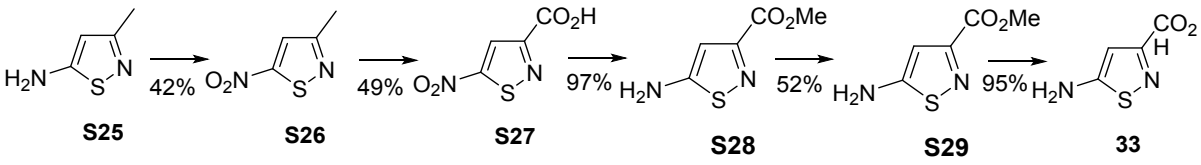
Diethyloxalic acid (6.77 mL, 50.0 mmol, 1.0 eq.) was added to a suspension of 18-crown-6 (1.19 g, 4.50 mmol, 0.09 eq.) and *t*-BuOK (5.61 g, 50.0 mmol, 1.0 eq.) in THF (35 mL) at 0 °C. The reaction mixture was heated to 60 °C and MeCN (2.63 mL, 50.0 mmol, 1.0 eq.) was added and stirring was continued for 40 min at this temperature. After cooling to room temperature, the precipitate was removed by filtration and was washed with Et_2O and dried to furnish potassium 1-cyano-3-ethoxy-3-oxoprop-1-en-2-olate **S23** (7.62 g, 42.5 mmol, 85%) as a light yellow solid.

^{1}H-NMR (400 MHz, DMSO-d_6): δ (ppm) = 4.08 (s, 1 H, 1-H), 4.01 (q, J = 7.0 Hz, 2 H, OEt), 1.18 (t, J = 7.0 Hz, 3 H, OEt); **^{13}C-NMR** (100 MHz, DMSO-d_6): δ (ppm) = 170.2 (C-2), 166.9 (CO), 125.0 (CN), 69.4 (C-1), 59.6 (OEt), 14.1 (OEt).

A suspension of enolate **S23** (2.93 g, 16.3 mmol, 1.0 eq.) in H_2O (8 mL) and EtOH (41 mL) was treated with conc. HCl (1.2 mL) at room temperature and stirred for 15 min. Then, methoxycarbonylhydrazine (1.54 g, 17.1 mmol, 1.05 eq.) were added and the suspension was stirred for 23.5 h. The mixture was concentrated under reduced pressureand taken up with a small amount of water followed by a saturated bicarbonate solution. After extraction with EtOAc (5 × 30 mL) the combined organic phases were dried $MgSO_4$), filtered and the solvent was removed under reduced pressure. The residue was purified by column chromatography (silica, PE/EtOAc = 3.5:1 → 2:1) to yield **S24a,b** (2.45 g, 11.5 mmol, 71%) as a light yellow solid which was directly employed in the next reaction. A solution of these pyrazoles (4.40 g, 20.6 mmol, 1.0 eq.) in THF (100 mL) was treated with LiOH · H_2O (8.66 g, 206 mmol, 1.0 eq.) in H_2O (33 mL) and stirred for 3 h at 50 °C. After cooling to room temperature the reaction mixture was neutralised by addition of 2 M HCl and the solvent was removed under reduced pressure. The residue was taken up in a small amount of water and filtered. The solid was recrystalised in semi concentrated HCl to yield **32** (1.82 g, 11.2 mmol, 54%) as a brownish solid.

^{1}H-NMR (200 MHz, DMSO-d_6): δ (ppm) = 6.65 (s, 1 H, 4-H); **^{13}C-NMR** (100 MHz, DMSO-d_6): δ (ppm)= 159.7 (CO), 141.7 (C-5), 136.4 (C-3), 101.3 (C-4); **$C_4H_5N_3O_2$ + H^+ (ESI)** HRMS: calc.: 128.0460; found: 128.0459; **mp**: >350 °C.

5-Aminoisothiazole-3-carboxylic acid (33)



Copper powder (11.8 g, 186 mmol, 2.0 eq.) and conc. HCl (0.2 mL) were added to a solution of sodium nitrite (19.2 g, 279 mmol, 3.0 eq.) in H_2O (140 mL) and the resulting mixture was treated dropwise with a solution of 5-amino-3-methylisothiazole hydrochloride (**S25**) (14.0 g, 93.0 mmol, 1.0 eq.) in an aqueous solution of 4 M HCl (40 mL). The reaction mixture was stirred at rt for 22 h. EtOAc (100 mL) was added and the mixture was passed through a pad silica, which was washed with EtOAc (2x 100 mL). The phases were separated and the aqueous phase was washed with EtOAc (2x 70 mL). The combined organic extracts were dried ($MgSO_4$), filtered and the solvent was removed under reduced pressure. Chromatographic purification on silica gel (PE/EtOAc = 50:1) yielded **S26** (5.70 g, 39.3 mmol, 42%) as a yellow oil.

^{1}H-NMR (400 MHz, $CDCl_3$): δ (ppm) = 7.64 (s, 1 H), 2.55 (s, 3 H); **^{13}C-NMR** (100 MHz, $CDCl_3$): δ (ppm) = 172.5 (C-5), 162.7 (C-3), 122.4 (C-4), 20.0 (Me).

3-Methyl-5-nitroisothiazol (**S26**) (297 mg, 2.06 mmol, 1.0 eq.) and chromium(VI) oxide (618 mg, 6.18 mmol, 3.0 eq.) in conc. H_2SO_4 (2 mL) was stirred for 3 d at room temperature. H_2O (10 mL) and EtOAc (15 mL) were added, the organic phase was separated and the aqueous phase was extracted with EtOAc (2 × 15 mL). The combined organic extracts were dried ($MgSO_4$), filtered and the solvent was removed under reduced pressure. Then, the residue was treated with an aqueous, saturated bicarbonate solution (10 mL) and EtOAc (10 mL). The organic phase was separated and the aqueous phase was acidified with conc. HCl to pH= 2. The aqueous phase was extracted with EtOAc (3 × 15 mL) and the combined organic phases were dried $MgSO_4$), filtered and the solvent was removed under reduced pressure to yield **S27** (174 mg, 1.00 mmol, 49%) as a light redish solid.

^{1}H-NMR (400 MHz, MeOH-d_4): δ (ppm) = 8.40 (s, 1 H, 4-H); **^{13}C-NMR** (100 MHz, MeOH-d_4): δ (ppm)= 174.9 (C-5), 162.2 (CO), 161.9 (C-3), 124.8 (C-4); **$C_4HN_2O_4S$ (ESI)** HRMS: calc.: 172.9657, found: 172.9657; **mp**: 169–171 °C.

A solution of carboxylic acid **S27** (1.56 g, 8.99 mmol, 1.0 eq.) and sulfuric acid in MeOH (50 mL) was heated under refluxing conditions for 5 h. After cooling to room temperature the mixture was

concentrated under reduced pressure. An aqueous, saturated bicarbonate solution was added until the evolution of gas ceased. EtOAc (30 mL) was added, the organic phase was separated and the aqueous phase was extracted with EtOAc (2 × 30 mL). The combined organic phases were dried ($MgSO_4$), filtered and the solvent was removed under reduced pressure to furnish **S28** (1.64 g, 8.72 mmol, 97%) as an orange-coloured solid.[S4]

^{1}H-NMR (400 MHz, $CDCl_3$): δ (ppm) = 8.43 (s, 1 H, 4-H), 4.02 (s, 3 H, Me); **^{13}C-NMR** (100 MHz, $CDCl_3$): δ (ppm) = 173.6 (C-5), 159.7 (CO), 158.9 (C-3), 123.8 (C-4), 53.5 (Me).

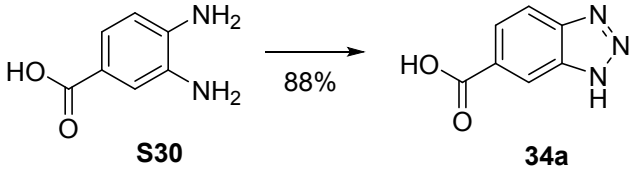
A solution of isothiazole **S28** (0.75 g, 3.99 mmol, 1.0 eq.) in acetic acid (15 mL) was treated with iron powder (2.23 g, 39.9 mmol, 10.0 eq.) and the mixture was stirred for 8 min at 75 °C. After cooling to room temperature, methanol was added and the mixture was passed through a pad of silica and the filtrate was concentrated under reduced mixture. Then, EtOAc (40 mL) and an aqueous saturated bicarbonate was added until the evolution of gas had ceased. The organic phase was separated and the aqueous phase was extracted with EtOAc (3 × 40 mL). The combined organic phases were dried ($MgSO_4$), filtered and the filtrate was concentrated under reduced pressure. The resulting residue was purified by column chromatography (silica; CH_2Cl_2/EtOAc= 4:1) to yield **S29** (0.33 g, 2.07 mmol, 52%) as a yellow solid.[S4]

^{1}H-NMR (400 MHz, $CDCl_3$): δ (ppm) = 6.88 (s, 1 H, 4-H), 4.61 (s_{br}, 2 H, NH_2), 3.91 (s, 3 H, Me); **^{13}C-NMR** (100 MHz, $CDCl_3$): δ (ppm) = 173.6 (C-5), 161.6 (CO), 158.3 (C-3), 107.2 (C-4), 52.7 (Me).

A solution of ester **S29** (0.27 g, 1.71 mmol, 1.0 eq.) in THF (1.3 mL) was treated with an aqueous solution NaOH (0.34 g, 8.55 mmol, 5.00 eq. in 4 mL H_2O) and stirred for 2 h at 50 °C. After cooling to room temperature the solution was concentrated under reduced pressure and the residue was taken up with a small amount of water. The aqueous phase was exracted with CH_2Cl_2 (2 × 10 mL) and the organic phase was separated and the aqueous phase was acidified with 1 M HCl (ca. pH= 4) and extracted with EtOAc (8 × 15 mL). The combined organic extracts were dried ($MgSO_4$), filtered and the solution was concentrated under reduced pressre to yield the title compound **33** (0.23 g, 1.62 mmol, 95%) as a brownish solid.

^{1}H-NMR (400 MHz, MeOH-d_4): δ (ppm)= 6.73 (s, 1 H, 4-H); **^{13}C-NMR** (100 MHz, MeOH-d_4): δ (ppm)= 177.5 (C-5), 164.1 (CO), 160.2 (C-3), 105.4 (C-4); **$C_4H_4N_2O_2S + H^+$ (ESI)** HRMS: calc.: 145.0072, found: 145.0072; **mp**: 180 °C (decomposition).

1*H*-Benzo[*d*][1,2,3]triazole-6-carboxylic acid (34a)

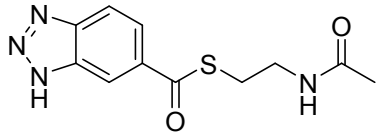


S30 **34a**

3,4-Diaminobenzoic acid **S30** (1.00 g, 65.7 mmol, 1.0 eq.) was dissolved in concentrated HCl (500 mL), cooled to 0 °C, followed by the dropwise addition of $NaNO_2$ (6.80 g dissolved in 100 mL H_2O, 98.6 mmol, 1.5 eq.). The mixture was stirred for 4 h at 0 °C and then NaN_3 (6.4 g dissolved in 100 mL H_2O, 98.6 mmol, 1.5 eq.) was added dropwise. After 3 h the mixture was warmed up to room temperature and stirred for 12 h. After extraction with ethyl acetate the combined, organic layers were dried over $MgSO_4$ and concentrated *in vacuo*. Purification by flash column chromatography (petroleum ether/ethyl acetate= 2:1) afforded benzotriazole **34a** (9.42 g, 57.7 mmol, 88 %) as a crystalline, yellow solid.

^{1}H NMR (400 MHz, MeOD-d_4, D_2HCOD = 3.31 ppm) δ 8.62 (s, 1H, H-Ar), 8.16 (d, 1H, *J* = 8.7 Hz, H-Ar), 7.91 (d, 1H, *J* = 8.7 Hz, H-Ar) ppm; **^{13}C NMR** (100 MHz, MeOD-d_4, MeOD-d_4= 49.0 ppm): δ 169.0 (s, COOH), 141.5 (s, C-Ar), 140.5 (s, C-Ar), 129.6 (s, C-Ar), 128.4 (d, C-Ar), 119.7 (d, C-Ar), 114.5 (d, C-Ar) ppm; **HRMS** (ESI) *m/z* for $C_7H_6N_3O_2$ $[M+H]^+$: cacl.: 164.0460, found: 164.0456; m.p. > 250 °C (decomposition).

S-(2-acetamidoethyl)-1*H*-benzo[d][1,2,3]triazole-6-carbothioate (34b)



Benzotriazole **34a** (0.20 g, 1.23 mmol, 1.0 eq.) was suspended under argon atmospere in DMF (5 mL) followed by addition of EDC*HCl (0.28 g, 1.47 mmol, 1.2 eq.), *N*-(2-mercaptoethyl)acetamide (0.16 mL, 1.47 mmol, 1.2 eq.) and DMAP (0.18 g, 1.47 mmol, 1.2 eq.). The reaction mixture was stirred at room temperature for 24 h, diluted with ethyl acetate and hydrolyzed by addition of water. The phases were separated, the aqueous phase was extracted with ethyl acetate and the combined, organic extracts were dried over magnesium sulfate and concentrated under reduced pressure. The crude product was purified by flash column chromatography (ethyl acetate/methanol= 4:1), followed by preparative HPLC (C18-P) (H_2O:MeOH= 95:5 {10 min}, gradient H_2O:MeOH = 95:5 → 60:40 {50 min}, H_2O:MeOH = 60:40 → 30:70 {30 min}, H_2O:MeOH = 30:70 → 0:100 {10 min}, 15 mL/min) (t_R = 73.1 min) afforded SNAC-ester **34b** (0.05 g, 0.19 mmol, 15 %).

^{1}H NMR (400 MHz, MeOD-d_4, D_2HCOD = 3.31 ppm) δ 8.61 (s, 1H, H-Ar), 8.10 (d, 1H, *J* = 8.7 Hz, H-Ar), 7.95 (d, 1H, *J* = 8.7 Hz, H-Ar), 3.55-3.45 (m, 2H, C*H*$_2$S), 3.29-3.25 (m, 2H, C*H*$_2$NH), 1.97 (s, 3H, $COCH_3$) ppm; **^{13}C NMR** (100 MHz, MeOD-d_4, MeOD-d_4 = 49.0 ppm): δ 192.1 (s, COSR), 173.6 (s, *C*OCH_3), 135.5 (s, C-Ar), 125.8 (s, C-Ar), 117.5 (d, C-Ar), 115.0 (d, C-Ar), 40.1 (t, *C*H_2S), 29.7 (t, *C*H_2NH), 22.5 (q, CO*C*H_3) ppm; **HRMS** (ESI) *m/z* for $C_{11}H_{13}N_4O_2S$ $[M+H]^+$: calc.: 265.0759, found: 265.0759.

3. Feeding experiments

3.1 General parameters: *Streptomyces hygroscopicus* mutant K390-61-1 was stored as stock cultures at -80°C as a cryoculture in the refrigerator. 100 µL of the cryoculture of K390-61-1 mutant was used to inoculate the GYP precultures which were incubated at 28°C for 2 days. Precultures were prepared in GYP-medium (40 mL/flask, 2.5 g/L yeast extract, 10 g/L peptone, 10 g/L glucose, 3 g/L xanthane gum, distilled water). Main cultures were prepared in GP-medium (40 g/L glucose, 2.5 g/L peptone, 2.5 g/L tryptone, 5 g/L oatmeal, 2.5 g/L yeast extract, 3 g/L xanthane gum, distilled water). Liquid culture fermentations were incubated at 28 °C with vigorous shaking (200 rpm) in 500 mL Erlenmeyer flasks equipped with a baffle. Main cultures were inoculated with 1 mL preculture per 30 mL culture broth. The production cultures were harvested after 5 d of fermentation and extracted twice with EtOAc. The EtOAc extracts were concentrated, and the residue was dissolved in MeOH (1 ml) and used directly for ESI-MS analysis.

3.2 Feeding experiments for small scale transformations

Benzoic acid derivatives and hetarenes (37.5 µmol, in 2 mL sterile solution in DMSO /H_2O= 1:1) were added in three 670 µL portions at 48, 72 and 96 h after inoculation to 30 mL of the main culture of *S. hygroscopicus* mutant K390-61-1. After having fed precursors according to the fermentation conditions described above, UPLC-ESI-HRMS analysis was employed for detecting new products as $[M+H]^+$ or [M+Na] peaks.

3.3 Feeding of benzoic acid 15 and formation of reblastatin derivatives 35 and 36: For scale-up fermentation 3-amino-5-cyclopropylbenzoic acid **(15)** (287.0 mg, 1.50 mmol, 20 mL sterile solution in DMSO / H_2O = 1:1) was fed in three equal portions at 48, 72 and 96 h after inoculation to sixteen 75 mL main cultures of *S. hygroscopicus* mutant K390-61-1. Extraction with EtOAc afforded 17-demethoxy-18-cyclopropyl-reblastatin (**35**) (1.3 mg, 2.3 µmol) and 17-demethyl-18-cyclopropyl-reblastatin (**36**) (0.3 mg, 0.5 µmol) after silica gel filtration and three HPLC purification steps.

HPLC conditions employed for the first purification step were a) column: Reprosil-Pur 120 C18 AQ, 250 x 25 mm, 5 µm, endc.; b) guard: Reprosil-Pur 120 C18 AQ, 30x20 mm, 10 µm, endc.; c) solvents: methanol / H_2O and d) program: H_2O:MeOH = 60:40 {5 min}, 60:40 → 50:50 {15 min}, H_2O:MeOH = 50:50 → 30:70 {65 min}, H_2O:MeOH = 30:70 → 0:100 {15 min}, H_2O:MeOH = 0:100 {10 min}, 15.0 ml/min) (t_R = 65.0 min). For the second purification step the conditions were a) column: Reprosil-Pur 120 C18-ISIS AQ, 250x8.0 mm, 5 µm, endc.; b) guard: Reprosil-Pur 120 C18-ISIS AQ, 40x8 mm, 5 µm, endc.; c) solvents: MeOH / H_2O and d) program: H_2O:MeOH = 80:20 → 65:35 {5 min}, H_2O:MeOH = 65:35 → 40:60 {65 min}, H_2O:MeOH = 40:60 → 0:100 {10 min}, H_2O:MeOH = 0:100 {10 min},

2.5 mL/min) (t_R = 63.0 min). For the third purification step the conditions were a) column: Reprosil-Pur 120 C18-ISIS AQ, 250x8.0 mm, 5 µm, endc.; b) guard: Reprosil-Pur 120 C18-ISIS AQ, 40x8 mm, 5 µm, endc.; c) solvents: methanol / H_2O and d) program: H_2O:MeOH = 80:20 → 65:35 {5 min}, H_2O:MeOH = 65:35 → 40:60 {80 min}, H_2O:MeOH = 40:60 → 0:100 {5 min}, 3.0 ml/min) (t_R = 64.4 min).

17-Demethoxy-18-cyclopropyl-reblastatin (35)

^{1}H NMR (500 MHz, T = 323 K, THF-d_8, THF-d_7 = 1.73 ppm): δ 8.43 (s, 1H, NH), 7.02 (s, 1H, Ar-H), 6.61 (s, 1H, Ar-H), 6.58 (s, 1H, Ar-H), 5.88-5.80 (m, 1H, 3-H), 5.69 (br. s, 2H, $CONH_2$), 5.30 (d, *J* = 9.9 Hz, 1H, 9-H), 5.06 (d, *J* = 5.8 Hz, 1H, 7-H), 4.54 (br. s., 1H, 11-OH), 3.54-3.48 (m, 1H, 11-H), 3.35 (s, 3H, 6-OMe), 3.31 (s, 3H, 12-OMe), 3.30-3.24 (m, 1H, 6-H), 3.15-3.08 (m, 1H, 12-H), 2.65 (dd, *J* = 13.2, 5.0 Hz, 1H, 15-Ha), 2.47-2.40 (m, 1H, 10-H), 2.37-2.25 (m, 1H, 15-Hb), 2.37-2.25 (m, 1H, 4-Ha), 2.17-2.05 (m, 1H, 4-Hb), 1.91-1.82 (m, 1H, 14-H), 1.91-1.82 (m, 1H, CH-cyclopropyl) 1.79 (s, 3H, 2-Me), 1.60-1.52 (m, 1H, 13-Ha), 1.48 (s, 3H, 8-Me), 1.40-1.32 (m, 2H, 5-H), 1.27-1.20 (m, 1H, 13-Hb), 1.00 (d, *J* = 6.6 Hz, 3H, 10-Me), 0.90-0.86 (m, 2H, CH_2-cyclopropyl), 0.82 (d, *J* = 6.8 Hz, 3H, 14-Me), 0.66-0.61 (m, 2H, CH_2-cyclopropyl) ppm; ^{13}C NMR (125 MHz, T= 323 K, THF-d_8, THF-d_8 = 25.5 ppm): δ 171.2 (s, C-1), 157.3 (s, *C*ONH_2), 145.4 (s, C-Ar), 141.8 (s, C-Ar), 141.3 (s, C-Ar), 134.7 (d, C-3), 133.0 (s, C-2), 133.0 (s, C-9), 131.7 (d, C-8), 124.3 (d, C-Ar), 123.5 (d, C-Ar), 116.3 (d, C-Ar), 82.7 (d, C-12), 81.6 (d, C-6), 81.3 (d, C-7), 75.1 (d, C-11), 58.9 (q, 6-OMe), 57.0 (q, 12-OMe), 43.9 (t, C-15), 35.5 (d, C-10), 34.6 (t, C-13), 32.8 (d, C-14), 31.0 (t, C-5), 25.5 (t, C-4), 19.8 (q, 14-Me), 17.3 (d, CH-cyclopropyl), 16.2 (q, 10-Me), 13.6 (q, 2-Me), 13.2 (q, 8-Me), 9.7 (t, CH_2-cyclopropyl), 9.6 (t, CH_2-cyclopropyl) ppm; HRMS (ESI) *m/z* for $C_{31}H_{46}N_2O_6Na$ $[M+Na]^+$: calculated: 565.3254 found: 565.3254.

17-Demethyl-18-cyclopropyl-reblastatin (36)

HPLC conditions employed for the first purification step were a) column: Reprosil-Pur 120 C18 AQ, 250 x 25 mm, 5 µm, endc.; b) guard: Reprosil-Pur 120 C18 AQ, 30x20 mm, 10 µm, endc.; c) solvents: methanol / H_2O and d) program: H_2O:MeOH = 60:40 {5 min}, 60:40 → 50:50 {15 min}, H_2O:MeOH = 50:50 → 30:70 {65 min}, H_2O:MeOH = 30:70 → 0:100 {15 min}, H_2O:MeOH = 0:100 {10 min}, 15.0 ml/min) (t_R = 65.0 min). For the second purification step the conditons were a) column: Reprosil-Pur 120 C18-ISIS AQ, 250x8.0 mm, 5 µm, endc.; b) guard: Reprosil-Pur 120 C18-ISIS AQ, 40x8 mm, 5 µm, endc.; c) solvents: MeOH / H_2O and d) program: H_2O:MeOH = 80:20 → 65:35 {5 min}, H_2O:MeOH = 65:35 → 40:60 {65 min}, H_2O:MeOH = 40:60 → 0:100 {10 min}, H_2O:MeOH = 0:100 {10 min}, 2.5 mL/min) (t_R = 61.0 min). For the third purification step the conditions were a) column: Reprosil-Pur 120 C18-ISIS AQ, 250x8.0 mm, 5 µm, endc.; b) guard: Reprosil-Pur 120 C18-ISIS AQ, 40x8 mm, 5 µm, endc.; c) solvents: MeOH / H_2O and d) program: H_2O:MeOH = 80:20 → 70:30 {5 min}, H_2O:MeOH = 70:30 → 40:60 {80 min}, H_2O:MeOH = 40:60 → 0:100 {5 min}, 3.0 ml/min) (t_R = 60.2 min).

3.4 Feeding of benzoic acid 22 and formation of reblastatin derivatives 37: For scale-up fermentation 2-amino-4-carboxythiophene (**22**) (215 mg, 1.50 mmol, in 20 mL sterile solution in DMSO / H_2O = 1:1) was fed in three equal portions at 48, 72 and 96 h after inoculation to sixteen 75 mL main cultures of *S. hygroscopicus* mutant K390-61-1. Extraction with EtOAc afforded thiageldanamycin-derivateve (**37**) (8.3 mg, 16.3 µmol) after silica gel filtration and three HPLC purification steps. HPLC conditions employed for the first purification step were a) column: Reprosil-Pur 120 C18 AQ, 250 x 25 mm, 5 µm, endc.; b) guard: Reprosil-Pur 120 C18 AQ, 30x20 mm, 10 µm, endc.; c) solvents: methanol / H_2O and d) program: H_2O:MeOH = 60:40 {5 min}, 60:40 → 50:50 {15 min}, H_2O:MeOH = 50:50 → 30:70 {65 min}, H_2O:MeOH = 30:70 → 0:100 {15 min}, H_2O:MeOH = 0:100 {10 min}, 15.0 ml/min) (t_R = 55.0 min). For the second purification step the conditions were a) column: Reprosil-Pur 120 C18-ISIS AQ, 250x8.0 mm, 5 µm, endc.; b) guard: Reprosil-Pur 120 C18-ISIS AQ, 40x8 mm, 5 µm, endc.; c) solvents: MeOH / H_2O and d) program: H_2O:MeOH = 80:20 → 65:35 {5 min}, H_2O:MeOH = 65:35 → 40:60 {65 min}, H_2O:MeOH = 40:60 → 0:100 {10 min}, H_2O:MeOH = 0:100 {10 min}, 2.5 mL/min) (t_R = 47.0 min). For the third purification step the conditions were a) column: Reprosil-Pur 120 CN AQ, 250x8.0 mm, 5 µm, endc.; b) guard: Reprosil-Pur 120 CN AQ, 40x8 mm, 5 µm, endc.; c) solvents: MeCN / H_2O and d) program: H_2O:MeCN = 90:10 → 85:15 {20 min}, H_2O:MeCN = 85:15 {40 min}, H_2O:MeCN = 85:15 → 75:25 {20 min}, H_2O:MeCN = 75:25 → 55:45 {20 min}, H_2O:MeCN = 55:45 → 0:100 {15 min}, 4.0 ml/min) (t_R = 40.0 min).

Thiophene-reblastatin (37)

^{1}H-NMR (500 MHz, T= 323 K, THF-d8, THF-d7 = 1.73 ppm): δ 8.61 (s, 1H, NH), 6.57 (s, 1H, Ar), 6.38 (s, 1H, Ar), 5.72 (br. s., 1H, 3-H), 5.57 (br. s, 2H, $CONH_2$), 5.22 (d, *J* = 10.0 Hz, 1H, 9-H), 4.99 (d, *J* = 7.0 Hz, 1H, 7-H), 3.56-3.51 (m, 1H, 11-H), 3.37 (s, 3H, 6-OMe), 3.31 (s, 3H, 12-OMe), 3.28-3.19 (m, 1H, 6-H), 3.11-3.05 (m, 1H, 12-H), 2.65 (dd, *J* = 13.6, 5.4 Hz, 1H, 15-Ha), 2.45-2.35 (m, 1H, 10-H), 2.45-2.35 (m, 1H, 15-Hb), 2.22-2.05 (m, 2H, 4-H), 2.05-1.91 (m, 1H, 14-H), 1.75 (s, 3H, 2-Me), 1.66-1.58 (m, 1H, 13-Ha), 1.55 (s, 3H, 8-Me), 1.24-1.14 (m, 2H, 5-H), 1.20-1.11 (m, 1H, 13-Hb), 1.00 (d, *J* = 6.6 Hz, 3H, 10-Me), 0.75 (d, *J* = 6.8 Hz, 3H, 14-Me) ppm; ^{13}C-NMR (125 MHz, T= 323 K, THF-d8, THF-d8 = 25.5 ppm): δ 172.3 (s, C-1), 156.9 (s, $CONH_2$), 143.2 (s, C-Ar), 139.6 (s, C-Ar), 135.3 (d, C-3), 134.0 (d, C-9), 131.8 (s, C-2), 131.7 (s, C-8), 124.4 (d, C-Ar), 117.3 (d, C-Ar), 82.1 (d, C-12), 81.8 (d, C-7), 81.4 (d, C-6), 74.2 (d, C-11), 59.1 (q, 6-OMe), 57.0 (q, 12-OMe), 39.0 (t, C-15), 35.7 (d, C-10), 33.3 (t, C-13), 31.4 (t, C-5), 30.5 (d, C-14), 24.5 (t, C-4), 18.7 (q, 14-Me), 17.9 (q, 10-Me), 14.1 (q, 2-Me), 12.7 (q, 8-Me) ppm; HRMS (ESI) *m/z* für $C_{26}H_{41}N_2O_6S$ $[M+H]^+$: calculated: 509.2685 found: 509.2666.

3.5 Feeding of benzoic acid 34 and formation of seco acid 38. For scale-up fermentation benzotriazole **34** (245 mg, 1.50 mmol), in 20 mL sterile solution in DMSO / H_2O = 1:1) was fed in three equal portions at 48, 72 and 96 h after inoculation to sixteen 75 mL main cultures of *S. hygroscopicus* mutant K390-61-1.

Extraction with EtOAc afforded *seco*-progeldanamycin acid derivative **38** (0.5 mg, 0.9 µmol) after silica gel filtration and three HPLC purification steps. HPLC conditions employed for the first purification step were a) column: Reprosil-Pur 120 C18-ISIS AQ, 250 x 25 mm, 5 µm, endc.; b) guard: Reprosil-Pur 120 C18-ISIS AQ, 30x20 mm, 10 µm, endc.; c) solvents: MeOH / H_2O and d) program: H_2O:MeOH = 60:40 {5 min}, 60:40 → 50:50 {15 min}, H_2O:MeOH = 50:50 → 30:70 {65 min}, H_2O:MeOH = 30:70 → 0:100 {15 min}, H_2O:MeOH = 0:100 {10 min}, 15.0 ml/min) (t_R = 55.0 min). For the second purification step the conditions were a) column: Reprosil-Pur 120 C18-ISIS AQ, 250x8.0 mm, 5 µm, endc.; b) guard: Reprosil-Pur 120 C18-ISIS AQ, 40x8 mm, 5 µm, endc.; c) solvents: methanol / H_2O and d) program: H_2O:MeOH = 80:20 → 65:35 {5 min}, H_2O:MeOH = 65:35 → 40:60 {65 min}, H_2O:MeOH = 40:60 → 0:100 {10 min}, H_2O:MeOH = 0:100 {10 min}, 2.5 mL/min) (t_R = 52.0 min). For the third purification step the conditions were a) column: Reprosil-Pur 120 CN AQ, 250x8.0 mm, 5 µm, endc.; b) guard: Reprosil-Pur 120 CN AQ, 40x8 mm, 5 µm, endc.; c) solvents: MeCN / H_2O and d) program: H_2O:MeCN = 90:10 → 85:15 {20 min}, H_2O:MeCN = 85:15 {40 min}, H_2O:MeCN = 85:15 → 75:25 {20 min}, H_2O:MeCN = 75:25 → 55:45 {20 min}, H_2O:MeCN = 55:45 → 0:100 {15 min}, 4.0 ml/min) (t_R = 53.5 min).

^{1}H-NMR (500 MHz, T = 323 K, THF-d8, THF-d7 = 1.73 ppm): δ 7.72 (d, *J* = 7.0 Hz, 1H, Ph), 7.56 (s, 1H, Ph), 7.20 (d, *J* = 8.0 Hz, 1H, Ph), 6.77-6.69 (m, 1H, 3-H), 5.53 (br. s, 2H, $CONH_2$), 5.28 (d, *J* = 9.4 Hz, 1H, 9-H), 4.90 (dd, *J* = 8.6, 2.9 Hz, 1H, 7-H), 3.86 (d, *J* = 6.3 Hz, 1H, 11-H), 3.42 (s, 3H, 6-OMe), 3.30 (s, 3H, 12-OMe), 3.35-3.25 (m, 1H, 6-H), 3.25-3.12 (m, 1H, 12-H), 2.84 (dd, *J* = 13.5, 5.7 Hz, 1H, 15-Ha), 2.79-2.69 (m, 1H, 10-H), 2.61-2.50 (m, 1H, 15-Hb), 2.28-2.17 (m, 2H, 4-H), 2.14-1.98 (m, 1H, 14-H), 1.80 (s, 3H, 2-Me), 1.75-1.02 (m, 1H, 5-Ha), 1.70-1.62 (m, 1H, 13-Ha), 1.60 (s, 3H, 8-Me), 1.50-1.36 (m, 1H, 13-Hb), 1.17-1.07 (m, 1H, 5-Hb), 0.97 (d, *J* = 6.6 Hz, 3H, 10-Me), 0.83 (d, *J* = 6.6 Hz, 3H, 14-Me) ppm; HRMS (ESI) *m/z* für $C_{28}H_{43}N_4O_7$ $[M+H]^+$: calculated: 547.3132 found: 547.3137.

5. Cell proliferation assay

Cell lines were obtained from DMSZ (KB-3-1 ACC 158; U-937 ACC 5; A-431 ACC 91) or ATCC (SK-OV-3 HTB-77; PC-3 CRL-1435). Growth inhibition was measured in microtiter plates. Sixty µL of serial dilutions of the test compounds were added to 120 µL aliquots of a cell suspension (50.000/mL) in 96-well plates and incubated at 37 °C under 10% CO_2 for 5 days. MTT [3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide] was used to measure growth and viability of cells which are capable of reducing it to a violet formazan product. Twenty µL MTT in phosphate buffered saline (PBS) were added to a final concentration of 0.5 mg/mL. After 2 h the precipitate of formazan crystals was centrifuged, and the supernatant discarded. The precipitate was washed with 100 µL PBS and dissolved in 100 µL isopropanol containing 0.4% hydrochloric acid. The microplates were measured at 595 nm using an

ELISA plate reader. All experiments were carried out in two parallel experiments, the percentage of viable cells was calculated as the mean with respect to the controls set to 100%. In the case of U-937 the WST-1 assay from Roche was employed.

6. Purification of heat shock proteins

Recombinant human Hsp90 α, Hsp90β, XcHtpG and HpHtpG were produced as previously reported.[20a-d] The protein expression was performed with E. coli BL21(DE3) to host the plasmid pET15.1 to host the human Hsp90 genes and pETSUMO containing the bacterial HtpG genes.[20b,20d] All protein purification steps were carried out at 4 °C and 0.005% (w/v) protease inhibitor (P-8465 from Sigma-Aldrich) were freshly added to lysis buffers (20mM Tris-HCl, pH 8.0, 500 mM KCl, 2 mM ß-mercaptoethanol, 2 mM imidazole, 10% (v/v) glycerol). Additionally, all buffers were supplemented with 2 mM ß-mercaptoethanol. Cell pellets were lysed by two French Press cycles at 12,000 p.s.i. and adjusted to 100 ml lysis buffer. Soluble proteins were separated from cellular debris by centrifugation at 25,000 g for 45 min at 4 °C. The recombinant protein was purified from the supernatant by metal chelating resin Talon from Jenabioscience after incubation for 2 h on ice. The N-terminal tag of the recombinant protein was removed by TEV protease or SUMO protease from Promega. The dialysis was performed against a 500-fold volume of buffer containing 20 mM Tris pH 8.0, 20 mM KCl, 2 mM ß-mercaptoethanol and 10% (v/v) glycerol at 4 °C for 24 h. The protein was concentrated with an YM30 centrifugal filter device, Amicon (EMD Millipore Corporation) and purified by SEC16/60 chromatography in 20 mM Tris-HCl pH 8.0, 500 mM KCl and 2 mM ß-mercaptoethanol. Finally, the protein was concentrated with a YM30 centrifugal filter device, Amicon (EMD Millipore Corporation) to 1 ml and diluted 2 times to 10 ml of storage buffer 20 mM Tris pH 7.5, 50 mM KCl, 2 mM ß-mercaptoethanol, 10% (v/v) glycerol, and samples were frozen at a concentration of 3 mg/ml in liquid nitrogen and stored at –80 °C.

7. Competitive microarray assay

Heat shock proteins (3 mg/mL each) were spotted in the storage buffer [Tris·HCl (pH 7.5, 20 mM), KCl (50 mM), ß-mercaptoethanol (2 mM), glycerol (10 %, v/v)] on the UniSart 3D nitro slide (Sartorius Stedim Biotech S.A.) with use of a contactless GeSim Nano-Plotter (GeSiM mbH, Großerkmannsdorf, Germany) with a nanotip pipette (volume of 800–1600 pL; 8 drops/dot).[20a-d] The slide was air-dried for 30 min at room temperature. Unspecific sites of printed proteins were blocked with BSA (1%, w/v) in the same buffer for 45 min and washed three times with storage buffer [Tris·HCl (pH7.5, 20 mM), KCl (50 mM), ß-mercaptoethanol (2 mM), glycerol (10 %, v/ v)] at room temperature. Before printed protein arrays were subjected to the ATP-binding and direct competitive assay, unspecific sites were blocked with 1% (w/v) BSA in the same buffer as before for 45 min at room temperature. For binding, the slides were incubated directly with 100 nM dye labelled ATP as indicated and inhibitors with concentrations as

indicated. The mix of binding buffer with 100 nM dye-labelled ATP (Cy3-ATP or Cy5-ATP, Jena Bioscience GmbH) in absence of any inhibitors served as control. In order to separate the subarrays, 16-well hybridisation chambers (Schott Nexterion, Mainz, Germany) were used. After incubation, the slides were washed three times for 5 min with binding buffer. Binding of dye-labelled ATP was determined by use of a Gene- Pix 4000B Laser Scanner (Molecular Devices, Inc.) with 532 nm excitation wavelength (for Cy3-ATP) or 635 nm excitation wavelength (for Cy5-ATP), laser power 10%, PMT gain 380 and calculated with ImaGene5 (BioDiscovery, Inc.). Evaluation of displacement was recorded in the form of the IC50 values. The dose–response curves were calculated with Origin 7G (OriginLab Corporation) and fitted with the nonlinear function logistic, A1=0, A2=1. Z´-factor calculations were performed as shown before [S5].

8. References and Notes (supporting information)

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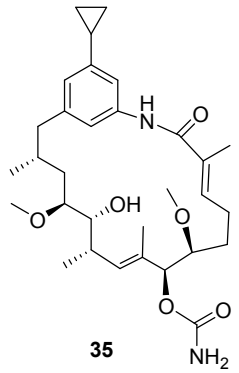
Attachment: Copies of ^{1}H- and ^{13}C-NMR spectra



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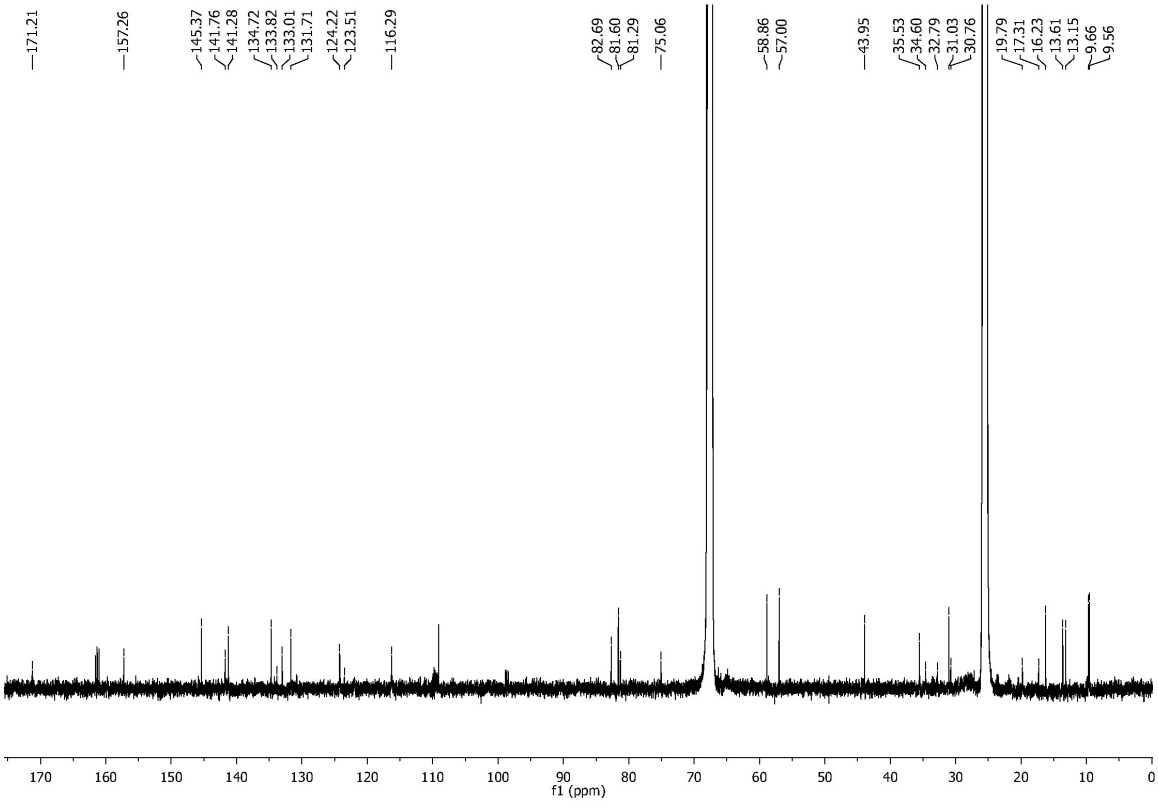

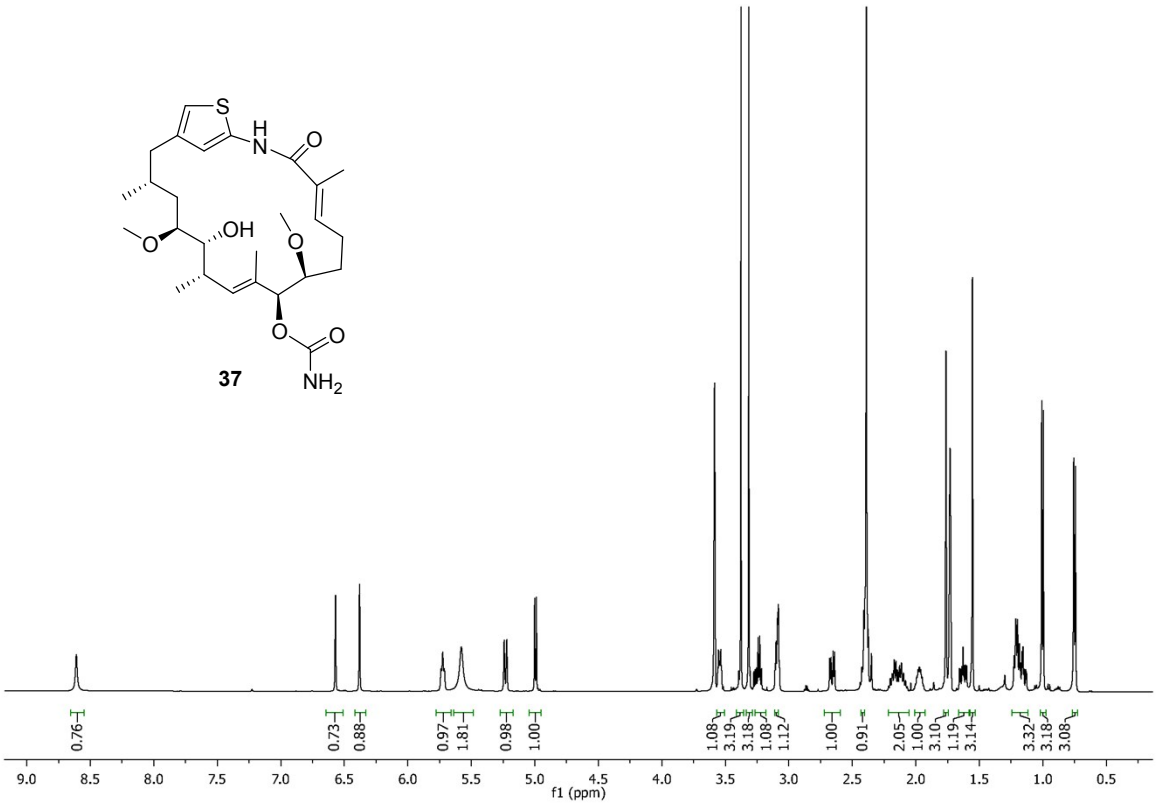
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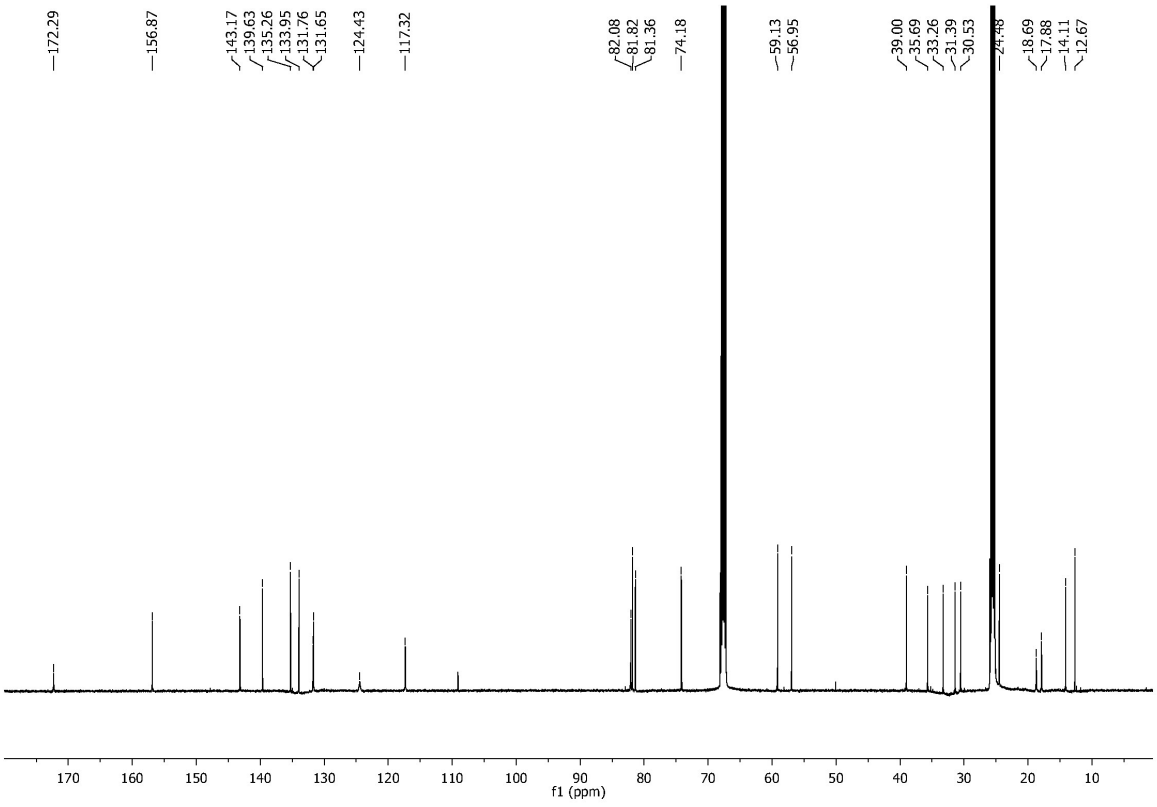
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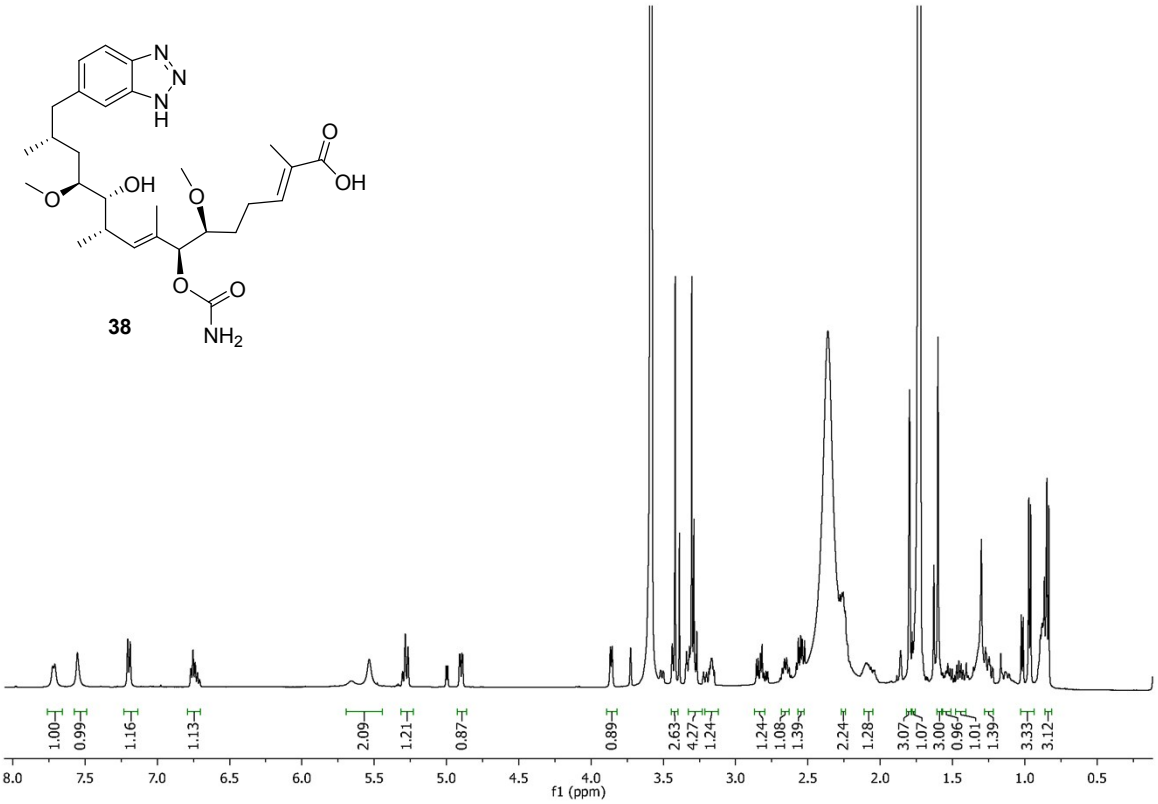
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