Species-specific involvement of aldehyde oxidase and xanthine oxidase in the metabolism of the pyrimidine-containing $mGlu_5$ negative allosteric modulator VU0424238 (auglurant)

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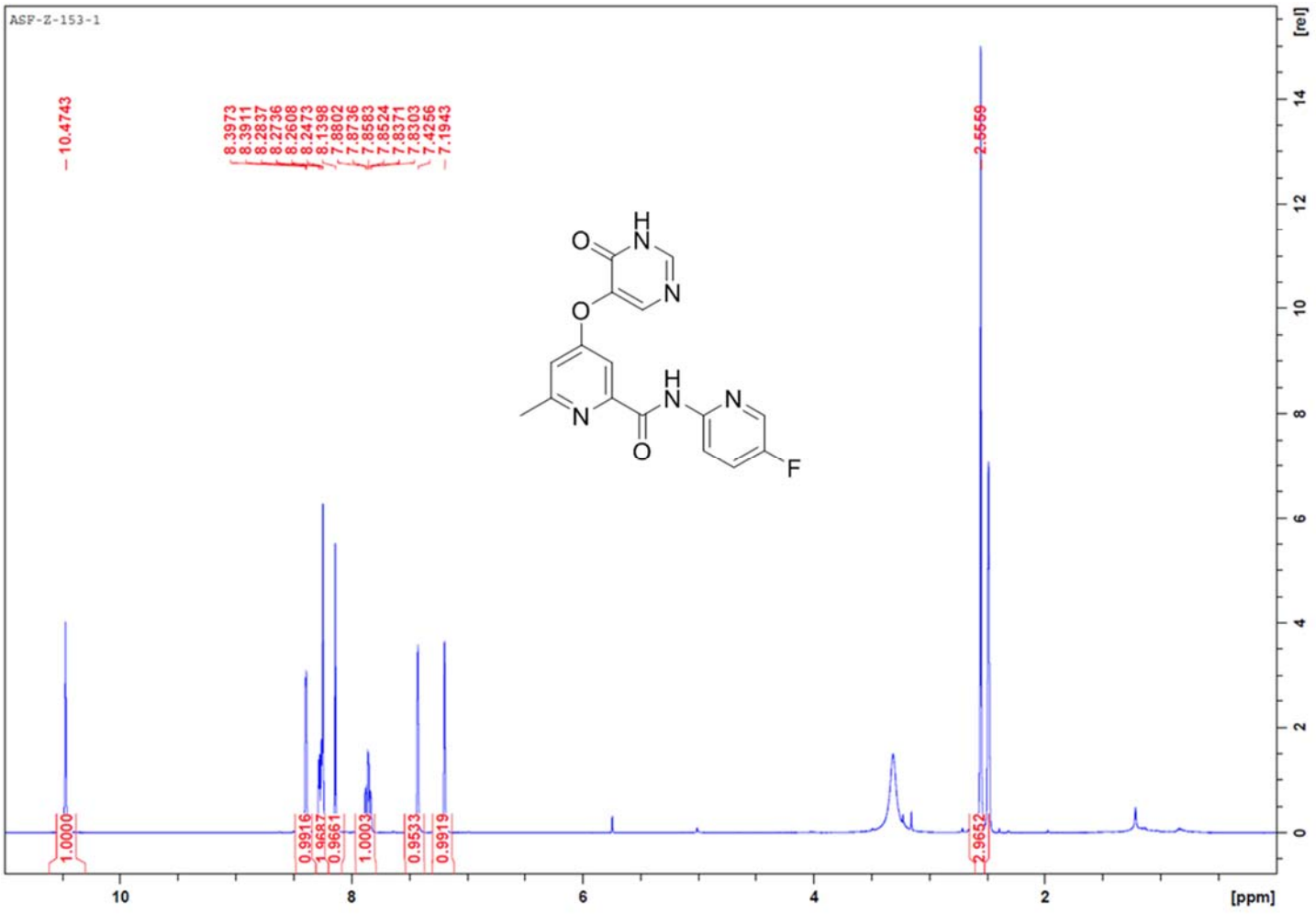
Drug Metabolism and Disposition

Supplemental Information

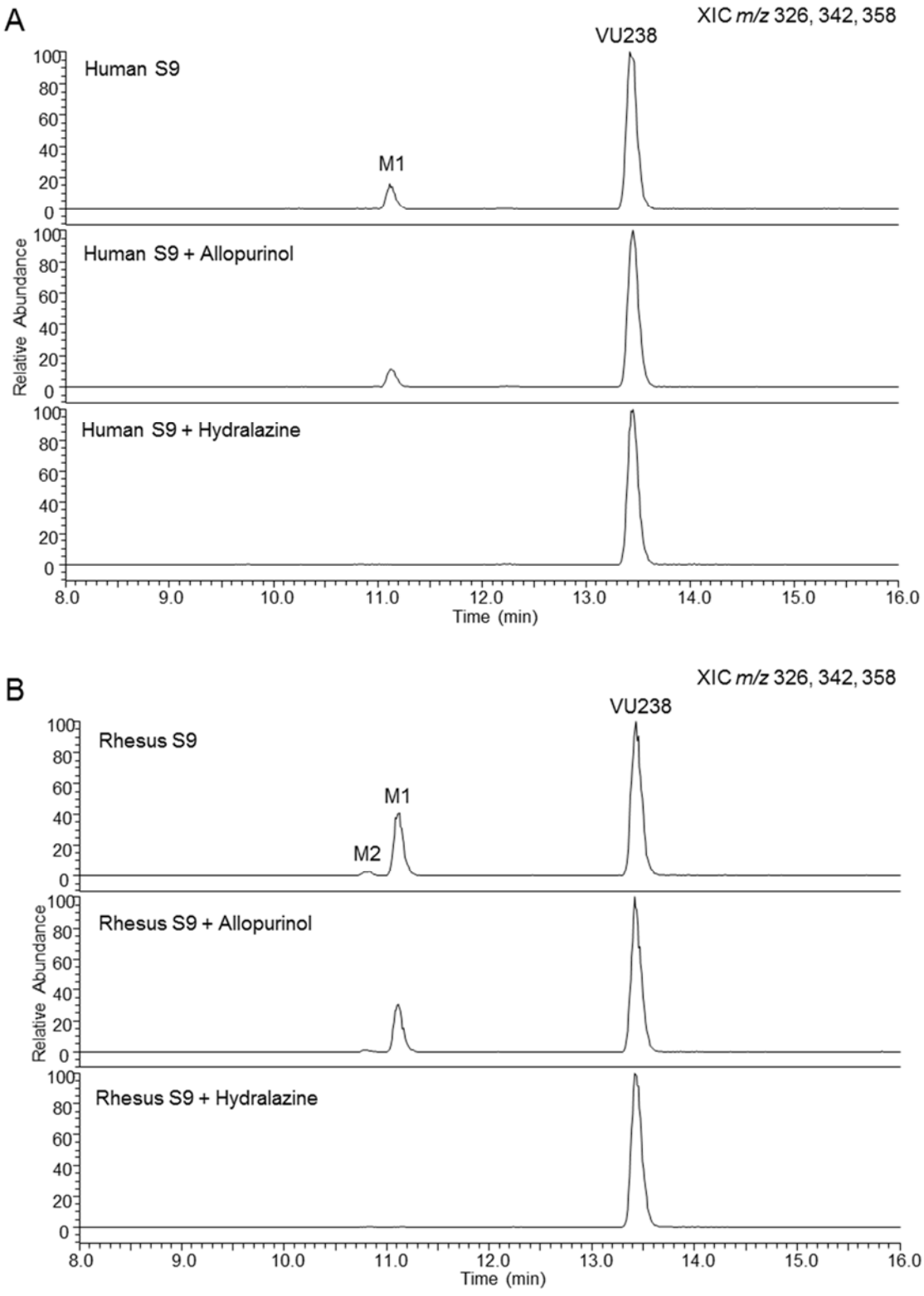
Supplemental Figure 1.

Proton NMR spectra for VU0652922 (M1). NMR spectra were recorded on a 400 MHz AMX Bruker NMR spectrometer.



Supplemental Figure 2.

Extracted ion chromatograms (XIC) obtained from extracts of incubations with VU238 (5 μM) in (A) mixed gender human hepatic S9 (2 mg/mL), (B) male rhesus monkey hepatic S9 (2 mg/mL), (C) male mouse hepatic S9 (2 mg/mL), (D) male guinea pig hepatic S9 (2 mg/mL), and (E) male minipig hepatic S9 (2mg/mL) demonstrating formation of M1 and M2 in the presence or absence of the XO inhibitor allopurinol (100 μM) or the AO inhibitor hydralazine (50 μM).



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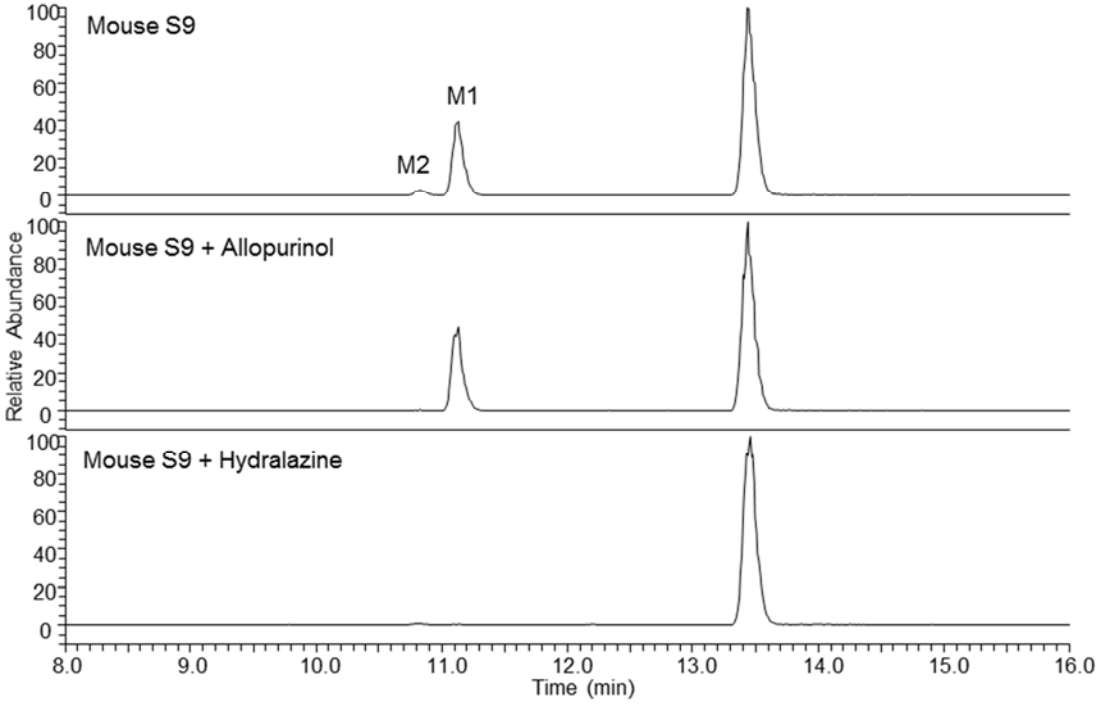
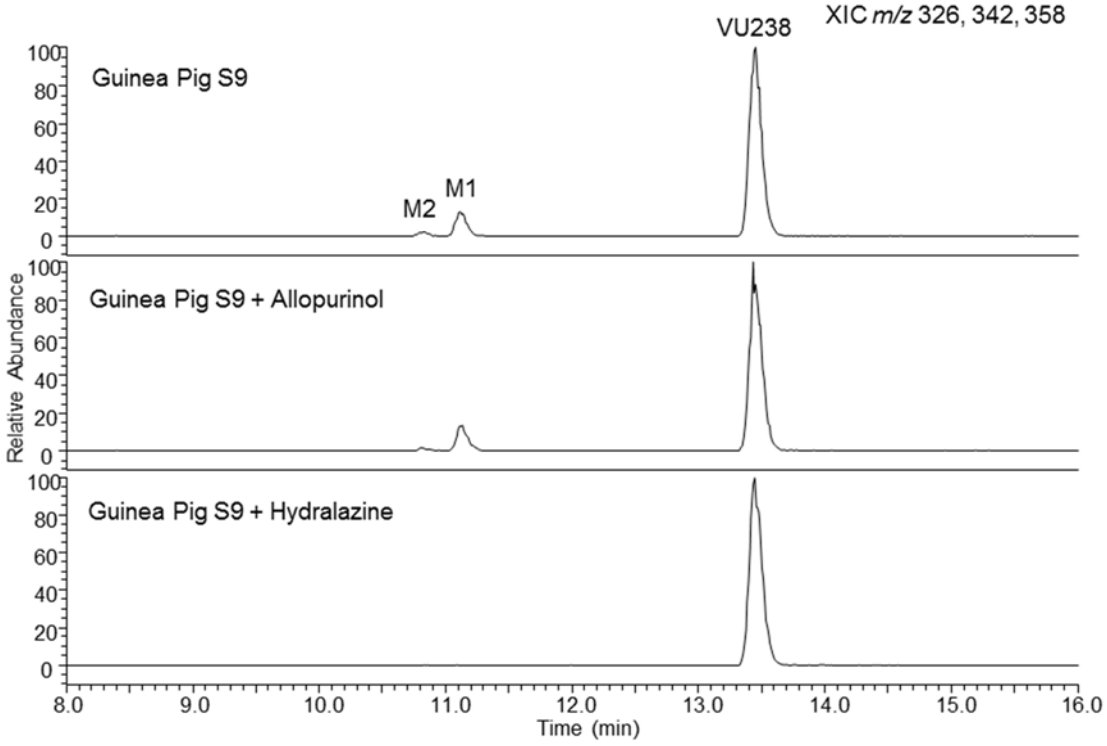
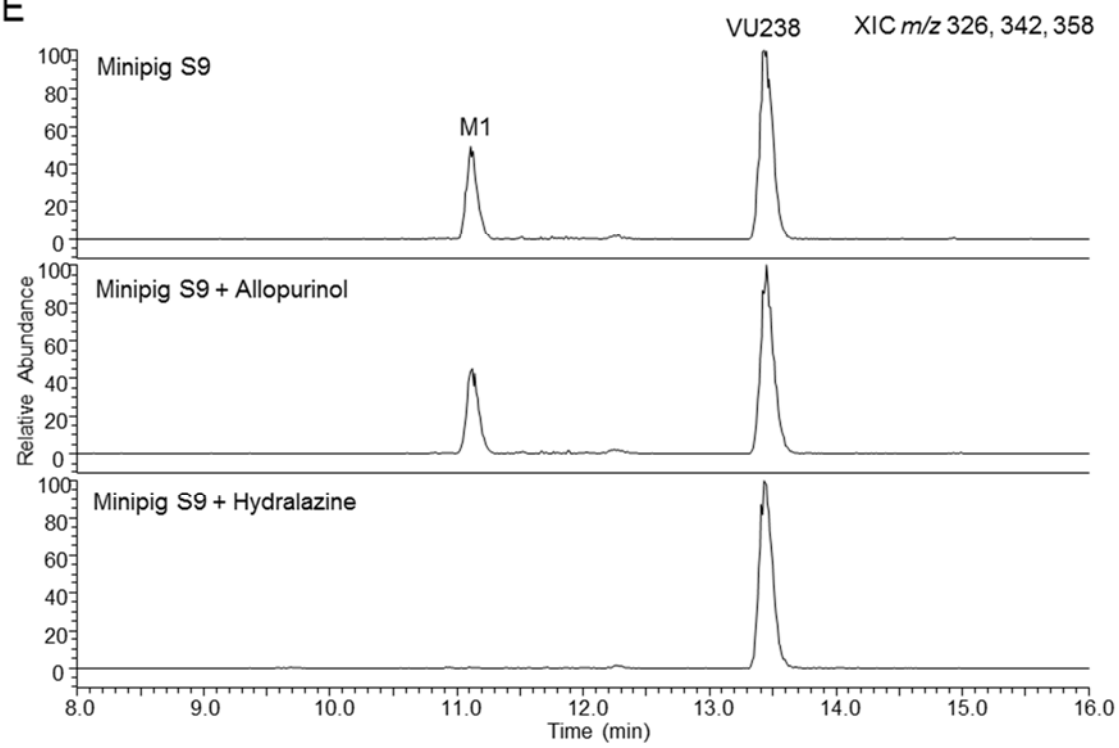

XIC m/z 326, 342, 358
VU238
Mouse S9
M1
M2
Mouse S9 + Allopurinol
Mouse S9 + Hydralazine
Relative Abundance
Time (min)

D

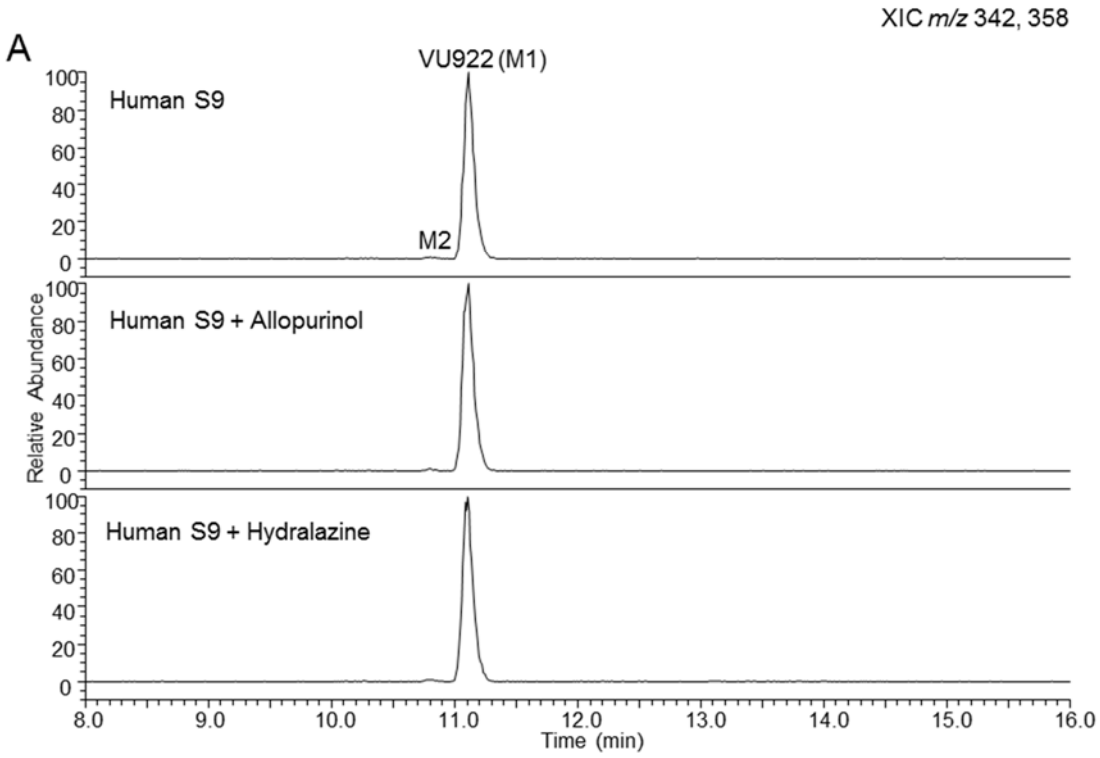

XIC m/z 326, 342, 358
VU238
Guinea Pig S9
M2
M1
Guinea Pig S9 + Allopurinol
Guinea Pig S9 + Hydralazine
Relative Abundance
Time (min)

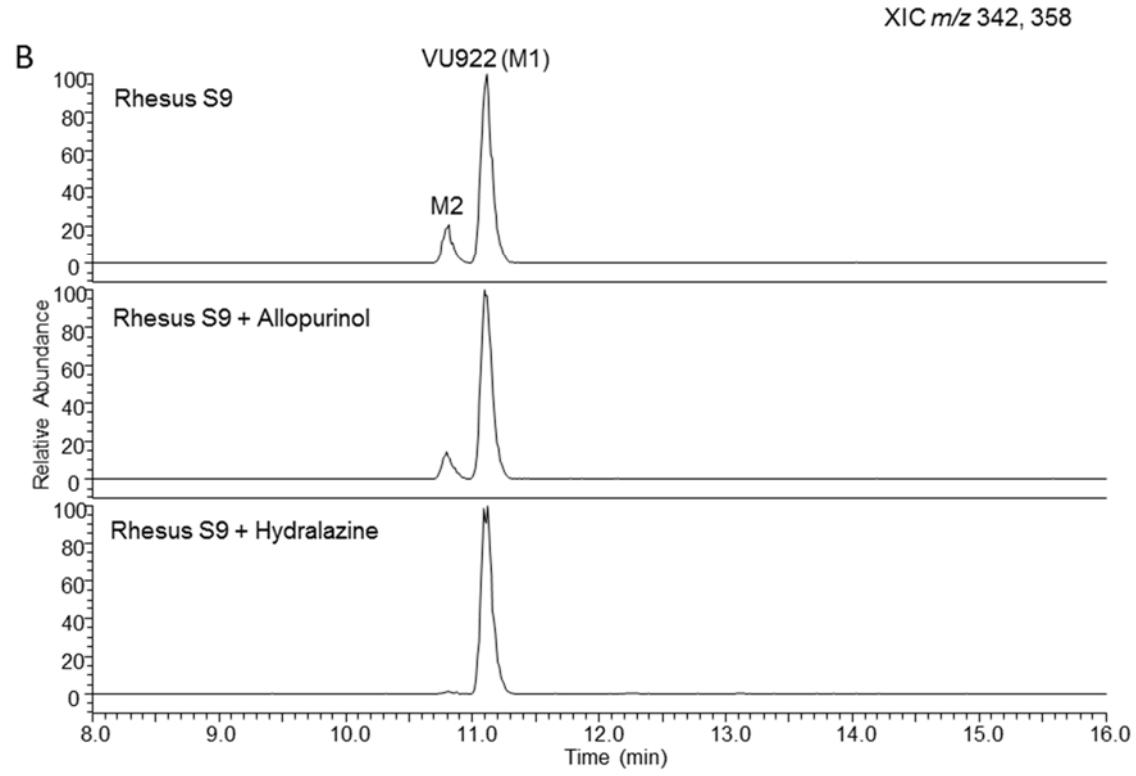
E

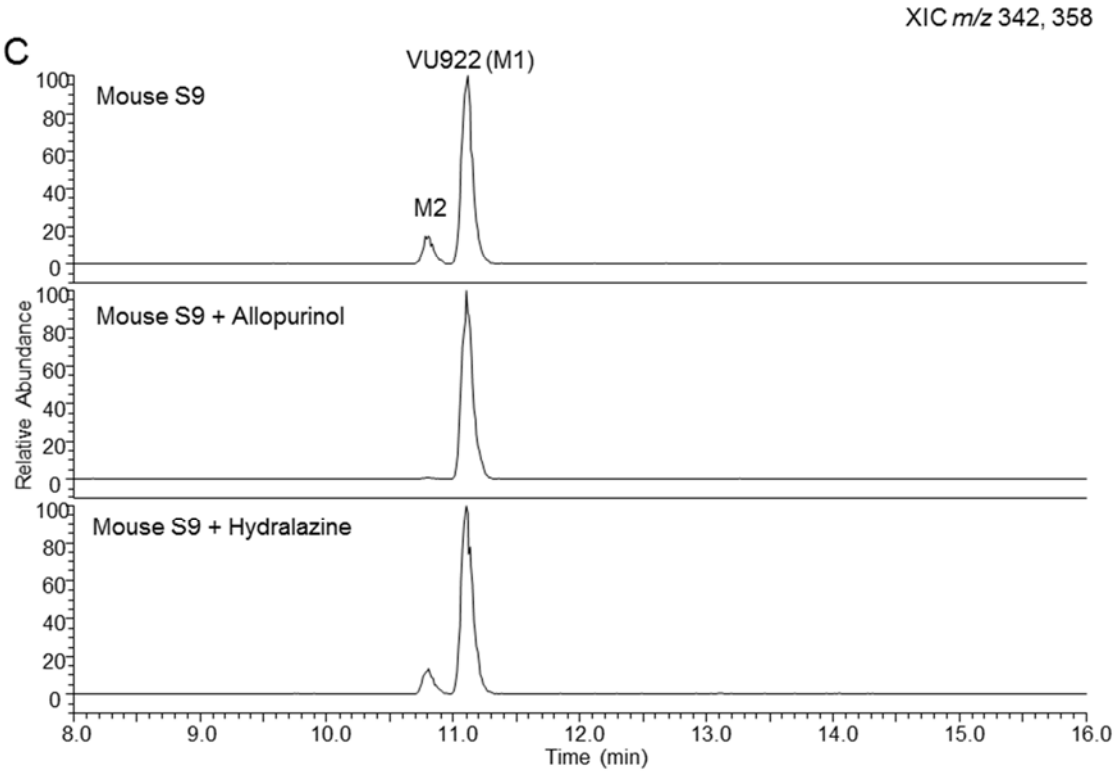

VU238
XIC m/z 326, 342, 358
Minipig S9
M1
Minipig S9 + Allopurinol
Minipig S9 + Hydralazine
Relative Abundance
Time (min)
100
80
60
40
20
0
8.0
9.0
10.0
11.0
12.0
13.0
14.0
15.0
16.0

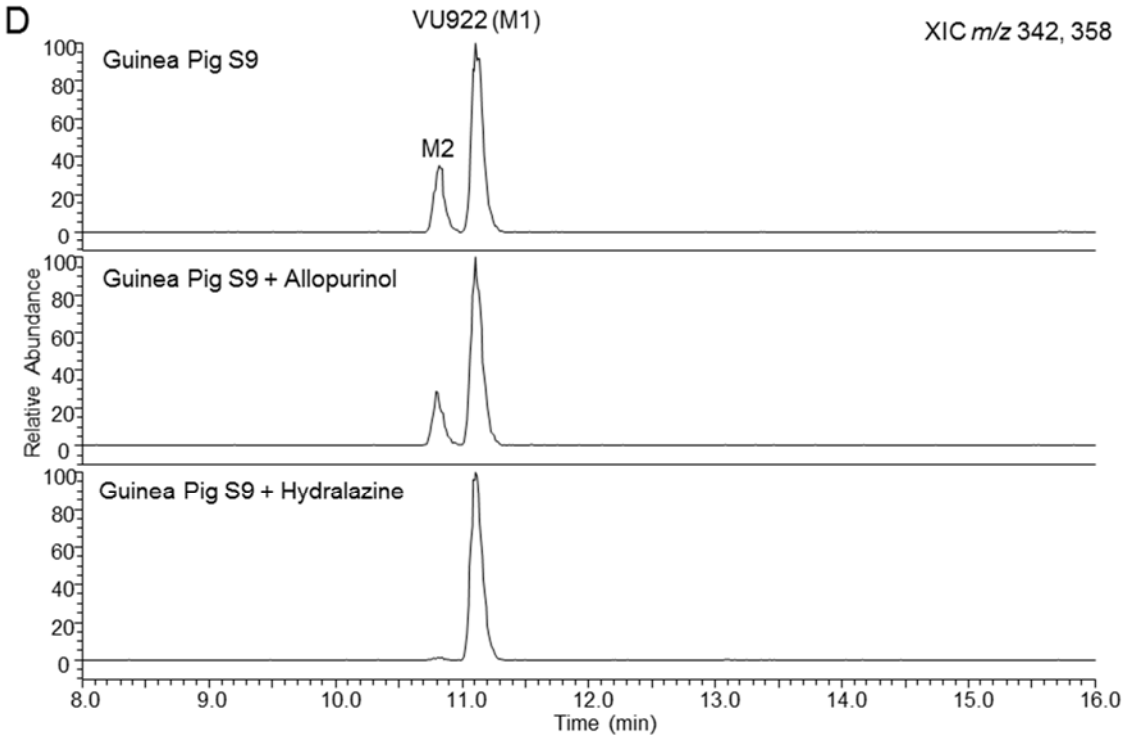
Supplemental Figure 3.

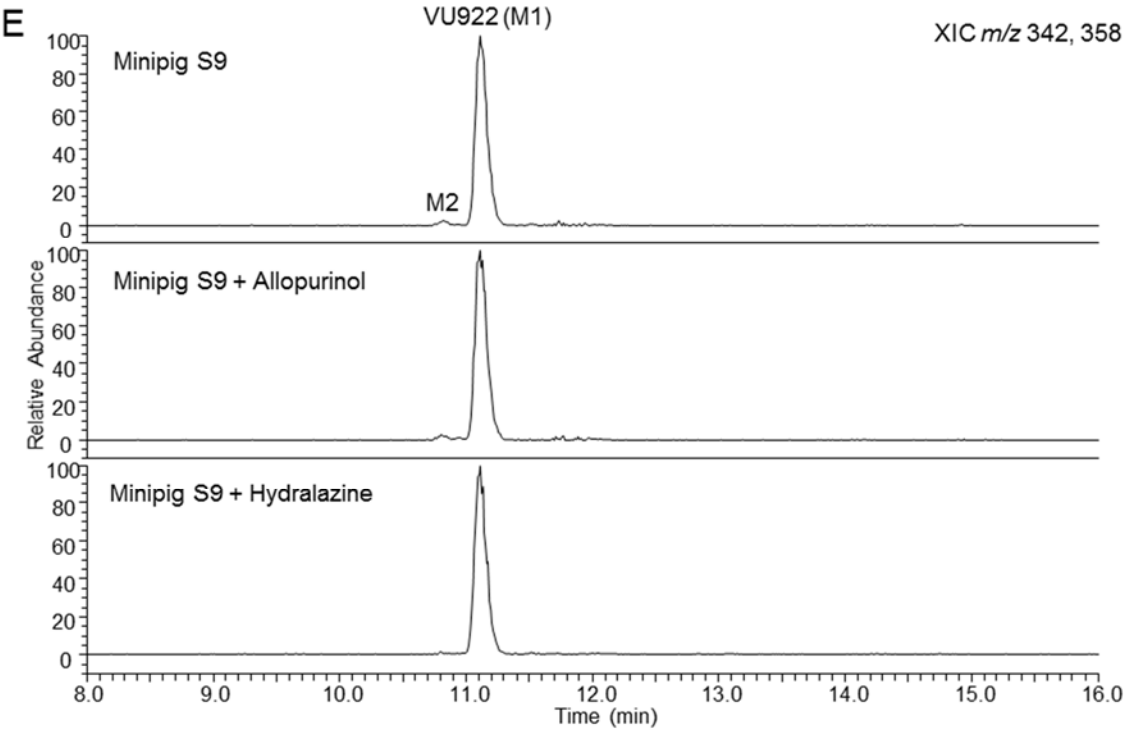
Extracted ion chromatograms (XIC) obtained from extracts of incubations with VU922 (5 μM) in (A) mixed gender human hepatic S9 (2 mg/mL), (B) male rhesus monkey hepatic S9 (2 mg/mL), (C) male mouse hepatic S9 (2 mg/mL), (D) male guinea pig hepatic S9 (2 mg/mL), and (E) male minipig hepatic S9 (2mg/mL) demonstrating formation of M2 in the presence or absence of the XO inhibitor allopurinol (100 μM) or the AO inhibitor hydralazine (50 μM).




XIC m/z 342, 358
B
VU922 (M1)
M2
Rhesus S9
Rhesus S9 + Allopurinol
Rhesus S9 + Hydralazine
Relative Abundance
Time (min)

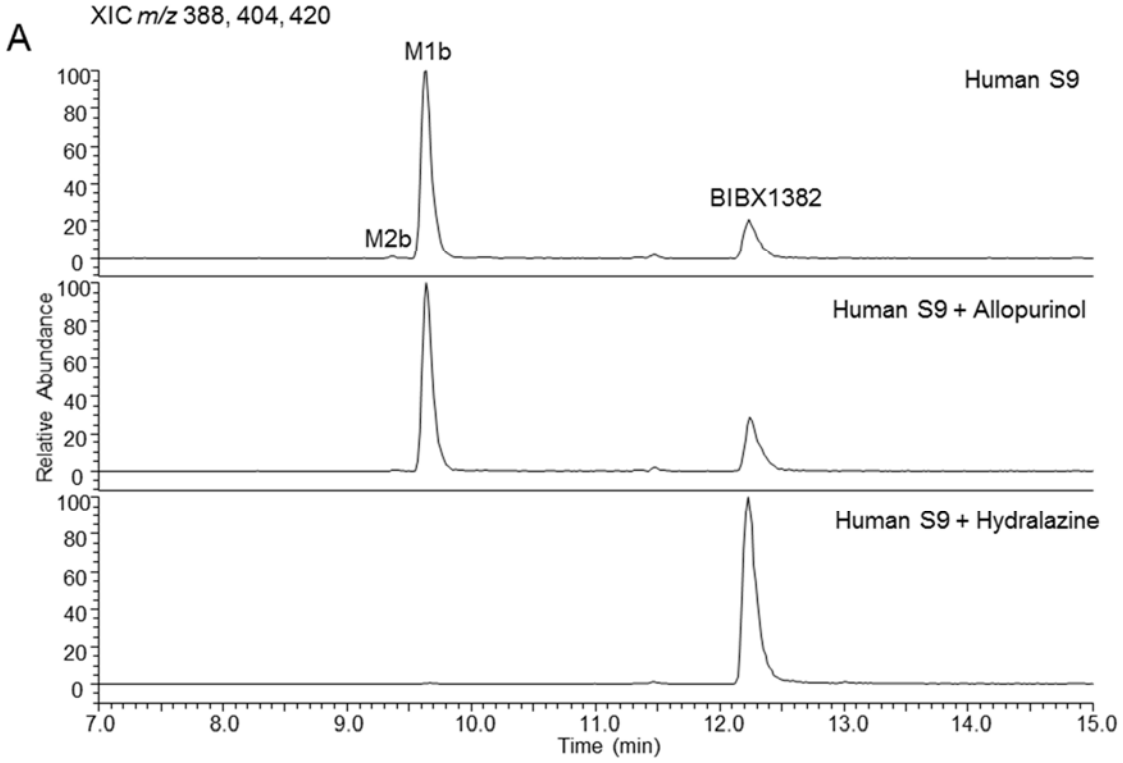

XIC m/z 342, 358
C
VU922 (M1)
M2
Mouse S9
Mouse S9 + Allopurinol
Mouse S9 + Hydralazine
Relative Abundance
Time (min)

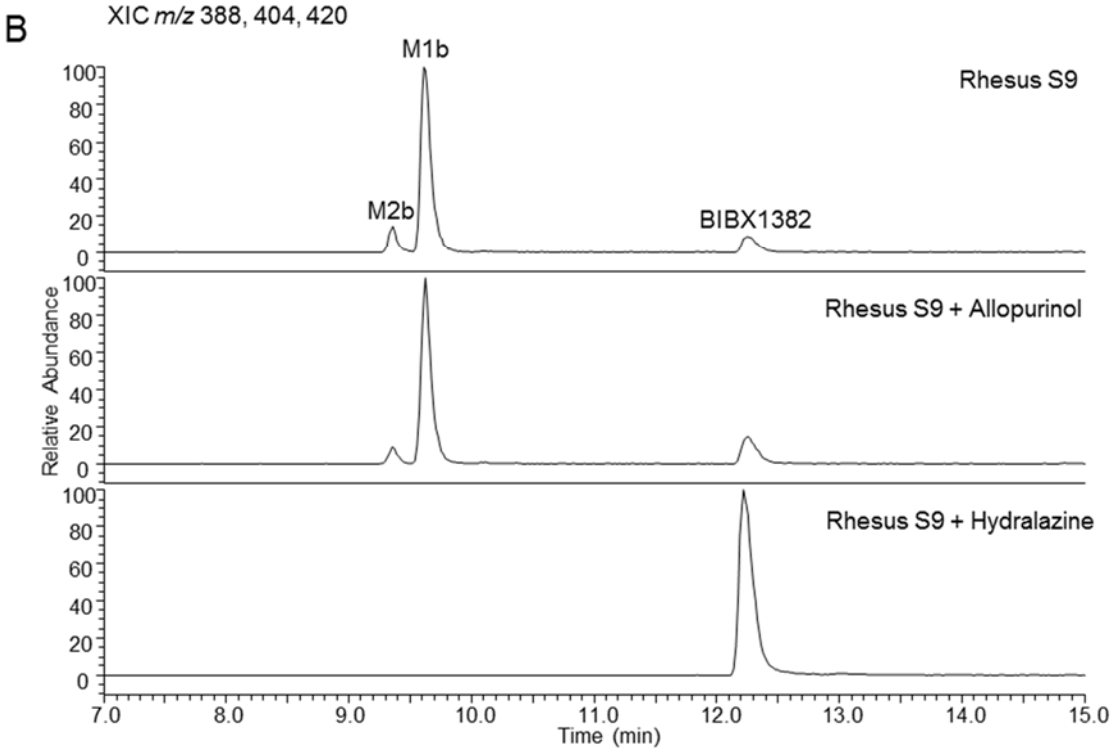

D
VU922 (M1)
XIC m/z 342, 358
Guinea Pig S9
M2
Guinea Pig S9 + Allopurinol
Guinea Pig S9 + Hydralazine
Relative Abundance
Time (min)

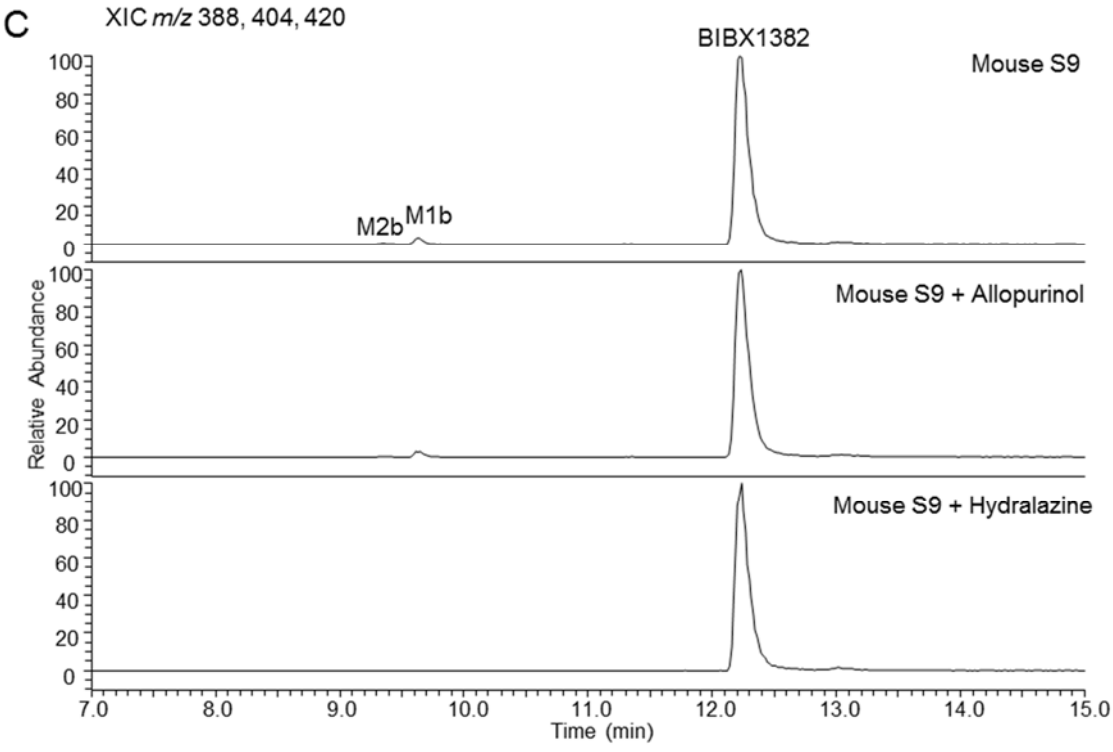

E
VU922 (M1)
XIC m/z 342, 358
Minipig S9
M2
Minipig S9 + Allopurinol
Minipig S9 + Hydralazine
Relative Abundance
Time (min)

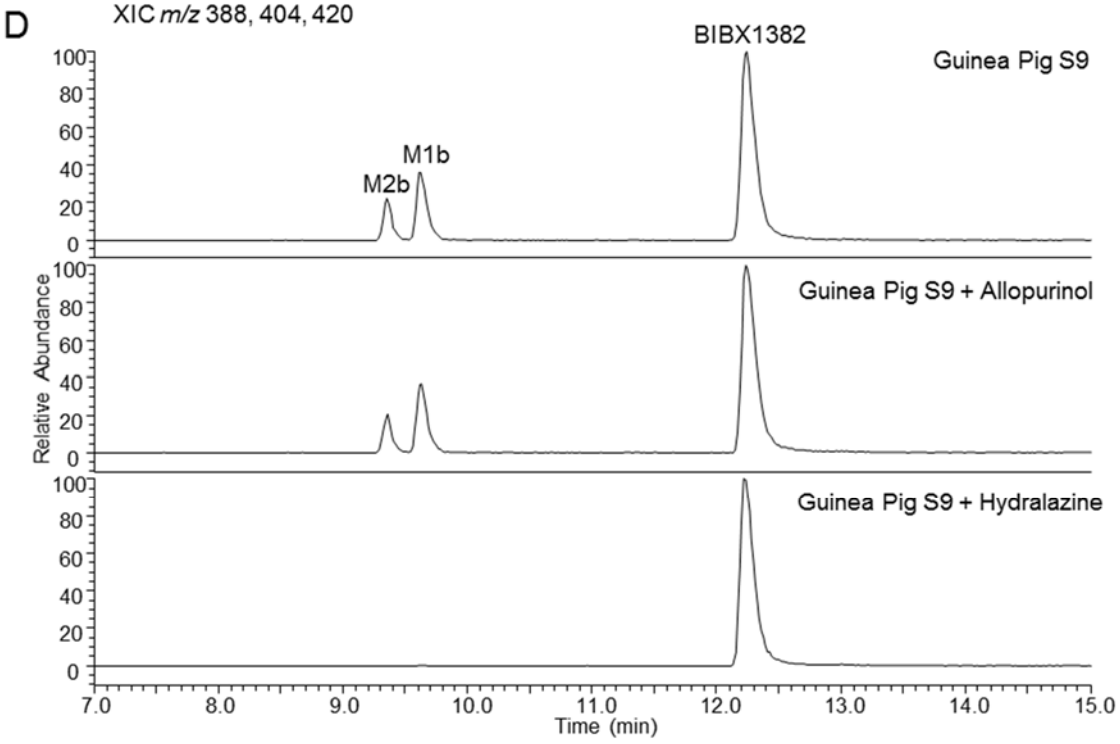
Supplemental Figure 4.

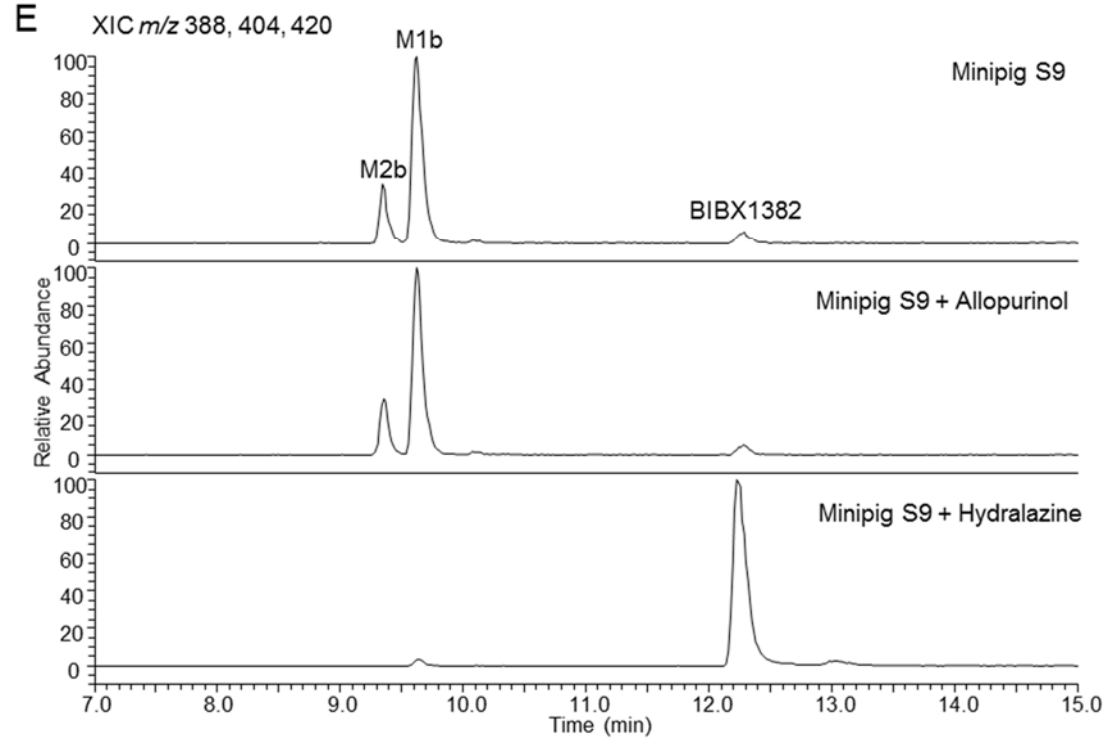
Extracted ion chromatograms (XIC) obtained from extracts of incubations with BIBX1382 (5 μM) in (A) mixed gender human hepatic S9 (2 mg/mL), (B) male rhesus monkey hepatic S9 (2 mg/mL), (C) male mouse hepatic S9 (2 mg/mL), (D) male guinea pig hepatic S9 (2 mg/mL), and (E) male minipig hepatic S9 (2mg/mL) demonstrating formation of M1b and M2b in the presence or absence of the XO inhibitor allopurinol (100 μM) or the AO inhibitor hydralazine (50 μM).




B
XIC m/z 388, 404, 420
M1b
M2b
BIBX1382
Rhesus S9
Rhesus S9 + Allopurinol
Rhesus S9 + Hydralazine
Relative Abundance
Time (min)


C
XIC m/z 388, 404, 420
BIBX1382
M2b
M1b
Mouse S9
Mouse S9 + Allopurinol
Mouse S9 + Hydralazine
Relative Abundance
Time (min)


D
XIC m/z 388, 404, 420
BIBX1382
Guinea Pig S9
M1b
M2b
Guinea Pig S9 + Allopurinol
Guinea Pig S9 + Hydralazine
Relative Abundance
Time (min)


E
XIC m/z 388, 404, 420
M1b
Minipig S9
M2b
BIBX1382
Minipig S9 + Allopurinol
Minipig S9 + Hydralazine
Relative Abundance
Time (min)

Supplemental Table 1.

Percent total MS peak area of parent drug and metabolites from biotransformation experiments with VU238, VU922, or BIBX1382 (5 μM) in hepatic S9 of multiple species (2 mg/mL) in the presence or absence of the XO inhibitor allopurinol (100 μM) or the AO inhibitor hydralazine (50 μM).

		VU238			**VU922 (M1)**		**BIBX1382**		
		VU238	M1	M2	M1	M2	BIBX1382	M1b	M2b
Human	Control	88.8%	11.2%	ND	99.4%	0.6%	22.1%	77.2%	0.7%
	Allopurinol	91.1%	8.9%	ND	99.3%	0.7%	28.5%	71.1%	0.5%
	Hydralazine	100%	ND	ND	>99%	n/d	99.8%	0.2%	ND
Cynomolgus Monkey	Control	43.6%	48.5%	7.9%	70.9%	29.1%	2.1%	78.6%	19.3%
	Allopurinol	51.0%	43.4%	5.7%	76.6%	23.4%	2.7%	82.5%	14.8%
	Hydralazine	100%	ND	ND	100%	ND	100%	ND	ND
Rhesus Monkey	Control	71.4%	26.9%	1.7%	84.6%	15.4%	10.5%	80.2%	9.3%
	Allopurinol	76.9%	22.3%	0.8%	88.8%	11.2%	17.3%	76.5%	6.2%
	Hydralazine	>99%	ND	n/d	>98%	n/d	100%	ND	ND
Rat	Control	41.4%	47.3%	11.3%	70.2%	29.8%	95.4%	1.2%	3.4%
	Allopurinol	44.0%	55.1%	0.9%	96.8%	3.2%	95.8%	1.3%	2.9%
	Hydralazine	>99%	ND	n/d	74.2%	25.8%	100%	ND	ND
Mouse	Control	72.2%	26.1%	1.7%	87.7%	12.3%	97.5%	2.2%	0.3%
	Allopurinol	70.9%	29.1%	ND	99.4%	0.6%	97.6%	2.1%	0.3%
	Hydralazine	>99%	ND	n/d	89.0%	11.0%	100%	ND	ND
Guinea Pig	Control	87.7%	10.7%	1.7%	75.9%	24.1%	71.8%	18.8%	9.4%
	Allopurinol	88.0%	11.1%	0.9%	79.6%	20.4%	72.4%	19.0%	8.5%
	Hydralazine	100%	ND	ND	>98%	n/d	99.9%	0.1%	ND
Minipig	Control	71.2%	28.8%	ND	98.3%	1.7%	5.6%	75.6%	18.8%
	Allopurinol	70.4%	29.6%	ND	98.0%	2.0%	5.2%	75.4%	19.4%
	Hydralazine	100%	ND	ND	>99%	n/d	97.5%	2.5%	ND
Female Cynomolgus Monkey	Control	89.3%	10.7%	ND	95.9%	4.1%	n/a	n/a	n/a
	Allopurinol	92.7%	7.3%	ND	97.1%	2.9%	n/a	n/a	n/a
	Hydralazine	>99%	ND	n/d	>99%	n/d	n/a	n/a	n/a
Female Rat	Control	97.2%	2.8%	ND	83.1%	16.9%	n/a	n/a	n/a
	Allopurinol	96.7%	3.3%	ND	100%	ND	n/a	n/a	n/a
	Hydralazine	>99%	ND	n/d	84.2%	15.8%	n/a	n/a	n/a

ND = not detected

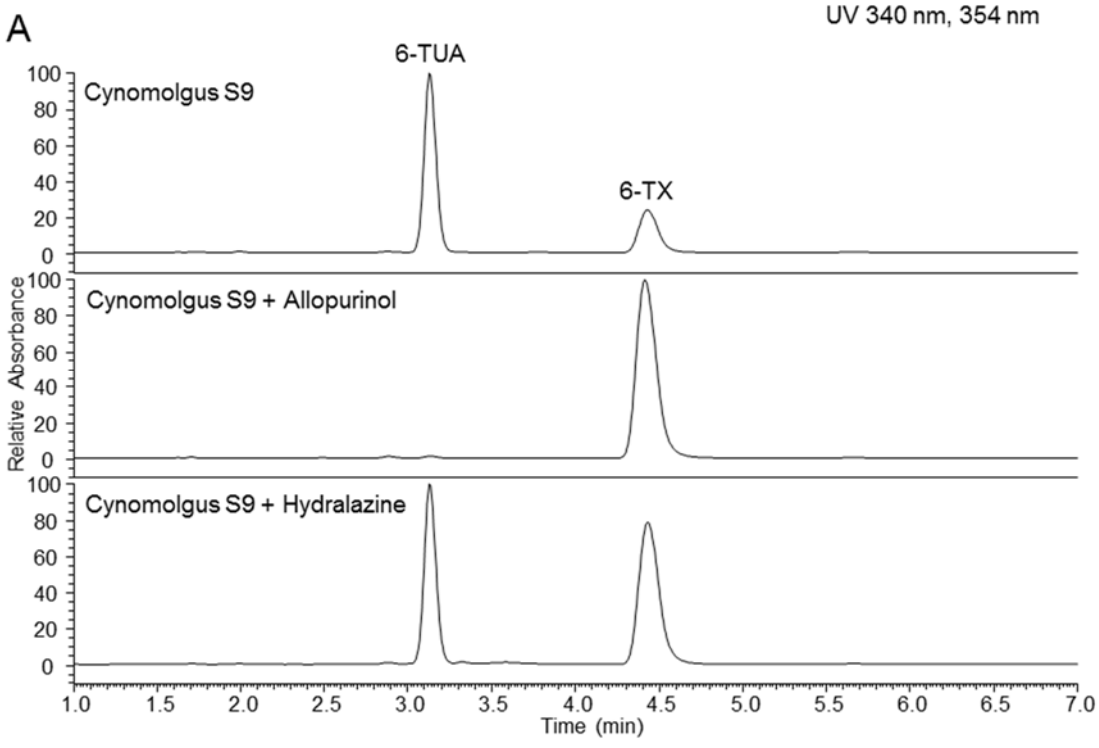
n/d = not determined due to presence of interfering analyte

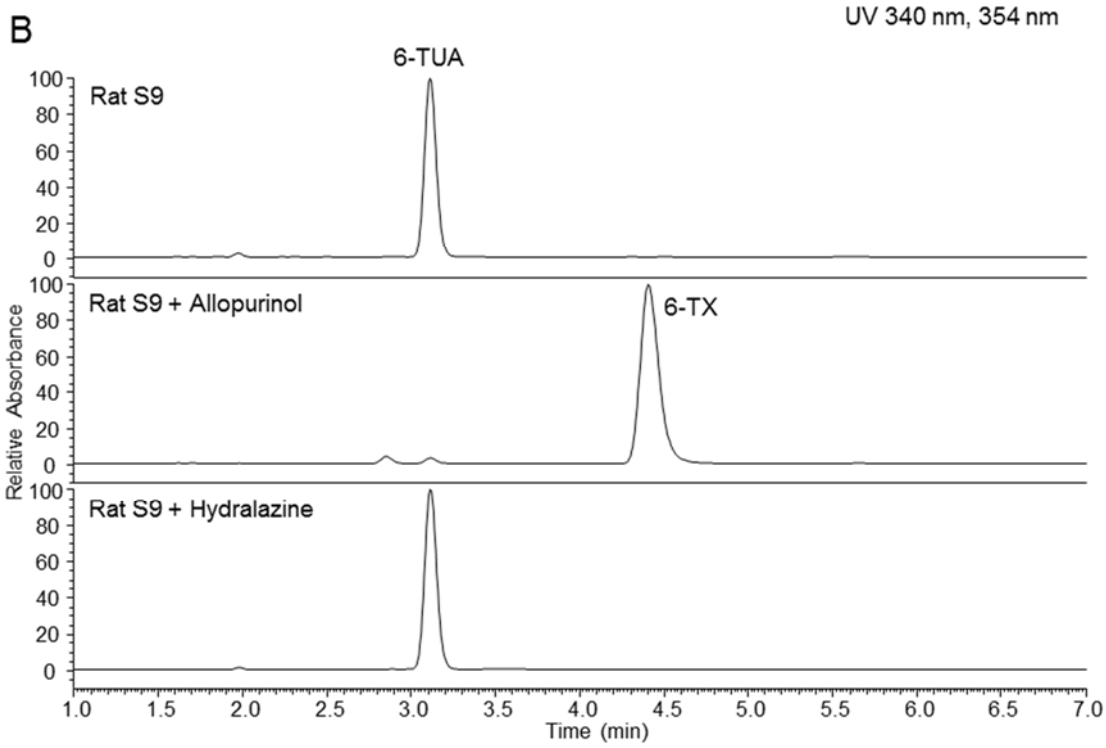
n/a = no data available

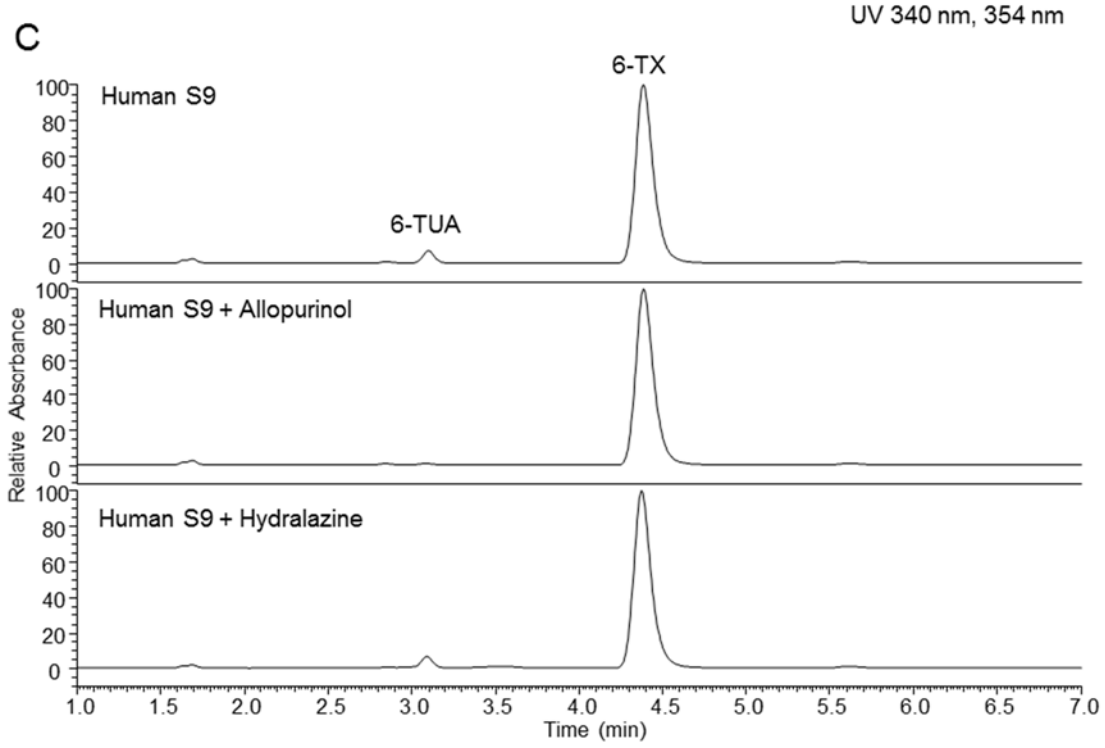
Supplemental Figure 5.

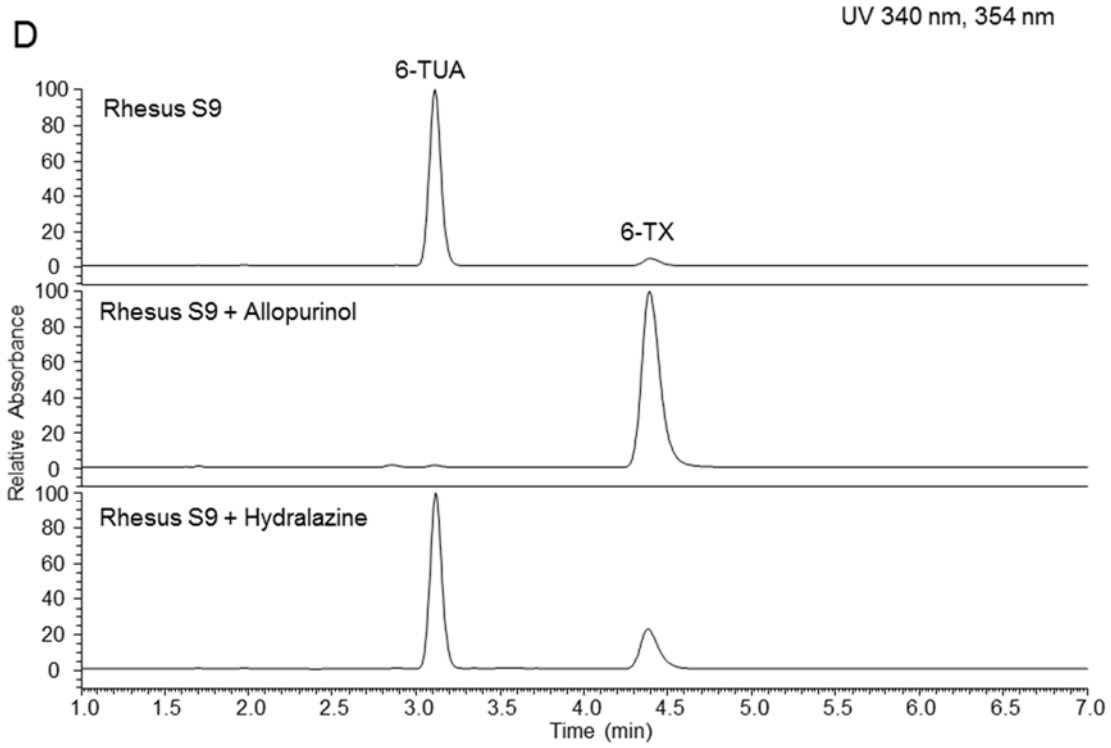
UV chromatograms obtained from extracts of 60-minute incubations with 6-thioxanthine (6-TX, 20 μM) in (A) male cynomolgus monkey hepatic S9 (2 mg/mL), (B) male rat hepatic S9 (2 mg/mL), (C) mixed gender human hepatic S9 (2 mg/mL), (D) male rhesus monkey hepatic S9 (2 mg/mL), (E) male mouse hepatic S9 (2mg/mL), (F) male guinea pig hepatic S9 (2mg/mL), and (G) male minipig hepatic S9 (2mg/mL) demonstrating formation of 6-thiouric acid (6-TUA) in the presence or absence of the XO inhibitor allopurinol (100 μM) or the AO inhibitor hydralazine (50 μM). Chromatograms depicted represent extracted UV plots of spectrum maximums at 340 nm (6-TX) and 354 nm (6-TUA).

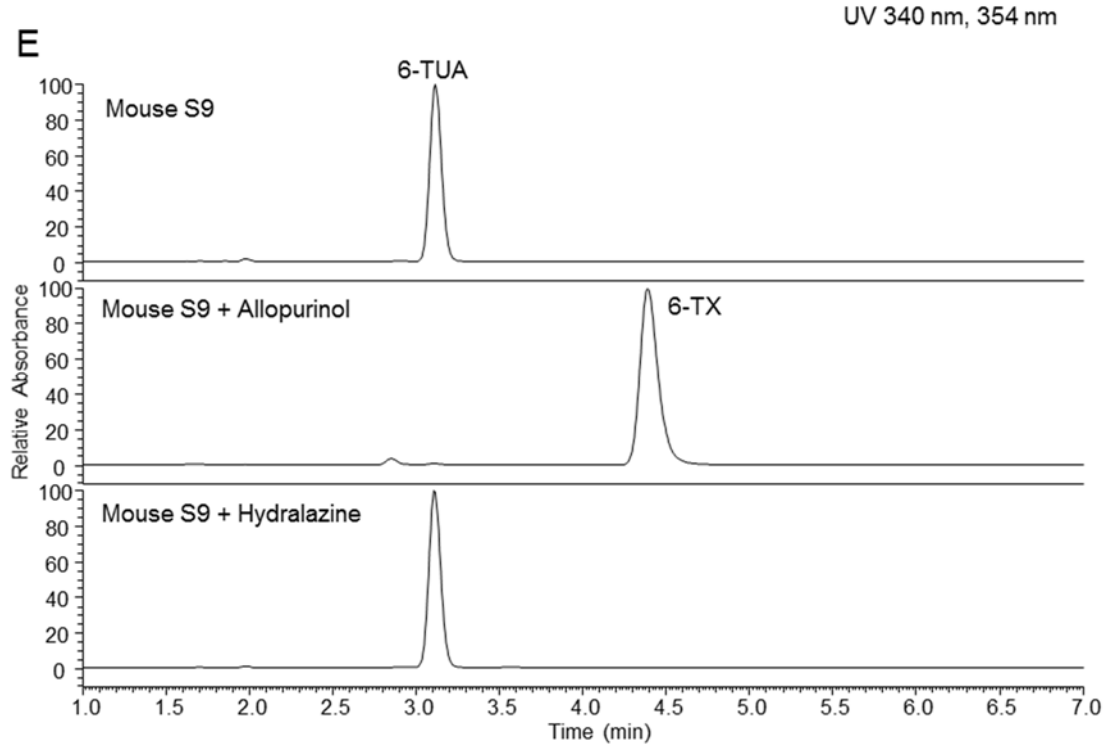
LC/UV/MS analysis of 6-TX and 6-TUA. Incubations were carried out as described in the materials and methods section. LC/UV/MS analysis of 6-TX and 6-TUA was performed with an Agilent 1290 Infinity HPLC system coupled to a LTQ XL ion trap mass spectrometer (ThermoFisher Scientific, Waltham, MA). Analytes were separated by gradient elution using a Zorbax Eclipse XDB C18 column (5 μm, 4.6 × 150 mm; Agilent Technologies). Solvent A was 0.1% formic acid in water (pH unadjusted), and solvent B was acetonitrile. The initial mobile phase was held at 95:5 A-B (v/v) for 5.5 minutes and by linear gradient was transitioned to 20:80 A-B over 1.5 minutes, held for 2 minutes, then transitioned back to starting conditions over 0.5 minutes for a total run time of 10 min. The flow rate was 0.800 ml/min. The HPLC eluent was first introduced into an Agilent 1290 diode array detector (spectrum scan 190 – 400 nm) followed by electrospray ionization-assisted introduction into a LTQ XL ion trap mass spectrometer operated in positive ionization mode. Ionization was assisted with sheath and auxiliary gas (ultra-pure nitrogen) set at 50 and 20 psi, respectively. The electrospray voltage was set at 5 kV with the heated ion transfer capillary set at 350 °C and 31 V. Data were analyzed using Thermo XCalibur 2.2 software. LC/UV/MS/MS analysis of analytes identified as 6-TX and 6-TUA are consistent with that of authentic standards of 6-TX and 6-TUA, respectively (data not shown).

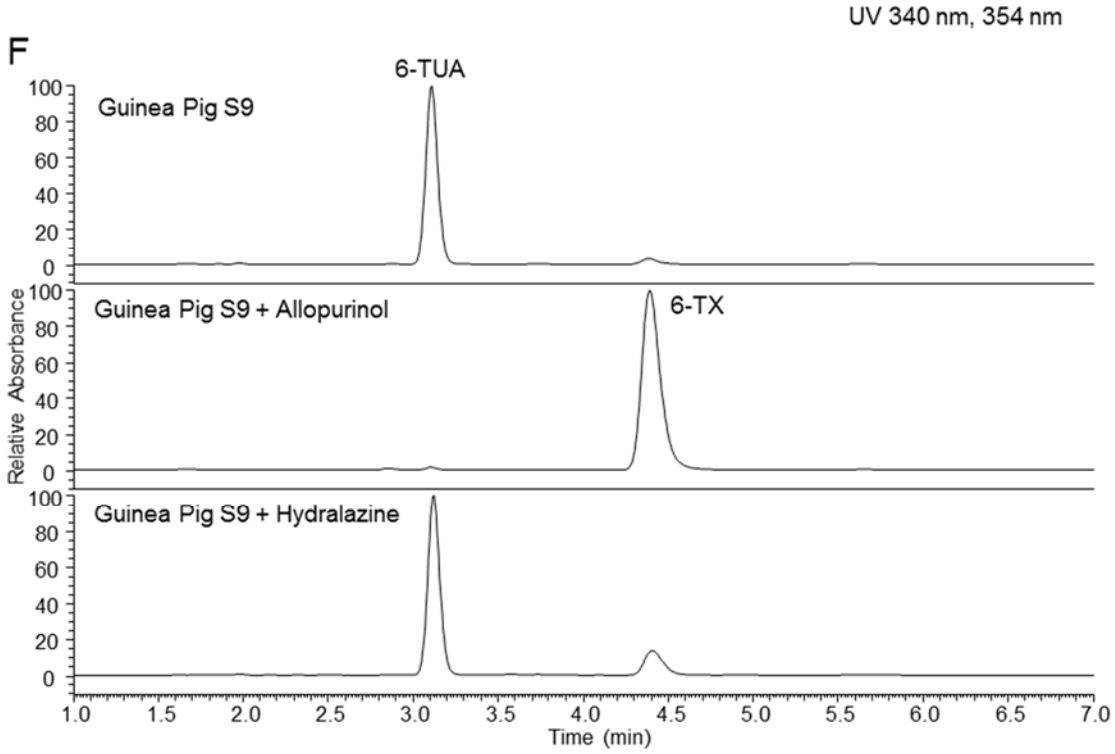


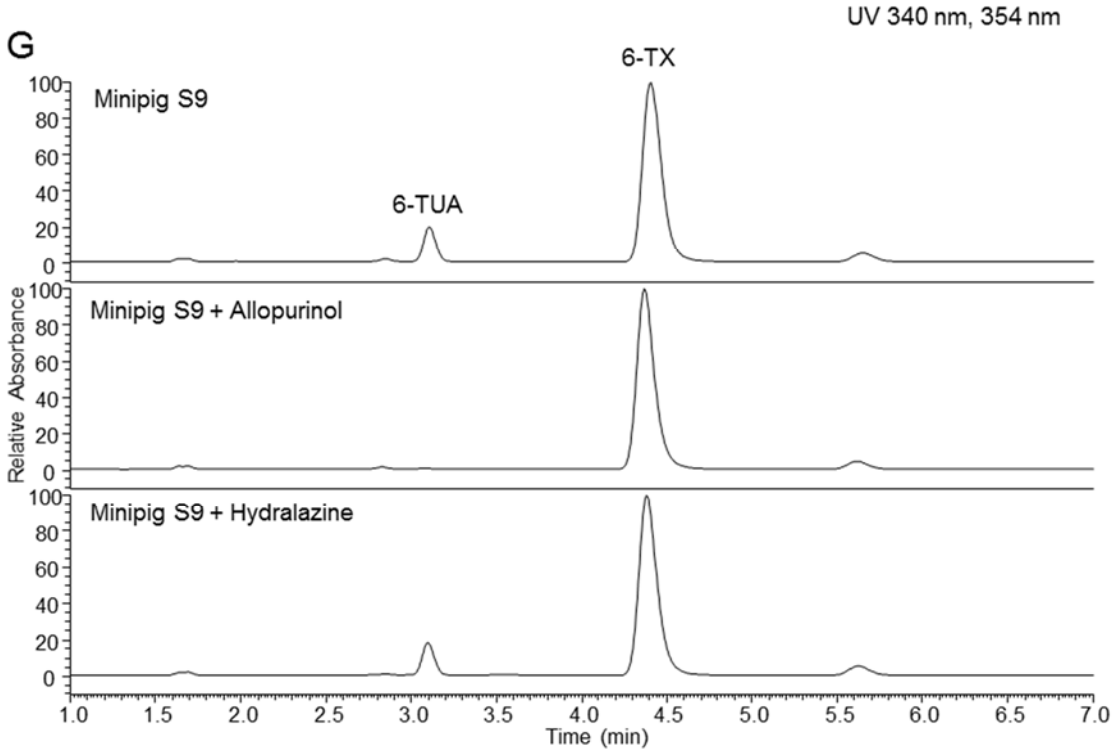

B
UV 340 nm, 354 nm
6-TUA
Rat S9
Rat S9 + Allopurinol
6-TX
Rat S9 + Hydralazine
Relative Absorbance
Time (min)


C
UV 340 nm, 354 nm
6-TX
Human S9
6-TUA
Human S9 + Allopurinol
Human S9 + Hydralazine
Relative Absorbance
Time (min)


D
UV 340 nm, 354 nm
6-TUA
6-TX
Rhesus S9
Rhesus S9 + Allopurinol
Rhesus S9 + Hydralazine
Relative Absorbance
Time (min)


E
UV 340 nm, 354 nm
6-TUA
6-TX
Mouse S9
Mouse S9 + Allopurinol
Mouse S9 + Hydralazine
Relative Absorbance
Time (min)


F
UV 340 nm, 354 nm
6-TUA
Guinea Pig S9
Guinea Pig S9 + Allopurinol
6-TX
Guinea Pig S9 + Hydralazine
Relative Absorbance
Time (min)


G
UV 340 nm, 354 nm
6-TX
Minipig S9
6-TUA
Minipig S9 + Allopurinol
Minipig S9 + Hydralazine
Relative Absorbance
Time (min)

Supplemental Table 2.

Percent total UV peak area of parent drug (6-TX) and metabolite (6-TUA) from biotransformation experiments (Supplemental Figure 5) with 6-TX (20 μM) in hepatic S9 of multiple species (2 mg/mL) in the presence or absence of the XO inhibitor allopurinol (100 μM) or the AO inhibitor hydralazine (50 μM). 6-TX, 6-thioxanthine; 6-TUA, 6-thiouric acid

		6-TX	6-TUA
Human	Control	95.7%	4.3%
	Allo	99.4%	0.6%
	Hyd	96.1%	3.9%
Cynomolgus	Control	27.5%	72.5%
	Allo	99.3%	0.7%
	Hyd	57.5%	42.5%
Rhesus	Control	5.7%	94.3%
	Allo	99.2%	0.8%
	Hyd	26.1%	73.9%
Rat	Control	ND	100.0%
	Allo	98.2%	1.8%
	Hyd	ND	100.0%
Mouse	Control	ND	100.0%
	Allo	99.6%	0.4%
	Hyd	ND	100.0%
Guinea Pig	Control	4.2%	95.8%
	Allo	99.1%	0.9%
	Hyd	17.6%	82.4%
Minipig	Control	88.9%	11.1%
	Allo	99.7%	0.3%
	Hyd	89.5%	10.5%

ND = not detected