Supporting Information

Heterodimeric GW7604 Derivatives: Modification of the Pharmacological Profile by Additional Interactions at the Coactivator Binding Site

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1. Synthesis of intermediates

Syntheses of GW7604 and methoxy-GW7604 was performed as described previously.¹

1.1. Synthesis of thioxo-quinazolinones

General procedure for synthesis of thioxo-quinazolinone carboxylic acids

Modifying the method from Sun et al.², 2-methoxycarbonylphenyl isothiocyanate (1 eq) and the respective amino carboxylic acid (1 eq) were dissolved in EtOH and refluxed for 2-3 days. After cooling the solution to ambient temperature and concentrating to half of the volume, the resulting precipitate was filtered off by suction and washed with ice-cold EtOH.

4-(4-Oxo-2-thioxodihydroquinazolin-3-yl)butanoic acid 1

1 was prepared following the general procedure with 580 mg of 2-methoxycarbonylphenyl isothiocyanate (3.00 mmol), 309 mg of 4-aminobutanoic acid (3.00 mmol) dissolved in 15 mL of EtOH. The mixture was refluxed for 2 days and yielded 1 as a pearl colored powder (538 mg, 2.04 mmol, 68%). ¹H NMR: (200 MHz, DMSO-d₆): δ 1.94 (p, 3J = 7.1 Hz, 2H, NCH₂CH₂CH₂COOH), 2.29 (t, 3J = 7.8 Hz, 2H, NCH₂CH₂CH₂COOH), 4.44 (t, 3J = 7.4 Hz, 2H, NCH₂CH₂CH₂COOH), 7.29-7.40 (m, 2H, ArH), 7.70-7.78 (m, 1H, ArH), 8.20 (d, 3J = 8.0 Hz, 1H, ArH).

HRMS (m/z): calculated for C₁₆H₂₀N₂O₃S [M-H]: 319.1122, found: 319.1735.

8-(4-Oxo-2-thioxodihydroquinazolin-3-yl)octanoic acid 2

2 was synthesized according to the general procedure with 386 mg of 2-methoxycarbonylphenyl isothiocyanate (2.00 mmol), 206 mg of 8-aminooctanoic acid (2.00 mmol) dissolved in 10 mL of EtOH. Reaction time was 3 days. 2 was obtained as an off-white powder (380 mg, 1.08 mmol, 56%). ¹H NMR: (200 MHz, DMSO-d₆): δ 1.28-1.39 (m, 6H, NCH₂CH₂CH₂CH₂CH₂CH₂COOH), 1.29-1.59 (m, 2H, NCH₂CH₂CH₂CH₂CH₂CH₂COOH), 1.59-1.83 (m, 2H, NCH₂CH₂CH₂CH₂CH₂CH₂COOH), 2.19 (t, 3J = 7.2 Hz, 2H, NCH₂CH₂CH₂CH₂CH₂CH₂CH₂COOH), 4.38 (t, 3J = 7.2 Hz, 2H, NCH₂CH₂CH₂CH₂CH₂CH₂CH₂COOH), 7.29-7.40 (m, 2H, ArH), 7.71-7.78 (m, 1H, ArH), 7.96 (d, 3J = 8.0 Hz, 1H, ArH). HRMS (m/z): calculated for C₁₆H₂₆N₂O₃S [M-H]: 319.1122, found: 319.1735.
General procedure for synthesis of thioxo-quinazolinone carboxamides

Similar to the previously mentioned general procedures for amide formation, PyBOP (1.0 eq) was dissolved in dry DCM and DIPEA (2.0 eq) together with the respective acid (1.0 eq) in dry DMF were added at 0 °C. After 5 min, the respective amine (1.1 eq) in dry DCM or DMF was supplemented dropwise. The mixture was stirred first at 0 °C for 30 min, then at rt for 20 h. The mixture was concentrated to a residue, which was dissolved in EA, washed with water (pH = 3-4; adjusted with 1N HCl) and further extracted twice with EA. The combined organic layers were washed with brine, dried over dry Na₂SO₄ and evaporated. Purification was carried out by flash column chromatography.

*N-[3-(Boc-amino)propyl]-4-[4-oxo-2-thioxodihydroquinazolin-3-yl]butanamide 3*

Synthesis of 3 was carried out according to the general procedure. 1 (120 mg, 0.45 mmol) was dissolved in 2.0 mL of dry DMF. 260 mg of PyBOP (1.1 eq, 0.50 mmol) in 2.0 mL of dry DCM, 0.16 mL of DIPEA (0.91 mmol) and 87 mg of N-Boc-1,3-propanediamine (0.50 mmol) in 0.5 mL of dry DCM were added. Flash column chromatography with PE and EA (83:17 → 100% EA) led to a waxy, white powder (175 mg, 0.42 mmol, 92%). ¹H NMR: (200 MHz, DMSO-d₆): δ 1.36 (s, 9H, COOC(CH₃)₃), 1.44-1.50 (m, 2H, NCH₂CH₂CH₂CONH), 1.87-1.99 (m, 2H, NHCH₂CH₂CH₂NH), 2.13 (t, ³J = 7.8 Hz, 2H, NCH₂CH₂CH₂CONH), 2.89-2.95 (m, 2H, NHCH₂CH₂CH₂NH), 3.00 (q, ³J = 6.2 Hz, 2H, NHCH₂CH₂CH₂NH), 4.41 (t, ³J = 7.0 Hz, 2H, NCH₂CH₂CH₂CONH), 6.74 (t, ³J = 5.4 Hz, 1H, NH), 7.30-7.50 (m, 2H, ArH), 7.70-7.82 (m, 2H, NH + ArH), 7.97 (m, 1H, ArH).

*N-[4-(Boc-amino)butyl]-8-[4-oxo-2-thioxodihydroquinazolin-3-yl]octanamide 4*

Following the general procedure, 2 (100 mg, 0.31 mmol) was dissolved in 2.0 mL of dry DMF. 162 mg of PyBOP (0.31 mmol) in 2.0 mL of dry DCM, 0.11 mL of DIPEA (0.62 mmol) and 65 mg of N-Boc-1,4-butanediamine (0.34 mmol) in 0.5 mL dry DCM were added. Flash column chromatography applying a gradient of PE and EA (3:1 → 100% EA) resulted in a white powder (95 mg, 0.19 mmol, 62%). ¹H NMR: (200 MHz, DMSO-d₆): δ 1.17-1.36 (m, 19H, CH₃+ COOC(CH₃)₃), 1.46-1.51 (m, 2H, CH₂), 1.59-1.67 (m, 2H, CH₂), 2.03 (t, ³J = 7.2 Hz, 2H, CH₂CONH), 2.87-2.89 (m, 2H, CH₂NCOOC(CH₃)₃), 2.98-3.00 (m, 2H, CONHCH₂), 4.38 (t, ³J = 7.6 Hz, 2H, NCH₂), 6.77 (brt, 1H, NH), 7.29-7.40 (m, 2H, ArH), 7.70-7.78 (m, 2H, NH + ArH), 7.96 (d, ³J = 8.0 Hz, 1H, ArH).
Ethyl 4-[4-(4-oxo-2-thioxodihydroquinazolin-3-yl)butanoyl]piperazin-1-yl]benzoate 5

5 was synthesized following the general procedure for preparation of thioxo-quinazolinone carboxamides. 1 (381 mg, 1.44 mmol) was dissolved in 4 mL of dry DMF. 0.40 mL of DIPEA (2.27 mmol), 786 mg of PyBOP (1.05 eq, 1.51 mmol) in 8 mL of dry DCM and 355 mg of ethyl 4-(piperazinyl)benzoate in 6 mL of dry DCM were added in this order. After 1 h, the clear solution turned into a cloudy suspension. The next day the solid was filtered by suction and washed with cold MeOH to yield 5 as a shiny white powder (530 mg, 1.1 mmol, 73%). \(^1\)H NMR: (200 MHz, DMSO-d<sub>6</sub>): δ 1.29 (t, \(^3\)J = 7.0 Hz, 3H, COOCH<sub>2</sub>CH<sub>3</sub>), 1.30-1.40 (m, 6H, CH<sub>2</sub>), 1.41-1.59 (m, 2H, CH<sub>2</sub>), 1.59-1.78 (m, 2H, CH<sub>2</sub>), 2.34 (t, \(^3\)J = 7.2 Hz, 2H, CH<sub>2</sub>CON(CH<sub>2</sub>CH<sub>2</sub>)<sub>N</sub>), 3.57-3.68 (m, 4H, CON(CH<sub>2</sub>CH<sub>2</sub>)<sub>N</sub>), 4.24 (q, \(^3\)J = 7.0 Hz, 2H, COOCH<sub>2</sub>CH<sub>3</sub>), 4.38 (t, \(^3\)J = 7.6 Hz, 2H, Ar-CH<sub>2</sub>), 6.98 (d, \(^3\)J = 8.8 Hz, 1H, ArH), 7.29-7.41 (m, 2H, ArH), 7.70-7.82 (m, 3H, ArH), 7.94 (d, \(^3\)J = 8.0 Hz, 1H, ArH).

Ethyl 4-[4-(8-(4-oxo-2-thioxodihydroquinazolin-3-yl)octanoyl]piperazin-1-yl]benzoate 6

6 was synthesized according to the general procedure for the preparation of thioxo-quinazolinone carboxamides using 372 mg of 2 (1.16 mmol) in 3.5 mL of dry DMF. 0.44 mL (1.25 mmol) of DIPEA, 650 mg of PyBOP (1.25 mmol) in 8 mL of dry DCM and 293 mg of ethyl 4-(piperazinyl)benzoate (1.25 mmol) in 6 mL of dry DCM were added in that order. The next day, water and 2.5N NaOH were added (to adjust pH = 8-9) and the residue was extracted 3× with EA. Then, the combined organic extracts were washed with H<sub>2</sub>O/1N HCl (pH = 2-3) whereupon the EA phase turned to cloudy. The aqueous phase was further extracted 2× with EA, then the combined organic extracts were washed with brine, dried over dry Na<sub>2</sub>SO<sub>4</sub> and evaporated. The crude product was washed with water and cold MeOH over a sintered glass funnel to remove salts and organic by-products, giving 6 as a white powder (344 mg, 0.64 mmol, 55%). \(^1\)H NMR: (200 MHz, DMSO-d<sub>6</sub>): δ 1.29 (t, \(^3\)J = 7.0 Hz, 3H, COOCH<sub>2</sub>CH<sub>3</sub>), 1.30-1.40 (m, 6H, CH<sub>2</sub>), 1.41-1.59 (m, 2H, CH<sub>2</sub>), 1.59-1.78 (m, 2H, CH<sub>2</sub>), 2.34 (t, \(^3\)J = 7.2 Hz, 2H, CH<sub>2</sub>CON(CH<sub>2</sub>CH<sub>2</sub>)<sub>N</sub>), 3.57-3.68 (m, 4H, CON(CH<sub>2</sub>CH<sub>2</sub>)<sub>N</sub>), 4.24 (q, \(^3\)J = 7.0 Hz, 2H, COOCH<sub>2</sub>CH<sub>3</sub>), 4.38 (t, \(^3\)J = 7.6 Hz, 2H, Ar-CH<sub>2</sub>), 6.98 (d, \(^3\)J = 8.8 Hz, 1H, ArH), 7.29-7.41 (m, 2H, ArH), 7.70-7.82 (m, 3H, ArH), 7.94 (d, \(^3\)J = 8.0 Hz, 1H, ArH).

4-[4-(4-Oxo-2-thioxodihydroquinazolin-3-yl)butanoyl]piperazin-1-yl]benzoic acid 7

For ester cleavage, 5 (400 mg, 0.82 mmol) was dissolved in 8 mL of EtOH/THF (1:1) and 4 mL of 2N KOH and the mixture was stirred for 24 h. The solution was concentrated, cooled and 8 mL of 1N HCl were added. The white powder was separated by vacuum filtration (376 mg, 0.82 mmol, quant.). \(^1\)H NMR: (200 MHz, DMSO-d<sub>6</sub>): δ 1.97 (p, \(^3\)J = 7.2 Hz, 2H, NCH<sub>2</sub>CH<sub>2</sub>CON), 2.46 (t, \(^3\)J = 7.6 Hz, 2H, NCH<sub>2</sub>CH<sub>2</sub>CH<sub>2</sub>CON), 3.22-3.33 (m, 4H, CON(CH<sub>2</sub>CH<sub>2</sub>)<sub>N</sub>), 4.46 (t, \(^3\)J = 6.0 Hz, 2H, NCH<sub>2</sub>), 6.98 (d, \(^3\)J = 8.8 Hz, 1H, ArH), 7.28-7.42 (m, 2H, ArH), 7.71-7.81 (m, 3H, ArH), 7.95 (d, \(^3\)J = 8.0 Hz, 1H, ArH).
4-[4-(8-(4-Oxo-2-thioxodihydroquinazolin-3-yl)octanoyl)piperazin-1-yl]benzoic acid 8

6 (340 mg, 0.82 mmol) was dissolved in 6.8 mL of EtOH/THF (1:1) and 3.4 mL of 2N KOH and the mixture was stirred for 24 h. The solution was concentrated, cooled and 4 mL of 2N HCl were added. The solids were collected by vacuum filtration and gave 8 as a white powder (349 mg, 0.69 mmol, 93%). $^1$H NMR: (200 MHz, DMSO-d$_6$): δ 1.17-1.39 (m, 6H, CH$_2$), 1.39-1.59 (m, 2H, CH$_2$), 1.59-1.78 (m, 2H, CH$_2$), 2.35 (t, $^3$J = 7.2 Hz, 2H, CH$_2$CON(CH$_2$CH$_2$)$_2$N), 3.56-3.75 (m, 4H, CON(CH$_2$CH$_2$)$_2$N), 4.38 (t, $^3$J = 7.7 Hz, 2H, NCH$_2$), 6.97 (d, $^3$J = 9.2 Hz, 1H, ArH), 7.27-7.41 (m, 2H, ArH), 7.70-7.80 (m, 3H, ArH), 7.96 (d, $^3$J = 7.4 Hz, 1H, ArH), 12.39 (brs, 2H, COOH + NH).

N-3-(Boc-aminopropyl)-4-[4-(4-oxo-2-thioxodihydroquinazolin-3-yl)butanoyl]piperazin-1-yl]benzamide 9

Following the general procedure for the synthesis of thioxo-quinazolinone carboxamides, 7 (200 mg, 0.44 mmol) was dissolved in 3 mL of dry DMF and 0.15 mL of DIPEA (0.88 mmol) was added, leading to an intense yellow colored solution. The color changed to red after addition of 230 mg (0.44 mmol) of PyBOP dissolved in 4 mL of dry DCM. At last, N-Boc-1,3-propanediamine (71 mg, 0.44 mmol) dissolved in 3 mL of dry DCM was added. The next day, the reaction was quenched with water whereupon the red color faded. The resulting precipitate was filtered off by vacuum suction and washed with cold MeOH (145 mg, 0.24 mmol, 54%). $^1$H NMR: (200 MHz, DMSO-d$_6$): δ 1.37 (s, 9H, COOC(CH$_3$)$_3$), 1.56 (p, $^3$J = 7.0 Hz, 2H, NHCH$_2$CH$_2$CH$_2$N), 1.97 (p, $^3$J = 6.6 Hz, 2H, NCH$_2$CH$_2$CH$_2$CON), 2.46 (t, $^3$J = 7.0 Hz, 2H, NCH$_2$CH$_2$CH$_2$CON), 2.95 (q, $^3$J = 6.2 Hz, 2H, NHCH$_2$CH$_2$CH$_2$NH), 3.23-3.35 (m, 4H, CON(CH$_2$CH$_2$)$_2$N), 3.54-3.66 (m, 4H, CON(CH$_2$CH$_2$)$_2$N), 4.46 (t, $^3$J = 6.8 Hz, 2H, NCH$_2$), 6.81 (brt, 1H, NH), 6.97 (d, $^3$J = 8.8 Hz, 2H, ArH), 7.28-7.40 (m, 2H, ArH), 7.71-7.75 (m, 3H, ArH), 7.94 (d, $^3$J = 7.6 Hz, 1H, ArH), 8.18 (brt, 1H, NH).

N-3-(Boc-aminopropyl)-4-[4-(8-(4-oxo-2-thioxodihydroquinazolin-3-yl)octanoyl)piperazin-1-yl]benzamide 10

10 was synthesized according to the general procedure for the synthesis of thioxo-quinazolinone carboxamides. 0.15 mL of DIPEA (0.87 mmol, 2 eq), 225 mg of PyBOP (0.43 mmol) in 4 mL of dry DCM and 76 mg of N-Boc-1,3-propanediamine (0.43 mmol) in 3 mL of dry DCM were added to 220 mg of 8 (0.43 mmol) in 3 mL of dry DMF. The following day, water and 2.5N NaOH were added (pH = 8-9) and the residue was extracted 3× with EA. Then, the combined organic extracts were washed with water/1N HCl (pH = 2-3). The aqueous phase was further extracted twice with EA and the combined organic extracts were evaporated to dryness. Purification was carried out by flash column chromatography applying a PE:EA:MeOH gradient (EA: 24% → 100%, then MeOH 0% → 5%), which afforded 10 as a white foam
(177 mg, 0.27 mmol, 62%). $^1$H NMR: (200 MHz, DMSO-d$_6$): $\delta$ 1.28-1.36 (m, 6H, CH$_2$), 1.37 (s, 9H, COOC(CH$_3$)$_3$), 1.47-1.66 (m, 6H, CH$_2$), 2.35 (t, $^3$J = 7.6 Hz, 2H, CH$_2$CON(CH$_2$CH$_2$)$_2$N), 2.89-3.00 (m, 2H, NHCH$_2$CH$_2$CH$_2$NH), 3.14-3.37 (m, 6H, CH$_2$), 3.53-3.66 (m, 4H, CON(CH$_2$CH$_2$)$_2$N), 4.39 (t, $^3$J = 8.0 Hz, 2H, NCH$_2$), 6.81 (t, $^2$J = 5.4 Hz, 2H, NH), 6.96 (d, $^2$J = 8.8 Hz, 2H, ArH), 7.29-7.41 (m, 2H, ArH), 7.71-7.78 (m, 3H, NH + ArH), 7.95 (d, $^2$J = 7.0 Hz, 1H, ArH), 8.17 (t, $^2$J = 5.6 Hz, 1H, NH).

3-[4-(4-Oxo-2-thioxo-dihydroquinazolin-3-yl)butanamido]propan-1-aminium trifluoroacetate 11

According to the general procedure for Boc-deprotection of amines (see 8.1.2.3), 160 mg of 3 (0.38 mmol) were suspended in 6 mL of dry DCM and 0.44 mL of TFA (5.71 mmol, 15 eq) were added dropwise. 11 was obtained as white powder (160 mg, 0.37 mmol, 99%). $^1$H NMR: (200 MHz, DMSO-d$_6$): $\delta$ 1.66 (p, $^1$J = 7.0 Hz, 2H, CH$_2$), 1.85-1.96 (m, 2H, CH$_2$), 2.17 (t, $^3$J = 7.3 Hz, 2H, NCH$_2$CH$_2$CH$_2$CH$_2$CONH), 2.73-2.83 (m, 2H, NHCH$_2$CH$_2$CH$_2$NH$_3^+$), 3.10 (q, $^3$J = 6.2 Hz, 2H, NHCH$_2$CH$_2$CH$_2$NH), 4.42 (t + brs, $^3$J = 7.2 Hz, 5H, NCH$_2$ + NH$_3^+$), 7.31-7.45 (m, 2H, ArH), 7.59-7.79 (m, 2H, ArH + NH), 7.95-8.04 (m, 2H, ArH + NH).

4-[8-(4-Oxo-2-thioxodihydroquinazolin-3-yl)octanamido]butan-1-aminium trifluoroacetate 12

According to the general procedure for Boc-deprotection of amines, 90 mg of 4 (0.18 mmol) were suspended in 3 mL of dry DCM and 0.26 mL of TFA (2.7 mmol, 18 eq) were slowly added whereupon the suspension turned clear. Workup resulted in a white solid (90 mg, 0.18 mmol, 99%). $^1$H NMR: (200 MHz, DMSO-d$_6$): $\delta$ 1.24-1.46 (m, 12H, CH$_2$), 1.60-1.81 (m, 2H, CH$_2$), 2.05 (t, $^3$J = 7.0 Hz, 2H, CH$_2$CONH), 2.80 (t, $^2$J = 6.6 Hz, 2H, CH$_2$NH$_3^+$), 3.04 (q, $^3$J = 5.8 Hz, 2H, CONHCH$_2$), 4.38 (t, $^3$J = 7.8 Hz, 2H, NCH$_2$), 7.31-7.41 (m, 2H, ArH), 7.51-7.82 (m, ArH + NH$_3^+$), 7.40 (d, $^2$J = 7.8 Hz, 1H, ArH).

3-[4-(4-(4-Oxo-2-thioxodihydroquinazolin-3-yl)butanoyl)piperazin-1-yl]benzamido]propan-1-aminium trifluoroacetate 13

13 was synthesized following the general procedure for Boc-deprotection with 140 mg of 9 (0.23 mmol), 4 mL of dry DCM and 0.27 mL of TFA (3.45 mmol, 15 eq). A white solid was obtained (143 mg, 0.23 mmol, quant.). $^1$H NMR: (200 MHz, DMSO-d$_6$): $\delta$ 1.78 (p, $^2$J = 6.8 Hz, 2H, NHCH$_2$CH$_2$CH$_2$NH$_3^+$), 1.98 (p, $^2$J = 7.3 Hz, 2H, NCH$_2$CH$_2$CH$_2$CON), 2.40-2.51 (m, 2H, NCH$_2$CH$_2$CH$_2$CON), 2.82 (q, $^3$J = 7.0 Hz, NHCH$_2$CH$_2$CH$_2$NH$_3^+$), 3.17-3.32 (m, 6H, CON(CH$_2$CH$_2$)$_2$N + NHCH$_2$CH$_2$CH$_2$NH$_3^+$), 3.66-3.81 (m, 4H, CON(CH$_2$CH$_2$)$_2$N), 4.46 (t, $^3$J = 7.2 Hz, 1H, NCH$_2$CH$_2$CH$_2$CON), 6.97 (d, $^3$J = 8.4 Hz, 2H, ArH), 7.29-7.41 (m, 2H, ArH), 7.62-7.83 (m, 6H, ArH + NH$_3^+$), 7.95 (d, $^2$J = 7.6 Hz, 1H, ArH), 8.38 (t, $^2$J = 5.8 Hz, 1H, NH).
3-[4-(4-(8-(4-Oxo-2-thioxodihydroquinazolin-3-yl)octanoyl)piperazin-1-yl)benzamido]propan-1-aminium trifluoroacetate 14

14 was synthesized according to the general procedure for Boc-deprotection, using 160 mg of 10 (0.24 mmol), 4.5 mL of dry DCM and an excess of TFA (1.0 mL). Workup resulted in a white powder (163 mg, 0.24 mmol, quant.). 1H NMR: (200 MHz, DMSO-d6): δ 1.24-1.41 (m, 6H, CH2), 1.51-1.81 (m, 6H, CH2), 2.35 (t, 1J = 7.4 Hz, 2H, CH2CON(CH2CH22N)), 2.80-2.89 (m, 2H, NHCH2CH22NH3+), 3.26-3.31 (m, 6H, CON(CH2CH22N + NHCH2CH22NH3+), 3.49-3.68 (m, 4H, CON(CH2CH22N), 6.98 (d, 3J = 8.8 Hz, 2H, ArH), 7.30-7.41 (m, 2H, ArH), 7.72-7.78 (m, 6H, ArH, + NH3+), 7.96 (d, 2J = 7.4 Hz, 1H, ArH), 8.38 (brt, 1H, NH).

1.2. Synthesis of 1H-benzo[d]imidazoles

Ethyl 3-(5-methoxy-1H-benzo[d]imidazole-2-yl)propanoate 19

19 was prepared adopting a described procedure. 4-Methoxybenzene-1,2-diamine (1.0 eq, 5.00 g, 36.2 mmol), succinic anhydride (1.2 eq, 4.35 g, 43.5 mmol) and dioxane (90 mL) were mixed and heated to 80 °C for 24 h. After cooling to rt, the mixture was concentrated to a sticky residue, which was dissolved in EtOH (90 mL). Concentrated H2SO4 (2 mL) was subsequently added dropwise and the mixture was heated again for 24 h to 80 °C. After cooling, the solution was concentrated, water was added (80 mL), the pH was adjusted to 8-9 with 1N NaOH and extracted three times with DCM (100 mL). The combined organic layers were washed with water (20 mL), brine, dried over Na2SO4 and evaporated. The crude product was dissolved in DCM, adsorbed on silica and purified by flash chromatography with DCM and MeOH (98:2 → 84:16) to yield 19 as a red-brown sticky solid (3.90 g, 15.6 mmol, 43%). 1H NMR: (200 MHz, CD3OD): δ 1.20 (t, 3J = 7.4 Hz, 3H, OCH2CH3), 2.87 (t, 1J = 7.2 Hz, 2H, CH2CH2COCH2CH3), 3.14 (t, 3J = 6.8 Hz, 2H, CH2CH2COCH2CH3), 3.82 (s, 3H, OCH3), 4.12 (q, 5J = 6.8 Hz, 3H, OCH2CH3), 6.82 (dd, 3J = 8.8 Hz, 4J = 2.4 Hz, 1H, ArH3), 7.00 (d, 4J = 2.6 Hz, 1H, ArH3), 7.36 (d, 3J = 8.8 Hz, 1H, ArH). 13C NMR: (50 MHz, CD3OD): δ 14.48, 25.03, 33.00, 56.20, 61.81, 98.46, 112.71, 157.84, 173.85.

3-(5-Methoxy-1H-benzo[d]imidazol-2-yl)propanoic acid hydrochloride 20

19 (780 mg, 3.14 mmol) was dissolved in 2 N HCl (13 mL) and heated to reflux for 4 h. Removal of water under reduced pressure led to 20 as dark brown crystals (806 mg, 3.14 mmol, quant.). 1H NMR: (200 MHz, CD3OD): δ 3.00 (t, 3J = 6.8 Hz, 2H, CH2CH2COOH), 3.37 (t, 3J = 6.0 Hz, 2H, CH2CH2COOH), 3.90 (s, 3H, OCH3), 7.14-7.20 (m, 2H, ArH6, ArH6), 7.61 (d, 5J = 8.7 Hz, 1H, ArH7).
General procedure for benzimidazole monoamide formation

Benzimidazole monoamide formation was performed based on the general procedure for homo diamide formation and was further optimized regarding temperate and work up. PyBOP (1.1 eq) dissolved in dry DCM was added to a solution of 20 (1.0 eq) in dry DMF at 0 °C under an argon atmosphere. The mixture was stirred for 5 min. Then, DIPEA (6.0 eq) was added dropwise followed by the addition of the respective mono N-Boc-protected diamine (1.0 eq) in dry DMF. The mixture was stirred first at 0 °C for 30 min, then at 40 °C for 20 h. Afterwards, the solvents were concentrated and the residue was dissolved in EA, washed with water (pH = 8-9; adjusted with 2N NaOH if needed), dried over Na2SO4 and evaporated. Purification was achieved by column chromatography with DCM and MeOH as eluent (93:7) followed by crystallization from MeOH/water.5,8

\[ N-[2-(Boc-amino)ethyl]-3-(5-methoxy-1H-benzo[\text{d}]imidazole-2-yl)propanamide 21 \]

21 was synthesized following the general procedure with 400 mg of 20 (1.56 mmol) in 2 mL of dry DMF, 315 mg of N-Boc-1,2-diaminoethane (1.56 mmol) in 0.4 mL of dry DMF, 1.52 mL of DIPEA (8.58 mmol) and 893 mg of PyBOP (1.72 mmol) in 4 mL of dry DCM. 21 was obtained as a pearl-colored powder (491 mg, 1.35 mmol, 87%). 1H NMR: (200 MHz, CD3OD): δ 1.41 (s, 9H, COOC(CH3)3), 2.72 (t, 3J = 7.4 Hz, 2H, CH2CH2CONH), 3.11-3.18 (m, 4H, CH2CH2CONH, CH2CH2NHCOOC(CH3)3), 3.21-3.27 (m, 2H, CH2CH2NHCOOC(CH3)3), 3.82 (s, 3H, OCMe), 3.81 (s, 3H, OCMe), 2.70 (t, J = 7.8 Hz, 2H, ArH), 7.01 (d, 4J = 2.2 Hz, 1H, ArH), 7.39 (d, 3J = 8.8 Hz, 1H, ArH).

\[ N-[3-(Boc-amino)propyl]-3-(5-methoxy-1H-benzo[d]imidazol-2-yl)propanamide 22 \]

22 was synthesized applying 160 mg of 20 (0.63 mmol) in 1.3 mL of dry DMF, 109 mg of N-Boc-1,3-diaminopropane (0.63 mmol) in 0.4 mL of dry DMF, 0.66 mL of DIPEA (3.8 mmol) and 358 mg of PyBOP (0.69 mmol) in 3.5 mL of dry DCM. 22 was obtained as a white powder (130 mg, 0.35 mmol, 55%). 1H NMR: (200 MHz, CD3OD): δ 1.43 (s, 9H, COOC(CH3)3), 1.58 (p, 3J = 6.6 Hz, 2H, CH2CH2CH2NHCOOC(CH3)3), 2.70 (t, 3J = 7.5 Hz, 2H, CH2CH2CONH), 2.98 (t, 3J = 6.6 Hz 2H, CH2CH2CONH), 3.13 (t, 3J = 7.8 Hz, 2H, CH2CH2CH2NHCOOC(CH3)3), 3.19 (t, 3J = 6.8 Hz, 2H, CH2CH2CH2NHCOOC(CH3)3), 3.81 (s, 3H, OCH3), 6.53 (brs, 1H, NCOOC(CH3)3), 6.82 (dd, 4J = 8.8 Hz, 4J = 2.2 Hz, 1H, ArH), 6.99 (d, 4J = 2.1 Hz, 1H, ArH), 7.36 (d, 3J = 8.8 Hz, 1H, ArH).
N-[4-(Boc-amino)butyl]-3-(5-methoxy-1H-benzo[d]imidazol-2-yl)propanamide 23

23 was synthesized using 215 mg of 20 (0.84 mmol) in 1.3 mL of dry DMF, 158 mg of N-Boc-1,4-diaminobutane (0.84 mmol) in 0.4 mL of dry DMF, 0.89 mL of DIPEA (5.0 mmol) and 523 mg of PyBOP (1.01 mmol) in 3 mL of dry DCM. 23 was obtained as a beige powder (210 mg, 0.54 mmol, 64%). 1H NMR: (200 MHz, CD3OD): δ 1.35-1.42 (m, 13H, CH2CH2CH2CH2NHCOC(CH3)3), 2.70 (t, J = 7.5 Hz, 2H, CH2CH2CONH), 2.97 (t, J = 6.1 Hz, 2H, CH2CH2CONH), 3.09-3.19 (m, 4H, CH2CH2CH2CH2NHCOC(CH3)3), 3.82 (s, 3H, OCMe3), 6.83 (dd, J = 8.8 Hz, J = 2.3 Hz, 1H, ArHδ), 7.00 (d, J = 2.2 Hz, 1H, ArHδ), 7.37 (d, J = 8.8 Hz, 1H, ArHγ).

N-[5-(Boc-amino)pentyl]-3-(5-methoxy-1H-benzo[d]imidazol-2-yl)propanamide 24

24 was prepared using 250 mg of 20 (0.97 mmol) in 1.5 mL of dry DMF, 197 mg of N-Boc-1,5-diaminopentane (0.97 mmol) in 0.4 mL of dry DMF, 1.03 mL of DIPEA (5.84 mmol) and 608 mg of PyBOP (1.17 mmol) in 4 mL of dry DCM. Column chromatography with a DCM and MeOH gradient (95:5 → 93:7) and crystallization (acetone/water) led to 20 as a pearl-brownish powder (170 mg, 0.42 mmol (43%). 1H NMR: (200 MHz, CD3OD): δ 1.30-1.55 (m, 15H, CH2CH2CH2CH2CH2NHCOC(CH3)3), 2.73 (t, J = 7.0 Hz, 2H, CH2CH2CONH), 2.88-3.27 (m, 6H, CH2CH2CH2CH2CH2NHCOC(CH3)3, CH2CH2CONH), 3.80 (s, 3H, OCMe3), 5.93 (brs, 1H, NHCOOC(CH3)3), 6.79 (dd, J = 8.7 Hz, J = 2.4 Hz, 1H, ArHδ), 7.06 (d, J = 2.4 Hz, 1H, ArHδ), 7.18 (brs, 1H, CH2CH2CONH), 7.40 (d, J = 8.7 Hz, 1H, ArHγ).

N-[6-(Boc-amino)hexyl]-3-(5-methoxy-1H-benzo[d]imidazol-2-yl)propanamide 25

25 was synthesized using 250 mg of 20 (0.97 mmol) in 1.5 mL of dry DMF, 211 mg of N-Boc-1,6-diaminohexane (0.97 mmol) in 0.4 mL dry DMF, 1.03 mL of DIPEA (5.84 mmol) and 608 mg of PyBOP (1.17 mmol) in 4 mL of dry DCM. Column chromatography and crystallization from acetone/water led to 25 as beige crystals (185 mg, 0.44 mmol, 45%). 1H NMR: (200 MHz, CD3OD): δ 1.22-1.38 (m, 4H, CH2), 1.39-1.51 (m, 13H, CH2 + COOC(CH3)3), 2.73 (t, J = 6.8 Hz, 2H, CH2CH2CONH), 2.97-3.07 (m, 2H, CH2CH2CONH), 3.12-3.23 (m, 4H, CH2CH2CH2CH2CH2NHCOC(CH3)3), 3.80 (s, 3H, OCMe3), 5.92 (brs, 1H, NHCOOC(CH3)3), 6.80 (dd, J = 8.8 Hz, J = 2.4 Hz, 1H, ArHδ), 7.06 (d, J = 2.4 Hz, 1H, ArHδ), 7.40 (d+brs, J = 8.8 Hz, 2H, ArHγ + CH2CH2CONH).

General procedure for Boc-deprotection of amines

N-Boc cleavage was performed revising literature procedures.7-9 TFA was added to the protected amine in dry DCM (DCM:TFA = 3:1) under an argon atmosphere at 0 °C. The reaction mixture was stirred for 2 h at 0 °C followed by the evaporation of the solvent under reduced pressure. The residue was treated with
MeOH and DCM several times to remove remaining TFA and then evaporated to dryness affording a brownish oil.

\[ N-(2\text{-Aminoethyl})-3-(5\text{-methoxy-1H-benzo[d]imidazol-2-yl})propanamide \text{ bis(trifluoroacetate) salt 26} \]

3.0 mL of TFA were added to 450 mg of 21 (1.24 mmol) in 9 mL of dry DCM (608 mg, 1.24 mmol, quant.).

\[ ^1H\text{ NMR: (200 MHz, DMSO-}d_6) \delta 2.66-2.98 \text{ (m, 4H, } CH_2CH_2CONH) \], 3.17-3.41 (m, 4H, \( CH_2CH_2NH_3^+ \)), 3.96 (s, 3H, OCH3), 5.31 (brs, 3H, NH3+), 7.11 (dd, \( ^3J = 9.0 \text{ Hz, } ^4J = 2.0 \text{ Hz, 1H, ArH}_5 \)), 7.23 (d, \( ^4J = 2.0 \text{ Hz, 1H, ArH}_6 \)), 7.66 (d, \( ^3J = 8.9 \text{ Hz, 1H, ArH}_7 \)), 7.86 (brs, 2H, NH3+), 8.31 (brt, 1H, CONH/CH2).

\[ N-(3\text{-Aminopropyl})-3-(5\text{-methoxy-1H-benzo[d]imidazol-2-yl})propanamide \text{ bis(trifluoroacetate) salt 27} \]

1.7 mL of TFA were added to 140 mg of 22 (0.37 mmol) in 5 mL of dry DCM (187 mg, 0.37 mmol, quant.).

\[ ^1H\text{ NMR: (400 MHz, (CD3)2CO) } \delta 1.98 \text{ (p, } ^3J = 6.8 \text{ Hz, 2H, } CH_2CH_2CH_2NH_3^+ \)), 2.41-2.68 (m, 2H, NH), 3.00 (t, \( ^3J = 6.8 \text{ Hz, 2H, } CH_2CH_2CONH \)), 3.32 (t, \( ^3J = 6.1 \text{ Hz, 2H, } CH_2CH_2CONH \)), 3.53 (t, \( ^3J = 6.7 \text{ Hz, 2H, } CH_2CH_2CH_2NH_3^+ \)), 3.83 (t, \( ^3J = 6.9 \text{ Hz, 2H, } CH_2CH_2CH_2NH_3^+ \)), 3.89 (s, 3H, OCH3), 7.14 (dd, \( ^3J = 9.0 \text{ Hz, } ^4J = 2.4 \text{ Hz, 1H, ArH}_7 \)), 7.35 (d, \( ^4J = 2.3 \text{ Hz, 1H, ArH}_7 \)), 7.72 (d, \( ^3J = 9.0 \text{ Hz, 1H, ArH}_6 \)), 8.14 (brs, 1H, NH).\n
\[ N-(4\text{-Aminobutyl})-3-(5\text{-methoxy-1H-benzo[d]imidazol-2-yl})propanamide \text{ bis(trifluoroacetate) salt 28} \]

2.2 mL of TFA were added to 206 mg of 23 (0.53 mmol) in 7 mL of dry DCM (273 mg, 0.53 mmol, quant.).

\[ ^1H\text{ NMR: (200 MHz, (CD3)2CO) } \delta 1.53 \text{ (p, 2H, } ^3J = 6.3 \text{ Hz, } CH_2CH_2CH_2CH_2NH_3^+ \)), 1.70 (p, 2H, \( ^3J = 6.2 \text{ Hz, } CH_2CH_2CH_2CH_2NH_3^+ \)), 2.44-2.58 (m, 2H, NH), 2.96 (t, \( ^3J = 7.3 \text{ Hz, 2H, } CH_2CH_2CONH \)), 3.19 (t, \( ^3J = 6.1 \text{ Hz, 2H, } CH_2CH_2CONH \)), 3.50 (t, \( ^3J = 7.3 \text{ Hz, 2H, } CH_2CH_2CH_2CH_2NH_3^+ \)), 3.73 (t, \( ^3J = 7.0 \text{ Hz, 2H, } CH_2CH_2CH_2CH_2NH_3^+ \)), 3.87 (s, 3H, OCH3), 7.10 (dd, \( ^3J = 9.0 \text{ Hz, } ^4J = 2.4 \text{ Hz, 1H, ArH}_6 \)), 7.36 (d, \( ^4J = 2.3 \text{ Hz, 1H, ArH}_7 \)), 7.70 (d, \( ^3J = 9.0 \text{ Hz, 1H, ArH}_6 \)), 7.96 (brs, 1H, NH).

\[ N-(5\text{-Aminopentyl})-3-(5\text{-methoxy-1H-benzo[d]imidazol-2-yl})propanamide \text{ bis(trifluoroacetate) salt 29} \]

1.8 mL of TFA were added to 156 mg of 24 (0.39 mmol) in 5 mL of dry DCM (208 mg, 0.39 mmol, quant.).

\[ ^1H\text{ NMR: (200 MHz, (CD3)2CO) } \delta 1.39-1.52 \text{ (m, 4H, } CH_2CH_2CH_2CH_2CH_2NH_3^+ \)), 1.75-1.86 (m, 2H, \( CH_2CH_2CH_2CH_2NH_3^+ \)), 2.97 (t, \( ^3J = 7.7 \text{ Hz, 2H, } CH_2CH_2CONH \)), 3.18-3.20 (m, 2H, \( CH_2CH_2CONH \)), 3.51 (t, \( ^3J = 7.6 \text{ Hz, 2H, } CH_2CH_2CH_2CH_2CH_2NH_3^+ \)), 3.73 (t, \( ^3J = 6.8 \text{ Hz, 2H, } CH_2CH_2CH_2CH_2NH_3^+ \)),
3.89 (s, 3H, OCH₃), 7.14 (dd, 3J = 8.9 Hz, 4J = 2.2 Hz, 1H, ArH₆), 7.36 (d, 4J = 2.4 Hz, 1H, ArH₄), 7.71 (d, 3J = 9.4 Hz, 1H, ArH₇), 7.73 (brs, 1H, NH).

**N-(6-Aminohexyl)-3-(5-methoxy-1H-benzo[d]imidazol-2-yl)propanamide bis(trifluoroacetate) salt 30**

2 mL of TFA were added to 251 mg of 25 (0.60 mmol) in 8 mL of dry DCM (327 mg, 0.60 mmol, quant.).

¹H NMR: (200 MHz, (CD₃)₂CO): δ 1.15-1.23 (m, 2H, CH₂CH₂CH₂CH₂CH₂CH₂NH₃⁺), 1.29-1.45 (m, 4H, CH₂CH₂CH₂CH₂CH₂CH₂NH₃⁺), 1.60-1.70 (m, 2H, CH₂CH₂CH₂CH₂CH₂CH₂CH₂NH₃⁺), 2.96 (t, 3J = 6.9 Hz, 2H, CH₂CH₂CONH), 3.07-3.22 (m, 2H, CH₂CH₂CONH), 3.51 (t, 3J = 6.9 Hz, 2H, CH₂CH₂CH₂CH₂CH₂CH₂CH₂NH₃⁺), 3.71 (t, 3J = 6.9 Hz, 2H, CH₂CH₂CH₂CH₂CH₂CH₂CH₂NH₃⁺), 3.88 (s, 3H, OCH₃), 7.11 (dd, 3J = 9.0 Hz, 4J = 2.4 Hz, 1H, ArH₆), 7.37 (d, 4J = 2.3 Hz, 1H, ArH₄), 7.72 (d, 3J = 9.0 Hz, 2H, ArH₇), 7.98 (brs, 1H, NH).
2. $^1$H and $^{13}$C NMR spectra of final compounds.

Figure S1: $^1$H (700 MHz) and $^{13}$C NMR (176 MHz) of compound 15 in CD$_3$OD.
Figure S2: $^1$H (700 MHz) and $^{13}$C NMR (176 MHz) of compound 16 in CD$_3$OD.
Figure S3: $^1$H (600 MHz) and $^{13}$C NMR (151 MHz) of compound 17 in DMSO-$d_6$. 
Figure S4: $^1$H-$^1$H COSY NMR spectrum of compound 17.

Figure S5: $^1$H-$^1$H NOESY NMR spectrum of compound 17.
Figure S6: $^1$H-$^{13}$C HSQC NMR spectrum of compound 17.
Figure S7: $^1$H-$^{13}$C HMBC NMR spectrum of compound 17.
Figure S8: $^1$H (700 MHz) and $^{13}$C NMR (176 MHz) of compound 18 in DMSO-$d_6$. 
Figure S9: $^1$H (700 MHz) and $^{13}$C NMR (176 MHz) of compound 31 in CD$_3$OD.
Figure S10: $^1$H (700 MHz) and $^{13}$C NMR (176 MHz) of compound 32 in CD$_3$OD.
Figure S11: \(^1\text{H} (600 \text{ MHz})\) and \(^{13}\text{C}\) NMR (151 MHz) of compound 34 in CD\(_3\)OD.
Figure S12: $^1$H (500 MHz) and $^{13}$C NMR (126 MHz) of compound 36 in CD$_3$OD.
Figure S13: $^1$H (500 MHz) and $^{13}$C NMR (126 MHz) of compound 38 in CD$_3$OD.
Figure S14: $^1$H (500 MHz) and $^{13}$C NMR (126 MHz) of compound 39 in CD$_3$OD.
Figure S15: $^1$H (700 MHz) and $^{13}$C NMR (176 MHz) of compound 40 in CD$_3$OD.
Figure S16: $^1$H (600 MHz) and $^{13}$C NMR (151 MHz) of compound 41 in CD$_3$OD.
Figure S17: $^1$H (500 MHz) and $^{13}$C NMR (126 MHz) of compound 42 in CD$_3$OD.
Figure S18: $^1$H (700 MHz) and $^{13}$C NMR (176 MHz) of compound 43 in CD$_3$OD.
3. HPLC chromatograms of final compounds

HPLC-Methods:

Compounds 15-18: ACN/water (0.1% TFA) gradient, flow rate: 1.6 mL/min, oven temperature: 30 °C.

Compound 31: ACN/water (Na₂SO₄, 20 mM (pH 3)) gradient, flow rate: 1.0 mL/min, oven temperature: 30 °C.

Compounds 32, 34, 36, 38: ACN/water (Na₂SO₄, 20 mM (pH 3)) gradient, flow rate: 1.2 mL/min, oven temperature: 30 °C.

Compound 39: ACN/water (0.1% TFA) gradient, flow rate: 1.2 mL/min, oven temperature: 30 °C.

Compound 40: (ACN/MeOH (1:1))/water (Na₂SO₄, 20 mM (pH 3)) gradient, flow rate: 1.0 mL/min, oven temperature: 30 °C.

Compounds 41-43: (ACN/MeOH (1:1))/water (Na₂SO₄, 20 mM (pH 3)) gradient, flow rate: 1.2 mL/min, oven temperature: 30 °C.
Figure S19: HPLC chromatogram of compound 15.

### Peak Table

| Peak# | Ret. Time | Area     | Height | Conc. | Unit | Area% |
|-------|-----------|----------|--------|-------|------|-------|
| 1     | 4.757     | 18584    | 1201   | 0.000 |      | 0.177 |
| 2     | 6.137     | 4111     | 653    | 0.000 |      | 0.039 |
| 3     | 6.357     | 9440     | 1480   | 0.000 |      | 0.090 |
| 4     | 6.929     | 8242     | 1290   | 0.000 |      | 0.079 |
| 5     | 7.780     | 7927     | 1000   | 0.000 |      | 0.076 |
| 6     | 8.388     | 38136    | 3865   | 0.000 |      | 0.364 |
| 7     | 8.560     | 10851    | 1829   | 0.000 |      | 0.103 |
| 8     | 9.127     | 3225421  | 527900 | 0.000 |      | 30.755|
| 9     | 9.431     | 7072161  | 1152985| 0.000 |      | 67.435|
| 10    | 9.977     | 10181    | 1387   | 0.000 |      | 0.097 |
| 11    | 10.187    | 13168    | 1788   | 0.000 |      | 0.126 |
| 12    | 10.821    | 15242    | 1778   | 0.000 |      | 0.145 |
| 13    | 11.377    | 28749    | 3652   | 0.000 |      | 0.274 |
| 14    | 17.617    | 11062    | 801    | 0.000 |      | 0.105 |
| 15    | 18.474    | 14123    | 873    | 0.000 |      | 0.135 |
| Total | 10487400  | 1702480  |        |       |      | 100.000|
Figure S20: HPLC chromatogram of compound 16.
Figure S21: HPLC chromatogram of compound 17.
Figure S22: HPLC chromatogram of compound 18.
Figure S23: HPLC chromatogram of compound 31.
Figure S24: HPLC chromatogram of compound 32.
**Figure S25**: HPLC chromatogram of compound 34.

| No. | RT  | Area   | Conc 1 | BC  |
|-----|-----|--------|--------|-----|
| 1   | 12.32 | 1027105 | 53,835 | MC  |
| 2   | 12.77 | 811802  | 42,550 | MC  |
| 3   | 13.31 | 37617   | 1,972  | MC  |
| 4   | 13.87 | 31345   | 1,643  | MC  |

|       |       |        |       |
|-------|-------|--------|-------|
|       |       | 1907869| 100,000|

Chrom Type: Fixed WL Chromatogram, 254 nm
Figure S26: HPLC chromatogram of compound 36.
Figure S27: HPLC chromatogram of compound 38.

Chrom Type: Fixed WL Chromatogram, 254 nm

| No. | RT (min) | Area   | Conc 1 | BC |
|-----|----------|--------|--------|----|
| 1   | 5.87     | 3418   | 0.137  | MC |
| 2   | 6.32     | 4013   | 0.161  | MC |
| 3   | 6.83     | 4413   | 0.177  | MC |
| 4   | 8.16     | 6036   | 0.242  | MC |
| 5   | 8.53     | 6043   | 0.242  | MC |
| 6   | 14.53    | 1368410| 54.764 | MC |
| 7   | 15.23    | 1106416| 44.279 | MC |

|                |        |
|----------------|--------|
| Sum of Areas  | 2498749|
| % of Sum      | 100.000|
Figure S28: HPLC chromatogram of compound 39.
Figure S29: HPLC chromatogram of compound 40.
Figure S30: HPLC chromatogram of compound 41.
Figure S31: HPLC chromatogram of compound 42.
Figure S32: HPLC chromatogram of compound 43.
4. Coactivator recruitment

Coactivator recruitment was tested using the LanthaScreen® TR-FRET ERα coactivator assay. The binding of the fluorescein-labeled PGC1α coactivator peptide to the ERα LBD protein labeled with terbium was investigated. As a control, the recruitment upon E2 treatment was assessed.

![Coactivator recruitment measured by TR-FRET](image)

**Figure S33:** Coactivator recruitment measured by TR-FRET of (a) thioxo-quinazolinones, (b) 5-methoxybenzimidazoles and (c) 5-hydroxybenzimidazoles. The synthesized compounds displayed no coactivator recruitment.
5. Crystal violet assay

To investigate the antiproliferative effects of the heterodimeric ligands, a crystal violet assay was performed using ER-positive (MCF-7), ER-negative (MDA-MB-231), and tamoxifen-resistant breast cancer (MCF-7TamR) cells.

![Graphs showing antiproliferative effects against ER-positive MCF-7 (blue), tamoxifen-resistant MCF-7TamR (red) and ER-negative MDA-MB-231 (orange) breast cancer cells. Values represent the mean ± SD of ≥ 3 independent experiments.](image-url)

**Figure S34:** Antiproliferative effects against ER-positive MCF-7 (blue), tamoxifen-resistant MCF-7TamR (red) and ER-negative MDA-MB-231 (orange) breast cancer cells. Values represent the mean ± SD of ≥ 3 independent experiments.
Figure S35: Time-dependent antiproliferative effects against ER-positive MCF-7 (blue) and ER-negative MDA-MB-231 (orange) breast cancer cells. Values represent the mean ± SD of ≥ 3 independent experiments.

6. Transactivation assay

The influence on the signal transduction upon ER binding was investigated with U2OS cells transiently transfected with pSG5-ERα (1 ng) or pSG5-ERβ (1 ng), the reporter plasmid p(ERE)2-luc+ (50 ng) and pRenilla-CMV (0.5 ng) for standardization. The compounds displayed no agonistic activity at 10 µM or 0.1 µM.

Figure S36: Luciferase reporter gene assay of selected heterodimeric compounds.
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