Degradation Products of Tryptophan in Cell Culture Media: Contribution to Color and Toxicity

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Supplementary Materials

Section S1. CIELAB color analysis

L* represents the lightness (0 = opaque, 100 = transparent), a* represents the color in the green (negative a* value) to red (positive a* value) field and b* represents the color in the blue (negative b* value) to yellow (positive b* value) field. ΔE* represents the overall difference in color between two samples and is calculated using the formula (1)

$$\Delta E^* = \sqrt{(L^*_2 - L^*_1)^2 + (a^*_2 - a^*_1)^2 + (b^*_2 - b^*_1)^2} \quad (1)$$

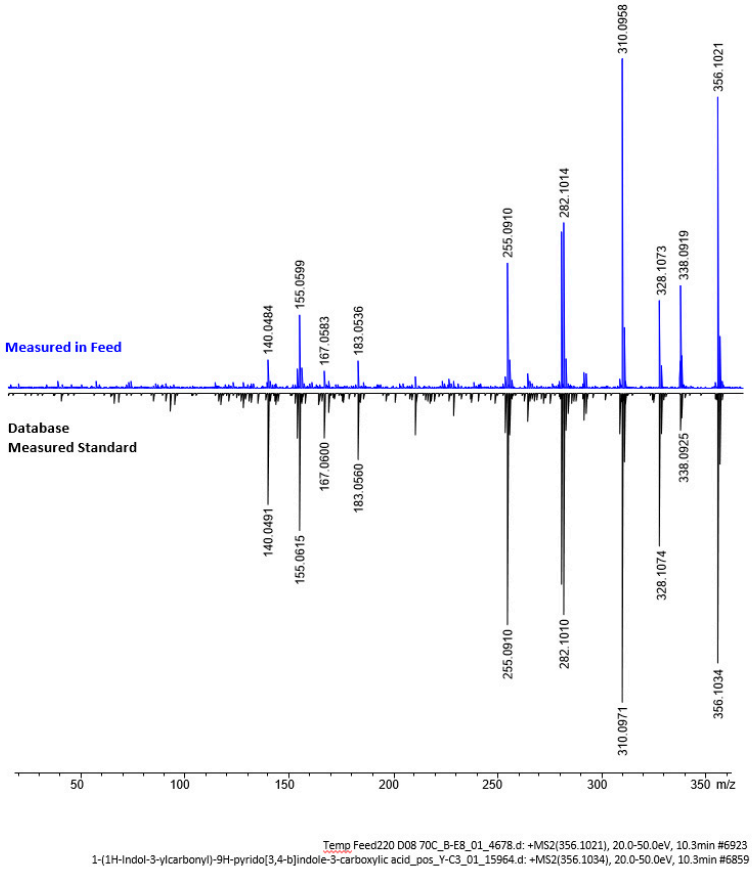
Section S2. LC-MS structure/tier assignments for features

Various levels of confidence were assigned to the annotations based on the initial recommendation of Sumner et al.[3] and some further published guidance document.[4] Briefly, tier 1 identification level was given to features where the retention time, the precursor mass and the fragmentation pattern matched a commercially available standard that was measured on the same LC-MS equipment and with the same method. Tier 2 annotations were obtained for features identified by comparing the MSMS fragmentation pattern with a spectral library (HMDB, METLIN or NIST). In a few cases, features were also annotated as tier 2 identification when the MSMS fragmentation pattern of the standard could not be confirmed using the experimental data (no MSMS) but for which the rt was correct (e.g. features of compounds **146**, **187**). Features were annotated as a tier 3 when a tentative structure matched the precursor mass and the MSMS fragmentation data could be annotated manually. Tier 4 correspond to features for which the precursor mass and the isotopic pattern were matched with a sum formula. Finally, Tier 5 were only identified by a unique retention time and a precursor mass and no tentative structure / sum formula can be reported.

Section S3. Supplementary Figures

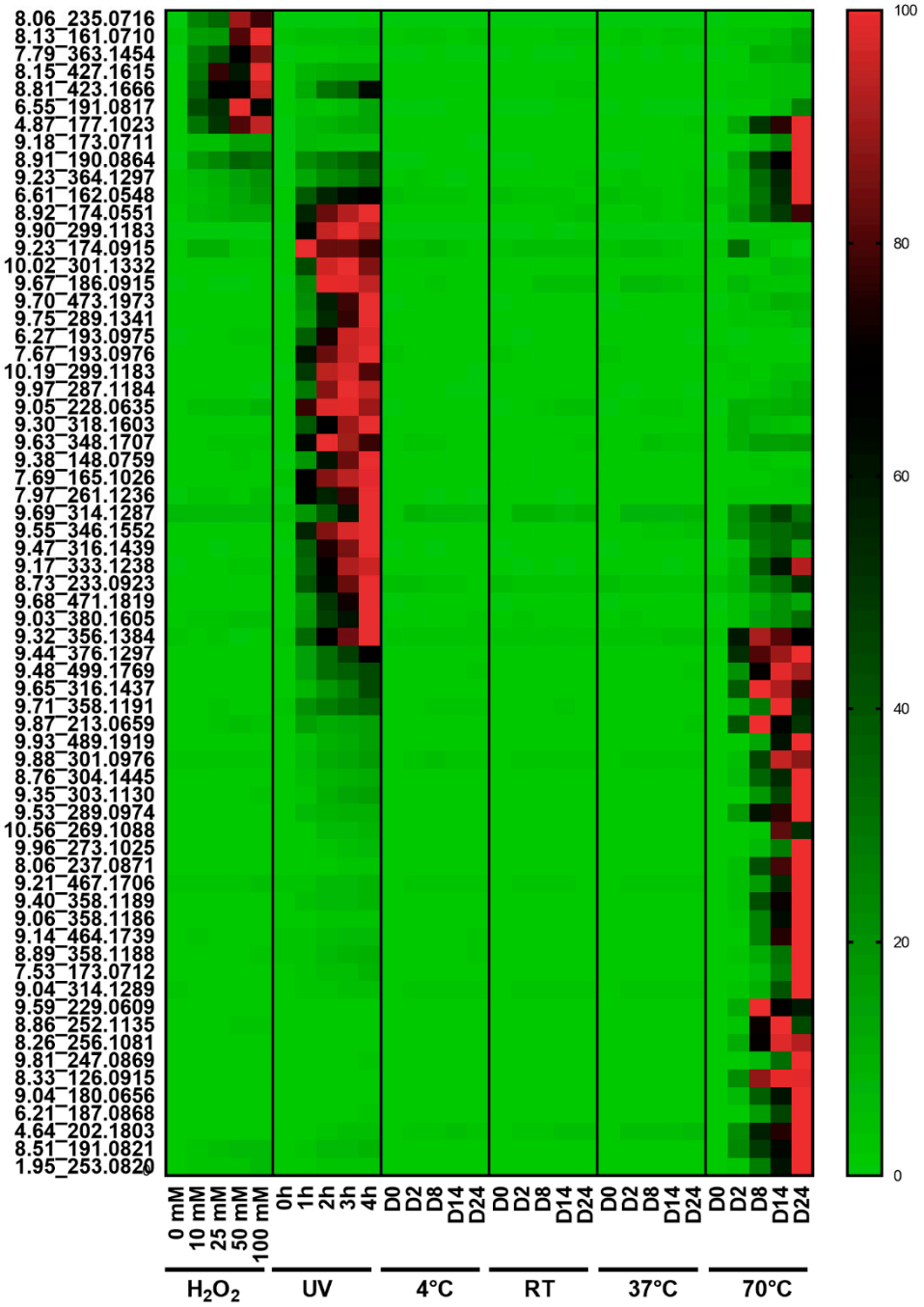
Supporting Figure S1. Example of Tier 1 level identification

Experimental data obtained in the feed (blue) compared to the data obtained for the std (black) for feature m/z = 356.1021, rt = 10.30 min, tier 1, identified as compound **31**.



Supplementary Figure S2. Heatmap of Tier 4-5 features that were significantly modulated in the aqueous Trp solutions.

Row titles depict rt_m/z for each feature.



Supplementary Table S1. LC-MS features identified in Trp-containing feed during 98-day storage at 37 °C.

Features are exclusively those with abundance over 30,000 and which were not detected in feed-Trp. All m/z represent the parent peaks detected as $[M+H]^+$ ions, unless indicated otherwise. Asterisks next to the compound numbers indicate features which were also detected in the water study (Fig 4). The *q*-value was determined between the feed condition and the feed lacking Trp using the area under the curve of the abundance over time. n/a = not applicable.

rt_m/z	Tier	Neutral Sum Formula	Compound #	Origin compound in feed (if known)	Abundance profile shape	Max. abundance	*q*-value
6.76_205.0972	1	C11H12N2O2	Trp	NA	decreased	4980057	2.41E-07
8.84_190.0864	4	C11H11NO2	n/a	Trp only	decreased	2324057	0.00201
4.75_237.0871	1	C11H12N2O4	4b*	Trp only	increased	594623	1.55E-06
4.22_237.0871	1	C11H12N2O4	4a*	Trp only	increased	580469	5.09E-06
3.66_221.0922	2	C11H12N2O3	7*	Trp only	increased	360091	3.15E-06
9.06_146.0602	1	C9H7NO	18*	Trp only	increased	302234	1.91E-05
7.73_146.0602	1	C9H7NO	20*	Trp only	increased	284365	2.23E-06
10.24_356.1032	1	C21H13N3O3	31*	Trp only	increased	202762	2.54E-06
8.73_360.1343	3	C21H17N3O3	30b*	Trp only	bell shape	177370	2.96E-06
4.82_221.0922	1	C11H12N2O3	6*	Trp only	increased	140580	4.97E-06
5.43_209.0924	1	C10H12N2O3	1*	Trp only	bell shape	139838	1.23E-06
5.93_221.0922	1	C11H12N2O3	3*	Trp only	decreased	139749	0.00683
8.07_231.1129	1	C13H14N2O2	22a*	Trp only	increased	128150	2.12E-04
9.14_173.0714	4	C10H8N2O	n/a	Trp only	bell shape	115920	1.21E-06
6.21_175.0869	3	C10H10N2O	40	Trp only	decreased	83558	0.0117
8.75_183.0927	1	C12H10N2	28*	Trp only	increased	86875	3.81E-07
5.81_237.0871	1	C11H12N2O4	2*	Trp only	decreased	75753	7.51E-06
9.19_334.1549	1	C20H19N3O2	14a*	Trp only	increased	64468	1.99E-04
10.10_296.1184	3	C20H13N3	36*	Trp only	increased	59784	2.07E-05
8.61_360.1342	1*	C21H17N3O3	30a	Trp only	bell shape	58239	9.52E-06
7.48_173.0711	4	C10H8N2O	n/a	Trp only	increased	57020	2.13E-04
9.76_312.1133	4	C20H13N3O	n/a	Trp only	increased	56607	9.30E-05
7.73_217.0975	1	C12H12N2O2	22*	Trp only	increased	52370	3.19E-06
9.37_314.1286	3	C20H15N3O	17*	Trp only	increased	51898	9.64E-06
4.99_174.0551	1	C10H7NO2	21*	Trp only	increased	49542	2.50E-05
6.91_247.1078	1	C13H14N2O3	25b*	Trp only	increased	48912	1.29E-05
8.35_231.1129	1	C13H14N2O2	23b*	Trp only	increased	44544	1.16E-05
9.95_340.1083	1	C21H13N3O2	38*	Trp only	increased	44022	6.07E-06
9.29_173.0716	4	C10H8N2O	n/a	Trp only	decreased	37305	7.25E-04
9.05_366.1446	3	C20H19N3O4	41	Trp only	increased	33732	2.60E-05
8.97_130.0650	2	C9H7N	8*	Trp only	increased	31284	1.15E-05
8.53_288.1708[a]	3	C16H22N3O2	43b	Choline	increased	279132	1.77E-06
8.41_288.1708[a]	3	C16H22N3O2	43a	Choline	increased	142758	4.76E-06
5.88_304.1657[a]	3	C16H22N3O3	44b	Choline	increased	82782	7.51E-06
8.50_455.1859	5	n/a	n/a	Thiamine	bell shape	56144	4.30E-06
5.53_304.1657[a]	3	C16H22N3O3	44a	Choline	increased	32364	3.29E-05
8.25_354.145	1	C19H19N3O4	42	Pyridoxine	increased	17201	1.29E-05
9.13_571.2122	5	n/a	n/a	Thiamine	increased	32235	1.12E-05
7.48_245.1284	4	C14H16N2O2	n/a	Leucine	increased	399111	4.49E-06
9.13_289.1548	3	C16H20N2O3	57	Leucine	increased	367727	3.61E-06

7.22_245.1284	4	C14H16N2O2	n/a	Isoleucine	increased	297299	2.91E-05
7.19_348.1665	3	C16H21N5O4	53b	Arginine	increased	225147	1.67E-04
6.52_231.1128	4	C13H14N2O2	n/a	Arginine	increased	191461	8.76E-07
6.94_245.1283	4	C14H16N2O2	n/a	Leucine	increased	144996	6.75E-05
7.35_245.1286	4	C14H16N2O2	n/a	Isoleucine	increased	139183	1.77E-05
8.26_289.1182	1*	C15H16N2O4	55	Glutamic acid	increased	101801	1.29E-05
6.18_348.1667	3	C16H21N5O4	53a	Arginine	increased	113533	1.05E-04
8.84_327.1089	3	C16H14N4O4	48	Histidine	increased	95428	1.29E-05
6.70_187.1231	4	C12H14N2	n/a	Proline	increased	87093	2.16E-05
5.06_295.1204	3	C16H14N4O2	50	Histidine	increased	80139	7.89E-06
9.14_273.1600	1	C16H20N2O2	56	Leucine	increased	67212	8.50E-06
8.32_314.1505	4	C17H19N3O3	n/a	Lysine	increased	70476	4.38E-06
5.91_347.1350	3	C16H18N4O5	49	Histidine	plateau	56107	2.12E-05
9.30_305.1490	3	C16H20N2O4	59	Leucine/Isoleucine	increased	53700	0.0274
6.21_231.1128	4	C13H14N2O2	n/a	Arginine	increased	52706	7.23E-06
7.09_316.1769	1	C16H21N5O2	52	Arginine	increased	52599	6.69E-06
9.02_255.1132	4	C15H14N2O2	n/a	Lysine	increased	36808	1.09E-05
8.63_275.1392	3	C15H18N2O3	58	Valine	increased	44580	6.02E-06
6.93_302.1136	3	C15H15N3O4	54	Asparagine	increased	43999	7.62E-06
8.45_291.0877	3	C16H10N4O2	51	Histidine	increased	35000	8.12E-06
6.40_231.1128	4	C13H14N2O2	n/a	Arginine	increased	36132	2.53E-05
5.17_313.1299	4	C16H16N4O3	n/a	Histidine	decreased	32295	0.0403
8.26_219.1493	4	C13H18N2O	n/a	Lysine	increased	31203	2.56E-06
8.74_227.0816	1	C13H10N2O2	45	n/a	increased	139010	1.09E-06
9.81_255.0765	1	C14H10N2O3	46	n/a	increased	125241	9.28E-04
8.48_175.0867	2	C10H10N2O	47	n/a	increased	118659	5.90E-06
6.37_271.1078	4	C15H14N2O3	n/a	n/a	increased	85358	2.17E-04
9.62_256.0718	4	C13H9N3O3	n/a	n/a	increased	81088	2.52E-06
7.16_438.1595	5	n/a	n/a	n/a	bell shape	61133	2.49E-06
7.27_285.1234	4	C16H16N2O3	n/a	n/a	increased	59718	1.06E-04
9.50_255.1133	4	C15H14N2O2	n/a	n/a	increased	59709	6.75E-05
8.89_308.1394	4	C18H17N3O2	n/a	n/a	increased	56190	3.72E-06
9.01_200.1285	4	C10H17NO3	n/a	n/a	increased	54363	3.84E-05
9.61_416.1970	4	C25H25N3O3	n/a	n/a	increased	52448	1.95E-05
9.36_402.1451	4	C23H19N3O4	n/a	n/a	increased	52048	2.07E-05
6.15_321.0958[b]	4	C15H14N4O3	n/a	n/a	bell shaped	50567	5.61E-05
9.43_307.0827	4	C16H10N4O3	n/a	n/a	increased	40987	7.89E-06
8.81_292.1446	4	C18H17N3O	n/a	n/a	bell shape	40812	0.0154
8.92_321.1444	4	C16H20N2O5	n/a	n/a	increased	39847	2.55E-04
8.20_263.0985	5	n/a	n/a	n/a	increased	38807	2.16E-05
8.74_321.1445	4	C16H20N2O5	n/a	n/a	increased	38908	1.27E-04
7.72_333.1824	5	n/a	n/a	n/a	decreased	39198	5.78E-06
8.46_263.1391	4	C14H19N2O3	n/a	n/a	increased	38908	6.21E-06
9.10_161.0720	4	C9H8N2O	n/a	n/a	only D0	38607	0.00332
8.75_309.1234	4	C18H16N2O3	n/a	n/a	increased	34524	1.39E-05

a. $m/z = [M]^+$
b. $m/z = [M+Na]^+$

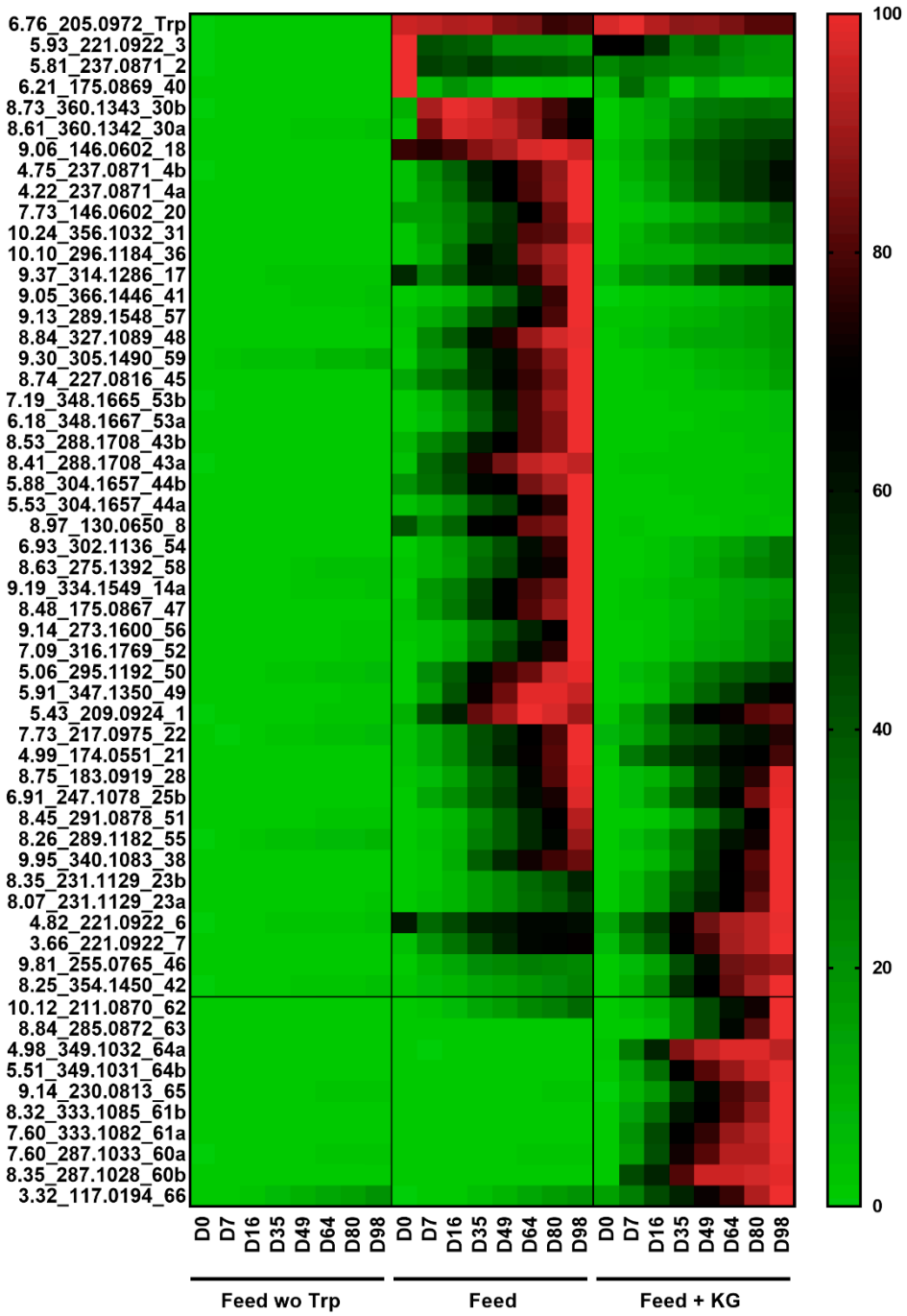
Supplementary Table S2. CHO cell toxicity assay

All compounds were tested in the CHOK1 GS cell line, and those which showed toxicity in that cell line were tested in the other three cell lines also. n/a = not applicable, as compound was not tested.

Compound #	[GI_{50}] (μM)			
	CHOK1 GS	CHOK1	CHOZN 6	CHOZN 10
39	< 0.13	0.2	1.1	1.05
34	24.0	10.6	12.3	13.1
31	34.6	21.6	13.0	14.4
12	64.5	115.8	54.5	92.3
16	76.6	74.9	88.5	57.6
5	118.2	158.0	76.7	75.5
38	118.4	123.5	98.9	114.0
27	127.4	74.8	98.5	64.5
29	156.7	388.8	>1000	347.4
28	170	74.9	65.5	77.3
18	295.4	226.4	236.0	257.5
24	342.7	311.9	314.4	306.0
1	>1000	n/a	n/a	n/a
2	>1000	n/a	n/a	n/a
3	>1000	n/a	n/a	n/a
4	>1000	n/a	n/a	n/a
6	>1000	n/a	n/a	n/a
8	>1000	n/a	n/a	n/a
9	>1000	n/a	n/a	n/a
10	>1000	n/a	n/a	n/a
11	>1000	n/a	n/a	n/a
14	>1000	n/a	n/a	n/a
19	>1000	n/a	n/a	n/a
20	>1000	n/a	n/a	n/a
21	>1000	n/a	n/a	n/a
22	>1000	n/a	n/a	n/a
23	>1000	n/a	n/a	n/a
25	>1000	n/a	n/a	n/a
32	>1000	n/a	n/a	n/a
42	>1000	n/a	n/a	n/a
45	>1000	n/a	n/a	n/a
46	>1000	n/a	n/a	n/a
47	>1000	n/a	n/a	n/a
56	>1000	n/a	n/a	n/a
60	>1000	n/a	n/a	n/a
62	>1000	n/a	n/a	n/a

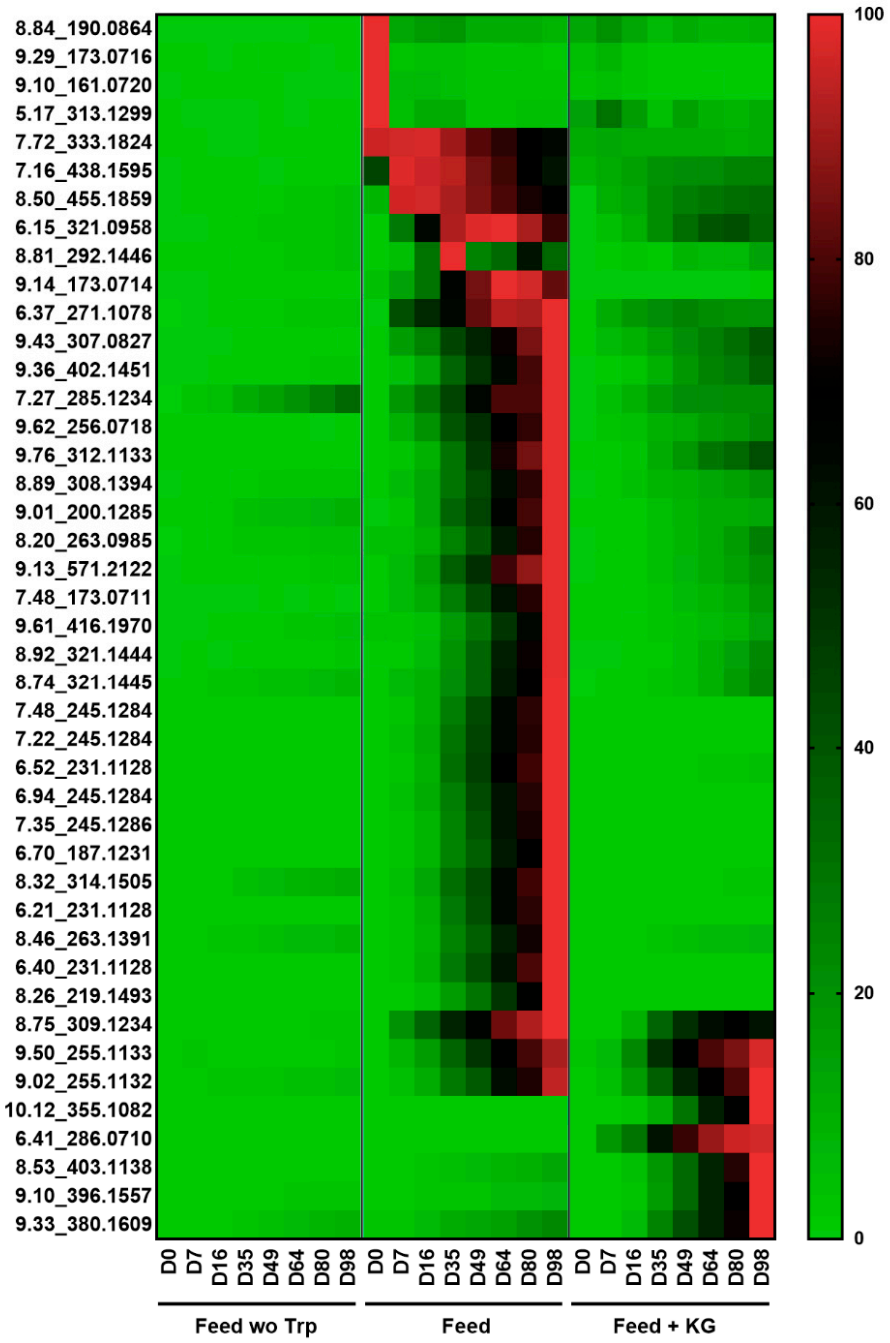
Supplementary Figure S3. Heatmap of Tier 1 to 3 features in the feed, feed-Trp and feed+aKG solutions stored at 37°C.

Row titles depict rt_m/z for each feature.



Supplementary Figure S4. Heatmap of Tier 4 and 5 features in the feed, feed-Trp and feed+aKG solutions stored at 37°C.

Row titles depict rt_m/z for each feature.



Supplementary Table S3. Commercial origin of compounds used as standards for Tier 1-2 feature identification.

Compound name	CAS	Compound #	Catalogue #	Supplier
L-Tryptophan	200-795-6	-	T0254	Sigma
N-Formylkynurenine	3978-11-8	**2**	T-205	TLC Pharmaceutical Standards
Kynurenine	343-65-7	**1**	61250	Sigma
Indole-3-acetaldehyde	2591-98-2	**12**	BN22755	Aurum Pharmatech
Indole-3-carboxaldehyde	487-89-8	**18**	129445	Sigma
2-amino-3-(2-oxo-2,3-dihydro-1h-indol-3-yl)propanic acid (Oia)	21704-80-3	**3**	FCH3581490	ChemSpace
3-(3-Hydroxy-2-oxo-2,3-dihydro-1H-indol-3-yl)-L-alanine (diOia)	184955-21-3	**4**	T-204	TLC Pharmaceutical Standards
1,2,3,3a,8,8a-Hexahydro-3a-hydroxypyrrolo[2,3-b]indole-2-carboxylic acid (PIC)	35169-97-2	**6**	CB0162344	Chemieliva
2,3,4,9-Tetrahydro-1H-beta-carboline-1-carboxylic acid	6052-68-2	**22**	S277886	Sigma
1-Methyl-2,3,4,9-tetrahydro-1H-beta-carboline-3-carboxylic acid	5470-37-1	**23**	H0500	Sigma
2-(3-Indolylmethyl)-L-tryptophan	149724-31-2	**14**	T-2014	TLC Pharmaceutical Standards
Tryptamine	61-54-1	**11**	193747	Sigma
4-Hydroxyquinoline	611-36-9	**20**	H58005	Sigma
Norharmane	244-63-3	**27**	N6252	Sigma
Harmane	486-84-0	**28**	103276	Sigma
1-Acetyl-β-carboline	50892-83-6	**62**	AG-B09388	Angel Pharmatech
1H-Pyrido[3,4-b]indole-1,3-dicarboxylic acid, 1-(2-carboxyethyl)-2,3,4,9-tetrahydro-, (1S-cis)-	122110-59-2	**60**	CE1002256	Chemieliva
2-Oxindole	59-48-3	**19**	O9808	Sigma
1-(1H-Indol-3-ylcarbonyl)-9H-pyrido[3,4-b]indole-3-carboxylic acid	863766-95-4	**31**	CC0472247	Chemieliva
9H-Pyrido[3,4-b]indole-1-carboxylic acid	26052-96-0	**5**	EN300-205447	Enamine
1-(1H-Indol-3-yl)-9H-pyrido[3,4-b]indole	155885-64-6	**34**	842D79	WuXi AppTec
3-Indoleacetamide	879-37-8	**47**	286281	Sigma
Quinoline	91-22-5	**8**	241571	Sigma
1H-Indol-3-yl-9H-pyrido[3,4-b]indol-1-ylmethanone	244295-64-5	**32**	9FE868	WuXi AppTec
2,3,4,9-Tetrahydro-1-(2-methylpropyl)-1H-pyrido[3,4-b]indole-3-carboxylic acid	146436-31-9	**56**	Amb17872939	Ambinter
4-Quinolinecarboxylic acid	486-74-8	**21**	174823	Sigma

1-methyl-9H-pyrido[3,4-b]indole-3-carboxylic acid	22329-38-0	**45**	MSC1806155A	Merck Serono Research
4,9-dihydro-3H-pyrido[3,4-b]indole-3-carboxylic acid	46501-39-7	**24**	CSC009997543	Chemspace
1-Phenyl-9H-pyrido[3,4-b]indole-3-carboxylic acid	374710-96-0	**16**	LN00360728	Labnetwork
1-Acetyl-3-carboxy-β-carboline	73818-29-8	**46**	CB0358346	Chemieliva
1H-Pyrido[3,4-b]indole-3-carboxylic acid, 2,3,4,9-tetrahydro-1-[3-hydroxy-5-(hydroxymethyl)-2-methyl-4-pyridinyl]-	158249-61-7	**42**	0DE6DA	Intonation Research Laboratories
2-Hydroxytryptamine	13078-93-8	**9**	CA0206490	Chemieliva
Tryptanthrin	13220-57-0	**39**	SML0310	Sigma
9H-Pyrido[3,4-b]indole-1-methanol	17337-22-3	**29**	CA0354102	Chemieliva
1H,2H,3H,3aH,8H,8aH-Pyrrolo[2,3-b]indol-3a-ol	58635-39-5	**10**	CA0659218	Chemieliva
2-[(E)-ethylideneamino]-3-(2-oxo-2,3-dihydro-1H-indol-3-yl)propanoic acid	No CAS	**25b**	5A768B	WuXi AppTec

Section S4. Synthesis of standards for LC-MS

All reagents were used as received from commercial suppliers. All NMR spectra were recorded at 298K on a 700MHz Bruker Advance III spectrometer equipped with a cryo cooled TCI probe. Chemical shifts were reported in parts per million (ppm), and the residual solvent peak was used as an internal reference: proton (chloroform δ 7.26, DMSO δ 2.50), carbon (chloroform δ 77.16, DMSO δ 39.52). Multiplicity was indicated as follows: s (singlet), d (doublet), t (triplet), q (quartet), m (multiplet), dd (doublet of doublet). HRMS was obtained using the Impact II mass spectrometer equipped with an ESI source (Bruker Daltonics, Bremen, Germany).

1-(1*H*-indole-3-carbonyl)-1,2,3,4-tetrahydro-β-carboline-3-carboxylic acid (30) Tetrahydro-β-carboline **30** was synthesized according to literature protocol,[5] and as per the literature precedent, was too unstable to allow NMR characterization. HRMS (ESI): m/z calcd for $C_{21}H_{18}N_3O_3$+H^+: 360.1343 $[M+H]^+$ 360.1340.

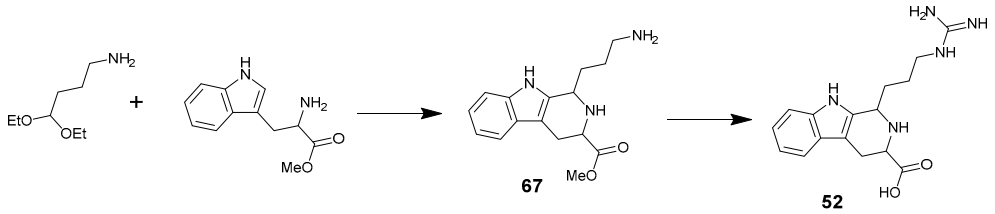
1-(Indol-3-yl)-β-carboline-3-carboxylic acid (33) β-Carboline **33** was synthesized according to literature protocol.[6] 1H NMR(700 MHz, d_6-DMSO) δ = 12.04 (2H, bs, NH), 8.87 (1H, s, Ar-H), 8.67 (1H, bs, Ar-H), 8.51 (1H, m, Ar-H), 8.44 (1H, m, Ar-H), 7.80 (1H, m, Ar-H), 7.64 (1H, m, Ar-H), 7.57 (1H, m, Ar-H), 7.36 (1H, m, Ar-H), 7.27 (1H, m, Ar-H), 7.21 (1H, m, Ar-H); 13C NMR (176 MHz, DMSO-d_6) δ = 166.25, 141.78, 139.11, 136.57, 135.19, 133.30, 128.97, 128.80, 127.73, 126.13, 122.47, 122.10, 122.03, 121.42, 120.69, 120.43, 114.68, 113.01, 111.82, 110.82; HRMS (ESI): m/z calcd for $C_{20}H_{14}N_3O_2$+H^+: 328.1081 $[M+H]^+$ 328.1081.

1-(Quinolin-4-yl)-β-carboline-3-carboxylic acid (38) 4-Quinonlinecarboxaldehyde (150 mg, 0.95 mmol) was added to a suspension of L-tryptophan (195 mg, 0.95 mmol) in ethanol (8 ml). Trifluoroacetic acid (0.80 ml, 10.38 mmol) was added and the solution was stirred for 6 days under nitrogen at room temperature, during which a precipitate formed. The precipitate was filtered and washed with ethyl acetate to afford the β-carboline **38** as a yellow-orange powder (100 mg, 31%). 1H NMR (400 MHz, d_6-DMSO) δ = 11.75 (1H, s, NH), 9.21 (1H, m, Ar-H), 9.08 (1H, s, Ar-H), 8.47 (1H, m, Ar-H), 8.24 (1H, m, Ar-H), 7.93-7.88 (2H, m, Ar-H), 7.75 (1H, m, Ar-H), 7.63-7.53 (3H, m, Ar-H), 7.36 (1H, m, Ar-H); 13C NMR (176 MHz, DMSO-d_6) δ = 166.68, 149.29, 141.52, 138.77, 137.35, 136.00, 130.82, 129.46, 129.11, 127.99, 127.89, 127.71, 126.29, 126.04, 122.52, 122.39, 121.07,

120.60, 120.43, 117.70, 112.53; HRMS (ESI): m/z calcd for $C_{21}H_{14}N_3O_4$ $+H^+$: 340.1081 $[M+H]^+$ 340.1080.

2-(N^{τ}-histidino)-4-(2-aminophenyl)-4-oxobutanoic acid (KYN-His adduct, 49) KYN-His adduct **49** was synthesized according to literature protocol,[7] and as per the literature precedent, was too unstable to allow NMR characterization. HRMS (ESI): m/z calcd for $C_{16}H_{18}N_4O_5$ $+H^+$: 347.1350 $[M+H]^+$ 347.1353.

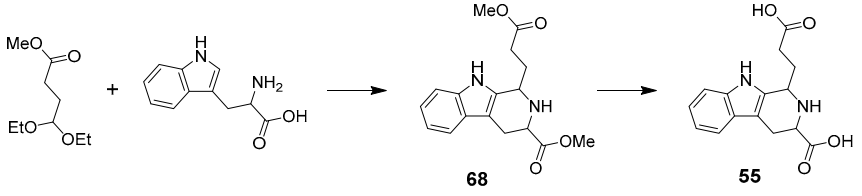
1-(3-Guanidinopropyl)-1,2,3,4-tetrahydro-*b*-carboline-3-carboxylic acid (**52**) was synthesized in two steps from L-tryptophan methyl ester.



Methyl 1-(3′-aminopropyl)-1,2,3,4-tetrahydro-β-carboline-3-carboxylate (67) A solution of 4-aminobutyraldehyde diethyl acetal (90%, 0.21 ml, 1.12 mmol) in dichloromethane (2 ml) was cooled to 0 °C. Trifluoroacetic acid (1 ml, 13 mmol) and water (1 ml) were added and the solution was stirred at 0 °C for 45 min. Tryptophan methyl ester (284.4 mg, 1.12 mmol) was added and the solution was allowed to warm to room temperature and stirred overnight. The solution was concentrated under reduced pressure and purified using flash chromatography (0-50% MeOH/EtOAc + 2% NH_4OH) to afford the tetrahydro-β-carboline **67** as a yellow oil (303 mg, 94%). δ_H (400 MHz, d_6-DMSO) 11.53, (1H, s, NH), 8.26 (2H, bs, NH2), 7.48 (1H, m, Ar-H), 7.38 (1H, m, Ar-H), 7.12 (1H, m, Ar-H), 7.02 (1H, m, Ar-H), 4.79 (1H, m, H1), 4.52 (1H, m, H3), 3.85 (3H, s, CH3), 3.30-3.10 (2H, m, H4), 2.91 (2H, m, H3′), 2.49-2.2.14 (2H, m, H2′), 2.03-1.80 (2H, m, H1′); 13C NMR (101 MHz, DMSO-d6) δ = 168.87, 136.46, 129.67, 125.52, 121.81, 119.11, 118.00, 111.44, 104.93, 55.05, 52.93, 52.78, 38.17, 27.80, 22.50, 22.19; HRMS (ESI): m/z calcd for $C_{16}H_{22}N_3O_2$ $+H^+$: 288.1707 $[M+H]^+$ 288.1701.

1-(3′-Guanidinopropyl)-1,2,3,4-tetrahydro-β-carboline-3-carboxylic acid (52) S-methylisothiouronium sulfate (1.45 g, 5.22 mmol) was added to a solution of NaOH(aq.) (1M, 5.2 ml, 5.22 mmol) in methanol (18 ml) at 0°C. The solution was stirred at 0°C for 5 min, upon which a precipitate formed. The precipitate was filtered off, and amine **67** (2.00 g, 6.96 mmol) was added to the filtrate. The solution was stirred at room temperature for 3 days. A solution of 25% ammonia (aq.) (4 ml) was added to the reaction mixture, which was then stirred at reflux for 2 h. The solution was concentrated by half using a rotary evaporator and then brought to pH 7 with aqueous HCl (10M), upon which a precipitate formed. The precipitate was filtered and triturated with hot methanol to afford carboxylic acid **52** as a white solid (88 mg, 4%) 1H NMR (400 MHz, d_6-DMSO) δ = 11.24 (1H, s, NH), 8.60 (1H, bs, NH), 7.60 (3H, bs, guanidino), 7.42 (1H, m, Ar-H), 7.36 (1H, m, Ar-H), 7.08 (1H, m, Ar-H),, 6.99 (1H, m, Ar-H), 4.60 (1H, m, H1), 3.78 (1H, m, H3), 3.21-2.83 (2H, m, H4), 3.20 (2H, m, H3′), 2.32-2.04 (2H, m, H2′), 1.87-1.62 (2H, m, H1′); 13C NMR (101 MHz, DMSO-d6) δ = 171.84, 157.30, 136.52, 131.25, 126.01, 121.36, 118.81, 117.88, 111.35, 106.85, 57.47, 52.59, 40.50, 28.63, 23.82, 23.23; HRMS (ESI): m/z calcd for $C_{16}H_{22}N_5O_2$ $+H^+$: 316.1768 $[M+H]^+$ 316.1763.

1-(2-Carboxyethyl)-1,2,3,4-tetrahydro-β-carboline-3-carboxylic acid (**55**) was synthesized in two steps from L-tryptophan methyl ester.



Methyl (1-(3-Methyl propanoate)-1,2,3,4-tetrahydro-β-carboline)-3-carboxylate (68) A solution of methyl 4,4-dimethoxybutyrate (200 mg, 1.2 mmol) in DCM (2 ml) was cooled to 0 °C. TFA (1 ml, 13.0 mmol) and water (1 ml) were added to the solution, and the solution was stirred at 0 °C for 45 min. L-Tryptophan methyl ester (305 mg, 1.2 mmol) was added to the solution, then the solution was allowed to warm to room temperature and stirred overnight. The reaction mixture was concentrated under reduced pressure and purified using column chromatography (10-100% MeOH/EtOAc) to afford dimethyl ester **68** as a pale yellow oil (154 mg, 41%). 1H NMR (400 MHz, d_6-DMSO) δ = 11.26 (1H, s, NH), 7.50 (1H, m, Ar-H), 7.39 (1H, m, Ar-H), 7.14 (1H, m, Ar-H), 7.04 (1H, m, Ar-H), 4.80 (1H, m, H1), 4.61-4.57 (1H, m, H3), 3.87 (3H, s, CH3), 3.66 (3H, s, CH3), 3.33-3.01 (2H, m, H4), 2.73-2.56 (2H, m, CH2), 2.62-2.18 (2H, m, CH2). 13C NMR (101 MHz, DMSO-d_6) δ = 172.52, 169.15, 136.54, 129.33, 125.55, 122.00, 119.25, 118.08, 111.50, 105.15, 55.09, 53.11, 53.06, 51.54, 28.86, 25.95, 22.38. HRMS (ESI): m/z calcd for $C_{17}H_{20}N_2O_4$+H^+: 317.1496 $[M+H]^+$ 317.1495.

1-(2´-Carboxyethyl)-1,2,3,4-tetrahydro-β-carboline-3-carboxylic acid (55) LiOH (238 mg, 9.7 mmol) was added to a solution of methyl carboxylate **68** (154 mg, 0.5 mmol) in 3:1 THF/water (16 ml) and the solution was stirred at reflux overnight. The solution was allowed to cool to room temperature and was neutralized using Amberlite IR120 H+ ion exchange resin. The solution was concentrated under reduced pressure to afford carboxylic acid **55** as pale yellow, waxy oil (142 mg, quant.). 1H NMR (400 MHz, d_6-DMSO) δ = 11.05 (1H, s, NH), 7.36 (1H, m, Ar-H), 7.30 (1H, m, Ar-H), 7.02 (1H, m, Ar-H), 6.94 (1H, m, Ar-H), 4.34 (1H, m, H1), 3.89 (1H, m, H3), 3.05-2.62 (2H, m, H4), 2.34-1.93 (4H, m, 2 × CH2). HRMS (ESI): m/z calcd for $C_{15}H_{16}N_2O_4$+H^+: 289.1183 $[M+H]^+$ 289.1179.

Section S5. References

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