Supporting Information

Design, Synthesis and Biological Evaluation of Conformationally Constrained Analogs of Naphthol AS-E as Inhibitors of CREB-mediated Gene Transcription

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General information on chemical synthesis and compound characterization. The solvents used for each reaction were purified from the Glass Contout solvent purification system. Melting points were determined in capillary tubes using Mel-Temp and are uncorrected. All ^{1}H and ^{13}C NMR spectra were obtained in Bruker Avance 400 MHz spectrometer using either $CDCl_3$ or DMSO-d_6 as solvent and the chemical shifts of the residual $CHCl_3$ (δ 7.24) or DMSO (δ 2.50) were taken as reference. The following abbreviations were used to describe the splitting pattern of individual peaks if applicable: s = singlet, d = doublet, t = triplet, q = quartet, m = multiplet. The coupling constants (*J*) were reported in Hertz (Hz). Silica gel flash chromatography was performed using 230–400 mesh silica gel (EMD). The mass spectra were obtained from a TSQ LC/MS system (Thermo Scientific) with electrospray operated either in positive or negative mode. All final compounds were confirmed to be of > 95% purity based on HPLC (Waters) analysis using an XBridge C18 column (4.6 x 150 mm) and detected at 254 nm. The mobile phases for HPLC are water and acetonitrile, both of which contain 0.1% TFA.

3-(*tert*-Butoxycarbonylamino)-2-naphthoic Acid (9). A suspension of 3-amino-2-naphthoic acid (**8**) (50 mg, 0.269 mmol) and K_2CO_3 (75 mg, 0.54 mmol) in dioxane/H_2O (4 mL/2 mL) was cooled to 0 ^{o}C. Then $(Boc)_2O$ (117 mg, 0.52 mmol) was added in one portion. The resulting mixture was allowed to warm up to room temperature and stirred at room temperature for 10 h. Then dioxane was evaporated *in vacuo* and the residue was dissolved in water (10 mL), which was then washed with EtOAc (3 × 10 mL). The aqueous solution was then cooled to 0 ^{o}C and acidified to pH = 4 with 1 N HCl. The product was extracted into CH_2Cl_2 (3 × 10 mL). The combined organic layers were washed with water (2 × 10 mL) and brine (2 × 10 mL). The organic solution was dried

over anhydrous Na_2SO_4, filtered and concentrated to give a yellow solid (65 mg, 87%): m.p. > 180 ^oC (dec). ^1H NMR (400 MHz, $CDCl_3$) δ 10.01 (s, 1 H), 8.85 (s, 1 H), 8.77 (s, 1 H), 7.85 (d, *J* = 8.8 Hz, 1 H), 7.83 (d, *J* = 8.8 Hz, 1 H), 7.57 (t, *J* = 7.6 Hz, 1 H), 7.42 (t, *J* = 7.6 Hz, 1 H), 1.61 (s, 9 H); ESI-MS *m/z* 285.9 $(M-H)^-$.

3-(*tert*-Butoxycarbonylamino-*N*-(4-chlorophenyl)-2-naphthamide (10). HBTU (66 mg, 0.174 mmol) was added to a stirred solution of acid **9** (50 mg, 0.174 mmol), 4-chloroaniline (25 mg, 0.21 mmol) and DIPEA (61 μL, 0.35 mmol) in CH_3CN (2 mL). The solution was stirred at room temperature for overnight. CH_3CN was evaporated and the residue was subjected to silica gel flash column chromatography, eluting with hexanes:EtOAc = 8:1 to yield a white solid (15 mg, 39%): ^1H NMR (400 MHz, $CDCl_3$) δ 9.50 (s, 1 H), 8.75 (s, 1 H), 8.11 (s, 1 H), 8.01 (s, 1 H), 7.82 (d, *J* = 7.6 Hz, 1 H), 7.81 (d, *J* = 6.8 Hz, 1 H), 7.61 (d, *J* = 8.8 Hz, 2 H), 7.55 (t, *J* = 8.0 Hz, 1 H), 7.43 (t, *J* = 8.0 Hz, 1 H), 7.40 (d, *J* = 8.8 Hz, 2 H), 1.56 (s, 9 H).

3-Amino-*N*-(4-chlorophenyl)-2-naphthamide (11). A solution of HCl/Et_2O (2 N, 1 mL) was added to a stirred solution of **10** (10 mg, 0.025 mmol) in CH_2Cl_2 (1 mL) at room temperature. The resulting mixture was stirred at room temperature for overnight. TLC indicated the reaction was not complete. The solvent was removed under reduced pressure. Then the residue was dissolved in CH_2Cl_2 (5 mL) and CF_3COOH (100 μL) was added. The reaction mixture was stirred at room temperature for 8 h. The solvent was removed under reduced pressure and the residue was treated with Et_2O (2 mL). The precipitate was collected by filtration and washed with dichloromethane to give a white solid. The solid was dissolved in water (2 mL) and basified with saturated aqueous $NaHCO_3$ solution. The precipitate was collected by filtration to give a yellow solid (5 mg,

69%): m.p. 246-248 °C (dec). ^{1}H NMR (400 MHz, DMSO-d_6) δ 10.58 (s, 1 H), 8.17 (s, 1 H), 7.80 (d, J = 8.8 Hz, 2 H), 7.78 (d, J = 7.2 Hz, 1 H), 7.57 (d, J = 8.4 Hz, 1 H), 7.43 (d, J = 8.8 Hz, 2 H), 7.38 (t, J = 8.0 Hz, 1 H), 7.20 (t, J = 8.0 Hz, 1 H), 7.07 (s, 1 H); ESI-MS *m/z* 294.8 $(M-H)^-$; HRESI-MS for $C_{17}H_{13}ClN_2O$-H, calcd 295.0633, found 295.0627.

Methyl 3-Fluoro-2-naphthoate (19). The methyl ester of **8** was prepared as described before.[1] A solution of $NaNO_2$ (347 mg, 5.0 mmol) in water (0.5 mL) was slowly added to a stirred suspension of methyl 3-amino-2-naphthoate (505 mg, 2.5 mmol) in HBF_4 (48%, 10 mL) in a plastic bottle at -15 °C. The reaction mixture was stirred for 20 min between -10 and 0 °C. The precipitate was collected by filtration, washed with HBF_4 (48%, 10 mL) and Et_2O (10 mL). The resulting yellow diazonium salt was air-dried overnight at room temperature. Then the diazonium salt was suspended in toluene (30 mL) and the mixture was heated under reflux for 3 h. The precipitate was removed by filtration and the filtrate was concentrated. The residue was then purified by column chromatography eluting with hexanes:EtOAc (10:1-4:1) to give a yellow solid 129.7 mg (25%): m.p. 68-69 °C. ^{1}H NMR (400 MHz, $CDCl_3$) δ 8.49 (d, J = 7.6 Hz, 1 H), 7.88 (d, J = 8.4 Hz, 1 H), 7.76 (d, J = 8.4 Hz, 1 H), 7.56 (t, J = 7.6 Hz, 1 H), 7.49 (d, J = 11.6 Hz, 1 H), 7.46 (t, J = 7.6 Hz, 1 H), 3.97 (s, 3 H); ^{13}C NMR (100 MHz, $CDCl_3$) δ 164.9 (d, $^3J_{CF}$ = 5 Hz), 158.3 (d, $^1J_{CF}$ = 256 Hz), 136.0 (d, $^3J_{CF}$ = 10 Hz), 134.1, 129.3, 129.2, 129.0, 126.8 (d, $^3J_{CF}$ = 5 Hz), 125.9, 118.7 (d, $^2J_{CF}$ = 15 Hz), 112.6 (d, $^2J_{CF}$ = 22 Hz), 52.4; ESI-MS *m/z* 204.9 $(M+H)^+$.

3-Fluoro-2-naphthaldehyde (20). DIBAL-H (1.2 M in toluene, 0.7 mL, 0.83 mmol) was slowly added to a stirred solution of ester **19** (142 mg, 0.7 mmol) in toluene (5 mL) at -78 °C. The reaction mixture was stirred at -78 °C for 30 min, when MeOH (0.2 mL) was

slowly added to quench the reaction. The reaction mixture was then diluted with EtOAc (70 mL), which was then washed with a saturated solution of Rochelle's salt (2 × 10 mL), H_2O (2 × 10 mL) and brine (2 × 10 mL). The organic solution was dried over anhydrous Na_2SO_4, filtered and concentrated. The residue was then subjected to column chromatography eluting with hexanes : EtOAc (10:1-4:1) to give a light yellow powder (76.5 mg, 76.5%): ^{1}H NMR (400 MHz, $CDCl_3$) δ 10.39 (s, 1 H), 8.35 (d, J = 6.8 Hz, 1 H), 7.90 (d, J = 8.0 Hz, 1 H), 7.76 (d, J = 8.4 Hz, 1 H), 7.58 (t, J = 7.6 Hz, 1 H), 7.45-7.48 (m, 2 H); ^{13}C NMR (100 MHz, $CDCl_3$) δ 158.3 (d, $^1J_{CF}$ = 250 Hz), 145.2 (d, $^3J_{CF}$ = 3 Hz), 134.5 (d, $^3J_{CF}$ = 9 Hz), 130.2, 128.5, 128.0 (d, $^3J_{CF}$ = 4 Hz), 127.8, 127.0, 125.8, 120.2 (d, $^2J_{CF}$ = 15 Hz), 111.7 (d, $^2J_{CF}$ = 21 Hz).

***N*-(4-Chlorophenyl)-3-fluoro-*N*′-hydroxy-2-naphthimidamide (22).** An aqueous solution of $NH_2OH{\cdot}HCl$ (400 mg, 5.7 mmol) in H_2O (0.5 mL) was added to a stirred solution aldehyde **20** (76.5 mg, 0.44 mmol) in EtOH (1.6 mL) at room temperature. The reaction mixture was then stirred at room temperature for overnight. EtOAc (100 mL) was added to dilute the reaction mixture, which was then washed with H_2O (2 × 10 mL) and brine (2 × 10 mL). The organic solution was dried over anhydrous Na_2SO_4, filtered and concentrated. The residue was then treated with CH_2Cl_2 (2 mL) and the precipitate was collected by filtration to provide the oxime as a white solid (36.8 mg). The mother liquid solution was concentrated and the residue was then subjected to column chromatography eluting with hexanes : EtOAc (10:1-4:1) to give another portion of oxime (11.2 mg). The combined yield of oxime was 58%: ^{1}H NMR (400 MHz, $CDCl_3$) δ 8.46 (s, 1 H), 8.20 (d, J = 7.2 Hz, 1 H), 8.02 (s, 1 H), 7.84 (d, J = 8.0 Hz, 1 H), 7.75 (d, J = 8.4 Hz, 1 H), 7.42-7.52 (m, 3 H); ^{13}C NMR (100 MHz, $CDCl_3$) δ 187.4 (d, $^3J_{CF}$ = 5

Hz), 160.0 (d, $^{1}J_{CF}$ = 253 Hz), 136.8 (d, $^{3}J_{CF}$ = 10 Hz), 131.9, 129.9, 129.7, 129.5, 127.1 (d, $^{3}J_{CF}$ = 5 Hz), 126.2, 124.0 (d, $^{2}J_{CF}$ = 13 Hz), 112.1 (d, $^{2}J_{CF}$ = 19 Hz). Half of NCS (25 mg, 0.19 mmol) was added to a stirred solution of the oxime (32.5 mg, 0.17 mmol) made above in DMF (0.5 mL) at room temperature. The reaction mixture was heated to 55 ^{o}C for 5 min, when the other half of NCS was added and the reaction mixture was stirred at 55 ^{o}C for another10 min. TLC indicated complete consumption of starting material. The reaction mixture was cooled to room temperature and H_2O (1 mL) was added to quench the reaction. EtOAc (80 mL) was added to dilute the reaction mixture. The organic layer was separated and washed with H_2O (2 × 10 mL) and brine (2 × 10 mL). The organic solution was dried over anhydrous Na_2SO_4, filtered and concentrated. The residue was then dissolved in THF (1 mL) and 4-chloroaniline (108.5 mg, 0.85 mmol) added at 0 ^{o}C. The resulting mixture was then stirred at room temperature for overnight, when it was diluted with EtOAc (100 mL). The organic layer was separated and washed with 1 N HCl (2 × 10 mL), H_2O (2 × 10 mL) and brine (2 × 10 mL). The organic solution was dried over anhydrous Na_2SO_4, filtered and concentrated. The residue was then subjected to column chromatography eluting with hexanes : EtOAc (10:1-3:1) to give a light brown solid (6 mg, 11% over two steps): ^{1}H NMR (400 MHz, $CDCl_3$) δ 8.04 (d, *J* = 7.2 Hz, 1 H), 7.85 (d, *J* = 8.0 Hz, 1 H), 7.74 (d, *J* = 8.0 Hz, 1 H), 7.54 (t, *J* = 7.6 Hz, 1 H), 7.47 (t, *J* = 7.6 Hz, 1 H), 7.34 (d, *J* = 11.2 Hz, 1 H), 6.99 (d, *J* = 8.8 Hz, 2 H), 6.60 (d, *J* = 9.2 Hz, 2 H); ^{13}C NMR (100 MHz, $CDCl_3$) δ 157.3 (d, $^{1}J_{CF}$ = 250 Hz), 143.4, 137.8, 134.7 (d, $^{3}J_{CF}$ = 9 Hz), 131.5 (d, $^{3}J_{CF}$ = 4 Hz), 130.0, 128.9, 128.5, 128.4, 128.0, 127.1 (d, $^{3}J_{CF}$ = 5 Hz), 125.9, 123.8, 122.0, 119.7 (d, $^{2}J_{CF}$ = 19 Hz).

3-Fluoro-2-naphthoic Acid (23). LiOH (46 mg, 1.9 mmol) was added to a stirred solution of **19** (129.7 mg, 0.64 mmol) in THF-MeOH-H_2O (2:2:1, 2.5 mL) at 0 ^oC. The reaction mixture was warmed up to room temperature and stirred at room temperature for 24 h. The organic solvents were removed *in vacuo* and the residue was dissolved in H_2O (1 mL). HCl (1 N) was added at 0 ^oC to adjust the pH to ~3.0. The precipitate was collected by filtration and washed with cold H_2O to give a pale yellow solid (103 mg, 85%): mp. ^1H NMR (400 MHz, $CDCl_3$) δ 8.64 (d, J = 7.2 Hz, 1 H), 7.94 (d, J = 8.4 Hz, 1 H), 7.80 (d, J = 8.4 Hz, 1 H), 7.61 (t, J = 7.6 Hz, 1 H), 7.55 (d, J = 12.0 Hz, 1 H), 7.51 (t, J = 7.6 Hz, 1 H); ^{13}C NMR (100 MHz, $CDCl_3$) δ 168.7, 158.7 (d, $^1J_{CF}$ = 256 Hz), 136.6 (d, $^3J_{CF}$ = 10 Hz), 135.3, 129.6, 129.3, 126.9 (d, $^3J_{CF}$ = 5 Hz), 126.1, 117.5 (d, $^2J_{CF}$ = 14 Hz), 112.9 (d, $^2J_{CF}$ = 22 Hz); ESI-MS *m/z* 189 $(M-H)^-$.

***N*-(4-Chlorophenyl)-3-fluoro-2-naphthamide (24).** BOP (34 mg, 0.077 mmol) and DIPEA (13.5 μL, 0.092 mmol) were sequentially added to a stirred solution of acid **23** (14.7 mg, 0.077 mmol) in CH_2Cl_2 (2 mL) at room temperature. The resulting mixture was stirred at room temperature for 5 min, when 4-chloroaniline (12 mg, 0.092 mmol) and an additional portion of DIPEA (20 μL, 0.12 mmol) were added. The reaction mixture was stirred at room temperature for overnight. EtOAc (70 mL) was added to dilute the reaction mixture, which was then washed with 1 N HCl (2 × 10 mL), H_2O (2 × 10 mL) and brine (2 × 10 mL). The organic solution was dried over anhydrous Na_2SO_4, filtered and concentrated. The residue was then subjected to column chromatography eluting with hexanes : EtOAc (10:1) to give a white powder (15 mg, 65%): ^1H NMR (400 MHz, $CDCl_3$) δ 8.73 (d, J = 8.0 Hz, 1 H), 8.61 and 8.57 (brs, 1 H, the NH appears as two peaks perhaps due to different conformations), 7.96 (d, J = 8.0 Hz, 1 H), 7.80 (d, J = 8.4 Hz, 1

H), 7.66 (d, J = 8.8 Hz, 2 H), 7.55-7.62 (m, 2 H), 7.51 (t, J = 7.6 Hz, 1 H), 7.34 (d, J = 8.4 Hz, 2 H); ^{13}C NMR (100 MHz, $CDCl_3$) δ 161.3, 157.3 (d, $^1J_{CF}$ = 242 Hz), 136.4, 135.5 (d, $^3J_{CF}$ = 10 Hz), 134.4, 130.1, 129.8, 129.4, 129.1, 126.8 (d, $^3J_{CF}$ = 4 Hz), 126.4, 121.8, 120.6 (d, $^2J_{CF}$ = 15 Hz), 112.2 (d, $^2J_{CF}$ = 25 Hz); ESI-MS *m/z* 297.8 $(M-H)^-$; HRESI-MS for $C_{17}H_{11}ClFNO$-H, calcd 298.0430, found 298.0422.

***N*-(4-Chlorophenyl)-3-fluoronaphthalene-2-carbothioamide (25).** A mixture of amide **24** (80 mg, 2.6 mmol) and Lawesson's reagent (160 mg, 3.9 mmol) in toluene (1 mL) was stirred under reflux for 12 h. The reaction mixture was cooled to room temperature and evaporation of toluene resulted in a residue, which was subjected to silica gel flash column chromatography, eluting with hexanes : ethyl acetate (5 : 1) to give a yellow solid (60 mg, 72%): m.p. 145 - 146 °C. ^{1}H NMR (400 MHz, $CDCl_3$) δ 9.41 (s, 1 H), 8.74 (d, J = 8.0 Hz, 1 H), 7.96 (d, J = 8.0 Hz, 1 H), 7.80 (t, J = 8.0 Hz, 1 H), 7.78 (d, J = 8.8 Hz, 2 H), 7.61–7.49 (m, 3 H), 7.44 (d, J = 8.8 Hz, 2 H).

Benzo[*g*]quinazolin-4-ol (26). 3-Amino-2-naphthoic acid (100 mg, 0.54 mmol) was mixed with formamidine acetate (168 mg, 1.6 mmol) and formamide (22 μL, 0.54 mmol). The reaction mixture was stirred at 160 °C for 2 h. After being cooled down to room temperature, the resulting solid was dissolved in hot 10% NaOH solution (10 mL). The clear solution was neutralized to pH = 7 by 2 N HCl. The precipitate was collected by filtration, washed with cold water and dried to give an off-white solid (75 mg, 72%): mp: 267 - 268 °C. ^{1}H NMR (400 MHz, DMSO-d_6) δ 12.1 (brs, 1 H), 8.87 (s, 1 H), 8.28 (s, 1 H), 8.25 (d, J = 8.0 Hz, 1 H), 8.15 (d, J = 8.4 Hz, 1 H), 8.10 (s, 1 H), 7.71 (t, J = 7.2 Hz, 1 H), 7.63 (t, J = 7.2 Hz, 1 H); ^{13}C NMR (100 MHz, DMSO-d_6) δ 161.7, 145.1, 144.7, 136.5, 131.4, 129.7, 129.0, 128.3, 127.7, 126.8, 125.2, 122.1.

4-Chlorobenzo[*g*]quinazoline (27). A solution of compound **26** (75 mg, 0.38 mmol) in $POCl_3$ (2 mL) was stirred at reflux for 24 h. The reaction mixture was cooled to room temperature and poured into an aqueous solution of NaOH (5%, 100 mL) at 0 ^oC. The precipitate was collected by filtration and washed with water (10 mL) to give a yellow solid (65 mg, 90%): mp: >291 ^oC (dec). ^1H NMR (400 MHz, $CDCl_3$) δ 9.04 (s, 1 H), 8.93 (s, 1 H), 8.66 (s, 1 H), 8.18 (d, *J* = 8.4 Hz, 1 H), 8.14 (d, *J* = 8.8 Hz, 1 H), 7.71 (td, *J* = 8.0, 1.2 Hz, 1 H), 7.66 (td, *J* = 8.4, 1.6 Hz, 1 H); ^{13}C NMR (100 MHz, $CDCl_3$) δ 164.0, 152.3, 145.4, 136.9, 132.8, 129.3, 129.0, 128.4, 127.6, 127.1, 126.7, 121.8.

***In vitro* KIX-KID *renilla* luciferase complementation assay.** RLucC-KIX (20 ng) and KID-RLucN-containing cell lysates (1.0 μg), both of which were prepared as described before,[2] were mixed together in 1 X *renilla* luciferase lysis buffer (Promega, Madison, WI) in the presence of different concentrations of different compounds. The final volume of the incubation mixture is 40 μL. The mixture was incubated at 4 ^oC for 20-24 h. Then residual *renilla* luciferase activity was measured by combining 5 μL of the incubation mixture with 30 μL of benzyl-coelenterazine (Nanolight, Pinetop, AZ) solution in PBS (pH 7.4, 10 μg/mL) and expressed as RLU. The IC_{50} was derived from non-linear regression analysis of the RLU-concentration curve in Prism 5.0 (La Jolla, CA)

CREB-reporter assay. HEK293T cells in a 10-cm plate were transfected with pCRE-RLuc (6.0 μg) with Lipofectamine™ 2000 (Invitrogen, Carlsbad, CA) according to manufacturer's protocol. After 3 h, the transfected cells were collected and replated into a 96-well plate (1-2 × 10^4 cells/well). The cells were allowed to attach to the bottom of the wells for overnight, when compounds of different concentrations were added to the cells. Forskolin (final concentration of 10 μM, LC Laboratories, Woburn, MA) was added 30

min after the addition of the compounds. The cells were then incubated at 37 ^{o}C for 4.5 h and the media were removed. The cells were then lysed in 1 X *renilla* luciferase lysis buffer (30 μL). To measure *renilla* luciferase activity, five μL of the lysate was combined with 30 μL of benzyl-coelenterazine solution in PBS (pH 7.4, 10 μg/mL). The sample protein concentration was determined by Dye Reagent Concentrate (Bio-Rad, Hercules, CA). The luciferase activity was normalized to protein content in each well and expressed as relative luciferase unit/μg protein (RLU/μg protein). The IC_{50} was derived from non-linear regression analysis of the RLU/μg protein-concentration curve in Prism 5.0 (La Jolla, CA).

Molecular modeling. All the molecular modeling studies were performed in Schrödinger suite of molecular modeling package (Portland, OR). A systematic conformational search was carried out for each molecule to locate their respective global minimum with MacroModel. The conformational search was following a systematic torsional sampling algorithm with MMFFs force filed, MMFFs charges and a constant dielectric constant of 1.0. The minimization algorithm was Polak-Ribier conjugate gradient (PRCG) with a gradient convergence threshold of 0.005 kJ/mol. The cLogP value was calculated from their global minimum using QirkProp. To calculate the molecular electrostatic potential surfaces, the structures were first optimized at the HF/3-21G level of theory and then at the HF/6-31G** level of theory in Jaguar 7.7. The conformational minima were confirmed by the absence of any negative frequencies. With these optimized structures, their electrostatic potential surfaces were calculated to map the total electron densities. All the surfaces were normalized to the same scale from -60 kcal/mol to +60 kcal/mol.

References:

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