Supporting Information

Application of Fragment Screening by X-ray Crystallography to the Discovery of Aminopyridines as Inhibitors of β-Secretase

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Supporting Information

HPLC Purity

Analytical reversed phase high performance liquid chromatography – mass spectrometry (LC-MS) was performed on a Waters HPLC Separation Module 2795 system equipped with an autosampler, a Micromass Platform LC mass spectrometer and a Waters 2996 photodiode array detector. Purity of the final compounds was determined by LC-MS using two different chromatographic methods. First method (Basic method) - Column: Phenomenex Gemini (5µm, 2.0 mm x 50 mm); Mobile phase A: 10 mM ammonium bicarbonate in water adjusted to pH 9.2 with ammonium hydroxide; Mobile phase B: acetonitrile; Flow rate: 0.8 mL/min; Elution profile: gradient elution from 5% to 95% B over 3.5 minutes followed by isocratic elution at 95% B for 1 minute. Second method (Polar method) - Column: Phenomenex Synergi MAX-RP 80A (4µm, 2.0 mm x 50 mm); Mobile phase A: 0.1% formic acid in water; Mobile phase B: 0.1% formic acid in acetonitrile; Flow rate: 0.8 mL/min; Elution profile: gradient elution from 0% to 50% B over 3 minutes followed by isocratic elution at 95% B for 1.5 minutes.

Purity of key compounds as determined by LC-MS (two methods)

	LC-MS : Basic method		**LC-MS : Polar method**	
Compound number	**Retention time (min)**	**Area under peak (%)**	**Retention time (min)**	**Area under peak (%)**
2	2.84	100	2.43	100
3	2.79	100	2.55	100
4	3.34	94.3	3.00	98.3
5	2.58	100	2.44	100
6a	2.56	100	2.10	97.2
6b	2.64	97.5	2.35	93.0
6c	3.00	91.3	2.45	93.6
7	3.07	100	3.04	93.1

8a	3.49	92.2	3.41	95.1
8b	3.08	100	2.96	100

Additional Compound NMR and MS Data

6-[2-(1H-Indol-6-yl)-ethyl]-pyridin-2-ylamine (3): From **12b**. Yellow oil (70%). ^{1}H NMR (400 MHz, DMSO-d_6): δ 2.79 (t, *J* = 7, 2H), 2.98 (t, *J* = 7, 2H), 5.82 (br s, 2H), 6.26 (d, *J* = 8, 1H), 6.35 –6.37 (m, 2H), 6.74 (dd, *J* = 8, 1.5, 1H), 7.20 (s, 1H), 7.23-7.27 (m, 2H), 7.42 (d, *J* = 8, 1H), 10.90 (br s, 1H). MS (+ES): *m/e* 238 (MH^+).

N~3~-[5-(1H-Indol-6-yl)-2-(pyridin-2-ylmethoxy)-benzyl]-pyridine-2,3-diamine (8b): From **18b**. Pale yellow solid (65%). ^{1}H NMR (400 MHz, DMSO-d_6): δ 4.43 (d, *J* = 5.5, 2H), 5.32 (s, 2H), 5.37 (t, *J* = 5.5, 1H), 5.55 (br s, 2H), 6.41 (br s, 2H), 6.58 (d, *J* = 7.5, 1H), 7.15-7.22 (m, 2H), 7.28 (d, *J* = 4, 1H), 7.33-7.38 (m, 2H), 7.50 (s, 2H), 7.56 (d, *J* = 8, 1H), 7.61 (s, 2H), 7.84 (td, *J* = 7.5, 1.5, 1H), 8.61 (d, *J* = 4, 1H), 11.11 (s, 1H). MS (+ES): *m/e* 422 (MH^+).

2-{6-[(E)-2-(1H-Indol-6-yl)-vinyl]-pyridin-2-yl}-isoindole-1,3-dione (11b): From **10c** and indole-6-carboxaldehyde. Pale yellow foam (60%). ^{1}H NMR (400 MHz, DMSO-d_6): δ 6.92 (d, *J* = 2, 1H), 7.82 (d, *J* = 17, 1H), 7.93 (m, 3H), 8.10 (d, *J* = 7, 1H), 8.16 (s, 1H), 8.23 (d, *J* = 7, 1H), 8.28 (d, *J* = 17, 1H), 8.50 (m, 2H), 8.55 (m, 3H), 11.79 (br s, 1H). MS (+ES): *m/e* 366 (MH^+).

2-{6-[(E)-2-(3-Bromo-phenyl)-vinyl]-pyridin-2-yl}-isoindole-1,3-dione (11c): From **10c** and 3-bromobenzaldehyde. Colourless solid (57%). ^{1}H NMR (400 MHz, DMSO-d_6): δ 7.36 (t, *J* = 8, 1H), 7.46-7.53 (m, 3H), 7.62 (d, *J* = 15, 1H), 7.70 (m, 2H), 7.92-7.98 (m, 3H), 8.00-8.09 (m, 3H). MS (+ES): *m/e* 405, 407 (MH^+).

2-{6-[2-(1H-Indol-6-yl)-ethyl]-pyridin-2-yl}-isoindole-1,3-dione (12b): From **11b**. Pale yellow foam (90%). ^{1}H NMR (400 MHz, DMSO-d_6): δ 3.10 (m, 4H), 6.35 (d, *J* = 2, 1H),

6.90 (d, *J* = 7, 1H), 7.27 (m, 2H), 7.35-7.46 (m, 3H), 7.92 (t, *J* = 7, 1H), 7.95 (m, 2H), 8.03 (m, 2H), 10.92 (br s, 1H). MS (+ES): *m/e* 368 (MH^+).

6-[(E)-2-(3-Bromo-phenyl)-vinyl]-pyridin-2-ylamine (13a): From **11c**. Pale yellow solid (84%). ^{1}H NMR (400 MHz, DMSO-d_6): δ 5.92 (br s, 2H), 6.39 (d, *J* = 7, 1H), 6.64 (d, *J* = 7, 1H), 7.17 (d, *J* = 15, 1H), 7.32-7.46 (m, 4H), 7.58 (d, *J* = 7, 1H), 7.82 (s, 1H). MS (+ES): *m/e* 275, 277 (MH^+).

N~3~-(3-benzyl-boronic acid)-pyridine-2,3-diamine (15b): From **14** and 3-formylphenyl boronic acid. Yellow solid (41%). ^{1}H NMR (400 MHz, MeOH-d_4): δ 4.39 (s, 2H), 6.62 (dd, *J* = 7.5, 5.4, 1H), 6.8 (d, *J* = 7.5, 1H), 7.22 (d, *J* = 5.4, 1H), 7.32 (t, *J* = 5.9, 1H), 7.41 (br d, 1H), 7.53 (br d, 1H), 7.68 (s, 1H). MS (+ES): *m/e* 244 (MH^+).

5-Bromo-2-(pyridin-2-ylmethoxy)-benzaldehyde (17b): From **16** and 2-chloromethylpyridine hydrochloride. Tan solid (89%). ^{1}H NMR (400 MHz, DMSO-d_6): δ 5.38 (s, 2H), 7.32 (d, *J* = 8, 1H), 7.37 (dd, *J* = 5, 2, 1H), 7.64 (d, *J* = 7, 1H), 7.78-7.90 (m, 3H), 8.60 (d, *J* = 2, 1H), 10.39 (s, 1H). MS (+ES): *m/e* 292, 294 (MH^+).

N~3~-[5-Bromo-2-(pyridin-2-ylmethoxy)-benzyl]-pyridine-2,3-diamine (18b): From **17b**. Pale yellow solid (42%). ^{1}H NMR (400 MHz, DMSO-d_6): δ 4.34 (d, *J* = 5.5, 2H), 5.28 (s, 2H), 5.37 (t, *J* = 5.5, 1H), 5.53 (br s, 2H), 6.37-6.46 (m, 2H), 7.06 (d, *J* = 9, 1H), 7.30 (d, *J* = 5, 1H), 7.34-7.39 (m, 3H), 7.57 (d, *J* = 8, 1H), 7.83 (td, *J* = 7.5, 1.5, 1H), 8.60 (d, *J* = 4.5, 1H). MS (+ES): *m/e* 385, 387 (MH^+).