Development of functionally selective, small molecule agonists at kappa opioid receptors

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Supplemental Data

CHEMICAL SYNTHESIS

General synthetic methods

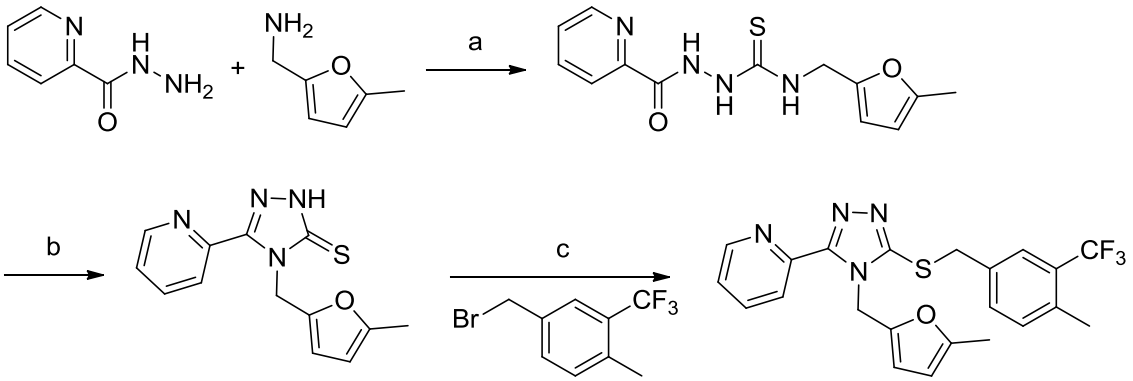
All chemicals were used as purchased from commercial suppliers. Methylene chloride, acetonitrile, toluene, ethyl ether and THF were dried by being passed through two packed columns of neutral alumina prior to use. ^{1}H and ^{13}C NMR spectra were recorded on a Bruker AM 400 spectrometer (operating at 400 and 100 MHz respectively) in $CDCl_3$ with 0.03% TMS as an internal standard, unless otherwise specified. Chemical shifts are reported in parts per million (ppm) downfield from TMS. ^{13}C multiplicities were determined with the aid of an APT pulse sequence, differentiating the signals for methyl and methane carbons as "d" from methylene and quarternary carbons as "u". The infrared (IR) spectra were acquired as thin films using a universal ATR sampling accessory on a PerkinElmer Spectrum 100 FT-IR spectrometer and the absorbtion frequencies are reported in cm^{-1}. Melting points were determined on a Stanford Research Systems Optimelt automated melting point system interfaced through a PC and are uncorrected. Microwave syntheses were conducted in a Biotage Initiator constant temperature microwave synthesizer.

HPLC/MS analysis was carried out with gradient elution (5% CH_3CN to 100% CH_3CN) on an Agilent 1200 RRLCwith a photodiode array UV detector and an Agilent 6224 TOF mass spectrometer (also used to produce high resolution mass spectra). HPLC purification was carried out by mass directed fractionation (MDF) with gradient elution (a narrow CH_3CN gradient was chosen based on the retention time of the target from LCMS analysis of the crude sample) on an Agilent 1200 instrument with photodiode array detector, an Agilent 6120 quadrupole mass spectrometer, and a HTPAL LEAP autosampler. Fractions were triggered using an MS and UV

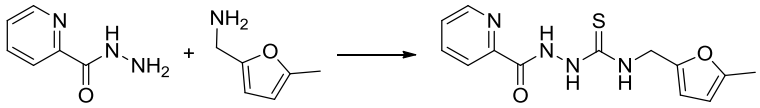
threshold determined by HPLC/MS analysis of the crude sample. One of two column/mobile phase conditions were chosen for both analysis and purification to promote the targets neutral state (0.02% formic acid with Waters Atlantis T3 5um, 19 x 150 mm; or pH 9.8 NH_4OH with Waters XBridge C18 5 um, 19 x 150 mm).

Triazole agonist compounds

Scheme S-1. Representative preparation of triazole analogues[a].

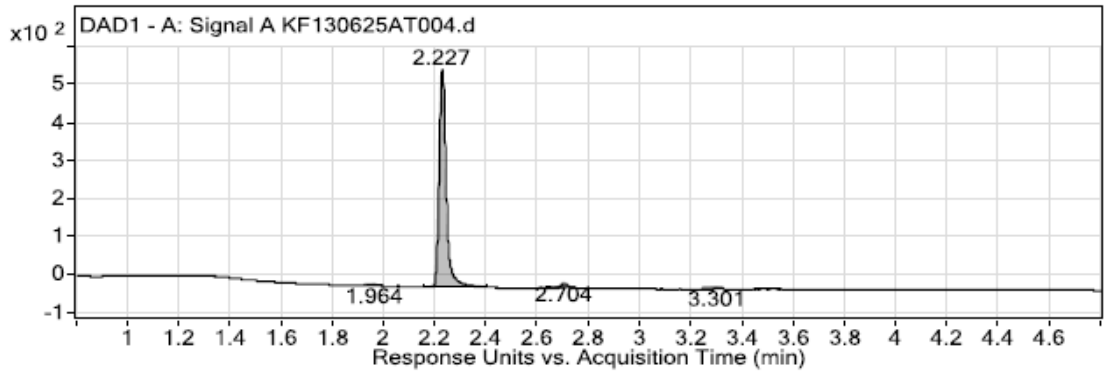


[a] Reagents and conditions: (a) di(2-pyridyl) thionocarbonate (64% yield); (b) NaOH, water, reflux (86% yield); (c) K_2CO_3, acetone, rt (63% yield).



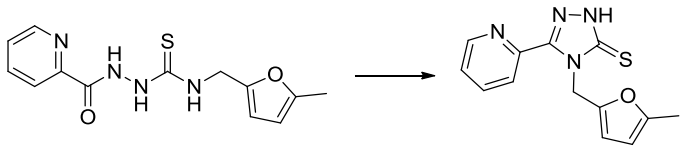
***N*-((5-Methylfuran-2-yl)methyl)-2-picolinoylhydrazinecarbothioamide**. To a solution of di(2-pyridyl) thionocarbonate (535 mg, 2.30 mmol, 1.05 equiv.) in THF (20 mL) was added 5-methyl furfurylamine (244 mg, 2.19 mmol). The solution was stirred at rt for 2 h and the solvent removed in vacuo. The residue was dissolved in ethanol (20 mL) and picolinyl hydrazide (301 mg, 2.19 mmol) was added. The reaction was stirred at 55 °C for 14 h and cooled to rt, precipitating the crude product. The precipitate was filtered, washed with a 2:1 mixture of ethyl

ether:ethanol (2 × 15 mL) and dried under vacuum to afford the hydrazinecarbothioamide (407 mg, 1.40 mmol, 64% yield, 97% HPLC purity) as a white solid, which was used without further purification. Mp = 172-173 ºC; ^{1}H NMR (DMSO-d6) δ 2.21 (s, 3 H), 4.61 (d, *J* = 8 Hz, 2 H), 5.97 (d, *J* = 1.6 Hz, 1 H), 6.14 (d, *J* = 2.4 Hz, 1 H), 7.62-7.65 (m, 1 H), 7.99-8.05 (m, 2 H), 8.41 (br s, 1 H), 8.66 (dd, *J* = 1.2, 4.8 Hz, 1 H), 9.50 (br s, 1 H), 10.60 (br s, 1 H); ^{13}C NMR (DMSO-d6) δ d 13.3, 106.3, 108.0, 122.5, 126.9, 137.6, 148.4; u 40.7, 149.4, 150.3, 181.7; IR (neat) 3302, 3267, 3197, 1659, 1544 cm^{-1}; HRMS (ESI) *m/z* calcd for $C_{13}H_{153}N_4O_2S$ ([M+H]$^{+}$), 291.0916, found 291.0915.



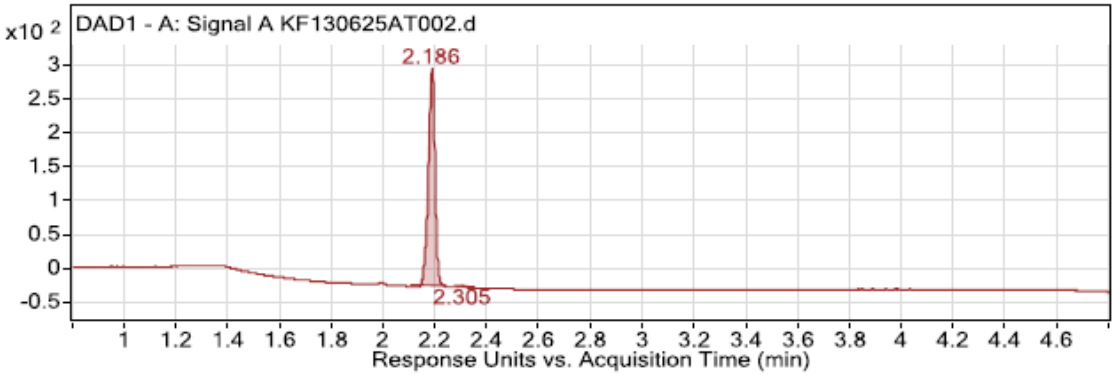
User Chromatogram Peak List

Compound Name	RT	Height	Height %	Area	Area %	Area Sum %	Symmetry	Width
	1.964	3.31	0.58	5.72	0.54	0.52	0.59	0.029
Cpd 1: 2.226	2.227	572.17	100	1067.55	100	96.81	0.73	0.028
	2.704	13.61	2.38	24.58	2.3	2.23	1.06	0.038
	3.301	2.96	0.52	4.82	0.45	0.44	0.88	0.031



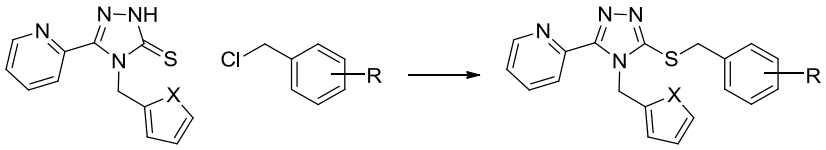
4-((5-Methylfuran-2-yl)methyl)-3-(pyridin-2-yl)-1H-1,2,4-triazole-5(4H)-thione. The above hydrazinecarbothioamide (407 mg, 1.40 mmol) was slurried in water (10 mL) and aqueous NaOH (4 N, 10 mL). The reaction was heated at 55 °C for 14 h, cooled to rt, and acidified to pH = 6 with aqueous HCl (2 N). The solid precipitate was filtered, washed with water (2 × 15 mL)

and dried under vacuum to afford the thione as a white solid (327 mg, 1.20 mmol, 86% yield, 99% HPLC purity), which was used without further purification. Mp = 143-145 ºC; ^{1}H NMR (DMSO-d6) δ 2.05 (s, 3 H), 5.78 (s, 2 H), 5.85 (dd, *J* = 1.2, 3.2 Hz, 1 H), 5.93 (d, *J* = 3.2 Hz, 1 H), 7.57 (ddd, *J* = 1.6, 4.8, 8.8 Hz, 1 H), 7.93-8.01 (m, 2 H), 8.74 (qd, *J* = 0.8, 4.8 Hz, 1 H), 14.18 (br s, 1 H); ^{13}C NMR (DMSO-d6) δ d 13.1, 106.4, 109.3, 123.0, 125.3, 137.8, 149.2; u 40.9, 145.7, 147.3, 148.5, 151.2, 168.4; IR (neat) 3108, 2932, 2780, 1588, 1562, 1549 cm^{-1}; HRMS (ESI) *m/z* calcd for $C_{13}H_{12}N_4OSNa$ ($[M+Na]^+$), 295.0630, found 295.0630.



User Chromatogram Peak List

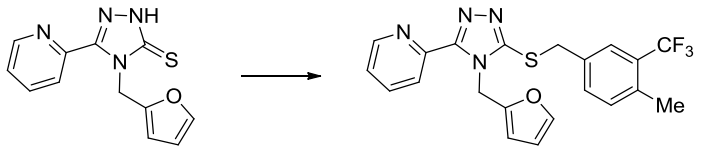
Compound Name	RT	Height	Height %	Area	Area %	Area Sum %	Symmetry	Width
Cpd 1: 2.176	2.186	321.62	100	585.23	100	99.24	1.26	0.028
	2.305	2.7	0.84	4.5	0.77	0.76	0.55	0.028



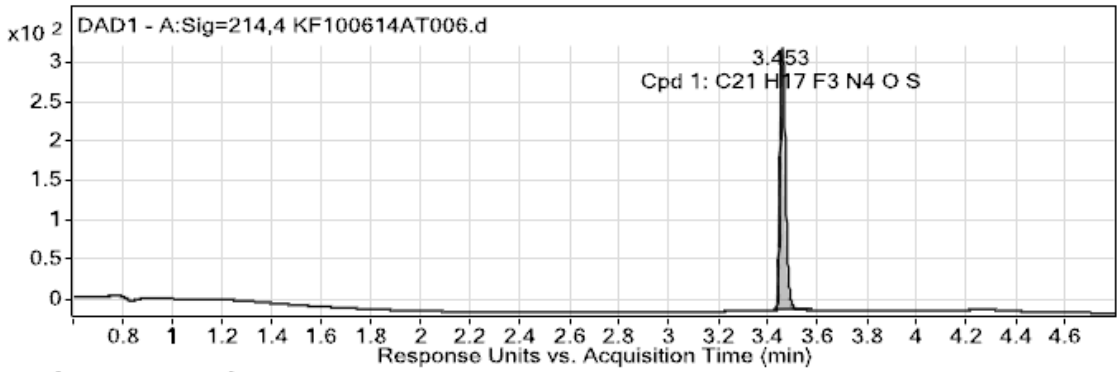
General procedure for the synthesis of series 1 analogues from thiones and benzyl halides.

The thione scaffold (0.1 to 0.3 mmol), K_2CO_3 (2 equiv) and the benzyl halide (1.2 equiv) were combined in acetone (15 mL/mmol substrate) and stirred at rt in a sealed vial. After 15 h, the solvent was removed and the residue washed with CH_2Cl_2 (2 × 3 mL) and filtered. The combined filtrates were evaporated down and either chromatographed on silica or subjected to mass-

directed, reverse phase preparative HPLC purification (MDF HPLC) as detailed in the general synthetic methods.

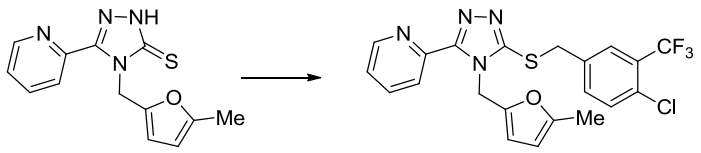


2-(4-(furan-2-ylmethyl)-5-((4-methyl-3-(trifluoromethyl)benzyl)thio)-4H-1,2,4-triazol-3-yl)pyridine (1.1). Following the general procedure and purification on silica gel, the known furan thione[1] (39 mg, 0.15 mmol) and 4-methyl-3-(trifluoromethyl)benzyl bromide (46 mg, 0.18 mmol) afforded the product as an off-white solid (51 mg, 0.19 mmol, 78% yield, 100% HPLC purity). ^{1}H NMR ($CDCl_3$) δ 2.45 (s, 3 H), 4.49 (s, 2 H), 5.82 (s, 2 H), 6.12 (dd, *J* = 0.8, 3.2 Hz, 1 H), 6.20 (dd, *J* = 1.2, 3.2 Hz, 1 H), 7.21 (d, *J* = 8.0 Hz, 1 H), 7.24 (dd, *J* = 0.8, 2.0 Hz, 1 H) 7.33 (ddd, *J* = 1.2, 4.8, 7.6 Hz, 1 H), 7.48 (dd, *J* = 1.2, 7.6 Hz, 1 H), 7.61 (d, *J* = 1.2 Hz, 1 H), 7.81 (dt, *J* = 2.0, 8.0 Hz, 1 H), 8.27 (td, *J* = 0.8, 8.0 Hz, 1 H), 8.64 (qd, *J* = 0.8, 4.8 Hz, 1 H); ^{13}C NMR ($CDCl_3$) δ d 19.2, 109.1, 110.5, 123.5, 124.3, 126.5 (q, *J* = 6 Hz), 132.4, 132.5, 137.1, 142.8, 148.6; u 37.4, 42.0, 123.1, 125.8, 129.3 (d, *J* = 26 Hz), 134.6, 136.3, 147.8, 149.1, 152.5, 152.6, 152.8; IR (neat) 2935, 1590, 1504 cm^{-1}; HRMS (ESI) *m/z* calcd for $C_{21}H_{18}F_3N_4OS$ ($[M+H]^+$), 431.1153, found 431.1155.

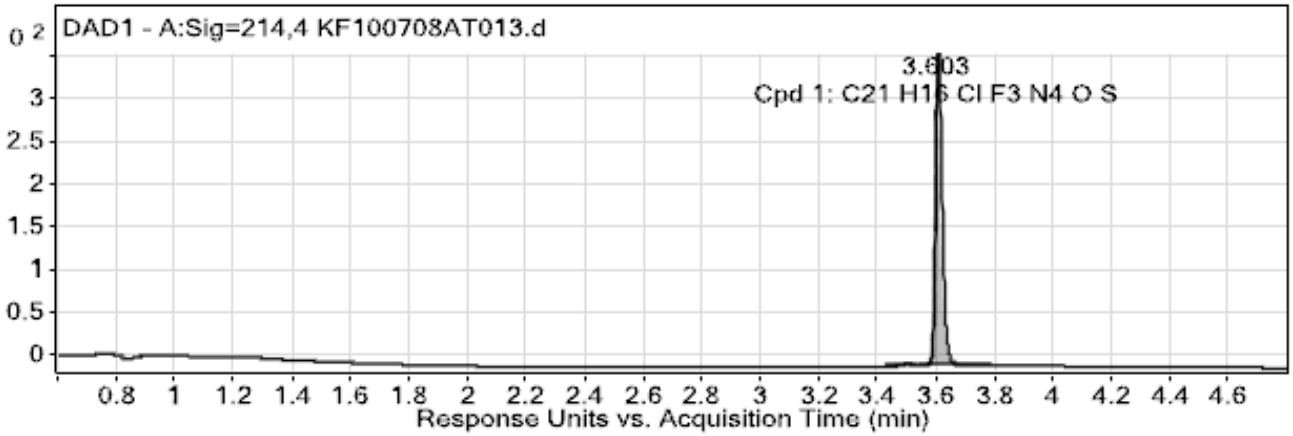


User Chromatogram Peak List

Peak #	Compound Name	RT	Height	Height %	Area	Area %	Area Sum %	Width
1	Cpd 1: C21 H17 F3 N4 O S	3.453	332.64	100	511.84	100	100	0.024

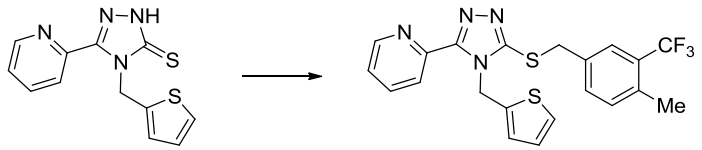


2-(5-((4-chloro-3-(trifluoromethyl)benzyl)thio)-4-((5-methylfuran-2-yl)methyl)-4H-1,2,4-triazol-3-yl)pyridine (1.2). Following the general procedure and purification using MDF HPLC, methylfuran thione (27 mg, 0.10 mmol) and 4-chloro-3-(trifluoromethyl)benzyl bromide (33 mg, 0.12 mmol) afforded the product as an off-white solid (26.5 mg, 0.057 mmol, 57% yield, 99% HPLC purity). ^{1}H NMR ($CDCl_3$) δ 2.14 (s, 3 H), 4.49 (s, 2 H), 5.77 (s, 2 H), 5.77 (m, 1 H), 5.97 (d, *J* = 2.8 Hz, 1 H), 7.34 (ddd, *J* = 1.2, 5.2, 7.6 Hz, 1 H), 7.41 (d, *J* = 8.0 Hz, 1 H), 7.58 (dd, *J* = 2.0, 8.4 Hz, 1 H), 7.72 (d, *J* = 2.0 Hz, 1 H), 7.81 (dt, *J* = 2.0, 8.0 Hz, 1 H), 8.24 (td, *J* = 0.8, 8.0 Hz, 1 H), 8.64 (qd, *J* = 0.8, 4.8 Hz, 1 H); ^{13}C NMR ($CDCl_3$) δ d 13.4, 106.2, 109.9, 123.4, 124.1, 128.2 (q, *J* = 5 Hz), 131.6, 133.6, 136.9, 148.5; u 36.5, 42.0, 121.2, 124.0, 126.6, 136.3, 146.8, 147.7, 152.0, 152.5, 152.8; IR (neat) 3054, 1590, 1568, 1480, 1463 cm^{-1}; HRMS (ESI) *m/z* calcd for $C_{21}H_{17}ClF_3N_4OS$ ($[M+H]^+$), 465.0764, found 465.0759.

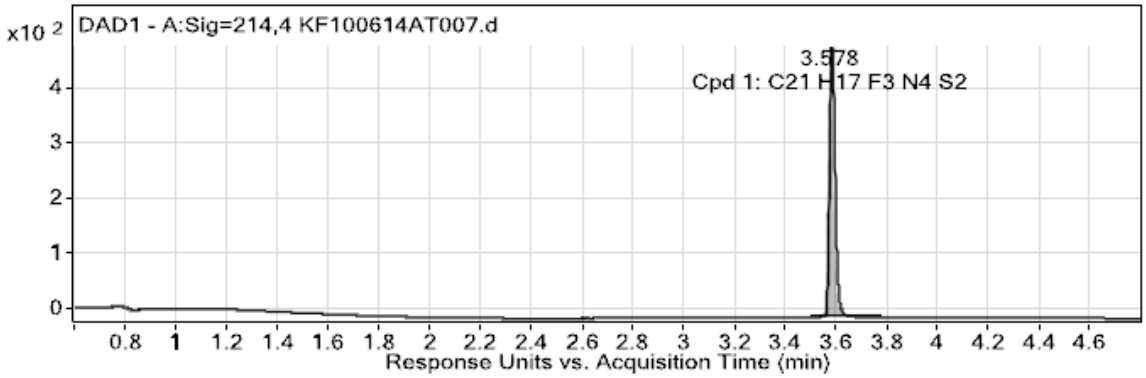


r Chromatogram Peak List

k #	Compound Name	RT	Height	Height %	Area	Area %	Area Sum %
1		3.499	2.55	0.7	4.01	0.69	0.69
2	Cpd 1: C21 H16 Cl F3 N4 O S	3.603	363.26	100	578.77	100	99.31

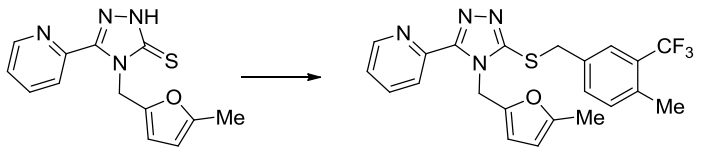


2-(5-((4-Methyl-3-(trifluoromethyl)benzyl)thio)-4-(thiophen-2-ylmethyl)-4H-1,2,4-triazol-3-yl)pyridine (1.3). Following the general procedure and purification on silica gel, the known thiophene thione[1] (35 mg, 0.13 mmol) and 4-methyl-3-(trifluoromethyl)benzyl bromide (39 mg, 0.15 mmol) afforded the product as an off-white solid (51 mg, 0.11 mmol, 90% yield, 100% HPLC purity). ^{1}H NMR ($CDCl_3$) δ 2.44 (s, 3 H), 4.49 (s, 2 H), 5.93 (s, 2 H), 6.85 (dd, *J* = 3.6, 5.2 Hz, 1 H), 6.99 (dd, *J* = 0.8, 3.6 Hz, 1 H), 7.15 (dd, *J* = 1.2, 5.2 Hz, 1 H), 7.20 (d, *J* = 8.0 Hz, 1 H), 7.35 (ddd, *J* = 0.8, 4.8, 7.6 Hz, 1 H), 7.48 (dd, *J* = 0.8, 8.0 Hz, 1 H), 7.61 (s, 1 H), 7.82 (dt, *J* = 1.6, 8.0 Hz, 1 H), 8.30 (d, *J* = 8.0 Hz, 1 H), 8.67 (d, *J* = 4.4 Hz, 1 H); ^{13}C NMR ($CDCl_3$) δ d 19.1, 123.3, 124.4, 126.55, 126.58, 127.8, 132.4, 132.5, 137.2, 148.6; u 37.3, 43.8, 123.0, 125.8, 129.3 (d, *J* = 30 Hz), 134.5, 136.3, 137.8, 147.7, 152.5; IR (neat) 2927, 1588, 1502 cm^{-1}; HRMS (ESI) *m/z* calcd for $C_{21}H_{18}F_3N_4OS_2$ ($[M+H]^+$), 447.0925, found 447.0929.

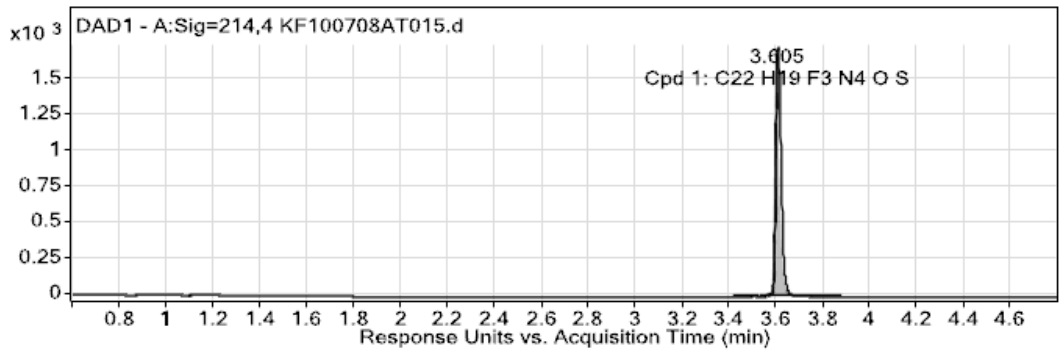


User Chromatogram Peak List

Peak #	Compound Name	RT	Height	Height %	Area	Area %	Area Sum %	Width
1	Cpd 1: C21 H17 F3 N4 S2	3.578	487.07	100	760.71	100	100	0.024

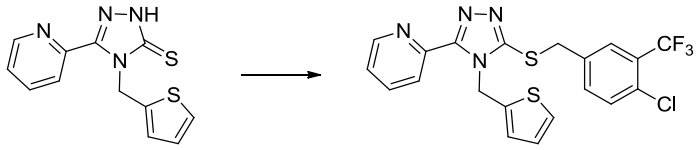


2-(5-((4-Methyl-3-(trifluoromethyl)benzyl)thio)-4-((5-methylfuran-2-yl)methyl)-4H-1,2,4-triazol-3-yl)pyridine (1.4). Following the general procedure and purification using MDF HPLC, methylfuran thione (27 mg, 0.10 mmol) and 4-methyl-3-(trifluoromethyl)benzyl bromide (30 mg, 0.12 mmol) afforded the product as an off-white solid (28.0 mg, 0.063 mmol, 63% yield, 99% HPLC purity). ^{1}H NMR ($CDCl_3$) δ 2.13 (s, 3 H), 2.44 (s, 3 H), 4.49 (s, 2 H), 5.76 (s, 2 H), 5.77 (m, 1 H), 5.97 (s, 1 H), 7.21 (d, *J* = 8.0 Hz, 1 H), 7.33 (ddd, *J* = 1.2, 4.8, 7.6 Hz, 1 H), 7.49 (dd, *J* = 1.2, 8.0 Hz, 1 H), 7.62 (d *J* = 1.6 Hz, 1 H), 7.80 (dt, *J* = 2.0, 8.0 Hz, 1 H), 8.25 (td, *J* = 1.2, 8.0 Hz, 1 H), 8.64 (qd, *J* = 0.8, 4.8 Hz, 1 H); ^{13}C NMR ($CDCl_3$) δ d 13.6, 19.2, 106.4, 110.0, 123.6, 124.2, 126.5 (q, *J* = 6 Hz), 132.4, 132.5, 137.1, 148.7; u 37.5, 42.1, 123.1, 125.8, 129.3 (d, *J* = 30 Hz), 134.7, 136.3, 147.1, 147.9, 152.6, 152.9; IR (neat) 2936, 1590, 1568, 1504 cm^{-1}; HRMS (ESI) *m/z* calcd for $C_{22}H_{20}F_3N_4OS$ ($[M+H]^+$), 445.1310, found 445.1314.

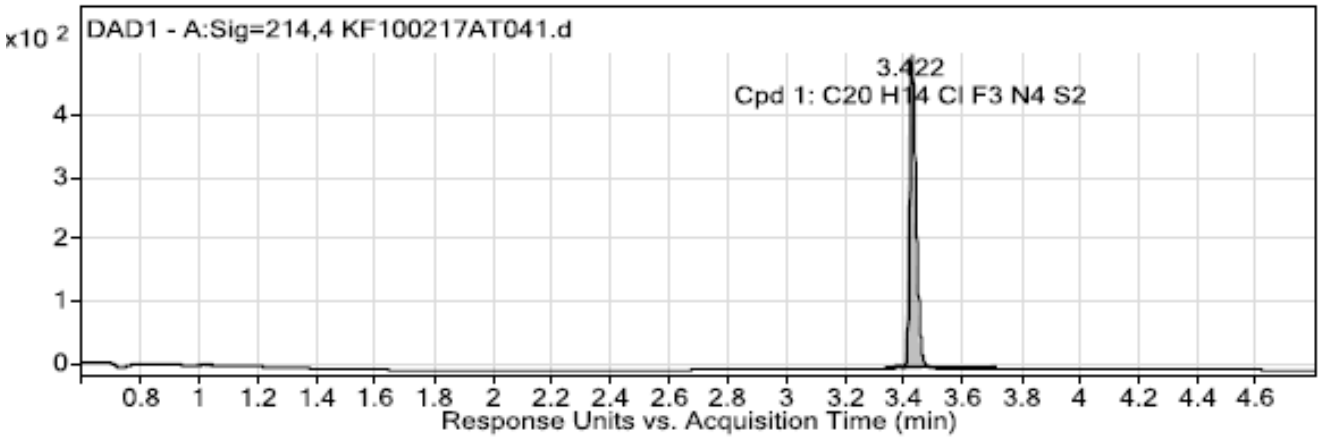


User Chromatogram Peak List

Peak #	Compound Name	RT	Height	Height %	Area	Area %	Area Sum %	Width
1		3.502	2.75	0.16	5.12	0.19	0.18	0.029
2		3.568	17.43	1.01	20.94	0.76	0.75	0.018
3	Cpd 1: C22 H19 F3 N4 O S	3.605	1728.95	100	2762.78	100	99.07	0.026



2-(5-((4-Chloro-3-(trifluoromethyl)benzyl)thio)-4-(thiophen-2-ylmethyl)-4*H*-1,2,4-triazol-3-yl)pyridine (1.5). Following the general procedure above and purification using reverse phase MDF HPLC, the known thiophene thione[1] (40 mg, 0.15 mmol) and 4-chloro-3-(trifluoromethyl)benzyl bromide (48 mg, 0.18 mmol) afforded the product as a white solid (58.4 mg, 0.13 mmol, 86% yield, 99% HPLC purity). ^{1}H NMR ($CDCl_3$) δ 4.50 (s, 2 H), 5.94 (s, 2 H), 6.86 (dd, *J* = 3.6, 5.2 Hz, 1 H), 6.98 (dd, *J* = 1.2, 5.2 Hz, 1 H), 7.16 (dd, *J* = 1.2, 5.2 Hz, 1 H), 7.36 (ddd, *J* = 1.2, 4.8, 7.6 Hz, 1 H), 7.39 (d, *J* = 8.4 Hz, 1 H), 7.55 (dd, *J* = 2.0, 8.0 Hz, 1 H), 7.71 (d, *J* = 2.0 Hz, 1 H), 7.82 (dt, *J* = 2.0, 8.0 Hz, 1 H), 8.30 (td, *J* = 1.2, 8.0 Hz, 1 H), 8.67 (qd, *J* = 1.2, 4.8 Hz, 1 H); ^{13}C NMR ($CDCl_3$) δ d 123.2, 124.3, 126.4, 126.5, 127.7, 128.1 (q, *J* = 5 Hz), 131.7, 133.7, 137.1, 148.5; u 36.4, 43.7, 121.3, 123.2, 128.5 (d, *J* = 29 Hz), 136.2, 137.6, 147.5, 151.7, 152.5; IR (neat) 3070, 1590, 1480, 1464 cm^{-1}; HRMS (ESI) *m/z* calcd for $C_{20}H_{15}ClF_3N_4S_2$ ($[M+H]^+$), 467.0379, found 467.0375.

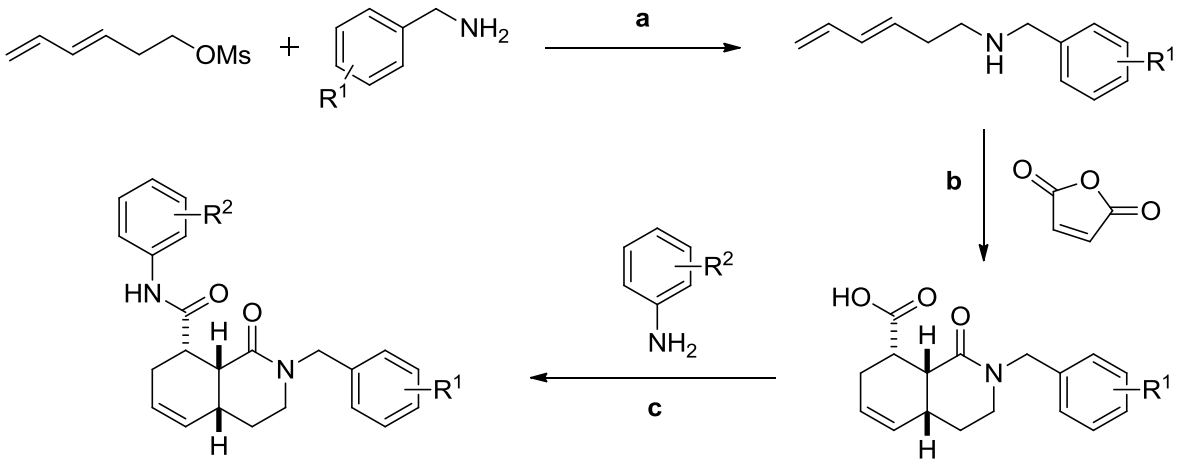


ser Chromatogram Peak List

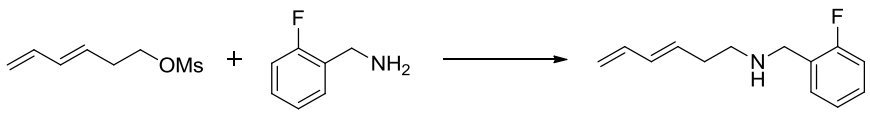
eak #	Compound Name	RT	Height	Height %	Area	Area %	Area Sum %
1		3.375	5.96	1.19	9.23	1.2	1.19
2	Cpd 1: C20 H14 Cl F3 N4 S2	3.422	503.08	100	767.49	100	98.81

Isoquinolinone agonist compounds

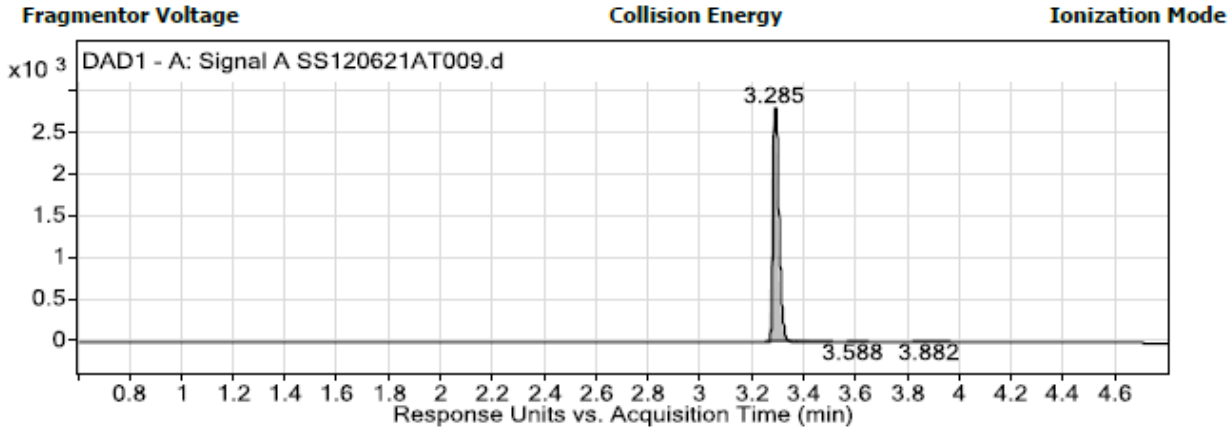
Scheme S-2. General preparation of the isoquinolinone analogues.[a]



[a] Reagents and conditions: (a) CH_3CN, µW, 130 °C, 1h; (b) $ClCH_2CH_2Cl$, µW, 165 °C, 1.5 h; (c) EDAC, DMAP, CH_2Cl_2, rt.



(E)-N-(2-Fluorobenzyl)hexa-3,5-dien-1-amine. To a solution of (*E*)-hexa-3,5-dien-1-yl methanesulfonate (4.0 g, 23 mmol) in dry acetonitrile (4.0 mL) was added 2-fluorobenzyl amine (8.5 g, 68 mmol). The solution was heated by microwave irradiation to 130 °C for 1 h. The reaction was diluted with 1N NaOH (75 mL) and extracted with CH_2Cl_2 (2 × 75 mL). The organic layers were combined, dried over Na_2SO_4, filtered, and concentrated. The oily residue was purified by silica gel chromatography to provide the diene product as a yellow oil (3.4 g, 17 mmol, 73% yield, 99% HPLC purity). ^{1}H NMR ($CDCl_3$) δ 1.48 (br s, 1H), 2.29 (q, *J* = 6.4 Hz, 2H), 2.70 (t, *J* = 6.9 Hz, 2H), 3.68 (s, 2H), 4.98 (d, *J* = 10.1 Hz, 1H), 5.12 (d, *J* = 16.64 Hz, 1H), 5.66 (dt, *J* = 14.8, 7.1 Hz, 1H), 6.09 – 6.18 (m, 1H), 6.20 – 6.40 (m, 1H), 6.92 – 7.06 (m, 1H), 7.08 – 7.14 (td, *J* = 7.4, 1.2 Hz, 1H), 7.16 – 7.26 (m, 1H), 7.28 – 7.40 (td, *J* = 7.5, 1.7 Hz, 1H); ^{13}C NMR ($CDCl_3$) δ 33.1, 47.2 (d, *J* = 3.0 Hz), 48.4, 115.3 (d, *J* = 22.0 Hz), 115.5, 124.0 (d, *J* = 4.0 Hz), 127.2 (d, *J* = 15.0 Hz), 128.6 (d, *J* = 8.2 Hz), 130.4 (d, *J* = 4.9 Hz), 132.3, 132.7, 137.0, 161.2 (d, *J* = 244.0 Hz); IR (neat) 2958, 1632 cm^{-1}; HRMS (ESI) *m/z* calcd for $C_{13}H_{16}FN$ ($[M+H]^+$), 206.1345, found 206.1358.



User Chromatogram Peak List

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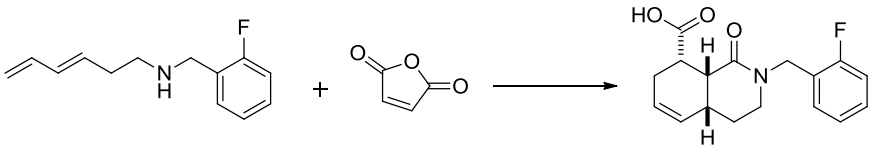
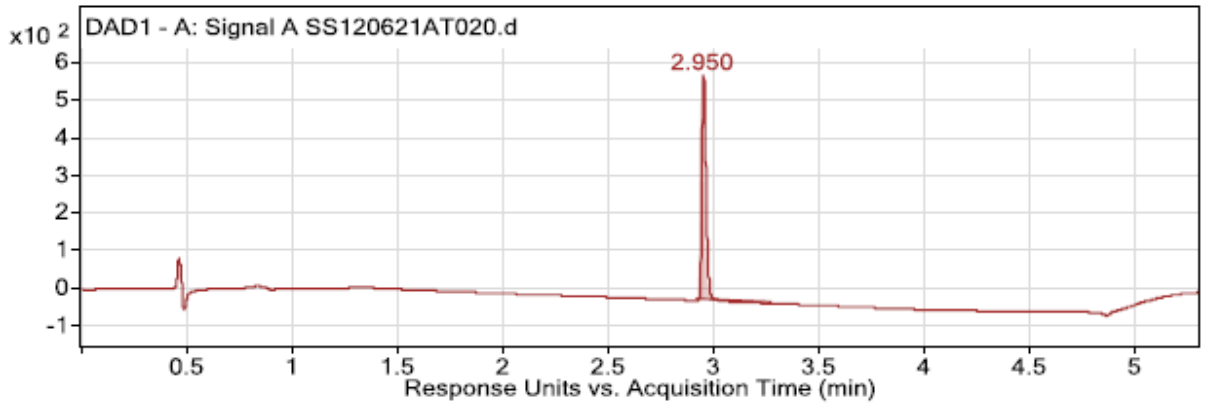
3.285	2818.09	100	4549.27	100	98.94	0.025
3.393	11.79	0.42	30.24	0.66	0.66	0.038
3.588	7.68	0.27	11.86	0.26	0.26	0.023
3.882	2.36	0.08	6.67	0.15	0.15	0.038



2-(2-Fluorobenzyl)-1-oxo-octahydroisoquinoline-8-carboxylic acid. Maleic anhydride (270 mg, 2.8 mmol) was added to a solution of the diene (513 mg, 2.5 mmol) in $ClCH_2CH_2Cl$ (5.0 mL) and heated to 165 °C by microwave irradiation for 1.5 h. The resulting crude product was loaded directly onto silica gel and chromatographed to afford the acid as a white solid (577 mg, 1.9 mmol, 76% yield, 100% HPLC purity). ^{1}H NMR ($CDCl_3$) δ 1.79 – 1.92 (m, 1H), 1.99 – 2.08 (m, 1H), 2.26 – 2.49 (m, 2H), 2.76 – 2.95 (m, 2H), 3.12 – 3.22 (m, 2H), 3.23 – 3.27 (m, 1H), 4.53 (d, *J* = 14.9 Hz, 1H), 4.77 (d, *J* = 14.9 Hz, 1H), 5.53 (d, *J* = 10.1 Hz, 1H), 5.79 – 5.95 (m, 1H), 7.00-7.06 (m, 1H), 7.09 (td, *J* = 7.5, 1.2 Hz, 1H), 7.14 – 7.27 (m, 2H), 13.2 (br s, 1H); ^{13}C NMR ($CDCl_3$) δ 25.1, 27.0, 34.7, 41.5, 44.28, 44.32, 44.7, 115.3 (d, *J* = 21.6 Hz), 123.0 (d, *J* = 14.8 Hz), 124.4 (d, *J* = 3.5 Hz), 127.4, 129.4 (d, *J* = 8.1 Hz), 129.5, 130.1 (d, *J* = 3.9 Hz), 161.0 (d, *J* = 244.9 Hz), 171.8, 176.2; IR (neat) 2929, 1704 cm^{-1}; HRMS (ESI) *m/z* calcd for $C_{17}H_{18}FNO_3$ ($[M+H]^+$), 304.1349, found 304.1367.

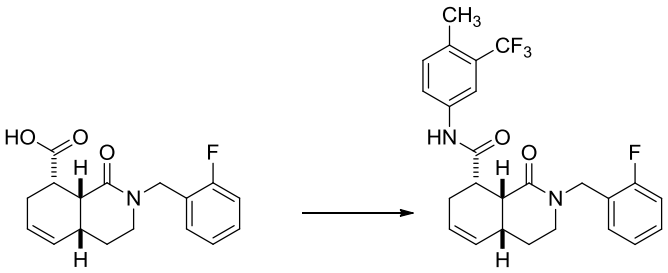


User Chromatogram Peak List

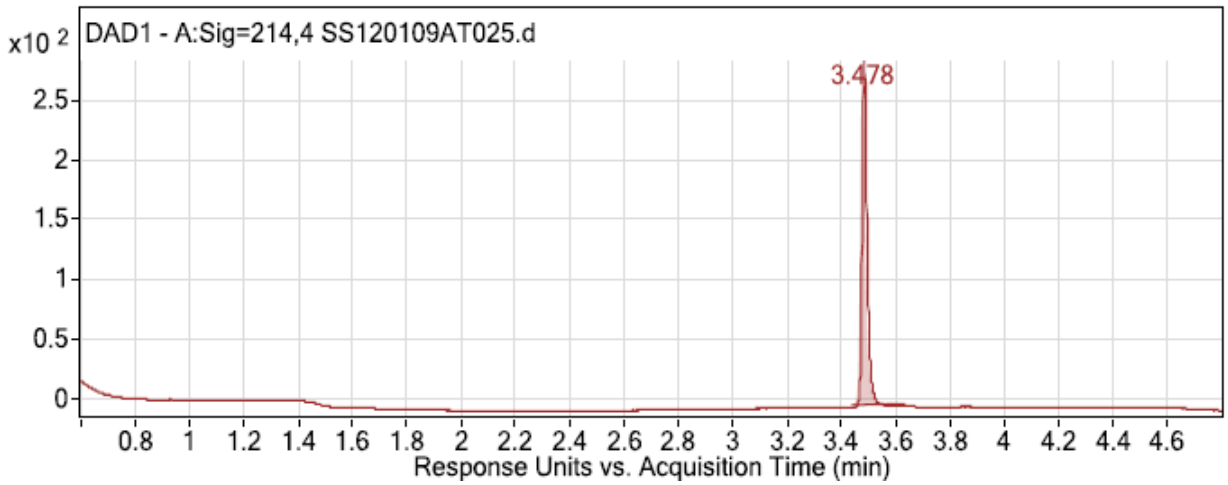
Compound Name	RT	Height	Height %	Area	Area %	Area Sum %	Width
Cpd 1: 2.949	2.95	590.14	100	856.63	100	100	0.023

General procedure for the synthesis of series 2 analogues from isoquinolinone acids and anilines.

1-Ethyl-3-(3-dimethylaminopropyl)carbodiimide (EDAC) (0.075 - 0.15 mmol, 1.5 equiv.) was added to a 24 position Bohdan Miniblock XT. A solution of the isoquinolinone acid (0.050 - 0.10 mmol) in CH_2Cl_2 (1.0 mL) and a solution of DMAP (0.010 - 0.020 mmol, 0.2 equiv.) and aniline (0.10 - 0.20 mmol, 2.0 equiv.) in CH_2Cl_2 (1.0 mL) were added. After stirring for 14 h at room temperature, the reactions were partitioned between water (2.0 mL) and CH_2Cl_2 in hydrophobic phase separator tubes. The aqueous layer was washed with CH_2Cl_2 (3 × 2.0 mL) and the combined organics evaporated. The crude material was subjected to mass-directed, reverse phase preparative HPLC purification as detailed in the general synthetic methods section.

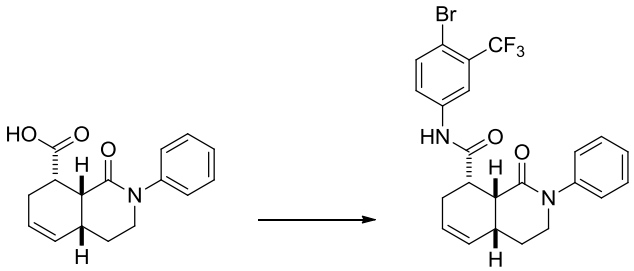


2-(2-Fluorobenzyl)-*N*-(4-methyl-3-(trifluoromethyl)phenyl)-1-oxo-octahydroisoquinoline-8-carboxamide (2.1). Following the general procedure, 2-(2-fluorobenzyl)-1-oxo-1,2,3,4,4a,7,8,8a-octahydroisoquinoline-8-carboxylic acid (20 mg, 0.066 mmol), 4-methyl-3-trifluoromethylaniline (23 mg, 0.13 mmol), EDAC (19 mg, 0.099 mmol), and DMAP (1.6 mg, 0.013 mmol) afforded the product as a white solid (22 mg, 0.047 mmol, 72% yield, 100% HPLC purity). ^{1}H NMR ($CDCl_3$) δ 1.83 – 1.95 (m, 1H), 1.96 – 2.10 (m, 1H), 2.37 – 2.55 (m, 5H), 2.82 – 3.01 (m, 2H), 3.19 – 3.26 (m, 3H), 4.55 (d, *J* = 15.1 Hz, 1H), 4.86 (d, *J* = 15.1 Hz, 1H), 5.61 (d, *J* = 9.96 Hz, 1H), 5.89 – 5.97 (m, 1H), 7.02 – 7.12 (m, 2H), 7.20 – 7.31 (m, 3H), 7.73 – 7.78 (m, 1H), 7.85 (d, *J* = 2.1 Hz, 1H), 11.12 (br s, 1H); ^{13}C NMR ($CDCl_3$) δ 18.8, 26.4, 27.2, 36.1, 42.4, 44.2, 44.3, 44.6, 115.4 (d, *J* = 22 Hz), 117.4 (q, *J* = 5.8 Hz), 122.8, 122.9 (d, *J* = 12 Hz), 123.4 (d, *J* = 15 Hz), 124.3 (d, *J* = 3.6 Hz), 125.7, 128.0, 129.2 (d, *J* = 8.0 Hz), 129.3, 129.7 (d, *J* = 4.0 Hz), 131.3 (d, *J* = 1.7 Hz), 132.3, 136.7, 161.0 (d, *J* = 244 Hz), 171.5, 172.5; IR (neat) 2932, 1671 cm^{-1}; HRMS (ESI) *m/z* calcd for $C_{25}H_{24}F_4N_2O_2$ ($[M+H]^+$), 461.1852, found 461.1811.

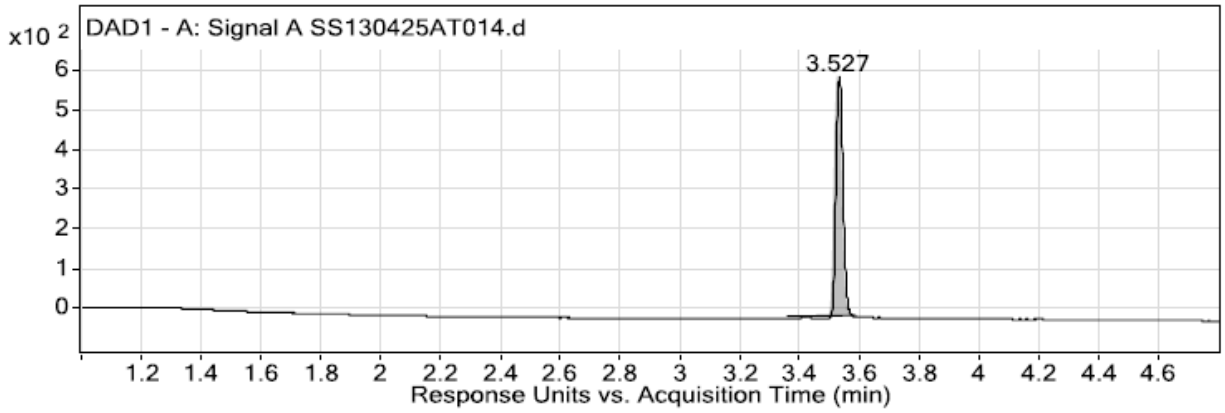


User Chromatogram Peak List

Peak #	RT	Height	Height %	Area	Area %	Area Sum %	Width
1	3.478	286.92	100	378.21	100	100	0.02



***N*-(4-Bromo-3-(trifluoromethyl)phenyl)-1-oxo-2-phenyl-octahydroisoquinoline-8-carboxamide (2.2).** Following the general procedure, the known 1-oxo-2-phenyl-1,2,3,4,4a,7,8,8a-octahydroisoquinoline-8-carboxylic acid[2] (20 mg, 0.073 mmol), 4-bromo-3-trifluoromethylaniline (35 mg, 0.15 mmol), EDAC (21 mg, 0.11 mmol), and DMAP (1.9 mg, 0.015 mmol) afforded the product as a white solid (24 mg, 0.049 mmol, 66% yield, 100% HPLC purity). ^{1}H NMR ($CDCl_3$) δ 1.94 – 2.05 (m, 1H), 2.18 (s, 1H), 2.42 – 2.54 (m, 2H), 2.91 – 3.02 (m, 2H), 3.33 (dd, *J* = 5.1, 2.1 Hz, 1H), 3.46 – 3.54 (m, 1H), 3.60 (td, *J* = 11.9, 4.6 Hz, 1H), 5.72 (d, *J* = 10.0 Hz, 1H), 5.96 – 6.04 (m, 1H), 7.11 – 7.18 (m, 2H), 7.29 – 7.35 (m, 1H), 7.39 – 7.45 (m, 2H), 7.51 – 7.57 (m, 1H), 7.64 (dd, *J* = 8.7, 2.5 Hz, 1H), 7.93 (d, *J* = 2.5 Hz, 1H), 11.04 (br s, 1H); ^{13}C NMR ($CDCl_3$) δ 26.3, 27.5, 36.1, 42.6, 48.1, 48.7, 112.9 (d, *J* = 1.7 Hz), 119.4(q, *J* = 5.7 Hz), 121.4, 124.1, 126.3, 127.7 (d, *J* = 32 Hz), 129.3, 129.5, 130.0, 130.3, 135.0, 138.2, 142.7, 171.9, 172.8; IR (neat) 2927, 1639 cm^{-1}; HRMS (ESI) *m/z* calcd for $C_{23}H_{20}BrF_3N_2O_2$ ($[M+H]^+$), 493.0739, found 493.0743.



User Chromatogram Peak List

RT	Height	Height %	Area	Area %	Area Sum %	Symmetry	Width
3.527	607.76	100	995.55	100	100	0.9	0.026

[1] Frankowski, K. J. et al. (2012) Discovery of Small Molecule Kappa Opioid Receptor Agonist and Antagonist Chemotypes through a HTS and Hit Refinement Strategy. *ACS Chem. Neurosci.* **3**, 221-236.

[2] Frankowski, K. J.; Ghosh, P.; Setola, V.; Tran, T. B.; Roth, B. L.; Aubé, J. (2010) N-Alkyl-octahydroisoquinolin-1-one-8-carboxamides: a Novel Class of Selective, Nonbasic, Nitrogen-Containing κ-Opioid Receptor Ligands. *ACS Med. Chem. Lett.* **1**, 189-193.