Fragment based Library Generation for the Discovery of A Peptidomimetic p53-Mdm4 Inhibitor

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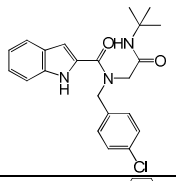
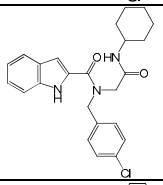
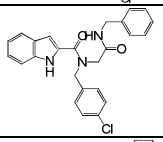
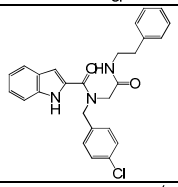
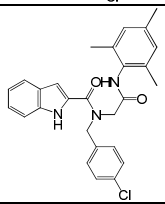
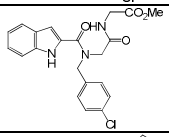
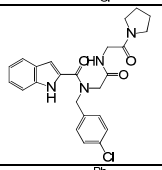
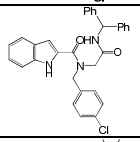
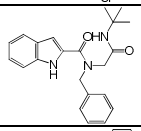
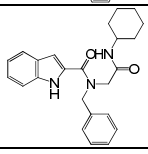
SUPPORTING INFORMATION

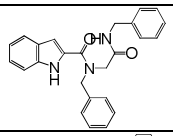
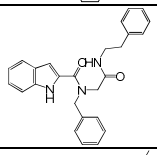
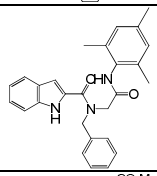
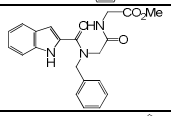
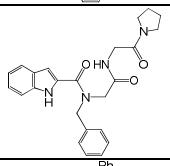
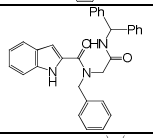
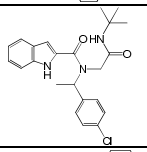
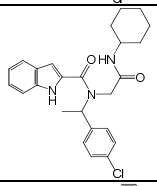
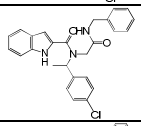
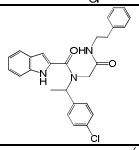
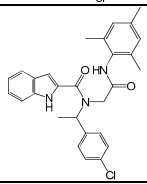
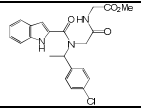
Methods

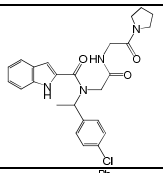
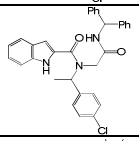
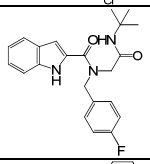
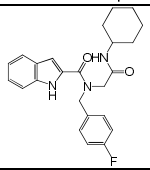
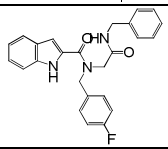
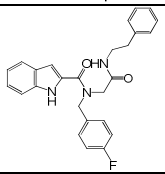
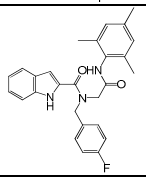
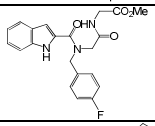
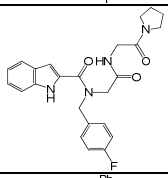
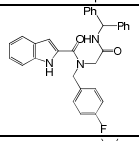
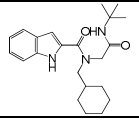
All reagents were purchased from commercial sources and used without further purification. Proton and carbon NMR spectra were determined on Bruker Avance™ 600 MHz NMR spectrometer. Chemical shifts are reported as δ values in parts per million (ppm) as referenced to residual solvent. ^{1}H NMR spectra are tabulated as follows: chemical shift, number of protons,

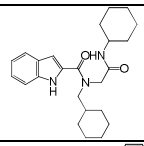
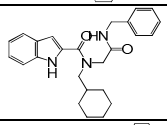
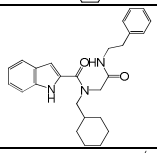
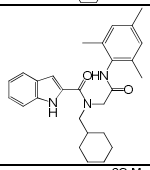
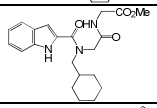
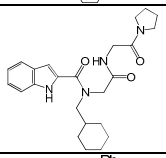
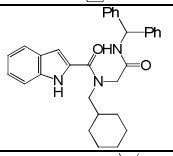
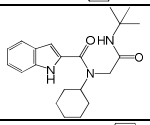
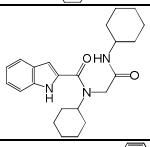
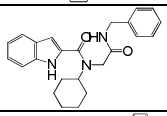
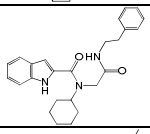
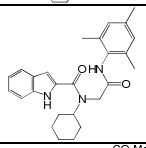
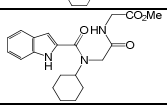
multiplicity (s = singlet, br.s = broad singlet, d = doublet, t = triplet, q = quartet, m = multiplet), and coupling constant. High Resolution Mass spectra were obtained at the University of Pittsburgh Mass Spectrometry facility. LC-MS analysis was performed on an SHIMADZU instrument (reverse-phase HPLC coupled to electrospray ionization-mass spectrometry), using an analytical C18 column (Dionex Acclaim 120 Å, 2.1 × 50 mm, 3.0 μm) coupled to an Applied Biosystems API2000 mass spectrometer (ESI-MS). Acetonitrile/water mixtures were used as the mobile phase for reverse-phase HPLC, the flow rate was maintained at 0.2 mL/min. The column temperature was kept at 40 ^{o}C. The UV detection was at 254 nm.

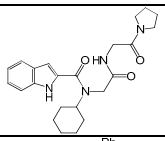
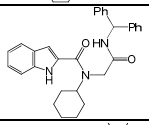
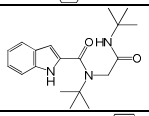
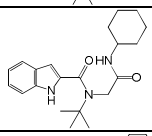
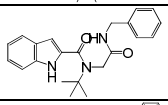
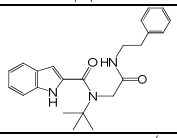
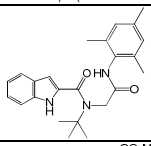
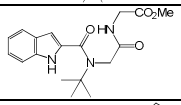
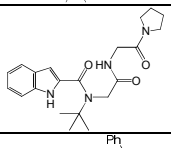
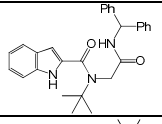
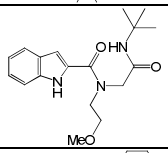
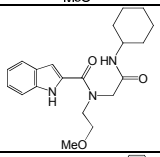
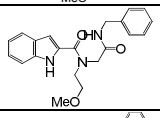
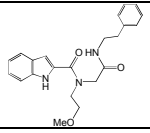
Table S1. Summary

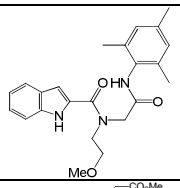
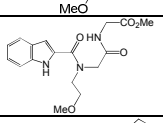
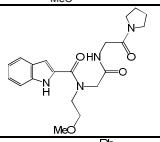
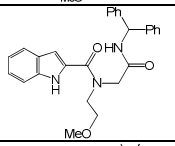
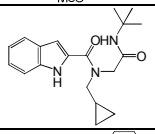
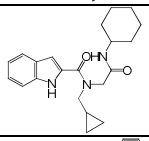
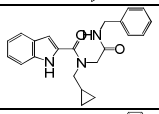
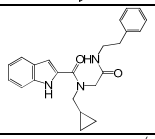
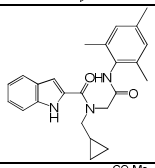
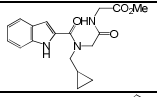
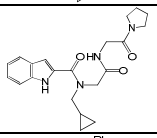
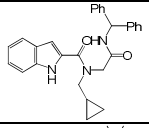
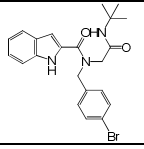
Compound	Structure	Mass (mg)	Yield (%)	LC/MS
A1		22.3	28	t_R = 11.61 min; *m/z* = 398.3 $[M+H]^+$
B1		20.6	24	t_R = 11.61 min; *m/z* = 424.2 $[M+H]^+$
C1		32.4	38	t_R = 11.48 min; *m/z* = 432.3 $[M+H]^+$
D1		15.6	18	t_R = 11.65 min; *m/z* = 446.2 $[M+H]^+$
E1		28.6	31	t_R = 12.01 min; *m/z* = 460.2 $[M+H]^+$
F1		34.7	42	t_R = 10.75 min; *m/z* = 414.3 $[M+H]^+$
G1		30.8	34	t_R = 10.63 min; *m/z* = 453.2 $[M+H]^+$
H1		19.7	19	t_R = 12.18 min; *m/z* = 508.2 $[M+H]^+$
A2		25.4	35	t_R = 11.18 min; *m/z* = 364.4 $[M+H]^+$
B2		30.1	39	t_R = 11.44 min; *m/z* = 390.4 $[M+H]^+$

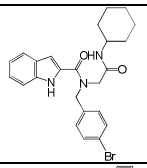
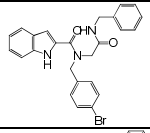
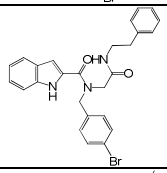
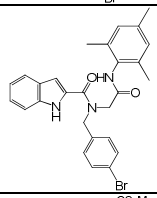
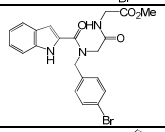
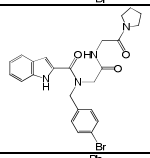
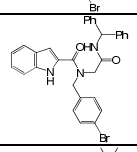
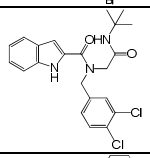
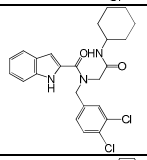
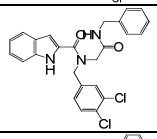
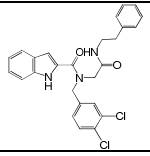
C2		10.5	13	t_R = 11.10 min; *m/z* = 398.4 $[M+H]^+$
D2		12.3	15	t_R = 11.32 min; *m/z* = 412.3 $[M+H]^+$
E2		12.8	15	t_R = 11.66 min; *m/z* = 426.4 $[M+H]^+$
F2		27.6	36	t_R = 10.31 min; *m/z* = 380.2 $[M+H]^+$
G2		34.2	41	t_R = 10.44 min; *m/z* = 419.4 $[M+H]^+$
H2		19.1	20	t_R = 11.87 min; *m/z* = 474.4 $[M+H]^+$
A3		41.6	51	t_R = 11.78 min; *m/z* = 412.2 $[M+H]^+$
B3		23	26	t_R = 11.98 min; *m/z* = 438.2 $[M+H]^+$
C3		5.5	6	t_R = 11.65 min; *m/z* = 446.3 $[M+H]^+$
D3		7.4	8	t_R = 11.82 min; *m/z* = 460.3 $[M+H]^+$
E3		17.2	18	t_R = 12.15 min; *m/z* = 474.3 $[M+H]^+$
F3		30.2	35	t_R = 10.90 min; *m/z* = 428.3 $[M+H]^+$

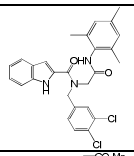
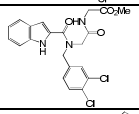
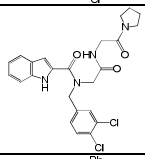
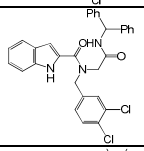
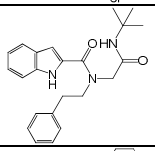
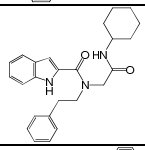
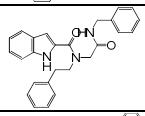
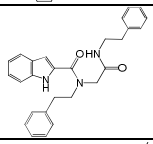
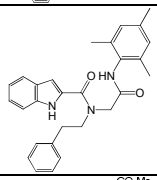
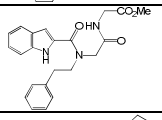
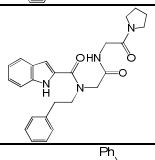
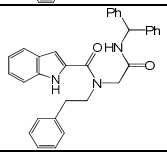
G3		37.7	40	t_R = 10.40 min; *m/z* = 467.4 $[M+H]^+$
H3		10.4	10	t_R = 12.33 min; *m/z* = 522.3 $[M+H]^+$
A4		23.9	31	t_R = 11.24 min; *m/z* = 382.2 $[M+H]^+$
B4		12.4	15	t_R = 11.48 min; *m/z* = 408.4 $[M+H]^+$
C4		21.2	26	t_R = 11.16 min; *m/z* = 416.3 $[M+H]^+$
D4		10.7	12	t_R = 11.38 min; *m/z* = 430.1 $[M+H]^+$
E4		49.2	56	t_R = 11.69 min; *m/z* = 444.4 $[M+H]^+$
F4		30.5	38	t_R = 10.39 min; *m/z* = 398.3 $[M+H]^+$
G4		19	22	t_R = 10.31 min; *m/z* = 437.4 $[M+H]^+$
H4		19.5	20	t_R = 11.88 min; *m/z* = 492.3 $[M+H]^+$
A5		21.1	29	t_R = 11.79 min; *m/z* = 370.5 $[M+H]^+$

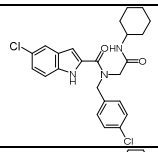
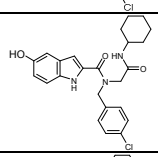
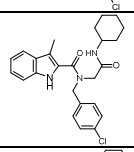
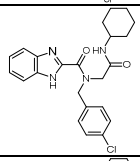
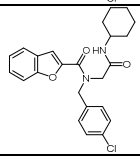
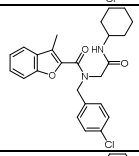
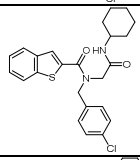
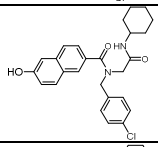
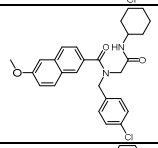
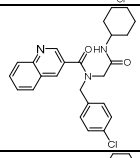
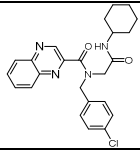
B5		10.6	13	t_R = 11.97 min; *m/z* = 396.5 $[M+H]^+$
C5		18.3	23	t_R = 11.62 min; *m/z* = 404.3 $[M+H]^+$
D5		27.9	33	t_R = 11.84 min; *m/z* = 418.2 $[M+H]^+$
E5		27.4	32	t_R = 12.18 min; *m/z* = 432.4 $[M+H]^+$
F5		17.6	23	t_R = 10.82 min; *m/z* = 386.3 $[M+H]^+$
G5		14.5	17	t_R = 10.68 min; *m/z* = 425.3 $[M+H]^+$
H5		26.3	27	t_R = 12.34 min; *m/z* = 480.4 $[M+H]^+$
A6		26.9	38	t_R = 11.42 min; *m/z* = 356.4 $[M+H]^+$
B6		14.7	19	t_R = 11.63 min; *m/z* = 382.2 $[M+H]^+$
C6		28.8	37	t_R = 11.28 min; *m/z* = 390.3 $[M+H]^+$
D6		32.8	41	t_R = 11.51 min; *m/z* = 404.2 $[M+H]^+$
E6		33	40	t_R = 11.88 min; *m/z* = 418.5 $[M+H]^+$
F6		23.7	32	t_R = 10.43 min; *m/z* = 372.3 $[M+H]^+$

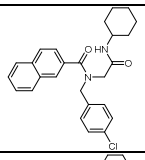
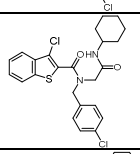
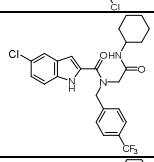
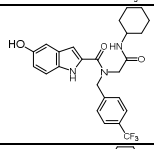
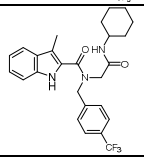
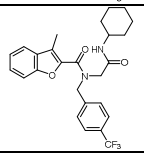
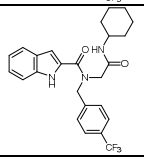
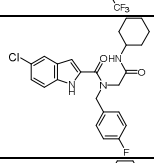
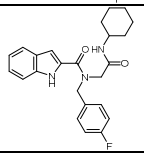
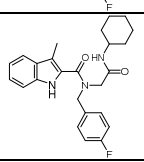
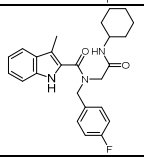
G6		21	26	t_R = 10.34 min; *m/z* = 411.4 $[M+H]^+$
H6		25.1	27	t_R = 12.07 min; *m/z* = 466.5 $[M+H]^+$
A7		7.2	11	t_R = 11.23 min; *m/z* = 330.5 $[M+H]^+$
B7		11.8	17	t_R = 11.44 min; *m/z* = 356.2 $[M+H]^+$
C7		15.7	22	t_R = 10.98 min; *m/z* = 364.3 $[M+H]^+$
D7		14.8	20	t_R = 11.22 min; *m/z* = 378.4 $[M+H]^+$
E7		28.8	37	t_R = 11.58 min; *m/z* = 392.3 $[M+H]^+$
F7		35	51	t_R = 9.95 min; *m/z* = 346.3 $[M+H]^+$
G7		22.1	29	t_R = 9.90 min; *m/z* = 385.3 $[M+H]^+$
H7		16.6	19	t_R = 11.81 min; *m/z* = 440.4 $[M+H]^+$
A8		8.3	13	t_R = 10.30 min; *m/z* = 332.2 $[M+H]^+$
B8		13.5	19	t_R = 10.60 min; *m/z* = 358.5 $[M+H]^+$
C8		9.6	13	t_R = 10.32 min; *m/z* = 366.2 $[M+H]^+$
D8		14.9	20	t_R = 10.56 min; *m/z* = 380.3 $[M+H]^+$

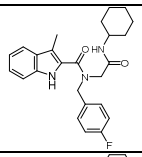
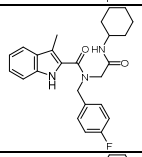
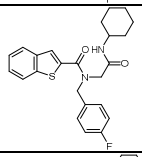
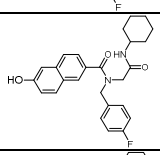
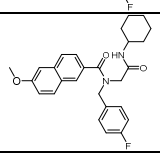
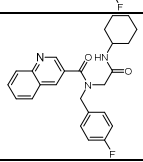
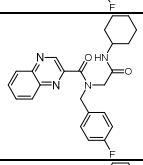
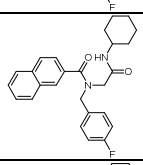
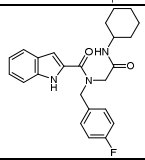
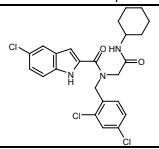
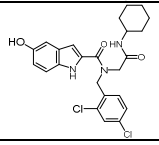
E8		36.5	46	t_R = 10.92 min; *m/z* = 394.3 $[M+H]^+$
F8		30.1	43	t_R = 9.35 min; *m/z* = 448.3 $[M+H]^+$
G8		32.6	42	t_R = 9.28 min; *m/z* = 387.2 $[M+H]^+$
H8		19.1	22	t_R = 11.48 min; *m/z* = 442.3 $[M+H]^+$
A9		21.7	33	t_R = 10.80 min; *m/z* = 328.4 $[M+H]^+$
B9		17.8	25	t_R = 11.03 min; *m/z* = 354.3 $[M+H]^+$
C9		13.8	19	t_R = 10.73 min; *m/z* = 362.3 $[M+H]^+$
D9		9.4	13	t_R = 10.93 min; *m/z* = 376.4 $[M+H]^+$
E9		29.4	38	t_R = 11.32 min; *m/z* = 390.4 $[M+H]^+$
F9		35.2	51	t_R = 9.84 min; *m/z* = 344.5 $[M+H]^+$
G9		25.8	34	t_R = 9.75 min; *m/z* = 383.2 $[M+H]^+$
H9		15.1	17	t_R = 11.58 min; *m/z* = 438.4 $[M+H]^+$
A10		30.2	34	t_R = 11.68 min; *m/z* = 442.1 $[M+H]^+$

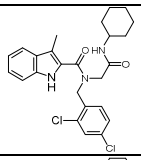
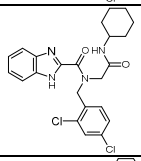
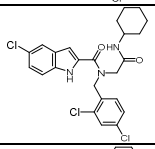
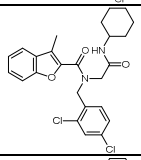
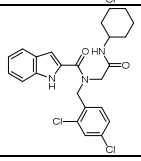
B10		29.1	31	t_R = 11.91 min; *m/z* = 468.3 $[M+H]^+$
C10		44.9	47	t_R = 11.58 min; *m/z* = 476.2 $[M+H]^+$
D10		24.3	25	t_R = 11.76 min; *m/z* = 490.2 $[M+H]^+$
E10		28.7	29	t_R = 12.11 min; *m/z* = 504.3 $[M+H]^+$
F10		28.3	31	t_R = 10.88 min; *m/z* = 458.2 $[M+H]^+$
G10		25.8	26	t_R = 10.73 min; *m/z* = 497.1 $[M+H]^+$
H10		17.9	16	t_R = 12.27 min; *m/z* = 552.3 $[M+H]^+$
A11		12.1	14	t_R = 11.93 min; *m/z* = 432.0 $[M+H]^+$
B11		51.4	56	t_R = 12.13 min; *m/z* = 458.4 $[M+H]^+$
C11		17.8	19	t_R = 11.80 min; *m/z* = 466.3 $[M+H]^+$
D11		22	23	t_R = 11.98 min; *m/z* = 480.3 $[M+H]^+$

E11		33.7	34	t_R = 12.28 min; *m/z* = 494.2 $[M+H]^+$
F11		38	42	t_R = 11.06 min; *m/z* = 448.1 $[M+H]^+$
G11		30.2	31	t_R = 10.91 min; *m/z* = 487.2 $[M+H]^+$
H11		22.4	21	t_R = 12.47 min; *m/z* = 542.3 $[M+H]^+$
A12		28.9	38	t_R = 11.32 min; *m/z* = 378.3 $[M+H]^+$
B12		28.5	35	t_R = 11.55 min; *m/z* = 404.3 $[M+H]^+$
C12		14.2	17	t_R = 11.23 min; *m/z* = 412.2 $[M+H]^+$
D12		41.7	49	t_R = 11.43 min; *m/z* = 426.3 $[M+H]^+$
E12		48.2	55	t_R = 11.76 min; *m/z* = 440.3 $[M+H]^+$
F12		19.9	25	t_R = 10.48 min; *m/z* = 394.2 $[M+H]^+$
G12		21	24	t_R = 10.34 min; *m/z* = 433.3 $[M+H]^+$
H12		31.3	32	t_R = 11.96 min; *m/z* = 488.3 $[M+H]^+$

Compound	Stucture	Mass (mg)	Yield (%)
I1		284	62
J1		106	24
K1		287	66
M1		140	33
N1		147	35
O1		213	49
P1		132	30
Q1		163	36
R1		366	79
S1		157	36
T1		230	53

U1		299	69
V1		241	51
I13		246	50
J13		16	3
K13		339	72
O13		203	43
W13		301	66
I14		299	68
J14		41	10
K14		169	40
L14		168	40

M14		239	22
N14		281	69
P14		22	5
Q14		72	17
R14		176	39
S14		239	57
T14		231	55
U14		30	7
W14		191	47
I15		310	68
J15		191	40

K15		315	67
M15		175	38
N15		292	64
O15		285	60
W15		258	56

Characterization of selected compounds

***N-(4-chlorobenzyl)-N-(2-(cyclohexylamino)-2-oxoethyl)-1H-indole-2-carboxamide (B1)*:**

HRMS: $C_{24}H_{26}N_3O_2ClNa$, 446.1611 (calcd.), 446.1634 (found).

^{1}H NMR (600 MHz, DMSO): 1.18-1.27 (m, 5H), 1.53-1.74 (m, 4H), 3.58 (m, 1H), 3.94 (m, 1H), 4.18 (m, 1H), 4.66 (m, 1H), 5.00 (m, 1H), 6.53 (m, 1H), 6.75 (m, 1H), 7.03 (m, 1H), 7.18 (m, 1H), 7.37-7.56 (m, 4H), 7.84 (m, 1H), 8.05 (m, 1H), 11.70 (m, 1H).

^{13}C NMR (150 MHz, DMSO): 24.4, 25.1, 32.2, 47.5, 49.7, 51.2, 54.9, 103.9, 111.99, 112.04, 119.8, 121.4, 123.5, 126.8, 128.3, 129.8, 131.8, 135.9, 136.3, 163.8, 166.9.

***N-(tert-butyl)-N-(2-(cyclohexylamino)-2-oxoethyl)-1H-indole-2-carboxamide (B7)*:**

HRMS: $C_{21}H_{29}N_3O_2Na$, 378.2157 (calcd.), 378.2140 (found).

^{1}H NMR (600 MHz, DMSO): 1.13-1.21 (m, 3H), 1.27-1.33 (m, 2H), 1.46 (s, 9H), 1.55-1.57 (m, 1H), 1.68-1.70 (m, 2H), 1.77-1.79 (m, 2H), 3.63-3.66 (m, 1H), 4.21 (s, 2H), 6.67 (s, 1H), 7.00-7.02 (t, 1H, J = 7.2Hz), 7.14-7.16 (t, 1H, J = 7.5 Hz), 7.37-7.38 (d, 2H, J = 8.4Hz), 7.53-7.54 (d, 1H, J = 8.4Hz), 7.93-7.94 (d, 1H, J = 7.8Hz), 11.52 (s, 1H).

^{13}C NMR (150 MHz, DMSO): 24.9, 25.7, 28.0, 32.6, 48.0, 50.6, 58.0, 100.0, 103.3, 112.4, 120.0, 121.7, 123.4, 127.2, 133.2, 136.2, 166.0, 169.9.

***N-(2-(benzylamino)-2-oxoethyl)-N-(tert-butyl)-1H-indole-2-carboxamide (C7)*:**

HRMS: $C_{22}H_{25}N_3O_2Na$, 386.1844 (calcd.), 386.1812 (found).

^{1}H NMR (600 MHz, DMSO): 1.47 (s, 9H), 4.32 (s, 2H), 4.37-4.38 (d, 2H), 6.60 (s, 1H), 7.02 (m, 1H), 7.15 (m, 1H), 7.26-7.39 (m, 5H), 7.37-7.39 (d, 1H, J = 8.4Hz), 7.47-7.48 (d, 1H, J = 8.4Hz), 8.58-8.60 (t, 1H, J = 6.0Hz), 11.51 (s, 1H).

^{13}C NMR (150 MHz, DMSO): 28.0, 42.8, 50.7, 58.0, 103.1, 112.4, 120.1, 121.7, 123.4, 127.2, 127.4, 127.9, 128.8, 133.0, 136.2, 139.8, 165.9, 170.9.

***N-(tert-butyl)-N-(2-(mesitylamino)-2-oxoethyl)-1H-indole-2-carboxamide (E7)*:**

HRMS: $C_{24}H_{30}N_3O_2$, 392.2338 (calcd.), 392.2341 (found).

^{1}H NMR (600 MHz, DMSO): 1.29 (s, 9H), 1.90 (s, 6H), 2.00 (s, 3H), 4.27 (s, 2H), 6.52 (s, 1H), 6.67 (s, 2H), 6.78-6.81 (t, 1H, J = 7.2Hz), 6.92-6.95 (t, 1H, J = 7.2Hz), 7.16-7.17 (d, 1H, J = 8.4Hz), 7.26-7.28 (d, 1H, J = 8.4Hz), 9.16 (s, 1H), 11.29 (s, 1H).

^{13}C NMR (150 MHz, DMSO): 17.9, 20.5, 27.5, 57.5, 62.8, 102.7, 111.9, 119.7, 121.0, 123.0, 126.7, 128.6, 131.9, 132.6, 134.8, 135.6, 135.8, 165.5, 169.3.

***N-(2-(cyclohexylamino)-2-oxoethyl)-N-phenethyl-1H-indole-2-carboxamide (B12)*:**

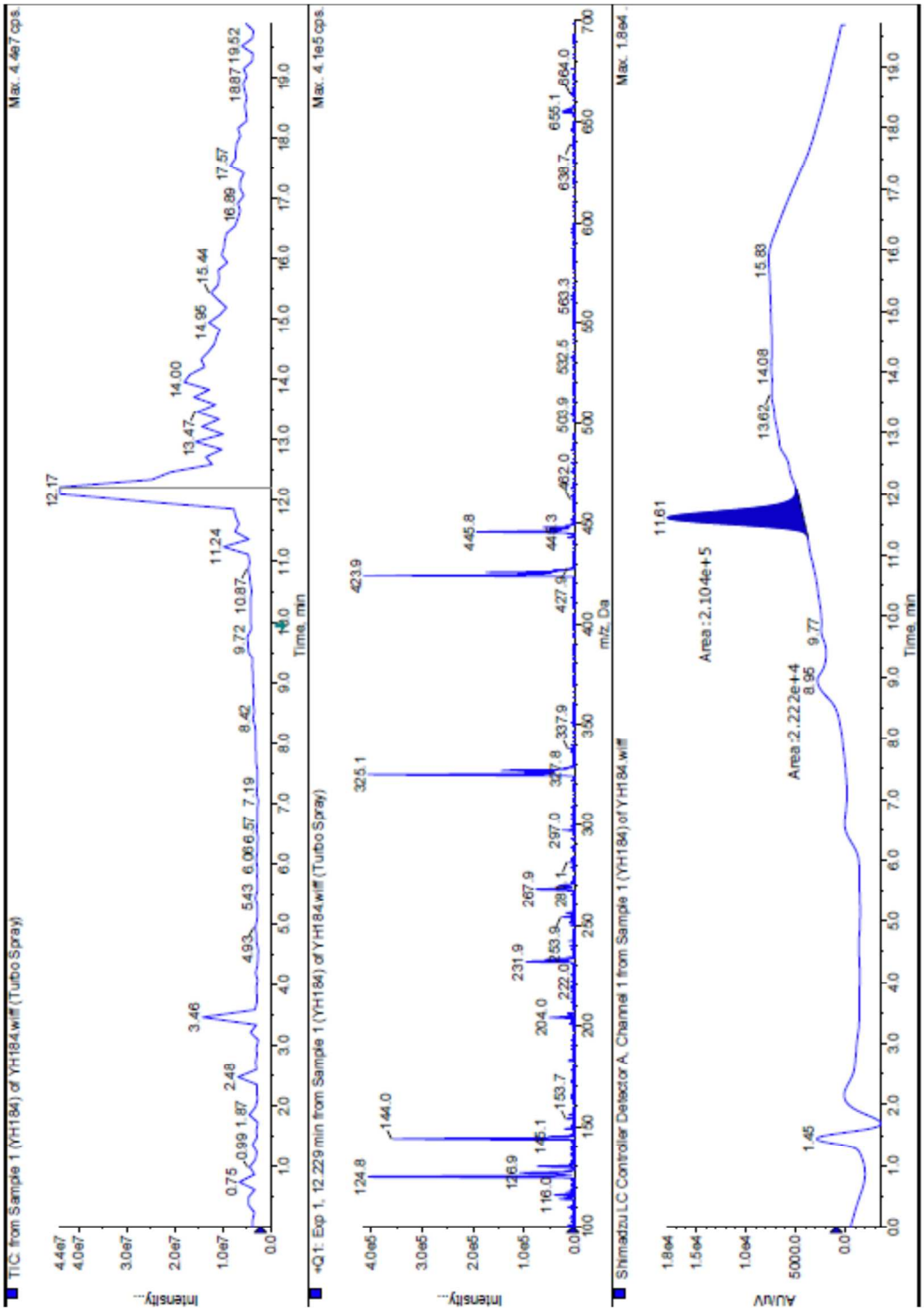
HRMS: $C_{25}H_{30}N_3O_2$, 404.2338 (calcd.), 404.2328 (found).

^{1}H NMR (600 MHz, DMSO): 1.13-1.29 (m, 6H), 1.55-1.57 (d, 1H, J = 12.6Hz), 1.68-1.70 (m, 2H), 1.75-1.77 (d, 2H, J = 12.0Hz), 2.92 (s, 2H), 3.03 (s, 1H), 3.64 (s, 1H), 3.89 (m, 1H), 4.09 (s, 1H) 4.24 (s, 1H), 6.68 (s, 1H) 7.03-7.05 (t, 1H, J = 7.32Hz), 7.18-7.26 (m, 2H), 7.26-7.36 (m, 4H), 7.42-7.44 (d, 1H, J = 8.4Hz), 7.57 (m, 1H), 8.11 (m, 1H), 11.64 (s, 1H).

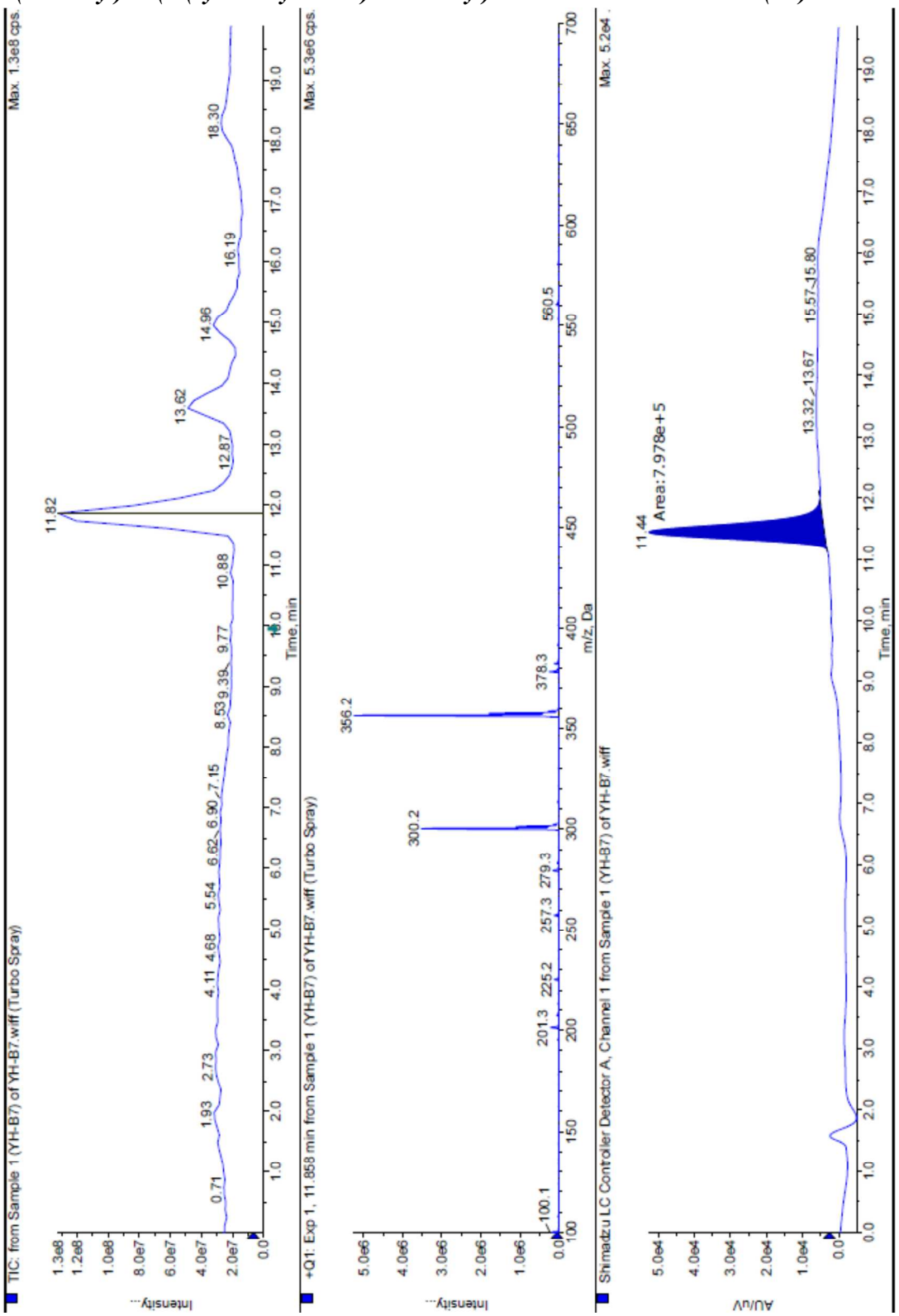
^{13}C NMR (150 MHz, DMSO): 24.5, 25.2, 32.3, 41.3, 47.6, 49.6, 52.0, 103.5, 112.0, 119.7, 121.3, 123.3, 126.2, 126.9, 128.5, 128.7, 135.8, 163.3, 167.4.

HPLC/MS Analysis

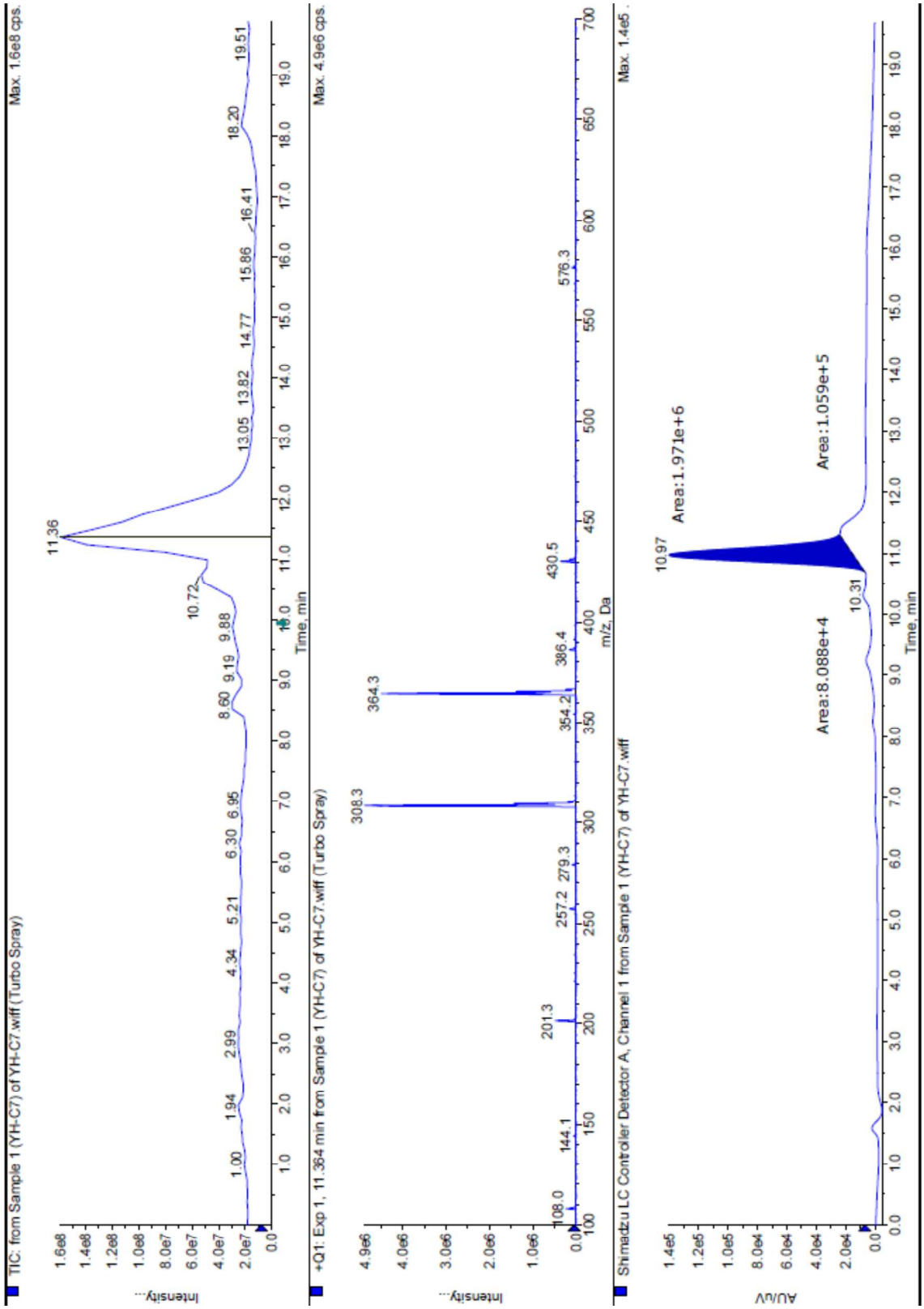
***N-(4-chlorobenzyl)-N-(2-(cyclohexylamino)-2-oxoethyl)-1H-indole-2-carboxamide (B1)*:**



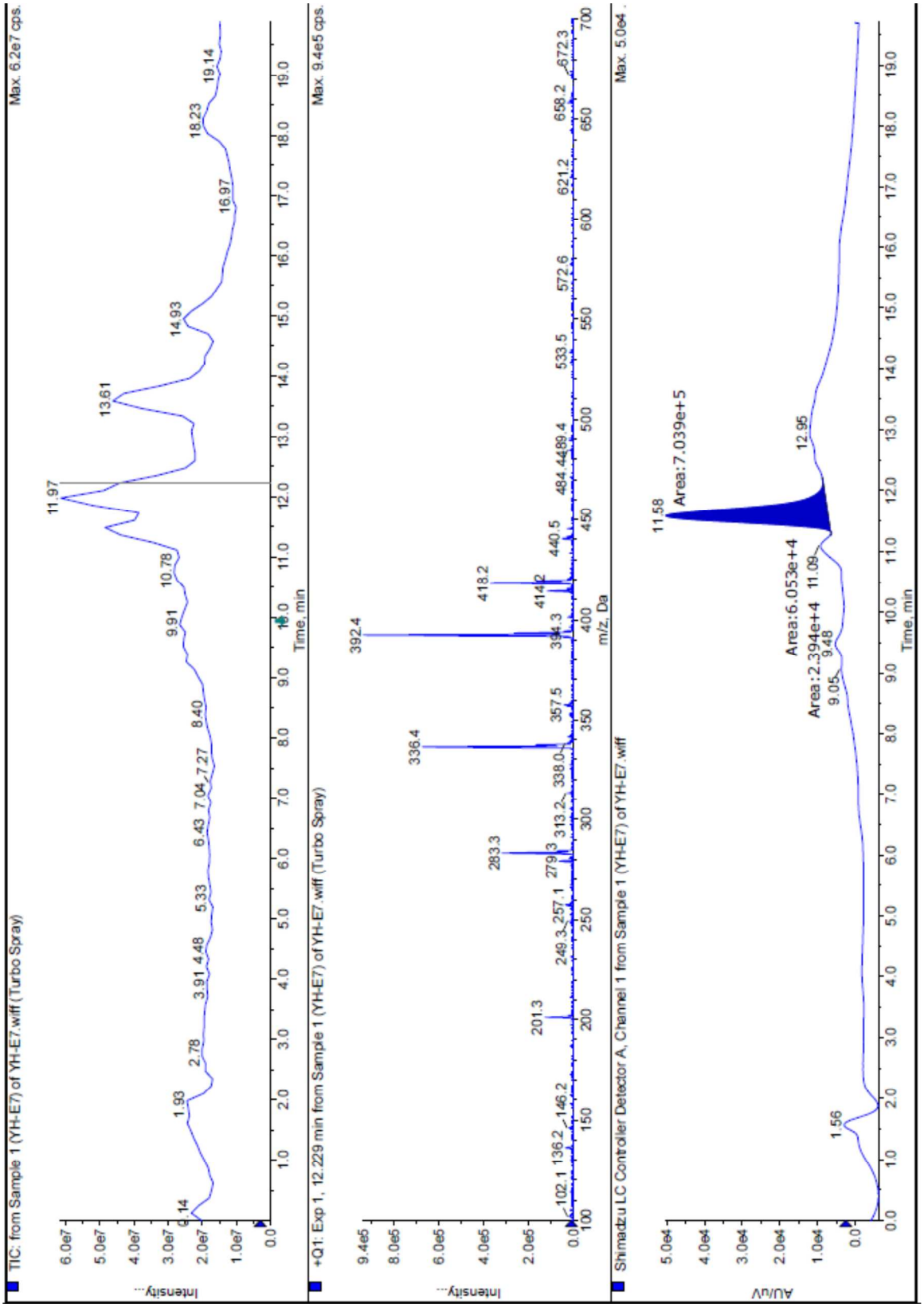
N-(tert-butyl)-N-(2-(cyclohexylamino)-2-oxoethyl)-1H-indole-2-carboxamide (B7):



N-(2-(benzylamino)-2-oxoethyl)-N-(tert-butyl)-1H-indole-2-carboxamide (C7):



N-(tert-butyl)-N-(2-(mesitylamino)-2-oxoethyl)-1H-indole-2-carboxamide (E7):



N-(2-(cyclohexylamino)-2-oxoethyl)-N-phenethyl-1H-indole-2-carboxamide (B12):