Supporting Information for

Discovery of Potent and Selective Inhibitors for ADAMTS-4 Through DNA-encoded Library Technology (ELT)

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Library synthesis

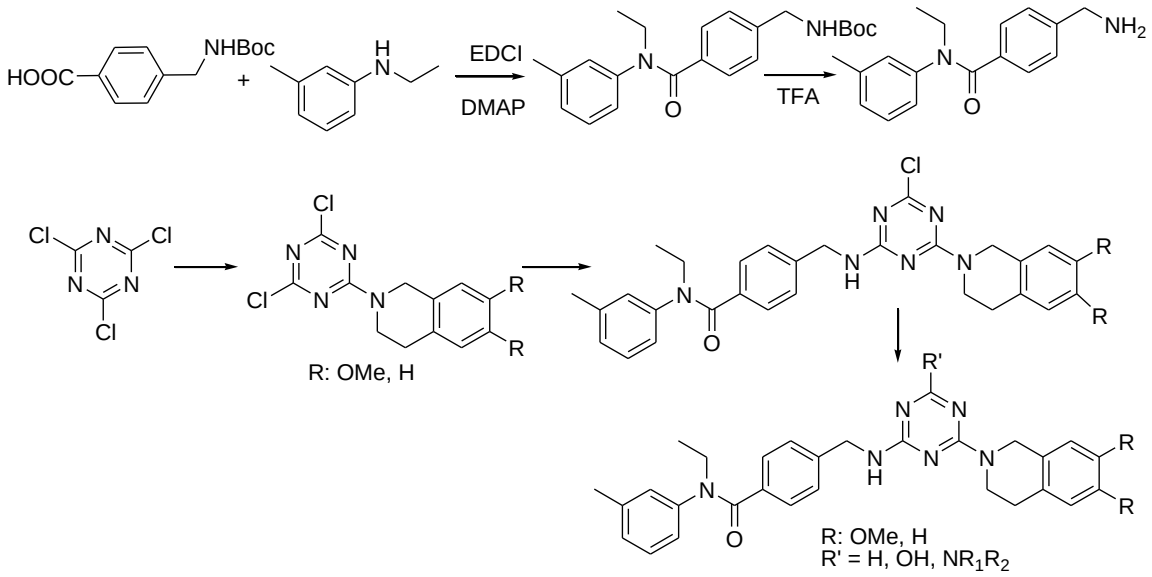
Please see supporting information in reference "*Nature Chemical Biology* **2009**, *5*, 647-654".

Chemical Synthesis.

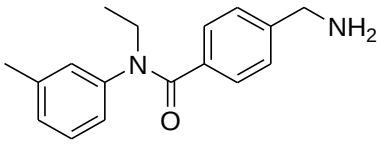
All reagents and solvents were of commercial quality and used without further purification unless indicated otherwise.

NMR spectra were recorded on a Varian Mercury 400 Plus. Chemical shifts are expressed in parts per million (ppm, δ units). Coupling constants (*J*) are in units of hertz (Hz). Splitting patterns describe apparent multiplicities and are designated as s (singlet), d (doublet), t (triplet), q (quartet), dd (double doublet), dt (double triplet), m (multiplet), br (broad). All mass spectra were performed under electrospray ionization (ESI) method. All the final compounds for biological assay were purified on Gilson system with a Phenomenex Luna 5 um C8(2), 100 mm x 21 mm 100A column with MeCN/H_2O (+ 0.1% TFA) solvents. High-resolution mass measurements were performed on Bruker MicroTOF electrospray mass spectrometer coupled with an Agilent 1100 HPLC system.

Scheme 1



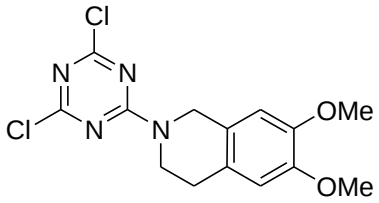
Preparation of 4-(aminomethyl)-*N*-ethyl-*N*-*m*-tolylbenzamide



A solution of 4-(Boc-aminomethyl)benzoic acid (1.0 g, 3.98 mmol, 1 equivalent), *N*-ethyl *m*-toluidine (614 mg, 4.54 mmol, 1.14 equivalents) and DMAP (98 mg, 0.80 mmol, 0.2 equivalent) in dichloromethane (15 mL) was cooled with stirring in an ice bath. 1-Ethyl-3-[3-(dimethylamino)propyl]carbodiimide hydrochloride (EDCI, 955 mg, 4.98 mmol, 1.25 equivalents) was added, and the reaction mixture was stirred at room temperature overnight. The solution was diluted with dichloromethane (50 mL), which was further washed with saturated sodium bicarbonate (25 mL), water (25 mL), salt (25 mL) and dried over Mg_2SO_4. The solvent was removed *in vacuo* to give *tert*-butyl 4-(ethyl(*m*-tolyl)carbamoyl)benzylcarbamate. Without purification, the acylation product was treated with 50% TFA in dichloromethane (25 mL) at

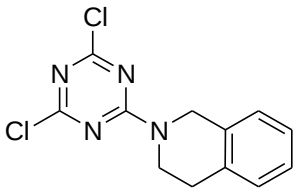
room temperature for 20 minutes. The reaction mixture was condensed to give the yellow oil which was further purified by RP-HPLC (Luna, 5µ C8(2), 100x21 mm, 10-40% CH_3CN/H_2O, 0.1% TFA, 20 min) to give the desired product as the TFA salt (1.172 g, 77% in 2 steps). MS: calcd for C17H20N2O+H^+ 269.36; found 269.20. ^{1}H NMR (DMSO-*d6*, 400 MHz) δ 8.21 (br. s., 3 H), 7.20 – 7.39 (m, 4H), 7.12 (t, *J* = 7.82 Hz, 1 H), 7.06 (s, 1 H), 7.00 (d, *J* = 7.42 Hz, 1 H), 6.87 (d, *J* = 7.82 Hz, 1 H), 3.97 (br. s., 2 H), 3.83 (q, *J* = 7.16 Hz, 2 H), 2.23 (s, 3 H), 1.09 (t, *J* = 7.03 Hz, 3 H). ^{13}C NMR (DMSO-*d6*, 400 MHz) δ 168.44, 142.41, 138.57, 136.65, 134.85, 128.74, 128.25, 128.18, 127.94, 127.40, 125.34, 44.54, 41.68, 20.69, 12.55.

Preparation of 2-(4,6-dichloro-1,3,5-triazin-2-yl)-6,7-dimethoxy-1,2,3,4-tetrahydroisoquinoline



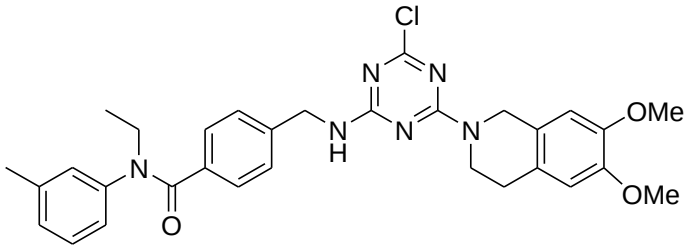
A mixture of cyanuric chloride (520 mg, 2.82 mmol, 1equivalent) in CH_3CN/H_2O (1/1, 20 mL) was cooled to 0 °C. 6,7-Dimethoxy-1,2,3,4-tetrahydroisoquinoline hydrochloride (647.2 mg, 2.82 mmol, 1 equivalent) was added. The reaction mixture was adjusted to a pH of about 9-10 using 1N NaOH. The reaction was stirred at cold for about 20 mins, during which a white solid was formed. The solid was collected by filtration to give the desired product with >95% purity (755.5 mg, 79% yield). MS: calcd for C14H14Cl2N4O2+H^+ 341.06; found 341.19. ^{1}H NMR ($CDCl_3$, 400 MHz) δ 6.68 (s, 1 H), 6.66 (s, 1 H), 4.86 (s, 2 H), 4.06 (t, *J* = 5.86 Hz, 2 H), 3.87 (s, 3 H), 3.87 (s, 3 H), 2.88 (t, *J* = 5.86 Hz, 2 H). ^{13}C NMR ($CDCl_3$, 400 MHz) δ 170.15, 170.09, 163.93, 148.09, 148.01, 125.82, 123.55, 111.26, 109.10, 55.99, 55.97, 45.95, 42.30, 27.98.

Preparation of 2-(4,6-dichloro-1,3,5-triazin-2-yl)-1,2,3,4-tetrahydroisoquinoline



A mixture of cyanuric chloride (544 mg, 2.95 mmol, 1 equivalent) in CH_3CN/H_2O (1/1, 20 mL) was cooled to 0 °C. 1,2,3,4-Tetrahydroisoquinoline hydrochloride (500.5 mg, 2.95 mmol, 1 equivalent) was added. The reaction mixture was adjusted to a pH of about 9-10 using 1N NaOH. The reaction was stirred at cold for 20 mins, during which a white solid was formed. The solid was collected by filtration to give the desired product with >95% purity (774.5 mg, 93.5% yield). MS: calcd for C12H10Cl2N4+H^+ 281.04; found 281.19. ^{1}H NMR ($CDCl_3$, 400 MHz) δ 7.18-7.26 (m, 4 H), 4.93 (s, 2 H), 4.07 (t, J = 6.25 Hz, 2H), 2.97 (t, J = 6.06 Hz, 2H). ^{13}C NMR ($CDCl_3$, 400 MHz) δ 170.19, 170.14, 164.01, 134.08, 131.87, 128.54, 127.19, 126.83, 126.45, 46.26, 42.31, 28.48.

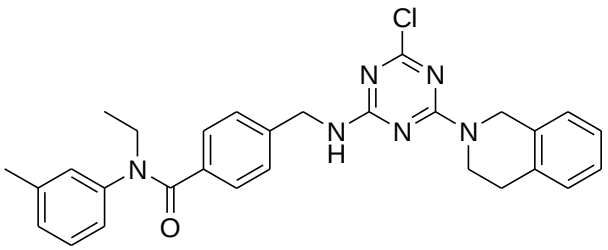
Preparation of 4-((4-chloro-6-(6,7-dimethoxy-3,4-dihydroisoquinolin-2(1*H*)-yl)-1,3,5-triazin-2-ylamino)methyl)-*N*-ethyl-*N*-*m*-tolylbenzamide (1g)



To a mixture of 2-(4,6-dichloro-1,3,5-triazin-2-yl)-6,7-dimethoxy-1,2,3,4-tetrahydroisoquinoline (0.15 mmol, 1 equivalent) in CH_3CN/H_2O (1/1, ~ 4 mL) was added 4-(aminomethyl)-*N*-ethyl-*N*-*m*-tolylbenzamide as the TFA salt (58 mg, 0.15 mmol, 1 equivalent). The pH of the reaction mixture was adjusted with 1N NaOH to about 9-10, and the reaction was stirred at room temperature overnight. LC-MS showed the complete conversion to the desired product. Without workup, the product in the reaction solution will be used directly for the next step. The pure product can be obtained through the purification with RP-HPLC (Luna, 5µ C8(2),

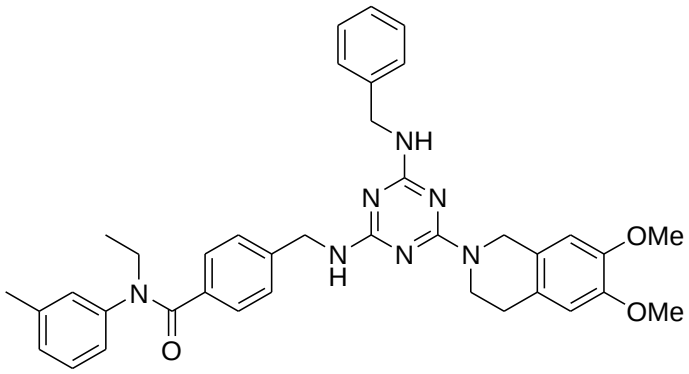
100x21mm, 40-95% CH_3CN/H_2O, 0.1% TFA, 20 min). MS: calcd for C31H33ClN6O3+H^+ 573.24; found 573.3.

Preparation of 4-((4-chloro-6-(3,4-dihydroisoquinolin-2(1*H*)-yl)-1,3,5-triazin-2-ylamino)methyl)-*N*-ethyl-*N*-*m*-tolylbenzamide



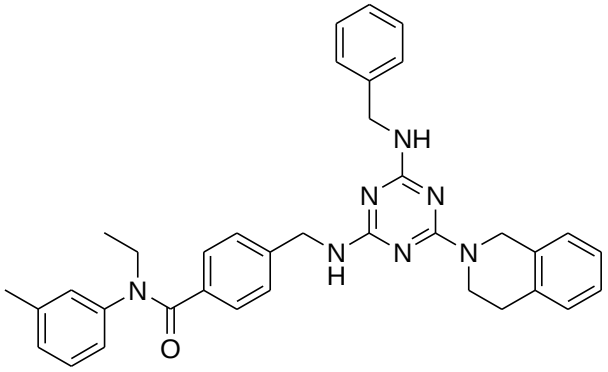
To a mixture of 2-(4,6-dichloro-1,3,5-triazin-2-yl)-1,2,3,4-tetrahydroisoquinoline (30 mg, 0.107 mmol, 1 equivalent) in CH_3CN/H_2O (1/1, 2 mL) was added 4-(aminomethyl)-*N*-ethyl-*N*-*m*-tolylbenzamide as the TFA salt (41 mg, 0.107 mmol, 1 equivalent). The pH of the reaction mixture was adjusted with 1N NaOH to about 9-10, and the reaction was stirred at room temperature overnight. LC-MS showed complete conversion to the desired product. Without workup, the product in the reaction solution will be used directly for the next step. The pure product can be obtained through the purification with RP-HPLC (Luna, 5µ C8(2), 100x21 mm, 40-95% CH_3CN/H_2O, 0.1% TFA, 20 min). MS: calcd for C29H29ClN6O+H^+ 513.22; found 513.36. ^{1}H NMR ($CDCl_3$, 400 MHz, mixture of rotamers) δ 9.93 (br. s., 3H), 9.39 (br. s., 1H), 7.20 - 7.33 (m, 4H), 7.01 - 7.20 (m, 5H), 6.95 (m, 1H), 6.86 (br. s., 1H), 6.77 (m, 1H), 4.93 (s, 1H), 4.76 (s, 1H), 4.54 (t, *J* = 6.64 Hz, 1H), 4.05 (t, *J* = 6.06 Hz, 1H), 3.95 (q, *J* = 6.25 Hz, 1H), 3.89 (t, *J* = 6.06 Hz, 1H), 2.93 (t, *J* = 5.86 Hz, 1H), 2.87 (t, *J* = 6.06 Hz), 2.24 (m, 3H), 1.21 (td, *J* = 7.03, 1.95 Hz, 3H). ^{13}C NMR ($CDCl_3$, 400 MHz) δ 170.64, 161.79, 161.66, 161.44, 161.40, 161.02, 160.63, 159.40, 159.35, 154.09, 142.29, 139.41, 139.36, 139.34, 138.87, 138.83, 138.39, 134.86, 134.79, 134.03, 134.01, 131.98, 131.54, 128.99, 128.96, 128.58, 128.46, 128.12, 128.10, 128.02, 128.00, 127.30, 127.23, 126.85, 126.73, 126.38, 126.36, 124.99, 46.47, 46.41, 45.92, 44.69, 44.59, 42.71, 42.51, 28.67, 28.19, 21.16, 12.75.

Preparation of 4-((4-(benzylamino)-6-(6,7-dimethoxy-3,4-dihydroisoquinolin-2(1*H*)-yl)-1,3,5-triazin-2-ylamino)methyl)-*N*-ethyl-*N*-*m*-tolylbenzamide (1e)



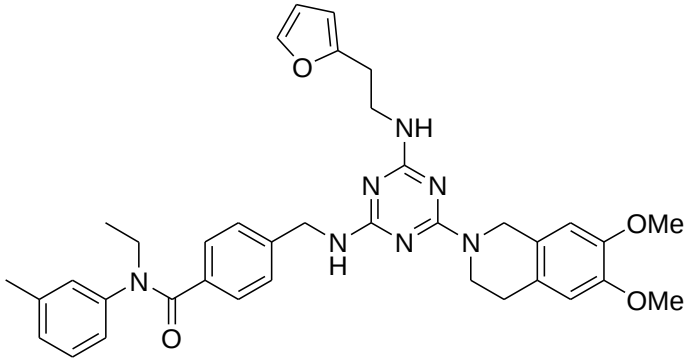
To the reaction solution for the preparation of 4-((4-chloro-6-(6,7-dimethoxy-3,4-dihydroisoquinolin-2(1*H*)-yl)-1,3,5-triazin-2-ylamino)methyl)-*N*-ethyl-*N*-*m*-tolylbenzamide (~0.03 mmol, 1 equivalent) in CH_3CN/H_2O (1/1, ~ 0.8 mL) was added benzylamine (22 µL, 0.2 mmol, 6.7 equivalents). The reaction mixture was heated at 80 °C for 2 hours. The solution was condensed down to give the crude product which was further purified with RP-HPLC (Luna, 5µ C8(2), 100x21 mm, 30-75% CH_3CN/H_2O, 0.1% TFA, 16 min). ^{1}H NMR (DMSO-*d6*, 400 MHz, 80 °C) δ 7.32 (m, 4H), 7.15 – 7.25 (m, 5H), 7.08 (t, J = 7.62 Hz, 1H), 6.92 – 6.95 (m, 2H), 6.84 (d, *J* = 7.82 Hz, 1H), 6.74 (s, 2H), 4.69 (br. s., 2H), 4.51 (br. s., 2H), 4.45 (br. s., 2H), 3.79 – 3.86 (m, 4H), 3.73 (m, 6H), 2.71 (t, *J* = 5.67 Hz, 2H), 2.17 (s, 3H), 1.10 (t, *J* = 7.42 Hz, 3H). ^{13}C NMR ($CDCl_3$, 400 MHz, mixture of rotamers) δ 169.81, 161.82, 155.86, 155.77, 148.01, 147.98, 147.91, 142.89, 139.18, 138.86, 138.78, 137.45, 137.43, 135.67, 135.56, 128.98, 128.89, 128.67, 128.65, 128.20, 127.69, 127.65, 127.62, 127.61, 126.75, 126.62, 126.22, 126.12, 125.07, 124.25, 124.17, 111.40, 111.36, 109.14, 109.12, 56.10, 56.06, 56.01, 45.91, 45.87, 45.75, 45.52, 44.54, 44.50, 44.12, 44.09, 42.22, 42.18, 28.15, 28.08, 21.22, 21.21, 12.92. HRMS $[M+H]^+$ calcd for [C38H41N7O3+H] 644.3344; found 644.3335.

Preparation of 4-((4-(benzylamino)-6-(3,4-dihydroisoquinolin-2(1*H*)-yl)-1,3,5-triazin-2-ylamino)methyl)-*N*-ethyl-*N*-*m*-tolylbenzamide (2a)



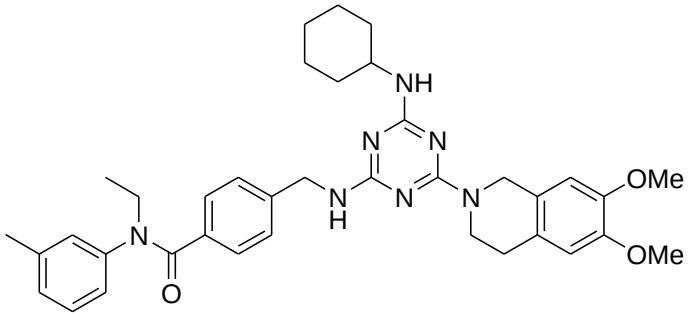
To the reaction solution for the preparation of 4-((4-chloro-6-(3,4-dihydroisoquinolin-2(1*H*)-yl)-1,3,5-triazin-2-ylamino)methyl)-*N*-ethyl-*N*-*m*-tolylbenzamide (~0.03 mmol, 1 equivalent) in NMP (1 mL) was added benzylamine (16.5 µL, 0.15 mmol, 5 equivalents). The reaction mixture was heated at 80 °C for 3-4 hours. The solvent was evaporated *in vacuo* and the residue was further purified with PR-HPLC to give the desired product (4.54 mg). MS: calcd for C36H37N7O+H^+ 584.73; found 584.38. ^{1}H NMR (DMSO-*d6*, 400 MHz, 80 °C) δ 7.25 – 7.35 (m, 4H), 7.14 – 7.26 (m, 9H), 7.08 (t, *J* = Hz, 1H), 6.92 – 6.95 (m, 2H), 6.84 (d, *J* = Hz, 1H), 4.77 (s, 2H), 4.49 (br. s., 2H), 4.43 (br. s., 2H), 3.86 (t, *J* = 5.86 Hz, 2H), 3.81 (q, *J* = 7.42 Hz, 2H), 2.79 (t, *J* = Hz, 2H), 2.17 (s, 3H), 1.10 (t, *J* = 7.42 Hz, 3H). ^{13}C NMR ($CDCl_3$, 400 MHz, mixture of rotamers) δ 169.71, 161.86, 155.87, 155.82, 155.79, 155.76, 142.93, 139.15, 138.82, 138.79, 137.44, 137.41, 135.65, 135.61, 134.41, 134.33, 132.55, 132.52, 129.00, 128.98, 128.87, 128.84, 128.66, 128.64, 128.56, 128.53, 128.21, 128.19, 127.69, 127.65, 127.59, 127.01, 126.99, 126.73, 126.67, 126.62, 126.37, 126.31, 125.09, 46.19, 46.15, 45.69, 45.49, 44.58, 44.49, 44.18, 44.08, 42.17, 28.57, 28.56, 21.21, 12.92.

Preparation of 4-((4-(6,7-dimethoxy-3,4-dihydroisoquinolin-2(1*H*)-yl)-6-(2-(furan-2-yl)ethylamino)-1,3,5-triazin-2-ylamino)methyl)-*N*-ethyl-*N*-*m*-tolylbenzamide (1b)



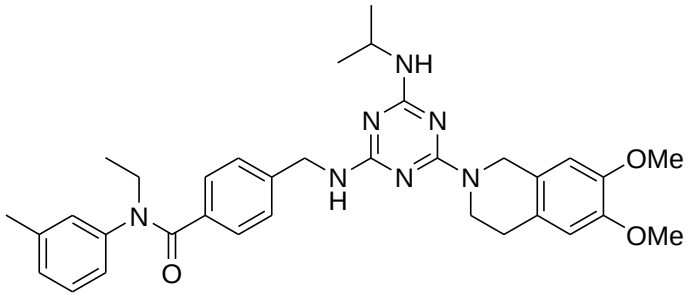
To the reaction solution for the preparation of 4-((4-chloro-6-(6,7-dimethoxy-3,4-dihydroisoquinolin-2(1*H*)-yl)-1,3,5-triazin-2-ylamino)methyl)-*N*-ethyl-*N*-*m*-tolylbenzamide (~0.03 mmol, 1 equivalent) in CH_3CN/H_2O (1/1, ~ 0.8 mL) was added 2-furan-2-yl-ethylamine (22 mg, 0.2 mmol, 6.7 equivalents). The reaction mixture was stirred at 80 °C for 2 hours. The solvent was evaporated and the residue was acidified and purified with RP-HPLC (Luna, 5µ C8(2), 100x21 mm, 30-80% CH_3CN/H_2O, 0.1% TFA, 16 mins) to give the desired product. HRMS $[M+H]^+$ calcd for [C37H41N7O4+H] 648.3293; found 648.3275.

Preparation of 4-((4-(cyclohexylamino)-6-(6,7-dimethoxy-3,4-dihydroisoquinolin-2(1*H*)-yl)-1,3,5-triazin-2-ylamino)methyl)-*N*-ethyl-*N*-*m*-tolylbenzamide (1d)



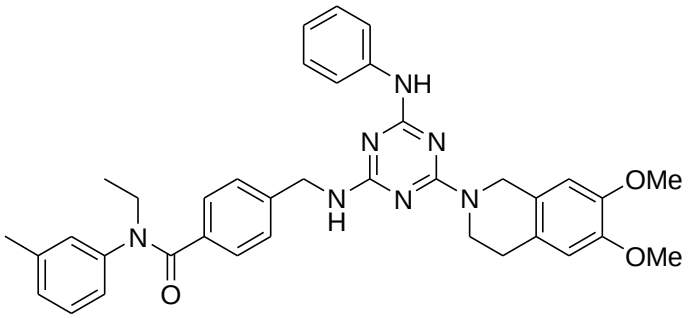
To the reaction solution for the preparation of 4-((4-chloro-6-(6,7-dimethoxy-3,4-dihydroisoquinolin-2(1*H*)-yl)-1,3,5-triazin-2-ylamino)methyl)-*N*-ethyl-*N*-*m*-tolylbenzamide (~0.03 mmol, 1 equivalent) in CH_3CN/H_2O (1/1, ~ 0.8 mL) was added cyclohexylamine (24 µL, 0.21 mmol, 7 equivalents). The reaction was heated at 80 °C for 2 hours. The solvent was evaporated *in vacuo* to give the residue which was further purified with RP-HPLC (Luna, 5µ C8(2), 100x21 mm, 30-80% CH_3CN/H_2O, 0.1% TFA, 16 mins). HRMS $[M+H]^+$ calcd for [C37H45N7O3+H] 636.3657; found 636.3641.

Preparation of 4-((4-(6,7-dimethoxy-3,4-dihydroisoquinolin-2(1*H*)-yl)-6-(isopropylamino)-1,3,5-triazin-2-ylamino)methyl)-*N*-ethyl-*N*-*m*-tolylbenzamide (1a)



To the reaction solution for the preparation of 4-((4-chloro-6-(6,7-dimethoxy-3,4-dihydroisoquinolin-2(1*H*)-yl)-1,3,5-triazin-2-ylamino)methyl)-*N*-ethyl-*N*-*m*-tolylbenzamide (~0.03 mmol, 1 equivalent) in NMP (1 mL) was added isopropylamine (9 mg, 0.15 mmol, 5 equivalents). The reaction mixture was heated at 80 °C for 3-4 hours. The solvent was evaporated *in vacuo* to give the residue which was further purified with RP-HPLC (Luna, 5µ C8(2), 100x21 mm, 36-75% CH_3CN/H_2O, 0.1% TFA, 18 mins). HRMS $[M+H]^+$ calcd for [C34H41N7O3+H] 596.3344; found 596.3321.

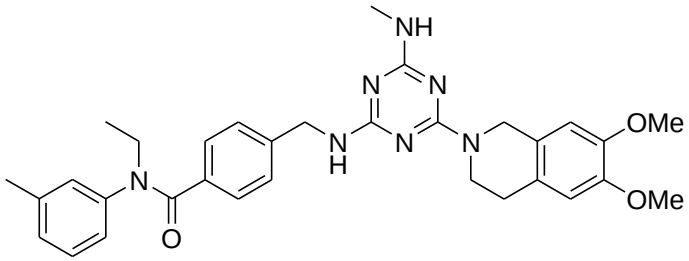
Preparation of 4-((4-(6,7-dimethoxy-3,4-dihydroisoquinolin-2(1*H*)-yl)-6-(phenylamino)-1,3,5-triazin-2-ylamino)methyl)-*N*-ethyl-*N*-*m*-tolylbenzamide (1c)



To the reaction solution for the preparation of 4-((4-chloro-6-(6,7-dimethoxy-3,4-dihydroisoquinolin-2(1*H*)-yl)-1,3,5-triazin-2-ylamino)methyl)-*N*-ethyl-*N*-*m*-tolylbenzamide (~0.03 mmol, 1 equivalent) in NMP (1 mL) was added aniline (14 mg, 0.15 mmol, 5 equivalents). The reaction mixture was heated at 80 °C for 3-4 hours. The solvent was evaporated

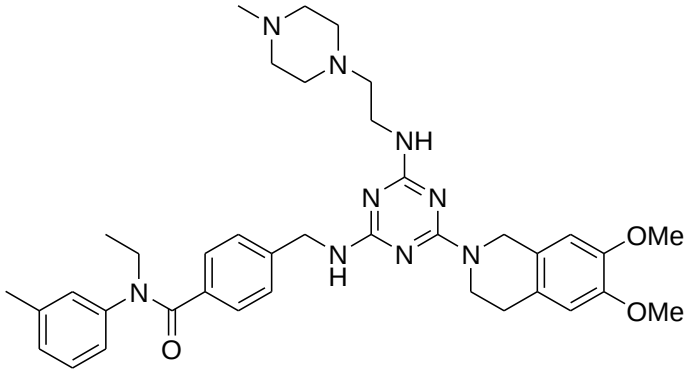
in vacuo to give the residue which was further purified with RP-HPLC (Luna, 5µ C8(2), 100x21 mm, 36-70% CH_3CN/H_2O, 0.1% TFA, 18 mins). HRMS $[M+H]^+$ calcd for [C37H39N7O3+H] 630.3187; found 630.3168.

Preparation of 4-((4-(6,7-dimethoxy-3,4-dihydroisoquinolin-2(1*H*)-yl)-6-(methylamino)-1,3,5-triazin-2-ylamino)methyl)-*N*-ethyl-*N*-*m*-tolylbenzamide (1f)



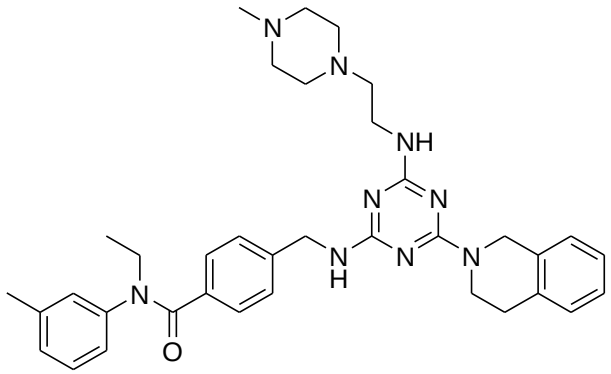
The above solution for the preparation of 4-((4-chloro-6-(6,7-dimethoxy-3,4-dihydroisoquinolin-2(1*H*)-yl)-1,3,5-triazin-2-ylamino)methyl)-*N*-ethyl-*N*-*m*-tolylbenzamide (~0.03 mmol, 1 equivalent) in CH_3CN/H_2O (1/1, ~ 0.8 mL) was treated with methylamine (40% in water, 16 µL, 0.20 mmol, 7 equivalents) at 80 °C for 2 hours. The reaction mixture was condensed down to give the crude product which was further purified with RP-HPLC (Luna, 5µ C8(2), 100x21 mm, 10-60% CH_3CN/H_2O, 0.1% TFA, 16 mins). HRMS $[M+H]^+$ calcd for [C32H37N7O3+H] 568.3031; found 568.3006.

Preparation of 4-((4-(6,7-dimethoxy-3,4-dihydroisoquinolin-2(1*H*)-yl)-6-(2-(4-methylpiperazin-1-yl)ethylamino)-1,3,5-triazin-2-ylamino)methyl)-*N*-ethyl-*N*-*m*-tolylbenzamide (1j)



The solution for the preparation of 4-((4-chloro-6-(6,7-dimethoxy-3,4-dihydroisoquinolin-2(1*H*)-yl)-1,3,5-triazin-2-ylamino)methyl)-*N*-ethyl-*N*-*m*-tolylbenzamide (~0.293 mmol, 1 equivalent) in CH_3CN/H_2O (1/1, 10 mL) was treated with 2-(4-methyl-piperazin-1-yl)-ethylamine (168 µL, 1.172 mmol, 4 equivalents) at 80 °C for 7 hours. The reaction mixture was condensed down to give the crude product which was further purified with RP-HPLC (Luna, 5µ C8(2), 100x21 mm, 10-60% CH_3CN/H_2O, 0.1% TFA, 16 mins) to give the desired product as a TFA salt (76.4 mg, 33% yield in 2 steps). ^{1}H NMR (DMSO-*d6*, 400 MHz, 80°C) δ 7.13 – 7.29 (m, 4H), 7.09 (m, 1H), 6.89 – 7.00 (m, 2H), 6.84 (d, J = 7.82 Hz, 1H), 6.75 (br. s., 2H), 4.70 (br. s., 2H), 4.45 (br. s., 2H), 3.76 – 3.94 (m, 4H), 3.74 (m, 7H), 3.13 (br. s., 4H), 2.81 (br. s., 3H) 2.73 (m, 9H), 2.18 (s, 3H), 1.09 (t, J = 7.03 Hz, 3H). HRMS $[M+H]^+$ calcd for [C38H49N9O3+H] 680.4031; found 680.4020.

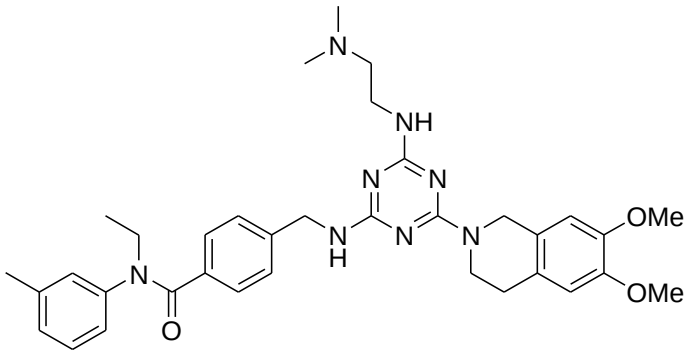
Preparation of 4-((4-(3,4-dihydroisoquinolin-2(1*H*)-yl)-6-(2-(4-methylpiperazin-1-yl)ethylamino)-1,3,5-triazin-2-ylamino)methyl)-*N*-ethyl-*N*-*m*-tolylbenzamide (2b)



To the solution of 4-((4-chloro-6-(3,4-dihydroisoquinolin-2(1*H*)-yl)-1,3,5-triazin-2-ylamino)methyl)-*N*-ethyl-*N*-*m*-tolylbenzamide (8 mg, 0.0156 mmol, 1 equivalent) in CH_3CN/H_2O (1/1, 1 mL) was added 2-(4-methyl-piperazin-1-yl)-ethylamine (20 mg, 0.142 mmol, 9 equivalents). The reaction was heated at 80 °C overnight. The reaction solution was condensed down to give the residue which was acidified and purified with RP-HPLC (Luna, 5µ C8(2), 100x21 mm, 20-80% CH_3CN/H_2O, 0.1% TFA, 20 mins) to give the desired product as a TFA salt. MS: calcd for C36H45N9O+H^+ 620.38; found 620.45. ^{1}H NMR (DMSO-*d6*, 400 MHz, 80 °C) δ 7.13-7.29 (m, 8H), 7.09 (t, J = 7.82 Hz, 1H), 6.96 (m, 2H), 6.85 (d, J = 7.82 Hz, 1H), 4.78 (s, 2H), 4.44 (s, 2H), 3.71 – 3.92 (m, 4H), 3.45 (br. s., 2H), 3.12 (br. s., 2H), 2.82 (m,

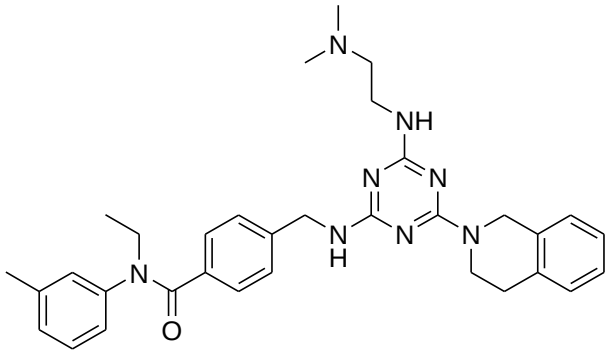
5H), 2.60 – 2.76 (m, 6H), 2.19 (s, 3H), 1.10 (t, J = 7.42 Hz, 3H). ^{13}C NMR ($CDCl_3$, 400 MHz, mixture of rotamers) δ 169.91, 155.50, 142.76, 139.23, 138.73, 138.69, 135.64, 135.59, 134.33, 134.31, 132.33, 128.93, 128.89, 128.87, 128.64, 128.52, 128.23, 128.21, 127.68, 127.10, 126.88, 126.76, 126.72, 126.36, 125.12, 54.41, 51.99, 48.86, 46.24, 46.21, 45.57, 44.25, 44.15, 43.16, 42.33, 42.20, 36.08, 28.61, 28.52, 21.20, 21.18, 12.87.

Preparation of 4-((4-(6,7-dimethoxy-3,4-dihydroisoquinolin-2(1*H*)-yl)-6-(2-(dimethylamino)ethylamino)-1,3,5-triazin-2-ylamino)methyl)-*N*-ethyl-*N*-*m*-tolylbenzamide (1k)



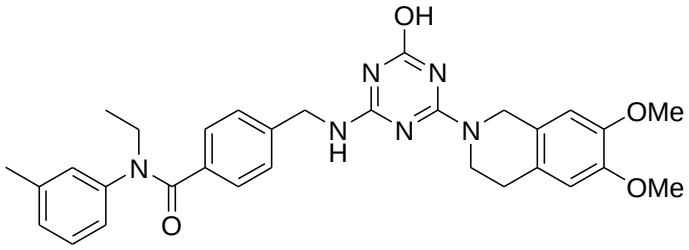
To the reaction solution for the preparation of 4-((4-chloro-6-(6,7-dimethoxy-3,4-dihydroisoquinolin-2(1*H*)-yl)-1,3,5-triazin-2-ylamino)methyl)-*N*-ethyl-*N*-*m*-tolylbenzamide (~0.058 mmol, 1 equivalent) in NMP (~1 mL) was added *N*,*N*-dimethylethylenediamine (52.8 mg, 0.6 mmol, ~10 equivalents). The reaction mixture was heated at 80 °C for 5 hours. The solution was neutralized with 1N HCl. The solvent was evaporated *in vacuo* and the crude was purified with RP-HPLC (Luna, 5µ C8(2), 100x21 mm, 20-95% CH_3CN/H_2O, 0.1% TFA, 20 mins) to give the desired product as a TFA salt (2.1 mg). MS: calcd for C35H44N8O3+H^+ 625.80; found 625.42.

Preparation of 4-((4-(3,4-dihydroisoquinolin-2(1*H*)-yl)-6-(2-(dimethylamino)ethylamino)-1,3,5-triazin-2-ylamino)methyl)-*N*-ethyl-*N*-*m*-tolylbenzamide (2c)



The solution of 4-((4-chloro-6-(3,4-dihydroisoquinolin-2(1*H*)-yl)-1,3,5-triazin-2-ylamino)methyl)-*N*-ethyl-*N*-*m*-tolylbenzamide (8 mg, 0.0156 mmol, 1 eq) in CH_3CN/H_2O (1/1, 2 mL) was treated with *N*,*N*-dimethylethylenediamine in CH_3CN (0.2 M, 650 µL, 0.13 mmol, 8.3 equivalents) at 80 °C overnight. The solvent was evaporated and the crude was purified with RP-HPLC (Luna, 5µ C8(2), 100x21 mm, 25-85% CH_3CN/H_2O, 0.1% TFA, 20 mins) to give the desired product as a TFA salt (10 mg, ~100%). MS: calcd for $C33H40N8O+H^+$ 565.33; found 565.39. ^{1}H NMR ($CDCl_3$, 400 MHz) δ 8.90 (br, 1 H), 7.96 (br, 1 H), 7.36 (m, 4 H), 7.22-7.14 (m, 4 H). ^{13}C NMR ($CDCl_3$, 400 MHz) δ 170.36, 170.31, 164.14, 148.30, 148.23, 126.04, 123.76, 111.48, 109.31, 56.19, 56.18, 46.16, 42.51, 28.18.

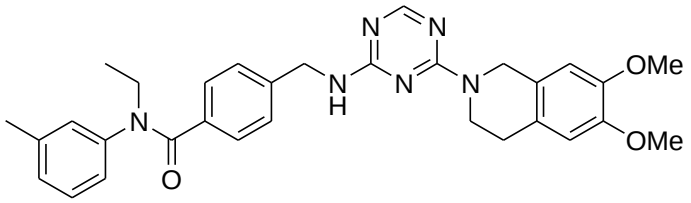
Preparation of 4-((4-(6,7-dimethoxy-3,4-dihydroisoquinolin-2(1*H*)-yl)-6-hydroxy-1,3,5-triazin-2-ylamino)methyl)-*N*-ethyl-*N*-*m*-tolylbenzamide (1h)



To the reaction solution for the preparation of 4-((4-chloro-6-(6,7-dimethoxy-3,4-dihydroisoquinolin-2(1*H*)-yl)-1,3,5-triazin-2-ylamino)methyl)-*N*-ethyl-*N*-*m*-tolylbenzamide (~0.04 mmol, 1 equivalent) in CH_3CN/H_2O (1/1, ~3 mL) was added 6N HCl (0.3 mL). The reaction mixture was heated at 80 °C overnight. The solvent was evaporated *in vacuo* and the

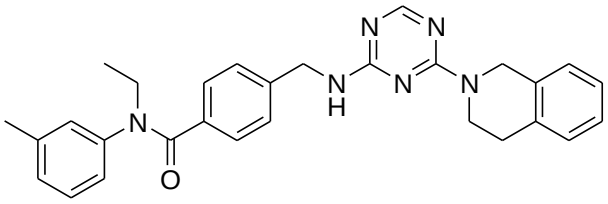
crude was purified with RP-HPLC (Luna, 5µ C8(2), 100x21 mm, 20-65% CH_3CN/H_2O, 0.1% TFA, 17 mins) to give the desired product (10.1 mg, 45% yield). MS: calcd for C31H34N6O4+H^+ 555.28; found 555.28. ^{1}H NMR (DMSO-*d6*, 400 MHz, 80 °C) δ 8.12 (br. s., 1H), 7.22 (m, 4H), 7.08 (t, *J* = 7.82 Hz, 1H), 6.90 – 7.00 (m, 2H), 6.85 (d, *J* = 7.82 Hz, 1H), 6.78 (s, 1H), 6.73 (s, 1H), 4.71 (s, 2H), 4.51 (d, *J* = 5.86 Hz, 2H), 3.78 – 3.86 (m, 4H), 3.74 (m, 6H), 2.77 (br. s., 2H), 2.18 (s, 3H), 1.10 (t, *J* = 7.03 Hz, 3H). ^{13}C NMR ($CDCl_3$, 400 MHz, mixture of rotamers) δ 169.80, 167.63, 156.20, 156.13, 154.19, 154.16, 149.84, 149.70, 148.56, 148.41, 148.30, 148.12, 142.71, 139.20, 138.23, 138.19, 135.76, 135.68, 128.95, 128.86, 128.17, 127.68, 126.78, 126.64, 125.98, 125.26, 125.08, 122.93, 121.74, 111.07, 109.25, 109.14, 56.16, 56.01, 55.96, 46.82, 46.51, 45.52, 44.85, 43.76, 43.60, 27.66, 27.47, 21.19, 21.17, 12.86. HRMS $[M+H]^+$ calcd for [C31H34N6O4+H] 555.2715; found 555.2690.

Preparation of 4-((4-(6,7-dimethoxy-3,4-dihydroisoquinolin-2(1*H*)-yl)-1,3,5-triazin-2-ylamino)methyl)-*N*-ethyl-*N*-*m*-tolylbenzamide (1i)



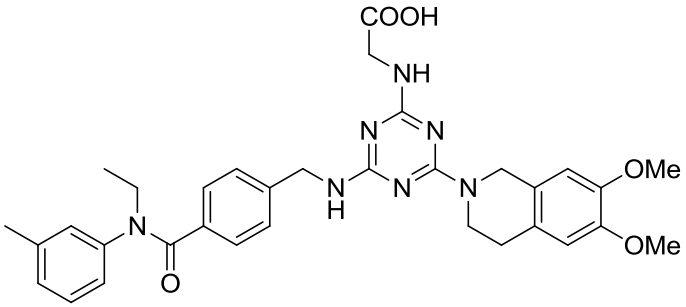
To the solution of 4-((4-chloro-6-(6,7-dimethoxy-3,4-dihydroisoquinolin-2(1*H*)-yl)-1,3,5-triazin-2-ylamino)methyl)-*N*-ethyl-*N*-*m*-tolylbenzamide (~2 mg) in MeOH (1 mL) was added 10% Pd/C (2 mg). The reaction mixture was stirred under an H_2 balloon at room temperature for 2 hours. The reaction solution was filtered through celite and concentrated. The crude product was purified with RP-HPLC to give the desired compound (0.25 mg). HRMS $[M+H]^+$ calcd for [C31H34N6O3+H] 539.2765; found 539.2744.

Preparation of 4-((4-(3,4-dihydroisoquinolin-2(1*H*)-yl)-1,3,5-triazin-2-ylamino)methyl)-*N*-ethyl-*N*-*m*-tolylbenzamide (2d)



To the solution of 4-((4-chloro-6-(3,4-dihydroisoquinolin-2(1*H*)-yl)-1,3,5-triazin-2-ylamino)methyl)-*N*-ethyl-*N*-*m*-tolylbenzamide (7 mg) in MeOH (0.8 mL) was added 10% Pd/C (2 mg). The reaction mixture was stirred under an H_2 balloon at room temperature for 1 hour. The reaction solution was filtered through celite and concentrated. The crude product was purified with RP-HPLC (Luna, 5µ C8(2), 100x21 mm, 30-85% CH_3CN/H_2O, 0.1% TFA, 17 mins) to give the desired compound (4 mg, 61.5%). HRMS $[M+H]^+$ calcd for [C29H30N6O+H] 479.2554; found 479.2542.

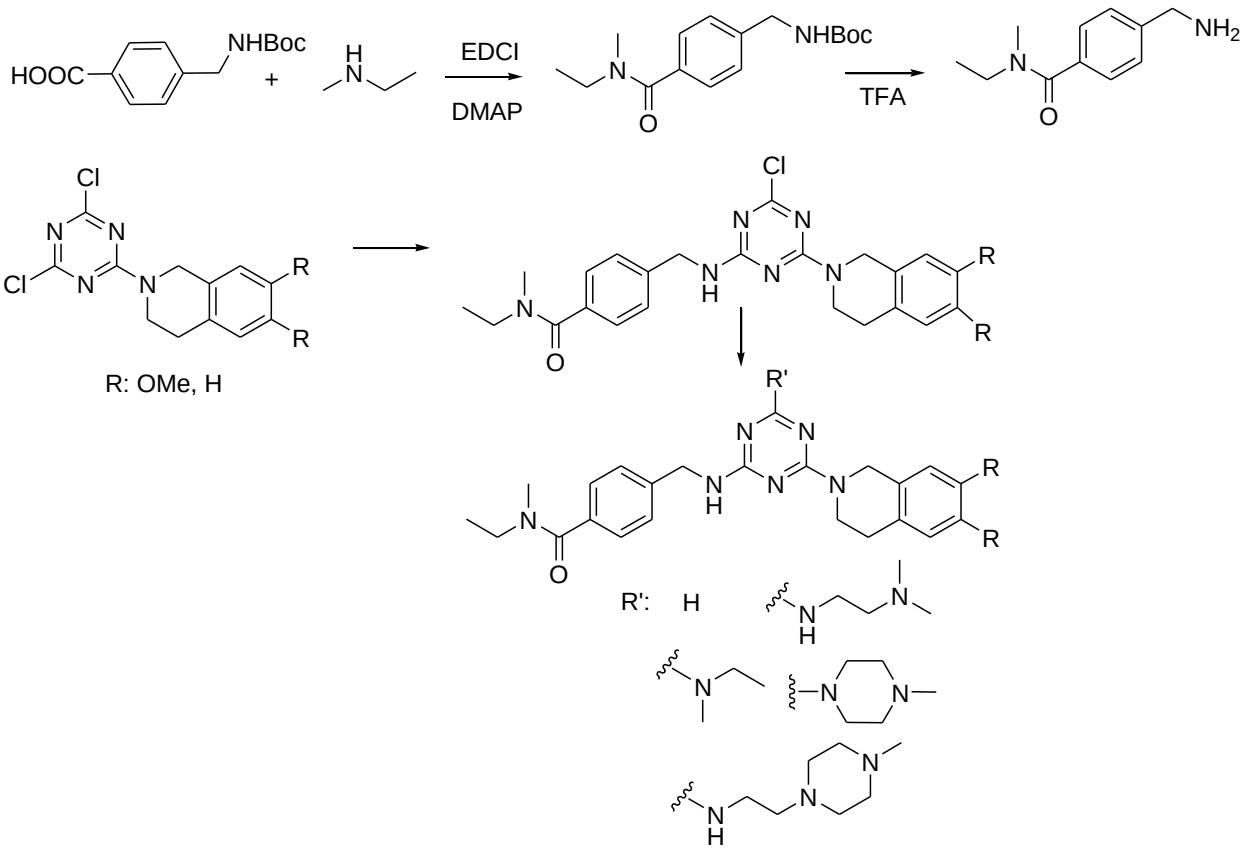
2-((4-(6,7-dimethoxy-3,4-dihydroisoquinolin-2(1H)-yl)-6-((4-(ethyl(m-tolyl)carbamoyl)benzyl)amino)-1,3,5-triazin-2-yl)amino)acetic acid (1l)



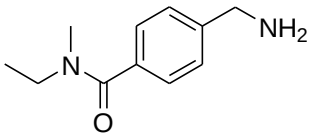
To the reaction solution for the preparation of 4-((4-chloro-6-(6,7-dimethoxy-3,4-dihydroisoquinolin-2(1*H*)-yl)-1,3,5-triazin-2-ylamino)methyl)-*N*-ethyl-*N*-*m*-tolylbenzamide (~0.044 mmol, 1 equivalent) in CH_3CN/H_2O (1/1, 5 mL) was added H-Glycine-OMe hydrochloride (55.25 mg, 0.44 mmol, 10 equivalents). The pH of the reaction solution was adjusted to around 9-10 by adding 1N NaOH. The reaction was heated at 80 °C overnight. Neutralize the solution with HCl. The solvent was evaporated *in vacuo* and the crude was purified with RP-HPLC (Luna, 5µ C8(2), 100x21mm, 20-85% CH_3CN/H_2O, 0.1% TFA, 20

mins) to give the desired product (14.25 mg, 58% yield). MS: calcd for C33H37N7O5+H^+ 612.29, found 612.3.

Scheme 2



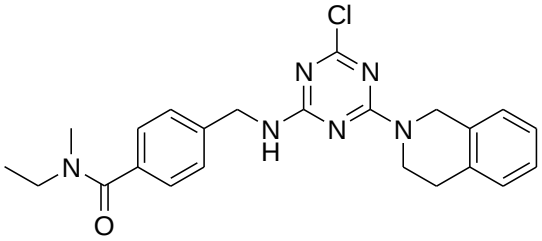
Preparation of 4-(aminomethyl)-*N*-ethyl-*N*-methylbenzamide



To a solution of 4-(Boc-aminomethyl)benzoic acid (0.534 g, 2.125 mmol, 1 equivalent) in DMF (8 ml) was added HATU (807 mg, 2.125 mmol, 1 equivalent) in DMF (5 mL), followed by adding DIEA (740 µl, 4.25 mmol, 2 equivalents). After being stirred at room temperature for 5 min, *N*-ethylmethylamine (219 µL, 2.55 mmol, 1.25 equivalents) was added. The reaction

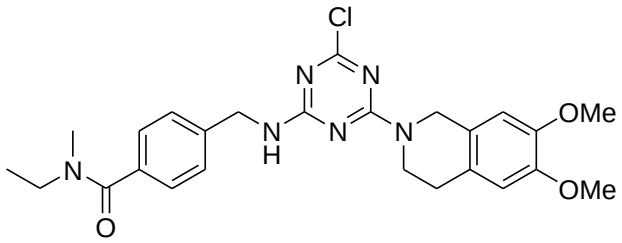
mixture was stirred at room temperature for 2 hours. The solvent was evaporated and the residue was redissolved in EtOAc, which was further washed with sat. $NaHCO_3$, water, salt and dried over $MgSO_4$. The solvent was removed *in vacuo* to give the crude which was purified with silicon chromatography (eluant: 60-70% EtOAc/Hexane). The product was collected and condensed to give the residue which was redissolved in dichloromethane (20 mL). TFA (20 mL) was added and the solution was stirred at room temperature for 1 hour. The solvents were evaporated to give the desired product which was lypholized to get rid of the extra TFA and would be used without further purification. MS: calcd for $C11H16N2O+H^+$ 193.14, found 193.03.

Preparation of 4-((4-chloro-6-(3,4-dihydroisoquinolin-2(1*H*)-yl)-1,3,5-triazin-2-ylamino)methyl)-*N*-ethyl-*N*-methylbenzamide



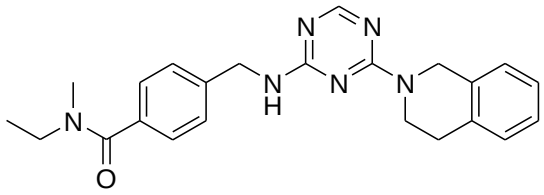
To a solution of 2-(4,6-dichloro-1,3,5-triazin-2-yl)-1,2,3,4-tetrahydroisoquinoline (54.7 mg, 0.195 mmol, 1 equivalent) in dichloromethane (2 mL) was added 4-(aminomethyl)-*N*-ethyl-*N*-methylbenzamide as the TFA salt (~80 mg, 0.261 mmol, 1.34 equivalents), followed by adding triethylamine (144 µL, 1 mmol, 5 equivalents). The reaction was stirred at room temperature for about 1 hour. The solvent was evaporated and the residue was washed with water to give the white solid which will be used directly for the next step. MS: calcd for $C23H25ClN6O+H^+$ 437.19, found 437.3.

Preparation of 4-((4-chloro-6-(6,7-dimethoxy-3,4-dihydroisoquinolin-2(1*H*)-yl)-1,3,5-triazin-2-ylamino)methyl)-*N*-ethyl-*N*-methylbenzamide



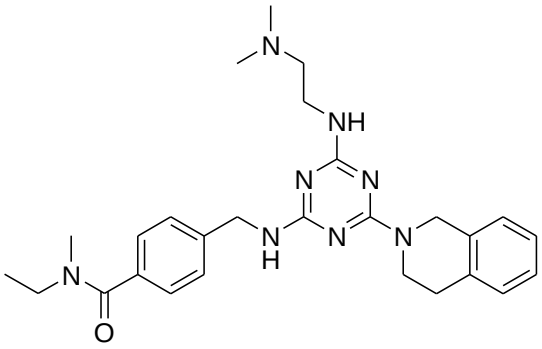
To a solution of 2-(4,6-dichloro-1,3,5-triazin-2-yl)-6,7-dimethoxy-1,2,3,4-tetrahydroisoquinoline (46.59 mg, 0.1366 mmol, 1 equivalent) in dichloromethane (2 mL) was added 4-(aminomethyl)-*N*-ethyl-*N*-methylbenzamide as the TFA salt (~70 mg, 0.23 mmol, 1.34 equivalents), followed by adding triethylamine (144 µL, 1 mmol, 5 equivalents). The reaction was stirred at room temperature for about 1 hour. The solvent was evaporated and the crude was purified with RP-HPLC (Luna, 5µ C8(2), 100x21mm, 30-75% CH_3CN/H_2O, 0.1% TFA, 20 mins) to give the desired product (30 mg, 44% yield). MS: calcd for C25H29ClN6O3+H^+ 497.21, found 497.3.

Preparation of 4-((4-(3,4-dihydroisoquinolin-2(1*H*)-yl)-1,3,5-triazin-2-ylamino)methyl)-*N*-ethyl-*N*-methylbenzamide (6b)



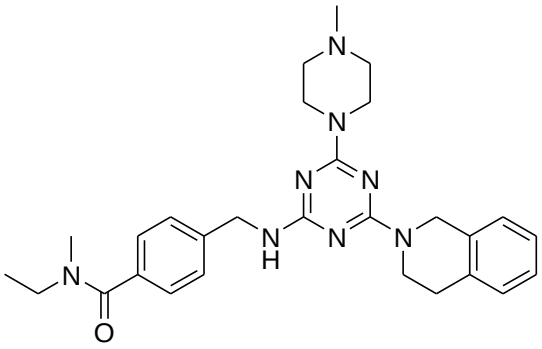
To a solution of crude 4-((4-chloro-6-(3,4-dihydroisoquinolin-2(1*H*)-yl)-1,3,5-triazin-2-ylamino)methyl)-*N*-ethyl-*N*-methylbenzamide (~25 mg, ~0.05 mmol, 1 equivalent) in MeOH (1 ml) and dichloromethane (0.5 mL) was added 10% Pd/C (~70 mg). Under an H_2 balloon, the reaction mixture was stirred at room temperature for 2.5 hours. The solid was filtered and the solution was condensed to give the crude which was further purified with RP-HPLC (Luna, 5µ C8(2), 100x21mm, 30-60% CH_3CN/H_2O, 0.1% TFA, 20 mins) to give the desired product (3.1 mg). HRMS $[M+H]^+$ calcd for [C23H26N6O+H] 403.2241; found 403.2262.

Preparation of 4-((4-(3,4-dihydroisoquinolin-2(1*H*)-yl)-6-(2-(dimethylamino)ethylamino)-1,3,5-triazin-2-ylamino)methyl)-*N*-ethyl-*N*-methylbenzamide (6a)



To a solution of crude 4-((4-chloro-6-(3,4-dihydroisoquinolin-2(1*H*)-yl)-1,3,5-triazin-2-ylamino)methyl)-*N*-ethyl-*N*-methylbenzamide (~25 mg, ~0.05 mmol, 1 equivalent) in NMP (1 mL) was added *N*,*N*-dimethylethylenediamine (200 µL, 1.8 mmol, 36 equivalents). The reaction mixture was heated at 80 °C overnight. After being neutralized with 1N HCl, the reaction solution was directly put on RP-HPLC for purification to give the desired product as a TFA salt (6.7 mg, ~22% yield in 2 steps). HRMS $[M+H]^+$ calcd for [C27H36N8O+H] 489.3085; found 489.3075.

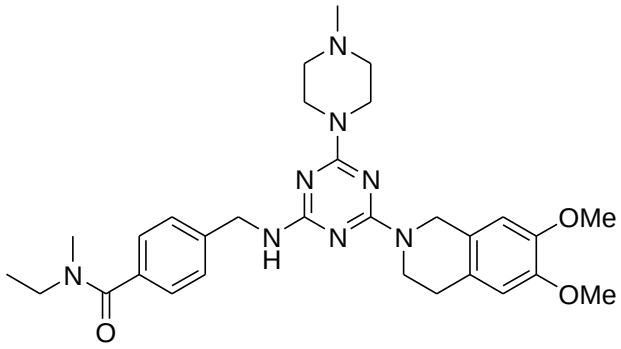
Preparation of 4-((4-(3,4-dihydroisoquinolin-2(1*H*)-yl)-6-(4-methylpiperazin-1-yl)-1,3,5-triazin-2-ylamino)methyl)-*N*-ethyl-*N*-methylbenzamide (6c)



To a solution of crude 4-((4-chloro-6-(3,4-dihydroisoquinolin-2(1*H*)-yl)-1,3,5-triazin-2-ylamino)methyl)-*N*-ethyl-*N*-methylbenzamide (~0.065 mmol, 1 equivalent) in CH_3CN/H_2O (1/1, 1 mL) was added 1-methylpiperazine (65 mg, 0.65 mmol, 10 equivalents). The reaction mixture was heated at 80 °C overnight. The reaction solution was neutralized and directly purified with

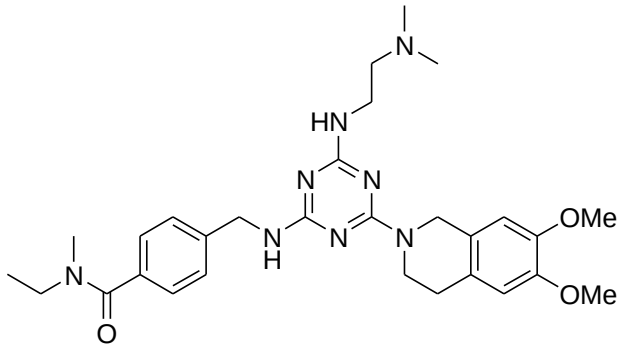
RP-HPLC (Luna, 5µ C8(2), 100x21mm, 10-50% CH_3CN/H_2O, 0.1% TFA, 19 mins) to give the desired product as a TFA salt (20 mg, ~40% yield in 2 steps). MS: calcd for C28H36N8O+H^+ 501.31, found 501.27.

Preparation of 4-((4-(6,7-dimethoxy-3,4-dihydroisoquinolin-2(1*H*)-yl)-6-(4-methylpiperazin-1-yl)-1,3,5-triazin-2-ylamino)methyl)-*N*-ethyl-*N*-methylbenzamide (5h)



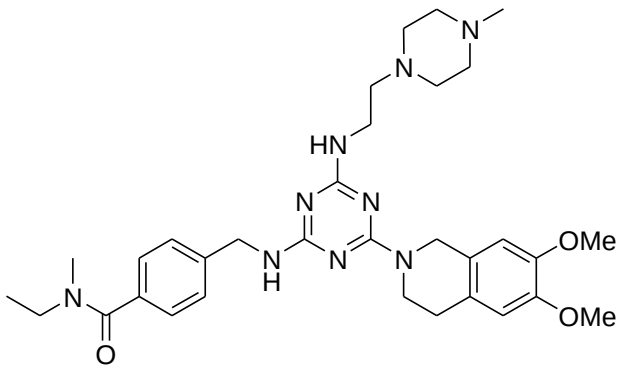
To a solution of 4-((4-chloro-6-(6,7-dimethoxy-3,4-dihydroisoquinolin-2(1*H*)-yl)-1,3,5-triazin-2-ylamino)methyl)-*N*-ethyl-*N*-methylbenzamide (6 mg, 0.012 mmol, 1 equivalent) in CH_3CN/H_2O (1/1, 1 mL) was added 1-methylpiperazine (12 mg, 0.12 mmol, 10 equivalents). The reaction mixture was heated at 80 °C overnight. The reaction solution was neutralized and directly purified with RP-HPLC (Luna, 5µ C8(2), 100x21mm, 4-40% CH_3CN/H_2O, 0.1% TFA, 19 mins) to give the desired product as a TFA salt (2.5 mg, 31% yield). HRMS $[M+H]^+$ calcd for [C30H40N8O3+H] 561.3296; found 561.3298.

Preparation of 4-((4-(6,7-dimethoxy-3,4-dihydroisoquinolin-2(1*H*)-yl)-6-(2-(dimethylamino)ethylamino)-1,3,5-triazin-2-ylamino)methyl)-*N*-ethyl-*N*-methylbenzamide (5f)



To a solution of 4-((4-chloro-6-(6,7-dimethoxy-3,4-dihydroisoquinolin-2(1*H*)-yl)-1,3,5-triazin-2-ylamino)methyl)-*N*-ethyl-*N*-methylbenzamide (6 mg, 0.012 mmol, 1 equivalent) in CH_3CN/H_2O (1/1, 1 mL) was added *N*,*N*-dimethylethylenediamine (10.6 mg, 0.12 mmol, 10 equivalents). The reaction mixture was heated at 80 °C overnight. The reaction solution was neutralized and directly purified with RP-HPLC (Luna, 5µ C8(2), 100x21mm, 4-40% CH_3CN/H_2O, 0.1% TFA, 19 mins) to give the desired product as a TFA salt (2 mg, 25% yield). HRMS $[M+H]^+$ calcd for [C29H40N8O3+H] 549.3296; found 549.3288.

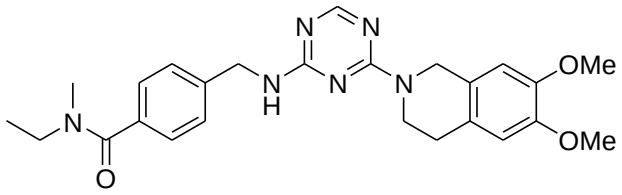
Preparation of 4-((4-(6,7-dimethoxy-3,4-dihydroisoquinolin-2(1*H*)-yl)-6-(2-(4-methylpiperazin-1-yl)ethylamino)-1,3,5-triazin-2-ylamino)methyl)-*N*-ethyl-*N*-methylbenzamide (5e)



To a solution of 4-((4-chloro-6-(6,7-dimethoxy-3,4-dihydroisoquinolin-2(1*H*)-yl)-1,3,5-triazin-2-ylamino)methyl)-*N*-ethyl-*N*-methylbenzamide (6 mg, 0.012 mmol, 1 equivalent) in

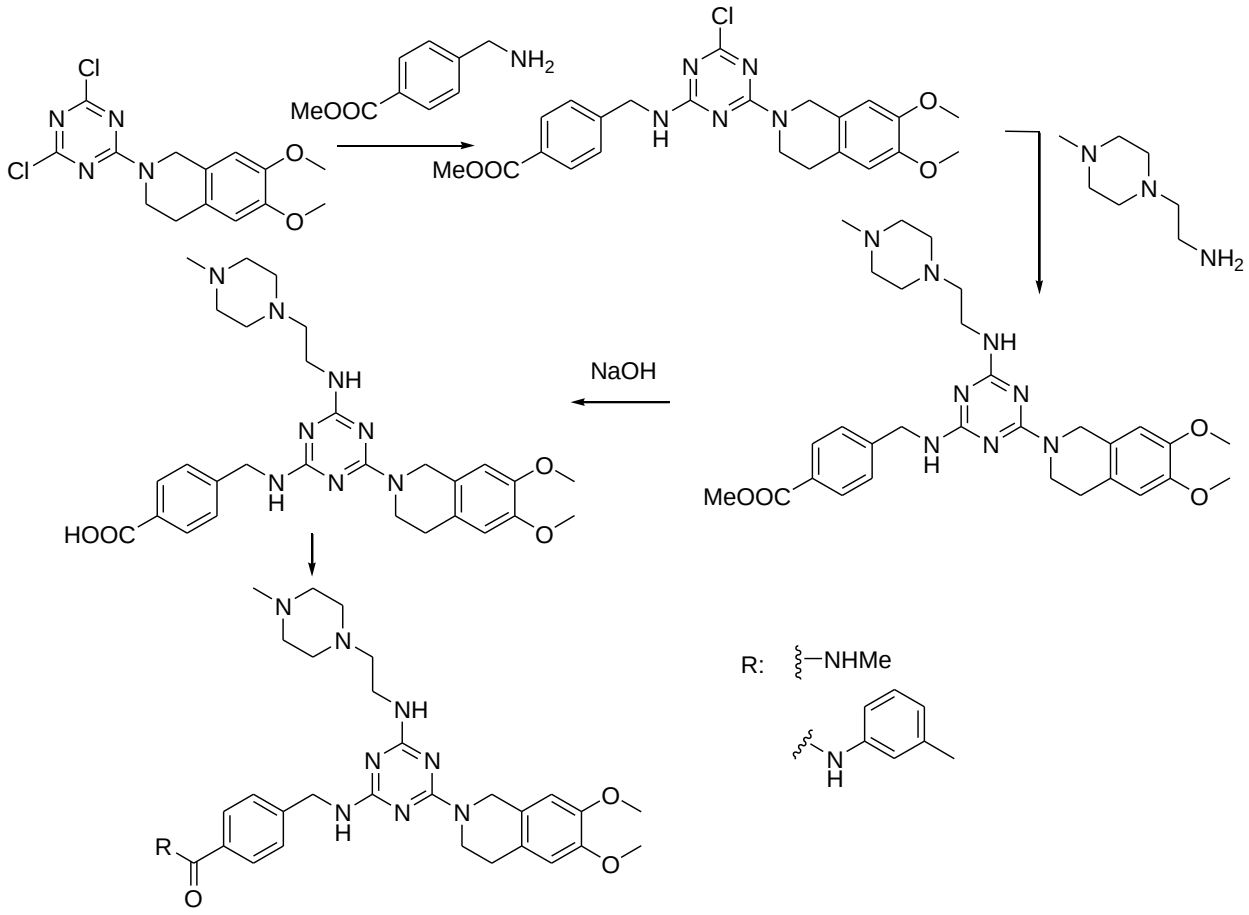
CH_3CN/H_2O (1/1, 1 mL) was added 2-(4-methyl-piperazin-1-yl)-ethylamine (17 mg, 0.12 mmol, 10 equivalents). The reaction mixture was heated at 80 °C overnight. The reaction solution was neutralized and directly purified with RP-HPLC (Luna, 5µ C8(2), 100x21mm, 4-40% CH_3CN/H_2O, 0.1% TFA, 19 mins) to give the desired product as a TFA salt (3.5 mg, 35% yied). HRMS $[M+H]^+$ calcd for [C32H45N9O3+H] 604.3718; found 604.3692.

Preparation of 4-((4-(6,7-dimethoxy-3,4-dihydroisoquinolin-2(1*H*)-yl)-1,3,5-triazin-2-ylamino)methyl)-*N*-ethyl-*N*-methylbenzamide (5g)

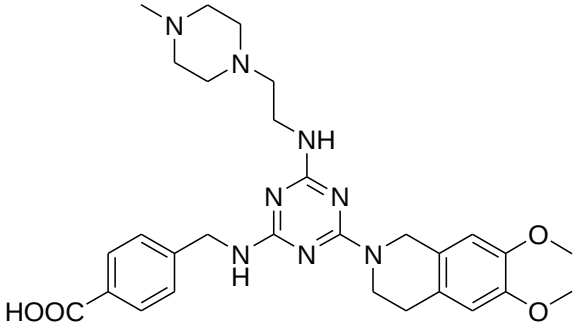


To a solution of 4-((4-chloro-6-(6,7-dimethoxy-3,4-dihydroisoquinolin-2(1*H*)-yl)-1,3,5-triazin-2-ylamino)methyl)-*N*-ethyl-*N*-methylbenzamide (12 mg, 0.024 mmol, 1 equivalent) in MeOH (1 mL) was added 10% Pd/C (~8 mg). Under an H_2 balloon, the reaction mixture was stirred at room temperature for 40 mins. The solid was filtered and the solution was condensed to give the crude which was further purified with RP-HPLC (Luna, 5µ C8(2), 100x21mm, 30-60% CH_3CN/H_2O, 0.1% TFA, 20 mins) to give the desired product (1.3 mg, 11.6% yield). MS: calcd for $C25H30N6O3+H^+$ 463.25, found 463.4.

Scheme 3



Preparation of 4-((4-(6,7-dimethoxy-3,4-dihydroisoquinolin-2(1*H*)-yl)-6-(2-(4-methylpiperazin-1-yl)ethylamino)-1,3,5-triazin-2-ylamino)methyl)benzoic acid (5c)

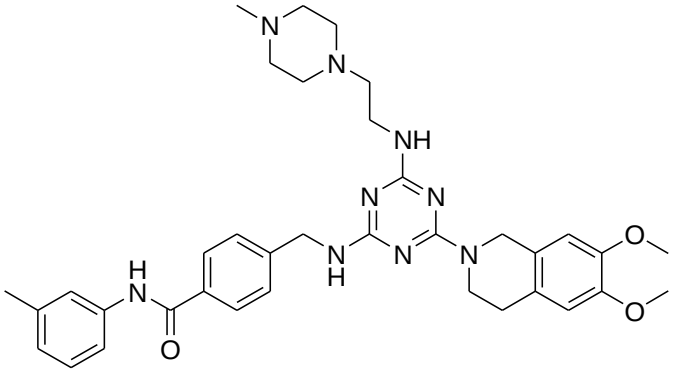


To the mixture of 2-(4,6-dichloro-1,3,5-triazin-2-yl)-6,7-dimethoxy-1,2,3,4-tetrahydroisoquinoline (100 mg, 0.293 mmol, 1 equivalent) in CH_3CN/H_2O (1/1, 8 mL) was

added 4-(aminomethyl)benzoic acid methyl ester (59.1 mg, 0.293 mmol, 1 equivalent). The pH of the reaction solution was kept at about 9-10 by adding 1N NaOH. The reaction was stirred at room temperature overnight. The solvent was removed *in vacuo* to give the crude product which would be used directly for the next step.

Methyl 4-((4-chloro-6-(6,7-dimethoxy-3,4-dihydroisoquinolin-2(1*H*)-yl)-1,3,5-triazin-2-ylamino)methyl)benzoate (0.15 mmol, 1 equivalent) was dissolved in NMP (3 mL). 2-(4-methyl-piperazin-1-yl)-ethylamine (125.88 mg, 0.88 mmol, 6 equivalents) was added, and the reaction mixture was heated at 80 °C for 5 hours. LC-MS showed the reaction has completed. 2/3 (0.1 mmol) of the reaction mixture was further treated with 1N NaOH (0.4 mL, 0.4 mmol, 4 equivalents). The reaction mixture was stirred at room temperature overnight. The reaction solution was neutralized and condensed to give the crude which was purified with RP-HPLC (Luna, 5μ C8(2), 100x21mm, 15-95% CH_3CN/H_2O, 0.1% TFA, 20 mins) to give the desired product as a TFA salt (26.2 mg, ~33% in 3 steps). MS: calcd for C29H38N8O4+H^+ 563.68, found 563.4.

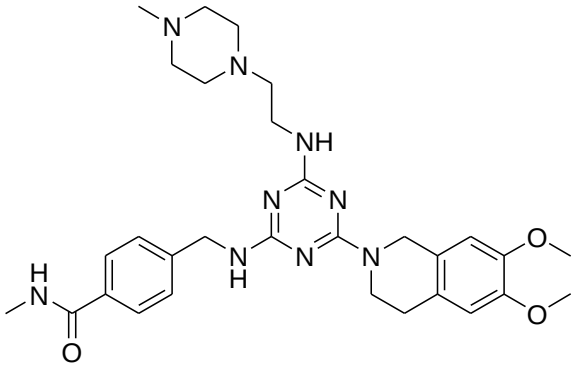
Preparation of 4-((4-(6,7-dimethoxy-3,4-dihydroisoquinolin-2(1*H*)-yl)-6-(2-(4-methylpiperazin-1-yl)ethylamino)-1,3,5-triazin-2-ylamino)methyl)-*N*-*m*-tolylbenzamide (5a)



A solution of 4-((4-(6,7-dimethoxy-3,4-dihydroisoquinolin-2(1*H*)-yl)-6-(2-(4-methylpiperazin-1-yl)ethylamino)-1,3,5-triazin-2-ylamino)methyl)benzoic acid (8 mg, 0.01 mmol, 1 equivalent), *m*-toluidine (1.76 μL, 0.0162 mmol, 1.6 equivalents)) and DMAP (0.3 mg, 0.003 mmol, 0.3 equivalents) in dichloromethane (1 mL) was cooled with stirring in an ice bath. 1-Ethyl-3-[3-(dimethylamino)propyl]carbodiimide hydrochloride (EDCI, 3.41 mg, 0.0178 mmol,

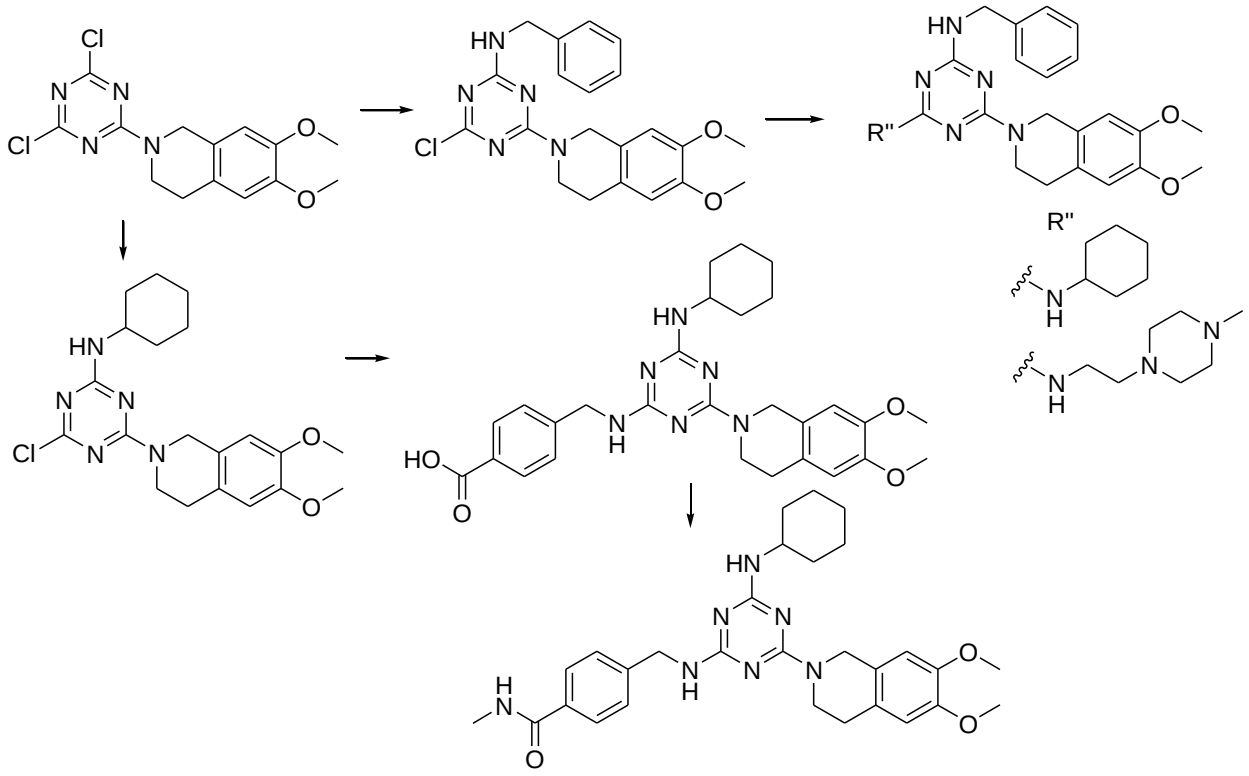
1.8 equivalents) was added, and the reaction mixture was stirred at 0 °C for 2 hours and at room temperature overnight. The solution was diluted with dichloromethane, which was further washed with saturated sodium bicarbonate, water, salt and dried over $MgSO_4$. The solvent was removed *in vacuo* to give the crude compound, which was further purified with RP-HPLC (Luna, 5μ C8(2), 100x21mm, 10-95% CH_3CN/H_2O, 0.1% TFA, 15 mins) to give the desired product as a TFA salt. MS: calcd for C36H45N9O3+H^+ 652.82, found 652.42.

Preparation of 4-((4-(6,7-dimethoxy-3,4-dihydroisoquinolin-2(1*H*)-yl)-6-(2-(4-methylpiperazin-1-yl)ethylamino)-1,3,5-triazin-2-ylamino)methyl)-*N*-methylbenzamide (5b)

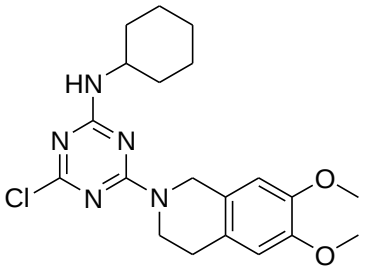


Following the same procedure as preparation of 4-((4-(6,7-dimethoxy-3,4-dihydroisoquinolin-2(1*H*)-yl)-6-(2-(4-methylpiperazin-1-yl)ethylamino)-1,3,5-triazin-2-ylamino)methyl)-*N*-*m*-tolylbenzamide, 4-((4-(6,7-dimethoxy-3,4-dihydroisoquinolin-2(1*H*)-yl)-6-(2-(4-methylpiperazin-1-yl)ethylamino)-1,3,5-triazin-2-ylamino)methyl)benzoic acid (8 mg, 0.01 mmol, 1 equivalent) in dichloromethane (1 mL) was coupled with methylamine (2.M in THF, 8 μL, 0.016 mmol, 1.6 equivalents) under the activation of EDCI (3.41 mg, 0.0178 mmol, 1.8 equivalents) and DMAP (0.3 mg, 0.003 mmol, 0.3 equivalent). The crude was purified with RP-HPLC (Luna, 5μ C8(2), 100x21mm, 10-95% CH_3CN/H_2O, 0.1% TFA, 15 mins) to give the desired product as a TFA salt (1.23 mg, 15% yield). MS: calcd for C30H42N9O3+H^+ 576.71, found 576.38.

Scheme 4

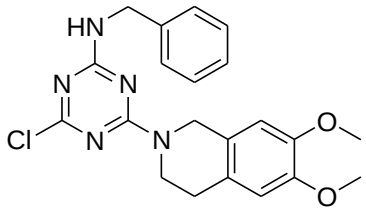


Preparation of 4-chloro-*N*-cyclohexyl-6-(6,7-dimethoxy-3,4-dihydroisoquinolin-2(1*H*)-yl)-1,3,5-triazin-2-amine



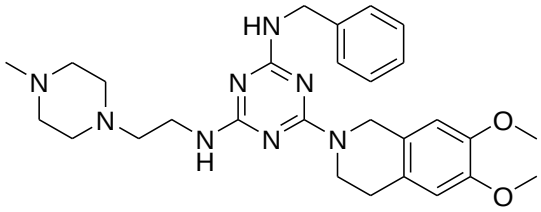
To the solution of 2-(4,6-dichloro-1,3,5-triazin-2-yl)-6,7-dimethoxy-1,2,3,4-tetrahydroisoquinoline (157 mg, 0.46 mmol, 1 equivalent) in CH_3CN/H_2O (1/1, 10 mL)was added cyclohexylamine (52.7 μL, 0.46 mmol, 1 equivalent). The pH of the reaction mixture was adjusted with 1N NaOH to about 9-10, and the reaction was stirred at room temperature for 1 hour. Without workup, the reaction solution will be used directly for the next step.

Preparation of *N*-benzyl-4-chloro-6-(6,7-dimethoxy-3,4-dihydroisoquinolin-2(1*H*)-yl)-1,3,5-triazin-2-amine



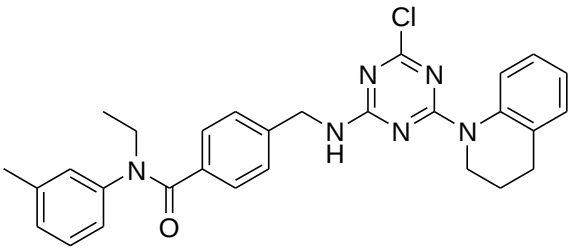
To the solution of 2-(4,6-dichloro-1,3,5-triazin-2-yl)-6,7-dimethoxy-1,2,3,4-tetrahydroisoquinoline (43 mg, 0.126 mmol, 1 equivalent) in CH_3CN/H_2O (1/1, 2 mL) was added benzylamine (13.7 μL, 0.126 mmol, 1 equivalent). The pH of the reaction mixture was adjusted to about 9-10 with 1N NaOH (126 μL, 1 equivalent), and the reaction was stirred at room temperature for 1 hour. Without workup, the reaction solution will be used directly for the next step.

Preparation of N^2-benzyl-6-(6,7-dimethoxy-3,4-dihydroisoquinolin-2(1*H*)-yl)-N^4-(2-(4-methylpiperazin-1-yl)ethyl)-1,3,5-triazine-2,4-diamine (5d)



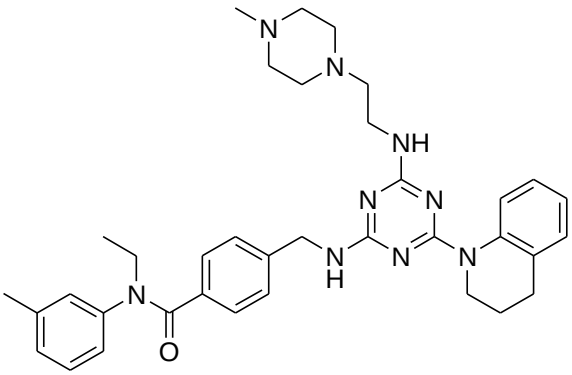
To the above reaction solution of *N*-benzyl-4-chloro-6-(6,7-dimethoxy-3,4-dihydroisoquinolin-2(1*H*)-yl)-1,3,5-triazin-2-amine (0.126 mmol, 1 equivalent) in CH_3CN/H_2O (1/1, 2 mL) was added 2-(4-methyl-piperazin-1-yl)-ethylamine (36 mg, 0.252 mmol, 2 equivalents), followed by adding 1N NaOH (126 μL, 0.126 mmol, 1 equivalent). The reaction mixture was heated at 80 °C overnight. The solvent was evaporated and the residue was acidified and purified with RP-HPLC (Luna, 5μ C8(2), 100x21mm, 10-60% CH_3CN/H_2O, 0.1% TFA, 17 mins) to give the desired product. MS: cacld for $C28H38N8O2+H^+$ 519.32, found 519.4.

Preparation of 4-((4-chloro-6-(3,4-dihydroquinolin-1(2*H*)-yl)-1,3,5-triazin-2-ylamino)methyl)-*N*-ethyl-*N*-*m*-tolylbenzamide



At 0 ^{o}C, the mixture of cyanuric chloride (50 mg, 0.375 mmol, 1 equivalent) in pH 9.4 borate buffer (0.5 M, 4 mL) was added 1,2,3,4-tetrahydroquinoline (69 mg, 0.375 mmol, 1 equivalent). The pH of the solution was adjusted to about 9-10 with 1N NaOH. The reaction was stirred at 4 ^{o}C overnight. 4-(Aminomethyl)-*N*-ethyl-*N*-*m*-tolylbenzamide as a TFA salt (143 mg, 0.375 mmol, 1 equivalent) was added, followed by adding 1N NaOH (30 µL, 0.75 mmol, 2 equivalents). The reaction was heated at 50 ^{o}C for 4-5 hrs. More 4-(Aminomethyl)-*N*-ethyl-*N*-*m*-tolylbenzamide was added to push the reaction to completion. The solvent was evaporated and the crude was purified with RP-HPLC (Luna, 5µ C8(2), 100x21mm, 50-98% CH_3CN/H_2O, 0.1% TFA, 20 mins) to give the desired compound (36.8 mg, 19% in 2 steps). MS: calcd for $C29H29ClN6O+H^+$ 513.22, found 513.30.

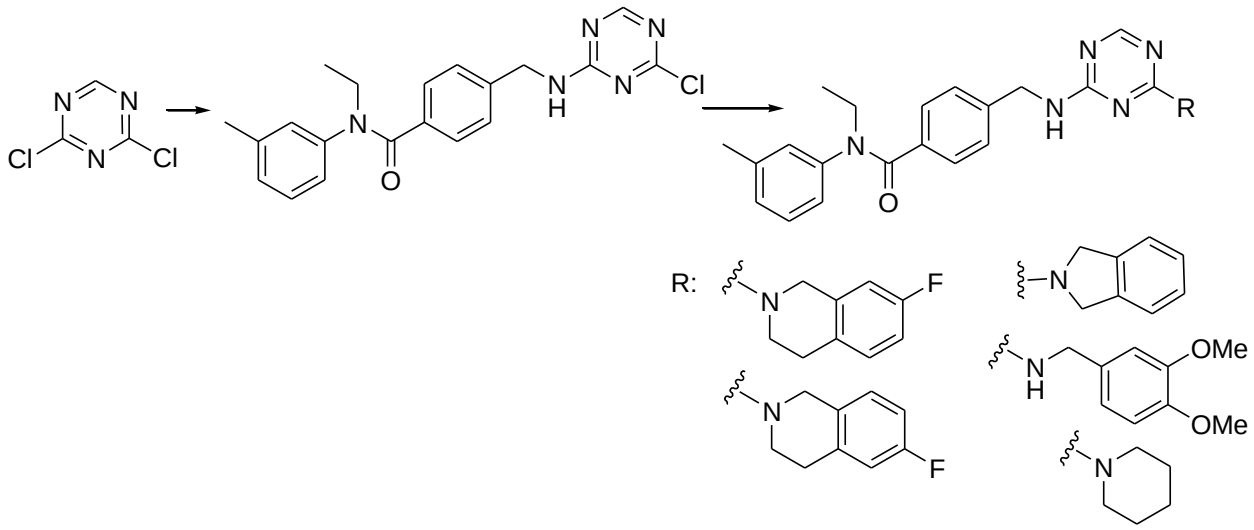
Preparation of 4-((4-(3,4-dihydroquinolin-1(2*H*)-yl)-6-(2-(4-methylpiperazin-1-yl)ethylamino)-1,3,5-triazin-2-ylamino)methyl)-*N*-ethyl-*N*-*m*-tolylbenzamide (3)



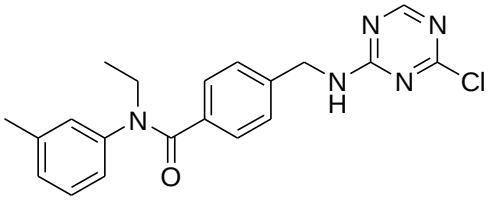
To the solution of 4-((4-chloro-6-(3,4-dihydroquinolin-1(2*H*)-yl)-1,3,5-triazin-2-ylamino)methyl)-*N*-ethyl-*N*-*m*-tolylbenzamide (20 mg, 0.039 mmol, 1 equivalent) in NMP (0.5 mL) was added 2-(4-methyl-piperazin-1-yl)-ethylamine (28 mg, 0.195 mmol, 5 equivalents). The reaction mixture was heated at 80 ^{o}C for about 3 hours. The reaction solution was acidified and directly purified with RP-HPLC (Luna, 5µ C8(2), 100x21mm, 40-95% CH_3CN/H_2O, 0.1%

TFA, 20 mins) to give the desired product as TFA salt (18.9 mg, 57%). MS: calcd for C36H45N9O+H$^+$ 620.83, found 620.5.

Scheme 6

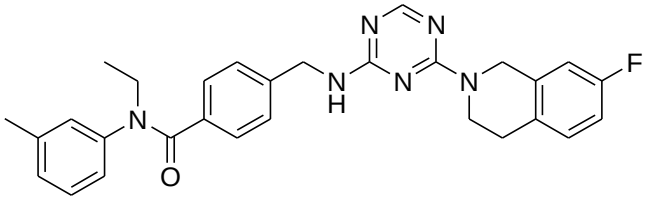


Preparation of 4-((4-chloro-1,3,5-triazin-2-ylamino)methyl)-*N*-ethyl-*N*-*m*-tolylbenzamide



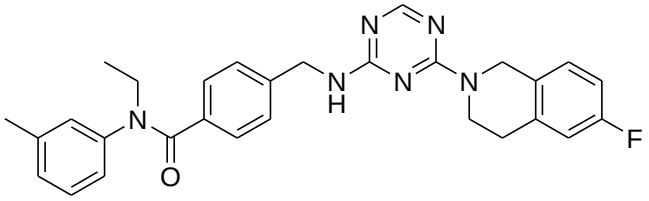
At 0 ^{o}C, the solution of 2,4-dichloro-1,3,5-triazine (95%, 20.4 mg, 0.13 mmol, 1 equivalent) and 4-(aminomethyl)-*N*-ethyl-*N*-*m*-tolylbenzamide as a TFA salt (52 mg, 0.136 mmol, 1 equivalent) in NMP (1.5 mL) was added diisopropylethylamine (59 µL, 0.34 mmol, 2.5 equivalents). The reaction was stirred at cold for 1-2 hours, followed by stirring at room temperature overnight. The reaction solution will be used directly for the next step. MS: calcd for C20H20ClN5O+H$^+$ 382.14, found 382.2.

Preparation of *N*-ethyl-4-((4-(7-fluoro-3,4-dihydroisoquinolin-2(1*H*)-yl)-1,3,5-triazin-2-ylamino)methyl)-*N*-*m*-tolylbenzamide (4b)



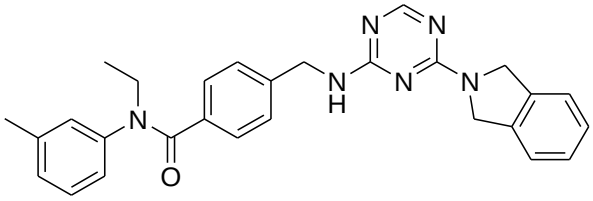
1/5 of the above reaction solution to prepare 4-((4-chloro-1,3,5-triazin-2-ylamino)methyl)-*N*-ethyl-*N*-*m*-tolylbenzamide (~0.025 mmol, 1 equivalent) was added the solution of 7-fluoro-1,2,3,4-tetrahydroisoquinoline (21 mg, 0.136 mmol, 5 equivalents) in NMP (0.5 mL). The reaction was heated at 80 °C for 1 hour. The reaction solution was neutralized and directly purified with RP-HPLC (Luna, 5µ C8(2), 100x21mm, 20-65% CH_3CN/H_2O, 0.1% TFA, 17 mins) to give the desired product (5.5 mg, ~46% in 2 steps). HRMS $[M+H]^+$ calcd for [C29H29FN6O+H] 497.2460; found 497.2432.

Preparation of *N*-ethyl-4-((4-(6-fluoro-3,4-dihydroisoquinolin-2(1*H*)-yl)-1,3,5-triazin-2-ylamino)methyl)-*N*-*m*-tolylbenzamide (4a)



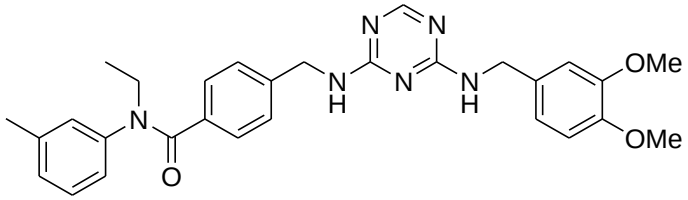
1/5 of the above reaction solution to prepare 4-((4-chloro-1,3,5-triazin-2-ylamino)methyl)-*N*-ethyl-*N*-*m*-tolylbenzamide (~0.025 mmol, 1 equivalent) was added the solution of 6-fluoro-1,2,3,4-tetrahydroisoquinoline (21 mg, 0.136 mmol, 5 equivalents) in NMP (0.5 mL). The reaction was heated at 80 ^oC for 1 hour. The reaction solution was neutralized and directly purified with RP-HPLC (Luna, 5µ C8(2), 100x21mm, 20-65% CH_3CN/H_2O, 0.1% TFA, 17 mins) to give the desired product (4.7 mg, ~39% in 2 steps). MS: calcd for $C29H29FN6O+H^+$ 496.25, found 497.36. HRMS $[M+H]^+$ calcd for [C29H29FN6O+H] 497.2460; found 497.2450.

Preparation of *N*-ethyl-4-((4-(isoindolin-2-yl)-1,3,5-triazin-2-ylamino)methyl)-*N*-*m*-tolylbenzamide (4c)



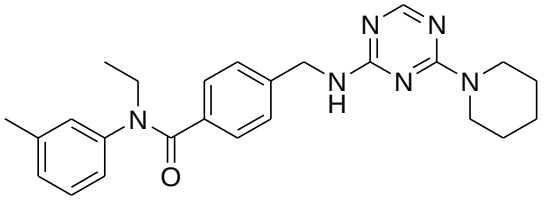
1/5 of the above reaction solution to prepare 4-((4-chloro-1,3,5-triazin-2-ylamino)methyl)-*N*-ethyl-*N*-*m*-tolylbenzamide (~0.025 mmol, 1 equivalent) was added the solution of isoindoline (15.4 µL, 0.136 mmol, 5 equivalents) in NMP (0.5 mL). The reaction was heated at 80 °C for 1 hour. The reaction solution was neutralized and directly purified with RP-HPLC (Luna, 5µ C8(2), 100x21mm, 20-65% CH_3CN/H_2O, 0.1% TFA, 17 mins) to give the desired product (5.3 mg, ~46% in 2 steps). HRMS $[M+H]^+$ calcd for [C28H28N6O+H] 465.2398; found 495.2390.

Preparation of 4-((4-(3,4-dimethoxybenzylamino)-1,3,5-triazin-2-ylamino)methyl)-*N*-ethyl-*N*-*m*-tolylbenzamide (4d)



1/5 of the above reaction solution to prepare 4-((4-chloro-1,3,5-triazin-2-ylamino)methyl)-*N*-ethyl-*N*-*m*-tolylbenzamide (~0.025 mmol, 1 equivalent) was added the solution of (3,4-dimethoxyphenyl)methanamine (22.7 mg, 0.136 mmol, 5 equivalents) in NMP (0.5 mL). The reaction was heated at 80 ^oC for 1 hour. The reaction solution was neutralized and directly purified with RP-HPLC (Luna, 5µ C8(2), 100x21mm, 20-65% CH_3CN/H_2O, 0.1% TFA, 17 mins) to give the desired product (5.3 mg, ~46% in 2 steps). MS: calcd for C29H32N6O3+H^+ 513.26, found 513.22. HRMS $[M+H]^+$ calcd for [C29H32N6O3+H] 513.2609; found 513.2620.

Preparation of *N*-ethyl-4-((4-(piperidin-1-yl)-1,3,5-triazin-2-ylamino)methyl)-*N*-*m*-tolylbenzamide



1/5 of the above reaction solution to prepare 4-((4-chloro-1,3,5-triazin-2-ylamino)methyl)-*N*-ethyl-*N*-*m*-tolylbenzamide (~0.025 mmol, 1 equivalent) was added the solution of piperidine (13.5 µL, 0.136 mmol, 5 equivalents) in NMP (0.5 mL). The reaction was heated at 80 ^{o}C for 1 hour. The reaction solution was neutralized and directly purified with RP-HPLC (Luna, 5µ C8(2), 100x21mm, 20-65% CH_3CN/H_2O, 0.1% TFA, 17 mins) to give the desired product (6.7 mg, ~62% in 2 steps). HRMS $[M+H]^+$ calcd for [C25H30N6O+H] 431.2554; found 431.2577.

Affinity Selection:

1x Selection Buffer used: 50mM Tris (pH 7.5), 150 mM NaCl, and 0.05% Brij35, 2 mM betamercaptoethanol BME, 1 mg/mL sheared salmon sperm DNA (sssDNA, Ambion). Selections were done using IMAC matrix tips (Phynexus) ADAMTS-4 Protein (Chemicon Cat #CC1028 aa213-575, 40.3kDa Cterm 6His). Each tip in each round of selection had 10 µg of protein loaded onto it (concentration of 4.4 µM)

Prepare tips: Wash tips with Blocking buffer (5% Casein in 1x Selection Buffer) 3 times and stored 12 hours at 4 °C with the same buffer. Rinse tips 2 times with Blocking Buffer. Exchange buffer once with 1mg/mL sssDNA in 1x selection buffer, intake 80 µL and store 12 hours, or until used, at 4 °C.

Selection Round 1: Immobilize 10 µg of ADAMTS-4 protein on a previously prepared IMAC resin tip (Phynexus), wash 4 times with selection buffer. No target controls are run for each target condition. Resuspend 5 nmol of DEL library in 60 µL of 1x Selection buffer. Pass this over the IMAC resin tip for 1 hour at RT. Wash 8 times with 1x Selection buffer. Wash 2 times with DNA free 1x Selection buffer. Heat elute in DNA Free 1x Selection buffer – 12 minutes at 80 °C. Post clear eluted material to remove denatured protein – (Pass eluted material over a fresh IMAC resin tip to remove any denatured protein – 10 mins at RT.) Repeat this step. Retain 1 µL of round 1 elution. Add sssDNA and buffer to bring the volume of the eluted material to 60 µL.

Selection Round 2: Repeat by binding fresh protein to a fresh IMAC tip. Wash tips 4 times with selection buffer. Repeat the above selection procedure using the eluted material from round 1. At the end of round 2, retain 5 µL of the elution. Post clear (twice) eluted material to remove denatured protein, as described above. Add sssDNA and buffer to bring the volume to 60 µL in order to begin round 3 of the selection.

Selection Round 3: Repeat by binding fresh protein to a fresh IMAC tip. Wash tips 4 times with selection buffer. Run selection round 3 as described above. (There is no post-clear step after Round 3 elution.)

At the end of round 3 elution, run a quantitative PCR to assess yield from each round of selection. Sequence the appropriate amount of round 3 elution target and no target control (454 contract sequencing).

ADAMTS-4 Assay. Zhang, Y. *et al* (**2004**) *Journal of Pharm. & Exp. Therapeutics 309(1)*, 348-355 first described an assay that can be used for measuring the biological activity of the ADAMTS-4 compounds. The concentration of the ADAMTS4 enzyme was 48 nM, the (WAAG-3R) substrate concentration was 25 µM. The buffer that was used contains 50 mM HEPES, pH = 7.5, 100 mM NaCl, 5 mM $CaCl_2$, 0.1% CHAPS, and 5% glycerol. 384 well (Greiner cat. # 781209) black plates were used. Serial dilutions of the compounds were made by first dissolving in 100% DMSO (10 mM) these were diluted serially and pre-incubated with the enzyme for 15 minutes. The final compound concentration was in 1% DMSO and 1× assay buffer. The substrate was added and the plate was read every 30 seconds for 1 hour at 37 ^{o}C. The excitation and emission wavelengths were 340 nm and 420 nm respectively. Percent inhibition was calculated by comparing the enzyme rate in the presence of inhibitor to the rate of the enzyme with 1% DMSO. The software package Graphpad Prism was used to calculate compound IC_{50}s by using a sigmoidal dose-response curve fitting routine. It is important to note that any IC_{50} values below the enzyme concentration are estimates. A reported hydroxamate inhibitor of aggrecanase (*Bioorg. & Med. Chem. Lett.* **2005**, *15*, 3385-3388) was used as a reference. Most of the compounds were tested in multiple independent assays and the final reported value was the mean of the independent assays. The only exceptions are compound **5b**, **5c** and **5d** in Table 3. Their analogs with cyclohexylamine at cycle 1 position (not disclosed in the manuscript) had similar activity and SAR as **5b**, **5c** and **5d**.

ADAMTS-5 Assay. This assay was run with the same basic protocol as that described above for ADAMTS-4. The differences include that amount of enzyme used (60 nM) while the amount of substrate used (25 μM) was the same. The remainder of the assay followed the same protocol as that for ADAMTS-4. Excitation and emission wavelengths were 340 nm and 420 nm correspondingly. IC_{50}s were determined as described above.

TACE Assay. Tace substrate (R&D ES003) and Tace enzyme **(**R&D 930-ADB) were used to assess cross reactivity of compounds generated from the ADAMTS-4 selection experiments. 1× TACE buffer was used for the assay: 25 mM Tris (pH 8.0), 2.5 μM $ZnCl_2$, and 0.1% Brij-35. The final concentration of TACE enzyme was 20 nM, while the substrate was 100 μM. Serially diluted compounds were pre-incubated with TACE enzyme for 15 minutes. Once the substrate was added the fluorescence was measured (Excitation: Emission; 355:405nm) every 30 seconds for 30 minutes. A derivative of reported hydroxamate inhibitor of TACE was used as a control (*Bioorg. & Med. Chem. Lett.* **2001**, *11*, 2975-2978).

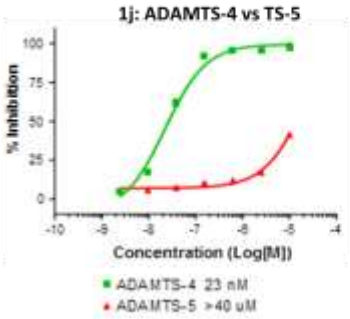
MMP-13 Assay. Matrix Metalloprotease-13 enzyme was purchased from R&D Systems (R&D 511-MM). The assay buffer was 50 mM HEPES (pH 7.5), 150 mM NaCl, 10 mM $CaCl_2$, 1 uM $ZnAc_2$, 600 μM CHAPS. Substrate was custom synthesized 5-FAM-TPGPLGL[Dap(DNP)]ARRK(5-TAMRA)-amide. Compounds were diluted serially in DMSO (final 1%) and added to the plate (Greiner #784076). The assay consisted of sequential addition of assay buffer, substrate (final 1 μM) and MMP-13 enzyme (final 480 pM) to the plate and incubated at room temperature for 45 minutes. The reaction was stopped by adding EDTA (final 10 mM). The assay plates was read at 485 nm excitation/530 nm emission with a 505 nm dichroic filter. IC50s were determined as described above. A reported hydroxamate inhibitor of MMP-13 was used as a control (*Bioorg. & Med. Chem. Lett.* **2005**, *15*, 3385-3388).

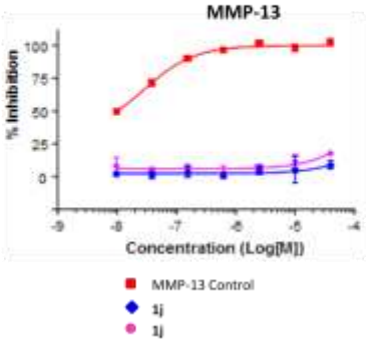
ADAMTS-13 assay:

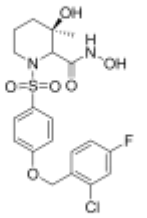
A diagnostic ELISA kit was purchased from DiaPharma (Cat.no. 5450501. The kit supplied 96 wells pre-coated with monoclonal anti-ADAMTS-13 antibody. It also includes standards, control plasma, activity substrate, and stopping solution. The kit is designed to run a ADAMTS-13 activity standard curve before running samples. Recombinant ADAMTS-13 was purchased from Abnova (Cat. no. H00011093-Q01). In addition, a second fluorescent substrate (FRETS-VWF73 Cat. no. 3224-s) was purchased from the Peptide Institute. This assay was run in 5 mM

Tris (pH 6.0), 25 mM $CaCl_2$, and 0.0005% Tween-20. The substrate was diluted in DMSO and H_2O according to the protocol. The fluorescence was read kinetically on a TECAN Safire2; excitation 340 nM, emission 450 nM. Pooled Normal Human plasma was purchased from Innovative Research (Cat. no. IPNP). EDTA (5 mM) was used as an inhibitor control. Compound IC50s were determined using a sigmoidal dose-response curve fitting routine (Graphpad Prism).

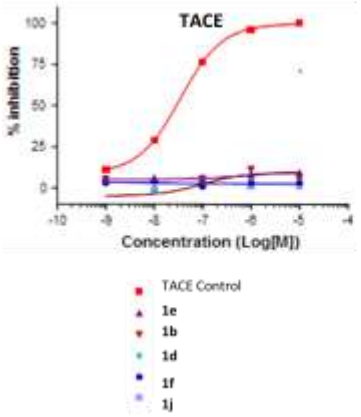
Selectivity Panel

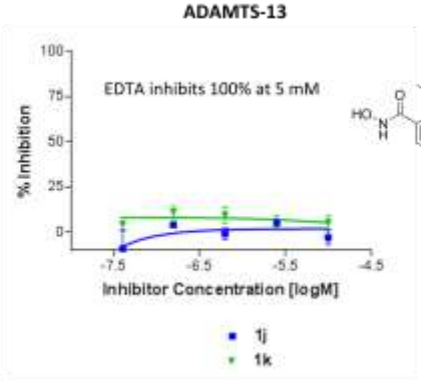


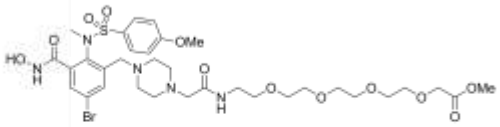




ADAMTS-4 control
MMP-13 control







TACE control