A structure-based approach towards identification of inhibitory fragments for eleven-nineteen-leukemia protein (ENL)

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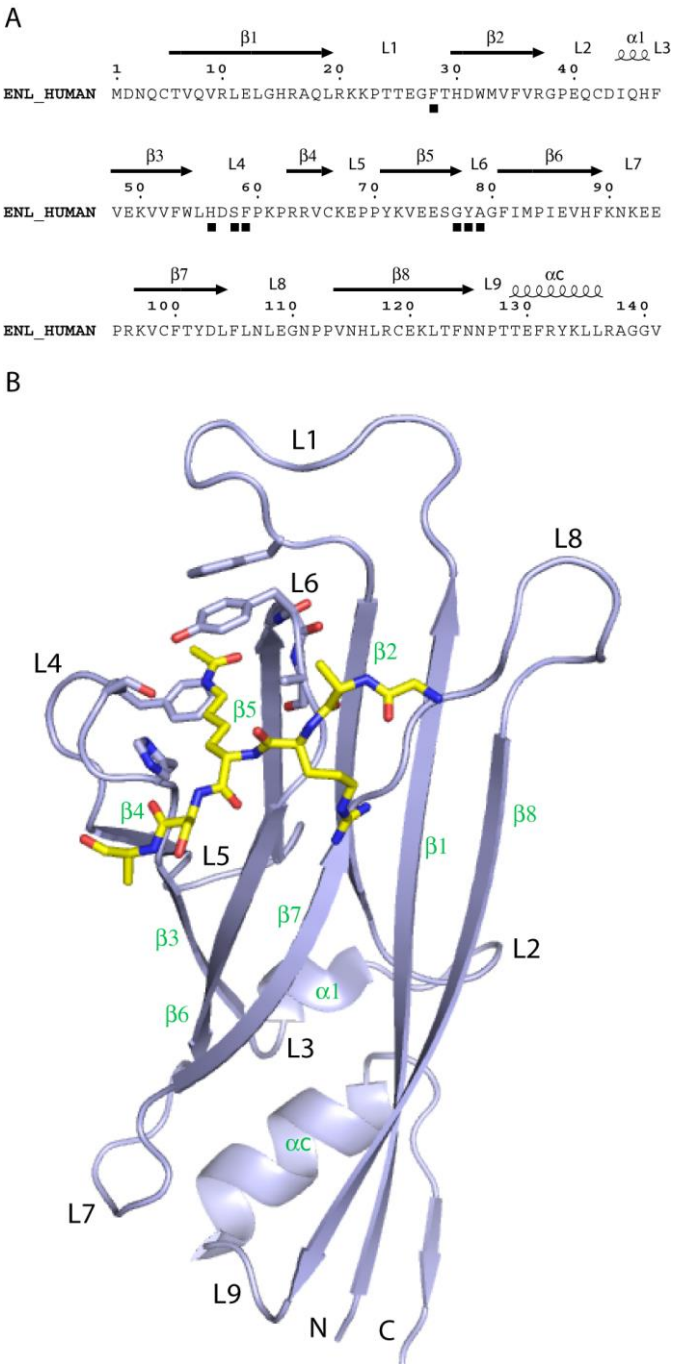
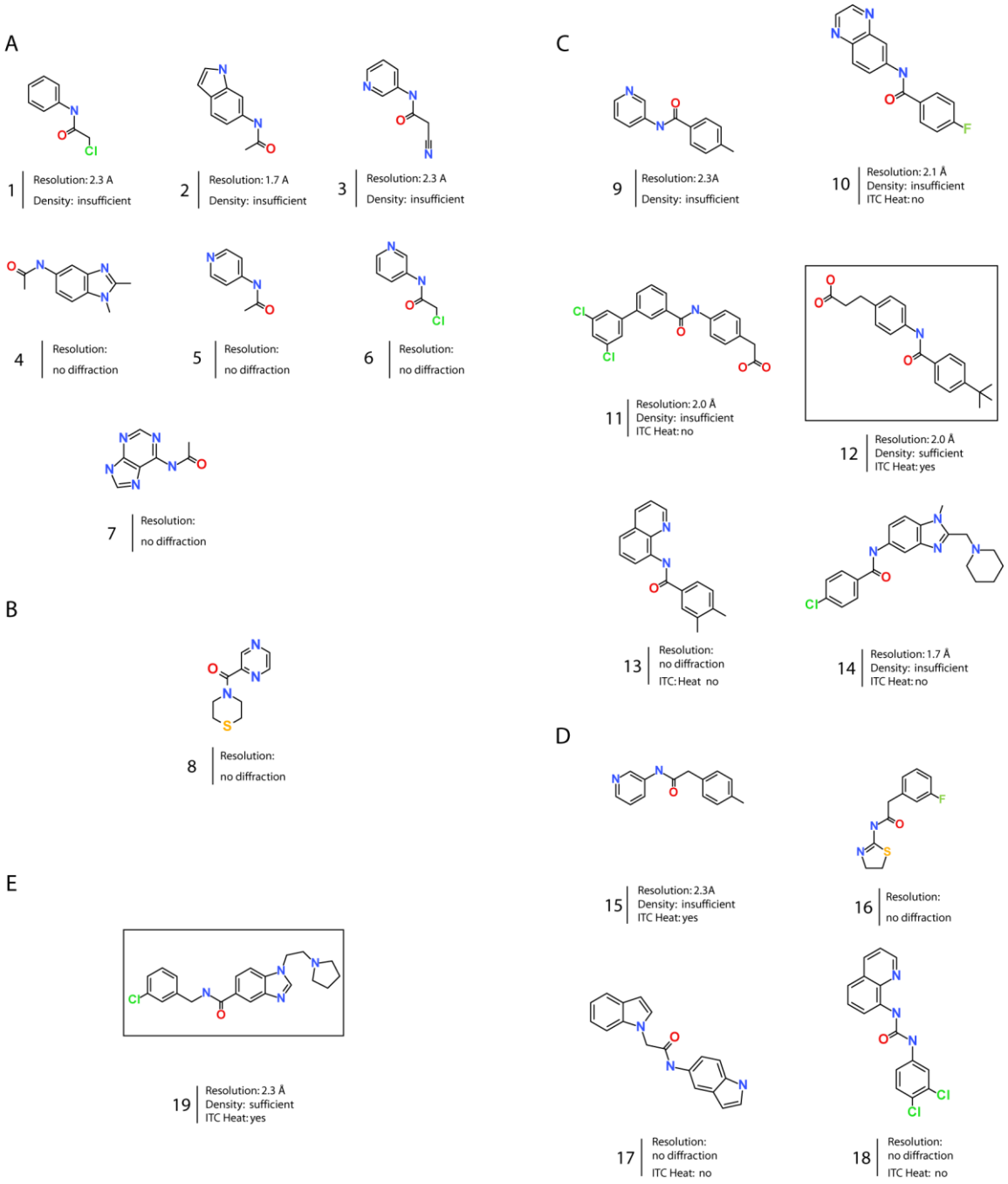
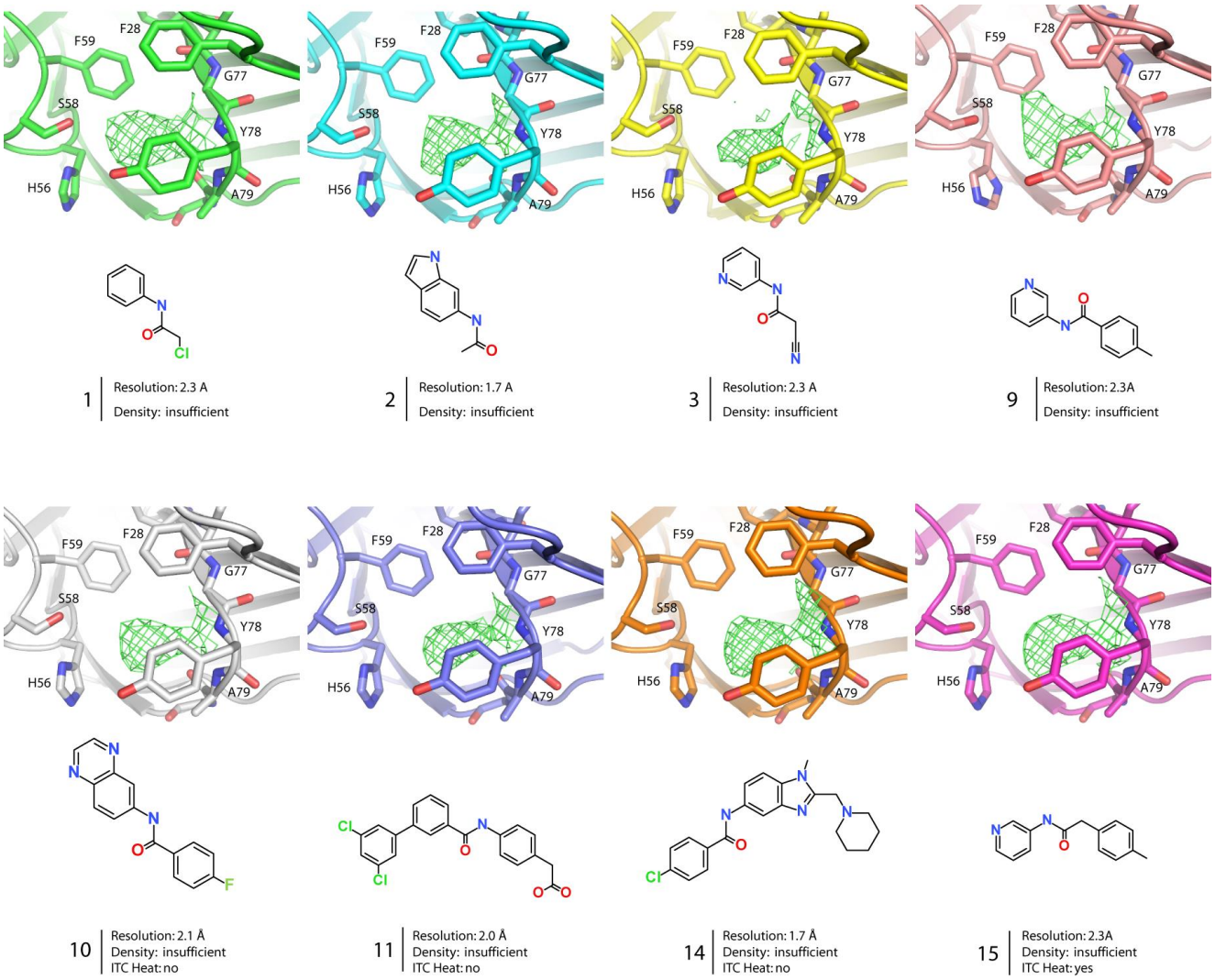


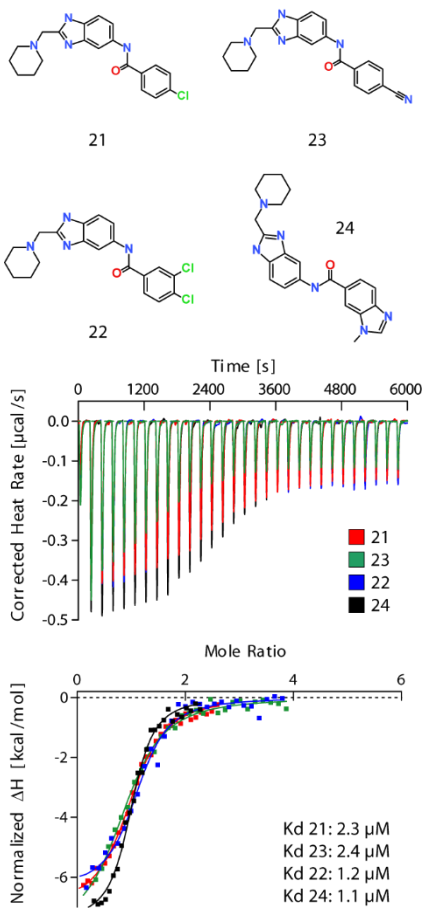
Supplementary Figure S1 Overall structures of YEATS domain. Shown are the seqence of ENL with secondary structure annotation (A) and overview of ENL structure (B).



Supplementary Figure S2 Structures of the selected 19 fragment-like compounds used in this study. Compounds were tested their binding using protein crystallography and ITC, of which the results are indicated.



Supplementary Figure S3 Analyses of electron density map within the binding pocket of ENL complexed with the studied ligands. In comparison to the apo structure, additional density in proximity to the binding site of the acetyl-lysine is observed in all complexes, suggesting the presence of the Kac mimetic amide group.



Supplementary Figure S4 ITC data for the interactions between ENL and compounds 21-24. Shown are the structures of initial hits (top) panel as well as isothermal titration calorimetry (ITC) data (lower panel). The ITC data are depicted as raw binding heats for compounds 21-24 colored as indicated in the figure, as well as normalized binding enthapies and fitted binding isotherms (Single binding site model). The dissociation constants (Kd) are given in the figure.

Compound synthesis

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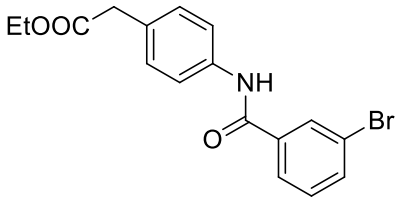
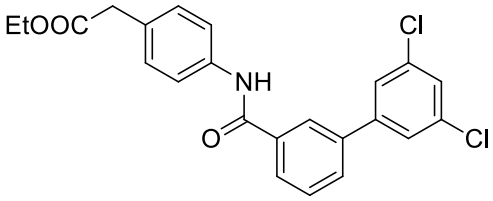


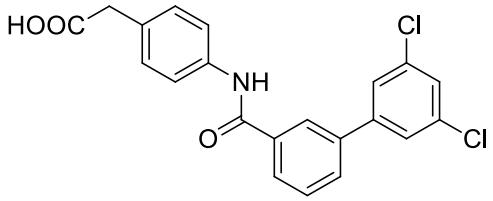
Step 1: Ethyl 2-(4-(3-bromobenzamido)phenyl)acetate

In a 50 mL round-bottomed flask purged with argon, Ethyl 4-aminophenylacetate (0.17 g, 0.98 mmol, 1.3 eq) was dissolved in $CHCl_3$ (abs., 20 mL) and cooled to 0 °C. 4-(Dimethylamino)pyridine (0.01 g, 0.08 mmol, 0.10 eq), 1-Ethyl-3-(3-dimethylaminopropyl)carbodiimid (0.16 g, 0.82 mmol, 1.1 eq) and 3-Bromobenzoic acid (0.15 g, 0.81 mmol, 1.0 eq) were added. The mixture was warmed to room temperature and afterwards stirred overnight under reflux. 5% aqueous hydrochloric acid (20 mL) was then added, phases were separated and the aqueous layer was extracted with EtOAc (3x20 mL). The combined organic layers were dried over Na_2SO_4 and the solvents were removed in vacuum. No further purification was performed to obtain the pale brown solid (0.27 g, 99%). ^{1}H NMR (250 MHz, DMSO-d_6) δ=10.32 (s, 1H), 8.14 (t, *J*=1.7 Hz, 1H), 8.00–7.90 (m, 1H), 7.82–7.75 (m, 1H), 7.70 (d, *J*=8.5 Hz, 2H), 7.50 (t, *J*=7.9 Hz, 1H), 7.25 (d, *J*=8.5 Hz, 2H), 4.08 (q, *J*=7.1 Hz, 2H), 3.63 (s, 2H), 1.19 (t, *J*=7.1 Hz, 3H). ^{13}C NMR (75 MHz, DMSO-d_6) δ=171.20, 163.86, 137.57, 137.05, 134.26, 130.65, 130.20, 129.93, 129.50, 126.83, 121.66, 120.43, 60.22, 14.08.



Step 2: Ethyl 2-(4-(3',5'-dichloro-[1,1'-biphenyl]-3-carboxamido)phenyl) acetate

In a 25 mL round-bottomed flask purged with argon, Ethyl 2-(4-(3-bromobenzamido)phenyl)acetate (0.27 g, 0.74 mmol, 1.0 eq), 3,5-dichlorophenylboronic acid (0.14 g, 0.74 mmol, 1.0 eq) and Cs_2CO_3 (0.6 g, 1.86 mmol, 2.5 eq) were dissolved in toluene (abs., 8 mL) and EtOH (abs., 0.8 mL) was added. The mixture was stirred for 30 min at room temperature. Tetrakis(triphenylphosphine)palladium$^{(0)}$ (0.09 g, 0.07 mmol, 0.1 eq) was added and the mixture was stirred at 80 °C for 5 h. After cooling to room temperature, H_2O (10 mL) was added, phases were separated, and the aqueous layer was extracted with EtOAc (3×10 mL). The combined organic layers were dried over Na_2SO_4 and the solvents were removed in vacuum. The crude product was purified by column chromatography on silica using hexane/EtOAc (80:20) as mobile phase to obtain the product as beige solid (0.08 g, 26%). R_f(hexane/EtOAc = 80:20) = 0.02. ^{1}H NMR (250 MHz, DMSO-d_6) δ=10.32 (s, 1H), 8.27 (s, 1H), 7.98 (t, *J*=7.0 Hz, 2H), 7.87 (d, *J*=1.8 Hz, 2H), 7.73 (d, *J*=8.4 Hz, 2H), 7.68–7.60 (m, 2H), 7.26 (d, *J*=8.4 Hz, 2H), 4.09 (q, *J*=7.1 Hz, 2H), 3.64 (s, 2H), 1.22–1.16 (m, 3H). ^{13}C NMR (75 MHz, DMSO-d_6) δ=171.37, 165.16, 143.12, 137.75, 137.38, 135.74, 134.88, 130.22, 130.00, 129.62, 129.48, 128.26, 127.35, 126.07, 125.76, 120.72, 60.37, 14.17.

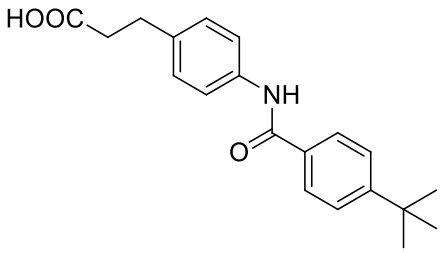


Step 3: 2-(4-(3',5'-Dichloro-[1,1'-biphenyl]-3-carboxamido)phenyl)acetic acid

In a 50 mL round-bottomed flask, Ethyl 2-(4-(3',5'-dichloro-[1,1'-biphenyl]-3-carboxamido)phenyl) acetate (0.08 g, 0.19 mmol, 1.0 eq) was dissolved in a mixture of THF (20 mL) and H_2O (2 mL). LiOH (0.03 g, 1.14 mmol, 6.0 eq) was added. The mixture was stirred overnight at 60 °C. 5% aqueous hydrochloric acid (20 mL) was then added, phases were separated and the aqueous layer was extracted with EtOAc (3x20 mL). The combined organic layers were dried over Na_2SO_4 and the solvents were removed in vacuum. The crude product was purified by crystallization from hexane/ethyl acetate to obtain the title compound as colorless solid (0.044 g, 58%). ^{1}H-NMR (500 MHz, DMSO-d_6): δ= 12.28 (s, 1H), 10.32 (s, 1H), 8.27 (s, 1H), 8.01-7.96 (m, 2H), 7.88 (d, *J*= 1.8 Hz, 2H), 7.72 (d, *J*= 8.5 Hz, 2H), 7.65 (dt, *J*= 15.6, 4.8 Hz, 2H), 7.26 (d, *J*= 8.5 Hz, 2H), 3.55 (s, 2H). ^{13}C-NMR (126 MHz, DMSO-d_6): δ= 172.82, 164.99, 143.06, 137.55, 137.30, 135.71, 134.79, 130.49, 130.15, 129.59, 129.36, 128.20, 127.28, 126.03, 125.72, 120.51, 30.43. HRMS (MALDI): *m/z* calculated 400.05018 for $C_{20}H_{24}NO_4$, found 400.04981 ($[M+H]^+$).

Compound purity was analyzed on a Varian ProStar HPLC (SpectraLab Scientific Inc., Markham, ON, Canada) equipped with a MultoHigh100 phenyl-5 µ 240 mm + 4 mm column (CS-Chromatographie Service GmbH, Langerwehe, Germany) using a gradient (H_2O/MeOH 80:20 + 0.1% formic acid isocratic for 5 min to MeOH + 0.1% formic acid after additional 45 min and MeOH + 0.1% formic acid for additional 10 min) at a flow rate of 1 mL/min and UV detection at 245 and 280 nm. The final compound for biological evaluation had a purity of ≥95%.

Reference: Schmidt, J.; Schierle, S.; Gellrich, L.; Kaiser, A.; Merk, D. Structural optimization and in vitro profiling of *N*-phenylbenzamide-based farnesoid X receptor antagonists. *Bioorg Med Chem* **2018**, *26*(14), 4240–53.



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3-(4-(4-(*tert*-Butylbenzamido)phenyl)propionic acid

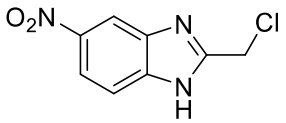
In a 50 mL round-bottomed flask purged with argon, 3-(4-Aminophenyl)propionic acid (0.17 g, 1.0 mmol, 1.0 eq) was dissolved in a mixture of THF (abs., 20 mL) and DMF (abs., 1 mL). Pyridine (0.24 mL, 3.0 mmol, 3.0 eq) was added before 4-*tert*-butylbenzoyl chloride (0.25 mL, 1.3 mmol, 1.3 eq) was added dropwise at room temperature. The mixture was stirred for two hours at room temperature. 5% aqueous hydrochloric acid (20 mL) was then added, phases were separated and the aqueous layer was extracted with EtOAc (3x20 mL). The combined organic layers were dried over Na_2SO_4 and the solvents were removed in vacuum. The crude product was purified by column chromatography on silica using hexane/EtOAc/acetic acid (74:24:2) as mobile phase to obtain the title compound as colorless solid (0.32 g, 98%). R_f(hexane/EtOAc/acetic acid = 74:24:2) = 0.31. ^{1}H-NMR (400 MHz, DMSO-d_6): δ = 12.12 (s, 1H), 10.09 (s, 1H), 7.87 (d, *J*= 8.5 Hz, 2H), 7.66 (d, *J*= 8.5 Hz, 2H), 7.53 (d, *J*= 8.5 Hz, 2H), 7.19 (d, *J*= 8.5 Hz, 2H), 2.79 (t, *J*= 7.6 Hz, 2H), 2.52 (t, *J*= 7.7 Hz, 2H), 1.32 (s, 9H). ^{13}C-NMR (100 MHz, DMSO-d_6): δ = 173.74, 165.29, 154.28, 137.24, 136.02, 132.31, 128.28, 127.44, 125.09, 120.29, 35.38, 34.64, 30.92, 29.85. HRMS (MALDI): *m/z* calculated 326.17507 for $C_{20}H_{24}NO_3$, found 326.17484 ($[M+H]^+$).

Compound purity was analyzed on a Varian ProStar HPLC (SpectraLab Scientific Inc., Markham, ON, Canada) equipped with a MultoHigh100 phenyl-5 µ 240 mm + 4 mm column (CS-Chromatographie Service GmbH, Langerwehe, Germany) using a gradient (H_2O/MeOH 80:20 + 0.1% formic acid isocratic for 5 min to MeOH + 0.1% formic acid after additional 45 min and MeOH + 0.1% formic acid for additional 10 min) at a flow rate of 1 mL/min and UV detection at 245 and 280 nm. The final compound for biological evaluation had a purity of ≥95%.

Reference: Schmidt, J.; Schierle, S.; Gellrich, L.; Kaiser, A.; Merk, D. Structural optimization and in vitro profiling of *N*-phenylbenzamide-based farnesoid X receptor antagonists. *Bioorg Med Chem* **2018**, *26*(14), 4240–53.

20-24

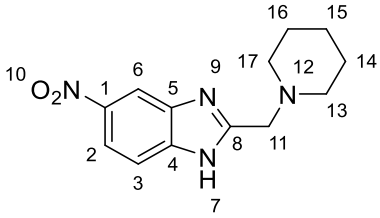
Step 1: 2-(chloromethyl)-5-nitro-1H-benzo[d]imidazole



4-nitrobenzene-1,2-diamine (10 g, 65.3 mmol, 1 eq) was dissolved in 4 *N* HCl solution (43.5 mL, 1.5 M) and had ethyl 2-chloroacetate (9.1 mL, 85 mmol, 1.3 eq) added dropwise before being heated to reflux for 6 hours. Upon reaction completion the mixture was cooled to 0°C with an ice bath and had ammonium hydroxide (19.3 mL, 174 mmol, 9 M) dropwise causing a red precipitate to form. The precipitate was filtered off and washed with H_2O (x 3) and dried in an oven vacuum (60°C) to give a dark red solid (13.15 g, 62.1 mmol, 95%) which was used without further purification.

LR-ESI-MS: $C_8H_7ClN_3O_2$ $[M+H]^+$ *m/z* found 212.20, calculated 212.02.

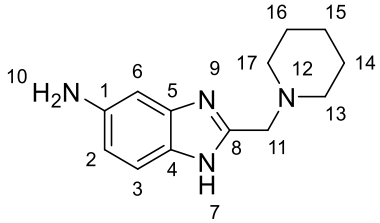
Step 2: 5-nitro-2-(piperidin-1-ylmethyl)-1H-benzo[d]imidazole



Initially a suspension of 2-(chloromethyl)-5-nitro-1H-benzo[d]imidazole (5 g, 23.6 mmol, 1 eq) and Na_2CO_3 (3.76 g, 35.4 mmol, 1.5 eq) in anhydrous MeCN (72 mL, 0.33 M). Piperidine (4.67 mL, 47.3 mmol, 2 eq) was added dropwise at room temperature. The reaction was allowed to stir at room temperature overnight. Upon reaction completion the suspension was filtered through a sintered frit and washed with acetone. The filtrate was concentrated to a residue which was purified using a Biotage LPLC KP Sil SNAP 25 g DCM:MeOH (0 to 20% MeOH) to give a red oil which was triturated with acetone to form an orange solid (2.245 g, 8.62 mmol, 37%).

Mpt: 149.3-151.3 °C; **ν_{max} (cm^{-1})** 2935, 2801, 1518, 1468, 1413, 1107, 884; **^{1}H NMR (400 MHz, DMSO-*d*$_6$) δ** 12.9 (s, 1H, 7), 8.4 (d, *J* = 2.3 Hz, 1H, 6), 8.1 (dd, *J* = 2.3, 8.9 Hz, 1H, 2), 7.6 (d, *J* = 8.9 Hz, 1H, 3), 3.7 (s, 2H, 11), 2.4 (t, *J* = 5.4 Hz, 4H, 13, 17), 1.5 (p, *J* = 5.6 Hz, 4H, 14, 16), 1.4 (q, *J* = 6.0 Hz, 2H, 15); **^{13}C NMR (101 MHz, DMSO-*d*$_6$) δ** 206.5 (1), 157.4 (8), 142.3 (2, 6), 117.4 (3), 56.5 (11), 54.2 (13, 17), 25.4 (14, 16), 23.6 (15); **LR-ESI-MS**: $C_{13}H_{17}N_4O_2$ $[M+H]^+$ *m/z* found 261.23, cald 261.14; **HR-ESI-MS**: $C_{13}H_{17}N_4O_2$ $[M+H]^+$ *m/z* found 261.1349, calculated 261.1352.

Step 3: 2-(piperidin-1-ylmethyl)-1H-benzo[d]imidazol-5-amine



Initially a stirred degassed solution of nitro-benzimidazole (2.057g, 7.90 mmol, 1 eq) and Pd (10% on Carbon, 10% mass) in MeOH (0.15 M) was carefully evacuated and backfilled with H_2 atmosphere (1 bar) and finally allowed to stir at room temperature overnight with a H_2 balloon attached. Upon reaction completion the suspension was filtered through celite with MeOH washings. The filtrate was then concentrated to an oil before being purified via Biotage LPLC using a KP-Sil-NH column eluting with Cy:EtOAc:MeOH (7:2:1) to give the corresponding aniline (1.511 g, 6.56 mmol, 83%) as a brown crystalline solid.

Mpt: 74.1-76.1 °C; **ν_{max} (cm^{-1})** 2931, 1632, 1428, 1336, 1105, 805, 624, 432; **^{1}H NMR (400 MHz, DMSO-*d*$_6$) δ** 7.2 (d, *J* = 8.5 Hz, 1H, 3), 6.6 (d, *J* = 2.1 Hz, 1H, 6), 6.5 (dd, *J* = 2.1, 8.5 Hz, 1H, 2), 3.6 (s, 2H, 11), 2.4 (d, *J* = 5.5 Hz, 4H, 13, 17), 1.5 (q, *J* = 5.6 Hz, 4H, 14, 16), 1.4 – 1.3 (m, 2H, 15); **^{13}C NMR (101 MHz, DMSO-*d*$_6$) δ** 148.9 (1), 144.2 (3), 110.9 (2, 6), 56.4 (11), 54.0 (13, 17), 25.3 (14, 16), 23.6 (15); **LR-ESI-MS**: $C_{13}H_{19}N_4$ $[M+H]^+$ *m/z* found 231.21, cald 231.16; **HR-ESI-MS**: $C_{13}H_{19}N_4$ $[M+H]^+$ *m/z* found 231.1602, cald 231.1610.

Step 4: derivatization of 2-(piperidin-1-ylmethyl)-1H-benzo[d]imidazol-5-amine (step 3 product) to final compounds

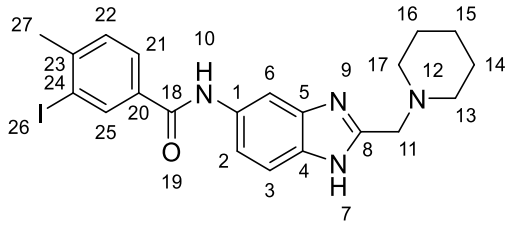
General Procedure A: Amide Synthesis

To a stirred solution of the corresponding anilines (1 eq) in CH_2Cl_2 (0.1 M, anhydrous) was added DIPEA-PS (200-400 mesh, 40% loading) (2 eq), the corresponding acid (1.2 eq) and HATU (1.2 eq). The suspension was stirred vigorously for 16 h before being concentrated onto silica gel and purified via Biotage LPLC using a mixture of DCM:DCM (20% MeOH) or CH:EA (10% MeOH). To give the products which were triturated to form solids with Et_2O or CH.

General Procedure B: Amide Synthesis

To a stirred solution of the corresponding anilines (1 eq) in CH_2Cl_2 (0.1 M, anhydrous) was added DIPEA-PS (200-400 mesh, 40% loading) (3 eq) and the corresponding acid chloride (1.5 eq). The suspension was stirred vigorously for 16 h before being concentrated onto silica gel and purified via Biotage LPLC using a mixture of DCM:DCM (20% MeOH) or CH:EA (10% MeOH). To give the products which were triturated to form solids with Et_2O or CH.

20: 3-iodo-4-methyl-*N*-(2-(piperidin-1-ylmethyl)-1H-benzo[d]imidazol-5-yl)benzamide

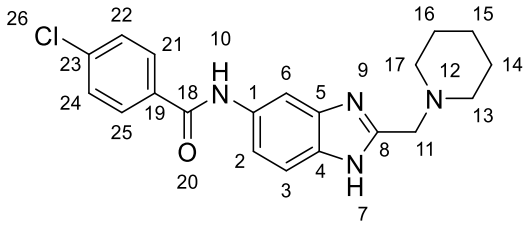


Synthesised according to general procedure **A** to give **20** (0.057 g, 0.121 mmol, 56 %) as a white solid.

Initially compound from step 3 (0.049 g, 0.216 mmol, 1 eq), 3-iodo-4-methylbenzoic acid (1.2 eq) and DIPEA-PS (200-400 mesh, 40% loading) (2 eq) were suspended in CH_2Cl_2 (0.1 M, anhydrous) before HATU (1.2 eq) was added in one portion. The suspension was stirred vigorously for 16 h before being concentrated onto silica gel and purified via Biotage LPLC using a mixture of DCM:MeOH (0 to 20% MeOH) to give a residue which was triturated to form a solid with cold Et_2O, **20** (0.057 g, 0.121 mmol, 56%).

Mpt: 156.5-158.5 °C; **ν_{max} (cm^{-1})** 2932, 1642, 1529, 1248,746, 556; **^{1}H NMR (400 MHz, DMSO-d_6) δ** 12.2 (s, 1H, 7), 10.2 (s, 1H, 10), 8.5 – 8.3 (m, 1H, 25), 8.0 (s, 1H, 3), 8.0 – 7.8 (m, 1H, 21), 7.6 – 7.3 (m, 3H, 2, 6, 22), 3.7 (s, 2H, 11), 2.5 – 2.3 (m, 7H, 13, 17, 27), 1.5 (p, *J* = 5.5 Hz, 4H, 14, 16), 1.4 (q, *J* = 6.0 Hz, 2H, 15); **^{13}C NMR (101 MHz, DMSO-d_6) δ** 163.4 (18), 144.4 (25), 139.2 (5), 137.4 (21), 134.3 (23), 130.0 (4), 129.7 (20), 129.2 (22), 127.7 (2, 6), 101.1 (24), 56.6 (11), 54.1 (13, 17), 27.5 (27), 25.4 (14, 16), 23.7 (15); **LR-ESI-MS**: $C_{21}H_{24}IN_4O$ $[M+H]^+$ *m/z* found 475.30, cald 475.09; **HR-ESI-MS**: $C_{21}H_{24}IN_4O$ $[M+H]^+$ *m/z* found 475.0960, cald 475.0995.

21: 4-chloro-*N*-(2-(piperidin-1-ylmethyl)-1H-benzo[d]imidazol-5-yl)benzamide 383 (EF00221a)

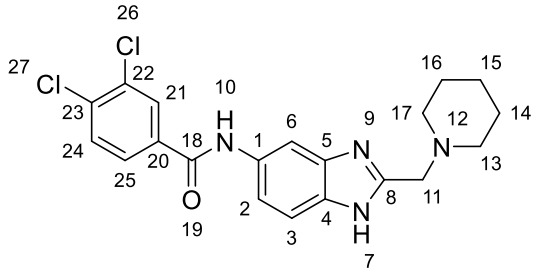


Synthesised according to general procedure **B** to give **21** (0.061 g, 0.166 mmol, 77 %) as a white solid.

Mpt: 122.3-124.3 °C; **ν_{max} (cm^{-1})** 2932, 1645, 1593, 1335, 1274, 843, 749; **^{1}H NMR (400 MHz, DMSO-d_6) δ** 13.2 (s, 1H, 10), 11.3 (s, 1H, 7), 9.1 – 8.8 (m, 3H, 3, 21, 25), 8.7 – 8.3 (m, 4H, 2, 6, 22, 24), 4.6 (s, 2H, 11), 3.5 (p, *J* = 1.8 Hz, 4H, 13, 17), 3.4 (t, *J* = 5.1 Hz, 4H, 14, 16), 2.4 (q, *J* = 6.0 Hz, 2H, 15); **^{13}C NMR (101 MHz, DMSO-d_6) δ** 164.1 (18), 136.2 (23), 133.9 (19), 131.1 (3), 129.6 (21, 25), 128.4 (22, 24), 128.4 (2,

6), 56.6 (11), 54.1 (13, 17), 25.4 (14, 16), 23.8 (15); **LR-ESI-MS**: $C_{20}H_{22}ClN_4O$ $[M+H]^+$ *m/z* found 369.27, cald 369.15; **HR-ESI-MS**: $C_{20}H_{22}ClN_4O$ $[M+H]^+$ *m/z* found 367.1467, cald 369.1482.

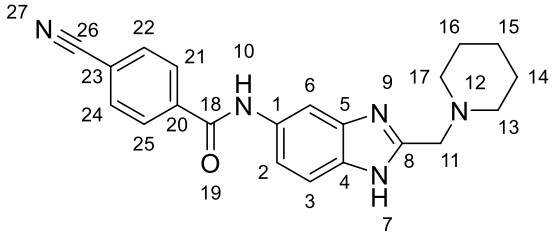
22: 3,4-dichloro-*N*-(2-(piperidin-1-ylmethyl)-1H-benzo[d]imidazol-5-yl)benzamide (417 (EF00223a)



Synthesised according to general procedure **B** to give **22** (0.031 g, 0.076 mmol, 35 %) as an off white solid.

Mpt: 142.2-144.2 °C; **ν_{max} (cm^{-1})** 2932, 1645, 1451, 1239, 1134, 1030, 746; **^{1}H NMR (400 MHz, DMSO-d_6) δ** 12.2 (s, 1H, 10), 10.4 (d, *J* = 19.7 Hz, 1H, 7), 8.2 (d, *J* = 2.1 Hz, 1H, 21), 8.1 – 7.9 (m, 2H, 6, 24), 7.8 (d, *J* = 8.4 Hz, 1H, 25), 7.5 – 7.3 (m, 2H, 2, 3), 3.6 (s, 2H, 11), 2.4 (t, *J* = 5.1 Hz, 4H, 13, 17), 1.5 (p, *J* = 5.5 Hz, 4H, 14, 16), 1.4 (d, *J* = 7.7 Hz, 2H, 15); **^{13}C NMR (101 MHz, DMSO-d_6) δ** 162.8 (18), 152.3 (8), 131.3 (21), 130.7 (25), 129.6 (23), 128.0 (22), 118.1 (3), 115.0 (2), 110.8 (6), 56.7 (11), 54.2 (13, 17), 25.4 (14, 16), 23.8 (15); **LR-ESI-MS**: $C_{20}H_{21}Cl_2N_4O$ $[M+H]^+$ *m/z* found 403.32, cald 403.11; **HR-ESI-MS**: $C_{20}H_{21}Cl_2N_4O$ $[M+H]^+$ *m/z* found 403.1072, cald 403.1092.

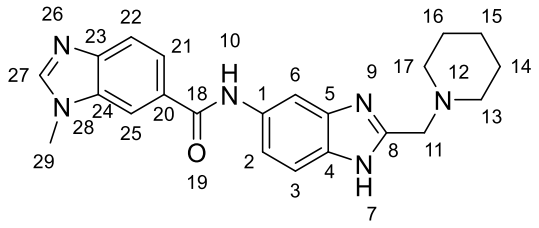
23: 4-cyano-*N*-(2-(piperidin-1-ylmethyl)-1H-benzo[d]imidazol-5-yl)benzamide 403 (EF00222a)



Synthesised according to general procedure **A** to give **23** (0.010 g, 0.028 mmol, 13 %) as a white solid.

Mpt: 133.1-135.1 °C; **ν_{max} (cm^{-1})** 2931, 1647, 1529, 1450, 1297, 1108, 808, 756, 621; **^{1}H NMR (400 MHz, DMSO-d_6) δ** 12.3 (s, 1H, 7), 10.5 (s, 1H, 10), 8.2 – 8.1 (m, 2H, 21, 25), 8.1 – 8.0 (m, 1H, 6), 8.0 – 8.0 (m, 2H, 22, 24), 7.6 – 7.3 (m, 2H, 2, 3), 3.6 (s, 2H, 11), 2.4 (t, *J* = 5.3 Hz, 4H, 13, 17), 1.5 (p, *J* = 5.5 Hz, 4H, 14, 16), 1.4 (dt, *J* = 4.7, 11.3 Hz, 2H, 15); **^{13}C NMR (101 MHz, DMSO-d_6) δ** 163.8 (18), 139.3 (20), 132.5 (21, 25), 128.5 (22, 24), 118.4 (23), 113.6 (26), 56.7 (11), 54.2 (13, 17), 25.5 (14, 16), 23.8 (15); **LR-ESI-MS**: $C_{21}H_{22}N_5O$ $[M+H]^+$ *m/z* found 360.64, cald 360.18; **HR-ESI-MS**: $C_{21}H_{22}N_5O$ $[M+H]^+$ *m/z* found 360.1811, cald 360.1824.

24: 1-methyl-*N*-(2-(piperidin-1-ylmethyl)-1H-benzo[d]imidazol-5-yl)-1H-benzo[d]imidazole-6-carboxamide 433 (EF00226a)



Synthesised according to general procedure **A** to give **24** (0.052 g, 0.135 mmol, 62 %) as an off white solid.

Mpt: 150.2-152.2 °C; **ν_{max} (cm^{-1})** 2932, 1642, 1527, 1415, 1248, 1220, 840, 732, 434; **^{1}H NMR (400 MHz, DMSO-d_6) δ** 12.2 (s, 1H, 10), 10.3 (d, *J* = 20.0 Hz, 1H, 7), 8.3 (q, *J* = 1.1 Hz, 1H, 27), 8.2 – 8.0 (m, 2H, 21, 25), 7.9 (dd, *J* = 0.8, 8.4 Hz, 1H, 3), 7.7 (dd, *J* = 1.4, 8.4 Hz, 1H, 6), 7.5 (dd, *J* = 8.4, 31.0 Hz, 2H, 2), 4.1 (s, 3H, 29), 3.7 (s, 2H, 11), 2.4 (d, *J* = 5.5 Hz, 4H, 13, 17), 1.5 (q, *J* = 5.6 Hz, 4H, 14, 16), 1.4 (d, *J* = 7.5 Hz, 2H, 15); **^{13}C NMR (101 MHz, DMSO-d_6) δ** 165.5 (18), 139.1 (27), 133.0 (20), 132.5 (21), 124.9 (22), 120.7 (2), 119.7 (3), 118.0 (25), 109.6 (6), 56.7 (11), 54.2 (13, 17), 35.6 (29), 25.5 (14, 16), 23.8 (15); **LR-ESI-MS**: $C_{22}H_{25}N_6O$ $[M+H]^+$ *m/z* found 389.42, cald 389.21; **HR-ESI-MS**: $C_{22}H_{25}N_6O$ $[M+H]^+$ *m/z* found 389.2071, cald 389.2090.

Purification and chromatography

Thin Layer Chromatography (TLC) was carried out using aluminium plates coated with 60 F_{254} silica gel. Plates were visualised using UV light (254 or 365 nm) or staining with Ninhydrin (1 M, EtOH) or 1% aq. $KMnO_4$. Normal-phase silica gel chromatography was carried out using Biotage Isolera One flash column chromatography system (LPLC). Reverse-phase high pressure liquid chromatography (RP-HPLC) was performed using a Waters system equipped with a Waters 2545 Binary Gradient Module, a SecurityGuard™ ULTRA cartridges for EVO-C18 UHPLC HPLC, Kinetex 5 µM EVO C18 100 Å 100 x 3.0 mm column and a Waters SQ Detector 2 using the stated eluent system. All compounds were determined to be of ≥95% ELSD purity using the stated Waters UHPLC using a 3-minute elution method, H_2O/MeCN (95:5 to 20:80, w/ NH_4OAc buffered to pH 6).

Characterization

Infrared spectroscopy was carried out with a Thermo Scientific Nicolet iS5 FT-IR spectrometer fitted with an iD7-ATR accessory, selected absorption maxima (ν_{max}) recorded in wavenumbers (cm^{-1}). NMR spectra were recorded using a Bruker Avance 400 MHz spectrometer using the deuterated solvent stated. Chemical shifts (δ) quoted in parts per million (ppm) and referenced to the residual solvent peak. Multiplicities are denoted as s- singlet, d- doublet, t- triplet, q- quartet and quin- quintet and derivatives thereof (br denotes a broad resonance peak). Coupling constants recorded as Hz and round to the nearest 0.1 Hz. Two-dimensional NMR experiments (COSY, HSQC, HMBC) were used to aid the assignment of ^{1}H and ^{13}C spectra. Low Resolution mass spectra were recorded on a Waters SQ Detector 2 (LC-MS). High Resolution Mass Spectrometry (HRMS) was recorded using an Agilent 6530 QTOF. Melting points were obtained using a Stuart SMP40 apparatus and are reported uncorrected in °C. Compound names were generated using ChemBioDraw Ultra v14 systematic naming. Atom numbering in structures is purely for the purposes of assignment and does not reflect IUPAC numbering conventions.

Supplementary table S1 Data collection and refinement statistics

Complex	ENL apo	ENL-Kac	ENL-12	ENL-19	ENL-20
PDB accession code	**6hq0**	**6hpz**	**6hpy**	**6hpx**	**6hpw**
Data Collection					
Resolution[a] (Å)	46.12-1.81 (1.84-1.81)	64.80-2.30 (2.38-2.30)	66.36-2.00 (2.05-2.00)	45.80-2.30 (2.38-2.30)	48.74-1.90 (1.94-1.90)
Spacegroup	$P4_32_12$	$P4_32_12$	$P4_32_12$	$P4_32_12$	$P4_32_12$
Cell dimensions	*a* = b = 49.4, *c* = 128.3 Å *α, β, γ* = 90.0˚	*a* = b = 49.3, *c* = 129.6 Å *α, β, γ* = 90.0˚	*a* = b = 49.0, *c* = 132.7 Å *α, β, γ* = 90.0˚	*a* = b = 48.4, *c* = 137.4 Å *α, β, γ* = 90.0˚	*a* = b = 48.7, *c* = 131.6 Å *α, β, γ* = 90.0˚
No. unique reflections[a]	15,436 (895)	7,707 (730)	11,691 (850)	7,846 (719)	13,292 (846)
Completeness[a] (%)	100.0 (99.8)	99.9 (100.0)	100.0 (100.0)	100.0 (100.0)	100.0 (100.0)
I/σI[a]	14.3 (3.2)	9.9 (2.1)	15.7 (7.7)	15.3 (7.0)	10.8 (2.5)
R_{merge}[a] (%)	0.090 (0.841)	0.147 (0.992)	0.079 (0.216)	0.057 (0.169)	0.115 (0.855)
Redundancy[a]	11.9 (10.2)	8.6 (8.6)	8.6 (8.9)	5.6 (5.8)	12.8 (12.8)
Refinement					
No. atoms in refinement (P/L/O)[b]	1,189/ 0/ 93	1,185/ 13/ 41	1,179/ 24/ 79	1,184/ 27/ 45	1,180/ 27/ 91
B factor (P/L/O)[b] (Å^2)	35/ 0/ 45	41/ 73/ 40	39/ 64/ 45	41/ 63/ 44	45/ 42/ 49
R_{fact} (%)	19.2	23.3	21.7	21.1	21.3
R_{free} (%)	23.7	29.6	26.0	26.1	28.1
rms deviation bond[c] (Å)	0.015	0.009	0.013	0.013	0.014
rms deviation angle[c] (°)	1.5	1.3	1.4	1.3	1.5
Molprobity Ramachandran					
Favour (%)	97.16	97.87	93.62	94.37	97.16
Allowed (%)	0.71	0	0	0	0
Crystallization condition	30% PEG 2000MME, 0.1M potassium bromide	25% PEG 1500	25% PEG3350, 0.2M ammonium sulfate, 0.1M bis-tris pH 5.5	25% PEG Smear Medium, 0.1M citrate pH 5.5	25% PEG3350, 0.2M ammonium acetate, 0.1M bis-tris pH 5.5

[a] Values in brackets show the statistics for the highest resolution shells.

[b] P/L/O indicate protein, ligand molecules presented in the active sites, and other (water and solvent molecules), respectively.

[c] rms indicates root-mean-square.