Phase I Metabolism of Desomorphine

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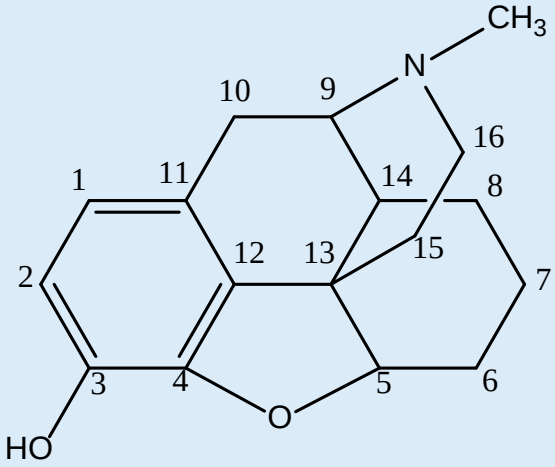
- The authors have no commercial disclosures

Desomorphine

- A semi-synthetic derivative of morphine
  - First synthesized in the early 1900s
- Narcotic analgesic
  - Mu receptor agonist



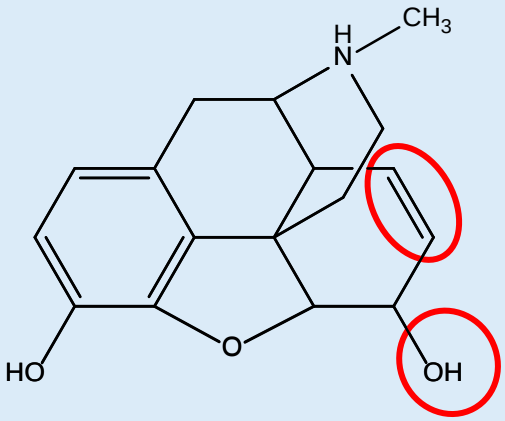
- Produced for a short time as a prescription pain killer
  - Permanently withdrawn from the market in 1952
  - Currently regarded worldwide as having no medical use
- Became a drug of abuse in the 2000s
  - Severe side effects include gangrene and necrotic ulcers

Pharmacology

- Animals
  - Desomorphine showed 10 times the depressant effects of morphine
  - Those dosed with desomorphine experienced less vomiting that those dosed with morphine
  - LD_{50} of pure desomorphine was found to be 27 mg/kg, greater than heroin but much less than morphine and codeine

- Humans
  - Duration of effect lasted no more than 3 hours regardless of dose and the typical abstinence syndrome of opioids observed

Phenanthrene-Type Opioids

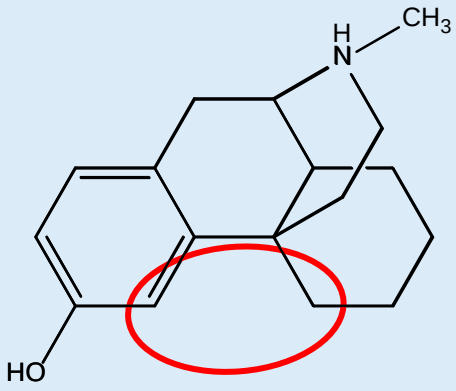


Normorphine

Morphine

CYP3A4

Projean et al 2003

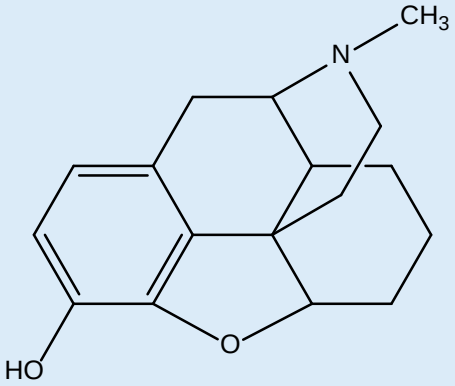


Norlevorphanol

Levorphanol

CYP3A4

Misra et al 1974



Desomorphine

Metabolism

- **Richter et al 2016**: supersomes, human liver microsomes, human liver cytosol and rat studies

- Phase I metabolites identified in HLMs:
  - Nordesomorphine, desomorphine-N-oxide, 5 hydroxydesomorphine isomers

- Recombinant human P450s investigated:
  - rCYP1A2, rCYP2A4, rCYP2B6, rCYP2C8, rCYP2C9, rCYP2C19, rCYP2D6, rCYP2E1, rCYP3A4, rCYP3A5

- **Metabolic activity observed only in rCYP3A4**
  - Formed nordesomorphine, desomorphine-*N*-oxide, 3 hydroxydesomorphine isomers

Reagents

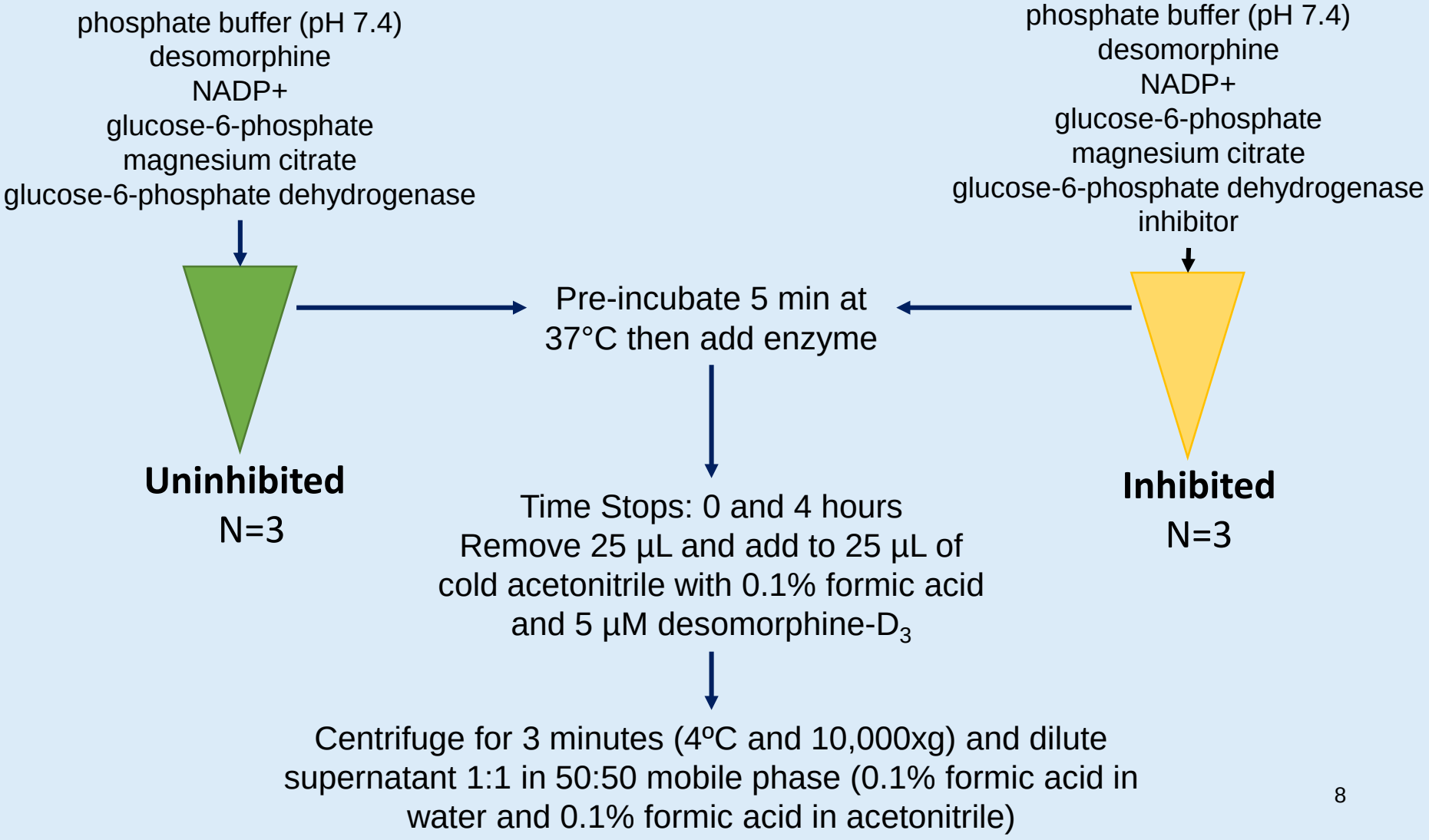
General Reaction

- Bactosomes – eight P450s
  - rCYP1A2, rCYP2B6, rCYP2C8, rCYP2C9, rCYP2C18, rCYP2C19, rCYP2D6, and rCYP3A4
- Control bactosomes (no CYP gene)
- Blanks (enzyme added but no drug)
- 500 µL reaction volume
  - 200 µM desomorphine
  - 50 pmol/mL enzyme
  - 100 µM pH 7.4 potassium phosphate buffer
  - 1.3 mM NADP+
  - 3.3 mM glucose-6-phosphate and magnesium citrate
  - 0.4 U/mL glucose-6-phosphate dehydrogenase

Inhibitors

- Ketoconazole – rCYP3A4, rCYP2C9, rCYP2C18, rCYP2C19
  - 20 µM in reaction
- Fluvoxamine – rCYP2D6, rCYP1A2
  - 20 µM in reaction
- Ticlopidine – rCYP2C8
  - 10 µM in reaction
- Montelukast – rCYP2B6
  - 10 µM in reaction

Incubation Setup



LC/Q-TOF-MS Conditions

Agilent Technologies 6530 Accurate-Mass Q-TOF LC/MS

LC Separation

- Poroshell 120 EC-C18 Column (2.1x100 mm, 2.7 µm)
- Mobile Phase A: 0.1% FA in diH_2O
- Mobile Phase B: 0.1% FA in ACN
- Flow Rate: 0.30 mL/min
- LC Gradient: 90% A (2 min), 63% A (6 min), 10% A (8 min)

Q/TOF Parameters

- Gas Temperature: 150°C
- Gas Flow Rate: 13 L/min
- Sheath Gas Temperature: 200°C
- Sheath Gas Flow Rate: 12 L/min
- Nebulizer Pressure 45 psig

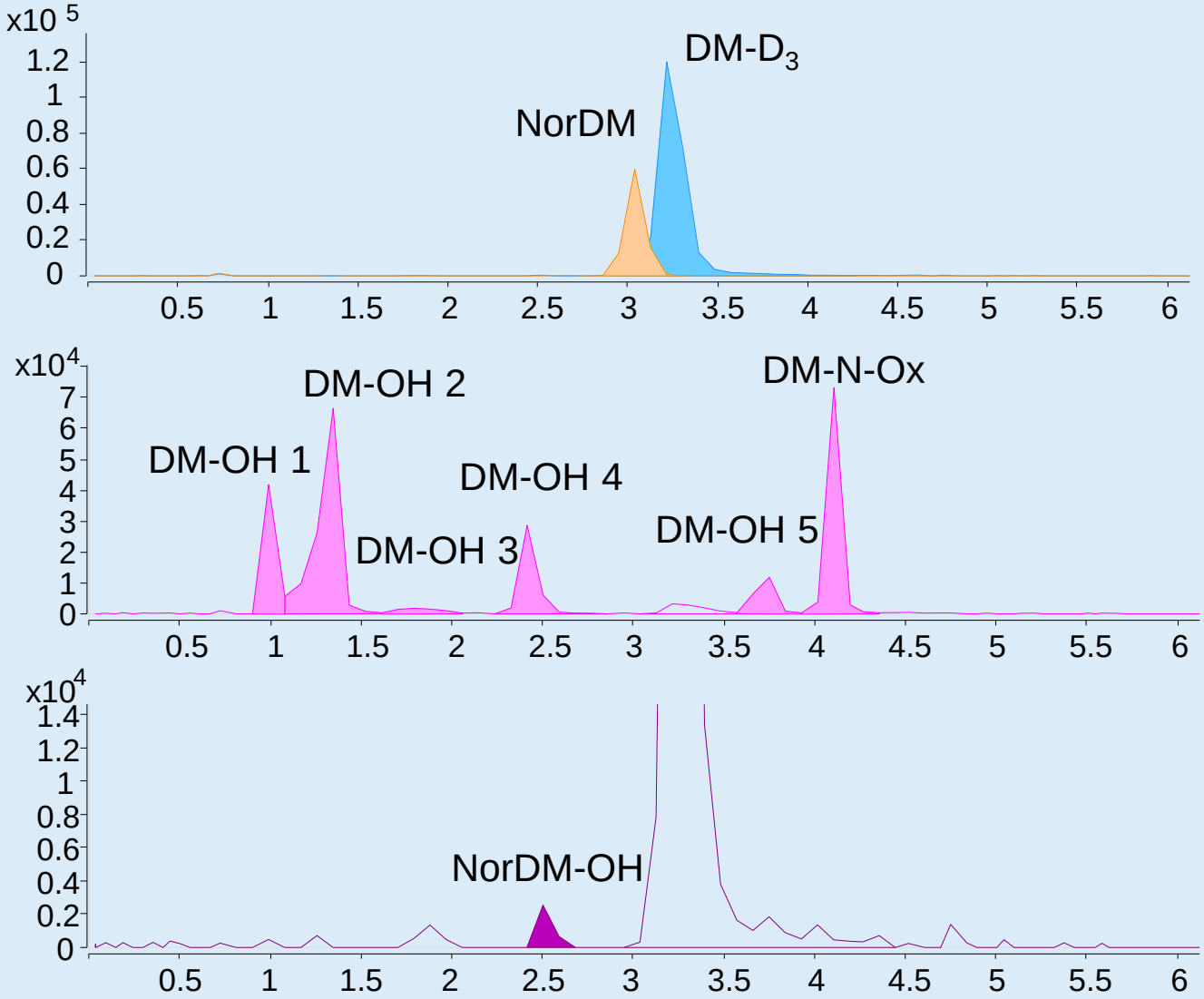
Mass Spectrometry

- Capillary Voltage: 4000 V
- Fragmentor Voltage: 150 V
- Nozzle Voltage: 1000 V
- Collision Energy: 30 eV, 40 eV, 50 eV
- MS Scan Rate: 8 spectra/sec
- MS/MS Scan Rate: 3 spectra/sec
- MS Scan Range: 100-1000 m/z
- ESI Mode: Positive

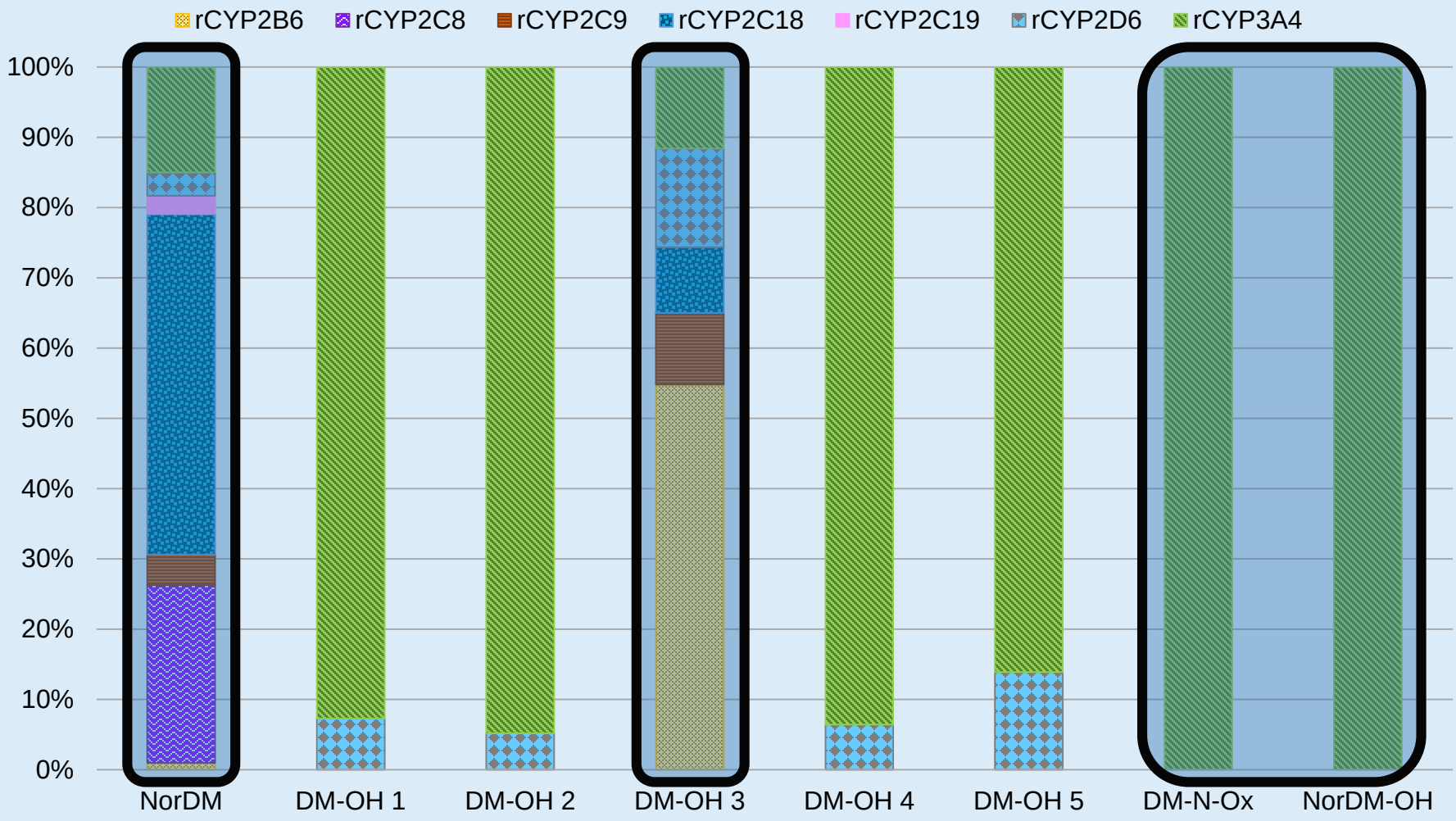
Acquisition

- Full Scan Auto
- Run Time: 8 minutes

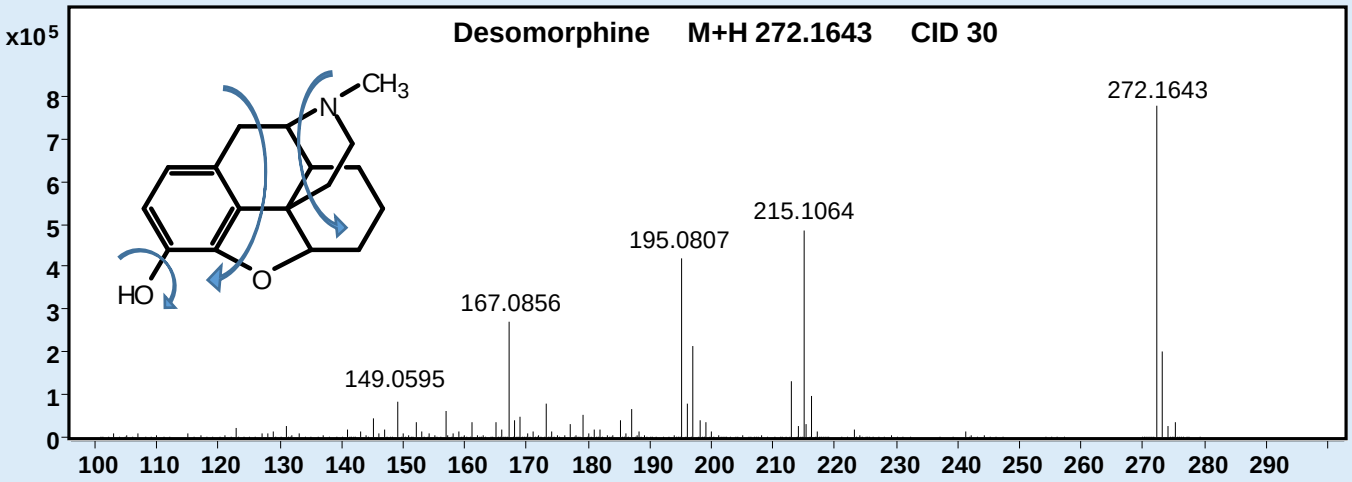
Extracted Ion Chromatograms



CYP Activity

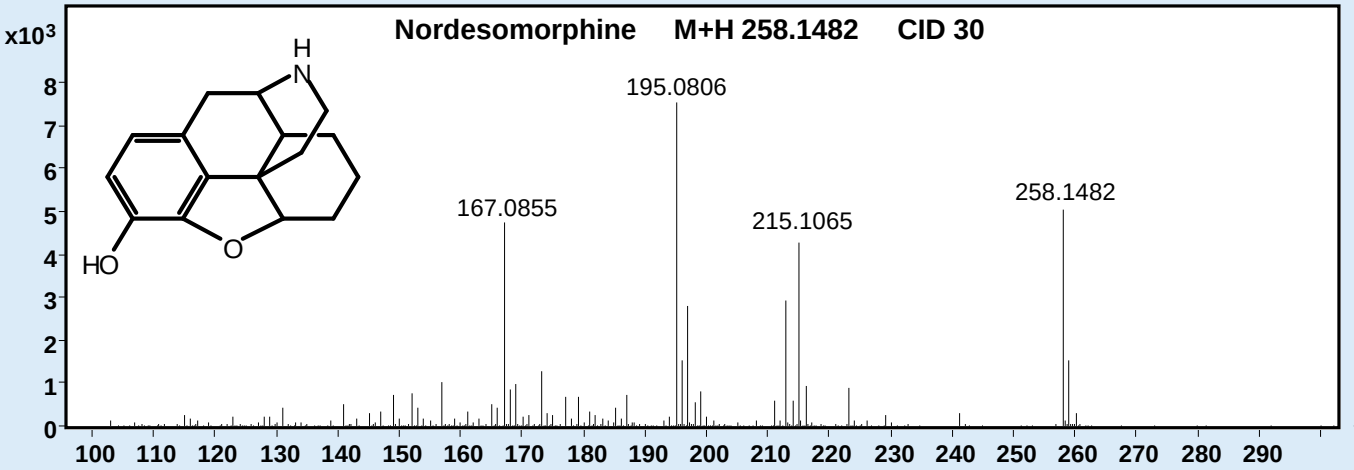


Desomorphine MS^2



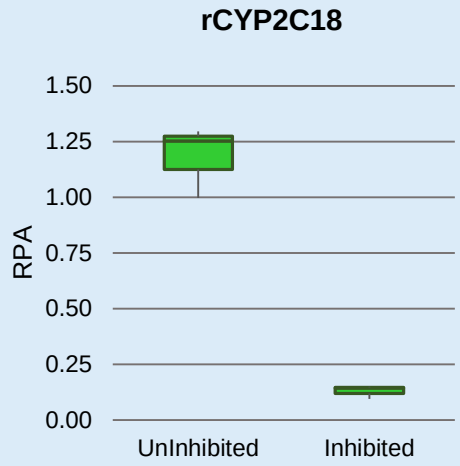
M/Z	Chemical Formula	Exact Mass	Accurate Mass	PPM
272	$C_{17}H_{22}NO_2^+$	272.1645	272.1643	0.91
215	$C_{14}H_{15}O_2^+$	215.1067	215.1064	1.00
195	$C_{14}H_{11}O^+$	195.0804	195.0807	1.08
167	$C_{13}H_{11}^+$	167.0855	167.0856	0.53
149	$C_9H_9O_2^+$	149.0597	149.0595	1.52

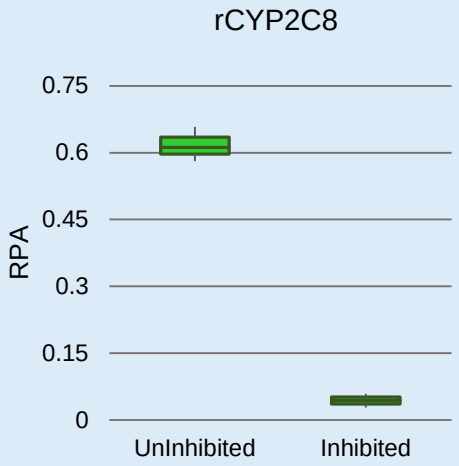
Nordesomorphine MS^2

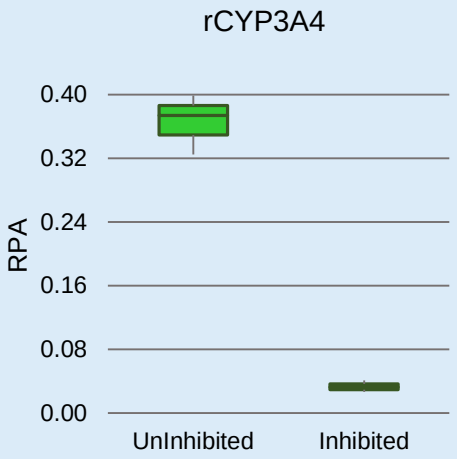


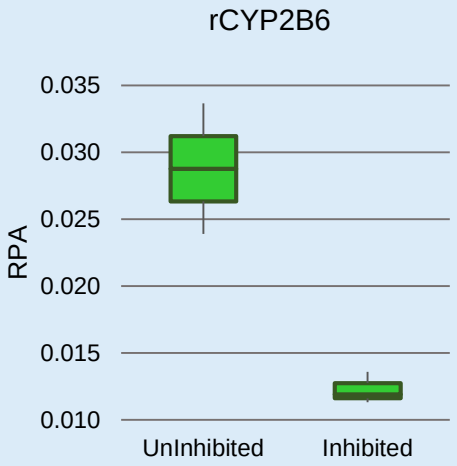
M/Z	Chemical Formula	Exact Mass	Accurate Mass	PPM
258	$C_{16}H_{20}NO_2^+$	258.1489	258.1482	2.54
215	$C_{14}H_{15}O_2^+$	215.1067	215.1065	0.74
195	$C_{14}H_{11}O^+$	195.0804	195.0806	1.04
167	$C_{13}H_{11}^+$	167.0855	167.0855	0.19

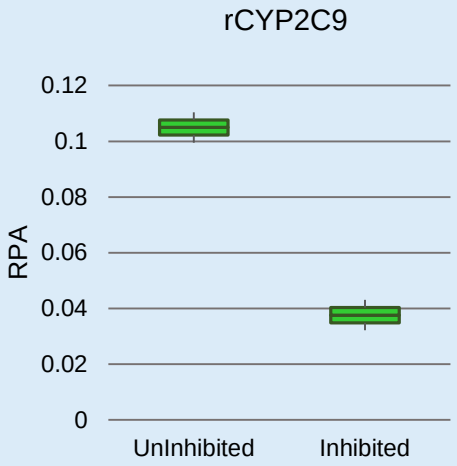
Nordesomorphine

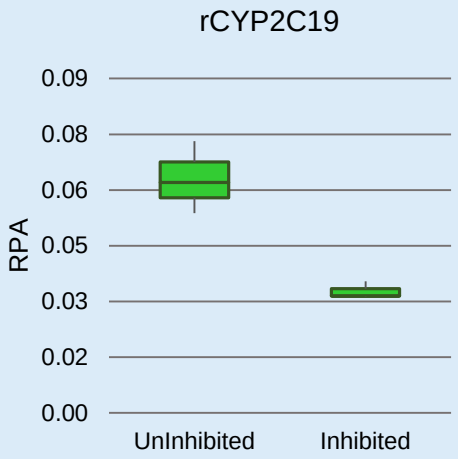


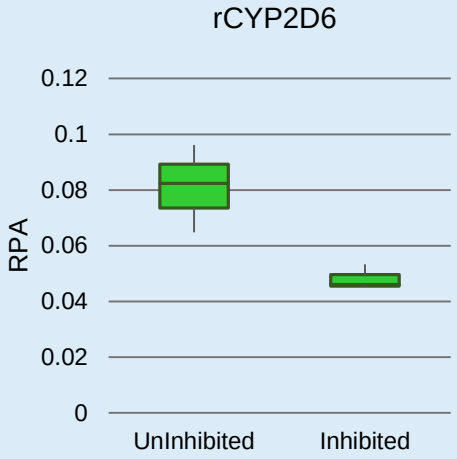




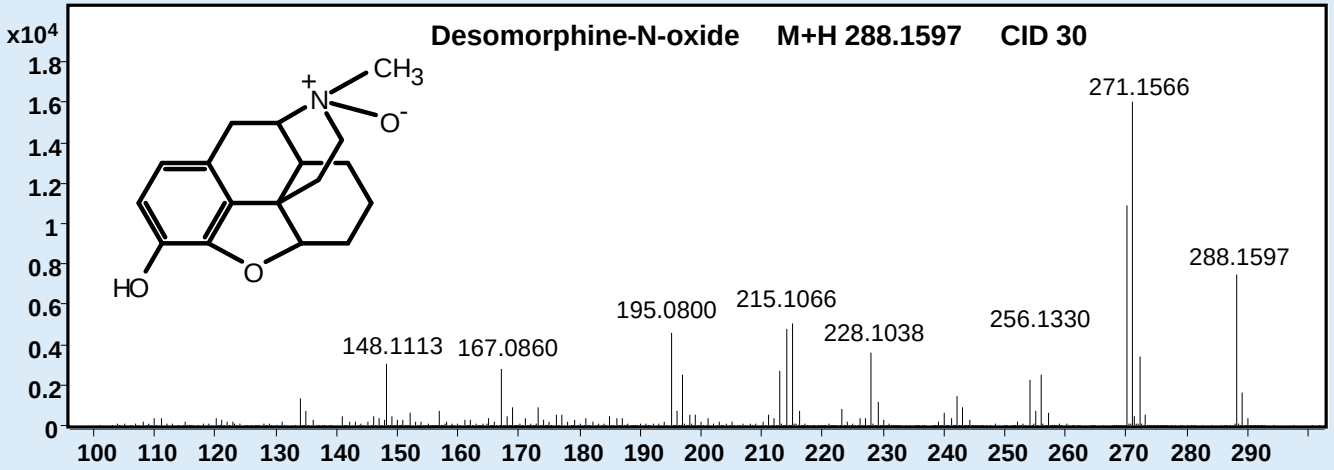




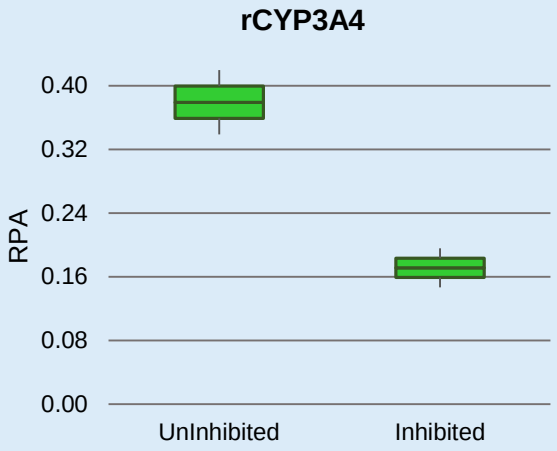




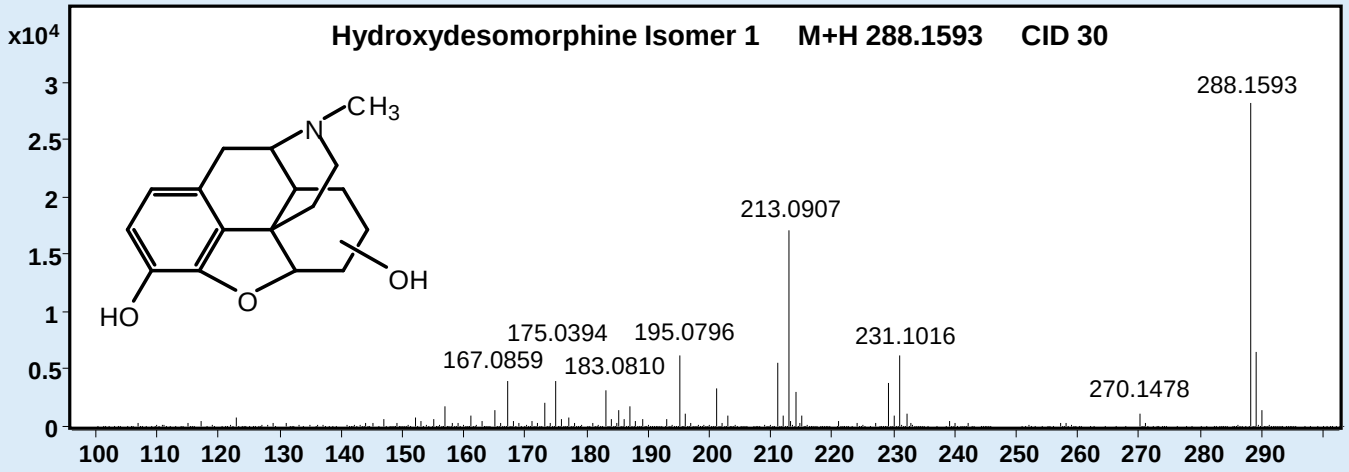
Desomorphine-N-oxide MS^2

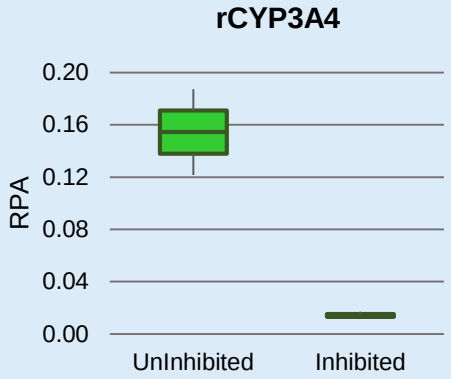


M/Z	Chemical Formula	Exact Mass	Accurate Mass	PPM
288	$C_{17}H_{22}NO_3^+$	288.1594	288.1597	1.11
271	$C_{17}H_{21}NO_2^+$	271.1567	271.1566	0.27
256	$C_{16}H_{18}NO_2^+$	256.1332	256.1330	0.77
228	$C_{14}H_{14}NO_2^+$	228.1019	228.1038	8.34
215	$C_{14}H_{15}O_2^+$	215.1067	215.1066	0.42
195	$C_{14}H_{11}O^+$	195.0804	195.0800	2.40
167	$C_{13}H_{11}^+$	167.0856	167.0860	2.88
148	$C_{10}H_{14}N^+$	148.1121	148.1113	5.46

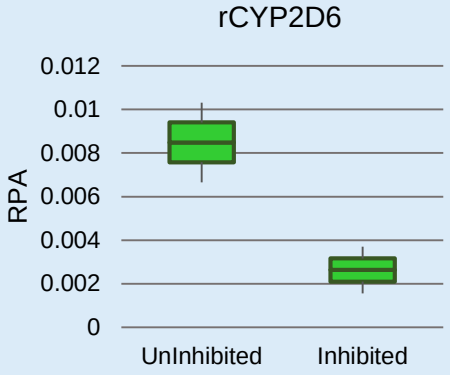


Hydroxydesomorphine Isomer 1 MS^2

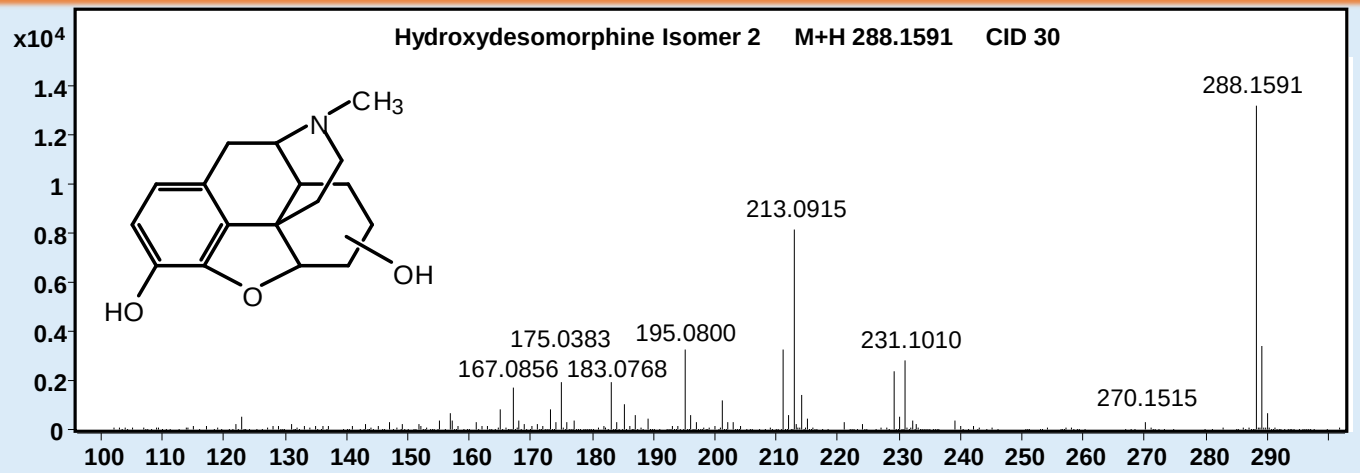




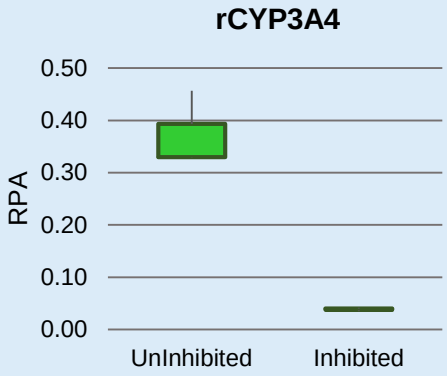
M/Z	Chemical Formula	Exact Mass	Accurate Mass	PPM
288	$C_{17}H_{22}NO_3^+$	288.1594	288.1593	0.25
270	$C_{17}H_{20}NO_2^+$	270.1489	270.1478	3.78
231	$C_{14}H_{15}O_3^+$	231.1016	231.1016	0.06
213	$C_{14}H_{13}O_2^+$	213.0910	213.0907	1.40
195	$C_{14}H_{11}O^+$	195.0804	195.0796	4.33
183	$C_{13}H_{11}O^+$	183.0804	183.0810	3.09
175	$C_{10}H_7O_3^+$	175.0390	175.0394	2.62
167	$C_{13}H_{11}^+$	167.0855	167.0859	2.02

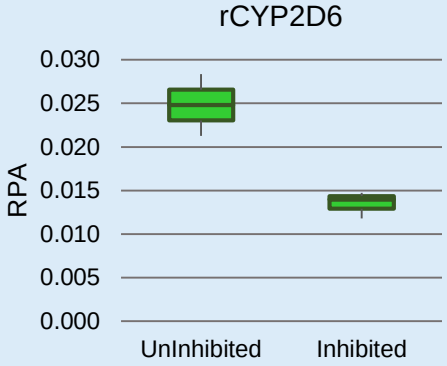


Hydroxydesomorphine Isomer 2 MS^2

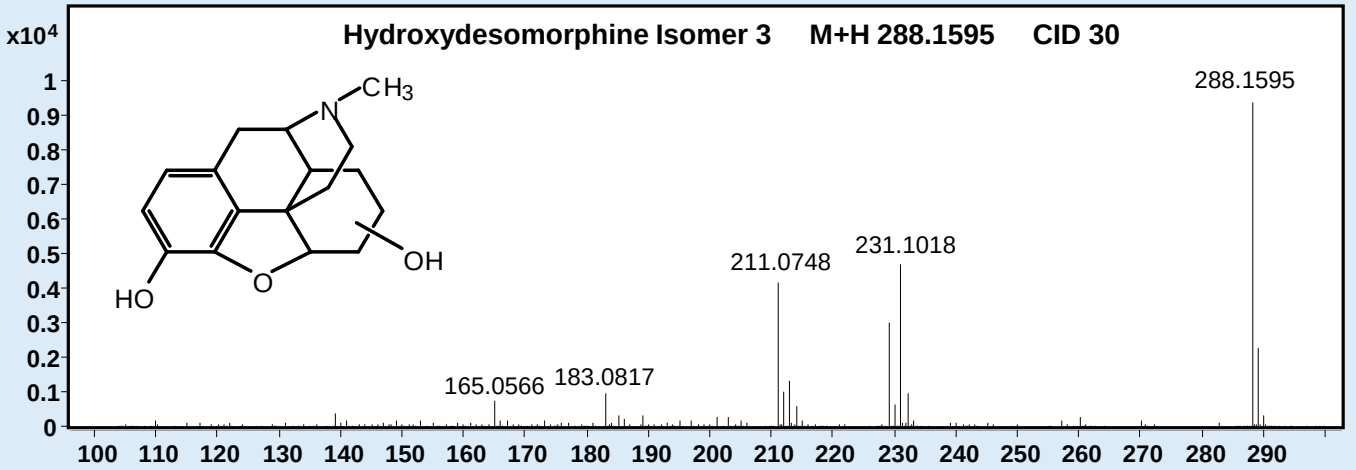


M/Z	Chemical Formula	Exact Mass	Accurate Mass	PPM
288	$C_{17}H_{22}NO_3^+$	288.1594	288.1591	1.07
270	$C_{17}H_{20}NO_2^+$	270.1489	270.1515	9.88
231	$C_{14}H_{15}O_3^+$	231.1016	231.1010	2.33
213	$C_{14}H_{13}O_2^+$	213.0910	213.0915	2.53
195	$C_{14}H_{11}O^+$	195.0804	195.0800	2.10
183	$C_{13}H_{11}O^+$	183.0804	183.0768	19.69
175	$C_{10}H_7O_3^+$	175.0390	175.0383	3.72
167	$C_{13}H_{11}^+$	167.0855	167.0856	0.59



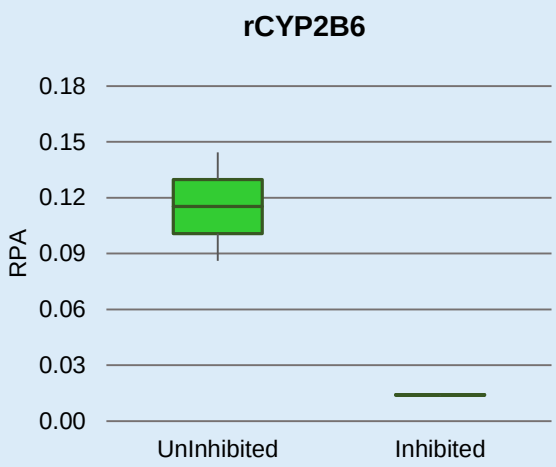


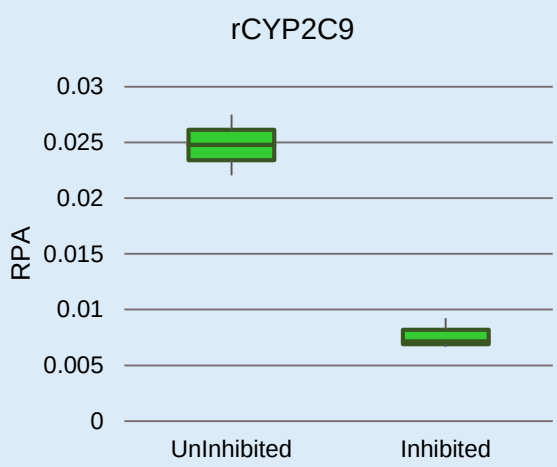
Hydroxydesomorphine Isomer 3 MS^2

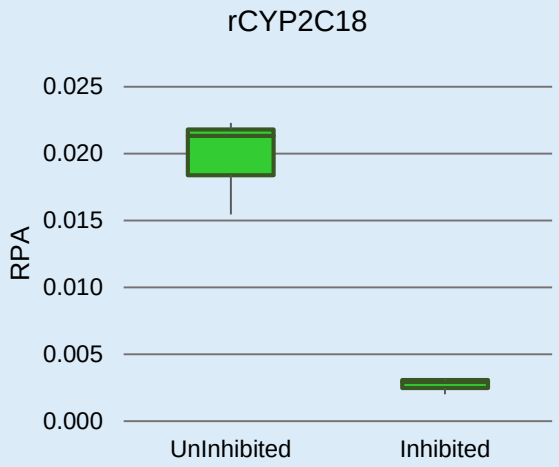


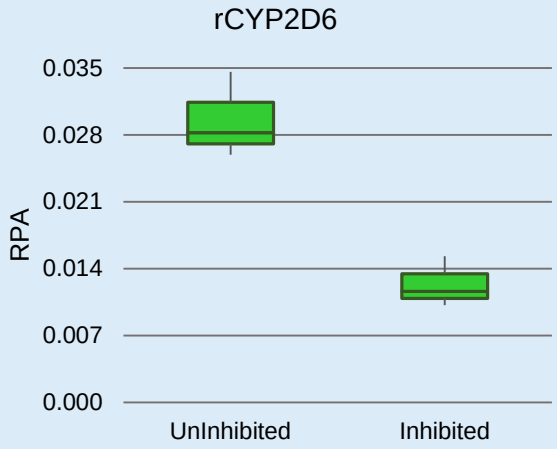
M/Z	Chemical Formula	Exact Mass	Accurate Mass	PPM
288	$C_{17}H_{22}NO_3^+$	288.1594	288.1595	0.38
231	$C_{14}H_{15}O_3^+$	231.1016	231.1018	0.92
211	$C_{14}H_{11}O_2^+$	211.0754	211.0748	2.67
183	$C_{13}H_{11}O^+$	183.0804	183.0817	7.09
165	$C_9H_9O_3^+$	165.0566	165.0583	0.99

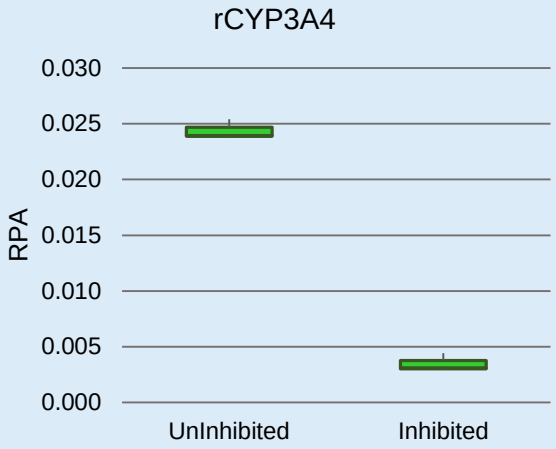
Hydroxydesomorphine Isomer 3



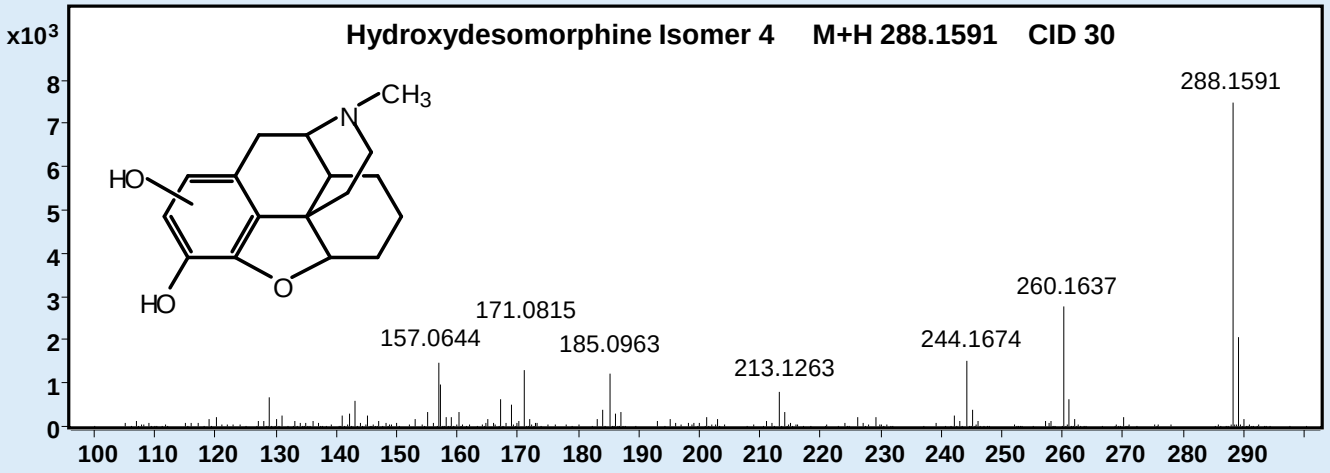


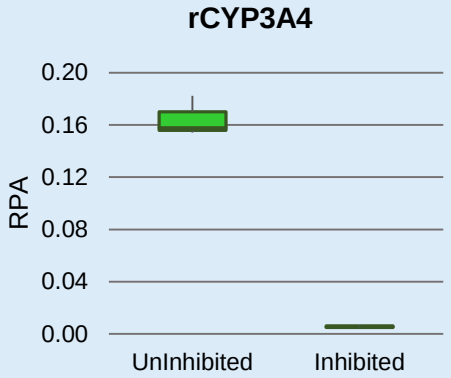


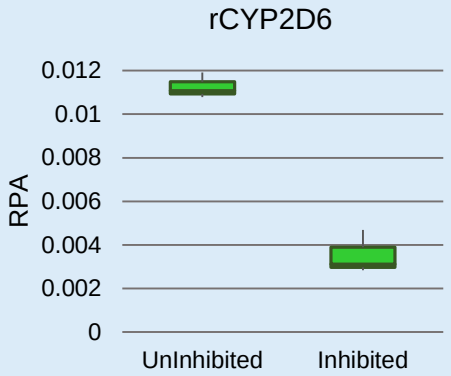




Hydroxydesomorphine Isomer 4 MS^2

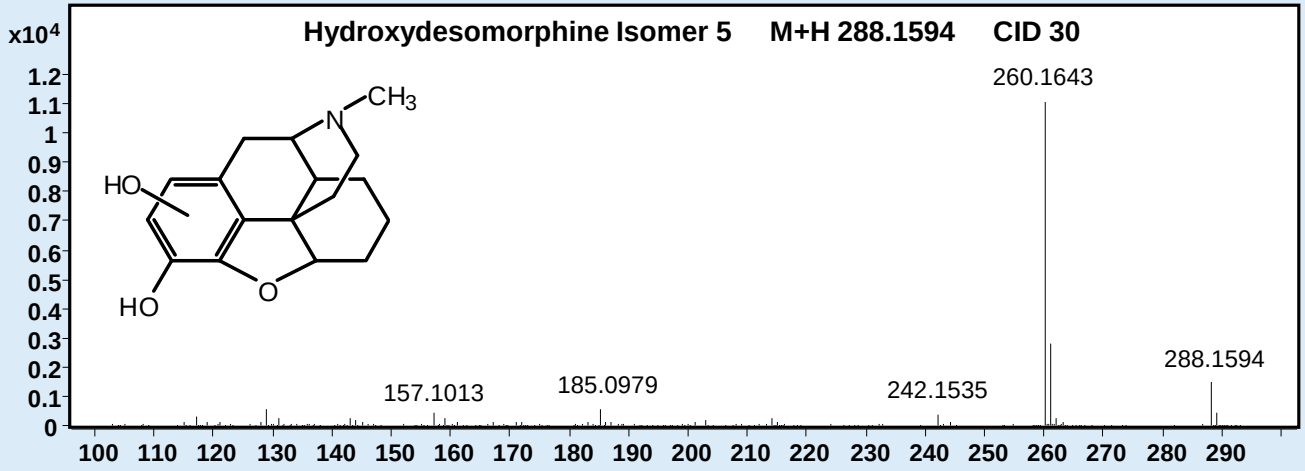




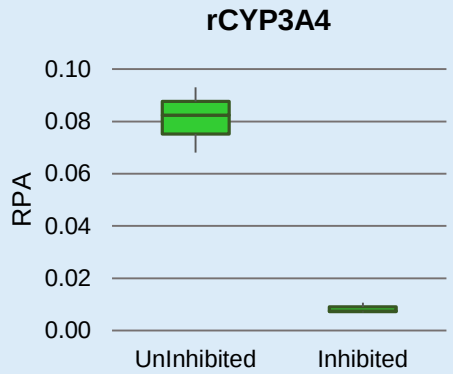


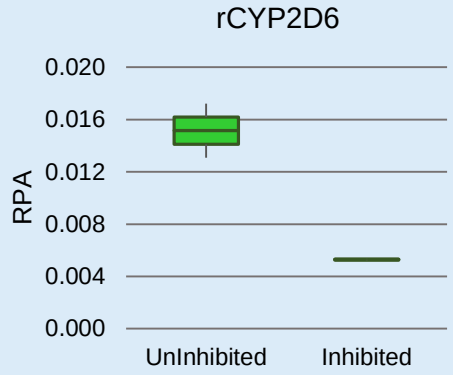
M/Z	Chemical Formula	Exact Mass	Accurate Mass	PPM
288	$C_{17}H_{22}NO_3^+$	288.1594	288.1591	1.20
260	$C_{16}H_{22}NO_2^+$	260.1645	260.1637	2.92
244	$C_{16}H_{22}NO^+$	244.1696	244.1674	8.92
213	$C_{15}H_{17}O^+$	213.1274	213.1263	5.19
185	$C_{13}H_{13}O^+$	185.0961	185.0963	1.19
171	$C_{12}H_{11}O^+$	171.0804	171.0815	6.45
157	$C_{11}H_9O^+$	157.0648	157.0644	2.69

Hydroxydesomorphine Isomer 5 MS^2

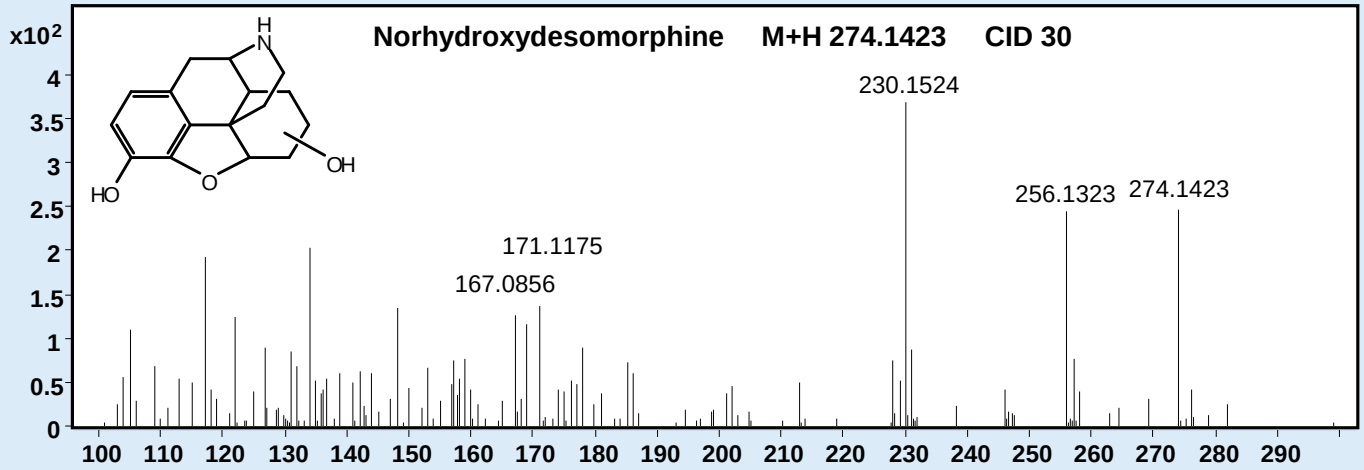


M/Z	Chemical Formula	Exact Mass	Accurate Mass	PPM
288	$C_{17}H_{22}NO_3^+$	288.1594	288.1594	0.03
260	$C_{16}H_{22}NO_2^+$	260.1645	260.1643	0.62
242	$C_{16}H_{20}NO^+$	242.1539	242.1535	1.95
185	$C_{13}H_{13}O^+$	185.0961	185.0979	9.85
157	$C_{12}H_{13}^+$	157.1012	157.1013	0.98

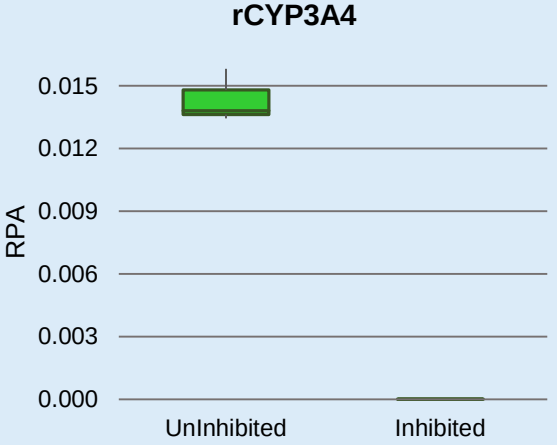




Norhydroxydesomorphine MS^2

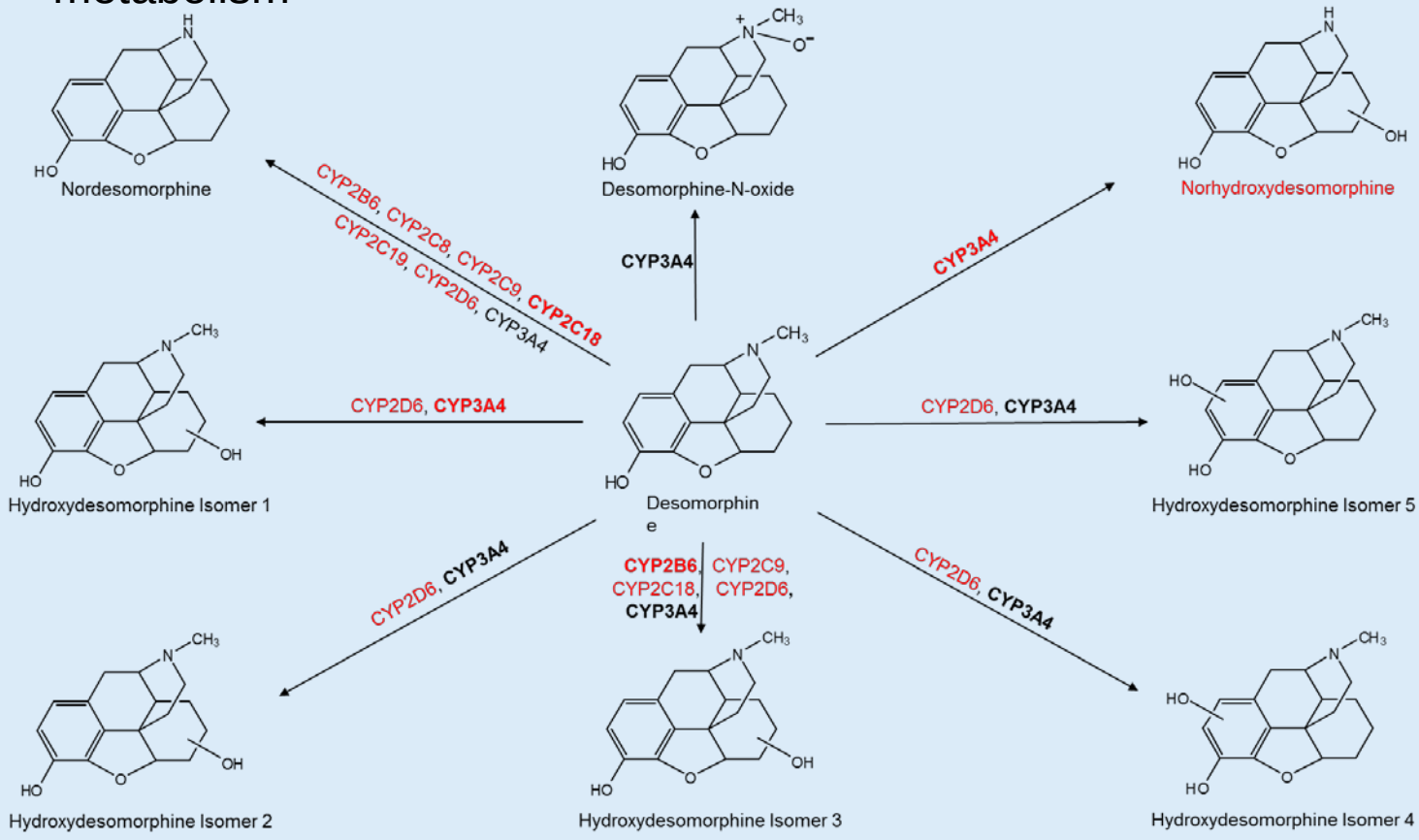


M/Z	Chemical Formula	Exact Mass	Accurate Mass	PPM
274	$C_{16}H_{20}NO_3^+$	274.1438	274.1423	5.21
256	$C_{16}H_{18}NO_2^+$	256.1332	256.1323	3.45
230	$C_{15}H_{20}NO^+$	230.1539	230.1524	6.83
171	$C_{13}H_{15}^+$	171.1168	171.1175	3.97
167	$C_{13}H_{11}^+$	167.0855	167.0856	0.29



Conclusions

- This study:
  - Identified a new Phase I metabolite of desomorphine
  - Suggests additional CYP isozymes may contribute to desomorphine's metabolism



Questions?

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