

Inhibitors for Human Glutaminyl Cyclase by Structure Based Design and Bio-isosteric Replacement

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Contents:

Detailed analytical data of the compounds (NMR, MS, high resolution MS, HPLC), yields, description of the homology model development including the model characteristic data, fitness and scoring results of the docking experiments, file report of the QSAR model generation process.

1-(3,4-Dimethoxyphenyl)-3-(3-(5-methyl-1H-imidazol-1-yl)propyl)thiourea 9.

6: 0.03 g (0.20 mmol); The purification was done using semi preparative HPLC. Yield: 0.041 g (51.4%); mp: oily at rt, ¹H-NMR (CDCl₃): δ 2.08-2.15 (m, 2H); 2.68 (s, 3H); 3.61-3.64 (m, 2H); 3.78 (s, 3H); 3.79 (s, 3H); 4.13-4.16 (m, 2H); 6.75-6.81 (br m, 2H); 6.93 (s, H); 7.24 (s, H); 7.3 (br s, H); 8.60 (br s, H); 8.68 (s, H); MS: m/z 335.4 (M+H)⁺; ESI-FTICR-MS: m/z 333.515387 ([M+H]⁺, calc. for C₁₆H₂₃O₂N₄S⁺ 335.15362); HPLC (214 nm, [A]): rt 18.32 min (100%).

1-(3,4-Dimethoxyphenyl)-3-(3-(4-methyl-1H-imidazol-1-yl)propyl)thiourea 10.

8: 0.070 g (0.50 mmol); The purification was done using semi preparative HPLC. Yield: 0.068 g (40.7%); mp: 139.5 °C; ¹H-NMR: (CDCl₃): δ 2.01-2.04 (m, 2H); 2.15 (s, 3H); 3.62-3.67 (m, 2H); 3.83 (s, 3H); 3.85-3.89 (m, 5H); 6.01 (br s, H); 6.70-6.75 (m, 2H); 6.84-6.86 (m, H); 7.24 (s, H); 7.35 (s, H); 7.72 (s, H); MS: m/z 335.1 (M+H)⁺; ESI-FTICR-MS: m/z 335.15361 ([M+H]⁺, calc. for C₁₆H₂₃O₂N₄S⁺ 335.15362); HPLC (214 nm, [A]): rt 17.20 min (96.0%).

1-(3,4-Dimethoxyphenyl)-N-(3-(5-methyl-1H-imidazol-1-yl)propyl)cyclopropanecarbothioamide 11.

6: 0.0028 g (0.204 mmol); Yield: 0.0032 g (43.6%); mp: 181.0 °C, ¹H-NMR (CDCl₃): δ 1.21-1.23 (m, 2H); 1.92-1.98 (br m, 4H); 2.11 (s, 3H); 3.58-3.63 (m, 2H); 3.74-3.80 (m, 2H); 3.85 (s, 3H); 3.87 (s, 3H); 6.71 (s, H); 6.81-6.90 (br m, 3H); 7.03 (br s, H); 7.27 (s, H); MS m/z 360.3 (M+H)⁺; ESI-FTICR-MS: m/z 360.17379 ([M+H]⁺, calc. for C₁₉H₂₆O₂N₃S⁺ 360.17402); HPLC (214 nm, [C]): rt 11.27 min (97.9%).

1-(3,4-Dimethoxyphenyl)-N-(3-(4-methyl-1H-imidazol-1-yl)propyl)cyclopropanecarbothioamide 12

8: 0.053 g (0.38 mmol); Yield: 0.116 (78.9%) mp: 67.0 °C; ¹H-NMR (CDCl₃): δ 1.21-1.23 (m, 2H); 1.94-2.01 (br m, 4H); 2.17 (s, 3H); 3.55-3.60 (m, 2H); 3.78-3.82 (m, 2H); 3.85 (s, 3H); 3.88 (s, 3H);

6.49 (s, H); 6.81-6.90 (br m, 3H); 7.24 (s, H); MS: m/z 360.3 (M+H)⁺; ESI-FTICR-MS: m/z 360.17383 ([M+H]⁺, calc. for C₁₉H₂₆O₂N₃S⁺ 360.17402); HPLC (214 nm, [C]): rt 11.17 min (100%).

(3-(1H-Imidazol-1-yl)propyl)-2-cyano-3-methylguanidine 13.

Yield: 0.13 g (15.8%); mp: 181.0-183.0 °C; ¹H-NMR δ 1.80-2.00 (br m, 2H); 2.60-2.70 (m, 3H); 3.00-3.10 (m, 2H); 3.85-4.00 (m, 2H); 6.80-7.00 (br m, 3H); 7.15 (s, H); 7.61 (s, H); MS: m/z 307.2 (M+H)⁺, 139.2 ([M-C₃H₃N₂][•])⁺; ESI-FTICR-MS: m/z 229.11679 ([M+Na]⁺, calc. for C₉H₁₄N₆Na⁺ 229.11722); HPLC (214 nm, [A]): rt 2.24 min (95.2%).

(3-(1H-Imidazol-1-yl)propyl)-2-cyano-3-(cyclopropylmethyl)guanidine 14.

Yield: 0.121g (11.7%); mp: 131.2 °C; ¹H-NMR: (CD₃OD) δ 0.23-0.26 (br m, 2H); 0.50-0.54 (br m, 2H); 1.00-1.09 (br m, H); 2.02-2.06 (m, 2H); 3.04-3.05 (m, 2H); 3.21-3.24 (m, 2H); 4.05-4.09 (m, 2H); 6.96 (s, H); 7.14 (s, H); 7.65 (s, H); MS: m/z 247.4 (M+H)⁺, 179.2 ([M-C₃H₃N₂][•])⁺; ESI-FTICR-MS: m/z 269.14821 ([M+Na]⁺, calc. for C₁₂H₁₈N₆Na⁺ 269.14852); HPLC (214 nm, [A]): 13.87 min (100%).

(3-(1H-Imidazol-1-yl)propyl)-2-cyano-3-phenylguanidine 15.

Yield: 0.58 g (54.0%); mp: 112.0-114.0 °C; ¹H-NMR: δ 1.90-2.00 (m, 2H); 3.10-3.20 (m, 2H); 3.90-4.00 (m, 2H); 6.87 (s, H); 7.13-7.16 (m, 2H); 7.20-7.22 (m, 3H); 7.32-7.34 (m, 2H); 7.61 (s, H); 8.98 (s, H); MS: m/z 269.2 (M+H)⁺; 201.3 ([M-C₃H₃N₂][•])⁺; ESI-FTICR-MS: m/z 291.13250 ([M+Na]⁺, calc. for C₁₄H₁₆N₆Na⁺ 291.13287); HPLC (214 nm, [A]): rt 20.88 min (95.1%).

(3-(1H-Imidazol-1-yl)propyl)-3-(4-bromophenyl)-2-cyanoguanidine 16.

Yield: 0.075 g (5.1%); mp: 161-163 °C; ¹H-NMR (CD₃OD): δ 2.00-2.07 (br m, 2H); 3.25-3.28 (br m, 2H); 4.04-4.08 (m, 2H); 6.96 (m, H); 7.13-7.18 (br m, 3H); 7.52-7.54 (m, 2H); 7.65 (s, H); MS: m/z 347.1 (M+H)⁺; ESI-FTICR-MS: m/z 371.04112([M+Na]⁺, calc. for C₁₄H₁₅N₆BrNa⁺ 371.04338); HPLC (214 nm, [A]): rt 21.68 min (94.9%).

(3-(1H-Imidazol-1-yl)propyl)-2-cyano-3-(4-(trifluoromethyl)phenyl)guanidine 17.

Yield: 0.045 g (3.2%); mp: 84-86 °C; ¹H-NMR (CD₃OD): δ 2.04-2.11 (m, 2H); 3.29-3.33 (m, 2H); 4.07-4.10 (m, 2H); 6.96 (s, H); 7.14 (s, H); 7.40-7.42 (m, 2H); 7.65-7.67 (m, 3H); MS: m/z 337.3 (M+H)⁺, 269.34 ([M-C₃H₃N₂])⁺; ESI-FTICR-MS: m/z 337.13832 ([M+H]⁺, calc. for C₁₅H₁₆N₆F₃⁺ 337.13831); HPLC (214 nm, [A]): rt 22.54 min (97.2%).

(3-(1H-Imidazol-1-yl)propyl)-2-cyano-3-(4-isopropylphenyl)guanidine 18.

Yield: 0.67 g (51.0%); mp: 149.4 °C; ¹H-NMR (CD₃OD): δ 1.24-1.26 (m, 6H); 1.98-2.05 (m, 2H); 2.88-2.94 (br m, H); 3.23-3.29 (m, 2H); 4.02-4.06 (m, 2H); 6.95 (s, H); 7.12-7.16 (br m, 3H); 7.28-7.29 (m, 2H); 7.64 (s, H); MS: m/z 311.4 (M+H)⁺, 243.4 ([M-C₃H₃N₂])⁺; ESI-FTICR-MS: m/z 311.19771 ([M+H]⁺, calc. for C₁₇H₂₃N₆⁺ 311.19787); HPLC (214 nm, [A]): rt 22.65 min (96.8%).

(3-(1H-Imidazol-1-yl)propyl)-2-cyano-3-(4-methoxyphenyl)guanidine 19.

Yield: 0.096 g (8.1%); mp: 154.0-156.0 °C; ¹H-NMR: δ 1.85-1.95 (m, 2H); 3.10-3.15 (m, 2H); 3.74 (s, 3H); 3.90-4.00 (m, 2H); 6.87 (s, H); 6.90-6.93 (m, 3H); 7.11-7.15 (m, 3H); 7.60 (s, H); 8.80 (s, H); MS: m/z 299.1 (M+H)⁺, 231.3 ([M-C₃H₃N₂])⁺; ESI-FTICR-MS: m/z 321.14338 ([M+Na]⁺, calc. for C₁₅H₁₈ON₆Na⁺ 321.14343); HPLC (214 nm, [A]): rt 13.33 min (94.9%).

(3-(1H-Imidazol-1-yl)propyl)-2-cyano-3-(3,4-dimethoxyphenyl)guanidine 20.

Yield: 0.15 g (11.4%); mp: 210.0-212.0 °C; ¹H-NMR: δ 2.00-2.03 (m, 2H); 3.31-3.36 (m, 2H); 3.76 (s, 3H); 3.81 (s, 3H); 4.05-4.09 (m, 2H); 6.93 (s, H); 7.05 (s, H); 7.25 (s, H); 7.68 (s, H); 7.75 (s, 2H); MS: m/z 329.4 (M+H)⁺, 261.1 ([M-C₃H₃N₂])⁺; ESI-FTICR-MS: m/z 329.17173 ([M+H]⁺, calc. for C₁₆H₂₁O₂N₆⁺ 329.17205); HPLC (270 nm, [A]): rt 13.95 min (100%).

(3-(1H-Imidazol-1-yl)propyl)-2-cyano-3-(2,3-dihydrobenzo[b][1,4]dioxin-7-yl)guanidine 21.

Yield: 0.13 g (9.3%); mp: 159.6 °C; ¹H-NMR (CD₃OD): δ 1.98-2.02 (m, 2H); 3.21-3.24 (m, 2H); 4.01-4.05 (m, 2H); 4.24 (s, 4H); 6.67-6.70 (br m, H); 6.73-6.74 (m, H); 6.84-6.86 (m, H); 6.95(s, H); 7.12 (s, H); 7.64 (s, H); 7.88 (s, H); MS: m/z 327.5 (M+H)⁺, 259.2 ([M-C₃H₃N₂][•])⁺; ESI-FTICR-MS: m/z 327.15624 ([M+H]⁺, calc. for C₁₆H₁₉O₂N₆⁺ 327.15640); HPLC (214 nm, [A]): rt: 15.55 min (97.9%).

2-Cyano(3-(5-methyl-1H-imidazol-1-yl)propyl)-3-(3,4-dimethylphenyl)guanidine 36.

6: 0.201 g (1.5 mmol); Yield: 0.16 g (34.3%); mp: 149.0 °C; ¹H-NMR (CDCl₃): δ 1.92-1.96 (m, 2H); 2.14 (s, 3H); 2.26 (s, 6H); 3.22-3.27 (m, 2H); 3.82-3.85 (m, 2H); 4.85-4.88 (m, H); 6.68 (s, H); 6.88-6.90 (m, H); 6.93 (s, H); 7.16-7.18 (m, H); 7.23 (s, H); 7.32 (s, H); MS: m/z 311.4 (M+H)⁺, 259.3 ([M-C₄H₅N₂][•])⁺; ESI-FTICR-MS: m/z 311.1970 ([M+H]⁺, calc. for C₁₇H₂₃N₆⁺ 311.19787); HPLC (214 nm, [C]): rt 11.29 min (100%).

(3,4-Dichlorophenyl)-2-cyano-3-(3-(5-methyl-1H-imidazol-1-yl)propyl)guanidine 37.

6: 0.201 g (1.5 mmol); Yield: (77.0%) mp: 142.2 °C; ¹H-NMR: δ 1H-NMR: 1.83-1.87 (m, 2H); 2.12 (s, 3H); 3.14-3.18 (m, 2H); 3.83-3.87 (m, 2H); 6.58 (s, H); 7.20-7.23 (m, H); 7.46-7.49 (m, 3H); 7.54-7.56 (m, H); 9.17 (s, H) MS: m/z 351.5 (M)⁺, 269.4 ([M-C₄H₅N₂][•])⁺; ESI-FTICR-MS: m/z 351.08869 ([M+H]⁺, calc. for C₁₅H₁₇N₆Cl₂⁺ 351.08863); HPLC (214 nm, [B]): rt 10.67 min (98.1%).

2-Cyano-1-[3-(5-methyl-1H-imidazol-1-yl)propyl]-4-phenylbenzene-1-guanidine 38.

6: 0.16 g (1.18 mmol); Yield: 0.25 g (60.0%); mp: 188.2 °C; ¹H-NMR: δ 1H-NMR: 1.83-1.90 (m, 2H); 2.12 (s, 3H); 3.15-3.20 (m, 2H); 3.84-3.88 (m, 2H); 6.58 (s, H); 7.28-7.33 (br m, 4H); 7.40-7.44 (m, 2H); 7.50 (s, H); 7.61-7.64 (m, 4H); 9.07 (s, H); MS: m/z 359.4 (M+H)⁺, 277.4 ([M-C₄H₅N₂][•])⁺; ESI-FTICR-MS: m/z 359.19807 ([M+H]⁺, calc. for C₂₁H₂₃N₆⁺ 359.19787); HPLC (214 nm, [C]): rt 14.19 min (99.1%).

(3-(5-Methyl-1H-imidazol-1-yl)propyl)-2-cyano-3-(2,3-dihydrobenzo[b][1,4]dioxin-7-yl)guanidine 39.

6: 0.201 g (1.5 mmol) Yield: 0.50 g (98.7%); mp: 146.5 °C ; ¹H-NMR: δ 1.80-1.87 (br m, 2H); 2.13 (s, 3H); 3.11-3.15 (m, 2H); 3.82-3.86 (m, 2H); 4.22 (s, 4H); 6.59 (s, H); 6.65-6.66 (m, H); 6.67-6.68 (m, H); 6.72-6.73 (m, H); 6.81-6.83 (m, H); 8.30 (s, H); 8.79 (s, H); MS: m/z 341.1 (M+H)⁺; ESI-FTICR-MS: m/z 341.17123 ([M+H]⁺, calc. for C₁₇H₂₁O₂N₆⁺ 341.17501); HPLC (214 nm, [C]): rt 8.30 min (98.7%).

2-Cyano(3,4,5-trimethoxyphenyl)-3-(3-(5-methyl-1H-imidazol-1-yl)propyl)guanidine 40.

6: 0.061 g (0.44 mmol); Yield: 0.13 g (80.0%); mp: 161.0 °C; ¹H-NMR(CDCl₃): δ 2.15-2.19 (m, 2H); 2.32 (s, 3H); 3.44-3.48 (m, 2H); 3.75 (s, 3H); 3.82 (s, 6H); 4.26-4.30 (m, 2H); 6.53 (s, H); 7.01 (s, H); 7.08-7.13 (m, H); 7.97 (s, H); 9.54 (s, H); MS: m/z 373.14 (M+H)⁺; ESI-FTICR-MS: m/z 373.20111 ([M+H]⁺, calc. for C₁₈H₂₅O₃N₆⁺ 373.20169); HPLC (214 nm, [C]): rt 7.69 min (99.1%).

(3-(4-Methyl-1H-imidazol-1-yl)propyl)-2-cyano-3-(2,3-dihydrobenzo[b][1,4]dioxin-7-yl)guanidine 51.

8: 0.083 g (0.6 mmol); Yield: 0.14 g (70.0%); mp: 191.2 °C; ¹H-NMR: δ 1.82-1.89 (m, 2H); 2.05 (s, 3H); 3.07-3.12 (m, 2H); 3.84-3.87 (m, 2H); 4.22 (s, 4H); 6.64-6.66 (m, H); 6.67 (s, H); 6.72-6.73 (m, 2H); 6.81-6.83 (m, H); 7.45 (s, H); 8.77 (s, H) ; MS: m/z 341.2 (M+H)⁺, 259.3 ([M-C₄H₅N₂][•]); ESI-FTICR-MS: m/z 341.17177 ([M+H]⁺, calc. for C₁₇H₂₁O₂N₆⁺ 341.17205); HPLC (214 nm, [C]): rt 8.64 min (100%).

N-(3-(1H-Imidazol-1-yl)propyl)-N-methyl-2-nitroethene-1,1-diamine 22.

Yield: 0.11 g (12.2%); mp: 160.5 °C; ¹H-NMR (CD₃OD): δ 2.11-2.14 (m, 2H); 2.87 (s, 3H); 3.20-3.29 (m, 2H); 4.11-4.15 (m, 2H); 6.68 (s, H); 6.98 (s, H); 7.15 (s, H); 7.66 (s, H); MS: m/z 226.4 (M+H)⁺; ESI-FTICR-MS: m/z 248.11156 ([M+Na]⁺, calc. for C₉H₁₅O₂N₅Na⁺ 248.11180); HPLC (214 nm, [A]): rt 5.39 min (96.9%).

N-(3-(1H-Imidazol-1-yl)propyl)-N-cyclohexyl-2-nitroethene-1,1-diamine 23.

Yield: 0.29 g (24.7%); mp: 166.0 °C; ¹H-NMR: δ 1.19-1.35 (br m, 6H); 1.50-1.53 (m, 2H); 1.60-1.63 (m, 2H); 1.77-1.79 (m, 2H); 1.91-1.95 (m, 2H); 3.02-3.15 (m, 2H); 3.95-4.00 (m, 2H); 6.49 (s, H); 6.86 (s, H); 7.14 (br s, 2H); 7.59 (s, H); 10.17 (br s, H); MS: m/z 294.1 (M+H)⁺; ESI-FTICR-MS: m/z 316.17459 ([M+Na]⁺, calc. for C₁₄H₂₃O₂N₅Na⁺ 316.17440); HPLC (214 nm, [A]): rt 14.48 min (100%).

N-(1-(3-(1H-Imidazol-1-yl)propylamino)-2-nitrovinyl)benzenamine 24.

Yield: 0.14 g (12.2%); mp: oily at rt; ¹H-NMR: δ 2.13-2.18 (m, 2H); 3.39-3.45 (m, 2H); 4.24-4.27 (m, 2H); 6.05-6.18 (br s, H); 7.18-7.26 (m, 2H); 7.27-7.32 (m, H); 7.39-7.46 (m, 2H); 7.65-7.68 (m, H); 7.77-7.80 (m, H); 9.12 (s, H); MS: m/z 288.2 (M+H)⁺; ESI-FTICR-MS: m/z 288.14550 ([M+H]⁺, calc. for C₁₄H₁₈O₂N₅⁺ 288.14550); HPLC (214 nm, [A]): rt 15.13 min (97.2%).

N-(1-(3-(1H-Imidazol-1-yl)propylamino)-2-nitrovinyl)-4-chlorobenzenamine 25.

Yield: 0.13 g (10.1%); mp: oily at rt; ¹H-NMR: δ 1.98-2.08 (m, 2H); 3.22-3.33 (m, 2H); 3.98-4.05 (m, 2H); 6.06-6.21 (br s, H); 6.85-6.89 (s, H); 7.13-7.17 (s, H); 7.22-7.28 (m, 2H); 7.44-7.48 (m, 2H); 7.60-7.62 (s, H); MS: m/z 322.2 (M+H)⁺; ESI-FTICR-MS: m/z 344.08830 ([M+Na]⁺, calc. for C₁₄H₁₆O₂N₅Na⁺ 344.08847); HPLC (214 nm, [A]): rt 17.28 min (95.1%).

N-(1-(3-(1H-Imidazol-1-yl)propylamino)-2-nitrovinyl)-4-(trifluoromethyl)-benzenamine 26.

Yield: 0.091 g (6.4%); mp: oily at rt; ¹H-NMR: δ 2.01-2.09 (m, 2H); 3.29-3.37 (br m, 2H); 3.99-4.06 (m, 2H); 6.22-6.32 (br s, H); 6.86-6.89 (s, H); 7.16-7.19 (s, H); 7.39-7.46 (m, 2H); 7.68-7.74 (m, 3H); MS: m/z 356.2 (M+H)⁺; ESI-FTICR-MS: m/z 356.13280 ([M+H]⁺, calc. for C₁₅H₁₇O₂N₅F₃⁺ 356.13289); HPLC (214 nm, [A]): rt 21.87 min (94.6%).

N-(1-(3-(1H-Imidazol-1-yl)propylamino)-2-nitrovinyl)naphthalen-1-amine 27.

Yield: 0.058 g (4.3%); mp: oily at rt; ¹H-NMR: δ 2.17-2.31 (br m, 2H); 3.32-3.65 (br m, 2H); 4.24-4.38 (br m, 2H); 7.41-7.52 (br m, H); 7.53-7.64 (br m; 4H); 7.66-7.73 (br m; H); 7.76-7.89 (br m; 3H); 7.94-

8.07 (m, 2H), 9.07-9.19 (s, H); 9.98-10.31 (br s, H); MS: m/z 338.2 ($M+H$)⁺; ESI-FTICR-MS: m/z 338.16121 ($[M+H]^+$, calc. for $C_{18}H_{20}O_{16}N_5^+$ 338.16115); HPLC (214 nm, [A]): rt 21.60 min (94.9%).

N-(1-(3-(1H-Imidazol-1-yl)propylamino)-2-nitrovinyl)-4-methoxybenzenamine 28.

Yield: 0.089 g (7.0%); mp: oily at rt; ¹H-NMR: δ 2.11-2.19 (m, 2H); 3.33-3.43 (br m, 2H); 3.74 (s, 3H); 4.22-4.28 (m, 2H); 6.08-6.21 (br s, H); 6.78-6.82 (m, 2H); 6.85-6.90 (m, H); 7.29-7.35 (m, H); 7.65-7.68 (m, H); 7.77-7.79 (m, H); 9.11 (s, H); MS: m/z 318.3 ($M+H$)⁺; ESI-FTICR-MS: m/z 318.15590. ($[M+H]^+$, calc. for $C_{15}H_{20}O_3N_5^+$ 318.15610); HPLC (214 nm, [A]): rt 14.31 min (100%).

N-(-1-(3-(1H-Imidazol-1-yl)propylamino)-2-nitrovinyl)-3,4-dimethoxy-benzenamine 29.

Yield: 0.13 g (9.4%); mp: oily at rt; ¹H-NMR: δ 2.08-2.26 (m, 2H); 3.28-3.53 (m, 2H); 3.68-3.80 (m, 6H); 4.19-4.32 (m, 2H); 5.94-6.24 (br s, H); 6.72-6.89 (br m, 2H); 6.94-7.04 (br m, H); 7.64-7.72 (br s, H); 7.75-7.84 (br s, H); 9.15 (s, H); MS: m/z 348.2 ($M+H$)⁺; ESI-FTICR-MS: m/z 348.16658 ($[M+H]^+$, calc. for $C_{16}H_{22}O_4N_5^+$ 348.16663); HPLC (214 nm, [A]): rt 13.09 min (97.7%).

N-(1-(3-(1H-Imidazol-1-yl)propylamino)-2-nitrovinyl)-2,3-dihydrobenzo[b][1,4]-dioxin-6-amine 30.

Yield: 0.13 g (11.3%); mp: 110.0-112.0 °C; ¹H-NMR: δ 1.97-2.07 (m, 2H); 3.20-3.30 (m, 2H); 3.97-4.03 (m, 2H); 4.23 (s, 4H); 5.98-6.15 (br s, 1H); 6.65-6.69 (m, H); 6.74-6.76 (m, H); 6.85-6.89 (m, 2H); 7.16 (s, H); 7.59(s, H); MS: m/z 346.1 ($M+H$)⁺; ESI-FTICR-MS: m/z 368.13290 ($[M+Na]^+$, calc. for $C_{16}H_{19}O_4N_5Na^+$ 368.13293); HPLC (214 nm, [A]): rt 12.51 min (94.7%).

N-(1-(3-(5-Methyl-1H-imidazol-1-yl)propylamino)-2-nitrovinyl)cyclohexanamine 41.

6: 0.47 g (3.4 mmol); Yield: 0.059 g (5.6%); mp: 44.0 °C; ¹H-NMR (CD_3DO): δ 1.16-1.57 (br m, 5H); 1.66-2.01 (br m, 5H); 2.03 (s, H); 2.16-2.26 (m, 2H); 2.37 (s, 3H); 3.29-3.31 (m, H); 3.36-3.37 (m, H);

3.51-3.54 (m, H); 3.59-3.64 (m, H); 3.65-3.72 (m, H); 4.24-4.32 (m, 2H); 7.31 (s, H); 8.86-8.87 (m, H); MS: m/z 308.2 (M+H)⁺; ESI-FTICR-MS: m/z 308.20782 ([M+H]⁺, calc. for C₁₅H₂₆O₃N₅⁺ 308.20810); HPLC (214 nm, [C]): rt 8.67 min (100%).

N-(1-(3-(5-Methyl-1H-imidazol-1-yl)propylamino)-2-nitrovinyl)-4-chloro-benzenamine 42.

6: 0.21 g (1.5 mmol); Yield: 0.31 g (61.5 %); mp: 175.0 °C; ¹H-NMR: δ 1.95-1.99 (m, 2H); 2.12 (s, 3H); 3.25-3.28 (m, 2H); 3.89-3.93 (m, 2H); 6.13 (br s, H); 6.59 (s, H); 7.24-7.25 (m, 2H); 7.45-7.48 (m, 3H); MS: m/z 336.1 (M+H)⁺; ESI-FTICR-MS: m/z 336.12199 ([M+H]⁺, calc. for C₁₅H₁₉O₂N₅Cl⁺ 343.12218); HPLC (214 nm, [C]): rt 9.89 min (100%).

N-(1-(3-(5-Methyl-1H-imidazol-1-yl)propylamino)-2-nitrovinyl)-4-(trifluoromethyl)benzenamine 43.

6: 0.21 g (1.5 mmol); Yield (after semi preparative HPLC): 0.0071 g (1.3%); mp: 152.0 °C; ¹H-NMR (CD₃OD): δ 2.26-2.28 (m, 2H); 2.39 (s, 3H); 3.52-3.54 (m, 2H); 4.28-4.32 (m, 2H); 7.32 (br s, H); 7.42, 7.46 (d, 2H); 7.75, 7.77 (d, 2H), 8.88 (s, H); MS: m/z 370.2 (M+H)⁺; ESI-FTICR-MS: m/z 370.14877 ([M+H]⁺, calc. for C₁₆H₁₉O₂N₅F₃⁺ 370.14854); HPLC (214 nm, [C]): rt 9.35 min (95.3%).

N-(1-(3-(5-Methyl-1H-imidazol-1-yl)propylamino)-2-nitrovinyl)naphthalen-1-amine 44.

6: 0.21 g (1.5 mmol); Yield: 0.19 g (36.7%); mp: 138.0 °C; ¹H-NMR (CD₃OD): δ 2.14-2.17 (m, 2H); 2.22 (s, 3H); 3.43-3.47 (m, 2H); 4.03-4.09 (m, 2H); 6.69 (s, H); 7.44-7.46 (m, H); 7.53-7.60 (br m, 5H); 7.85-7.88 (m, H); 7.95-7.98 (m, H); MS: m/z 352.4 (M+H)⁺; ESI-FTICR-MS: m/z 352.18674 ([M+H]⁺, calc. for C₁₉H₂₂N₅O₂⁺ 352.18733); HPLC (214 nm, [C]): rt 10.61 min (99.2%).

N-(1-(3-(5-Methyl-1H-imidazol-1-yl)propylamino)-2-nitrovinyl)-2,3-dihydrobenzo[b][1,4]-dioxin-6-amine 45.

6: 0.21 g (1.5 mmol); Yield: 0.31 g (57.5%); mp: 198.0 °C; ¹H-NMR (CD₃OD): δ 2.10-2.14 (m, 2H); 2.22 (s, 3H); 3.30-3.31 (m, 2H); 4.03-4.07 (m, 2H); 4.24 (s, 4H); 6.37 (br s, H); 6.68-6.70 (m, 2H); 6.73-6.74 (m, H); 7.56 (s, H); 7.87 (s, H); MS: m/z 360.2 (M+H)⁺; ESI-FTICR-MS: m/z 382.14793 ([M+Na]⁺, calc. for C₁₇H₂₁O₄N₅Na⁺ 382.14858); HPLC (214 nm, [C]): rt 8.43 min (100%).

N-(1-(3-(4-Methyl-1H-imidazol-1-yl)propylamino)-2-nitrovinyl)-2,3-dihydrobenzo[b][1,4]-dioxin-6-amine 52.

8: 0.13 g (0.9 mmol); Yield: 0.077 g (23.8%); mp: 155.0 °C; ¹H-NMR (CD₃OD): δ 2.14-2.17 (m, 5H); 3.32-3.35 (m, 2H); 4.05-4.09 (m, 2H); 4.26 (s, 4H); 6.68-6.72 (m, H); 6.74-76 (m, H); 6.85 (s, H); 6.88-6.91 (m, H); 7.74 (s, H); MS: m/z 360.4 (M+H)⁺; ESI-FTICR-MS: m/z 382.14844 ([M+H]⁺, calc. for C₁₇H₂₁O₄N₅Na⁺ 382.14858); HPLC (214 nm, [C]): rt 7.15 min (96.9%).

3-(3-(1H-Imidazol-1-yl)propyl)-2,3-dihydro-2-thioxoquinazolin-4(1H)-one 31.

Yield: 0.40 g (35.1%); mp: oily at rt; ¹H-NMR: δ 2.10-2.17 (m, 2H); 4.07-4.08 (m, 2H); 4.38-4.41 (m, 2H); 6.86 (s, H); 7.18 (s, H); 7.29-7.37 (br m, 2H); 7.64 (s, H); 7.70-7.73 (m, H); 7.93-7.95 (m, H); MS: m/z 286.6 (M+H)⁺, 219.4 ([M-C₃H₃N₂][•]); ESI-FTICR-MS: m/z 287.09591 ([M+H]⁺, calc. for C₁₄H₁₅ON₄S₂⁺ 287.09611); HPLC (214 nm, [A]): rt 18.11 min (100%).

3-(3-(1H-Imidazol-1-yl)propyl)-2,3-dihydro-7-methyl-2-thioxothieno[3,2-d]pyrimidin-4(1H)-one 32.

Yield: 0.18 g (18.4%); mp: 226.0 °C; ¹H-NMR: δ 2.12-2.17 (m, 2H); 2.27 (s, 3H); 4.04-4.07 (m, 2H); 4.38-4.41 (m, 2H); 6.89 (s, H); 7.20 (s, H); 7.69 (s, H); 7.76 (s, H); MS: m/z 307.1 (M+H)⁺, 239.1 ([M-C₃H₃N₂][•]); ESI-FTICR-MS: m/z 307.06802 ([M+H]⁺, calc. for C₁₃H₁₅ON₄S₂⁺ 307.06818); HPLC (214 nm, [A]): rt 18.89 min (93.3%).

3-[3-(1H-Imidazol-1-yl)propyl]-2-thioxo-2,3,5,6,7,8-hexahydro[1]benzothieno[2,3-d]pyrimidin-4(1H)-one 33.

Yield: 0.19 g (13.7%); mp: 119.0 °C; ¹H-NMR: δ 1.71-1.74 (br m, 4H); 2.09-2.13 (m, 2H); 2.62 (s, 2H); 2.75 (2H); 4.04-4.07 (m, 2H); 4.31-4.34 (m, 2H); 6.93 (s, H); 7.24 (s, H); 7.78 (s, H); 8.28 (s, H); MS: m/z 347.1 (M+H)⁺, 279.4 ([M-C₃H₃N₂])⁺; ESI-FTICR-MS: m/z 347.09949 ([M+H]⁺, calc. for C₁₆H₁₉ON₄S₂⁺ 347.09948); HPLC (214 nm. [A]): rt 23.07 min (94.9%).

3-[3-(1H-Imidazol-1-yl)propyl]-2-thioxo-1,2,3,5,6,7-hexahydro-4H-cyclopenta[4,5]thieno[2,3-d]pyrimidin-4-one 34.

3-(1H-imidazol-1-yl)propan-1-amine: 0.19 g (1.5 mmol); Yield: 0.020 g (4.0%) ; mp: 245.0 °C; ¹H-NMR (CD₃COOD): δ 2.54-2.56 (br m, 4H); 2.81-3.02 (br m, 4H); 4.50-4.64 (br m, 5H); 7.50 (s, H); 8.76 (s, H); MS: m/z 333.4 (M+H)⁺, 265.3 ([M-C₃H₃N₂])⁺; ESI-FTICR-MS: m/z 333.08382 ([M+H]⁺, calc. for C₁₅H₁₇ON₄S₂⁺ 333.08383); HPLC (214 nm. [C]): rt 9.88 min (99.9%).

3-[3-(1H-Imidazol-1-yl)propyl]-2-thioxo-2,3,5,6,7,8,9,10-octahydrocycloocta[4,5]thieno[2,3-d]pyrimidin-4(1H)-one 35.

3-(1H-imidazol-1-yl)propan-1-amine: 0.19 g (1.5 mmol); Yield: 0.005g (0.9%); mp: 228.0 °C; ¹H-NMR (CF₃COOD, 500 MHz): δ 1.39-1.41 (m, 2H); 1.47-1.52 (m, 2H); 1.58-1.62 (m, 2H); 1.80-1.86 (br m, 4H); 2.64-2.71 (m, 2H); 2.96-3.01 (m, 2H); 3.14-3.19 (m, 2H); 4.75-4.81 (m, 2H); 5.78 (s, H); 7.58 (s, H); 7.67 (s, H); 8.87 (s, H); MS: m/z 375.1 (M+H)⁺, 307.4 ([M-C₃H₃N₂])⁺; ESI-FTICR-MS: m/z 375.13121 ([M+H]⁺, calc. for C₁₈H₂₃ON₄S₂⁺ 375.13078); HPLC (214 nm. [C]): rt 12.70 min (98.6%).

2,3-Dihydro-3-(3-(5-methyl-1H-imidazol-1-yl)propyl)-2-thioxoquinazolin-4(1H)-one 46.

6: 0.20 g (1.43 mmol); Yield: 0.35 g (81.5%); mp: 228.0 °C; ¹H-NMR (500 MHz): δ 2.04-2.10 (m, 2H); 2.14 (s, 3H); 3.94-3.97 (m, 2H); 4.37-4.40 (m, 2H); 6.59 (s, H); 7.29-7.37 (br m, 2H); 7.55 (s, H); 7.69-7.73 (m, H); 7.93-7.94 (m, H); 12.91 (s, H); MS: m/z 301.2 (M+H)⁺, 219.4 ([M-C₄H₅N₂])⁺;

ESI-FTICR-MS: m/z 301.11180 ($[M+H]^+$, calc. for $C_{15}H_{17}ON_4S_1^+$ 301.11176); HPLC (214 nm, [C]): rt 10.53 min (100%).

3-[3-(5-Methyl-1H-imidazol-1-yl)propyl]-2-thioxo-2,3,5,6,7,8-hexahydro[1]benzothieno[2,3-d]pyrimidin-4(1H)-one 47.

6: 0.091 g (0.655 mmol); Yield: 0.20 g (84.8%); mp: 250.0 °C; 1H -NMR (CF_3COOD): δ 1.85-2.05 (br m, 4H); 2.49 (s, 3H); 2.52-2.54 (m, 2H); 2.78-2.80 (m, 2H); 2.95-3.00 (m, 2H); 4.00-4.10 (m, 2H); 4.70-4.80 (m, 2H); 7.23 (s, 2H); 8.73 (s, H); MS: m/z 361.2 ($M+H$) $^+$, 279.1 ($[M-C_4H_5N_2]^\bullet$) $^+$; ESI-FTICR-MS: m/z 361.11508 ($[M+H]^+$, calc. for $C_{17}H_{21}ON_4S_2^+$ 361.11513); HPLC (214 nm, [C]): rt 14.75 min (100%).

3-[3-(5-Methyl-1H-imidazol-1-yl)propyl]-2-thioxo-2,3,5,6,7,8,9,10-octahydrocycloocta[4,5]-thieno[2,3-d]pyrimidin-4(1H)-one 48.

8: 0.049 g (0.355 mmol); Yield: 0.11 g (76.2%); mp: 237.1 °C; 1H -NMR (CF_3COOD): δ 1.40-1.45 (m, 2H); 1.52-1.61 (m, 2H); 1.74-1.78 (m, 2H); 2.49 (s, 3H); 2.53-2.57 (m, 2H); 2.89-2.93 (m, 2H); 3.06-3.14 (m, 2H); 4.41-4.45 (m, 2H); 4.71-4.75 (m, 2H); 7.24 (s, H); 8.73 (s, H); MS: m/z 389.3 ($M+H$) $^+$; ESI-FTICR-MS: m/z 389.14661 ($[M+H]^+$, calc. for $C_{19}H_{25}ON_4S_2^+$ 389.14643); HPLC (214 nm, [C]): rt 17.65 min (93.1%).

6-Benzyl-2,3-dihydro-3-(3-(5-methyl-1H-imidazol-1-yl)propyl)-2-thioxothieno[2,3-d]pyrimidin-4(1H)-one 49.

6: 0.048 g (0.345 mmol); Yield: 0.09 g (65.8%); mp: 230.2°C; 1H -NMR (CF_3COOD): δ 2.47 (s, 3H); 2.53-2.56 (m, 2H); 4.15 (s, 2H); 4.39-4.42 (m, 2H); 4.70-4.74 (m, 2H); 7.07 (s, H); 7.23-7.34 (br m, 6H); 8.76 (s, H); MS: m/z 397.3 ($M+H$) $^+$; ESI-FTICR-MS: m/z 397.11503 ($[M+H]^+$, calc. for $C_{20}H_{21}ON_4S_2^+$ 397.11513); HPLC (214 nm, [C]): rt 16.21 min (100%).

2,3-Dihydro-6-methyl-3-(3-(5-methyl-1H-imidazol-1-yl)propyl)-5-phenyl-2-thioxothieno[2,3-d]pyrimidin-4(1H)-one 50.

6: 0.048 g (0.345 mmol); Yield: 0.115 g (84.1%); mp: 148.5 °C; ¹H-NMR (CF₃COOD): δ 2.33 (s, 3H); 2.44 (s, 3H); 2.46-2.50 (m, 2H); 4.33-4.37 (m, 2H); 4.63-4.68 (m, 2H); 7.20 (s, H); 7.24-7.27 8 (br m, 3H); 7.45 (s, 3H); 8.66 (s, H); MS: m/z 397.3 (M+H)⁺; ESI-FTICR-MS: m/z 397.11506 ([M+H]⁺, calc. for C₂₀H₂₁ON₄S₂⁺ 397.11513); HPLC (214 nm, [C]): rt 16.16 min (100%).

2,3-Dihydro-3-(3-(4-methyl-1H-imidazol-1-yl)propyl)-2-thioxoquinazolin-4(1H)-one 53.

8: 0.067 g (0.48 mmol); Yield: 0.105 g (73.0%); mp: 217.5 °C; ¹H-NMR (CD₃COOD): δ 2.36 (s, 3H); 2.50 - 2.53 (m, 2H); 4.35 - 4.39 (m, 2H); 4.65 - 4.69 (m, 2H); 7.16 (s, H); 7.35 - 7.37 (m, 2H); 7.43 - 7.47 (m, H); 7.78 - 7.82 (m, H); 8.10 - 8.12 (m, H); 8.56 (s, H); 8.62 (s, H); MS: m/z 301.4 (M+H)⁺, 219.4 ([M-C₄H₅N₂][•])⁺; ESI-FTICR-MS: m/z 301.11174 ([M+H]⁺, calc. for C₁₅H₁₇ON₄S⁺ 301.11176); HPLC (214 nm, [C]): rt 8.47 min (97.1%).

Molecular Modeling

Zinc transfer

For the positioning of the Zinc 1 into the existing hQC-model¹² the pdb structures 1moi and 1rtq (apAP) were taken from www.rcsb.org. Using the MOE software, the structures were aligned, whereas 1rtq was kept fixed. The substitution matrix was set as blossom62 and the „Enable Structural Alignment“ option was disabled. All of the other settings were used as default. Next, the Zinc 1 coordinating side chains (H330, E202 and D159 for 1moi and H256, E152 and D117 for 1rtq) were used as templates for the further refinement of the superposition of both protein structures. Thereby the side chains of 1rtq were fixed. Now all atoms of the template molecule 1rtq, except Zinc 1 and the metal coordinating water molecule, were removed. The „protonate 3D“ functionality of MOE was used for the proton attachment. For doing so, only the side chains of the protein were kept flexible and in the protonate3D.dat file the basic pK_a 14.5 for histidine was deleted. An energy minimization step followed by using the Charmm27 force field, keeping the backbone fixed and using the default parameters.

Loop Modeling

The side chain of Q304 was rotated, followed by an energy minimization of the 3 amino acids I303-D305 using Charmm27 with default parameters, all other atoms were kept fixed. Next, a MD-simulation of the whole loop beginning at S288 and ending at D305 was performed, by keeping all other atoms fixed. A simulated annealing was performed heating slowly from 100 K to 350 K in 100 ps, holding the temperature for 100 ps, followed by cooling down to 0 K in 50 ps.

Inhibitor Modeling

1-Methylimidazole was superimposed to the water molecule coordinating the zinc, whereas the N3 of imidazole was placed at the position of the water-oxygen. The water was deleted and the inhibitor was minimized using the “merck force field” (mff94x) keeping all other atoms fixed. Then all residues

containing atoms in a distance of 4.5 Å from the inhibitor were switched to the flexible mode with the exception of the zinc and the chelating amino acids. All other atoms of the protein were kept fixed. On the inhibitor site only the N3 of the imidazole residue was fixed, all other atoms were handled as flexible. The following MD-simulation was done by equilibrating the system for 10 ps at 300 K, simulating for 100 ps at the same temperature and cooling down to 0 K in 30 ps.

In the next step the phenyl ring of 1-benzylimidazole was attached to 1-methylimidazole, followed by an energy minimization as described for 1-methylimidazole. Again, all atoms in the 4.5 Å range around the inhibitor were handled as described before, and the imidazole ring was kept fixed for the following MD-simulation with the same temperature gradient as described for 1-methylimidazole.

Compound **1** was built by alignment of the alkyl chain with the phenyl ring atoms of 1-benzylimidazole. Again, only the inhibitor was energy minimized, followed by the same steps as described above. The final MD-simulation was performed as a simulated annealing by keeping the imidazole and the atoms C₁ and C₂ of the propyl-chain fixed and heating the system from 100 K to 350 K in 100 ps. This temperature was kept for 100 ps, followed by cooling down to 0 K in the 50 ps. The quality of the resulting complex was checked with PROCHECK. Outliers in loop regions, indicated by the Ramachandran-plot, were manually removed by rotating the corresponding dihedral angles and a subsequent energy minimization. The resulting complex was again checked by a Ramachandran- and a ProSa-plot. At the end, the homology model (for characteristics see Table S1) was superimposed with the template structure of apAP (1rtq) and the rmsd of the center of masses was calculated residue-wise using a script available from <http://svl.chemcomp.com/index.php>.

Table S1. Characteristics of the hQC homology model

Analysis method	Plot statistics		
Ramachandran	Residues in most favored regions	221	77.3%
	Residues in additional allowed regions	56	19.6%
	Residues in generously allowed regions	7	2.4%
	Residues in disallowed regions	2	0.7%
	Number of non-glycine and non-proline residues	286	100%
	Number of end-residues (excl. Gly and Pro)	3	
	Number of glycine residues	16	
	Number of proline residues	22	
	Total number of residues	327	
	Z-score of combined energy	-6.77	
Prosa	Z-score of pair energy (C ^β -interactions)	-5.62	
	Z-score of surface energy (C ^β)	-4.51	

Table S2. Fitness values and clash terms for the compounds **1**, **9**, **10**, **2**, **11** and **12** docked in the homology model using ChemScore as scoring function.

compound class	Cpd	best ranked solution		average of 20 solutions	
		Score	Clash	Score	Clash
Thiourea	1	22.01	1.86	19.47	2.06
	9	23.04	2.31	20.85	1.98
	10	20.83	3.76	17.15	4.85
Thioamide	2	28.39	0.52	25.79	2.36
	11	29.68	2.04	27.16	2.36
	12	26.51	4.06	24.24	5.03

Table S2. Fitness values and clash terms for the compounds **1**, **9**, **10**, **2**, **11** and **12** docked in the homology model using ChemScore as scoring function.

Table S3. Compounds used for the training and external validation of the QSAR-model. The measured and calculated/predicted activities are displayed as [%] of inhibition of the A β _{3(pE)-40/42}-peptide formation

Training set			Test set		
compound	measured	calculated	compound	measured	predicted
01	55.3	72.6	9	95.7	89.5
50	64.1	79.0	10	45.5	55.9
46	87.0	94.0	12	71.8	88.0
24	5.9	5.0	42	66.1	52.8
40	37.6	20.0	49	63.0	81.6
11	112.7	96.8	51	1.3	-4.9
43	63.7	53.0			
52	-15.9	-12.1			
47	89.7	100.3			
18	24.5	24.0			
38	57.5	54.1			
41	47.5	48.6			
33	45.3	28.0			
02	87.4	93.6			
39	32.2	41.1			
53	49.8	31.9			
45	27.2	30.8			
36	59.3	70.0			

Figure S1. Ramachandran plot⁴⁹ of the final hQC homology model. The plot statistics itself are given in Table S1

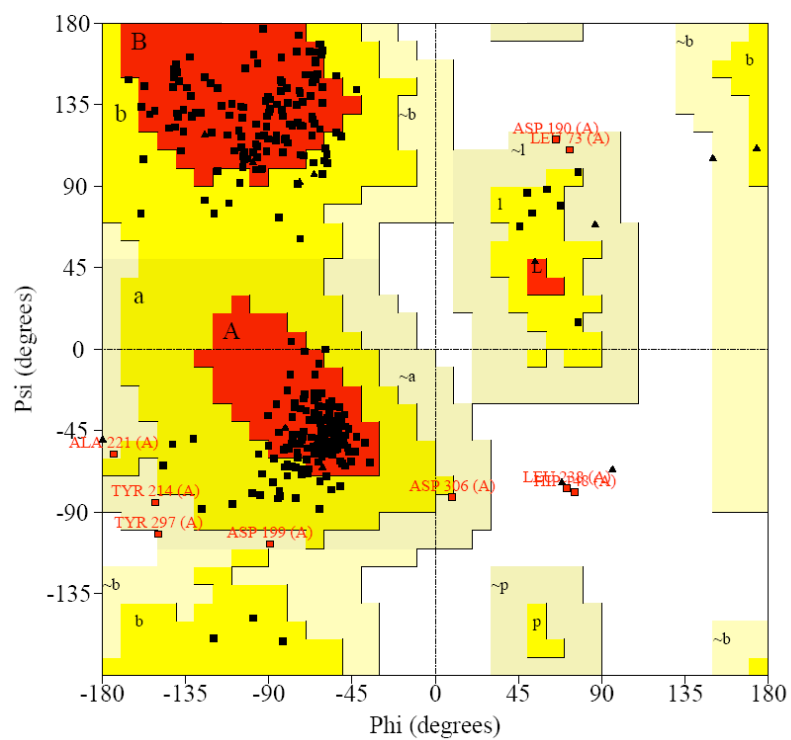


Figure S2. ProSa plots⁴⁷ of the template model 1moi¹² (dotted line) and the final hQC homology model (solid line). The incorporation of the zinc ion into the protein model and the subsequent MD-simulations led to an energetically more preferred model.

