Electronic Supplementary Material (ESI) for ChemComm.

Electronic Supplementary Information

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Synthetic procedures, structural and spectral data

General

For full details on ligand docking and bioassay results, see supporting information. ^{1}H NMR spectra were recorded at 300 MHz (JEOL ECLIPSE+) or 400 MHz (Bruker Avance III) with $CDCl_3$ or [D_6]DMSO as solvent and tetramethylsilane as internal standard. ^{13}C NMR spectra were recorded at 75 MHz (JEOL ECLIPSE+) or 100.6 MHz (Bruker Avance III) with $CDCl_3$ or [D_6]DMSO as solvent and tetramethylsilane as internal standard. Mass spectra were obtained with a mass spectrometer Agilent 1100, 70 eV. IR spectra were measured with a Spectrum One FT-IR spectrophotometer. High resolution electron spray (ES) mass spectra were obtained with an Agilent Technologies 6210 series time-of-flight instrument. Melting points of crystalline compounds were measured with a Büchi 540 apparatus or with a Kofler Bench, type WME Heizbank of Wagner & Munz. The purity of all tested compounds was assessed by HRMS analysis and/or HPLC analysis, confirming a purity of ≥95%.

Synthesis of 6-bromobenzothiophene-3-carbaldehyde 9[17]

Benzothiophene-3-carbaldehyde (811 mg, 5 mmol, 1 equiv) **3a** was dissolved in acetonitrile (15 mL) and to this solution was slowly added bromine (1,29 mL, 25 mmol, 5 equiv). The resulting reaction mixture was stirred at room temperature for 18 hour after which it was partitioned between an aqueous sodium bicarbonate solution (50 mL) and EtOAc (50 mL). To this biphasic solution was added dropwise, under vigorous stirring, a saturated aqueous sodium thiosulfate solution until discoloration of the organic medium. The organic layer was separated, and the aqueous layer was extracted with EtOAc (25 mL). The organic fractions were combined, dried ($MgSO_4$), filtered and concentrated under vacuum. Purification through column chromatography (R_f 0.14, EtOAc/PE: 1/13) yielded 6-bromobenzothiophene-3-carbaldehyde **9** (482 mg, 2 mmol, 40%) as a white powder.

9: 6-bromobenzothiophene-3-carbaldehyde 40% as white powder; R_f 0.14 (EtOAc/PE: 1/13); m.p 111°C; **^{1}H-NMR** (400 MHz, $CDCl_3$): δ = 7.63 (dd, *J* = 8.7, 1.7 Hz, 1H); 8.04 (d, *J* = 1.7 Hz, 1H); 8.30 (s, 1H); 8.56 (d, *J* = 8.7 Hz, 1H); 10.12 (s, 1H); **^{13}C-NMR** (100.6 MHz, $CDCl_3$): δ = 120.2, 125.0, 125.9, 129.6, 134.0, 136.1, 141.8, 143.2 and 185.1; **IR** (cm^{-1}): v = 1662 (C=O); Elemental Analysis Calcd (%) for C_9H_5BrOS: C 44.84 H 2.09; Found: C 45.17 H 1.71.

Synthesis of 5- and 6-phenylbenzothiophene-3-carbaldehyde 4 and 10

The synthesis of 5-phenylbenzothiophene-3-carbaldehyde **4** will be used as an example for the synthesis of 5- and 6-phenylbenzothiophene-3-carbaldehydes **4** and **10**. 5-Bromobenzothiophene-3-carbaldehyde **3b** (482 mg, 2 mmol, 1 equiv) was dissolved in toluene (15 mL) and to this solution were added an aqueous solution of sodium carbonate (7 mL, 2M) and a solution of phenylboronic acid (488 mg, 4 mmol, 2 equiv) in ethanol (7 mL). This mixture was flushed with nitrogen for 10 minutes before tetrakis(triphenylphosphine)palladium(0) (92 mg, 0.08 mmol, 0.04 equiv) was added and the reaction mixture was heated to its boiling temperature for 8 hour. The reaction mixture was poured in to brine (20 mL) and three times extracted with EtOAc (20 mL). The combined organic fraction was thereafter three times washed with brine (15 mL), dried ($MgSO_4$), filtered and evaporated under vacuum. Purification through column chromatography (R_f 0.35, EtOAc/PE 1/5) yielded 5-phenylbenzothiophene-3-carbaldehyde **4** (343 mg, 1.44 mmol, 72%) as an orange powder.

4: 5-phenylbenzothiophene-3-carbaldehyde 72% as orange powder; R_f 0.35 (EtOAc/PE 1/5); m.p. 102°C; **^{1}H-NMR** (300 MHz, $CDCl_3$): δ = 7.36-7.41 (m, 1H); 7.46-7.51 (m, 2H); 7.69-7.72 (m, 3H); 7.94 (d, *J* = 8.3 Hz, 1H); 8.35 and 8.92 (2 × s, 2 × 1H); 10.17 (s, 1H); **^{13}C-NMR** (75 MHz, $CDCl_3$): δ = 122.7, 123.3, 125.9, 127.6, 127.7, 129.0, 135.9, 136.7, 139.5, 139.8, 140.8, 144.0 and 185.5; **IR** (cm^{-1}): v = 1671 (C=O); **MS** (70 eV): m/z (%) = 239 (35) [M^+ + H]; **HRMS** (ESI) Anal. Calcd. for $C_{15}H_{11}OS$ 239.0525 [M^+ + H] Found 239.0524.

10: 6-phenylbenzothiophene-3-carbaldehyde 96% as orange powder; R_f 0.20 (EtOAc/PE 1/13); m.p. 94°C; **^{1}H-NMR** (400 MHz, $CDCl_3$): δ = 7.37-7.41 (m, 1H); 7.46-7.50 (m, 2H); 7.65-7.68 (m, 3H); 7.76 (dd, *J* = 8.4, 1.4 Hz, 1H); 8.08 (d, *J* = 1.4 Hz, 1H); 8.33 (s, 1H); 8.72 (d, *J* = 8.4 Hz, 1H); 10.16 (s, 1H); **^{13}C-NMR** (100.6 MHz, $CDCl_3$): δ = 120.6, 125.0, 125.8, 127.4, 127.7, 129.0, 134.2, 136.4, 139.6, 140.5, 141.3, 143.2 and 185.4; **IR** (cm^{-1}): v = 1662 (C=O); **MS** (70 eV): m/z (%) = 239 (100) [M^+ + H]; **HRMS** (ESI) Anal. Calcd. for $C_{15}H_{11}OS$ 239.0525 [M^+ + H] Found 239.0524.

Synthesis of methyl 4-aminobenzoate esters 5a-d and 11a-b

The synthesis of 3-[(4-methoxycarbonylphenyl)aminomethyl]benzothiophene **5a** will be used as an example for the synthesis of secondary amines **5a-d** and **11a-b**. Benzothiophene-3-carbaldehyde **3a** (406 mg, 2.5 mmol, 1

equiv) was dissolved in ethanol (15 mL) and to this solution were added glacial acetic acid (751 mg, 12.5 mmol, 5 equiv) and methyl 4-aminobenzoate (454 mg, 3 mmol, 1.2 equiv). This reaction mixture was stirred for one hour at refluxing conditions after which it was cooled to 0°C. Sodium cyanoborohydride (471 mg, 7.5 mmol, 3 equiv) was added and the reaction mixture was allowed to warm to room temperature. After one hour the mixture was poured in to brine (15 mL) and three times extracted with EtOAc (15 mL). The combined organic fraction was thereafter three times washed with brine (15 mL), dried ($MgSO_4$), filtered and evaporated under vacuum. Purification through recrystallization from ethanol yielded 3-[(4-methoxycarbonylphenyl)aminomethyl]-benzothiophene **5a** (520 mg, 1.75 mmol, 70%) as a white powder. For secondary amine **5b** a solvent mixture of ethanol/CH_2Cl_2 (1/1) was used as solvent for the reaction.

5a: 3-[(4-methoxycarbonylphenyl)aminomethyl]benzothiophene 70% as white powder; recrystallization from EtOH; m.p. 127°C; **^{1}H-NMR** (300 MHz, $CDCl_3$): δ = 3.84 (s, 3H); 4.48 (s(broad), 1H); 4.58 (d, *J* = 5.0 Hz, 2H); 6.62 (d, *J* = 8.8 Hz, 2H); 7.32 (s, 1H); 7.35-7.43, 7.73-7.81 and 7.86-7.90 (3 × m, 2H, 1H and 3H); **^{13}C-NMR** (75 MHz, $CDCl_3$): δ = 42.2, 51.7, 111.8, 119.0, 121.7, 123.2, 124.1, 124.4, 124.8, 131.7, 132.8, 137.8, 141.0, 151.7 and 167.4; **IR** (cm^{-1}): v = 3379 (NH); 1685 (C=O); **MS** (70 eV): m/z (%) = 296 (100) [M^- - H]; **HRMS** (ESI) Anal. Calcd. for $C_{17}H_{14}NO_2S$ 296.0751 [M^- - H], Found 296.0760.

5b: 5-bromo-3-[(4-methoxycarbonylphenyl)aminomethyl]benzothiophene 70% as brown powder; recrystallization from EtOH; m.p. 143°C; **^{1}H-NMR** (300 MHz, $CDCl_3$): δ = 3.86 (s, 3H); 4.48 (s(broad), 1H); 4.56 (s, 2H); 6.64 (d, *J* = 8.5 Hz, 2H); 7.37 (s, 1H); 7.48 (d, *J* = 8.3 Hz, 1H); 7.74 (d, *J* = 8.3 Hz, 1H); 7.89 (d, *J* = 8.5 Hz, 2H); 7.92 (s, 1H); **^{13}C-NMR** (75 MHz, $CDCl_3$): δ = 42.1, 51.7, 111.9, 118.6, 119.3, 124.4, 124.5, 125.8, 127.9, 131.7, 132.3, 139.4, 139.6, 151.5 and 167.3; **IR** (cm^{-1}): v = 3376 (NH); 1683 (C=O); **MS** (70 eV): m/z (%) = 374/6 (100) [M^- - H]; **HRMS** (ESI) Anal. Calcd. for $C_{17}H_{13}BrNO_2S$ 373.9856 [M^- - H], Found 373.9869.

5c: 3-[(4-methoxycarbonylphenyl)aminomethyl]-5-phenylbenzothiophene 75% as brown powder; R_f 0.29 (EtOAc/PE 1/5); m.p. 154°C; **^{1}H-NMR** (300 MHz, $CDCl_3$): δ = 3.85 (s, 3H); 4.49 (s(broad), 1H); 4.65 (d, *J* = 4.9 Hz, 2H); 6.65 (d, *J* = 8.8 Hz, 2H); 7.33-7.48, 7.62-7.65 and 7.88-7.96 (3 × m, 4H, 3H and 4H); **^{13}C-NMR** (75 MHz, $CDCl_3$): δ = 42.3, 51.7, 111.9, 119.1, 120.0, 123.4, 124.5, 124.9, 127.5, 127.6, 129.0, 131.7, 133.0, 138.1, 138.3, 140.0, 141.2, 151.7 and 167.3; **IR** (cm^{-1}): v = 3389 (NH); 1697 (C=O); **MS** (70 eV): m/z (%) = 372 (25) [M^- - H]; **HRMS** (ESI) Anal. Calcd. for $C_{23}H_{18}NO_2S$ 372.1064 [M^- - H], Found 372.1069.

5d: 3-[(4-methoxycarbonyl-2-methylphenyl)aminomethyl]benzothiophene 50% as white powder; recrystallization from EtOH; m.p. 148°C; **^{1}H-NMR** (300 MHz, $CDCl_3$): δ = 2.15 (s, 3H); 3.85 (s, 3H); 4.29 (s(broad), 1H); 4.64 (s, 2H); 6.66 (d, *J* = 8.8 Hz, 1H); 7.33 (s, 1H); 7.37-7.43 and 7.78-7.89 (2 × m, 2H and 4H); **^{13}C-NMR** (75 MHz, $CDCl_3$): δ = 17.5, 42.4, 51.7, 108.9, 118.5, 121.2, 121.7, 123.2, 124.2, 124.5, 124.8, 129.9, 131.7, 132.8, 137.8, 141.0, 149.8 and 167.6; **IR** (cm^{-1}): v = 3442 (NH); 1702 (C=O); **MS** (70 eV): m/z (%) = 310 (100) [M^- - H]; **HRMS** (ESI) Anal. Calcd. for $C_{18}H_{16}NO_2S$ 310.0907 [M^- - H], Found 310.0917.

11a: 6-bromo-3-[(4-methoxycarbonylphenyl)aminomethyl]-benzothiophene 50% as light yellow powder; recrystallization from EtOH; m.p. 155°C; **^{1}H-NMR** (400 MHz, [D_6]DMSO): δ = 3.74 (s, 3H); 4.57 (d, *J* = 5.5 Hz, 2H); 6.70 (d, *J* = 8.8 Hz, 2H); 7.12 (t, *J* = 5.5 Hz, 1H); 7.58 (dd, *J* = 8.6, 1.8 Hz, 1H); 7.65 (s, 1H); 7.69 (d, *J* = 8.8 Hz, 2H); 7.89 (d, *J* = 8.6 Hz, 1H); 8.30 (d, *J* = 1.8 Hz, 1H); **^{13}C-NMR** (100.6 MHz, [D_6]DMSO): δ **=** 41.1, 51.7, 111.8, 116.6, 118.1, 124.2, 125.7, 125.8, 127.7, 131.4, 133.8, 137.4, 142.4, 153.0 and 166.8; **IR** (cm^{-1}): v = 3346 (NH); 1672 (C=O); **MS** (70 eV): m/z (%) = 374/6 (20) [M^- - H]; **HRMS** (ESI) Anal. Calcd. for $C_{17}H_{13}BrNO_2S$ 373.9856 [M^- - H], Found 373.9862.

11b: 3-[(4-methoxycarbonylphenyl)aminomethyl]-6-phenyl-benzothiophene 66% as white powder; recrystallization from EtOH; m.p. 169°C; **^{1}H-NMR** (400 MHz, $CDCl_3$): δ = 3.86 (s, 3H); 4.48 (t, *J* = 5.0 Hz, 1H); 4.64 (d, *J* = 5.0 Hz, 2H); 6.66 (d, *J* = 8.8 Hz, 2H); 7.36-7.40 (m, 2H); 7.48 (t, *J* = 7.6 Hz, 2H); 7.64-7.67 (m, 3H); 7.85 (d, *J* = 8.4 Hz, 1H); 7.90 (d, *J* = 8.8 Hz, 2H); 8.09 (d, *J* = 1.2 Hz, 1H); **^{13}C-NMR** (100.6 MHz, $CDCl_3$): δ = 42.2, 51.6, 111.7, 119.0, 121.4, 121.8, 124.1, 124.4, 127.4, 127.5, 128.9, 131.6, 132.5, 136.8, 138.2, 140.8, 141.6, 151.6 and 167.3; **IR** (cm^{-1}): v = 3390 (NH); 1683 (C=O); **MS** (70 eV): m/z (%) = 396 (30) [M^+ + Na].

Synthesis of tertiary amines 6a-c and 12a-b

The synthesis of 3-[*N*-benzyl-*N*-(4-methoxycarbonylphenyl)aminomethyl]-benzothiophene **6a** will be used as an example for the synthesis of tertiary amines **6a-c** and **12a-b**. 3-[(4-Methoxycarbonylphenyl)aminomethyl]-benzothiophene **5a** (297 mg, 1 mmol, 1 equiv) was dissolved in DMF (10 mL) and to this solution was sodium hydride (40 mg, 60 % dispersion in mineral oil, 1 mmol, 1 equiv) added. The reaction mixture was stirred for 30 minutes at room temperature under nitrogen atmosphere after which benzyl bromide (342 mg, 2 mmol, 2 equiv) and potassium iodide (5 mg) were added. After two hours the reaction mixture was poured in to brine (20 mL) and three times extracted with EtOAc (20 mL). The combined organic fraction was thereafter three times washed with brine (15 mL), dried ($MgSO_4$), filtered and evaporated under vacuum. Purification through column

chromatography (R_f 0.30, EtOAc/PE 1/5) yielded 3-[*N*-benzyl-*N*-(4-methoxycarbonylphenyl)-aminomethyl]benzothiophene **6a** (271 mg,0.7 mmol, 70%) as a yellow powder.

6a: 3-[*N*-benzyl-*N*-(4-methoxycarbonylphenyl)aminomethyl]-benzothiophene 70% as yellow powder; R_f 0.30 (EtOAc/PE 1/5); m.p. 126°C; **^{1}H-NMR** (300 MHz, $CDCl_3$): δ = 3.84 (s, 3H); 4.76 and 4.87 (2 × s, 2 × 2H); 6.76 (d, *J* = 8.8 Hz, 2H); 7.11 (s, 1H); 7.21-7.40, 7.65-7.68 and 7.85-7.90 (3 × m, 7H, 1H and 3H); **^{13}C-NMR** (75 MHz, $CDCl_3$): δ = 49.6, 51.7, 54.0, 111.5, 118.3, 121.3, 122.8, 123.2, 124.3, 124.8, 126.6, 127.4, 129.0, 131.4, 131.6, 137.5, 137.6, 141.3, 152.4 and 167.3; **IR** (cm^{-1}): v = 1701 (C=O); **MS** (70 eV): m/z (%) = 388 (85) [M^+ + H]; **HRMS** (ESI) Anal. Calcd. for $C_{24}H_{22}NO_2S$ 388.1366 [M^+ + H], Found 388.1374.

6b: 5-bromo-3-[*N*-benzyl-*N*-(4-methoxycarbonylphenyl)aminomethyl]-benzothiophene 79% as yellow powder; R_f 0.37 (EtOAc/PE 1/5); m.p. 130°C; **^{1}H-NMR** (300 MHz, $CDCl_3$): δ = 3.84 (s, 3H); 4.76 and 4.87 (2 × s, 2 × 2H); 6.76 (d, *J* = 8.8 Hz, 2H); 7.11 (s, 1H); 7.22-7.41, 7.65-7.68 and 7.85-7.91 (3 × m, 6H, 1H and 3H); **^{13}C-NMR** (75 MHz, $CDCl_3$): δ = 49.4, 51.7, 54.1, 111.5, 118.5, 124.2, 124.5, 126.6, 127.5, 127.8, 129.0, 130.9, 131.6, 137.3, 139.2, 139.9, 152.2 and 167.3; **IR** (cm^{-1}): v = 1702 (C=O); **MS** (70 eV): m/z (%) = 466/8 (100) [M^+ + H]; **HRMS** (ESI) Anal. Calcd. for $C_{24}H_{21}BrNO_2S$ 466.0471 [M^+ + H], Found 466.0483.

6c: 3-[*N*-benzyl-*N*-(4-methoxycarbonylphenyl)aminomethyl]-5-phenyl-benzothiophene 65% as yellow powder; R_f 0.31 (EtOAc/PE 1/5); m.p. 82°C; **^{1}H-NMR** (300 MHz, $CDCl_3$): δ = 3.83 (s, 3H); 4.76 and 4.90 (2 × s, 2 × 2H); 6.76 (d, *J* = 8.8 Hz, 2H); 7.13 (s, 1H); 7.22-7.46, 7.59-7.63 and 7.83-7.93 (3 × m, 8H, 3H and 4H); **^{13}C-NMR** (75 MHz, $CDCl_3$): δ = 49.7, 51.7, 54.1, 111.6, 118.4, 119.8, 123.4, 123.5, 124.5, 126.6, 127.4, 127.5, 129.0, 131.6, 131.7, 137.5, 138.0, 138.2, 140.4, 141.2, 152.4 and 167.3; **IR** (cm^{-1}): v = 1702 (C=O); **MS** (70 eV): m/z (%) = 464 (70) [M^+ + H]; **HRMS** (ESI) Anal. Calcd. for $C_{30}H_{26}NO_2S$ 464.1679 [M^+ + H], Found 464.1698.

12a: 6-bromo-3-[*N*-benzyl-*N*-(4-methoxycarbonylphenyl)aminomethyl]-benzothiophene 87% as white powder; R_f 0.21 (EtOAc/PE 1/10); m.p. 63°C; **^{1}H-NMR** (400 MHz, [D_6]DMSO): δ = 3.74 (s, 3H); 4.83 and 5.01 (2 × s, 2 × 2H); 6.79 (d, *J* = 9.1 Hz, 2H); 7.24-2.28 and 7.32-7.36 (2 × m, 3H and 2H); 7.38 (s, 1H); 7.58 (dd, *J* = 8.6, 1.8 Hz, 1H); 7.71 (d, *J* = 9.1 Hz, 2H); 7.80 (d, *J* = 8.6 Hz, 1H); 8.31 (d, *J* = 1.8 Hz, 1H); **^{13}C-NMR** (100.6 MHz, [D_6]DMSO): **δ =** 49.7, 51.8, 54.1, 112.1, 117.1, 118.2, 124.0, 124.6, 125.9, 127.0, 127.4, 127.7, 129.1, 131.3, 132.4, 137.0, 138.5, 142.6, 152.2 and 166.6; **IR** (cm^{-1}): v = 1702 (C=O); **MS** (70 eV): m/z (%) = 466/8 (100) [M^+ + H]; **HRMS** (ESI) Anal. Calcd. for $C_{24}H_{21}BrNO_2S$ 466.0471 [M^+ + H], Found 466.0482.

12b: 3-[*N*-benzyl-*N*-(4-methoxycarbonylphenyl)aminomethyl]-6-phenyl-benzothiophene 91% as white powder; R_f 0.13 (EtOAc/PE 1/10); m.p. 66°C; **^{1}H-NMR** (400 MHz, $CDCl_3$): δ = 3.84 (s, 3H); 4.76 and 4.88 (2 × s, 2 × 2H); 6.77 (d, *J* = 9.1 Hz, 2H); 7.11 (s, 1H); .7.23-7.39, 7.45-7.48, 7.61-7.66 and 7.70-7.72 (4 × m, 6H, 2H, 3H and 1H); 7.87 (d, *J* = 9.1 Hz, 2H); 8.08 (d, *J* = 1.0 Hz, 1H); **^{13}C-NMR** (100.6 MHz, $CDCl_3$): **δ =** 49.5, 51.6, 53.9, 111.5, 118.2, 121.4, 121.5, 123.1, 124.0, 126.5, 2 × 127.4, 127.5, 2 × 128.9, 131.2, 131.6, 136.6, 137.4, 138.1, 140.9, 142.0, 152.3 and 167.2; **IR** (cm^{-1}): v = 1702 (C=O); **MS** (70 eV): m/z (%) = 464 (100) [M^+ + H]; **HRMS** (ESI) Anal. Calcd. for $C_{30}H_{26}NO_2S$ 464.1679 [M^+ + H], Found 464.1672.

Synthesis of 3-[(4-methoxycarbonylphenyl)iminomethyl]indole 16

Indole-3-carbaldehyde **15** (435 mg, 3 mmol, 1 equiv), methyl 4-aminobenzoate (544 mg, 3.6 mmol, 1.2 equiv) and *p*-toluenesulfonic acid monohydrate (29 mg, 0.15 mmol, 0.05 equiv) were added to toluene (25 mL) in a Dean Stark apparatus. After 18 hour refluxing the mixture was extracted with EtOAc (25 mL) and washed with a saturated aqueous solution of sodium bicarbonate (25 mL), water (25 mL) and brine (25 mL). Drying ($MgSO_4$), filtering and evaporating of the organic layer yielded a yellow crude reaction mixture which was recrystallized from EtOAc/hexane to obtain 3-[(4-methoxycarbonylphenyl)iminomethyl]indole **16** (710 mg, 2.55 mmol, 85%) as a light yellow powder.

16: 3-[(4-methoxycarbonylphenyl)iminomethyl]indole 85% as light yellow powder; recrystallization from EtOAc/hexane; m.p. 159°C; **^{1}H-NMR** (400 MHz, $CDCl_3$): δ = 3.92 (s, 3H); 7.24 (d, *J* = 8.2 Hz, 2H); 7.29-7.31 and 7.38-7.40 (2 × m, 2H and 1H); 7.64 (s, 1H); 8.07 (d, *J* = 8.2 Hz, 2H); 8.49-8.51 (m, 1H); 8.62 (s, 1H); 8.83 (s(broad), 1H); **^{13}C-NMR** (100.6 MHz, $CDCl_3$): **δ =** 52.0, 111.4, 116.5, 120.9, 122.1, 122.3, 123.9, 125.1, 126.3, 130.9, 131.2, 136.9, 155.7, 157.7 and 167.2; **IR** (cm^{-1}): v = 3292 (NH); 1698 (C=O); **MS** (70 eV): m/z (%) = 279 (100) [M^+ + H]; **HRMS** (ESI) Anal. Calcd. for $C_{17}H_{15}N_2O_2$ 279.1128 [M^+ + H], Found 279.1135.

Synthesis of 3-[(4-methoxycarbonylphenyl)aminomethyl]indole 17

3-[(4-Methoxycarbonylphenyl)iminomethyl]indole **16** (417 mg, 1.5 mmol, 1 equiv) was dissolved in methanol (20 mL). To this solution was sodium borohydride (284 mg, 7.5 mmol, 5 equiv) added after which the mixture was heated to its boiling point. After 90 minutes of stirring the mixture was cooled to room temperature and quenched with water. The obtained mixture was extracted with EtOAc (2 x 25 mL), washed with water (25 mL) and brine (25

mL), dried ($MgSO_4$), filtered and evaporated under vacuum. After recrystallization from EtOAc/hexane 3-[(4-methoxycarbonylphenyl)-aminomethyl]indole **17** (370 mg, 1.32 mmol, 88%) was obtained as a yellow powder.

17: 3-[(4-methoxycarbonylphenyl)aminomethyl]indole 88% as yellow powder; recrystallization from EtOAc/hexane; m.p. 115.5°C; **^{1}H-NMR** (400 MHz, [D_6]DMSO): δ = 3.74 (s, 3H); 4.45 (d, *J* = 5.3 Hz, 2H); 6.72 (d, *J* = 8.8 Hz, 2H); 6.87 (t, *J* = 5.3 Hz, 1H); 7.01 and 7.10 (2 × t, *J* = 7.5 Hz, 2 × 1H); 7.35-7.39 and 7.62-7.64 (2 × m, 2H and 1H); 7.70 (d, *J* = 8.8 Hz, 2H); 10.95 (s(broad), 1H); **^{13}C-NMR** (100.6 MHz, [D_6]DMSO): δ = 38.7, 51.6, 111.6, 111.9, 112.2, 116.0, 119.0, 119.2, 121.6, 124.4, 127.1, 131.3, 136.9, 153.4 and 166.9; **IR** (cm^{-1}): ν = 3360 (NH); 1685 (C=O); **MS** (70 eV): m/z (%) = 279 (20) [M^- - H]; **HRMS** (ESI) Anal. Calcd. for $C_{17}H_{15}N_2O_2$ 279.1139 [M^- - H], Found 279.1146.

Synthesis of hydroxamic acids 7a-d, 8a-c, 13a-b, 14a-b and 18

The synthesis of 3-[(4-hydroxycarbamoylphenyl)aminomethyl]benzothiophene **7a** will be used as an example for the synthesis of hydroxamic acids **7a-d, 8a-c, 13a-b, 14a-b** and **18**. 3-[(4-Methoxycarbonylphenyl)aminomethyl]-benzothiophene **6a** (400 mg, 1.35 mmol, 1 equiv) was dissolved in ethanol (10 mL) and to this solution was firstly hydroxylamine (8.3 mL, 50% in water, 135 mmol, 100 equiv) added and secondly potassium hydroxide (16.9 mL, 4M in methanol, 67.5 mmol, 50 equiv). The resulting mixture was stirred for an additional 10 minutes at room temperature before it was poured in a saturated aqueous solution of sodium bicarbonate (10 mL). This aqueous solution was extracted two times with ethyl acetate, after which the combined organic fractions were washed with water (10 mL) and brine (10 mL). After drying ($MgSO_4$), filtering and evaporating a very viscous colorless liquid was obtained which was recrystallized overnight from $CHCl_3$ to obtain 3-[(4-hydroxycarbamoylphenyl)aminomethyl]benzothiophene **7a** (161 mg, 0.54 mmol, 40%) as a white powder. For hydroxamic acids **7b-d** and **8a-c** the mixture was stirred for 10 minutes in ethanol at refluxing conditions and for hydroxamic acids **13a-b**, **14a-b** and **18** the mixture was stirred for 10 minutes in THF at room temperature.

7a: 3-[(4-hydroxycarbamoylphenyl)aminomethyl]benzothiophene 40% as white powder; crystallization from $CHCl_3$; m.p. 191°C; **^{1}H-NMR** (300 MHz, [D_6]DMSO): δ = 4.53 (d, *J* = 5.5 Hz, 2H); 6.64 (d, *J* = 8.6 Hz, 2H); 6.78 (t, *J* = 5.5 Hz, 1H); 7.35-7.43 (m, 2H); 7.50 (d, *J* = 8.6 Hz, 2H); 7.59 (s, 1H); 7.90 – 8.00 (m, 2H); 8.67 (s(broad), 1H); 10.76 (s(broad), 1H); **^{13}C-NMR** (75 MHz, [D_6]DMSO): δ = 41.4, 111.7, 120.1, 122.7, 123.5, 124.5, 124.7, 125.0, 128.8, 134.5, 138.5, 140.6, 151.6, and 165.5; **IR** (cm^{-1}): ν = 3380, 3255, 3105 (NH/OH); ν = 1600 (C=O); **MS** (70 eV): m/z (%) = 299 (100) [M^+ + H]; **HRMS** (ESI) Anal. Calcd. for $C_{16}H_{15}N_2O_2S$ 299.0849 [M^+ + H], Found 299.0862.

7b: 5-bromo-3-[(4-hydroxycarbamoylphenyl)aminomethyl]-benzothiophene 85% as white powder; crystallization from $CHCl_3$; m.p. 199°C; **^{1}H-NMR** (300 MHz, [D_6]DMSO): δ = 4.52 (d, *J* = 5.5 Hz, 2H); 6.64 (d, *J* = 8.5 Hz, 2H); 6.82 (t, *J* = 5.5 Hz, 1H); 7.50 (d, *J* = 8.5 Hz, 2H and 1H); 7.69 (s, 1H); 7.96 (d, *J* = 8.5 Hz, 1H); 8.17 (s, 1H); 8.67 (s(broad), 1H); 10,75 (s(broad), 1H); **^{13}C-NMR** (75 MHz, [D_6]DMSO): δ = 41.2, 111.8, 118.2, 120.2, 125.3, 125.5, 126.7, 127.7, 128.8, 134.1, 139.6, 140.4, 151.5 and 165.5; **IR** (cm^{-1}): ν = 3376, 3235 (NH/OH); 1604 (C=O); **MS** (70 eV): m/z (%) = 377/9 (100) [M^+ + H]; **HRMS** (ESI) Anal. Calcd. for $C_{16}H_{14}BrN_2O_2S$ 376.9954 [M^+ + H], Found 376.9943.

7c: 3-[(4-hydroxycarbamoylphenyl)aminomethyl]-5-phenyl-benzothiophene 33% as white powder; crystallization from $CHCl_3$; m.p. 181°C; **^{1}H-NMR** (300 MHz, [D_6]DMSO): δ = 4.62 (d, *J* = 5.5 Hz, 2H); 6.68 (d, *J* = 8.8 Hz, 2H); 6.87 (t, *J* = 5.5 Hz, 1H); 7.35-7.40, 7.46-7.53 and 7.65-7.76 (3 × m, 1H, 4H and 4H); 8.05 (d, *J* = 8.8 Hz, 1H); 8.19 (s, 1H); 8.67 (s(broad), 1H); 10.76 (s(broad), 1H); **^{13}C-NMR** (75 MHz, [D_6]DMSO): δ = 41.4, 111.8, 120.0, 120.8, 123.9, 124.1, 125.3, 127.7, 127.9, 128.8, 129.5, 134.8, 137.1, 139.2, 139.8, 140.9, 151.7 and 165.5; **IR** (cm^{-1}): ν = 3403, 3202 (NH/OH); 1608 (C=O); **MS** (70 eV): m/z (%) = 375 (100) [M^+ + H]; **HRMS** (ESI) Anal. Calcd. for $C_{22}H_{19}N_2O_2S$ 375.1162 [M^+ + H], Found 375.1171.

7d: 3-[(4-hydroxycarbamoyl-2-methylphenyl)aminomethyl]-benzothiophene 40% as white powder; crystallization from $CHCl_3$; m.p. 190°C; **^{1}H-NMR** (300 MHz, [D_6]-DMSO): δ = 2.16 (s, 3H); 4.63 (d, *J* = 5.5 Hz, 2H); 6.17 (t, *J* = 5.5 Hz, 1H); 6.51 (d, *J* = 8.2 Hz, 1H); 7.36-7.43 (m, 4H); 7.51 (s, 1H); 7.96 (d, *J* = 7.7 Hz, 1H); 8.05 (d, *J* = 7.7 Hz, 1H); 8.66 (s(broad), 1H); 10,73 (s(broad), 1H); **^{13}C-NMR** (75 MHz, [D_6]-DMSO): δ = 18.5, 41.7, 109.0, 119.9, 121.6, 122.7, 123.5, 124.1, 124.6, 125.0, 126.5, 129.4, 134.7, 138.4, 140.7, 149.2 and 165.6; **IR** (cm^{-1}): ν = 3396, 3366, 3260 (NH/OH); ν = 1604 (C=O); **MS** (70 eV): m/z (%) = 313 (100) [M^+ + H]; **HRMS** (ESI) Anal. Calcd. for $C_{17}H_{17}N_2O_2S$ 313.1005 [M^+ + H], Found 313.1019.

8a: 3-[*N*-benzyl-*N*-(4-hydroxycarbamoylphenyl)aminomethyl]-benzothiophene 17% as brown powder; R_f 0.14 (CH_2Cl_2/MeOH/Et_3N 95/5/2); m.p. 178°C; **^{1}H-NMR** (300 MHz, $CDCl_3$): δ = 4.74 and 4.85 (2 × s, 2 × 2H); 6.74-6.76 (m, 2H); 7.09 (s, 1H); 7.20-7.40, 7.57-7.67 and 7.87-7.90 (3 × m, 7H, 3H and 1H); **^{13}C-NMR** (75 MHz, $CDCl_3$): δ **=** 49.5, 54.0, 111.9, 118.6, 121.4, 122.8, 123.2, 124.3, 124.8, 126.6, 127.4, 128.8, 128.9, 131.4, 137.5,

137.6, 141.3, 151.8 and 167.4; **IR** (cm^{-1}): v = 3059 (NH/OH); 1604 (C=O); **MS** (70 eV): m/z (%) = 389 (100) [M^+ + H]; **HRMS** (ESI) Anal. Calcd. for $C_{23}H_{21}N_2O_2S$ 389.1318 [M^+ + H], Found 389.1334.

8b: 5-bromo-3-[*N*-benzyl-*N*-(4-hydroxycarbamoylphenyl)aminomethyl]-benzothiophene 13% as a white powder; R_f 0.14 (CH_2Cl_2/MeOH/Et_3N 95/5/2); m.p. 179°C; **^{1}H-NMR** (300 MHz, [D_6]acetone): δ = 4.84 and 5.03 (2 × s, 2 × 2H); 6.82 (d, *J* = 8.8 Hz, 2H); 7.24-7.37 (m, 6H); 7.53 (d, *J* = 8.8 Hz, 1H); 7.66 (d, *J* = 8.8 Hz, 2H); 7.93 (d, *J* = 8.8 Hz, 1H); 8.05 (s, 1H); 10.45 (s(broad), 1H); **^{13}C-NMR** (75 MHz, [D_6]acetone): δ = 49.6, 54.1, 111.9, 117.9, 119.7, 124.7, 124.8, 126.7, 127.0, 127.5, 128.4, 128.7, 132.2, 138.5, 139.8, 140.0, 151.2 and 165.7; **IR** (cm^{-1}): v = 3199 (NH/OH); 1605 (C=O); **MS** (70 eV): m/z (%) = 465/7 (22) [M^- - H]; **HRMS** (ESI) Anal. Calcd. for $C_{23}H_{18}BrN_2O_2S$ 465.0278 [M^- - H], Found 465.0287.

8c: 3-[*N*-benzyl-*N*-(4-hydroxycarbamoylphenyl)aminomethyl]-5-phenyl-benzothiophene 13% as yellow powder; R_f 0.14 (CH_2Cl_2/MeOH/Et_3N 95/5/2); m.p. 126°C; **^{1}H-NMR** (300 MHz, $CDCl_3$): δ = 4.73 and 4.88 (2 × s, 2 × 2H); 6.73-3.79 (m, 2H); 7.11-7.46 and 7.59-7.63 (2 × m, 10H and 4H); 7.81 (s, 1H); 7.92 (d, *J* = 8.3 Hz, 1H); 8.63 (s(broad), 1H); **^{13}C-NMR** (75 MHz, $CDCl_3$): δ = 49.7, 54.0, 112.0, 118.4, 119.8, 123.4, 123.5, 124.4, 126.6, 127.4, 127.5, 128.8, 129.0, 131.5, 137.4, 137.9, 138.1, 140.3, 141.1, 152.0 and 167.6. **IR** (cm^{-1}): v = 3198 (NH/OH); 1604 (C=O); **MS** (70 eV): m/z (%) = 465 (100) [M^+ + H]; **HRMS** (ESI) Anal. Calcd. for $C_{29}H_{25}N_2O_2S$ 465.1631 [M^+ + H], Found 465.1639.

13a: 6-bromo-3-[(4-hydroxycarbamoylphenyl)aminomethyl]-benzothiophene 80% as white powder; crystallization from $CHCl_3$; m.p. 179.5°C; **^{1}H-NMR** (400 MHz, [D_6]DMSO): δ = 4.54 (d, *J* = 5.5 Hz, 2H); 6.65 (d, *J* = 8.6 Hz, 2H); 6.80 (t, *J* = 5.5 Hz, 1H); 7.52 (d, *J* = 8.6 Hz, 2H); 7.58 (dd, *J* = 8.6, 1.7 Hz, 1H); 7.64 (s, 1H); 7.90 (d, *J* = 8.6 Hz, 1H); 8.30 (d, *J* = 1.6 Hz, 1H); 8.69 (s(broad), 1H); 10,78 (s(broad), 1H); **^{13}C-NMR** (100.6 MHz, [D_6]-DMSO): δ = 41.2, 111.6, 118.0, 120.0, 124.2, 125.5, 125.8, 127.6, 128.7, 134.2, 137.4, 142.4, 151.5 and 165.3; **IR** (cm^{-1}): v = 3417, 3220 (NH/OH); 1604 (C=O); **MS** (70 eV): m/z (%) = 375/7 (100) [M^- - H]; **HRMS** (ESI) Anal. Calcd. for $C_{16}H_{12}BrN_2O_2S$ 374.9808 [M^- - H], Found 374.9815.

13b: 3-[(4-hydroxycarbamoylphenyl)aminomethyl]-6-phenyl-benzothiophene 68% as white powder; crystallization from $CHCl_3$; m.p. 190.5°C; **^{1}H-NMR** (400 MHz, [D_6]DMSO): δ = 4.58 (d, *J* = 5.6 Hz, 2H); 6.68 (d, *J* = 8.8 Hz, 2H); 6.82 (t, *J* = 5.6 Hz, 1H); 7.39 (t, *J* = 7.3 Hz, 1H); 7.48-7.55 (m, 4H); 7.64 (s, 1H); 7.72-7.78 (m, 3H); 8.03 (d, *J* = 8.4 Hz , 1H); 8.67 (s(broad), 1H); 10.77 (s(broad), 1H); **^{13}C-NMR** (100.6 MHz, [D_6]DMSO): δ = 41.4, 111.7, 120.0, 121.3, 123.0, 123.7, 125.0, 127.4, 127.9, 128.7, 129.5, 134.2, 137.1, 137.7, 140.5, 141.5, 151.5 and 165.4; **IR** (cm^{-1}): v = 3411, 3254 (NH/OH); 1604 (C=O); **MS** (70 eV): m/z (%) = 375 (35) [M^+ + H]; **HRMS** (ESI) Anal. Calcd. for $C_{22}H_{19}N_2O_2S$ 375.1162 [M^+ + H], Found 375.1154.

14a: 6-bromo-3-[*N*-benzyl-*N*-(4-hydroxycarbamoylphenyl)aminomethyl]-benzothiophene 56% as light brown powder; recrystallization from $CHCl_3$/ether; m.p. 97.5°C; **^{1}H-NMR** (400 MHz, $CDCl_3$): δ = 4.73 and 4.83 (2 × s, 2 × 2H); 6.76 (d, *J* = 8.2 Hz, 2H); 7.08 (s, 1H); 7.21 (d, *J* = 7.1 Hz, 2H); 7.29-7.36 (m, 3H); 7.50 (s, 2H); 7.59 (d, *J* = 8.2 Hz, 2H); 8.02 (s, 1H); **^{13}C-NMR** (100.6 MHz, $CDCl_3$): δ **=** 49.3, 53.9, 111.9, 118.2, 118.8, 122.3, 123.2, 125.6, 126.4, 127.4, 127.7, 128.7, 128.9, 131.1, 136.2, 137.1, 142.6, 151.8 and 167.6; **IR** (cm^{-1}): v = 3061 (NH/OH); 1602 (C=O); **MS** (70 eV): m/z (%) = 467/9 (100) [M^+ + H]; **HRMS** (ESI) Anal. Calcd. for $C_{23}H_{18}BrN_2O_2S$ 467.0423 [M^+ + H], Found 467.0423.

14b: 3-[*N*-benzyl-*N*-(4-hydroxycarbamoylphenyl)aminomethyl]-6-phenyl-benzothiophene 74% as white powder; recrystallization from $CHCl_3$/ether; m.p. 102°C; **^{1}H-NMR** (400 MHz, [D_6]DMSO): δ = 4.82 and 5.02 (2 × s, 2 × 2H); 6.75 (d, *J* = 9.0 Hz, 2H); 7.24-7.41 (m, 7H); 7.50 (t, *J* = 7.6 Hz, 2H); 7.55 (d, *J* = 9.0 Hz, 2H); 7.74 (dd, *J* = 8.4, 1.6 Hz, 1H); 7.77 (d, *J* = 7.3 Hz, 2H); 7.94 (d, *J* = 8.4 Hz, 1H); 8.33 (s, 1H); 8.76 (s(broad), 1H); 10.82 (s(broad), 1H); **^{13}C-NMR** (100.6 MHz, [D_6]DMSO): δ = 49.9, 54.1, 112.0, 120.4, 121.4, 122.8, 123.8, 124.1, 127.0, 127.3, 127.4, 127.9, 128.7, 129.0, 129.5, 132.8, 137.2, 137.3, 139.0, 140.4, 141.7, 150.7 and 165.1; **IR** (cm^{-1}): v = 3026 (NH/OH); 1602 (C=O); **MS** (70 eV): m/z (%) = 465 (100) [M^+ + H]; **HRMS** (ESI) Anal. Calcd. for $C_{29}H_{25}N_2O_2S$ 465.1631 [M^+ + H], Found 465.1640.

18: 3-[(4-hydroxycarbamoylphenyl)aminomethyl]indole 77% as yellow powder; crystallization from $CHCl_3$; m.p. 132.5°C; **^{1}H-NMR** (400 MHz, [D_6]DMSO): δ = 4.40 (d, *J* = 5.3 Hz, 2H); 6.52 (t, *J* = 5.3 Hz, 1H); 6.66 (d, *J* = 8.8 Hz, 2H); 6.97-7.01, 7.06-7.10 and 7.33-7.37 (3 × m, 1H, 1H and 2H); 7.51 (d, *J* = 8.8 Hz, 2H); 7.62 (d, *J* = 7.8 Hz, 1H); 8.66, 10.74 and 10.93 (3 × s(broad), 3 × 1H); **^{13}C-NMR** (100.6 MHz, [D_6]DMSO): δ = 38.8, 111.5, 111.9, 112.5, 118.9, 119.2, 119.4, 121.6, 124.3, 127.1, 128.6, 136.8, 151.8 and 165.5; **IR** (cm^{-1}): v = 3407 (NH/OH); 1601 (C=O); **MS** (70 eV): m/z (%) = 282 (20) [M^+ + H]; **HRMS** (ESI) Anal. Calcd. for $C_{16}H_{16}N_3O_2$ 282.1237 [M^+ + H], Found 282.1236.

Bioassay results (performed by Cerep – www.cerep.com)

Assay	Compound	Test Concentration (M)	% of Control Values		
			1st	2nd	Mean
HDAC6 (h)	7a	3.0E-10	105.8	109.0	107.4
HDAC6 (h)	7a	3.0E-09	92.0	94.1	93.0
HDAC6 (h)	7a	3.0E-08	36.4	30.9	33.7
HDAC6 (h)	7a	3.0E-07	4.9	5.0	4.9
HDAC6 (h)	7a	3.0E-06	0.5	0.6	0.6
HDAC6 (h)	7a	3.0E-05	0.3	0.3	0.3
HDAC6 (h)	7b	3.0E-10	106.2	99.8	103.0
HDAC6 (h)	7b	3.0E-09	101.7	102.2	102.0
HDAC6 (h)	7b	3.0E-08	58.5	53.9	56.2
HDAC6 (h)	7b	3.0E-07	30.6	13.4	22.0
HDAC6 (h)	7b	3.0E-06	2.5	2.9	2.7
HDAC6 (h)	7b	3.0E-05	2.0	2.1	2.1
HDAC6 (h)	7c	3.0E-09	102.5	105.3	103.9
HDAC6 (h)	7c	3.0E-08	102.3	98.7	100.5
HDAC6 (h)	7c	1.0E-07	83.6	82.7	83.1
HDAC6 (h)	7c	3.0E-07	53.8	50.1	51.9
HDAC6 (h)	7c	3.0E-06	14.1	21.5	17.8
HDAC6 (h)	7c	3.0E-05	0.7	1.0	0.8
HDAC6 (h)	7d	1.0E-08	103.4	106.2	104.8
HDAC6 (h)	7d	1.0E-07	99.4	96.8	98.1
HDAC6 (h)	7d	1.0E-06	72.2	69.6	70.9
HDAC6 (h)	7d	3.0E-06	48.0	45.4	46.7
HDAC6 (h)	7d	1.0E-05	29.4	18.7	24.0
HDAC6 (h)	7d	1.0E-04	2.0	3.0	2.5
HDAC6 (h)	8a	3.0E-09	107.8	101.5	104.6
HDAC6 (h)	8a	3.0E-08	100.1	100.2	100.2
HDAC6 (h)	8a	1.0E-07	89.3	90.9	90.1
HDAC6 (h)	8a	3.0E-07	61.4	65.3	63.4
HDAC6 (h)	8a	3.0E-06	18.7	18.3	18.5
HDAC6 (h)	8a	3.0E-05	3.7	2.9	3.3
HDAC6 (h)	8b	1.0E-08	100.5	101.4	101.0
HDAC6 (h)	8b	1.0E-07	83.3	95.6	89.4
HDAC6 (h)	8b	1.0E-06	47.6	50.9	49.2
HDAC6 (h)	8b	3.0E-06	27.3	26.8	27.0
HDAC6 (h)	8b	1.0E-05	19.6	14.0	16.8
HDAC6 (h)	8b	1.0E-04	2.4	1.7	2.0
HDAC6 (h)	13a	3.0E-10	103.3	101.8	102.5
HDAC6 (h)	13a	3.0E-09	98.9	96.8	97.8

HDAC6 (h)	13a	3.0E-08	75.9	66.6	71.3
HDAC6 (h)	13a	3.0E-07	14.1	17.8	16.0
HDAC6 (h)	13a	3.0E-06	1.6	2.2	1.9
HDAC6 (h)	13a	3.0E-05	0.5	0.6	0.5
HDAC6 (h)	13b	3.0E-09	103.8	96.9	100.3
HDAC6 (h)	13b	3.0E-08	96.9	93.0	95.0
HDAC6 (h)	13b	1.0E-07	83.7	79.5	81.6
HDAC6 (h)	13b	3.0E-07	83.1	58.9	71.0
HDAC6 (h)	13b	3.0E-06	27.8	16.7	22.3
HDAC6 (h)	13b	3.0E-05	7.3	7.5	7.4
HDAC6 (h)	14a	1.0E-08	102.9	96.3	99.6
HDAC6 (h)	14a	1.0E-07	98.3	94.2	96.3
HDAC6 (h)	14a	1.0E-06	65.7	69.9	67.8
HDAC6 (h)	14a	3.0E-06	44.4	40.5	42.5
HDAC6 (h)	14a	1.0E-05	15.6	18.3	17.0
HDAC6 (h)	14a	1.0E-04	4.7	3.2	4.0
HDAC6 (h)	18	3.0E-10	105.3	100.1	102.7
HDAC6 (h)	18	3.0E-09	104.2	95.3	99.7
HDAC6 (h)	18	3.0E-08	85.5	81.1	83.3
HDAC6 (h)	18	3.0E-07	41.7	45.0	43.3
HDAC6 (h)	18	3.0E-06	8.6	7.2	7.9
HDAC6 (h)	18	3.0E-05	0.4	0.2	0.3

Assay	Compound	Test Concentration (M)	% of Control Values		
			1st	2nd	Mean
HDAC1 (h)	7a	3.0E-08	106.3	97.4	101.9
HDAC1 (h)	7a	3.0E-07	101.5	94.4	97.9
HDAC1 (h)	7a	1.0E-06	89.7	85.1	87.4
HDAC1 (h)	7a	3.0E-06	72.1	68.5	70.3
HDAC1 (h)	7a	1.0E-05	48.5	44.0	46.3
HDAC1 (h)	7a	1.0E-04	7.5	7.2	7.3
HDAC1 (h)	7b	3.0E-08	106.0	102.6	104.3
HDAC1 (h)	7b	3.0E-07	98.9	104.9	101.9
HDAC1 (h)	7b	1.0E-06	98.4	96.4	97.4
HDAC1 (h)	7b	3.0E-06	90.0	89.0	89.5
HDAC1 (h)	7b	1.0E-05	71.1	66.1	68.6
HDAC1 (h)	7b	1.0E-04	82.4	87.5	85.0
HDAC1 (h)	13a	3.0E-08	109.7	109.3	109.5
HDAC1 (h)	13a	3.0E-07	166.6	57.6	112.1
HDAC1 (h)	13a	1.0E-06	82.1	90.5	86.3
HDAC1 (h)	13a	3.0E-06	64.9	53.2	59.0
HDAC1 (h)	13a	1.0E-05	30.7	34.4	32.5

HDAC1 (h)	13a	1.0E-04	13.3	1.7	7.5
HDAC2 (h)	7a	3.0E-08	99.2	100.4	99.8
HDAC2 (h)	7a	3.0E-07	100.3	100.5	100.4
HDAC2 (h)	7a	1.0E-06	97.3	97.0	97.1
HDAC2 (h)	7a	3.0E-06	91.5	88.0	89.7
HDAC2 (h)	7a	1.0E-05	80.7	69.6	75.2
HDAC2 (h)	7a	1.0E-04	26.4	22.5	24.4
HDAC2 (h)	7b	3.0E-08	104.6	99.9	102.2
HDAC2 (h)	7b	3.0E-07	106.2	104.2	105.2
HDAC2 (h)	7b	1.0E-06	98.0	100.4	99.2
HDAC2 (h)	7b	3.0E-06	97.5	102.4	99.9
HDAC2 (h)	7b	1.0E-05	90.2	86.7	88.4
HDAC2 (h)	7b	1.0E-04	87.4	76.4	81.9
HDAC2 (h)	13a	3.0E-08	105.7	102.8	104.3
HDAC2 (h)	13a	3.0E-07	105.1	103.9	104.5
HDAC2 (h)	13a	1.0E-06	98.2	100.6	99.4
HDAC2 (h)	13a	3.0E-06	96.0	77.4	86.7
HDAC2 (h)	13a	1.0E-05	74.4	66.1	70.2
HDAC2 (h)	13a	1.0E-04	15.8	23.7	19.7
HDAC3 (h)	7a	3.0E-08	107.4	106.3	106.9
HDAC3 (h)	7a	3.0E-07	104.0	104.8	104.4
HDAC3 (h)	7a	1.0E-06	102.4	90.4	96.4
HDAC3 (h)	7a	3.0E-06	81.0	75.0	78.0
HDAC3 (h)	7a	1.0E-05	58.7	54.2	56.4
HDAC3 (h)	7a	1.0E-04	9.8	9.1	9.5
HDAC3 (h)	7b	3.0E-08	101.0	100.8	100.9
HDAC3 (h)	7b	3.0E-07	96.1	102.4	99.2
HDAC3 (h)	7b	1.0E-06	97.2	90.0	93.6
HDAC3 (h)	7b	3.0E-06	84.3	91.7	88.0
HDAC3 (h)	7b	1.0E-05	60.0	62.2	61.1
HDAC3 (h)	7b	1.0E-04	71.9	77.8	74.8
HDAC3 (h)	13a	3.0E-08	98.8	100.8	99.8
HDAC3 (h)	13a	3.0E-07	100.9	103.3	102.1
HDAC3 (h)	13a	1.0E-06	83.9	88.4	86.2
HDAC3 (h)	13a	3.0E-06	68.1	55.4	61.8
HDAC3 (h)	13a	1.0E-05	35.8	53.0	44.4
HDAC3 (h)	13a	1.0E-04	6.2	4.7	5.4
HDAC4 (h)	7a	3.0E-08	105.8	104.0	104.9
HDAC4 (h)	7a	3.0E-07	93.2	91.8	92.5
HDAC4 (h)	7a	1.0E-06	92.5	88.6	90.5
HDAC4 (h)	7a	3.0E-06	71.7	72.8	72.2
HDAC4 (h)	7a	1.0E-05	51.0	59.9	55.5
HDAC4 (h)	7a	1.0E-04	9.5	10.1	9.8
HDAC4 (h)	7b	3.0E-08	106.4	98.3	102.3
HDAC4 (h)	7b	3.0E-07	97.0	96.9	97.0

HDAC4 (h)	7b	1.0E-06	105.9	99.9	102.9
HDAC4 (h)	7b	3.0E-06	87.5	97.6	92.6
HDAC4 (h)	7b	1.0E-05	77.1	73.6	75.3
HDAC4 (h)	7b	1.0E-04	78.9	82.7	80.8
HDAC4 (h)	13a	3.0E-08	88.5	101.0	94.8
HDAC4 (h)	13a	3.0E-07	100.2	101.7	101.0
HDAC4 (h)	13a	1.0E-06	99.6	122.6	111.1
HDAC4 (h)	13a	3.0E-06	111.0	78.7	94.9
HDAC4 (h)	13a	1.0E-05	63.5	97.0	80.3
HDAC4 (h)	13a	1.0E-04	22.2	19.3	20.8
HDAC5 (h)	7a	3.0E-08	109.5	108.4	108.9
HDAC5 (h)	7a	3.0E-07	105.9	102.8	104.3
HDAC5 (h)	7a	1.0E-06	96.3	103.1	99.7
HDAC5 (h)	7a	3.0E-06	92.9	87.0	89.9
HDAC5 (h)	7a	1.0E-05	70.3	62.7	66.5
HDAC5 (h)	7a	1.0E-04	15.8	17.7	16.8
HDAC5 (h)	7b	3.0E-08	105.7	104.8	105.2
HDAC5 (h)	7b	3.0E-07	106.1	105.6	105.9
HDAC5 (h)	7b	1.0E-06	97.2	108.6	102.9
HDAC5 (h)	7b	3.0E-06	98.6	97.8	98.2
HDAC5 (h)	7b	1.0E-05	74.9	88.3	81.6
HDAC5 (h)	7b	1.0E-04	89.4	87.5	88.4
HDAC5 (h)	13a	3.0E-08	105.1	103.7	104.4
HDAC5 (h)	13a	3.0E-07	101.1	104.0	102.5
HDAC5 (h)	13a	1.0E-06	99.4	101.7	100.5
HDAC5 (h)	13a	3.0E-06	96.2	102.9	99.5
HDAC5 (h)	13a	1.0E-05	79.7	91.4	85.6
HDAC5 (h)	13a	1.0E-04	33.6	28.4	31.0
HDAC7 (h)	7a	3.0E-08	90.8	92.1	91.5
HDAC7 (h)	7a	3.0E-07	89.8	90.6	90.2
HDAC7 (h)	7a	1.0E-06	76.6	83.3	80.0
HDAC7 (h)	7a	3.0E-06	59.7	61.8	60.8
HDAC7 (h)	7a	1.0E-05	29.3	33.7	31.5
HDAC7 (h)	7a	1.0E-04	6.5	4.1	5.3
HDAC7 (h)	7b	3.0E-08	100.3	101.1	100.7
HDAC7 (h)	7b	3.0E-07	103.8	100.9	102.3
HDAC7 (h)	7b	1.0E-06	92.6	86.7	89.7
HDAC7 (h)	7b	3.0E-06	88.2	76.1	82.1
HDAC7 (h)	7b	1.0E-05	49.5	56.6	53.1
HDAC7 (h)	7b	1.0E-04	64.4	71.9	68.2
HDAC7 (h)	13a	3.0E-08	105.1	99.9	102.5
HDAC7 (h)	13a	3.0E-07	93.3	91.0	92.2
HDAC7 (h)	13a	1.0E-06	78.6	101.1	89.8
HDAC7 (h)	13a	3.0E-06	52.1	88.1	88.1
HDAC7 (h)	13a	1.0E-05	59.0	42.8	50.9

HDAC7 (h)	13a	1.0E-04	12.1	10.3	11.2
HDAC8 (h)	7a	3.0E-08	108.9	95.7	102.3
HDAC8 (h)	7a	3.0E-07	81.8	84.9	83.3
HDAC8 (h)	7a	1.0E-06	61.6	60.7	61.2
HDAC8 (h)	7a	3.0E-06	40.1	24.6	32.3
HDAC8 (h)	7a	1.0E-05	12.4	8.8	10.6
HDAC8 (h)	7a	1.0E-04	-16.1	-1.5	-8.8
HDAC8 (h)	7b	3.0E-08	106.4	109.4	107.9
HDAC8 (h)	7b	3.0E-07	98.2	92.0	95.1
HDAC8 (h)	7b	1.0E-06	61.7	80.0	70.8
HDAC8 (h)	7b	3.0E-06	70.3	41.2	55.8
HDAC8 (h)	7b	1.0E-05	21.8	29.3	25.6
HDAC8 (h)	7b	1.0E-04	18.4	22.0	20.2
HDAC8 (h)	13a	3.0E-08	108.7	111.5	110.1
HDAC8 (h)	13a	3.0E-07	101.3	104.4	102.9
HDAC8 (h)	13a	1.0E-06	65.9	62.0	64.0
HDAC8 (h)	13a	3.0E-06	49.9	63.0	56.5
HDAC8 (h)	13a	1.0E-05	17.4	31.6	24.5
HDAC8 (h)	13a	1.0E-04	7.9	8.8	8.3
HDAC9 (h)	7a	3.0E-08	101.8	105.3	103.5
HDAC9 (h)	7a	3.0E-07	93.2	99.8	96.5
HDAC9 (h)	7a	1.0E-06	85.3	95.3	90.3
HDAC9 (h)	7a	3.0E-06	66.8	66.2	66.5
HDAC9 (h)	7a	1.0E-05	50.7	42.6	46.7
HDAC9 (h)	7a	1.0E-04	10.5	15.3	12.9
HDAC9 (h)	7b	3.0E-08	108.0	97.4	102.7
HDAC9 (h)	7b	3.0E-07	99.5	103.8	101.6
HDAC9 (h)	7b	1.0E-06	95.7	110.0	102.8
HDAC9 (h)	7b	3.0E-06	98.1	82.8	90.4
HDAC9 (h)	7b	1.0E-05	78.2	87.4	82.8
HDAC9 (h)	7b	1.0E-04	79.7	77.1	78.4
HDAC9 (h)	13a	3.0E-08	113.8	115.3	114.6
HDAC9 (h)	13a	3.0E-07	99.0	101.0	100.0
HDAC9 (h)	13a	1.0E-06	95.7	96.8	96.2
HDAC9 (h)	13a	3.0E-06	88.7	107.4	98.0
HDAC9 (h)	13a	1.0E-05	64.7	82.0	73.4
HDAC9 (h)	13a	1.0E-04	26.2	23.2	24.7
HDAC10 (h)	7a	3.0E-08	111.1	111.4	111.3
HDAC10 (h)	7a	3.0E-07	102.3	103.5	102.9
HDAC10 (h)	7a	1.0E-06	100.1	91.9	96.0
HDAC10 (h)	7a	3.0E-06	72.8	73.3	73.1
HDAC10 (h)	7a	1.0E-05	61.9	61.0	61.4
HDAC10 (h)	7a	1.0E-04	12.5	15.0	13.8
HDAC10 (h)	7b	3.0E-08	107.4	105.5	106.4
HDAC10 (h)	7b	3.0E-07	91.8	104.6	98.2

HDAC10 (h)	7b	1.0E-06	96.8	95.8	96.3
HDAC10 (h)	7b	3.0E-06	76.7	89.5	83.1
HDAC10 (h)	7b	1.0E-05	62.2	64.9	63.5
HDAC10 (h)	7b	1.0E-04	54.9	47.3	51.1
HDAC10 (h)	13a	3.0E-08	104.2	95.2	99.7
HDAC10 (h)	13a	3.0E-07	97.4	97.5	97.4
HDAC10 (h)	13a	1.0E-06	95.6	89.2	92.4
HDAC10 (h)	13a	3.0E-06	68.1	83.5	75.8
HDAC10 (h)	13a	1.0E-05	44.5	37.0	40.7
HDAC10 (h)	13a	1.0E-04	13.4	3.9	8.6
HDAC11 (h)	7a	3.0E-07	105.5	104.8	105.1
HDAC11 (h)	7a	3.0E-06	95.1	103.7	99.4
HDAC11 (h)	7a	3.0E-05	54.4	54.0	54.2
HDAC11 (h)	7a	1.0E-04	23.4	21.8	22.6
HDAC11 (h)	7a	3.0E-04	48.7	12.4	12.4
HDAC11 (h)	7a	1.0E-03	1.0	-0.3	0.3
HDAC11 (h)	7b	3.0E-07	102.5	97.4	100.0
HDAC11 (h)	7b	3.0E-06	47.7	61.2	61.2
HDAC11 (h)	7b	3.0E-05	54.2	43.8	49.0
HDAC11 (h)	7b	1.0E-04	25.1	27.8	26.4
HDAC11 (h)	7b	3.0E-04	19.6	18.9	19.3
HDAC11 (h)	7b	1.0E-03	11.8	10.6	11.2
HDAC11 (h)	13a	3.0E-07	81.5	80.3	80.9
HDAC11 (h)	13a	3.0E-06	41.0	38.4	39.7
HDAC11 (h)	13a	3.0E-05	12.1	12.4	12.3
HDAC11 (h)	13a	1.0E-04	11.4	11.2	11.3
HDAC11 (h)	13a	3.0E-04	14.9	16.5	15.7
HDAC11 (h)	13a	1.0E-03	11.6	12.2	11.9

Bioassay results (performed by Laboratory of Neurobiology and Vesalius Research Center, VIB)

General

Values represent the normalized ratio Acetyl α-Tubulin/α-Tubulin against Tubastatin A (Tub A) in an established neuronal cell line (Neuro-2a cells: ATCC N° CCL-131). Neuro-2a cells are treated overnight with different concentrations of the HDAC6 inhibitors and the effect on the acetylation level of α-tubulin is determined by using Western blot.

Cell culture Western Blot

Mouse neuroblastoma (Neuro-2a) cells were grown in a 1:1 mix of D-MEM (Dulbecco's Modified Eagle Medium) and F12 medium supplemented with glutamax (Invitrogen), 100 µg/ml streptomycin, 100 U/ml penicillin (Invitrogen), 10% fetal calf serum (Greiner Bio-one), 1% non-essential amino acids (Invitrogen) and 1.6% $NaHCO_3$ (Invitrogen) at 37 °C and 7.5% CO_2. To split the cells, cells were washed with Versene (Invitrogen) and dissociated with 0.05% Trypsine-EDTA (Invitrogen). The Neuro-2a cells were treated overnight at 37°C with dosages ranging from 10 nM up to 1 µM of either Tubastatin A (Sigma-Aldrich) or the candidate HDAC6 inhibitors.

Western Blot

For sodium dodecyl sulfate–polyacrylamide gel electrophoresis (SDS-PAGE) analysis, transfected cells were collected using the EpiQuik Total Histone Extraction Kit (EpiGentek) according to manufacturer's instructions. Protein concentrations were determined using microBCA kit (Thermo Fisher Scientific Inc., Pittsburgh, PA, USA) according to manufacturer's instructions. Before resolving the samples on a 12% SDS–PAGE gel, samples containing equal amounts of protein were supplemented with reducing sample buffer (Thermo Scientific) and boiled at 95 °C for 5 min. After electrophoresis, the proteins were transferred to a polyvinylidene difluoride (PVDF) membrane (Millipore Corp., Bedford, MA, USA). The non-specific binding was blocked by incubation of the membrane in 5% bovine serum albumin (BSA), diluted in Tris Buffered Saline Tween (TBST, 50 mM TRIS, 150 mM NaCl, 0,1% Tween-20 (Applichem, Darmstadt, Duitsland) for 1h at room temperature followed by incubation with primary antibodies overnight. The antibodies, diluted in TBS-T, were directed against α-tubulin (1/5000, T6199, Sigma-Aldrich), and acetylated α-tubulin (1/5000, T6793 monoclonal, Sigma-Aldrich). The secondary antibodies, coupled to alkaline phosphatase (anti-mouse or anti-rabbit, 1/5000, Sigma-Aldrich) were used. Blots were visualized by adding the ECF substrate (Enhanced Chemical Fluorescence, GE Healthcare, Uppsala, Sweden) and imaged with the ImageQuant_LAS 4000. A mild reblotting buffer (Millipore) was applied to strip the blots. ImageQuant TL version 7.0-software was used to quantify the blots.

Compound 7a

	DMSO	Tub A 10 nM	Tub A 50 nM	Tub A 100 nM	Tub A 500 nM	Tub A 1 µM	DMSO	7a 10 nM	7a 50 nM	7a 100 nM	7a 500 nM	7a 1 µM
N=1	14.23	83.86	36.39	45.55	59.89	100.00	28.03	71.28	125.02	165.81	302.05	27.25
N=2	2.66	10.75	34.60	49.66	53.59	100.00	0.27	0.32	3.46	11.57	95.17	86.25
N=3	8.30	4.96	44.96	41.30	79.01	100.00	5.88	12.65	21.17	27.16	42.76	61.02
N=4	5.02	9.20	23.91	39.48	72.46	100.00	10.86	11.83	37.17	65.07	116.96	187.26
Average	**7.55**	**27.19**	**34.97**	**44.00**	**66.24**	**100.00**	**11.26**	**24.02**	**46.70**	**67.40**	**139.23**	**90.45**

Compound 7b

	DMSO	Tub A 10 nM	Tub A 50 nM	Tub A 100 nM	Tub A 500 nM	Tub A 1 µM	DMSO	7b 10 nM	7b 50 nM	7b 100 nM	7b 500 nM	7b 1 µM
N=1	9.04	15.47	32.35	47.79	67.73	100.00	9.72	17.56	24.55	36.05	85.99	99.53
N=2	5.31	2.70	13.55	31.44	55.71	100.00	1.31	1.78	5.36	7.24	34.97	83.22
N=3	1.95	4.28	24.46	29.63	73.97	100.00	3.87	3.74	14.84	23.85	61.59	69.97
N=4	7.25	12.67	30.11	56.84	95.18	100.00	9.30	10.34	18.25	41.80	72.25	80.03
Average	**5.89**	**8.78**	**25.12**	**41.43**	**73.15**	**100.00**	**6.05**	**8.35**	**15.75**	**27.24**	**63.70**	**83.19**

Compound 13a

	DMSO	Tub A 10 nM	Tub A 50 nM	Tub A 100 nM	Tub A 500 nM	Tub A 1 µM	DMSO	13a 10 nM	13a 50 nM	13a 100 nM	13a 500 nM	13a 1 µM
N=1	4.71	8.42	22.76	31.46	56.60	100.00	6.81	15.09	22.81	27.19	62.72	52.31
N=2	6.04	2.13	3.52	10.65	41.12	100.00	2.28	1.36	3.33	7.80	45.50	36.45
N=3	4.01	5.26	36.54	40.93	86.74	100.00	4.58	6.21	9.02	19.35	74.04	131.29
N=4	6.57	11.55	32.61	53.68	96.97	100.00	10.11	10.74	20.54	38.48	97.60	101.71
Average	**5.33**	**6.84**	**23.86**	**34.18**	**70.36**	**100.00**	**5.95**	**8.35**	**13.93**	**23.21**	**69.97**	**80.44**

Bioassay results (performed by Cytokine Receptor Lab, VIB)

General

Figure 4

A549 cells with the stably integrated recombinant reporter gene p(GRE)2-50-luc (**A**) were pre-incubated with respective solvents, the selective Glucocorticoid Receptor modulator CpdA (10 µM), **7a** (1µM or 10µM), **7b** (1µM or 10µM), **13a** (1µM or 10µM) for 1h after which the synthetic glucocorticoid dexamethasone (DEX,1µM) was added, where indicated, for 5h. A549 cells with the stably integrated recombinant reporter gene p(IL6κB)350hu.IL6P-luc (**B**)[1] or Collagenase-luc (**C**) were pre-incubated with respective solvents, DEX (1µM), CpdA (10 µM), **7a** (1µM or 10 µM), **7b** (1µM or 10 µM), **13a** (1µM or 10 µM) for 1h after which TNF (2000 units/ml) or PMA (20 nM) were added, where indicated, for 5h. Cell lysates were assayed for luciferase activities. Promoter activities are expressed as relative induction factor calculated as percentage of maximal DEX (**A**), TNF (**B**) or PMA (**C**) responses. Averaged results of four independent experiments are shown ± SD. ****p < 0.0001; *** p < 0.001; ** p < 0.01; * p < 0.05 Assays were performed in triplicate, and results are averages of at least four independent experiments and shown ± S.D. ****p < 0.0001; *** p < 0.001; ** p < 0.01; * p < 0.05. Statistical significance was determined on the averaged results, and analysis performed using one-way ANOVA tests followed by a Tukey multiple comparison post test.

Figure 5

A549 cells with the stably integrated recombinant reporter gene p(GRE)2-50-luc (**A**) were pre-incubated with respective solvents, the selective Glucocorticoid Receptor modulator CpdA (10 µM), Tubastatin A (0,5, 1, 5, 10, 50 µM) for 1h after which the synthetic glucocorticoid dexamethasone (DEX, 1µM) was added, where indicated, for 5h. An extra DMSO control at the highest dose was included (1/200), whereas the level of DMSO for the other concentrations corresponded to the one-before highest dose, i.e. of the 10 µM set-up. A549 cells with the stably integrated recombinant reporter gene p(IL6κB)350hu.IL6P-luc (**B**) or Collagenase-luc (**C**) were pre-incubated with respective solvents, DEX (1µM), CpdA (10 µM), Tubastatin A (0,5, 1, 5, 10, 50 µM) for 1h after which TNF (2000 units/ml) or PMA (20 nM) were added, where indicated, for 5h. Cell lysates were assayed for luciferase activities. Promoter activities are expressed as relative induction factor calculated as percentage of maximal DEX (A), TNF (B) or PMA (C) responses. Averaged results of two independent experiments are shown ± SD. ****p < 0.0001; *** p < 0.001; ** p < 0.01; * p < 0.05 Assays were performed in triplicate, and results are averages of two independent experiments and shown ± S.D. ****p < 0.0001; *** p < 0.001; ** p < 0.01; * p < 0.05. Statistical significance was determined on the averaged results, and analysis performed using one-way ANOVA tests followed by a Tukey multiple comparison post test.

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Ligand docking – experimental details

All manipulations were performed with the molecular modelling program YASARA and the YASARA/WHATIF twinset[2,3] and the figure was created with PyMol v1.3.[4] The HDAC6 sequence was obtained from the UniProt database (www.uniprot.org; UniProt entry Q9UBN7). To increase the accuracy of the model, the sequence was limited to the major functional domain of HDAC6 (Gly482-Gly800). Possible templates were identified by running 3 PSI-BLAST iterations to extract a position specific scoring matrix (PSSM) from UniRef90, and then searching the PDB for a match. To aid the alignment of the HDAC6 sequence and templates, and the modelling of the loops, a secondary structure prediction was performed, followed by multiple sequence alignments. All side chains were ionised or kept neutral according to their predicted pKa values. Initial models were created from different templates (pdb entry 2VQW, 2VQQ and 3C10), each with several alignment variations and up to hundred conformations tried per loop. After the side-chains had been built, optimised and fine-tuned, all newly modelled parts were subjected to a combined steepest descent and simulated annealing minimisation, i.e. the backbone atoms of aligned residues were kept fixed to preserve the folding, followed by a full unrestrained simulated annealing minimisation for the entire model. The final model was obtained as a hybrid model of the best parts of the initial models, and checked once more for anomalies like incorrect configurations or colliding side chains. Furthermore, it was structurally aligned with known HDAC crystal structures to check if the chelating residues and the zinc atom were arranged correctly.

The HDAC inhibitor structures were created with YASARA Structure and energy minimised with the AMBER03 force field.[5] The grid box used for docking had a dimension of 25 x 25 x 25 angstrom, and comprised the entire catalytic cavity including the Zn ion and the outer surface of the active site entrance. Docking was performed with AutoDock VINA[6] and default parameters. Ligands were allowed to freely rotate during docking. The first conformer from the cluster that has its zinc binding group in the vicinity of the zinc ion, was selected as the binding mode for further analysis. The associated cluster was moreover always the highest populated and had the highest average binding energy, proving that the selected docking pose is highly preferred. Docking was in addition redone with a grid covering the whole protein extended by 5 Å on each side (~60x60x60) and the results were consistent with those obtained with the smaller grid. The docking experiments thus showed that the preferred binding mode is the one in which the phenylhydroxamate group occupies the tubular access channel (with the zinc binding group close to the zinc atom) and the cap group interacts with the protein surface. For the most potent inhibitors (**7a**, **7b** and **13a**), the cap group was somehow sandwiched between Phe620 and Phe680, interacting via pi-pi stacking and hydrophobic contacts. Similar interactions were found for the cap group of the less potent N-substituted compounds, with additional contacts between the extra phenyl group and Phe679 and Leu749. There is accordingly no obvious reason for the lower *in vitro* activity. The selectivity for HDAC6 in contrast could be captured by molecular docking experiments. To that end, the most potent and selective HDAC6 inhibitors (**7a**, **7b** and **13a**) were docked in HDAC2 (class I) and HDAC4 (class IIa) and predicted binding energies were compared with HDAC6 (class IIb) (Figure S1). A clear preference for HDAC6 was observed, with significantly lower affinities for the other isoforms (in accordance with the *in vitro* tests).

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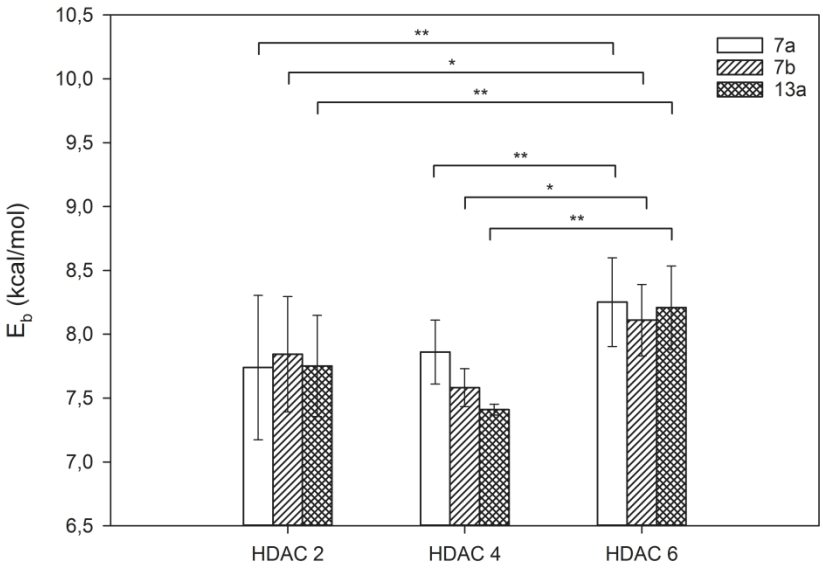


Figure S1 Predicted binding energies for the most potent and selective HDAC6 inhibitors (**7a**, **7b** and **13a**) against HDAC2 (class I), HDAC4 (class IIa) and HDAC6 (class IIb) (structures used for ligand docking: pdb entry 4LY1 chain A (HDAC2), 4CBY chain A (HDAC4) and the model created in this study (HDAC6); average binding energy from the cluster having its zinc binding group in the vicinity of the zinc ion (the higher the E_b the better the binding); ** $p<0.01$, * $p<0.05$).

Ames Fluctuation Assay 7a (performed by Eurofins Cerep Panlabs)

Bacterial cytotoxicity results 7a

Assay	Test Concentration (M)	Cytotoxicity [1,2,3] (% of control)
Bacterial cytotoxicity (TA98 - S9)	6.0E-07	96
Bacterial cytotoxicity (TA98 - S9)	1.2E-06	93
Bacterial cytotoxicity (TA98 - S9)	2.5E-06	87
Bacterial cytotoxicity (TA98 - S9)	5.0E-06	87
Bacterial cytotoxicity (TA98 - S9)	1.0E-05	87
Bacterial cytotoxicity (TA98 - S9)	2.5E-05	69
Bacterial cytotoxicity (TA98 - S9)	5.0E-05	55
Bacterial cytotoxicity (TA98 - S9)	1.0E-04	31
Bacterial cytotoxicity (TA100 - S9)	6.0E-07	92
Bacterial cytotoxicity (TA100 - S9)	1.2E-06	107
Bacterial cytotoxicity (TA100 - S9)	2.5E-06	96
Bacterial cytotoxicity (TA100 - S9)	5.0E-06	92
Bacterial cytotoxicity (TA100 - S9)	1.0E-05	81
Bacterial cytotoxicity (TA100 - S9)	2.5E-05	67
Bacterial cytotoxicity (TA100 - S9)	5.0E-05	55
Bacterial cytotoxicity (TA100 - S9)	1.0E-04	41
Bacterial cytotoxicity (TA1535 - S9)	6.0E-07	100
Bacterial cytotoxicity (TA1535 - S9)	1.2E-06	98
Bacterial cytotoxicity (TA1535 - S9)	2.5E-06	98
Bacterial cytotoxicity (TA1535 - S9)	5.0E-06	99
Bacterial cytotoxicity (TA1535 - S9)	1.0E-05	100
Bacterial cytotoxicity (TA1535 - S9)	2.5E-05	75
Bacterial cytotoxicity (TA1535 - S9)	5.0E-05	57
Bacterial cytotoxicity (TA1535 - S9)	1.0E-04	44
Bacterial cytotoxicity (TA1537 - S9)	6.0E-07	104
Bacterial cytotoxicity (TA1537 - S9)	1.2E-06	100
Bacterial cytotoxicity (TA1537 - S9)	2.5E-06	92
Bacterial cytotoxicity (TA1537 - S9)	5.0E-06	92

Bacterial cytotoxicity (TA1537 - S9)	1.0E-05	93
Bacterial cytotoxicity (TA1537 - S9)	2.5E-05	70
Bacterial cytotoxicity (TA1537 - S9)	5.0E-05	41
Bacterial cytotoxicity (TA1537 - S9)	1.0E-04	30

1. Cytotoxicity is presented as % of control growth.
2. A cytotoxicity value of less than 60 % is considered as toxic at the respective concentration.
3. Reference compound: Mytomycin C

Ames results 7a

Assay	Test Concentration (M)	Count(# of wells)	PositiveSignificance(- to +++)	FisherExact Test(p-value)
Ames fluctuation test (TA98 - S9)	5.0E-06	1	-	0.1019
Ames fluctuation test (TA98 - S9)	1.0E-05	1	-	0.1019
Ames fluctuation test (TA98 - S9)	5.0E-05	5	-	1.0000
Ames fluctuation test (TA98 - S9)	1.0E-04	0	-	0.0280
Ames fluctuation test (TA98 + S9)	5.0E-06	5	-	0.5000
Ames fluctuation test (TA98 + S9)	1.0E-05	4	-	1.0000
Ames fluctuation test (TA98 + S9)	5.0E-05	4	-	1.0000
Ames fluctuation test (TA98 + S9)	1.0E-04	3	-	0.5000
Ames fluctuation test (TA100 - S9)	5.0E-06	2	-	0.5000
Ames fluctuation test (TA100 - S9)	1.0E-05	0	-	0.5000
Ames fluctuation test (TA100 - S9)	5.0E-05	0	-	0.5000
Ames fluctuation test (TA100 - S9)	1.0E-04	0	-	0.5000
Ames fluctuation test (TA100 + S9)	5.0E-06	10	-	0.5000
Ames fluctuation test (TA100 + S9)	1.0E-05	5	-	0.1932
Ames fluctuation test (TA100 + S9)	5.0E-05	4	-	0.1161
Ames fluctuation test (TA100 + S9)	1.0E-04	1	-	0.0077
Ames fluctuation test (TA1535 - S9)	5.0E-06	1	-	1.0000
Ames fluctuation test (TA1535 - S9)	1.0E-05	0	-	0.5000
Ames fluctuation test (TA1535 - S9)	5.0E-05	0	-	0.5000
Ames fluctuation test (TA1535 - S9)	1.0E-04	0	-	0.5000
Ames fluctuation test (TA1535 + S9)	5.0E-06	4	-	0.5000
Ames fluctuation test (TA1535 + S9)	1.0E-05	3	-	0.3572
Ames fluctuation test (TA1535 + S9)	5.0E-05	0	-	0.0280
Ames fluctuation test	1.0E-04	1	-	0.1019

(TA1535 + S9)				
Ames fluctuation test (TA1537-S9)	5.0E-06	0	-	0.5000
Ames fluctuation test (TA1537-S9)	1.0E-05	0	-	0.5000
Ames fluctuation test (TA1537-S9)	5.0E-05	0	-	0.5000
Ames fluctuation test (TA1537-S9)	1.0E-04	0	-	0.5000
Ames fluctuation test (TA1537 + S9)	5.0E-06	1	-	0.5000
Ames fluctuation test (TA1537 + S9)	1.0E-05	0	-	1.0000
Ames fluctuation test (TA1537 + S9)	5.0E-05	1	-	0.5000
Ames fluctuation test (TA1537 + S9)	1.0E-04	1	-	0.5000

Ames background results

Assay	Count(# of wells)
Ames fluctuation test (TA98 - S9)	5
Ames fluctuation test (TA98 + S9)	4
Ames fluctuation test (TA100 - S9)	1
Ames fluctuation test (TA100 + S9)	9
Ames fluctuation test (TA1535 - S9)	1
Ames fluctuation test (TA1535 + S9)	5
Ames fluctuation test (TA1537-S9)	1
Ames fluctuation test (TA1537 + S9)	1

Ames reference compounds results

Assay	Compound I.D.	Count(# of wells)	PositiveSignificance(- to +++)	FisherExact Test(p-value)
Ames fluctuation test (TA98 - S9)	Streptozotocin	15	+	0.0111
Ames fluctuation test (TA98 - S9)	9-Aminoacridine	2	-	0.2176
Ames fluctuation test (TA98 - S9)	2-Aminoanthracene	0	-	0.0280
Ames fluctuation test (TA98 - S9)	Quercetin	17	++	0.0033
Ames fluctuation test (TA98 + S9)	Streptozotocin	15	++	0.0046
Ames fluctuation test (TA98 + S9)	9-Aminoacridine	10	-	0.0732
Ames fluctuation test (TA98 + S9)	2-Aminoanthracene	47	+++	0.0000
Ames fluctuation test (TA98 + S9)	Quercetin	47	+++	0.0000
Ames fluctuation test (TA100 - S9)	Streptozotocin	43	+++	0.0000
Ames fluctuation test (TA100 - S9)	9-Aminoacridine	1	-	1.0000
Ames fluctuation test (TA100 - S9)	2-Aminoanthracene	2	-	0.5000

Ames fluctuation test (TA100 - S9)	Quercetin	0	-	0.5000
Ames fluctuation test (TA100 + S9)	Streptozotocin	30	+++	0.0000
Ames fluctuation test (TA100 + S9)	9-Aminoacridine	11	-	0.4011
Ames fluctuation test (TA100 + S9)	2-Aminoanthracene	31	+++	0.0000
Ames fluctuation test (TA100 + S9)	Quercetin	10	-	0.5000
Ames fluctuation test (TA1535 - S9)	Streptozotocin	48	+++	0.0000
Ames fluctuation test (TA1535 - S9)	9-Aminoacridine	1	-	1.0000
Ames fluctuation test (TA1535 - S9)	2-Aminoanthracene	1	-	1.0000
Ames fluctuation test (TA1535 - S9)	Quercetin	0	-	0.5000
Ames fluctuation test (TA1535 + S9)	Streptozotocin	48	+++	0.0000
Ames fluctuation test (TA1535 + S9)	9-Aminoacridine	7	-	0.3795
Ames fluctuation test (TA1535 + S9)	2-Aminoanthracene	42	+++	0.0000
Ames fluctuation test (TA1535 + S9)	Quercetin	4	-	0.5000
Ames fluctuation test (TA1537-S9)	Streptozotocin	4	-	0.1808
Ames fluctuation test (TA1537-S9)	9-Aminoacridine	25	+++	0.0000
Ames fluctuation test (TA1537-S9)	2-Aminoanthracene	0	-	0.5000
Ames fluctuation test (TA1537-S9)	Quercetin	11	++	0.0018
Ames fluctuation test (TA1537 + S9)	Streptozotocin	2	-	0.2474
Ames fluctuation test (TA1537 + S9)	9-Aminoacridine	7	++	0.0062
Ames fluctuation test (TA1537 + S9)	2-Aminoanthracene	25	+++	0.0000
Ames fluctuation test (TA1537 + S9)	Quercetin	3	-	0.1211

Table 1

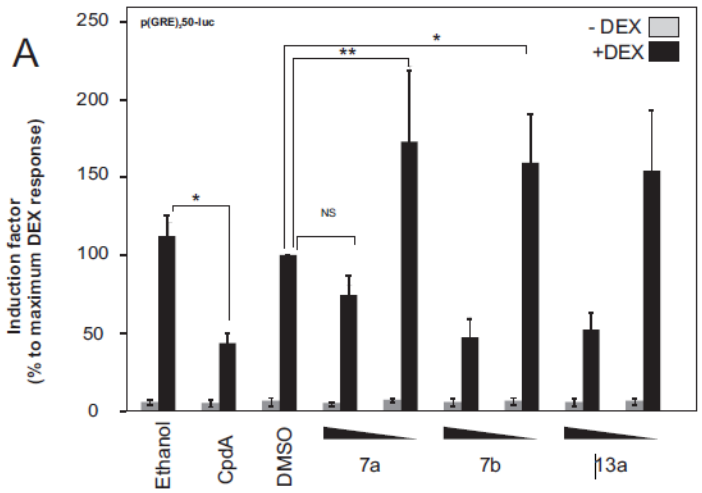
Table 1 *In vitro* enzyme inhibition data: HDAC6 inhibition[a]

Compound	R^1	R^2	% Inhibition (10 µM)	IC_{50} (µM)
7a	H	H	99.8	0.014
7b	Br	H	99.2	0.037
7c	Ph	H	95.1	0.31
7d	H	Me	73.4	2.4
8a	H	-	89.9	0.47
8b	Br	-	84.9	0.85
8c	Ph	-	47.9	N.D.[b]
13a	Br	-	99.3	0.064
13b	Ph	-	89.8	0.66
14a	Br	-	70.7	2.1
14b	Ph	-	61.1	N.D.[b]
18	-	-	99.0	0.2

[a] Reference compound: Trichostatin A (IC_{50} = 0.014 µM)

[b] Not Determined (< 70% inhibition at 10 µM)

Figure 4



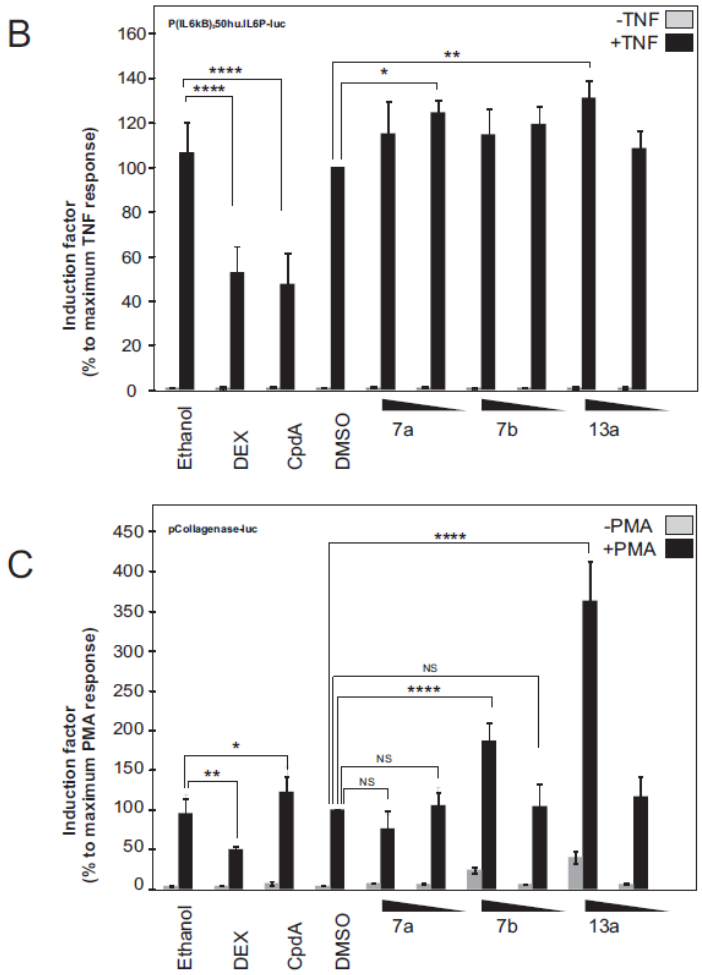


Figure 4 Influence of HDAC6 inhibitors **7a**, **7b** and **13a** on the transcriptional level of a GRE-dependent luciferase reporter gene construct (A), an NF-κB-dependent recombinant promoter construct (B) and an AP1-dependent luciferase reporter gene construct (C).

Figure 5

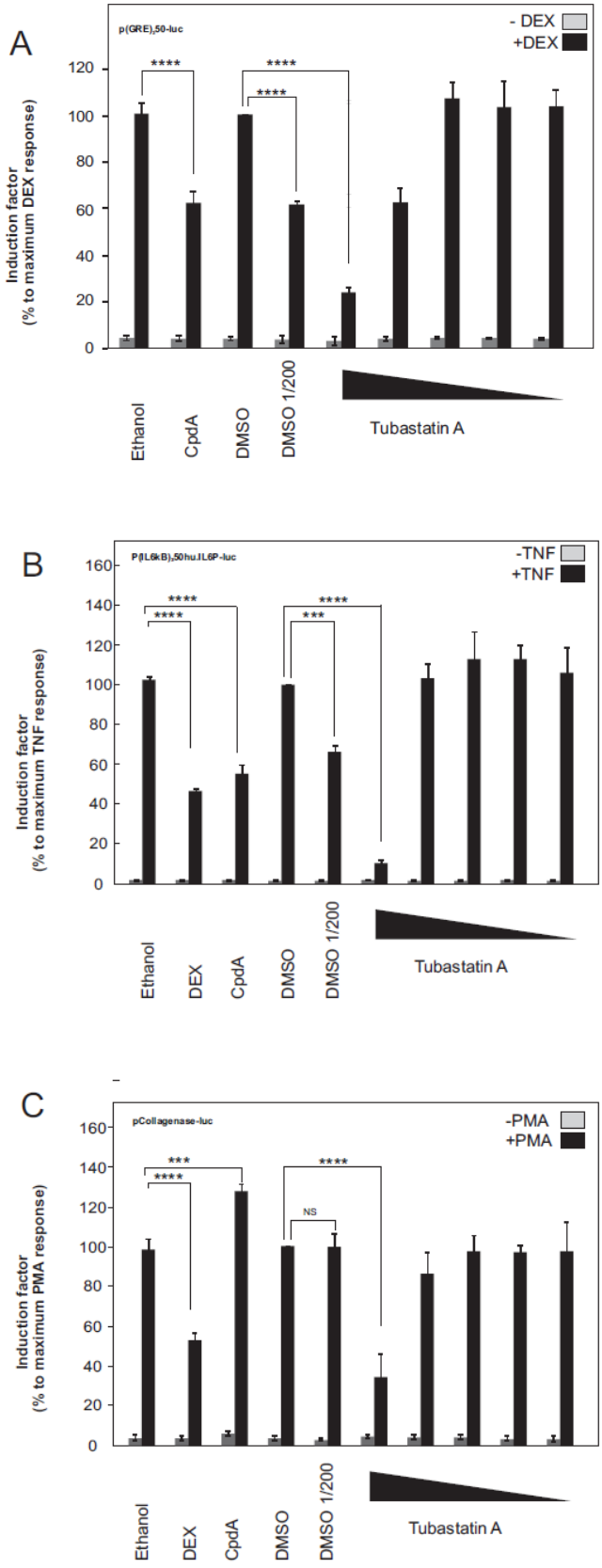


Figure 5 Influence of HDAC6 inhibitor Tubastatin A on the transcriptional level of a GRE-dependent luciferase reporter gene construct (A), an NF-κB-dependent recombinant promoter construct (B) and an AP1-dependent luciferase reporter gene construct (C).