Supporting Information

Lateral Flow Assessment and Unanticipated Toxicity of Kratom

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Figure S1. ^{1}H NMR spectrum of mitragynine

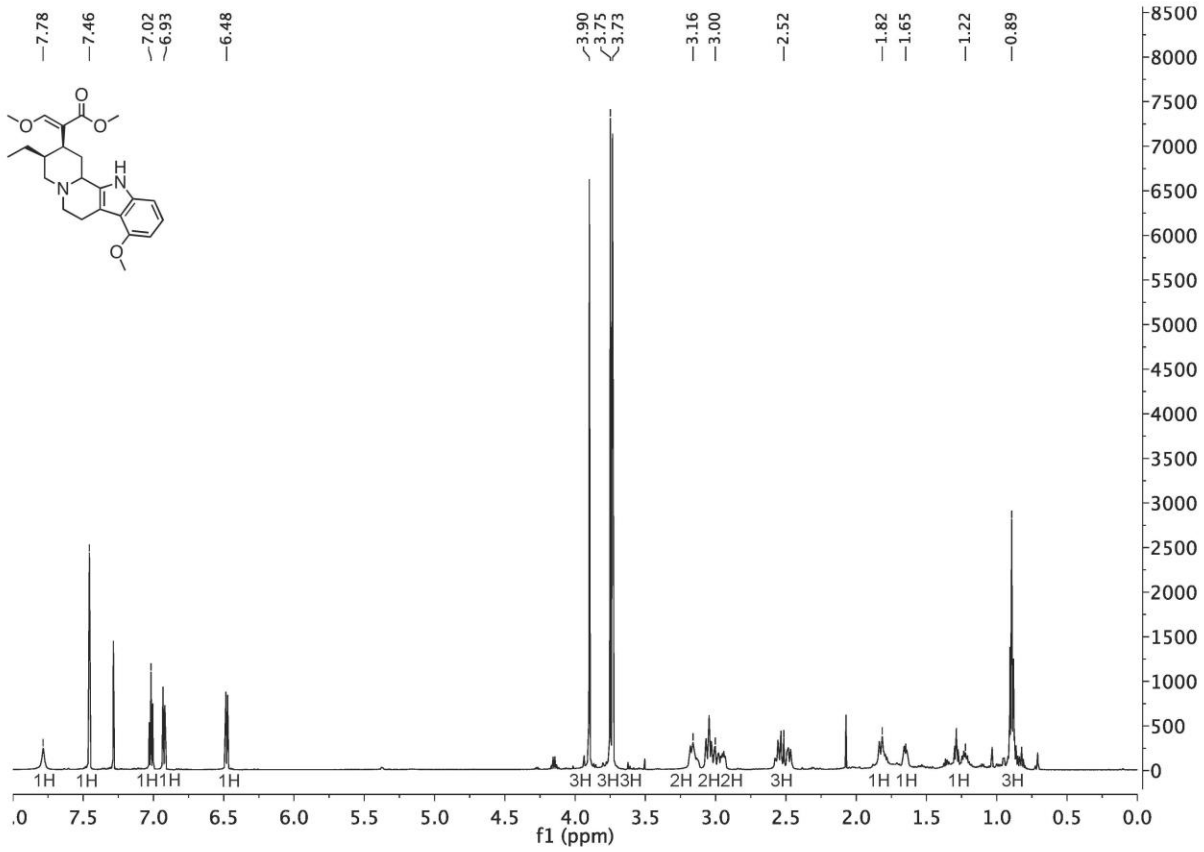

Figure S2. ^{13}C NMR spectrum of mitragynine

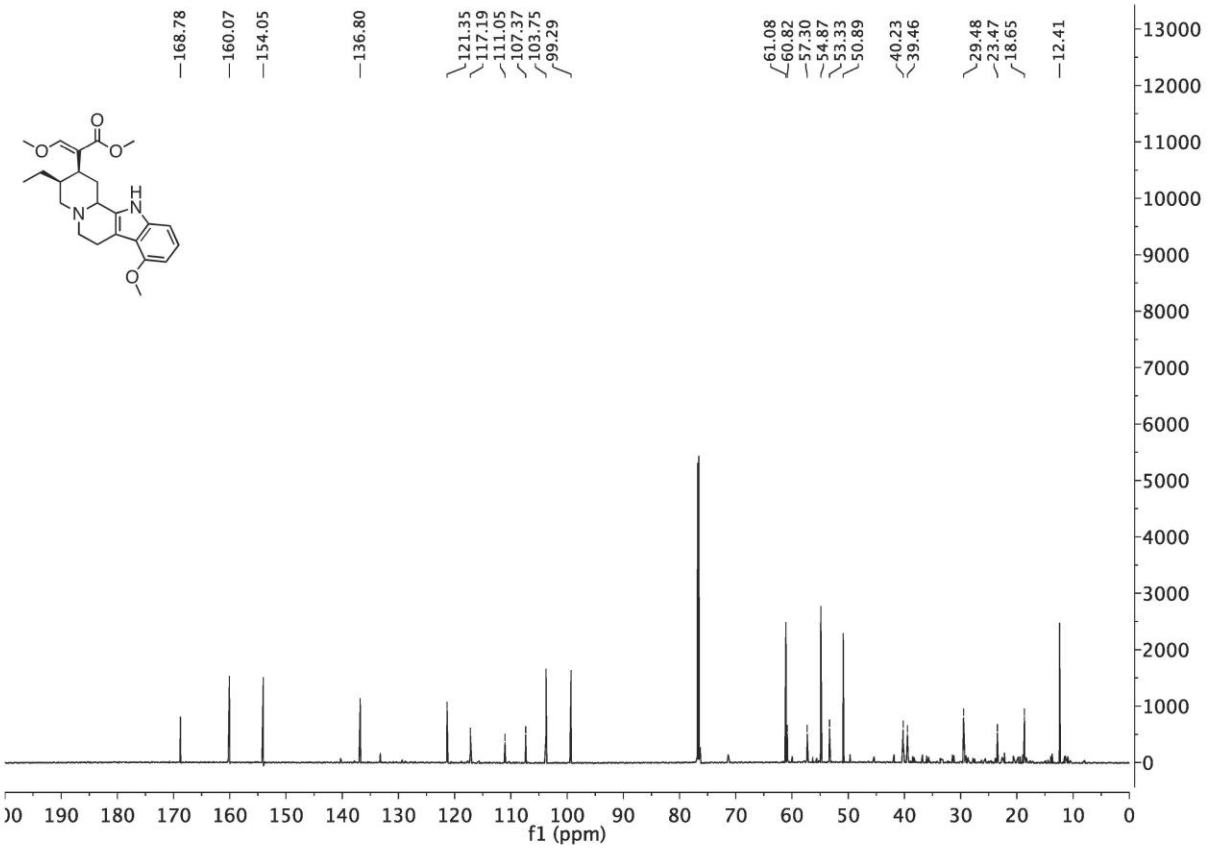

Figure S3. ^{1}H NMR spectrum of **2**

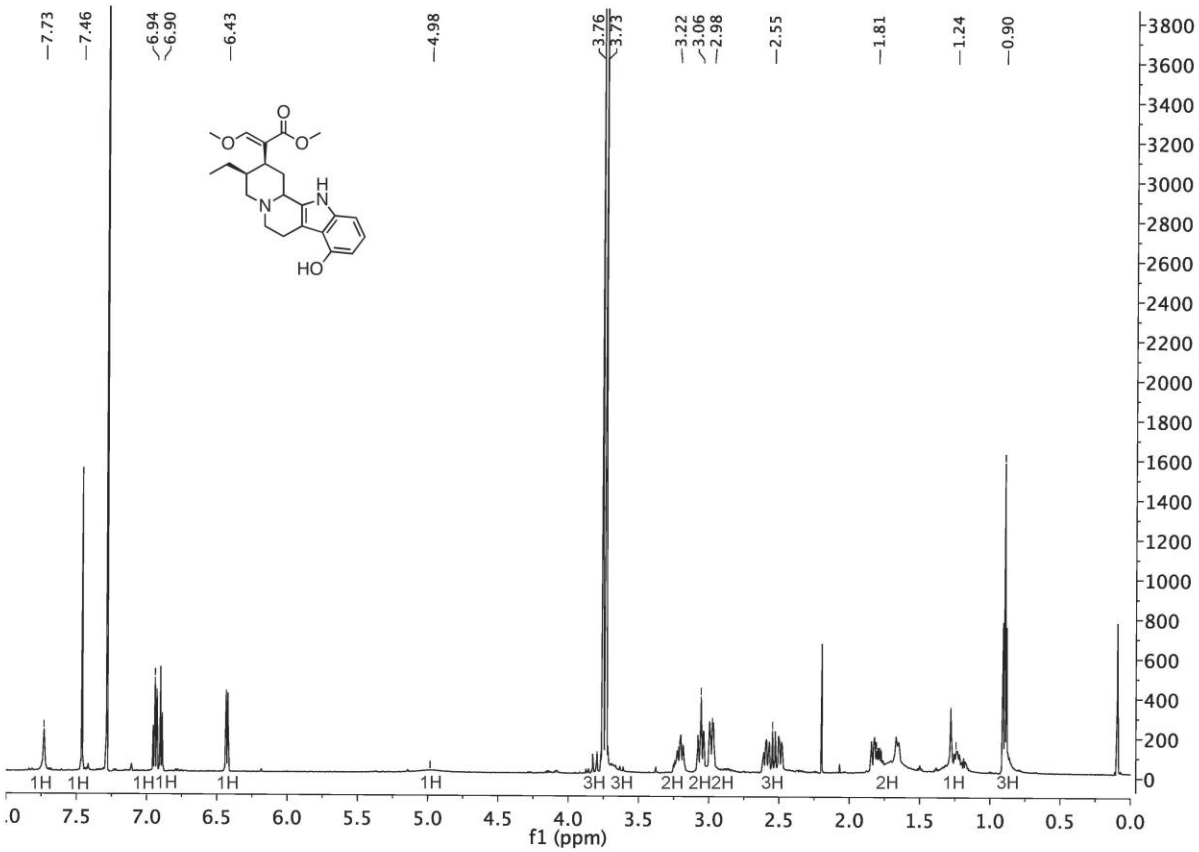

Figure S4. ^{13}C NMR spectrum of **2**

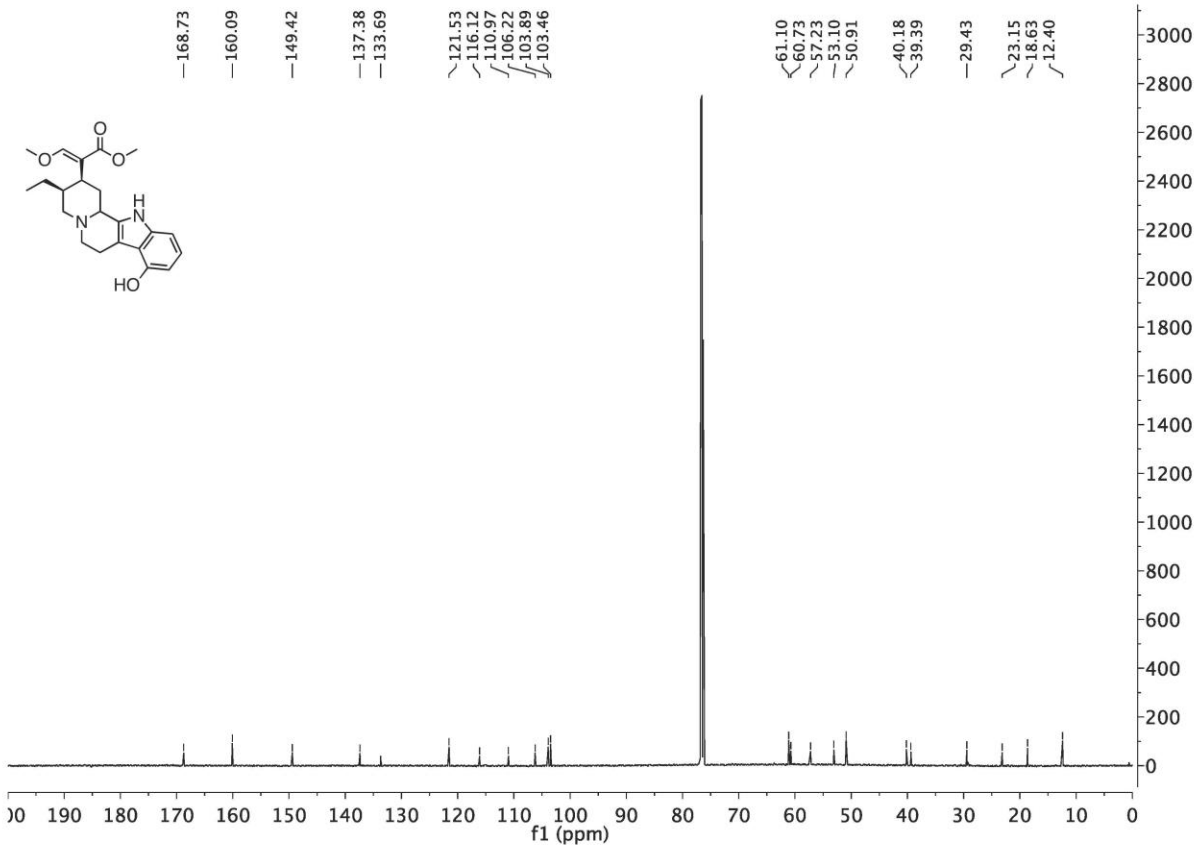

Figure S5. ^{1}H NMR spectrum of **3**

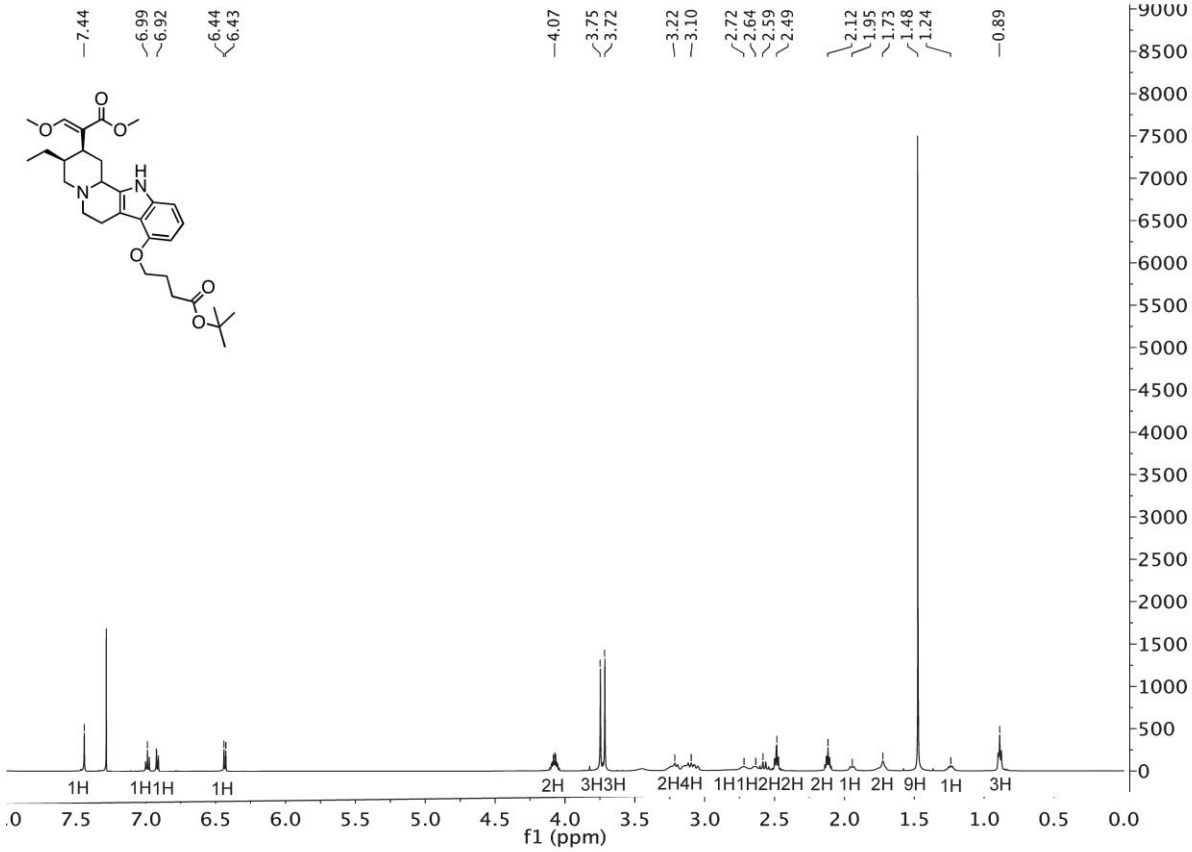

Figure S6. ^{13}C NMR spectrum of **3**

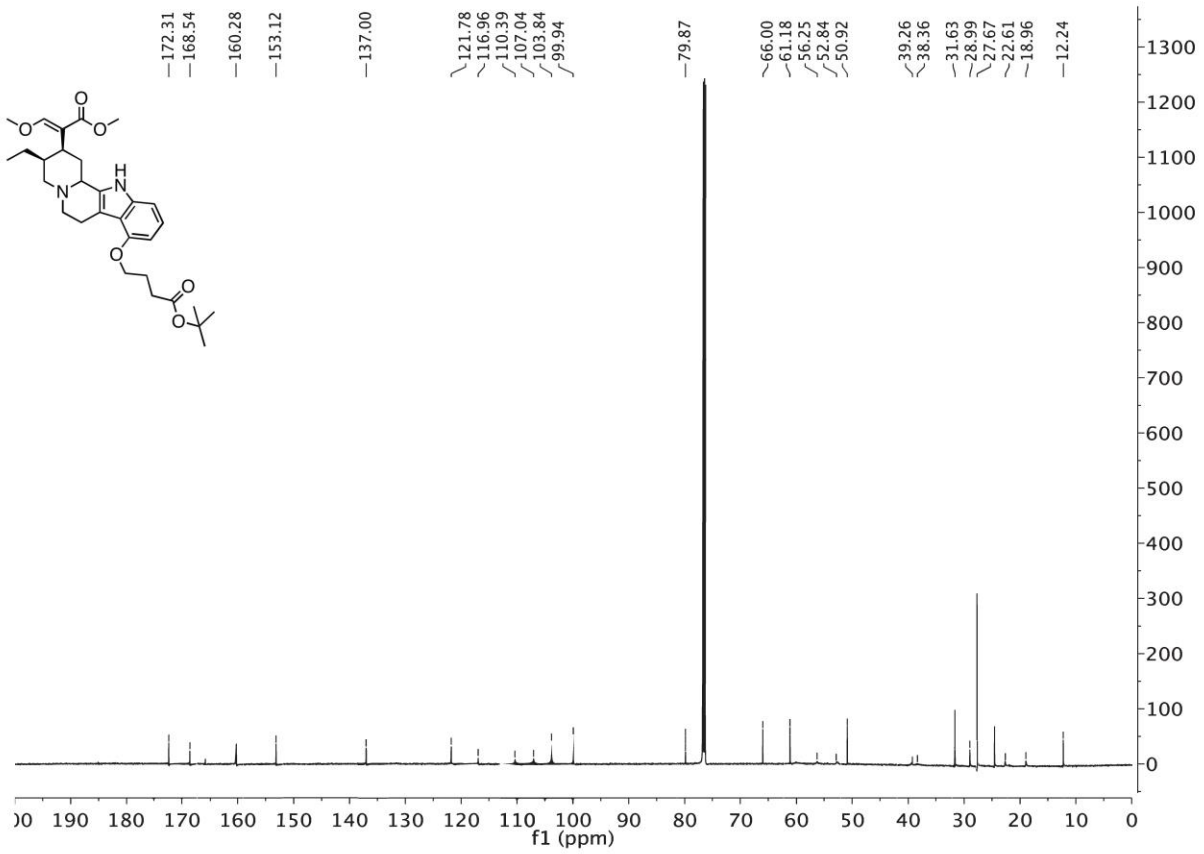

Figure S7. HPLC chromatogram and mass trace of Mit-hapten (**4)**

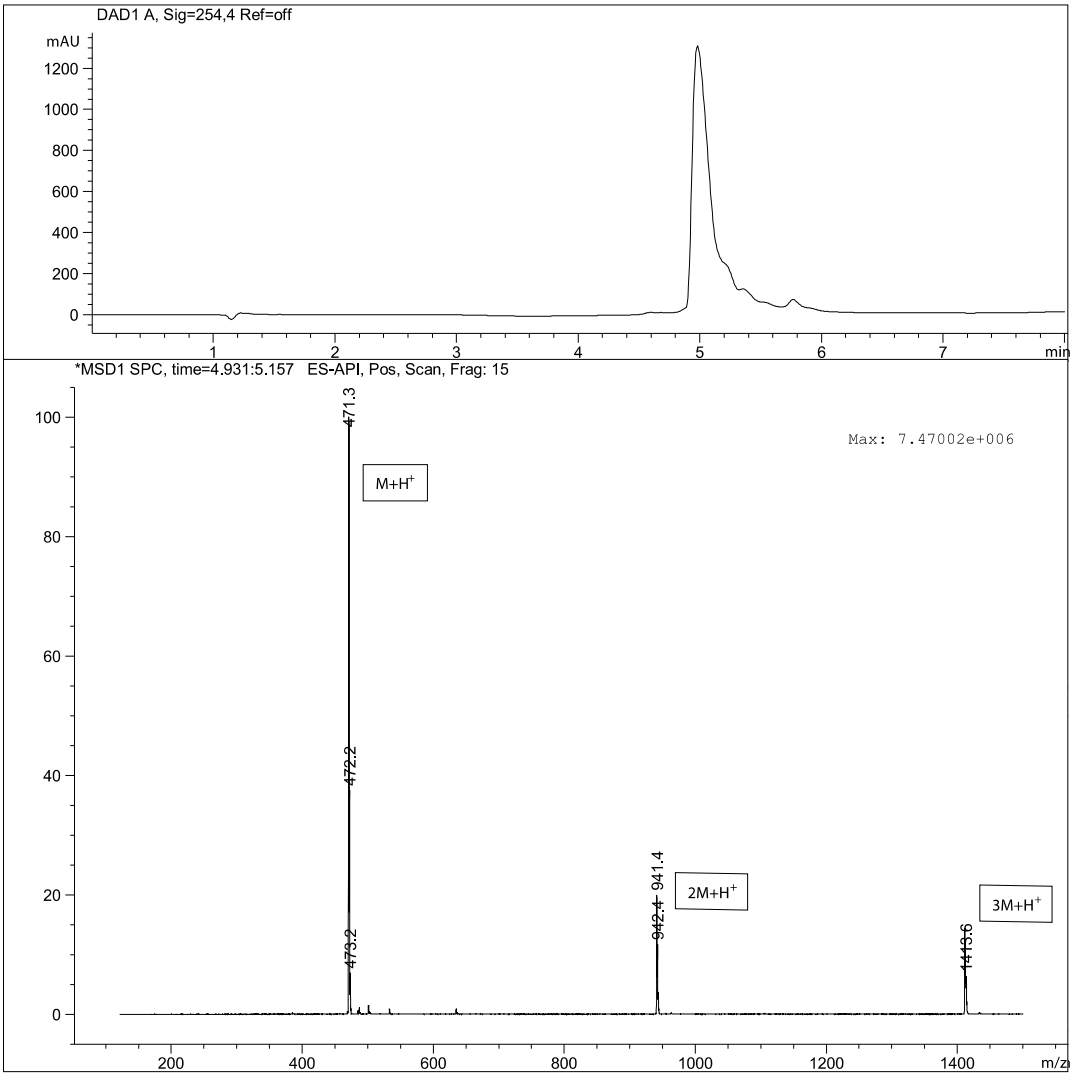

Figure S8. ^{1}H NMR spectrum of 7-hydroxymitragynine

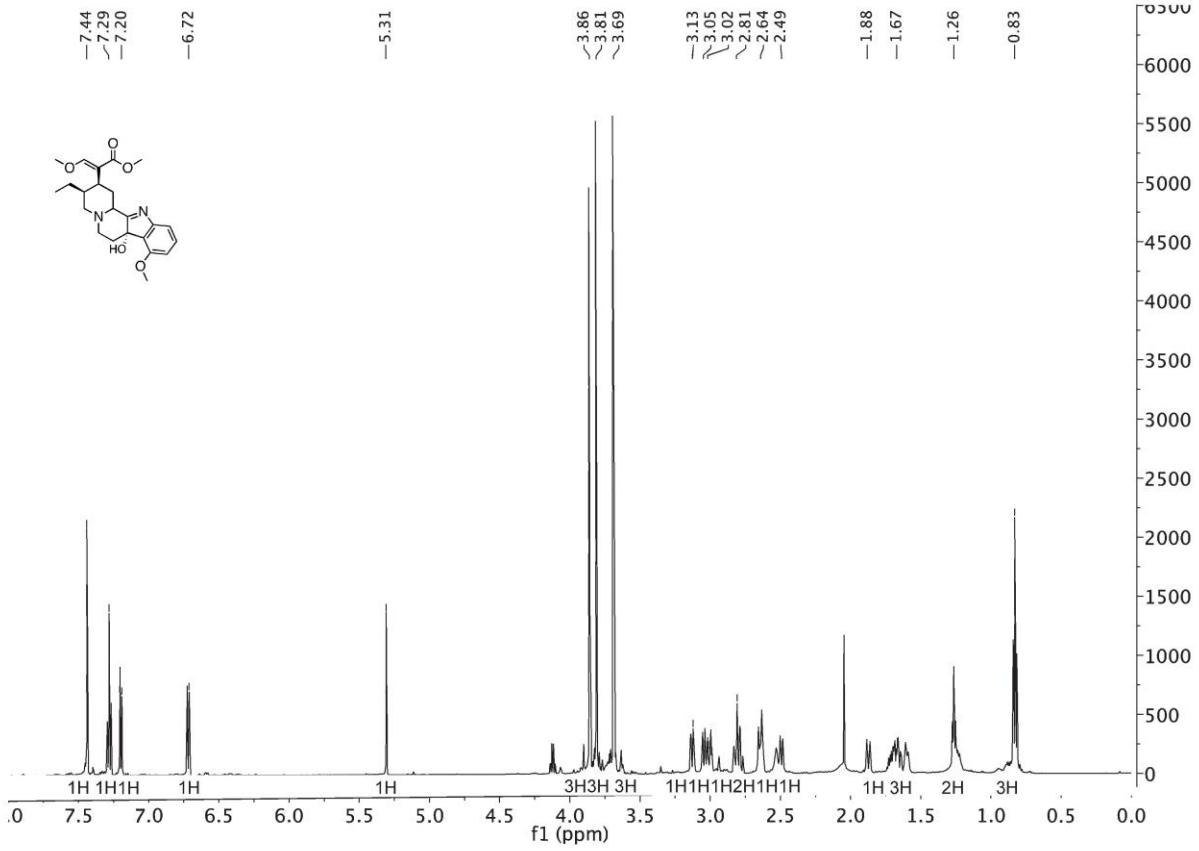

Figure S9. ^{13}C NMR spectrum of 7-hydroxymitragynine

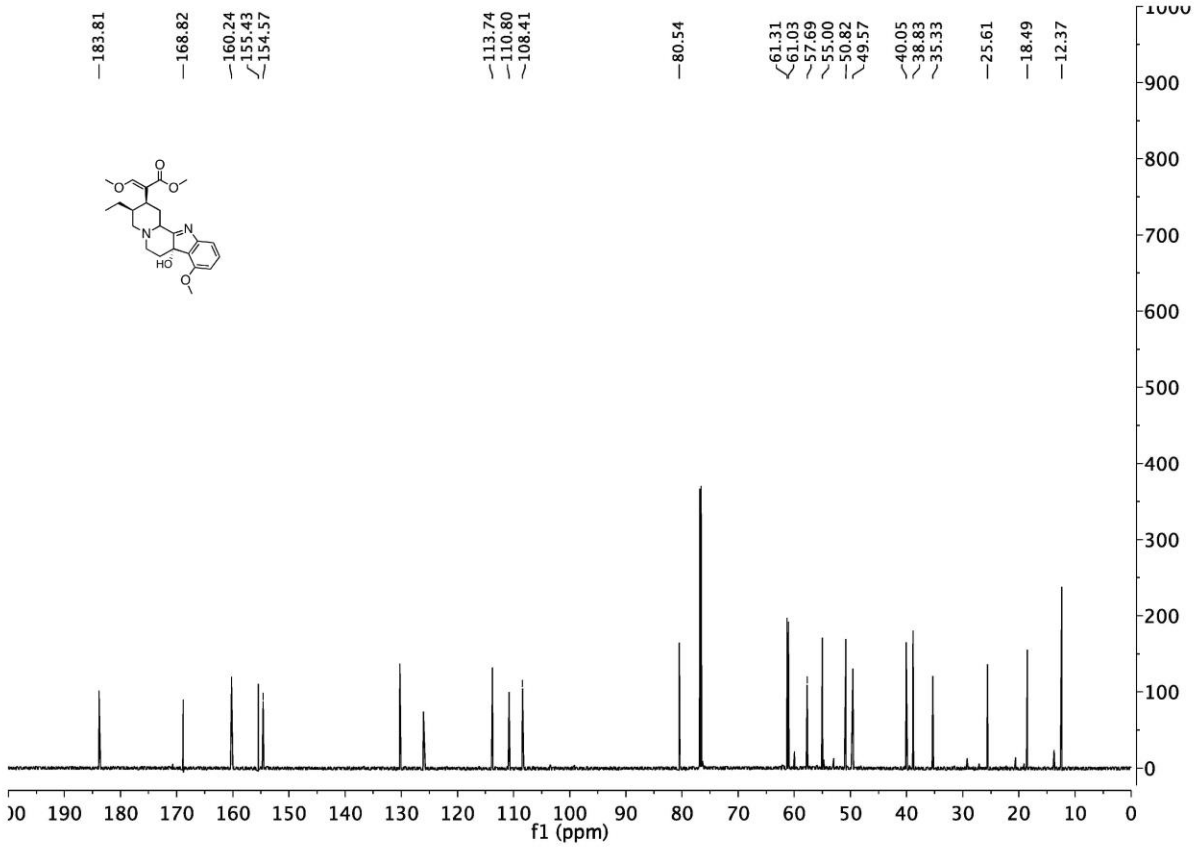

Figure S10. MALDI-TOF MS spectrum of unconjugated BSA

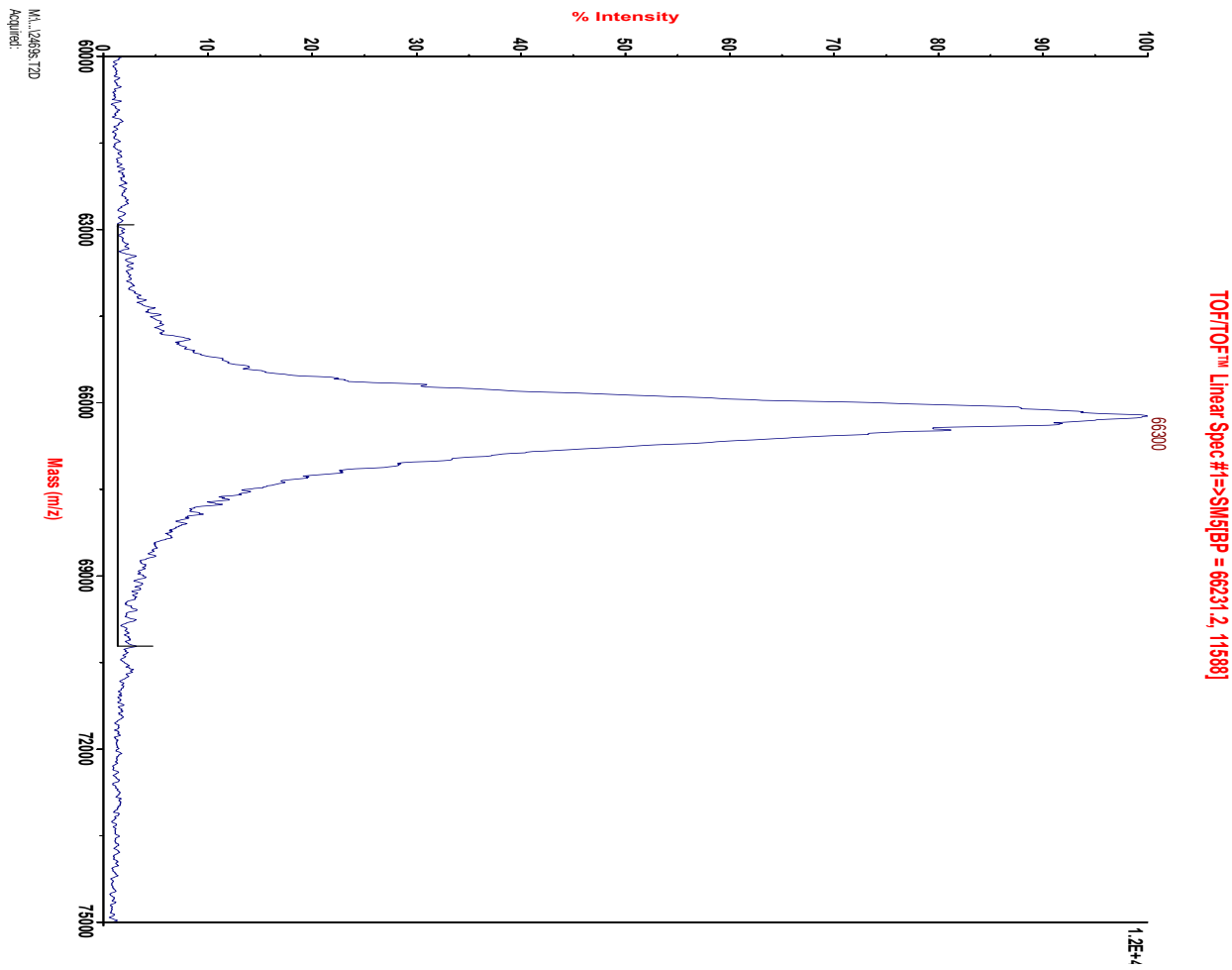

Figure S11. MS-ESI(+) spectrum of unconjugated BSA

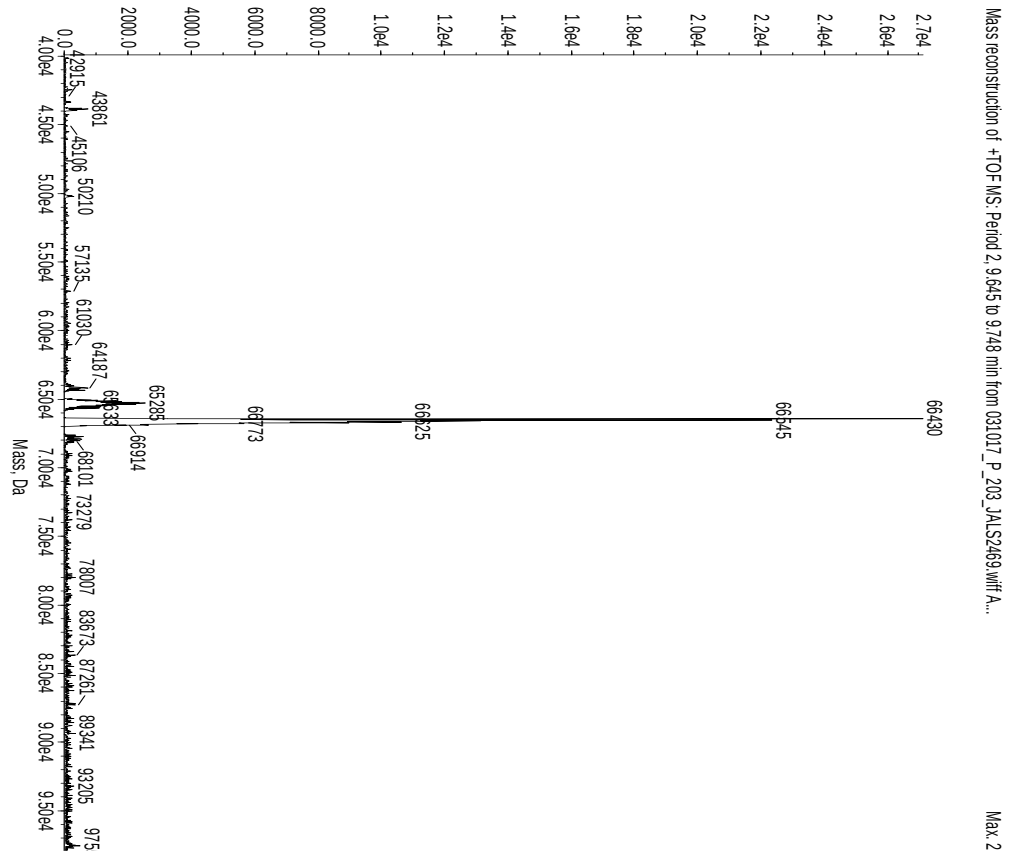

Figure S12. MALDI-TOF MS spectrum of Mit-BSA

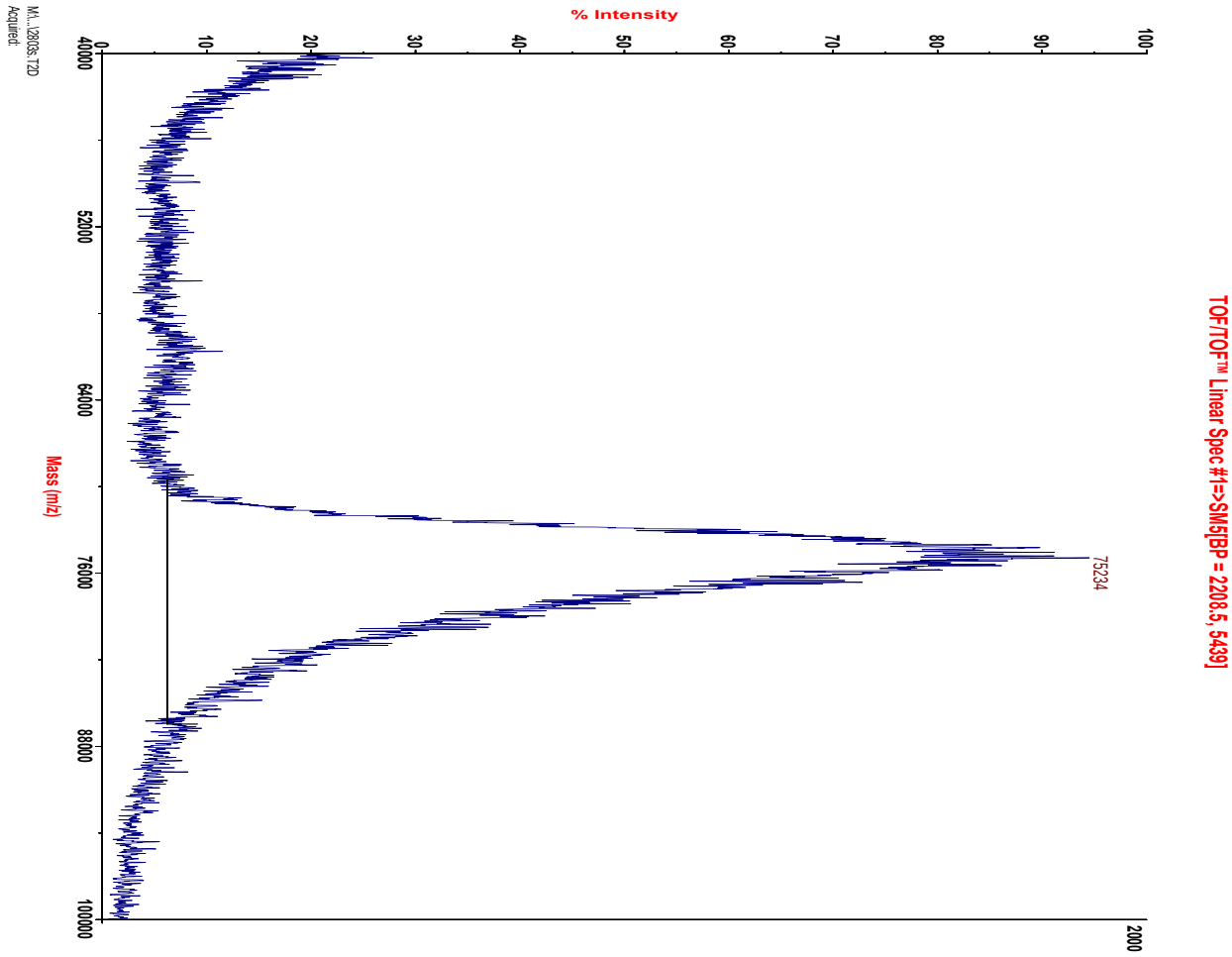

Figure S13. MALDI-TOF MS spectrum of Mit-FliC

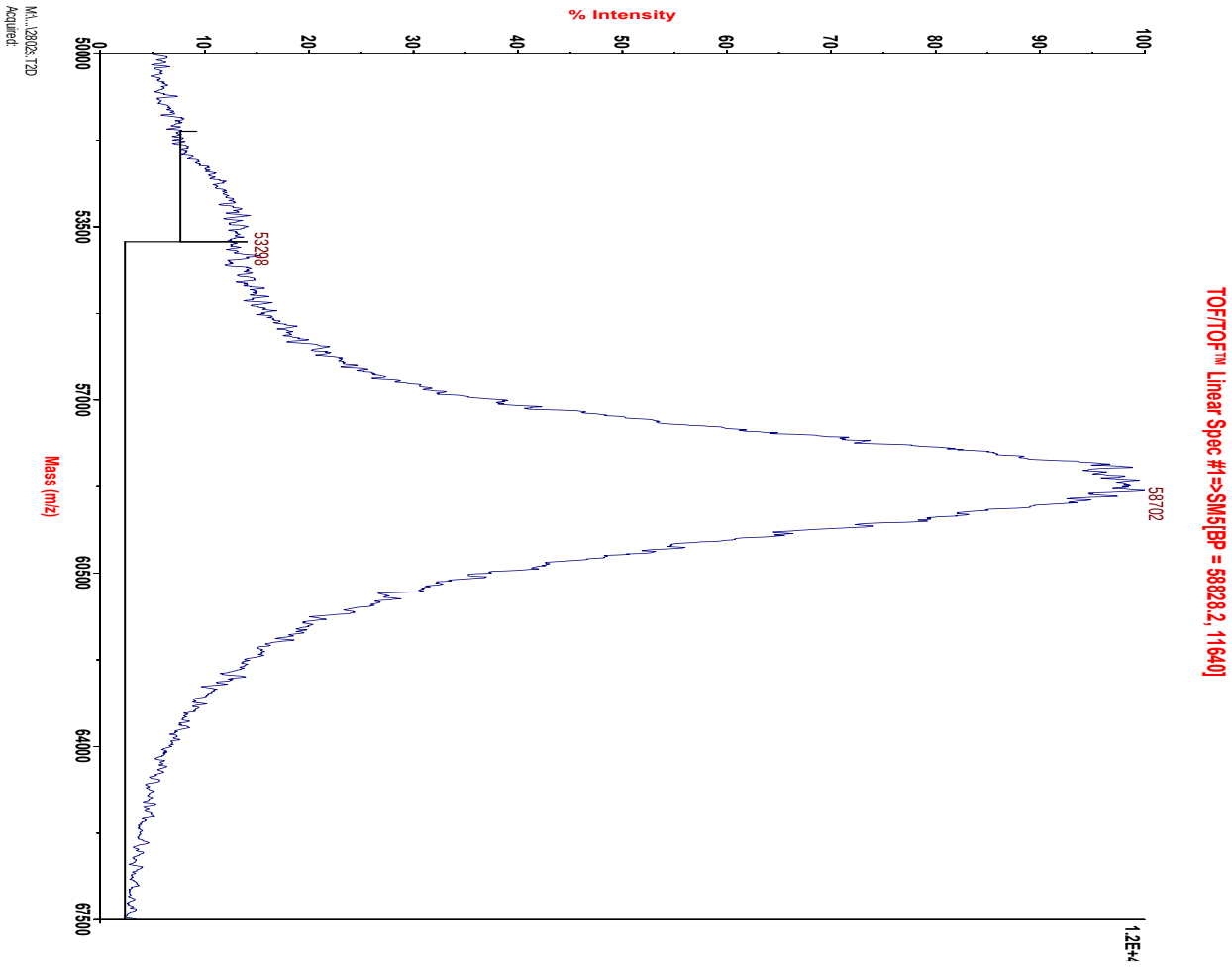

Figure S14. Survival curve and LD_{50} determination of mitragynine administered intravenously

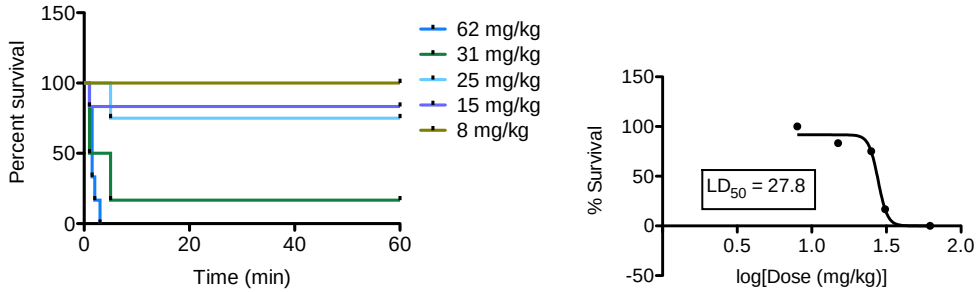

Figure S15. Survival curve and LD_{50} determination of 7-hydroxymitragynine administered intravenously

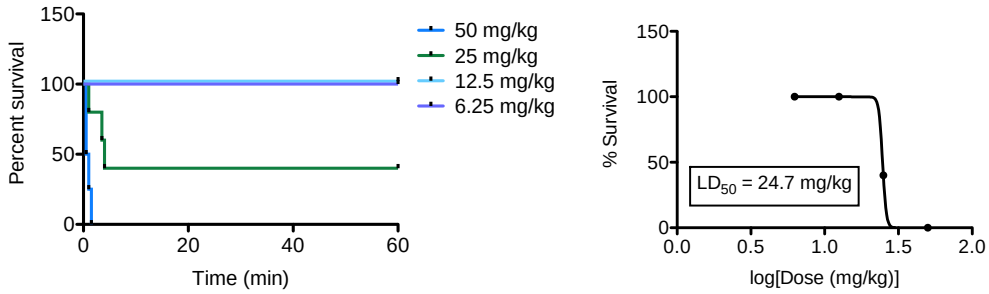

Figure S16. Survival curve and LD_{50} determination of heroin administered intravenously

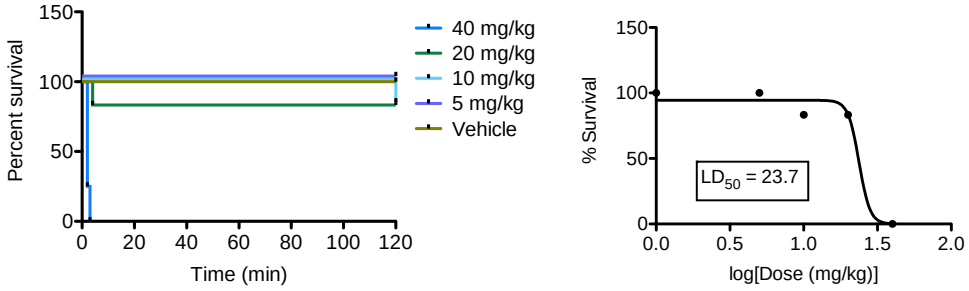

Figure S17. Survival curve and LD_{50} determination of orally administered mitragynine

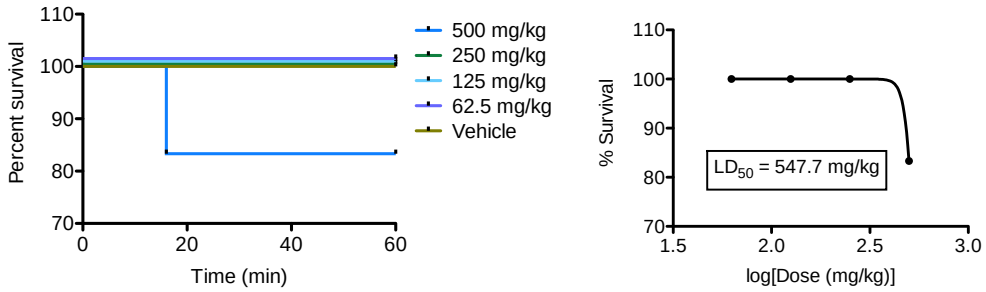

Figure S18. Survival curve of orally administered 7-hydroxymitragynine

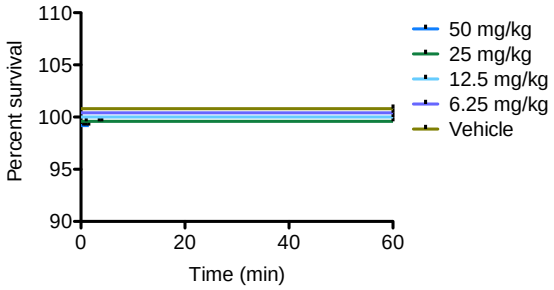

Table S1. Hybridoma panel with midpoint titers and IC_{50} value range

Entry	mAb	Mit-BSA Midpoint Titer	Mit Isotype	GαM Midpoint Titer	GαM Isotype	IC_{50} (μM)
1	1F12	4-8	kg1	256	kg1	1-10
2	7D12	512-1024	lg2b	256-512	k, l, g1, g2b, g3	1-10
3	11C2	1024-2048	lg2b	256-512	k, l, g1, g2b	0.1-1
4	13G12	256	kg1	256-512	kg1	~ 1
5	16A1	64-128	kg2a	64	kg2a	1-10
6	16C7	256+	kg2a	256+	kg2a	>>10
7	17B7	64-128	kg2b	256+	kg1, 2b	0.1-1
8	17E8	256+	kg2a	256	kg2a	>>10
9	17G7	256+	kg2a	128-256	kg2a	>>10
10	17H11	256+	kg1	256+	kg1	1-10
11	19A4	256+	kg2b	256+	kg2b	>>10
12	21B2	64	kg2a	64	kg2a, m	>>10
13	21F10	256+	kg1	64	kg1	1-10
14	22D8	64	kg1	64	kg1	>>10
15	23A4	256+	kg1	256	kg1	>>10
16	23D7	128	kg1	128	kg1	1-10
17	23D9	256+	kg2a	128	kg2a	~ 1
18	24H7	128	kg2a	64	kg2a	~ 1
19	24H8	256	kg1	256	kg1	1-10
20	24H9	256+	kg1	128-256	kg1	>>10
21	25A7	128	kg2a	32-64	kg2a	~ 1
22	25G11	256+	kg1	256+	kg1	~ 1
23	26B8	256+	kg2b	256+	kg2b	>>10
24	27H9	128	kg2a	64	kg2a	1-10
25	28B6	256+	kg1	256+	kg1	>>10
26	28C12	256+	kg2a	128-256	kg2a	~ 1
27	28E1	256	kg1	128	kg1	>>10
28	28E9	128	kg1	64-128	kg1	~ 1
29	28F5	256+	kg1	256+	kg1	>>10
30	28G12	256+	kg2a	128	kg2a	1-10
31	29B7	128-256	kg1	128-256	kg1	0.1-1
32	29F3	256+	lg1	256	lg1	~ 1

GαM – Goat anti Mouse

Table S2. Competitive ELISA IC_{50} values for select hybridomas

mAb	Urine IC_{50} (µg/mL)	Buffer IC_{50} (µg/mL)
11C2	0.037(±0.000)	0.446(±0.251)
17B7	1.45(±0.000)	0.607(±0.321)
29B7	3.28(±2.23)	1.23(±0.000)
13G12	0.376(±0.012)	0.247(±0.021)
23D9	1.50(±0.121)	0.390(±0.006)
24H7	0.186(±0.013)	0.550(±484)
25A7	2.22(±0.781)	0.233(±0.133)
25G11	6.17(±3.27)	0.508(±0.000)
28C12	0.852(±0.05)	0.100(±0.002)
28E9	-	0.569(±0.113)
29F3	0.912(±0.043)	0.098(±0.0004)
17H11	0.997(±0.000)	0.347(±0.028)

Table S3. Post mortem urine mitragynine levels from several toxicology reports

Urine Concentration (µg/mL)	Reference
0.167	Nelsen, J. L., et al, *J Med Toxicol* **2010,** *6* (4), 424-6.
1.2	Holler, J. M., et al, *J Anal Toxicol* **2011,** *35* (1), 54-9.
3.47	Karinen, R., et al, *Forensic Sci Int* **2014,** *245*, e29-32.
0.37	McIntyre, I. M., et al, *J Anal Toxicol* **2015,** *39* (2), 152-5.
>0.4	Domingo, O., et al, *Forensic Sci Int* **2017,** *271*, e1-e7.

Figure S19. Image of lateral flow strip and peak measurement read-out from Leelu

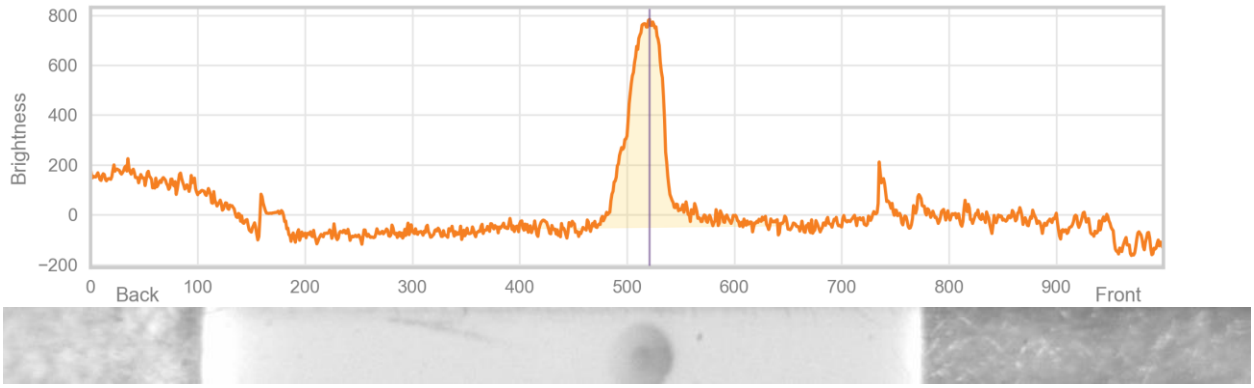

Figure S20. Standard curve for 7-hydroxymitragynine and MIT29B7 strips in human urine

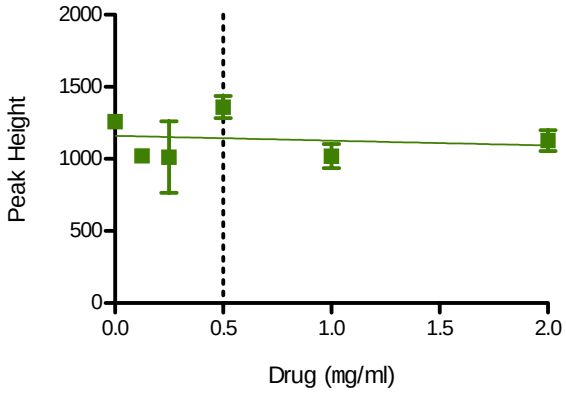

Table S4. Physiochemical properties of mitragynine

Compound	pKa	Method	Source
Mitragynine	8.11 ± 0.11	UV Spectrophotometer	Ramanathan, S.; et. al., Understanding the physicochemical properties of mitragynine, a principal alkaloid of Mitragyna speciosa, for preclinical evaluation. *Molecules* **2015,** *20* (3), 4915-27.
Mitragynine	8.08 ± 0.04	Microplate Spectrophotometer	Ramanathan, S.; et. al., Understanding the physicochemical properties of mitragynine, a principal alkaloid of Mitragyna speciosa, for preclinical evaluation. *Molecules* **2015,** *20* (3), 4915-27.
Compound	**Solubility**	**Solvent**	**Source**
Mitragynine	88.9 ± 1.6 µg/ml	Buffer, pH 7	Ramanathan, S.; et. al., Understanding the physicochemical properties of mitragynine, a principal alkaloid of Mitragyna speciosa, for preclinical evaluation. *Molecules* **2015,** *20* (3), 4915-27.
Mitragynine	64.6 ± 1.2 µg/ml	Water	Ramanathan, S.; et. al., Understanding the physicochemical properties of mitragynine, a principal alkaloid of Mitragyna speciosa, for preclinical evaluation. *Molecules* **2015,** *20* (3), 4915-27.
Compound	**logP**	**logD**	**Source**
Mitragynine	1.70	0.78	Ramanathan, S.; et. al., Understanding the physicochemical properties of mitragynine, a principal alkaloid of Mitragyna speciosa, for preclinical evaluation. *Molecules* **2015,** *20* (3), 4915-27.

Table S5. Bioavailability properties of mitragynine and 7-hydroxymitragynine

Compound	Monolayer permeability (% Transport)	Cell type	Source
Mitragynine (10 μM)	11.4 ± 0.8	Caco-2	Manda, V. K.; et. al., Evaluation of in vitro absorption, distribution, metabolism, and excretion (ADME) properties of mitragynine, 7-hydroxymitragynine, and mitraphylline. *Planta Med* **2014,** *80* (7), 568-76.
Mitragynine (10 μM)	6.8 ± 0.6	MDR-MDCK	Manda, V. K.; et. al., Evaluation of in vitro absorption, distribution, metabolism, and excretion (ADME) properties of mitragynine, 7-hydroxymitragynine, and mitraphylline. *Planta Med* **2014,** *80* (7), 568-76.
7-Hydroxymitragynine (10 μM)	7.8 ± 0.8	Caco-2	Manda, V. K.; et. al., Evaluation of in vitro absorption, distribution, metabolism, and excretion (ADME) properties of mitragynine, 7-hydroxymitragynine, and mitraphylline. *Planta Med* **2014,** *80* (7), 568-76.
7-Hydroxymitragynine (10 μM)	5.6 ± 0.9	MDR-MDCK	Manda, V. K.; et. al., Evaluation of in vitro absorption, distribution, metabolism, and excretion (ADME) properties of mitragynine, 7-hydroxymitragynine, and mitraphylline. *Planta Med* **2014,** *80* (7), 568-76.

Compound	Metabolism ($t_{1/2}$)	Method	Source
Mitragynine (1.5 mg/kg)	2.9 ± 2.1 hr	IV delivery in Rats	Parthasarathy, S.; et. al., Determination of mitragynine in plasma with solid-phase extraction and rapid HPLC-UV analysis, and its application to a pharmacokinetic study in rat. *Anal Bioanal Chem* **2010,** *397* (5), 2023-30.
Mitragynine (50 mg/kg)	6.6 ± 1.3 hr	Oral delivery in Rats	Parthasarathy, S.; et. al., Determination of mitragynine in plasma with solid-phase extraction and rapid HPLC-UV analysis, and its application to a pharmacokinetic study in rat. *Anal Bioanal Chem* **2010,** *397* (5), 2023-30.
7-Hydroxymitragynine	24 min	Human liver microsomes	Manda, V. K.; et. al., Evaluation of in vitro absorption, distribution, metabolism, and excretion (ADME) properties of mitragynine, 7-hydroxymitragynine, and mitraphylline. *Planta Med* **2014,** *80* (7), 568-76.

Compound	Stability in SGF (%RD)	Incubation Time	Source
Mitragynine	-22.5%	30	Ramanathan, S.; et. al., Understanding the physicochemical properties of mitragynine, a principal alkaloid of Mitragyna speciosa, for preclinical evaluation. *Molecules* **2015,** *20* (3), 4915-27.
Mitragynine	-8.9%	30	Manda, V. K.; et. al., Evaluation of in vitro absorption, distribution, metabolism, and excretion (ADME) properties of mitragynine, 7-hydroxymitragynine, and mitraphylline. *Planta Med* **2014,** *80* (7), 568-76.
7-Hydroxymitragynine	-7.6%	30	Manda, V. K.; et. al., Evaluation of in vitro absorption, distribution, metabolism, and excretion (ADME) properties of mitragynine, 7-hydroxymitragynine, and mitraphylline. *Planta Med* **2014,** *80* (7), 568-76.

Compound	Stability in SIF (%RD)	Incubation Time	Source
Mitragynine	+4.43%	30	Ramanathan, S.; et. al., Understanding the physicochemical properties of mitragynine, a principal alkaloid of Mitragyna speciosa, for preclinical evaluation. *Molecules* **2015,** *20* (3), 4915-27.
Mitragynine	-0.96%	30	Manda, V. K.; et. al., Evaluation of in vitro absorption, distribution, metabolism, and excretion (ADME) properties of mitragynine, 7-hydroxymitragynine, and mitraphylline. *Planta Med* **2014,** *80* (7), 568-76.
7-Hydroxymitragynine	-1.23%	30	Manda, V. K.; et. al., Evaluation of in vitro absorption, distribution, metabolism, and excretion (ADME) properties of mitragynine, 7-hydroxymitragynine, and mitraphylline. *Planta Med* **2014,** *80* (7), 568-76.

Compound	Plasma Protein Binding (% Free Drug Concentration)	P-glycoprotein inhibition (EC_{50})	Source
Mitragynine (15 μM)	6.2 ± 1.3	18.2 ± 3.6 μM	Manda, V. K.; et. al., Evaluation of in vitro absorption, distribution, metabolism, and excretion (ADME) properties of mitragynine, 7-hydroxymitragynine, and mitraphylline. *Planta Med* **2014,** *80* (7), 568-76.
7-Hydroxymitragynine (15 μM)	10.3 ± 1.5	32.4 ± 1.9 μM	Manda, V. K.; et. al., Evaluation of in vitro absorption, distribution, metabolism, and excretion (ADME) properties of mitragynine, 7-hydroxymitragynine, and mitraphylline. *Planta Med* **2014,** *80* (7), 568-76.