Electronic Supplementary Material (ESI) for MedChemComm.

Supporting Information

Pyrazolo[3,4-*d*]pyrimidines as Sigma-1 Receptor Antagonists. Part 1: 4-Acylamino Derivatives.

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Analytical data: purity, NMR, HRMS

In vitro tests

In vivo tests

Analytical data

Analytical LC Method

Method A: Column XBridge C18 4.6 x 50 mm, 2.5 µm; flow rate 1.5 mL/min; A: Ammonium bicarbonate 10 mM, B: ACN; Gradient: 0.5 min in 98% A, 98 to 5% A in 5.4 min, 2 min in 5% A.

Method B: Column XBridge C18 4.6 x 50 mm, 2.5 µm; flow rate 2 mL/min; A: Ammonium bicarbonate 10 mM, B: ACN; Gradient: 0.5 min in 98% A, 98 to 5% A in 3.7 min, 2 min in 5% A.

comp	PURITY (%)	Rt (min)	Method	FORM	NMR	HRMS $[M+H]^+$ (diff ppm)
8a	99	2.09	A	Maleate	^{1}H NMR (300 MHz, CD_3OD) δ 8.63 (1H, s), 8.61 (1H, s), 6.22 (2H, s), 4.79 (2H, t, *J* = 5.9 Hz), 3.65 (2H, t, *J* = 5.9 Hz), 3.38 – 3.22 (4H, m), 2.21 – 1.90 (5H, m), 1.13 – 1.06 (2H, m), 1.06 – 0.96 (2H, m).	301.1771 (-0.25)
8b	98	3.36	A	Maleate	^{1}H NMR (300 MHz, CD_3OD) δ 8.72 (1H, s), 8.64 (1H, s), 6.25 (2H, s), 4.96 – 4.71 (2H, m), 3.84 (2H, t, *J* = 5.7 Hz), 3.48 (1H, p, *J* = 8.4 Hz), 4.05 – 2.93 (4H, m), 2.52 – 2.21 (4H, m), 2.19 – 1.88 (6H, m).	315.1930 (2.96)
8c	99	2.51	B	Maleate	^{1}H NMR (300 MHz, CD_3OD) δ 8.68 (1H, s), 8.65 (1H, s), 6.24 (2H, s), 4.91 – 4.67 (2H, m), 3.83 (2H, t, *J* = 5.6 Hz), 3.84 – 2.76 (4H, m), 3.11 – 2.99 (1H, m), 2.23 – 1.57 (12H, m).	329.2084 (0.03)
8d	99	2.72	B	Maleate	^{1}H NMR (400 MHz, CD_3OD) δ 8.67 (1H, s), 8.65 (1H, s), 6.25 (2H, s), 4.85 (2H, t, *J* = 5.7 Hz), 3.84 (2H, t, *J* = 5.6 Hz), 3.91 – 3.60 (2H, m), 3.42 – 3.05 (2H, m), 2.60 (1H, tt, *J* = 3.5, 11.5 Hz), 2.27 – 1.81 (8H, m), 1.81 – 1.70 (1H, m), 1.66 – 1.48 (2H, m), 1.49 – 1.22 (3H, m).	343.2241 (-0.06)
9a	97	2.80	B	Base	^{1}H NMR (300 MHz, $CDCl_3$) δ 8.68 (1H, s), 8.56 (1H, s), 8.37 (1H, s), 4.59 (2H, t, *J* = 7.1 Hz), 2.86 (2H, t, *J* = 7.1 Hz), 2.55 – 2.42 (4H, m),	331.2239 (0.41)

					1.58 – 1.46 (4H, m), 1.43 – 1.36 (2H, m), 1.39 (9H, s).	
9b	100	4.47	A	Hydrochloride	^{1}H NMR (300 MHz, CD_3OD) δ 8.79 (1H, s), 8.75 (1H, s), 4.96 (2H, t, *J* = 6.0 Hz), 3.84 – 3.67 (4H, m), 3.06 (2H, t, *J* = 12.4 Hz), 2.22 – 2.05 (9H, m), 2.03 – 1.71 (11H, m), 1.64 – 1.45 (1H, m).	409.2708 (0.46)
9c	99	4.17	A	Hydrochloride	^{1}H NMR (300 MHz, CD_3OD) δ 8.73 (1H, s), 8.73 (1H, s), 4.93 (2H, t, *J* = 5.9 Hz), 3.83 – 3.65 (4H, m), 3.06 (2H, t, *J* = 12.2 Hz), 2.93 (1H, t, *J* = 6.9 Hz), 2.47 – 2.36 (2H, m), 2.22 – 2.06 (4H, m), 2.03 – 1.90 (4H, m), 1.89 – 1.65 (7H, m), 1.66 – 1.44 (1H, m).	395.2552 (-0.37)
9d	100	3.89	A	Hydrochloride	^{1}H NMR (300 MHz, CD_3OD) δ 8.76 (1H, s), 8.74 (1H, s), 7.72 (1H, d, *J* = 8.3 Hz), 7.69 (1H, d, *J* = 1.9 Hz), 7.54 (1H, dd, *J* = 2.0, 8.3 Hz), 4.97 (2H, t, *J* = 5.9 Hz), 3.86 – 3.68 (4H, m), 3.07 (2H, td, *J* = 2.2, 12.3 Hz), 2.05 – 1.91 (2H, m), 1.90 – 1.68 (3H, m), 1.64 – 1.47 (1H, m).	419.1147 (-0.2)
10a	95	3.76	A	Hydrochloride	^{1}H NMR (300 MHz, CD_3OD) δ 8.76 (1H, s), 8.75 (1H, s), 4.94 (2H, t, *J* = 6.0 Hz), 3.83 (2H, t, *J* = 6.0 Hz), 3.69 – 3.56 (2H, m), 3.40 – 3.27 (2H, m), 2.10 – 1.82 (4H, m), 1.82 – 1.67 (4H, m), 1.40 (9H, s).	345.2397 (-0.05)
10b	100	2.89	A	Hydrochloride	^{1}H NMR (300 MHz, CD_3OD) δ 8.79 (1H, s), 8.75 (1H, s), 4.95 (2H, t, *J* = 6.0 Hz), 3.83 (2H, t, *J* = 6.0 Hz), 3.71 – 3.55 (2H, m), 3.41 – 3.19 (2H, m), 2.18 – 2.06 (9H, m), 2.02 – 1.81 (10H, m), 1.79 – 1.69 (4H, m).	423.2867 (-0.03)
10c	98	4.56	A	Hydrochloride	^{1}H NMR (400 MHz, $CDCl_3$) δ 8.77 (1H, s), 8.59 (1H, s), 8.34 (1H, s), 6.21 (2H, s), 4.89 (2H, t, *J* = 6.2 Hz), 3.70	409.2709 (0.4)

					(2H, t, *J* = 6.2 Hz), 3.67 – 3.35 (2H, m), 3.35 – 2.91 (2H, m), 2.87 (1H, t, *J* = 6.8 Hz), 2.50 – 2.40 (2H, m), 2.21 – 2.12 (3H, m), 2.03 – 1.81 (8H, m), 1.81 – 1.62 (10H, m).	
10d	99	3.86	A	Maleate	^{1}H NMR (300 MHz, DMSO) δ 11.31 (1H, s), 9.22 (1H, s), 8.70 (1H, s), 8.56 (1H, s), 6.05 (2H, s), 4.79 (2H, t, *J* = 6.3 Hz), 3.66 (2H, t, *J* = 6.3 Hz), 3.21 – 3.02 (4H, m), 2.08 – 1.31 (17H, m).	357.2395 (-0.68)
10e	100	4.03	A	Hydrochloride	^{1}H NMR (300 MHz, CD_3OD) δ 8.73 (1H, s), 8.72 (1H, s), 4.94 (2H, t, *J* = 6.0 Hz), 3.83 (2H, t, *J* = 6.0 Hz), 3.72 – 3.54 (2H, m), 3.41 – 3.18 (2H, m), 2.67 (1H, tt, *J* = 3.3, 11.4 Hz), 2.17 – 1.82 (8H, m), 1.82 – 1.69 (5H, m), 1.67 – 1.50 (2H, m), 1.50 – 1.22 (3H, m).	371.2558 (1.11)
10f	100	3.13	B	Maleate	^{1}H NMR (300 MHz, CD_3OD) δ 8.67 (1H, s), 8.66 (1H, s), 6.26 (2H, s), 4.89 (2H, t, *J* = 5.9 Hz), 3.80 (2H, t, *J* = 5.9 Hz), 3.69 – 2.90 (8H, m), 2.82 – 2.64 (1H, m), 2.28 – 1.81 (12H, m).	407.2365 (0.09)
10g	95	3.02	A	Hydrochloride	^{1}H NMR (300 MHz, CD_3OD) δ 8.77 (1H, s), 8.74 (1H, s), 4.95 (2H, t, *J* = 6.0 Hz), 4.04 (2H, dt, *J* = 3.0, 11.1 Hz), 3.83 (2H, t, *J* = 6.0 Hz), 3.76 – 3.44 (4H, m), 3.44 – 3.18 (2H, m), 3.04 – 2.86 (1H, m), 2.12 – 1.63 (12H, m).	373.2345 (-0.53)
10h	95	4.53	A	Citrate	^{1}H NMR (300 MHz, CD_3OD) δ 8.63 (1H, s), 8.61 (1H, s), 4.83 (2H, t, *J* = 5.9 Hz), 3.74 (2H, t, *J* = 5.9 Hz), 3.45 – 3.36 (4H, m), 2.91 – 2.69 (5H, m), 2.00 – 1.82 (4H, m), 1.82 – 1.67 (4H, m), 1.36 (6H, s), 1.29 (6H, s).	385.2708 (-0.49)

10i	98	4.19	A	Hydrochloride	^{1}H NMR (300 MHz, CD_3OD) δ 8.75 (2H, s), 7.70 (1H, d, *J* = 8.4 Hz), 7.68 (1H, d, *J* = 2.0 Hz), 7.54 (1H, dd, *J* = 2.0, 8.3 Hz), 4.95 (2H, t, *J* = 5.9 Hz), 3.84 (2H, t, *J* = 5.9 Hz), 3.64 (2H, ddd, *J* = 2.6, 7.6, 13.6 Hz), 3.42 – 3.29 (2H, m), 2.08 – 1.82 (4H, m), 1.82 – 1.69 (4H, m).	433.1297 (-1.67)
10j	99	3.83	A	Citrate	^{1}H NMR (400 MHz, CD_3OD) δ 8.72 (1H, s), 8.67 (1H, s), 8.15 (2H, dd, *J* = 5.2, 9.0 Hz), 7.31 (2H, dd, *J* = 8.6, 9.0 Hz), 4.91 – 4.85 (2H, m), 3.72 (2H, t, *J* = 6.0 Hz), 3.44 – 3.35 (4H, m), 2.78 (4H, q, *J* = 15.3 Hz), 1.96 – 1.83 (4H, m), 1.77 – 1.68 (4H, m).	383.1990 (-0.05)
11a	95	3.75	B	Base	^{1}H NMR (300 MHz, CD_3OD) δ 8.61 (1H, s), 8.54 (1H, s), 4.61 (2H, t, *J* = 6.8 Hz), 3.16 – 3.03 (2H, m), 2.89 (2H, t, *J* = 6.8 Hz), 2.02 (2H, td, *J* = 2.1, 12.0 Hz), 1.74 – 1.60 (2H, m), 1.38 (9H, s), 1.26 (2H, qd, *J* = 3.7, 12.4 Hz), 1.09 – 0.97 (1H, m), 0.85 (9H, s).	387.2862 (1.14)
11b	98	4.28	A	Hydrochloride	^{1}H NMR (300 MHz, CD_3OD) δ 8.75 (1H, s), 8.74 (1H, s), 4.91 (2H, t, *J* = 7.3 Hz), 3.91 (2H, p, *J* = 6.6 Hz), 3.76 (2H, t, *J* = 7.2 Hz), 3.66 (1H, s), 1.45 (12H, d, *J* = 6.6 Hz), 1.40 (9H, s).	347.2553 (0.18)
11c	98	2.89	A	Hydrochloride	^{1}H NMR (300 MHz, CD_3OD) δ 8.78 (1H, s), 8.76 (1H, s), 4.98 (2H, t, *J* = 5.8 Hz), 4.07 (2H, d, *J* = 13.2 Hz), 3.84 (2H, t, *J* = 5.8 Hz), 3.81 – 3.62 (4H, m), 3.35 – 3.17 (2H, m), 1.41 (9H, s).	333.2033 (-0.1)
11d	95	2.39	B	Base	^{1}H NMR (300 MHz, $CDCl_3$) δ 8.70 (1H, s), 8.57 (1H, s), 8.33 (1H, s), 4.79 – 4.50 (2H, m), 3.70 (2H, t, *J* = 6.1 Hz), 3.73 – 3.53 (2H, m), 3.26 – 3.06 (2H, m), 2.97 – 2.70 (4H,m), 1.98 – 1.76 (2H, m), 1.39 (9H, s).	347.2189 (-0.25)

11e	97	2.11	B	Maleate	^{1}H NMR (400 MHz, CD_3OD) δ 8.61 (1H, s), 8.54 (1H, s), 6.28 (4H, s), 4.62 (2H, t, *J* = 6.0 Hz), 3.37 – 2.74 (6H, m), 3.01 (2H, t, *J* = 6.0 Hz), 2.80 (3H, s), 2.70 – 2.24 (2H, m), 1.38 (9H, s).	346.2349 (-0.3)
11f	95	2.07	B	Base	^{1}H NMR (300 MHz, $CDCl_3$) δ 8.69 (1H, s), 8.57 (1H, s), 8.33 (1H, s), 4.55 (2H, t, *J* = 6.7 Hz), 3.08 (2H, t, *J* = 6.7 Hz), 2.86 – 2.73 (4H, m), 2.64 – 2.49 (4H, m), 2.32 (3H, s), 1.87 – 1.69 (2H, m), 1.39 (9H, s).	360.2502 (-1.29)
11g	95	2.38	B	Base	^{1}H NMR (400 MHz, CD_3OD) δ 8.61 (1H, s), 8.53 (1H, s), 4.50 (2H, t, *J* = 6.8 Hz), 2.49 – 2.32 (6H, m), 2.21 – 2.08 (2H, m), 1.65 – 1.51 (4H, m), 1.50 – 1.41 (2H, m), 1.38 (9H, s).	345.2400 (-0.65)
11h	99	2.46	B	Maleate	^{1}H NMR (400 MHz, CD_3OD) δ 8.64 (1H, s), 8.58 (1H, s), 6.26 (2H, s), 4.59 (2H, t, *J* = 6.4 Hz), 3.57 – 3.36 (2H, m), 3.28 – 3.08 (4H, m), 2.48 – 2.30 (2H, m), 2.02 – 1.78 (4H, m), 1.78 – 1.65 (4H, m), 1.37 (9H, s).	359.2554 (-0.08)
11i	92	2.41	B	Maleate	^{1}H NMR (300 MHz, CD_3OD) δ 8.62 (1H, s), 8.55 (1H, s), 6.24 (2H, s), 4.53 (2H, t, *J* = 6.6 Hz), 3.30 – 3.22 (4H, m), 3.22 – 3.11 (2H, m), 2.02 (2H, p, *J* = 7.0 Hz), 1.93 – 1.80 (4H, m), 1.80 – 1.59 (6H, m), 1.38 (9H, s).	373.2706 (-1.2)
11j	100	3.20	B	Maleate	^{1}H NMR (400 MHz, CD_3OD) δ 8.63 (1H, s), 8.55 (1H, s), 6.25 (2H, s), 4.54 (2H, t, *J* = 6.6 Hz), 3.63 – 3.46 (2H, m), 3.21 – 3.04 (2H, m), 2.87 (2H, t, *J* = 12.6 Hz), 2.08 – 1.91 (4H, m), 1.76 – 1.65 (2H, m), 1.56 – 1.42 (2H, m), 1.38	415.3178 (-0.4)

					(9H, s), 1.41 – 1.30 (1H, m), 0.91 (9H, s).	
12a	99	5.22	A	Hydrochloride	^{1}H NMR (300 MHz, CD_3OD) δ 8.72 (1H, s), 8.72 (1H, s), 4.89 (2H, t, *J* = 7.2 Hz), 3.91 (2H, p, *J* = 6.7 Hz), 3.75 (2H, t, *J* = 7.2 Hz), 2.17 – 2.11 (3H, m), 2.11 – 2.06 (6H, m), 1.89 – 1.81 (6H, m), 1.45 (12H, dd, *J* = 2.8, 6.7 Hz).	425.3025 (-0.28)
12b	95	3.82	A	Maleate	^{1}H NMR (300 MHz, CD_3OD) δ 8.61 (1H, s), 8.54 (1H, s), 6.29 (4H, s), 4.62 (2H, t, *J* = 6.0 Hz), 3.49 – 3.32 (1H, m), 3.47 – 2.75 (6H, m), 3.01 (2H, t, *J* = 6.0 Hz), 2.68 – 2.29 (2H, m), 2.17 – 2.10 (3H, m), 2.10 – 2.03 (6H, m), 1.95 – 1.66 (6H, m), 1.30 (6H, d, *J* = 6.6 Hz).	452.3130 (-0.41)
12c	97	3.81	A	Hydrochloride	^{1}H NMR (300 MHz, CD_3OD) δ 8.78 (1H, s), 8.75 (1H, s), 4.98 (2H, t, *J* = 5.8 Hz), 4.18 – 4.00 (2H, m), 3.84 (2H, t, *J* = 5.8 Hz), 3.81 – 3.64 (4H, m), 3.33 – 3.20 (2H, m), 2.17 – 2.12 (3H, m), 2.12 – 2.07 (6H, m), 1.89 – 1.80 (6H, m).	411.2500 (-0.84)
13a	97	2.11	B	Maleate	^{1}H NMR (400 MHz, CD_3OD) δ 8.61 (1H, s), 8.54 (1H, s), 6.28 (4H, s), 4.62 (2H, t, *J* = 6.0 Hz), 3.01 (2H, t, *J* = 6.1 Hz), 3.55 – 2.25 (8H, m), 2.80 (3H, s), 1.38 (9H, s).	346.2349 (-0.3)
13b	99	2.62	B	Base	^{1}H NMR (400 MHz, CD_3OD) δ 8.55 (1H, s), 8.34 (1H, s), 4.59 (2H, t, *J* = 6.9 Hz), 3.34 (2H, t, *J* = 7.0 Hz), 2.88 (2H, t, *J* = 6.8 Hz), 2.62 – 2.44 (4H, m), 1.66 (2H, h, *J* = 7.3 Hz), 1.54 (4H, p, *J* = 5.5 Hz), 1.49 – 1.37 (2H, m), 1.02 (3H, t, *J* = 7.4 Hz).	332.2193 (-0.11)
13c	100	3.56	A	Hydrochloride	^{1}H NMR (400MHz, CD_3OD) δ 8.68 (1H, s), 8.68 (1H, s), 4.98 (2H, t, *J*=6.0 Hz), 4.20 (1H, p, *J*=6.3 Hz),	358.2354 (-0.41)

					3.77 (2H, t, *J*=6.0 Hz), 3.72 (2H, d, *J*=12.1 Hz), 3.07 (2H, td, *J*=12.3, 3.1 Hz), 2.12 – 1.44 (14H, m)	
13d	96	3.81	B	Hydrochloride	^{1}H NMR (300 MHz, CD_3OD) δ 8.65 (1H, s), 8.56 (1H, s), 4.93 (2H, t, *J* = 6.0 Hz), 3.81 – 3.64 (4H, m), 3.51 – 3.40 (1H, m), 3.06 (3H, t, *J* = 12.1 Hz), 2.07 – 1.06 (16H, m).	372.2504 (-0.61)
13e	98	4.55	A	Hydrochloride	^{1}H NMR (300 MHz, CD_3OD) δ 8.65 (1H, s), 8.59 (1H, s), 4.95 (2H, t, *J* = 6.0 Hz), 3.82 – 3.65 (4H, m), 3.06 (2H, t, *J* = 12.3 Hz), 2.16 – 2.09 (9H, m), 2.04 – 1.91 (2H, m), 1.91 – 1.69 (9H, m), 1.65 – 1.46 (1H, m).	424.2815 (-0.92)
13f	98	4.57	A	Hydrochloride	^{1}H NMR (300 MHz, CD_3OD) δ 8.73 (1H, s), 8.53 (1H, s), 8.35 (1H, d, *J* = 8.9 Hz), 7.56 (1H, d, *J* = 2.4 Hz), 7.37 (1H, dd, *J* = 2.4, 8.9 Hz), 4.94 (2H, t, *J* = 6.0 Hz), 3.82 – 3.68 (4H, m), 3.07 (2H, t, *J* = 12.3 Hz), 2.05 – 1.91 (2H, m), 1.91 – 1.66 (3H, m), 1.65 – 1.45 (1H, m).	434.1258 (0.1)
14a	99	3.15	A	Hydrochloride	^{1}H NMR (300 MHz, CD_3OD) δ 8.68 (1H, s), 8.61 (1H, s), 4.95 (2H, t, *J* = 6.0 Hz), 3.83 (2H, t, *J* = 6.0 Hz), 3.63 (2H, ddd, *J* = 2.9, 7.4, 13.6 Hz), 3.40 (2H, q, *J* = 7.3 Hz), 3.36 – 3.27 (2H, m), 2.14 – 1.84 (4H, m), 1.84 – 1.66 (4H, m), 1.25 (3H, t, *J* = 7.2 Hz).	332.2190 (-1.15)
14b	97	3.53	A	Maleate	^{1}H NMR (300 MHz, CD_3OD) δ 8.61 (1H, s), 8.45 (1H, s), 6.27 (4H, s), 4.98 – 4.71 (2H, m), 3.80 (2H, t, *J* = 5.9 Hz), 3.68 – 3.14 (6H, m), 2.06 – 1.83 (4H, m), 1.83 – 1.73 (4H, m), 1.66 (2H, h, *J* = 7.2 Hz), 1.01 (3H, t, *J* = 7.4 Hz).	346.2349 (-0.16)

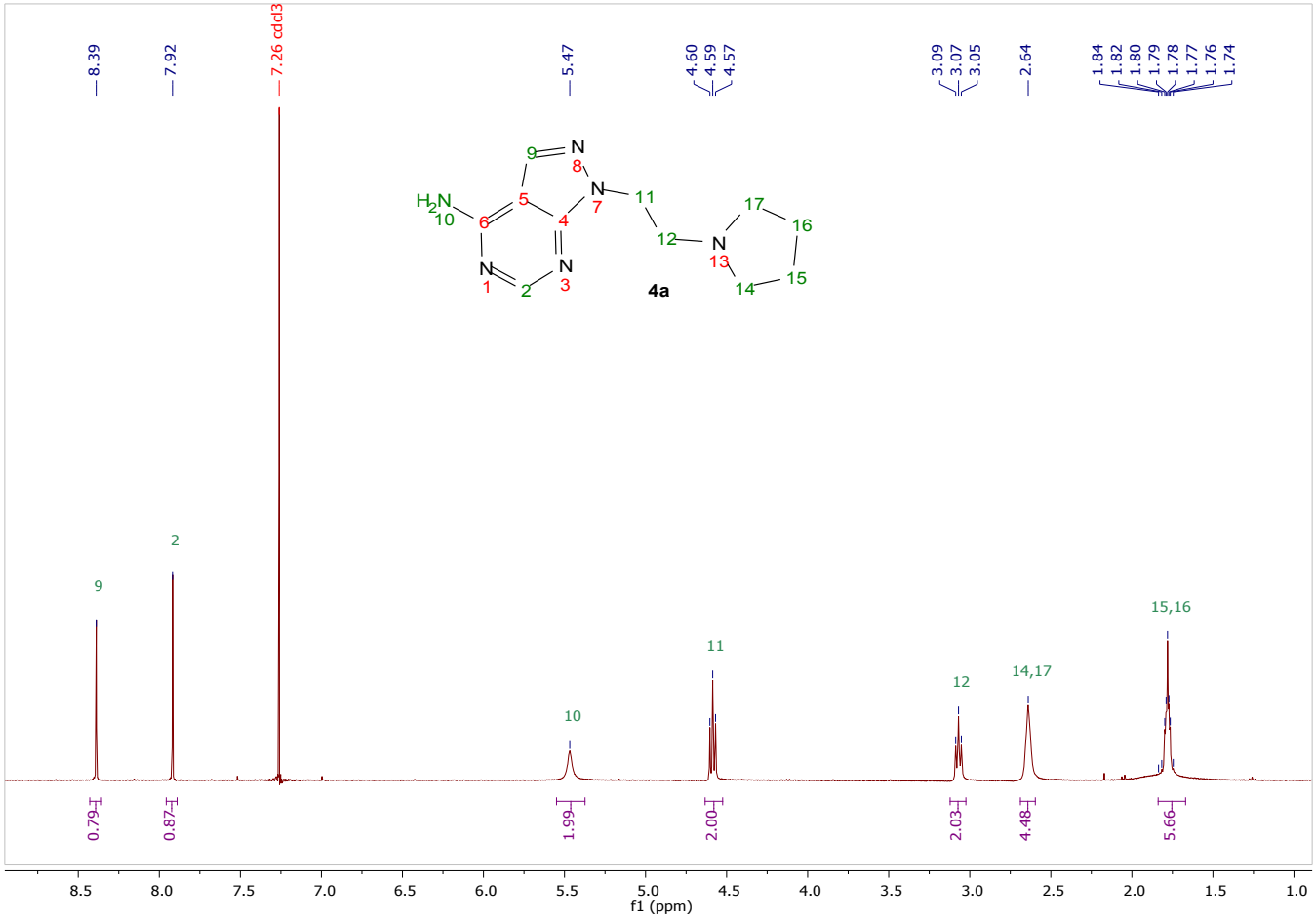
14c	96	2.84	B	Hydrochloride	^{1}H NMR (300 MHz, CD_3OD) δ 8.67 (1H, s), 8.61 (1H, s), 4.95 (2H, t, *J* = 6.0 Hz), 4.04 (1H, p, *J* = 6.6 Hz), 3.83 (2H, t, *J* = 6.0 Hz), 3.71 – 3.55 (2H, m), 3.41 – 3.23 (2H, m), 2.12 – 1.85 (4H, m), 1.85 – 1.64 (4H, m), 1.29 (6H, d, *J* = 6.6 Hz).	346.2344 (-1.7)
14d	95	3.13	B	Hydrochloride	^{1}H NMR (300 MHz, CD_3OD) δ 8.67 (1H, s), 8.60 (1H, s), 4.95 (2H, t, *J* = 6.0 Hz), 3.83 (2H, t, *J* = 6.0 Hz), 3.63 (2H, ddd, *J* = 2.8, 7.4, 13.5 Hz), 3.42 – 3.30 (4H, m), 2.14 – 1.84 (4H, m), 1.84 – 1.69 (4H, m), 1.62 (2H, p, *J* = 7.0 Hz), 1.45 (2H, h, *J* = 7.1 Hz), 0.99 (3H, t, *J* = 7.3 Hz).	360.2506 (-0.2)
14e	98	2.63	B	Hydrochloride	^{1}H NMR (400 MHz, CD_3OD) δ 8.67 (1H, s), 8.58 (1H, s), 4.94 (2H, t, *J* = 6.0 Hz), 3.83 (2H, t, *J* = 6.0 Hz), 3.62 (2H, ddd, *J* = 2.6, 7.7, 13.5 Hz), 3.38 – 3.30 (2H, m), 2.78 (1H, tt, *J* = 3.8, 7.2 Hz), 2.06 – 1.82 (4H, m), 1.82 – 1.68 (4H, m), 0.91 – 0.80 (2H, m), 0.70 – 0.61 (2H, m).	344.2193 (-0.24)
14f	99	3.83	A	Hydrochloride	^{1}H NMR (300 MHz, CD_3OD) δ 8.67 (1H, s), 8.61 (1H, s), 4.95 (2H, t, *J* = 5.9 Hz), 4.20 (1H, p, *J* = 6.3 Hz), 3.83 (2H, t, *J* = 6.0 Hz), 3.63 (2H, ddd, *J* = 2.3, 7.3, 13.4 Hz), 3.39 – 3.31 (2H, m), 2.14 – 1.85 (6H, m), 1.85 – 1.46 (10H, m).	372.2505 (0.37)
14g	95	4.12	A	Hydrochloride	^{1}H NMR (400 MHz, CD_3OD) δ 8.66 (1H, s), 8.58 (1H, s), 4.94 (2H, t, *J* = 6.0 Hz), 3.83 (2H, t, *J* = 6.0 Hz), 3.79 – 3.70 (1H, m), 3.62 (2H, ddd, *J* = 2.5, 7.6, 13.4 Hz), 3.38 – 3.29 (2H, m), 2.09 – 1.84 (6H, m), 1.84 – 1.70 (6H, m), 1.70 – 1.61 (1H, m), 1.52 – 1.26 (5H, m).	386.2565 (1.84)

14h	95	3.93	A	Hydrochloride	^{1}H NMR (300 MHz, DMSO) δ 10.53 (1H, s), 10.26 (1H, s), 8.98 (1H, s), 8.61 (1H, s), 8.58 (1H, s), 4.82 (2H, t, *J* = 6.5 Hz), 3.86 – 3.62 (2H, m), 3.52 – 3.36 (2H, m), 3.27 – 3.07 (2H, m), 1.90 – 1.71 (4H, m), 1.71 – 1.47 (4H, m), 1.38 (9H, s).	360.2507 (0.13)
15a	100	3.15	B	Base	^{1}H NMR (300 MHz, CD_3OD) δ 8.58 (1H, s), 8.43 (1H, s), 4.57 (2H, t, *J* = 6.6 Hz), 3.77 (2H, q, *J* = 7.3 Hz), 3.10 (2H, t, *J* = 6.6 Hz), 2.75 (4H, t, *J* = 5.3 Hz), 1.71 – 1.45 (8H, m), 1.34 (3H, t, *J* = 7.3 Hz).	348.1963 (-0.5)
15b	100	3.45	B	Base	^{1}H NMR (300 MHz, $CDCl_3$) δ 11.82 (1H, s), 8.54 (1H, s), 8.35 (1H, s), 8.05 (1H, s), 4.71 – 4.40 (2H, m), 3.74 (2H, q, *J* = 6.6 Hz), 3.23 – 2.95 (2H, m), 2.79 – 2.53 (4H, m), 1.81 (2H, h, *J* = 7.4 Hz), 1.70 – 1.40 (8H, m), 1.07 (3H, t, *J* = 7.4 Hz).	362.2100 (-0.49)
15c	100	3.70	B	Maleate	^{1}H NMR (400MHz, CD_3OD, 400 MHz) δ 8.62 (1H, s), 8.52 (1H, s), 6.22 (2H, s), 4.88 (2H, t, *J*=5.9 Hz), 3.79 (2H, t, *J*=5.9 Hz), 3.75 (2H, t, *J*=7.1 Hz), 3.49 – 3.44 (4H, m), 1.95 – 1.90 (4H, m), 1.80 – 1.68 (6H, m), 1.56 – 1.42 (2H, m), 1.01 (3H, t, *J*=7.4 Hz).	376.2279 (0.18)
15d	100	3.21	B	Hydrochloride	^{1}H NMR (300 MHz, CD_3OD) δ 8.63 (1H, s), 8.54 (1H, s), 4.89 (2H, t, *J* = 6.1 Hz), 3.80 (2H, t, *J* = 5.9 Hz), 3.71 – 3.54 (2H, m), 3.45 – 3.27 (2H, m), 3.22 (1H, tt, *J* = 4.1, 7.5 Hz), 1.04 – 0.90 (2H, m), 0.81 – 0.68 (2H, m).	360.1963 (-0.59)
20	98	3.60	A	Base	^{1}H NMR (300 MHz, CD_3OD) δ 8.69 (1H, s), 8.67 (1H, s), 4.69 (2H, t, *J* = 5.4 Hz), 3.95 (2H, t, *J* = 5.4 Hz), 3.47 (2H, q, *J* = 7.0 Hz), 2.69 (1H, tt, *J* = 3.4, 11.4 Hz), 2.10 – 1.97 (2H, m), 1.97 – 1.83 (2H, m), 1.83 –	318.1921 (0.5)

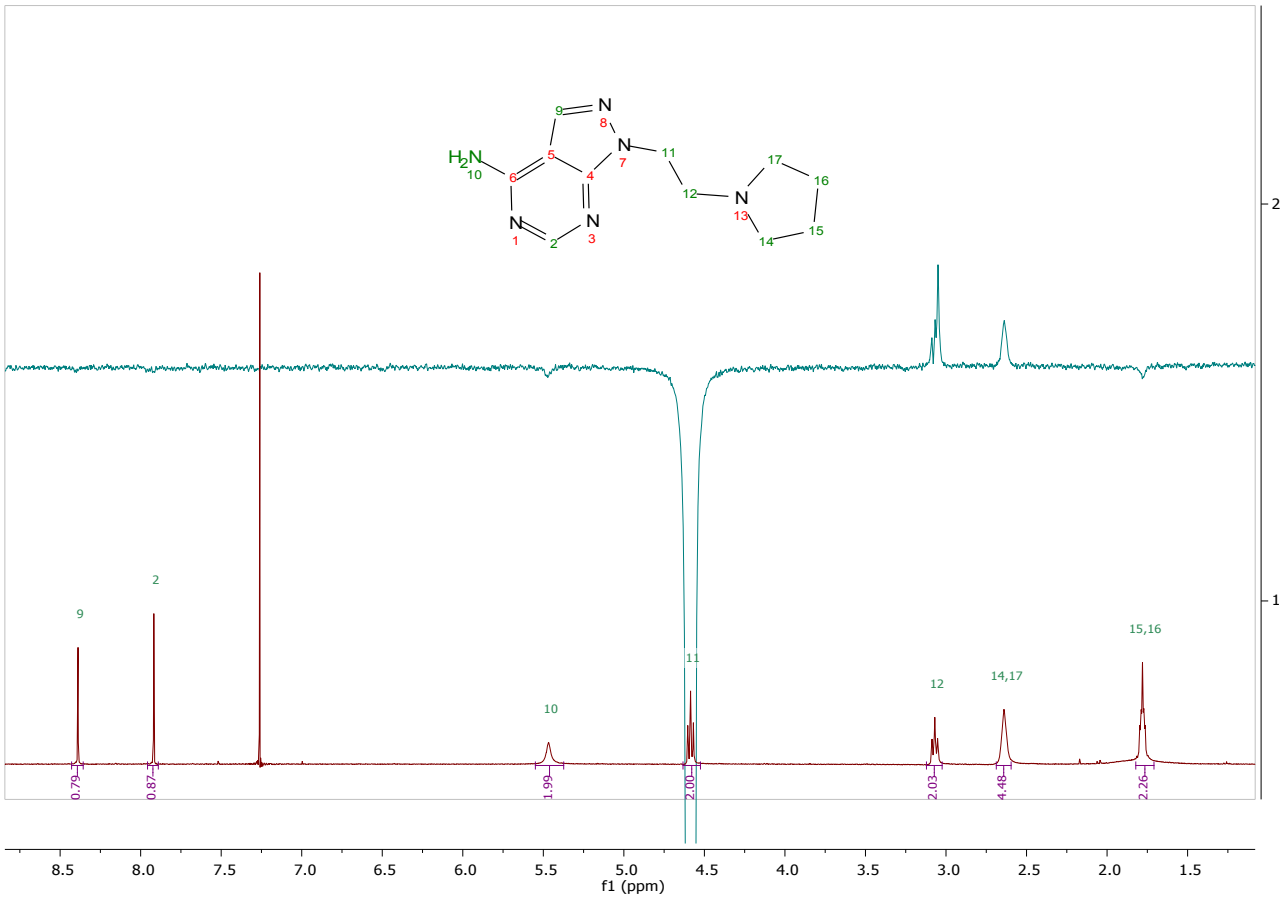
					1.71 (1H, m), 1.69 – 1.51 (2H, m), 1.51 – 1.23 (3H, m), 1.04 (3H, t, *J* = 7.0 Hz).	
21	95	2.00	A	Base	^{1}H NMR (300 MHz, DMSO) δ 8.51 (1H, d, *J* = 1.9 Hz), 8.48 (1H, dd, *J* = 1.4, 4.8 Hz), 8.21 (1H, s), 8.12 (1H, s), 7.73 (2H, s), 7.60 (1H, dt, *J* = 1.7, 7.9 Hz), 7.33 (1H, dd, *J* = 4.9, 7.8 Hz), 5.54 (2H, s).	227.1038 (0.84)

Spectra of Compound 4a

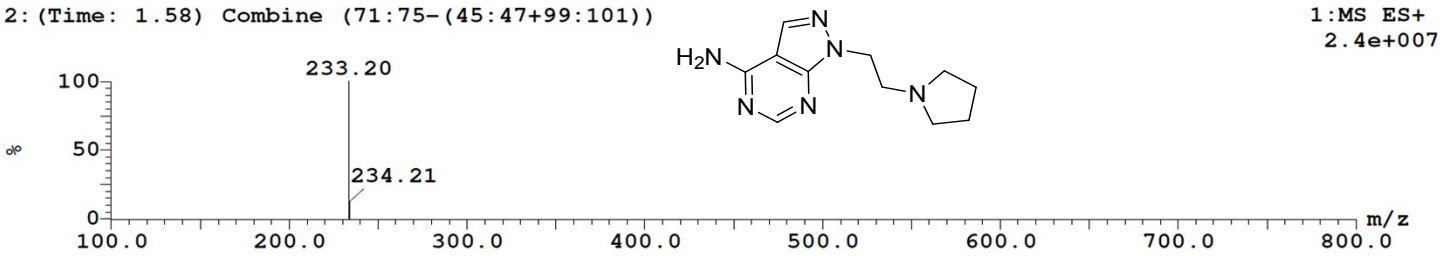
^{1}H-NMR spectrum



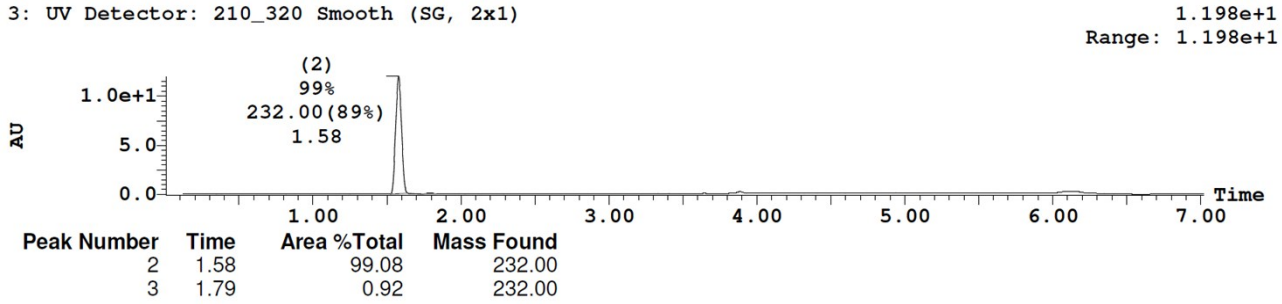
NOE 1D Spectrum



MS Spectrum



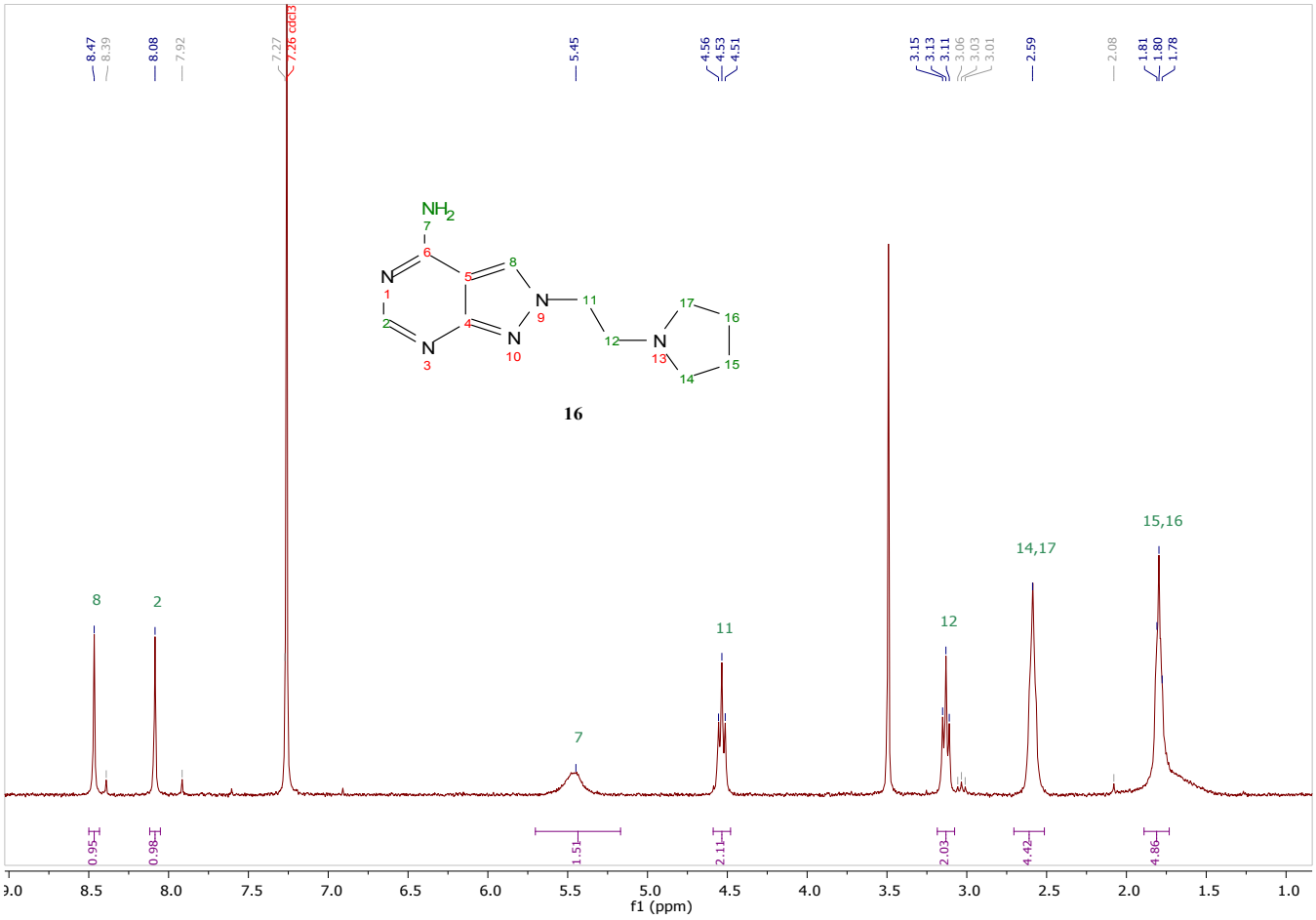
PDA Chromatogram



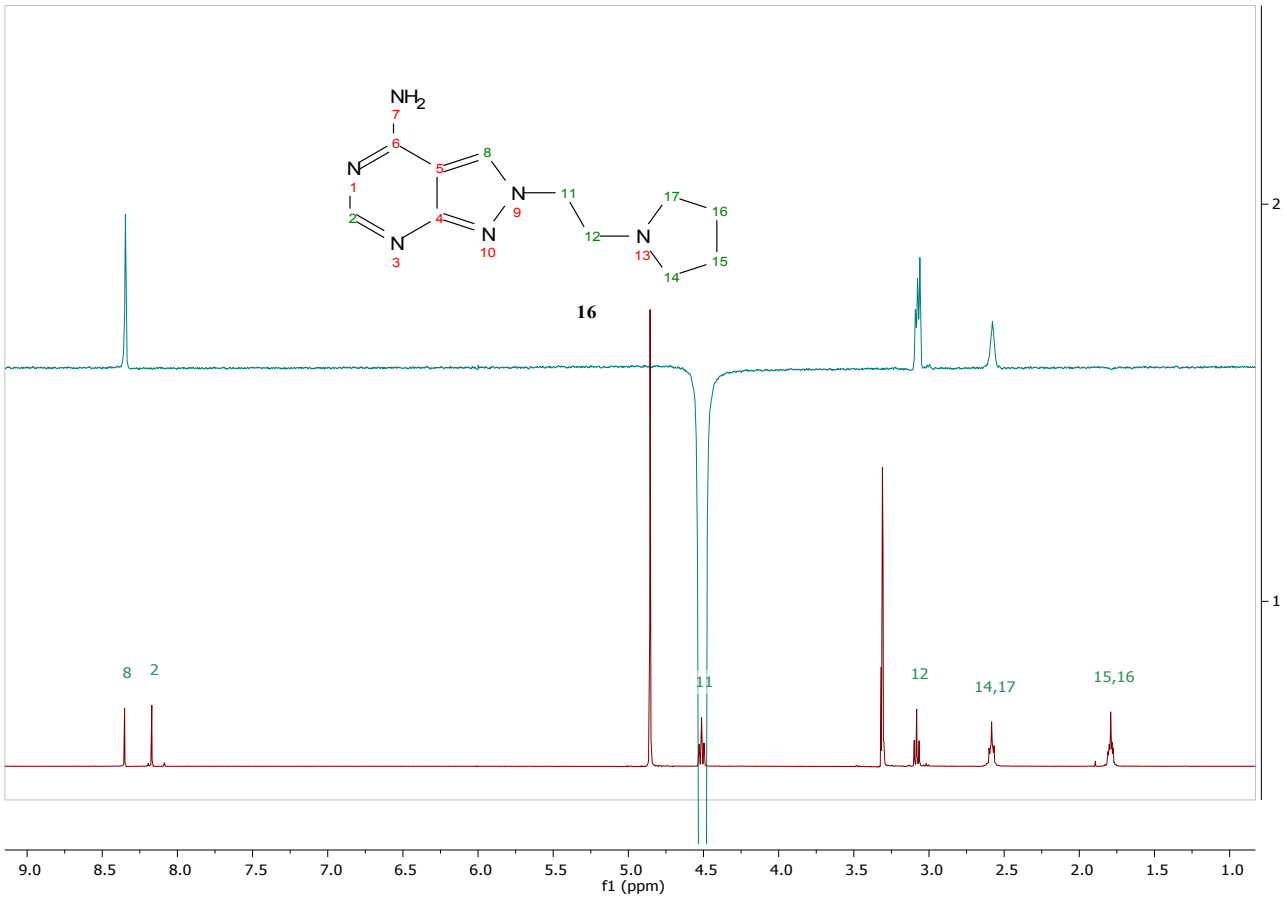
Peak Number	Time	Area %Total	Mass Found
2	1.58	99.08	232.00
3	1.79	0.92	232.00

Spectra of Compound 16

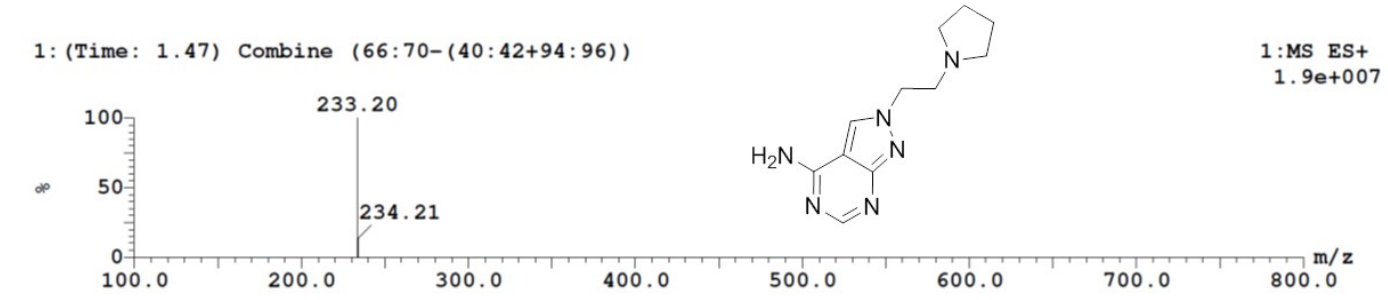
^{1}H-NMR spectrum



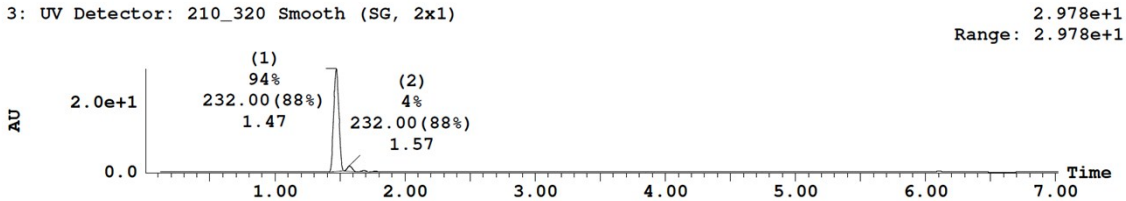
NOE 1D Spectrum



MS Spectrum



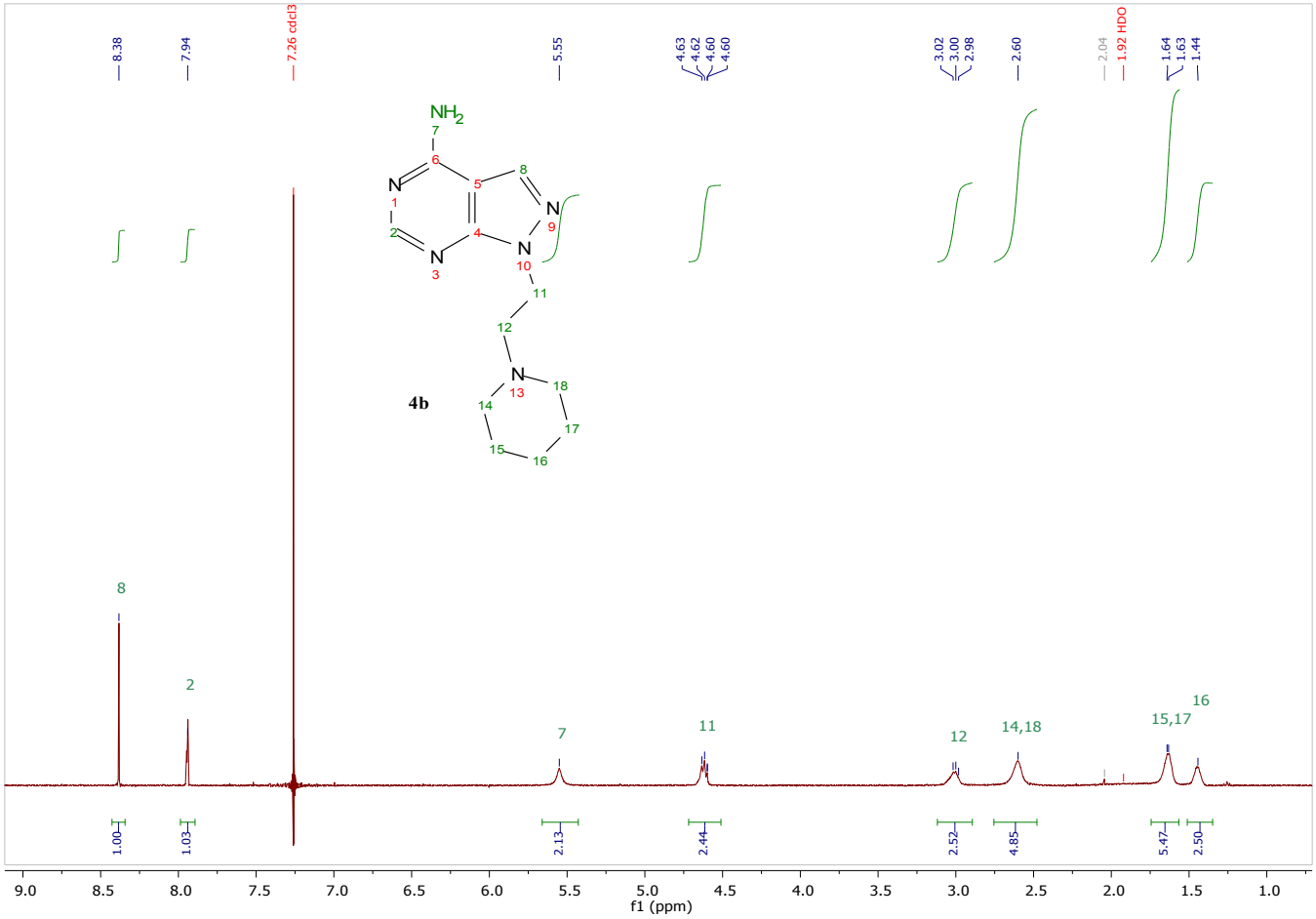
PDA Chromatogram



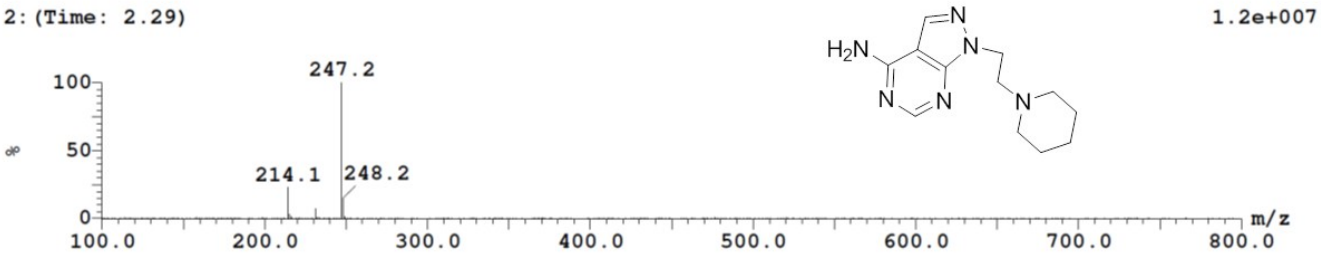
Peak Number	Time	Area %Total	Mass Found
1	1.47	93.63	232.00
2	1.57	4.40	232.00
3	1.68	1.25	232.00
4	1.77	0.71	232.00

Spectra of Compound 4b

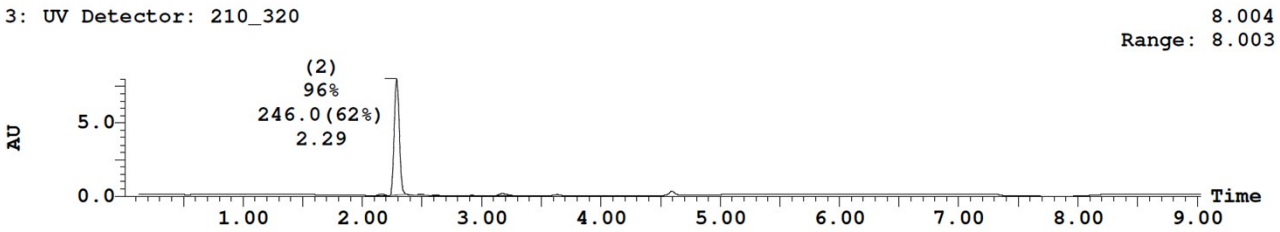
^{1}H-NMR spectrum



MS Spectrum



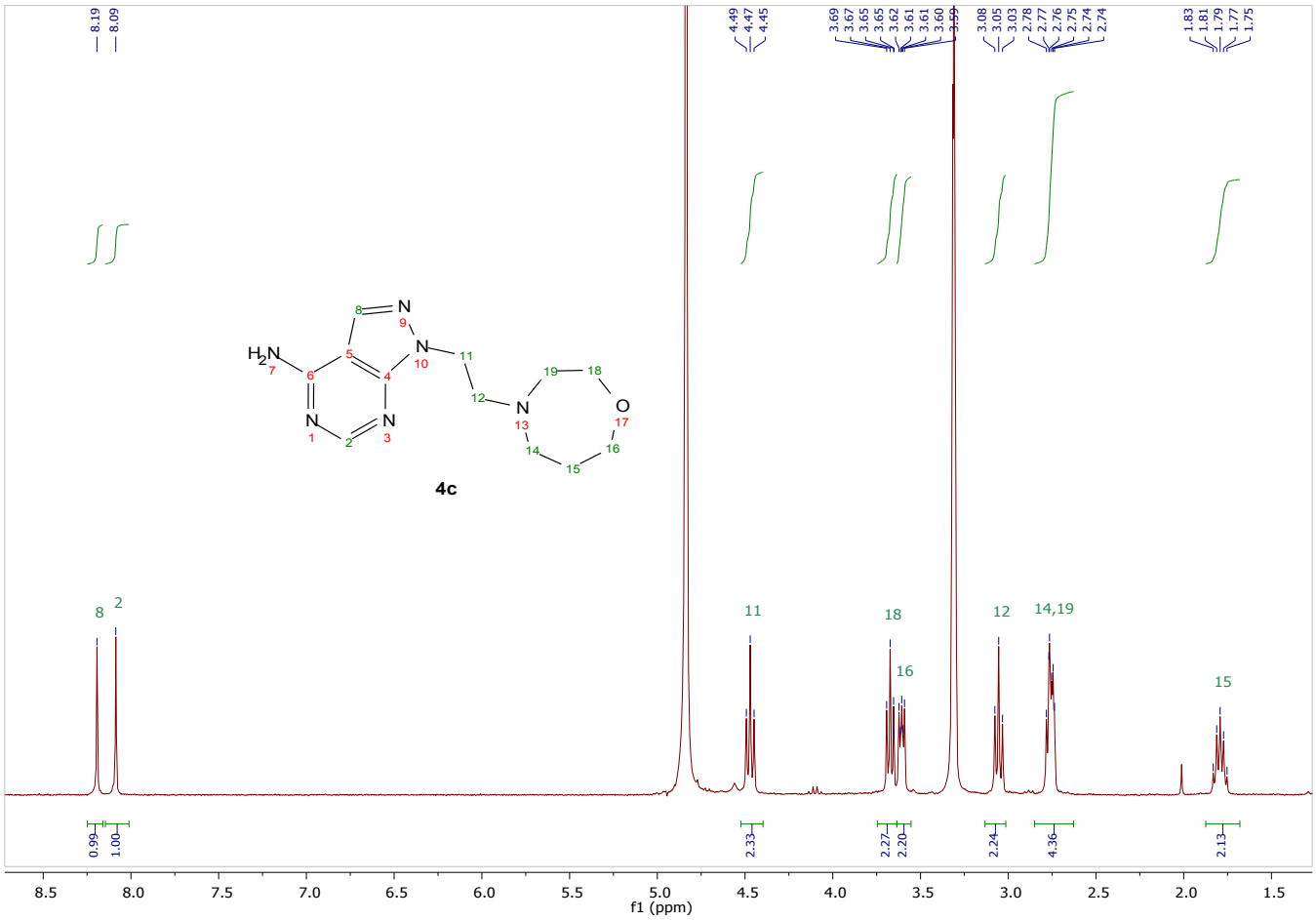
PDA Chromatogram



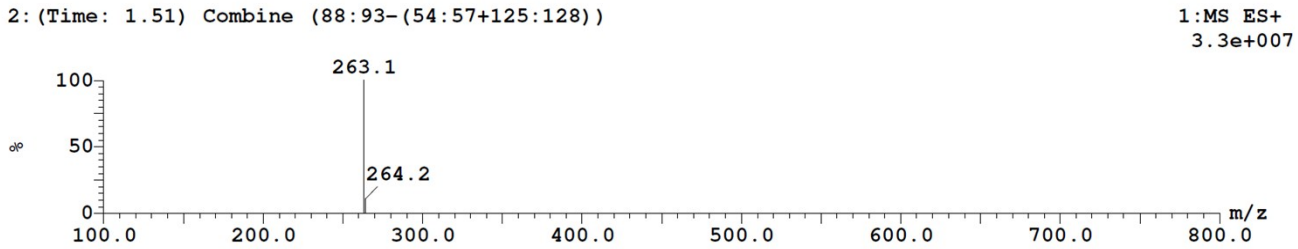
Peak Number	Time	Area %Total	Mass Found
1	2.16	1.12	
2	2.29	95.67	246.00
4	2.49	0.78	246.00
5	3.17	2.43	

Spectra of Compound 4c

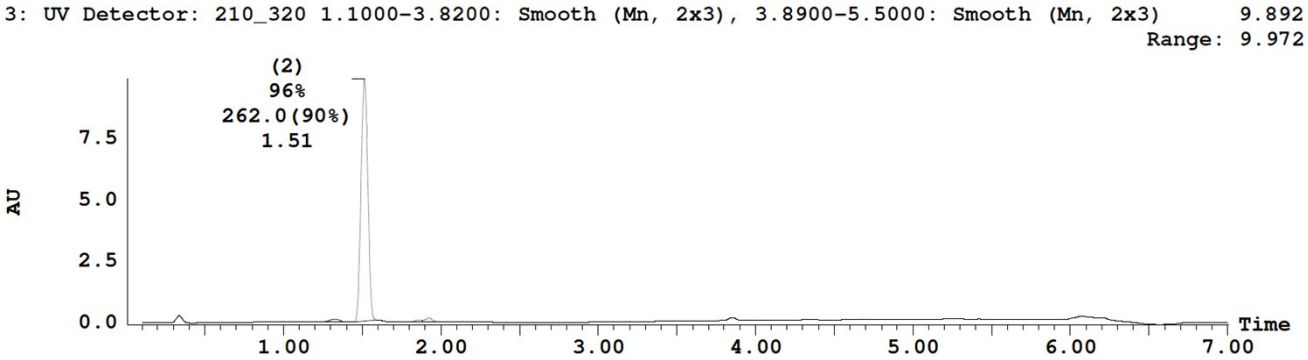
^{1}H-NMR spectrum



MS Spectrum



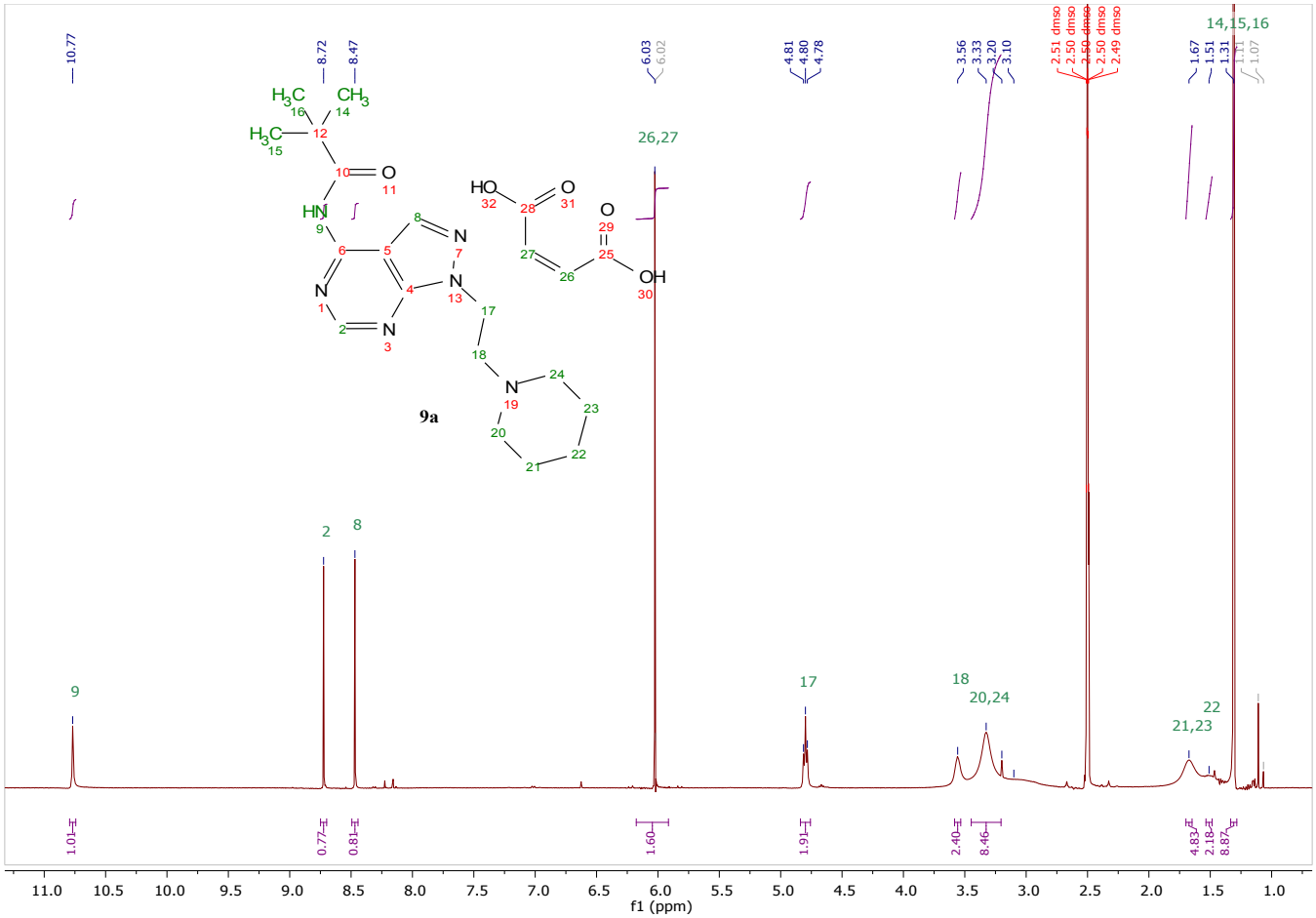
PDA Chromatogram



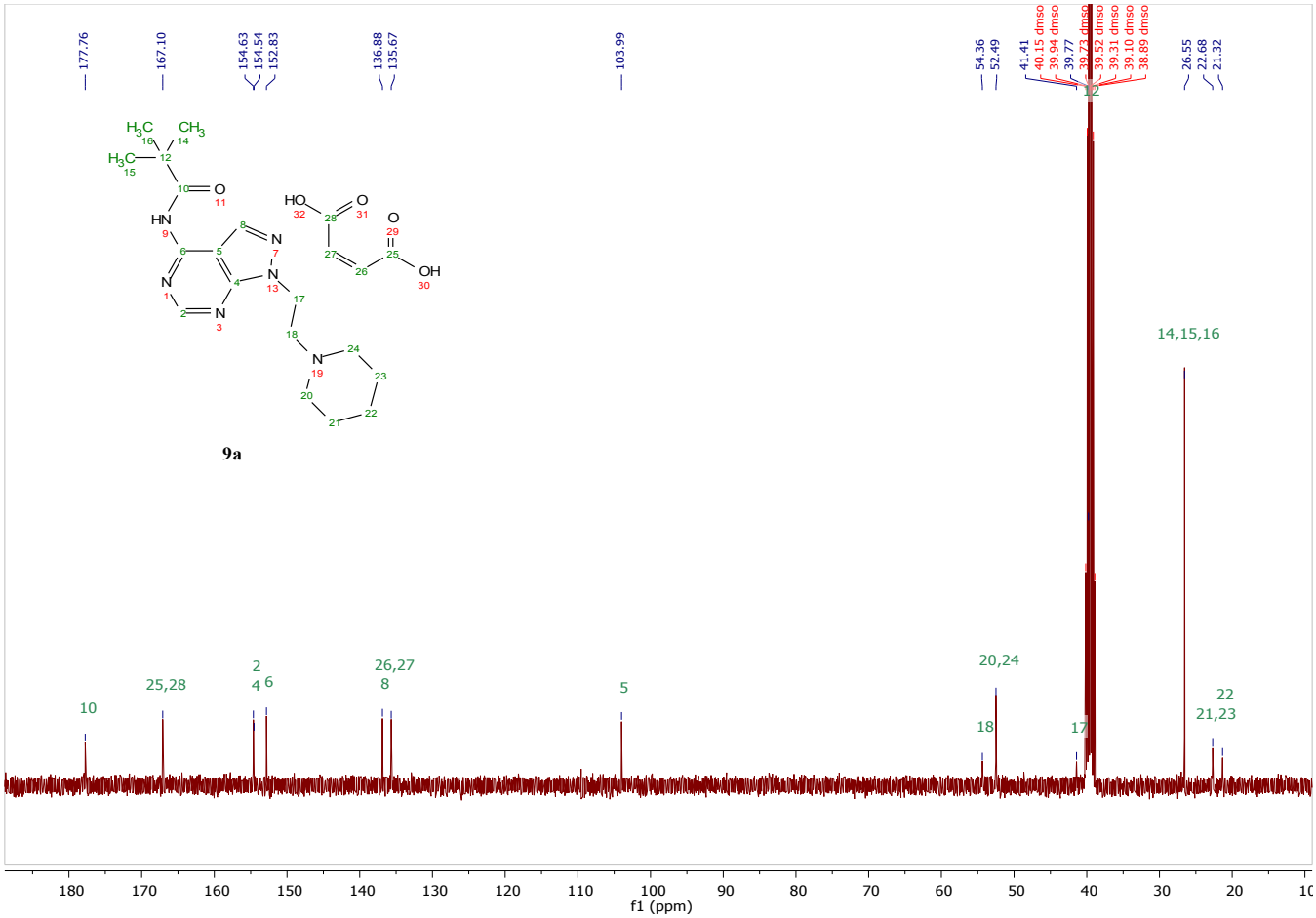
Peak Number	Time	Area %Total	Mass Found
1	1.33	1.93	
2	1.51	95.62	262.00
4	1.86	0.81	262.00
5	1.92	1.64	262.00

Spectra of Compound 9a

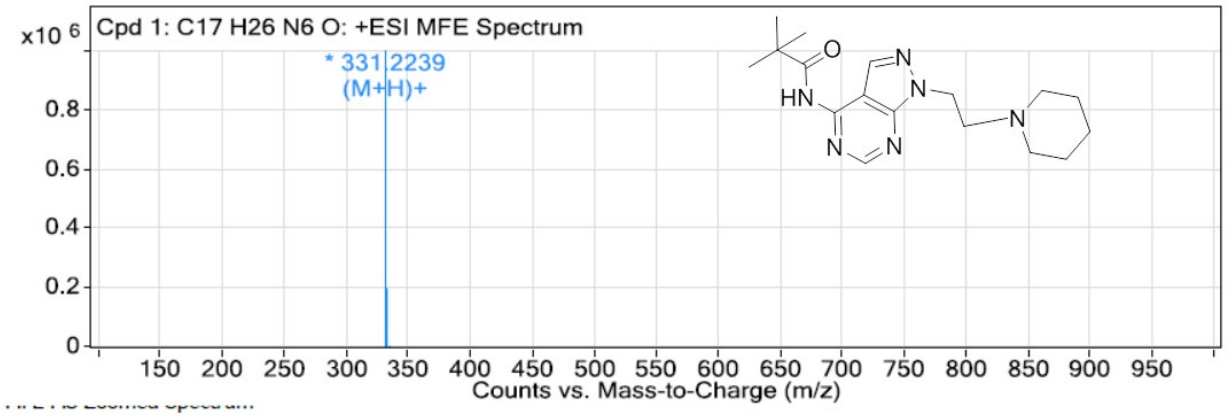
^{1}H-NMR spectrum



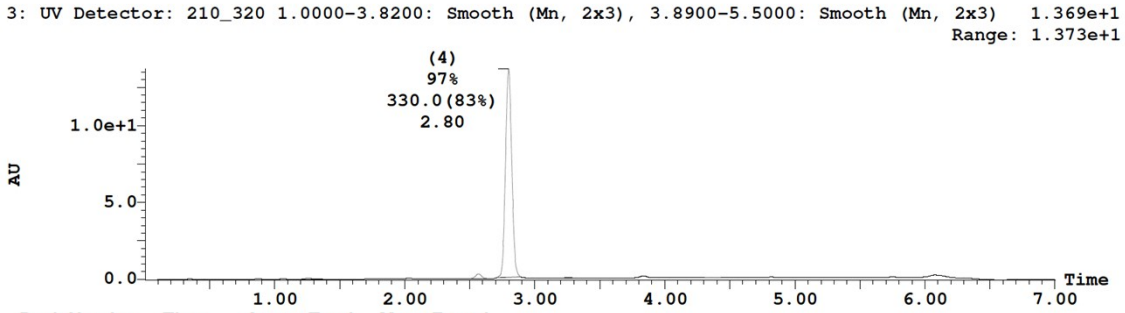
^{13}C-NMR spectrum



HRMS Spectrum



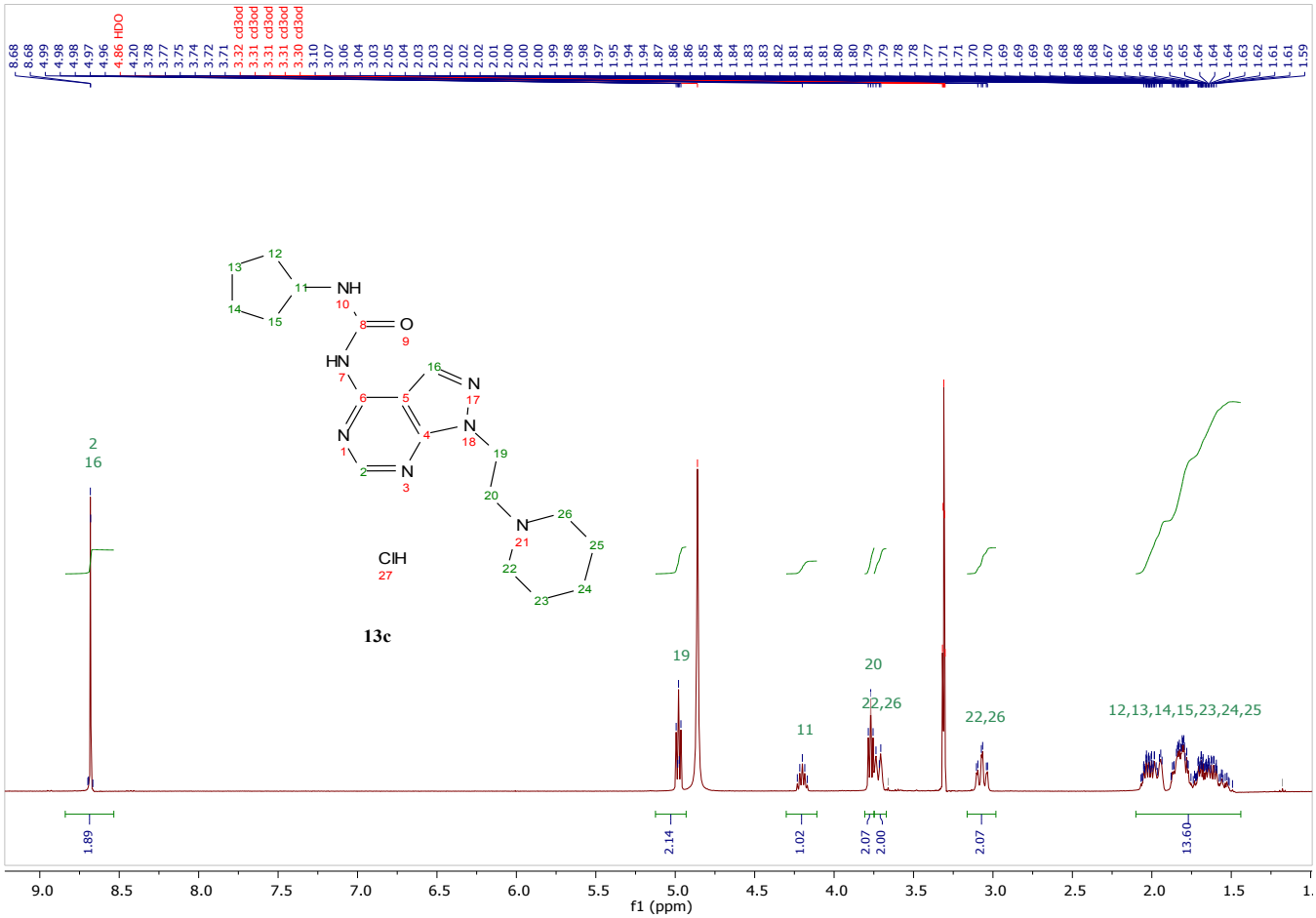
PDA Chromatogram



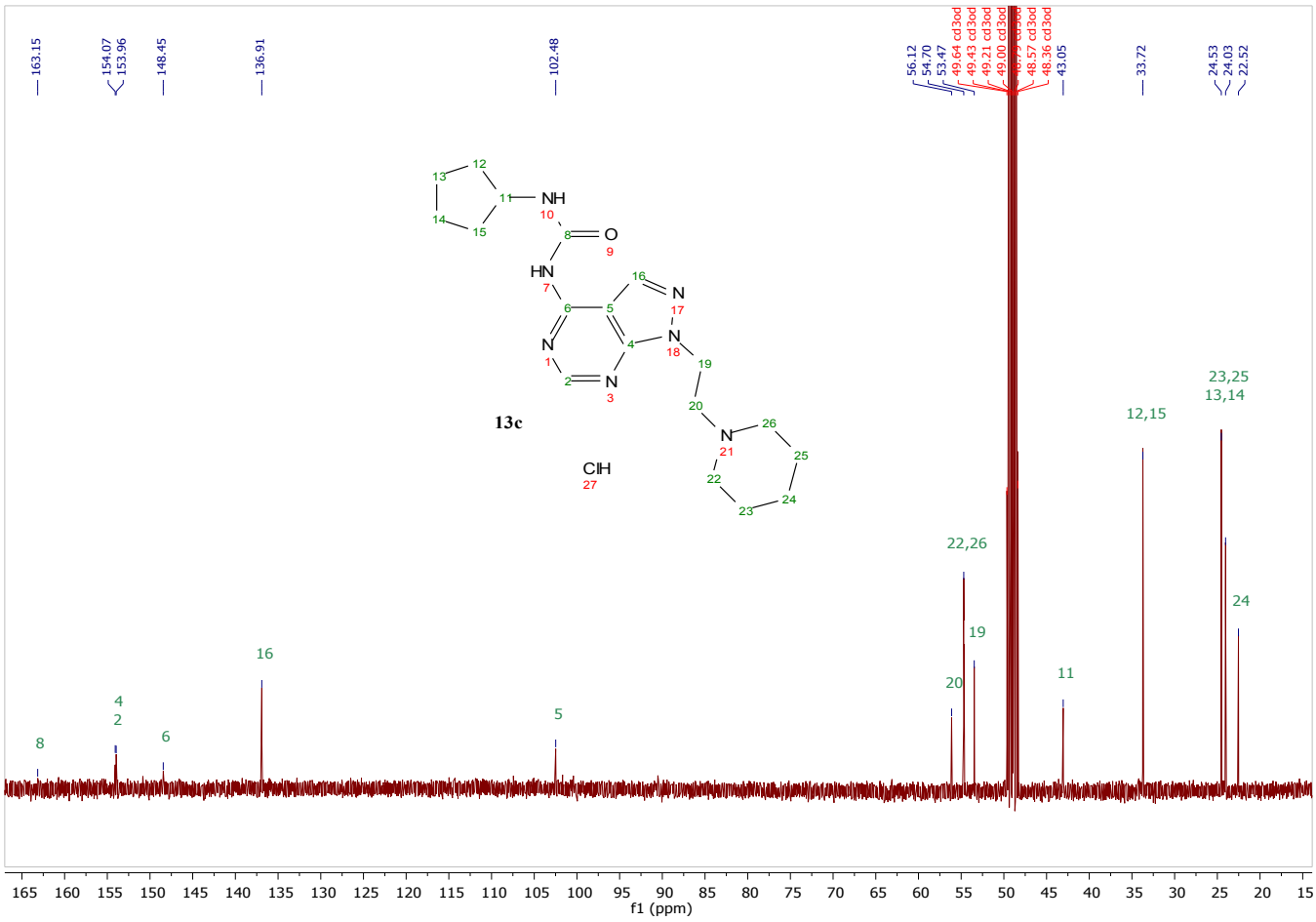
Peak Number	Time	Area %Total	Mass Found
1	1.26	0.50	
2	2.57	2.01	330.00
4	2.80	96.77	330.00
6	3.26	0.42	
7	4.33	0.30	

Spectra of Compound 13c

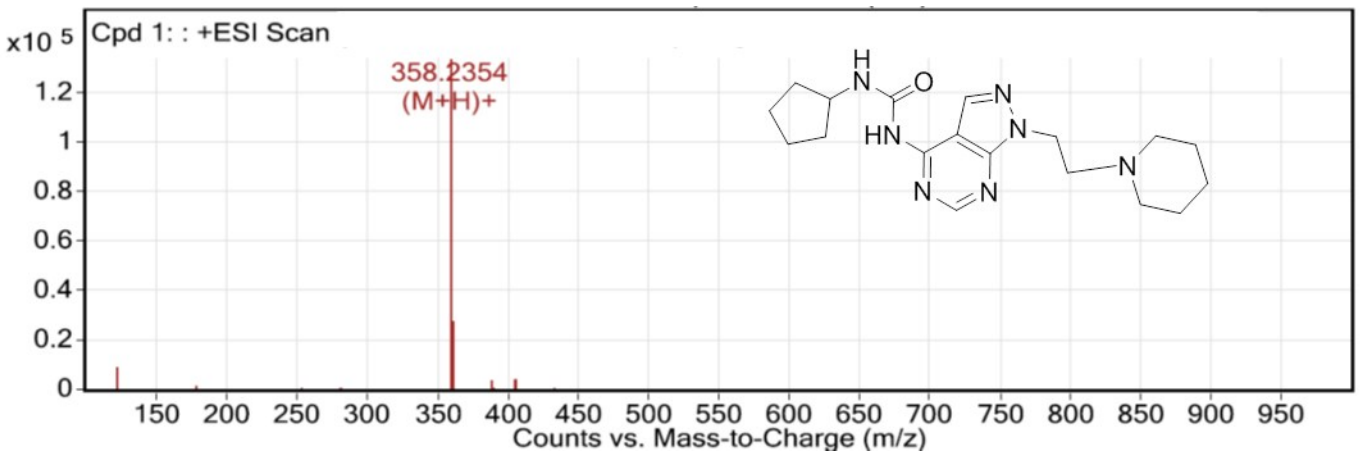
^{1}H-NMR spectrum



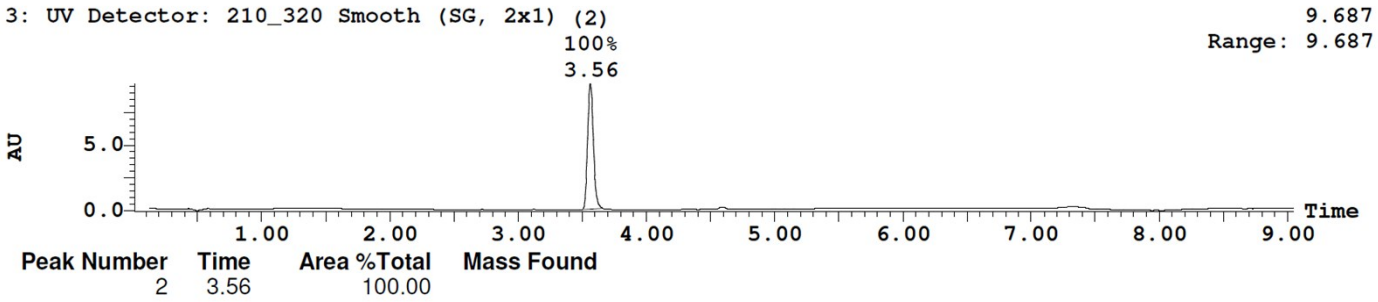
^{13}C-NMR spectrum



HRMS Spectrum



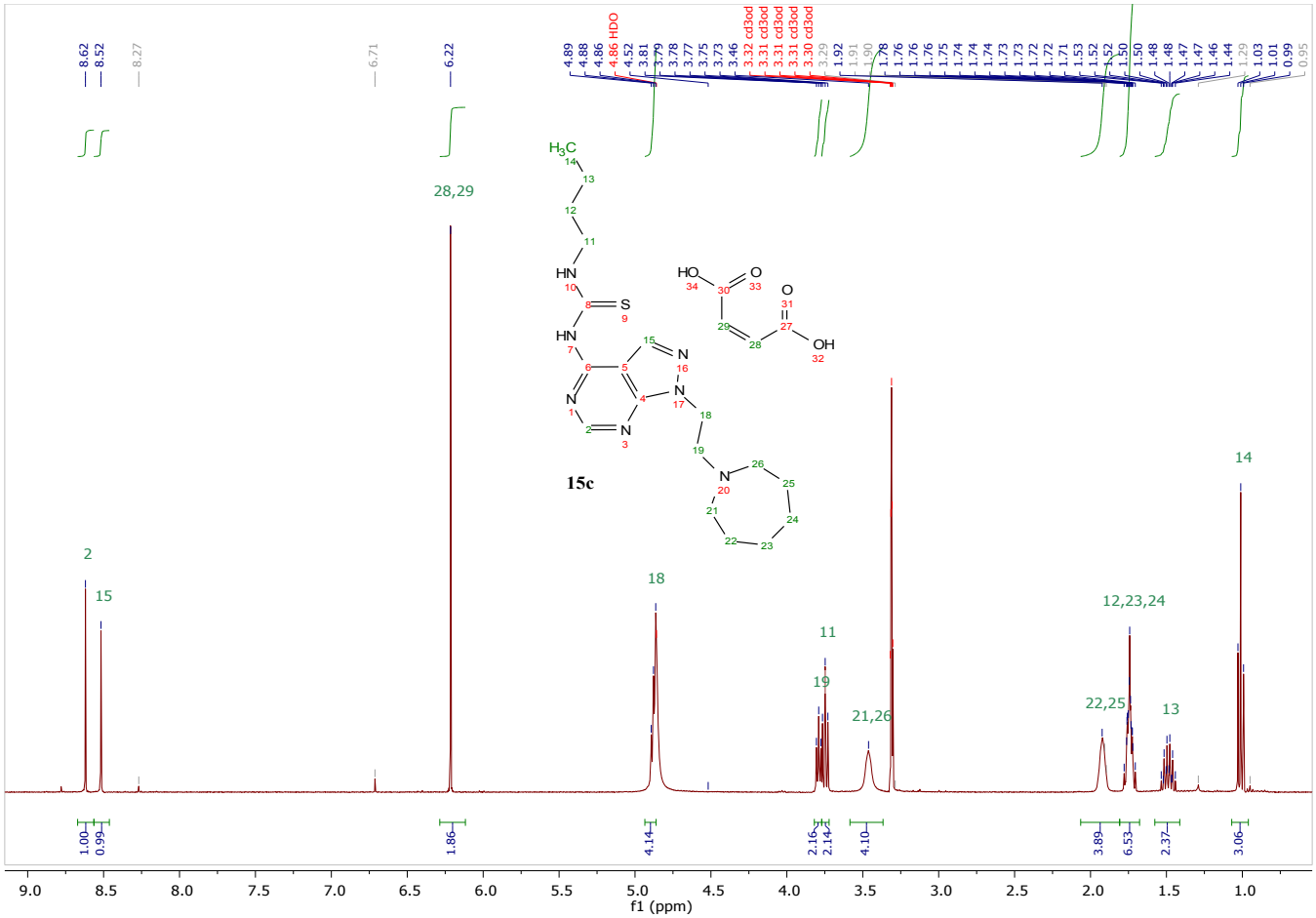
PDA Chromatogram



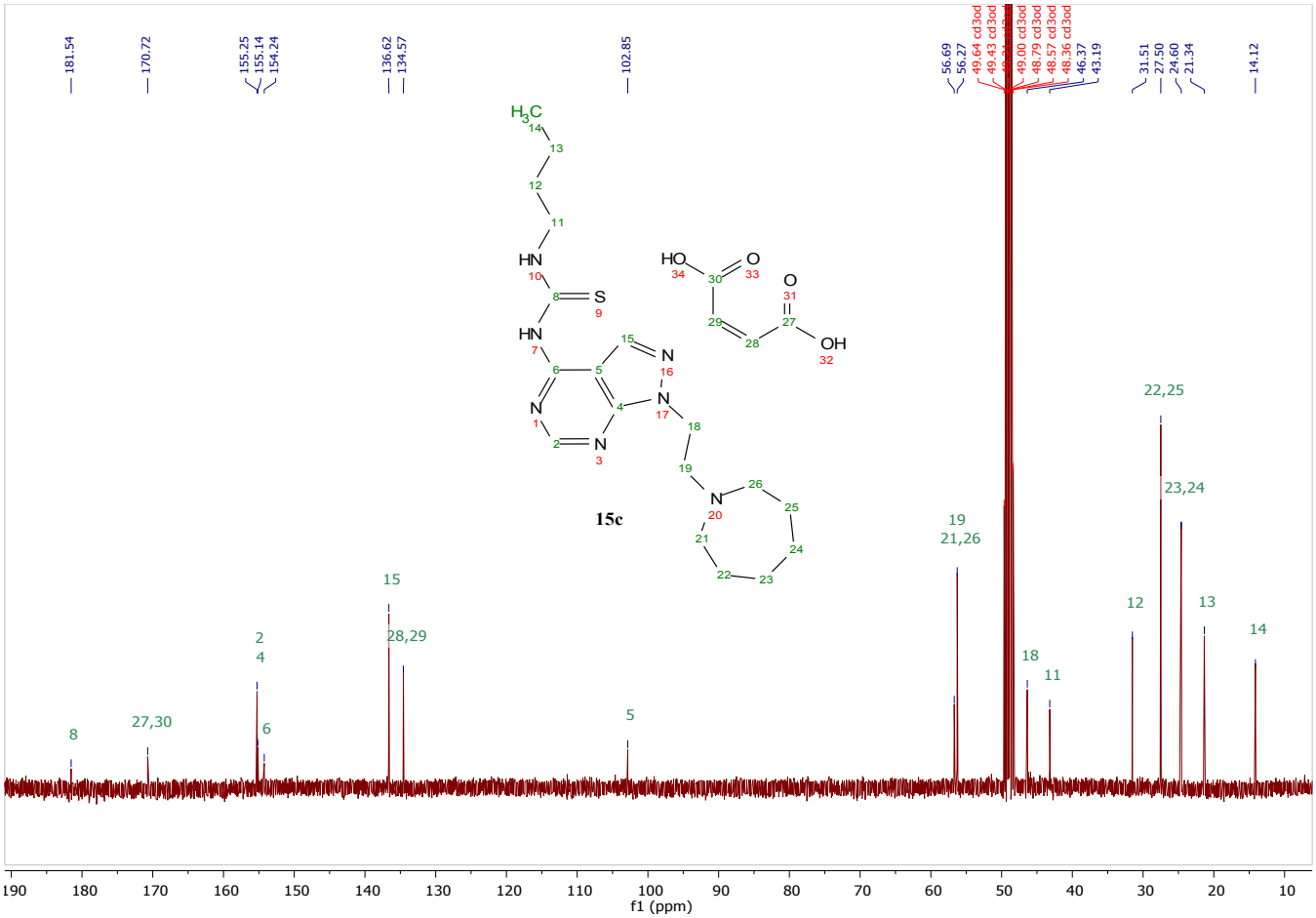
Peak Number	Time	Area %Total	Mass Found
2	3.56	100.00	

Spectra of Compound 15c

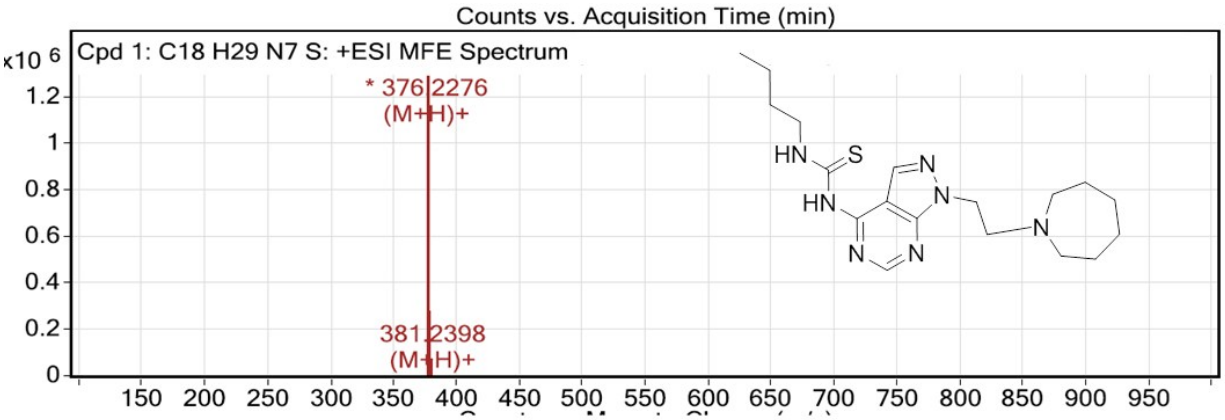
^{1}H-NMR spectrum



^{13}C-NMR spectrum

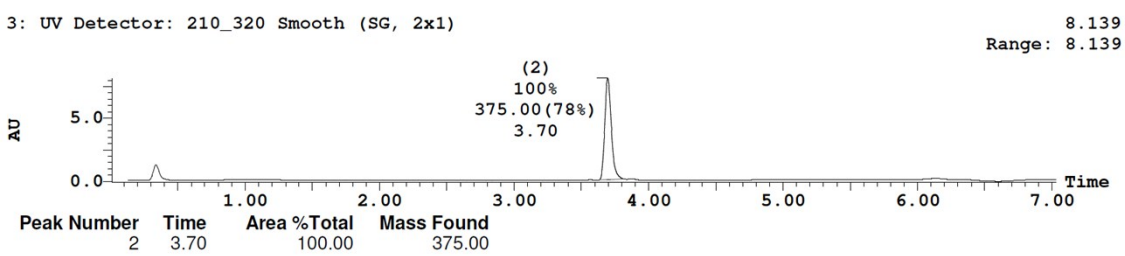


HRMS Spectrum



P

DA Chromatogram



In vitro tests

Human Sigma-1 receptor radioligand assay. The binding properties of the test compounds to human σ_1R, were studied in transfected HEK-293 membranes using [^{3}H]-(+)-pentazocine (Perkin Elmer, NET-1056) as the radioligand. The assay was carried out with 7 μg of membrane suspension, [^{3}H]-(+)-pentazocine (5 nM) in either absence or presence of either buffer or 10 μM Haloperidol for total and non-specific binding, respectively. Binding buffer contained Tris-HCl (50 mM, at pH 8). Plates were incubated at 37 °C for 120 minutes. After the incubation period, the reaction mix was transferred to MultiScreen HTS, FC plates (Millipore), filtered and plates were washed (3 times) with ice-cold Tris–HCl (10 mM, pH 7.4). Filters were dried and counted at approximately 40% efficiency in a MicroBeta scintillation counter (Perkin-Elmer) using EcoScint liquid scintillation cocktail.

Guinea pig Sigma-2 receptor radioligand assay. The binding properties of test compounds to guinea pig σ_2R were studied in guinea pig brain membranes. [^{3}H]-di-*o*-tolylguanidine (DTG) (Perkin Elmer, Code NET-986) was used as the radioligand. The assay was carried out with 200 μg of membrane suspension, [^{3}H]-DTG (10 nM) in either absence or presence of either buffer or 10 μM Haloperidol for total and non-specific binding, respectively. Binding buffer contained Tris-HCl (50 mM, pH 8) and sigma 1 receptor was blocked with (+)-SKF10047 at 400 nM. Plates were incubated at 25 °C for 120 minutes. After the incubation period, the reaction mix was transferred to MultiScreen HTS, FC plates (Millipore), filtered and plates were washed 3 times with ice-cold 50 mM Tris–HCl (pH 7.4). Filters were dried and counted at approximately 40% efficiency in a MicroBeta scintillation counter (Perkin-Elmer) using EcoScint liquid scintillation cocktail.

***In vitro* metabolic stability in human liver microsomes.** The assay was carried out in a robotic liquid handling system (Freedom Evo, Tecan). All incubations were performed individually for each test compound. Compounds (1 μM) were incubated in 96-well plates at 37 ºC during 1 h under standard incubation conditions: sodium-potassium phosphate buffer (50 mM, pH 7.4), $MgCl_2$ (3 mM), the NADPH-regenerating system and CYP content (0.3 nmol/mL). At preset times (0, 10, 20, 40 and 60 min) aliquots of the reaction mixture were stopped with an equal volume of cold acetonitrile. Upon centrifugation of the resultant mixture, supernatants were analyzed by a generic UPLC-MS/MS method.

Metabolic stability was determined by the disappearance of compound over time. Ln-linear plots of the % of compound remaining based on chromatographic peak area *versus* time were plotted, and the slope was calculated by linear fitting of the curve. The *in vitro* metabolic half-life ($t_{1/2}$) was estimated by using the equation 0.693/k where k is the biotransformation rate constant and corresponds to the slope of the ln-linear curve. The microsomal intrinsic clearance (Cl_{int}) was calculated using the equation: Cl_{int} = 0.693/[$t_{1/2}$ x (mg of microsomal protein/volume of incubation)].

hERG inhibition. Automated patch clamp electrophysiology studies using QPatch. CHO cells stably expressing hERG channels (Millipore) were cultured in F12 HAM medium supplemented with 10% FBS and 400µg/L Geneticin. The extracellular Ringer's solution consisted of (in mM): 2 CaCl2, 1 MgCl2, 10 HEPES, 4 KCl, 145 NaCl, 10 glucose, pH 7.4, 305 mOsm. The intracellular Ringer's solution consisted of (in mM): 5.37 CaCl2, 1.75 MgCl2, 31.25/10 KOH/EGTA, 10 HEPES, 210 KCl, pH 7.2, 295 mOsm. 4 mM Na2-ATP was added to intracellular Ringer's solution shortly before use. Whole-cell currents were measured with a QPatch system (Sophion) in response to continuously executed voltage protocols as per manufacturer's recommendations. Upon onset of the voltage protocol, cells were maintained at a holding potential (Vh) of -80 mV, then clamped briefly to -50 mV (20 ms), subsequently depolarized to 20 mV for 4800 ms, and finally re-polarized to -50 mV for 5000 ms, at which potential the peak outward tail current was measured. Finally, the voltage returned to Vh for 3100 ms. Thus, voltage protocols were repeated each 15 seconds. For each cell, extracellular solution was applied previous to increasing concentrations of the tested compound.

In vivo tests. *Animals*. CD1 mice (Charles River, France) from 6 to 8 weeks old were used. Male mice were used for the formalin test and partial sciatic nerve ligation model and female mice for the capsaicin test. Animals had access to food and water *ad libitum* and were kept in controlled laboratory conditions with the temperature at 21 ± 1 °C and a light-dark cycle of 12 h. Experimental behavioural testing was carried out in a soundproof and air-regulated experimental rooms and was done blind with respect to treatment. Experimental procedures and animal husbandry were conducted according to European guidelines regarding protection of animals used for experimental and other scientific purposes (Council Directive of Nov 24, 1986, 86/609/ECC) and received approval by the local Ethical Committee.

Formalin test. A diluted 2.5 % formalin solution (20 µL, 0.92% formaldehyde) was injected into the midplantar surface of the right hind paw of the mouse. Formalin- induced nociceptive behavior was quantified as the time spent licking or biting the injected paw during two different periods individually recorded: the first period was recorded 0−5 min after formalin injection and was considered indicative of phase I formalin-evoked nociception; the second period was recorded 15−30 min after formalin injection (phase II). Mice (n = 8 per group) received i.p. administration of a 10 mL/kg volume of vehicle 0.5% hydroxypropyl methyl cellulose (HPMC, Sigma-Aldrich) or test compound 30 min before i.pl. formalin injection. Antinociception values werecalculated with the following equation: antinociception (%) = [(LTV – LTD) – LTV] × 100, where LTV and LTD represent the licking-biting time in vehicle- and drug-treated animals, respectively.

Capsaicin test. Capsaicin (8-methyl-N-vanillyl-6-nonamide) was purchased from Sigma-Aldrich and dissolved in 1% DMSO in physiological saline to a concentration of 0.05 µg/µl. Mice were habituated for 2 h in individual test compartments placed on an elevated mesh-bottomed platform with a 0.5 cm^2 grid to provide access to the ventral surface of the hind paws. Then, animals were given an i.pl. capsaicin injection (1 µg in 20 µL of 1% DMSO) into the midplantar surface of the right hind paw. Fifteen minutes after the administration, mechanical stimulation was applied onto the plantar surface of the right hind paw. A nonflexible filament (0.5 mm diameter) was electronically (dynamic plantar

aesthesiometer, Ugo Basile, Italy) driven into the ventral side of the paw previously injected, at least 5 mm away from the injection site. The automated device exerts a constant upward pressure of 0.5 g (4.90 mN) onto the plantar surface. When a paw withdrawal response occurred, the stimulus was automatically terminated and the response latency time was automatically recorded. A cut-off time of 50 s was used. In all experiments, the filament was applied three times, separated by intervals of 0.5 min, and the mean value was considered the withdrawal latency time. Mice (n = 8−24 per group) received vehicle 0.5% HPMC or test compound via i.p. in a volume of 10 mL/kg, 30 min before capsaicin injection, and withdrawal latencies to mechanical stimulation were determined 15 min after capsaicin injection(45 min after the treatment). The effect of the treatments was calculated with the following equation: reduction of mechanical hypersensitivity (%) = [(LTD − LTV)/(CT − LTV)] × 100, where LTD and LTV are the latency times in drug- and vehicle-treated animals, respectively, and CT is the cut-off time (50 s).

Partial sciatic nerve ligation model. Mice were anaesthetized with isoflurane (induction: 3%; surgery: 1%) and the common sciatic nerve was exposed at the level of the mid-thigh of the right paw. At about 1 cm, proximally to the nerve trifurcation, tight ligation was created around 33-50% of the sciatic nerve, leaving the rest of the nerve "uninjured". Care was taken to ensure that the ligation was not too tight so as to occlude the perineural blood flow. The muscle was then stitched, and the incision was closed with wound clips. Allodynia to mechanical and thermal (cold) stimuli and thermal (heat) hyperalgesia were used as outcome measures of neuropathic pain by using von Frey test, cold plate test and plantar test, respectively (as described below). Animals were first habituated for 1 h to experimental test once daily for 4 days. After the habituation period, baseline responses were established during 2 consecutive days. One day after baseline measurements, sciatic nerve injury was induced and mice were tested on days 5 and 10 after the surgical procedure to monitor the development of neuropathic pain-related behaviour. On day 10, neuropathic pain-related behaviour was already apparent and mice received a vehicle injection. On days 11, 12, and 13, mice received i.p. administration of **9a** following a Latin square

design (n = 8). Three tests were performed: von Frey test first (30 min after treatment), plantar test second (45 min after treatment) and cold plate third (60 min after treatment).

Mechanical allodynia was quantified by measuring the hind paw withdrawal response to von Frey filament stimulation. Briefly, animals were placed in a Plexiglas® box (20 cm high, 9 cm diameter) with a wire grid bottom through which the von Frey filaments (bending force range from 0.008 to 2 g) (North Coast Medical, Inc., San Jose CA, USA) was applied by using the updown paradigm as previously described.[36] The filament of 0.4 g was first applied. Then, the strength of the next filament was decreased when animal responded or increased when animal did not respond. This up-down procedure was stopped 4 measures after the first change in animal responding (i.e. from response to no response or from no response to response). The threshold of response was then calculated by using the up-down Excel program generously provided by Basbaum's laboratory (UCSF, San Francisco, CA, USA). Clear paw withdrawal, shaking or licking was considered as a nociceptive-like response. Both ipsilateral and contralateral hind paws were tested.

The effect of the treatments on mechanical hypersensitivity induced by partial sciatic nerve ligation was calculated with the following equation: reduction of mechanical hypersensitivity (%) = [(PD − Pd10)/(Baseline − Pd10)] × 100, where PD is the pressure (grams) required for the threshold of response in the von Frey filament stimulation in drug-treated animals, Pd10 is the pressure on day 10 and Baseline is the measure before surgery.

Thermal (heat) hyperalgesia was assessed with a plantar test apparatus (Ugo Basile), by measuring hind paw withdrawal latency in response to radiant heat. Briefly, mice were placed into compartment enclosures on a glass surface. The heat source was then positioned under the plantar surface of the hind paw and activated with a light beam intensity chosen based on preliminary studies to give baseline latencies from 8 to 9 s in control mice. The digital timer connected to the heat source automatically recorded the response latency for paw withdrawal to the nearest 0.1 s. A cutoff time of 20 s was imposed to prevent tissue damage in the absence of response. The mean withdrawal latencies for the ipsilateral hind paw were determined from the average of three separate trials done at 5 min intervals.

The effect of treatments on thermal (heat) hypersensitivity induced by the operation was calculated with the following equation: reduction of thermal hypersensitivity (%) = [(LTD− LTd10)/(B − LTd10)] × 100, where LTD is the latency time (seconds) in drug-treated animals, LTd10 is the latency time on day 10 and Baseline is the measure before surgery.

Cold allodynia was assessed using a hot-/cold-plate analgesia meter (Columbus, OH, USA). A glass cylinder (19 cm high, 19 cm diameter) was used to keep the mice on the cold surface of the plate, which was maintained at a temperature of 5 ± 0.1 °C. The number of elevations of each hind paw was then recorded for 5 min. A score was calculated by subtracting the number of elevations of the right hind paw (ipsilateral) from left hind paw (contralateral). A positive difference score will indicate development of cold allodynia. The effect of treatments on cold allodynia induced by the operation was calculated with the following equation: reduction of thermal allodynia (%) = [(SD− Sd10)/(SB − Sd10)] × 100, where SD is the calculated score in drug-treated animals, Sd10 is the calculated score on day 10 and SB is the score measure calculated before surgery. Finally, on day 14 after surgery, mice were administered with vehicle and were tested again in the absence of active compound (data not shown).