

Supplementary Materials

To

Automated Radiosynthesis of [¹⁸F]FluoFAPI and its' Dosimetry and Single Acute Dose Toxicological Evaluation

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Content:

- 1) NMR and other characterization of compounds **4(a,b)**, **5(a,b)**, **1**, **6b** and **2**
 - 2) Radio- and UV-HPLC chromatograms of
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 - 3) Biodistribution data of [¹⁸F]FluoFAPI **1** in Sprague-Dawley rats
 - 4) Single Acute Dose Toxicological Data
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- 1) NMR and other characterization of compounds **4(a,b)**, **5(a,b)**, **1**, **6b** and **2**.

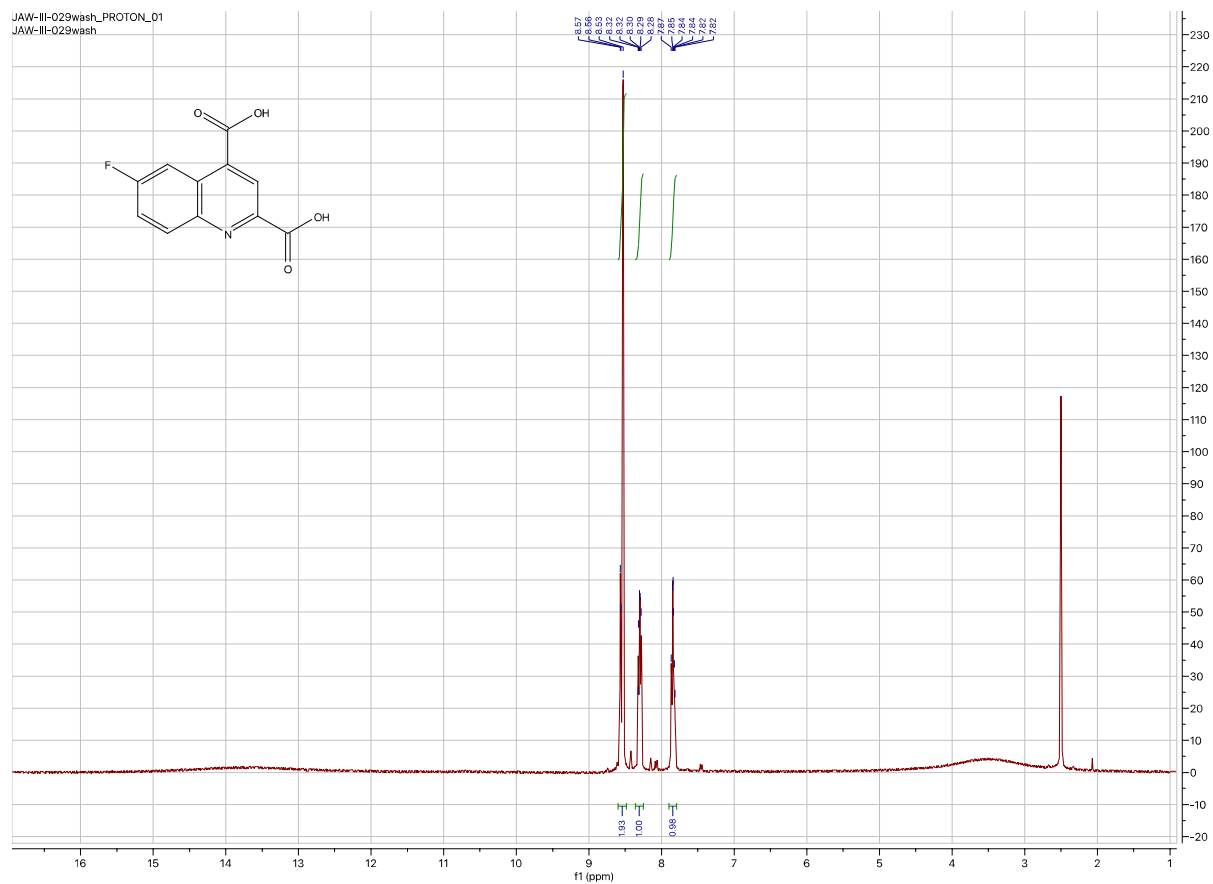


Figure S1. ^1H NMR of compound 4a.

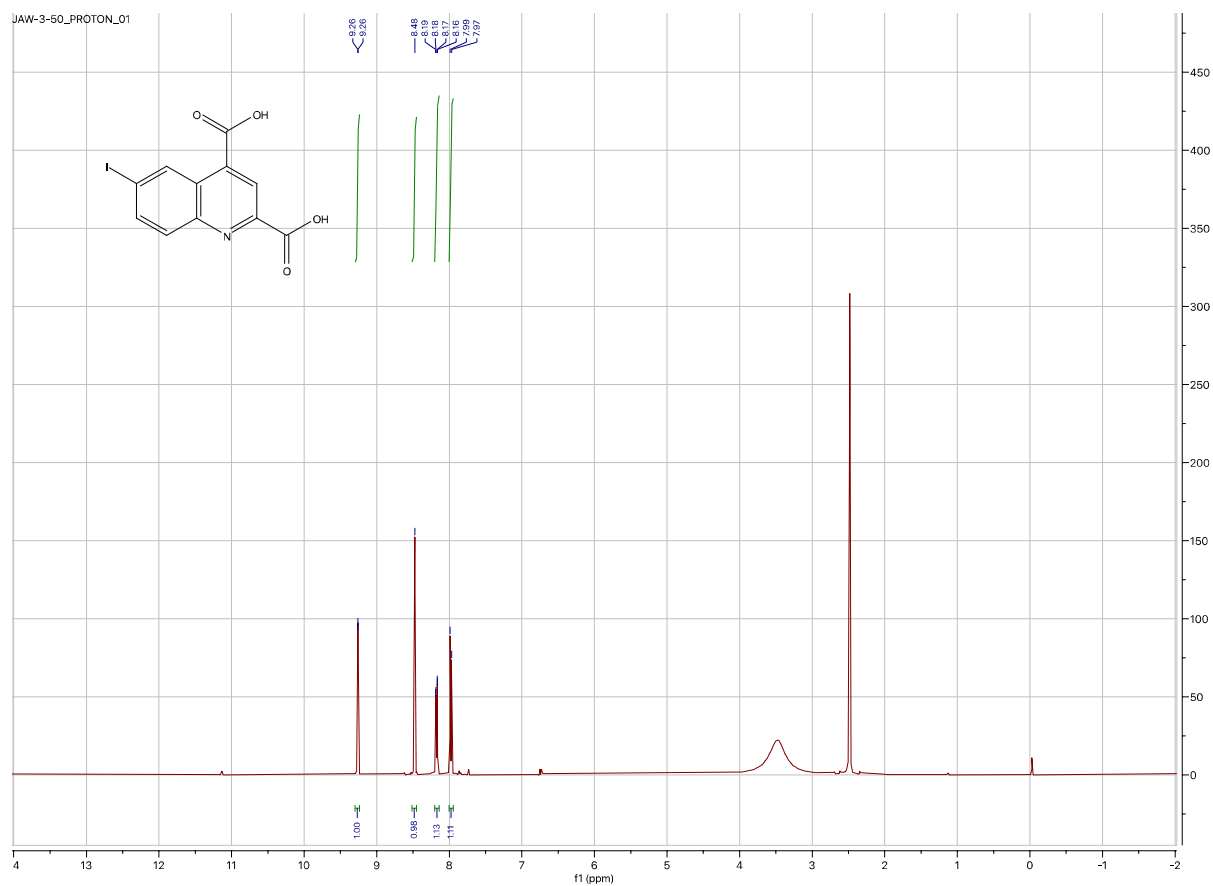


Figure S2. ^1H NMR of compound **4b**.

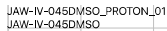


Figure S3. ^1H NMR of compound **5a**.



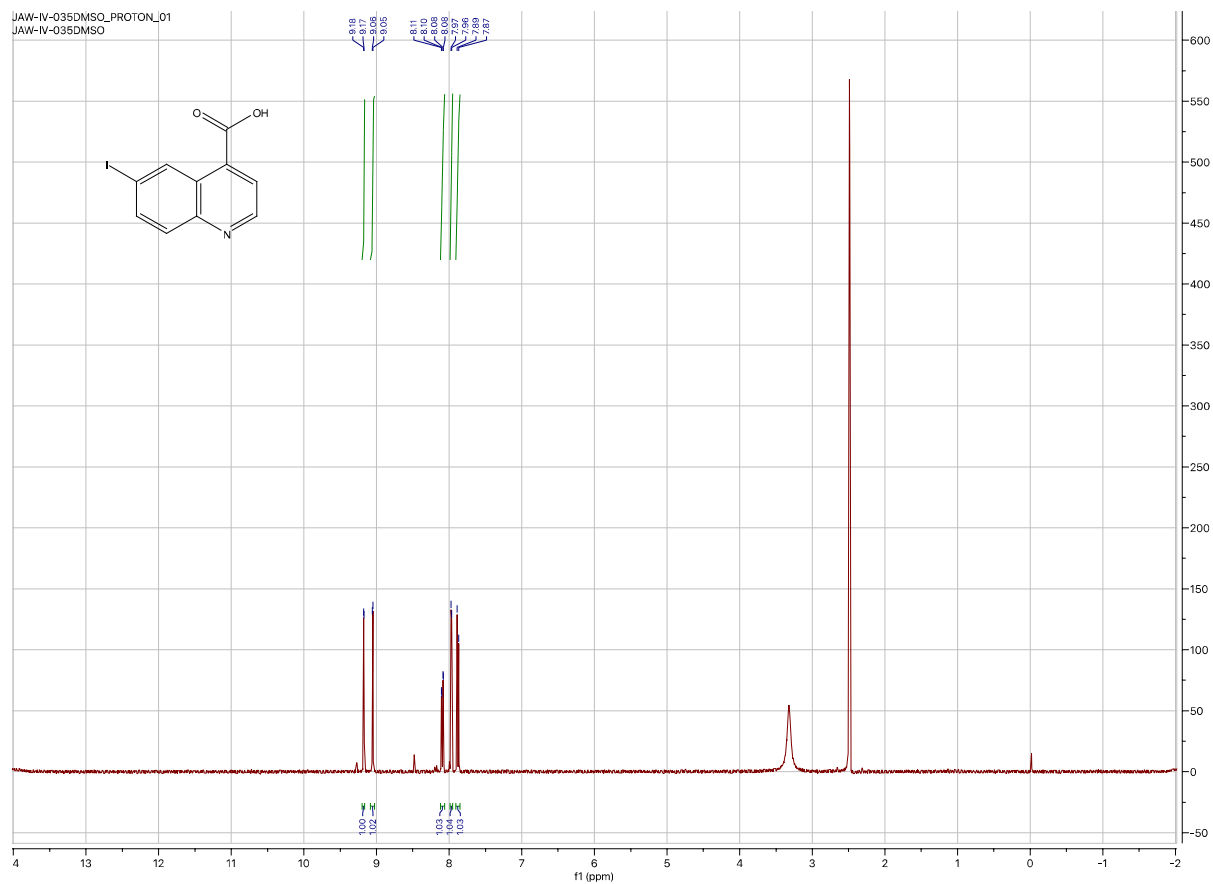


Figure S5. ^1H NMR of compound **5b**.

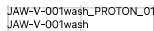


Figure S6. ^1H NMR of FluoFAPI 1.

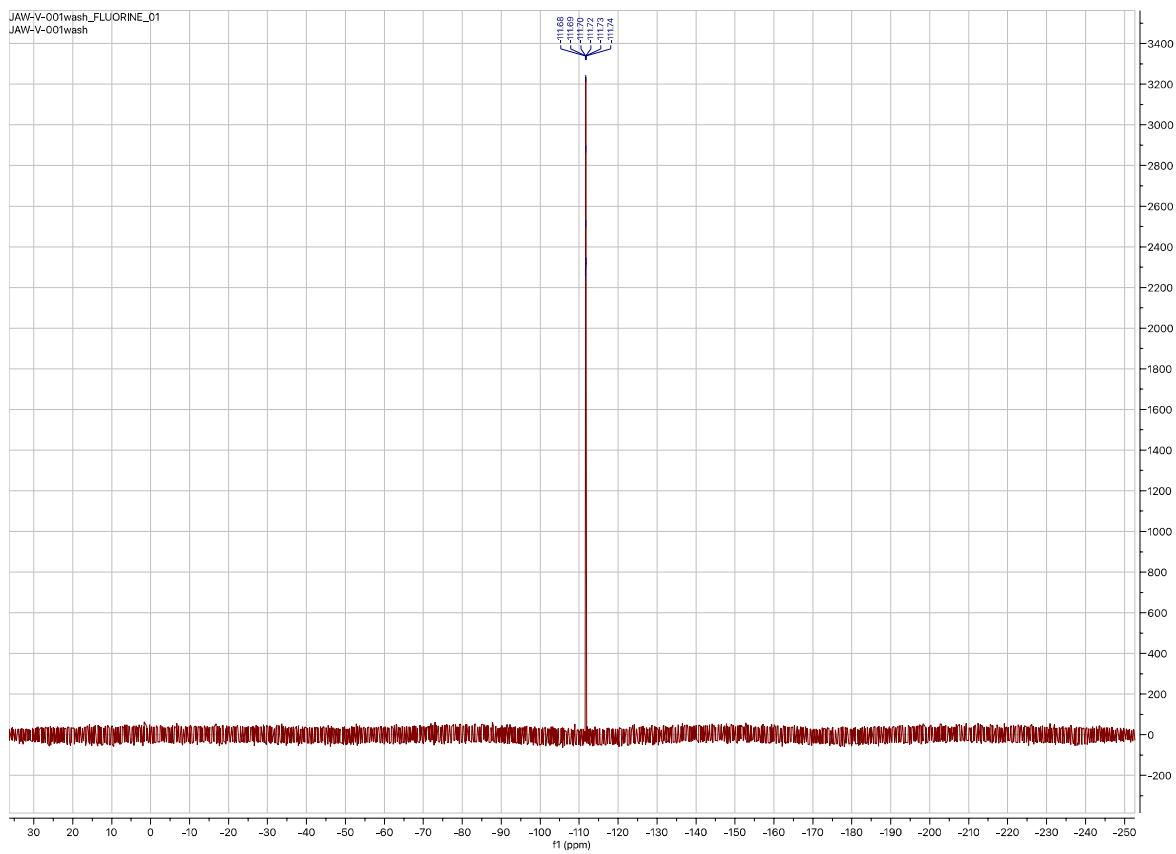


Figure S7. ^{19}F NMR of FluoFAP1 1.

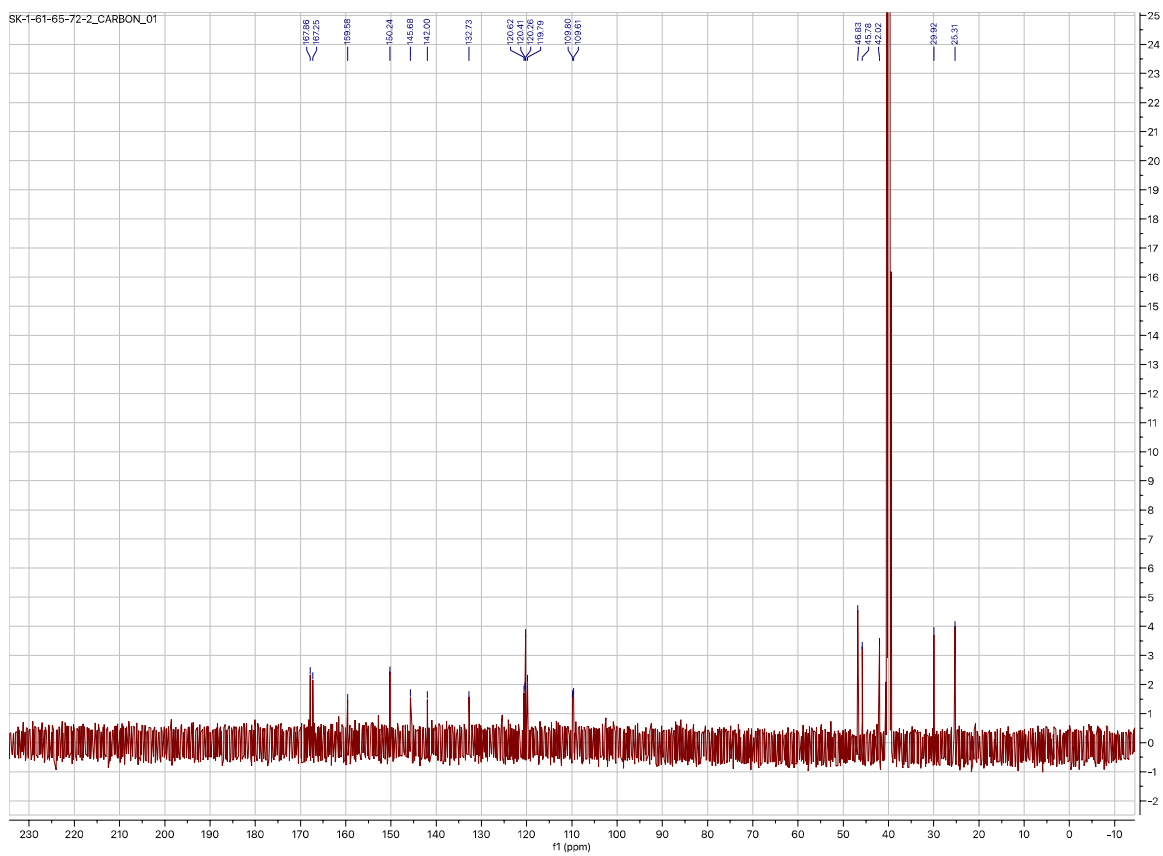


Figure S8. ^{13}C NMR of FluoFAP1 1.

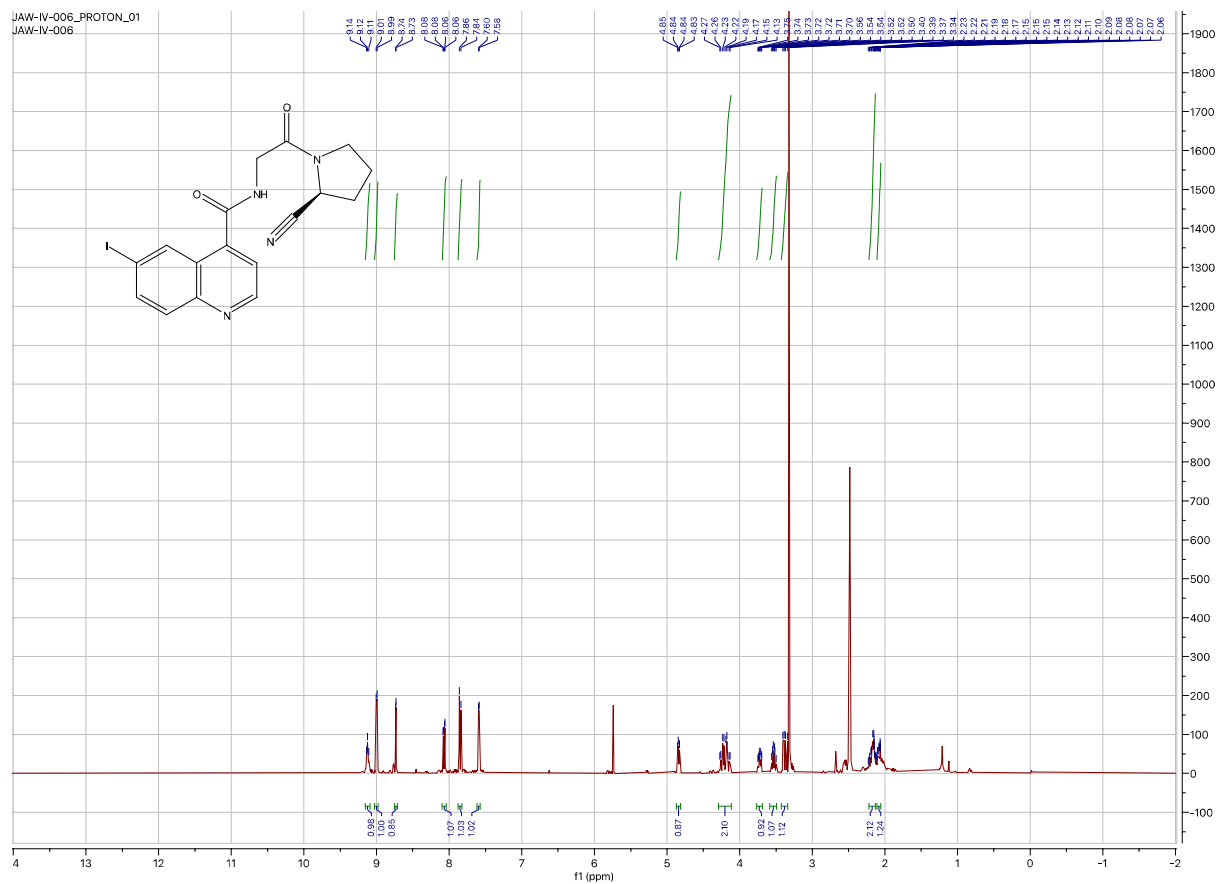


Figure S9. ^1H NMR of Compound 6b.

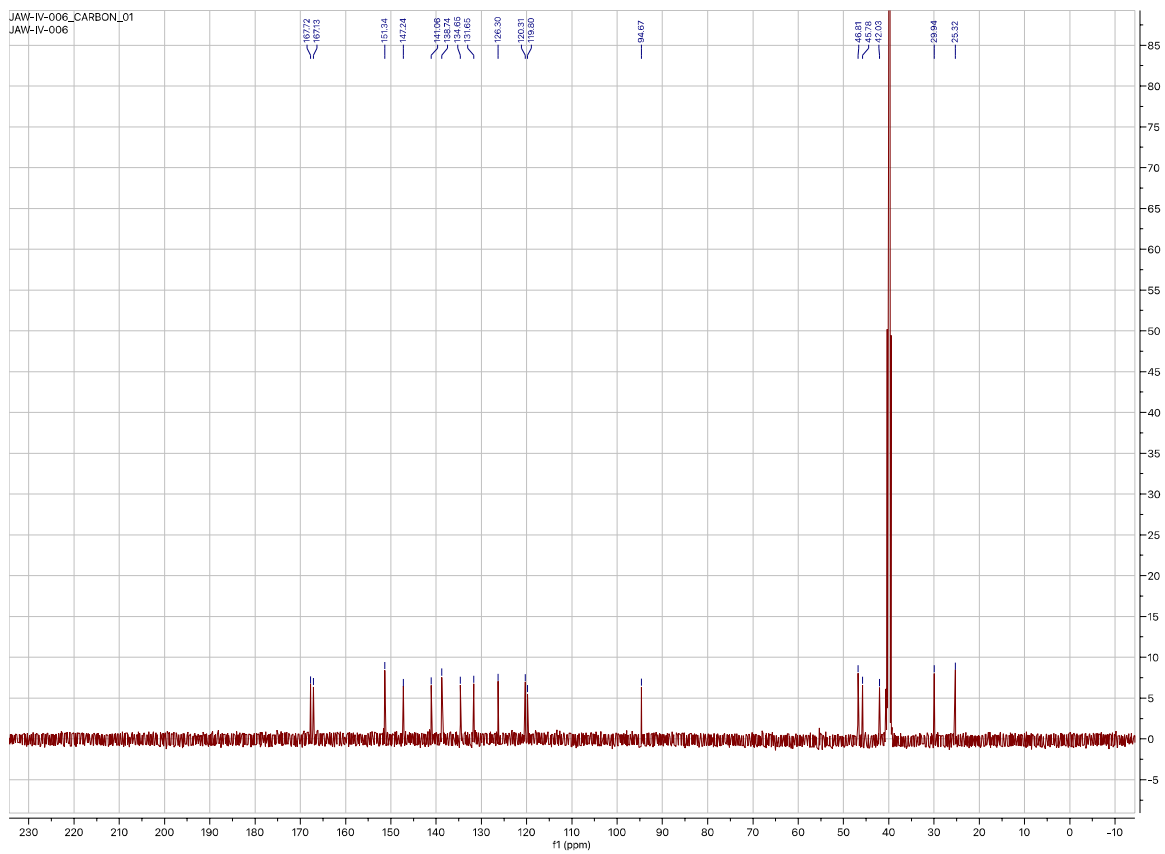


Figure S10. ^{13}C NMR of Compound **6b**.



Figure S11. ^1H NMR of Me_3SnFAPI **2**.

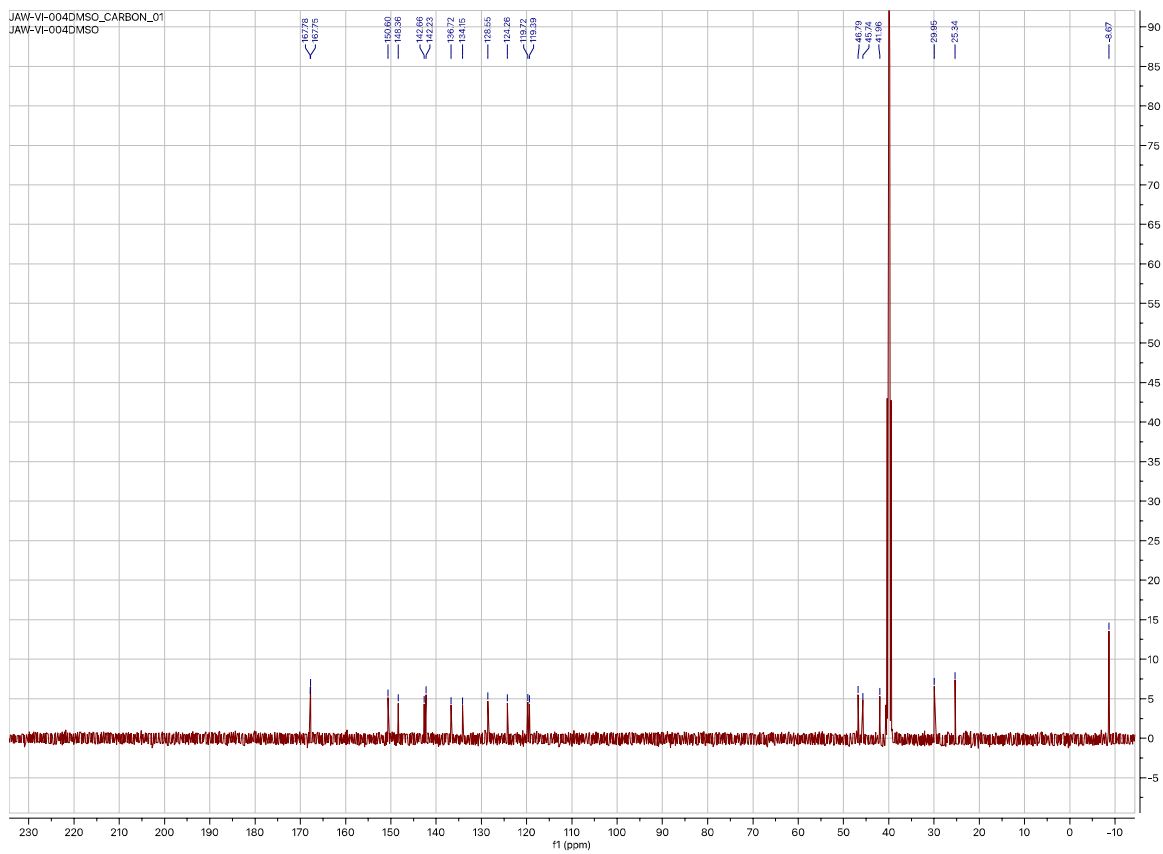


Figure S12. ^{13}C NMR of $\text{Me}_3\text{SnFAPI 2}$.

Solvent: MeOH

Ionization Method: ESI+

Analyzer: TOF

Mass Calculator				
Base formula (M)				
C20 H24 N4 O2 Sn				
Species to calculate				
☒ Positive ions ☐ Negative ions				
☒ Neutral ☐ Radical ☒ +H				
Species	Calc m/z	Mono m/z	Diff (ppm)	Defect
M	464.0947	472.0921		0.0947
(M+H)+	465.102	473.0994		0.102

Peak of Interest

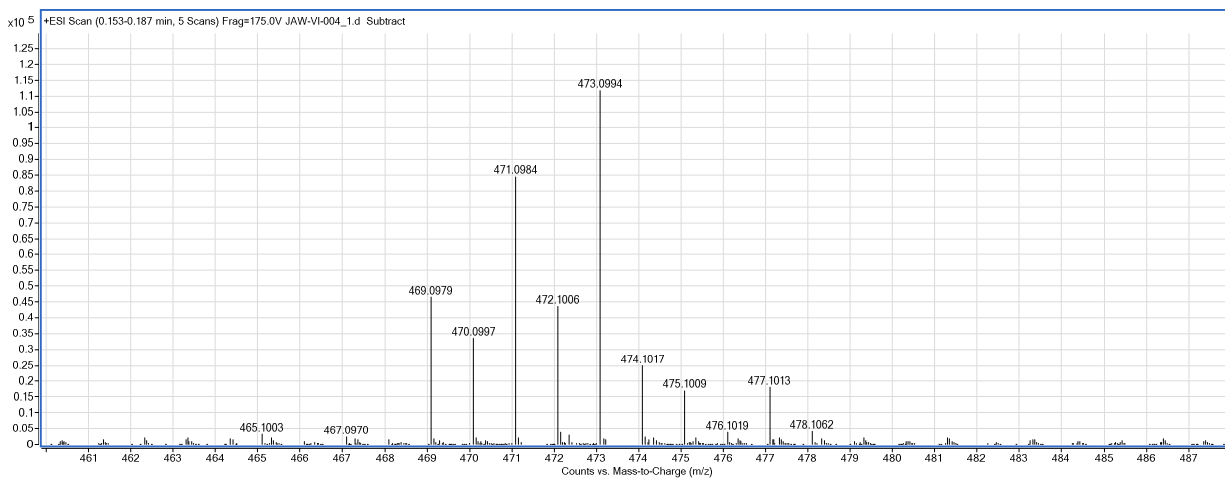


Figure S13. HRMS of Me₃SnFAPI **2**.

2) Radio- and UV-HPLC chromatograms of:

2i) Semi-preparative separation of [^{18}F]FluoFAPI 1

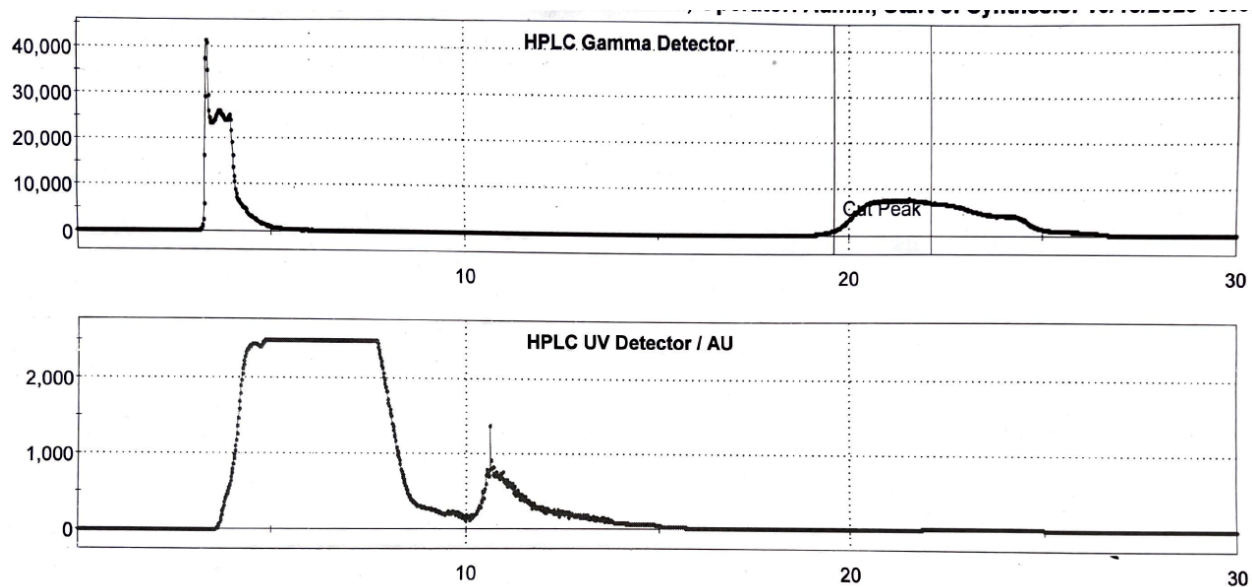
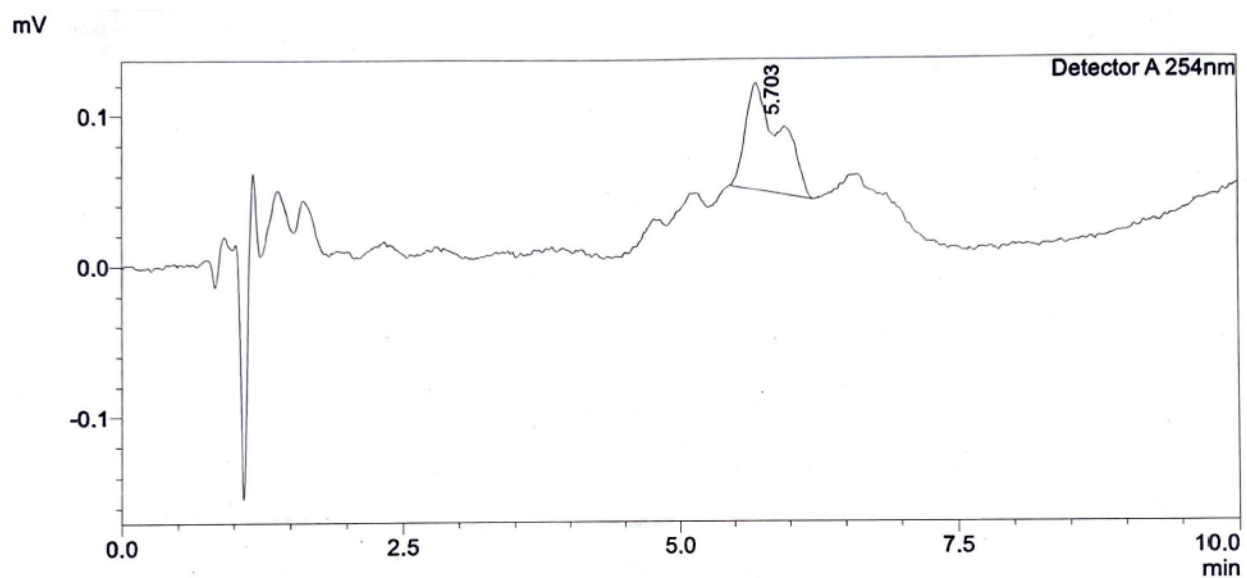


Figure S14. UV- and radio-chromatogram of the semi-preparative HPLC separation of [^{18}F]FluoFAPI 1.

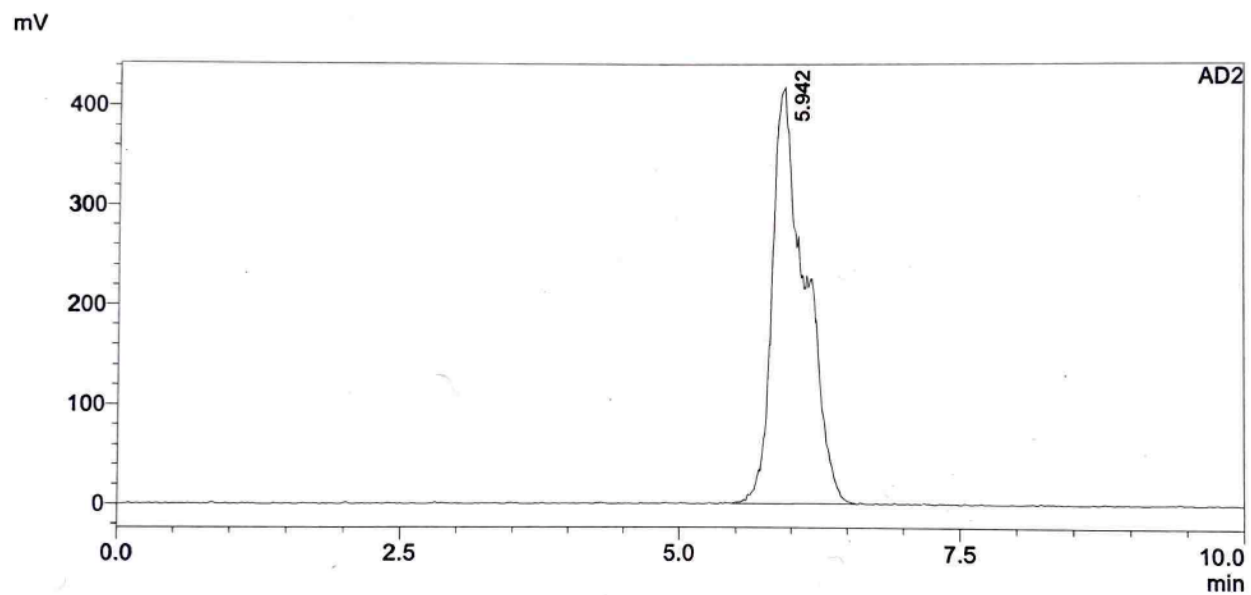
Column: Gemini 5 μ NXC18 110 Å, 250X10; Buffer: 55% MeCN, 10 mM NH_4HCO_3 , pH 10.0
Flow rate: 4 ml/min @ rt; UV: 254 nm; Retention time: ~20 min

2ii) Final formulated dose [^{18}F]FluoFAPI **1**



Detector A 254nm

Peak#	Ret. Time	Area	Height	Conc.	Unit	Mark	Name
1	5.703	1545	71	100.000		M	
Total		1545	71				



AD2

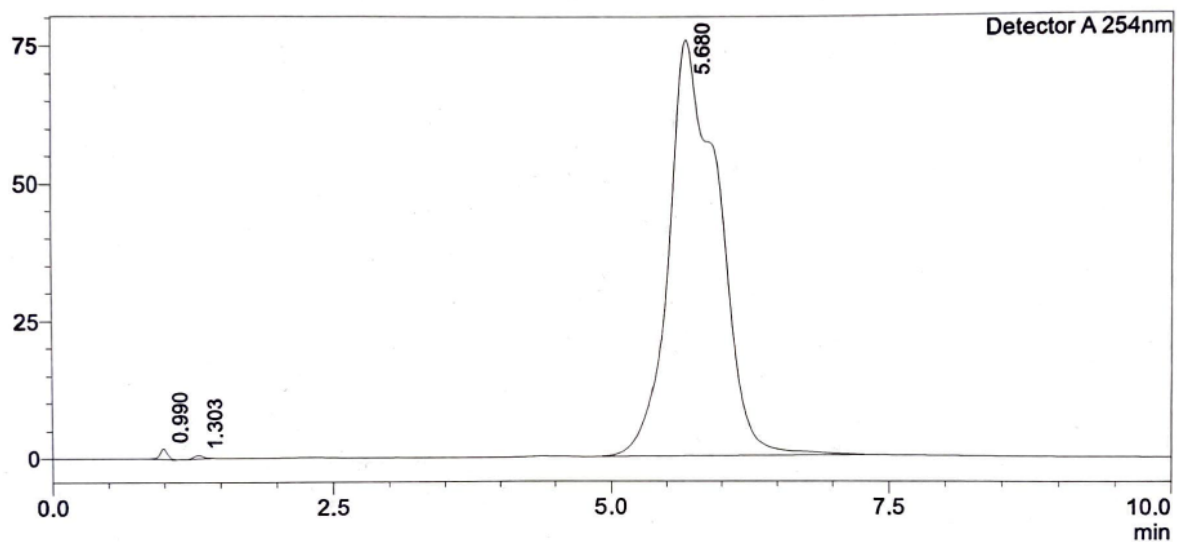
Peak#	Ret. Time	Area	Height	Conc.	Unit	Mark	Name
1	5.942	8720139	419005	100.000			

Figure S15. Analytical HPLC UV- and radio-chromatogram of [^{18}F]FluoFAPI **1**: Final formulated dose.

Column: Luna 5 μ C18(2), 150X4.6; Buffer: 20% MeCN, 10 mM NH₄OAc, pH 5.0 Flow rate: 2 ml/min @ 40 °C; UV: 254 nm; Retention time: ~5.94 min

2iii) Final formulated dose co-inject [¹⁸F]FluoFAPI 1

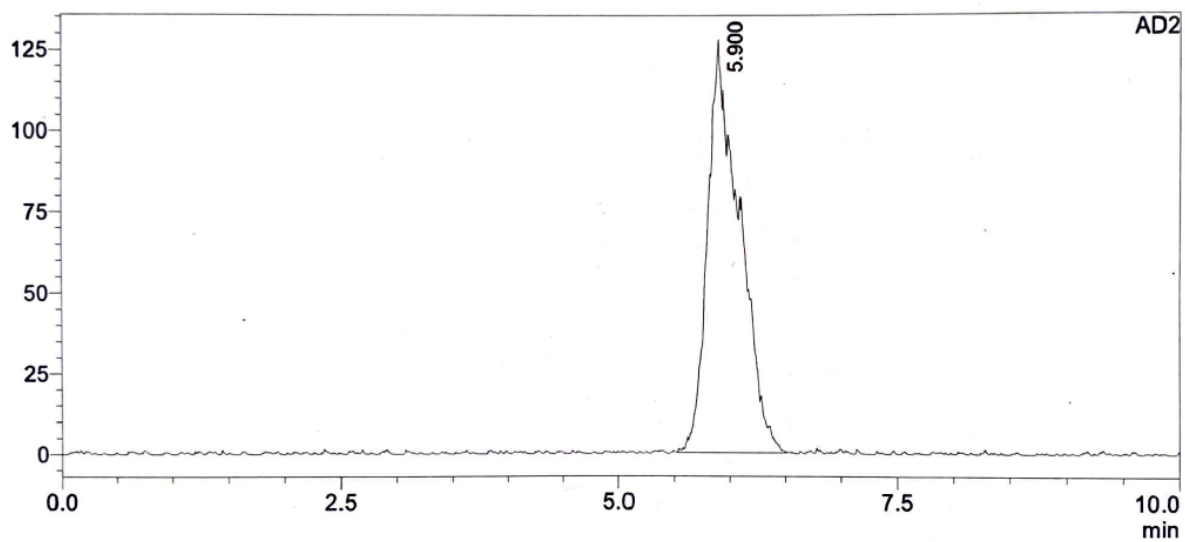
mV



Detector A 254nm

Peak#	Ret. Time	Area	Height	Conc.	Unit	Mark	Name
1	0.990	9090	1979	0.000			
2	1.303	3993	678	0.000			
3	5.680	2412126	75842	0.000			
Total		2425209	78499				

mV



AD2

Peak#	Ret. Time	Area	Height	Conc.	Unit	Mark	Name
1	5.900	2583008	128241	100.000			
Total		2583008	128241				

Figure S16. Analytical HPLC UV- and radio-chromatogram of [^{18}F]FluoFAPI **1**: Co-inject final formulated dose.

Column: Luna 5 μ C18(2), 150X4.6; Buffer: 20% MeCN, 10 mM NH₄OAc, pH 5.0 Flow rate: 2 ml/min @ 40 °C; UV: 254 nm; Retention time: ~5.90 min

3) Biodistribution data of [^{18}F]FluoFAPI **1** in Sprague-Dawley rats

Gender Avg Biodistribution

0.166667 hrs

Organ	%ID/g	±	S.E.
Brain	0.0347%	±	0.0067%
Eyes	0.1814%	±	0.0311%
Heart	0.4097%	±	0.0351%
Lungs	0.3438%	±	0.0334%
Liver	0.4987%	±	0.0620%
Pancreas	0.5010%	±	0.0427%
Spleen	0.3239%	±	0.0453%
Adrenals	0.3257%	±	0.0350%
Kidney	0.8577%	±	0.1466%
Adipose	0.0744%	±	0.0101%
Stomach	0.4059%	±	0.0566%
CO Stomach	0.0859%	±	0.0321%
Small Int	0.6419%	±	0.0777%
CO Small Int	0.3234%	±	0.0724%
Caecum	0.3656%	±	0.0713%
CO Caecum	0.1158%	±	0.0239%
Lg Int	0.4403%	±	0.0722%
CO Lg Int	0.1194%	±	0.0408%
Ovaries	0.3103%	±	0.0908%
Uterus	0.3507%	±	0.1027%
Testes	0.0614%	±	0.0011%
Muscles	0.2101%	±	0.0339%
Bone	0.3196%	±	0.0482%
Blood	0.3101%	±	0.0207%
Total	0.3898%	±	0.0594%
Carcass	0.5089%	±	0.0278%

	0.333333	hrs		
Organ	%ID/g	±	S.E.	
Brain	0.0400%	±	0.0086%	
Eyes	0.1791%	±	0.0352%	
Heart	0.3099%	±	0.0240%	
Lungs	0.2657%	±	0.0187%	
Liver	0.3710%	±	0.0308%	
Pancreas	0.3607%	±	0.0169%	
Spleen	0.2329%	±	0.0199%	
Adrenals	0.2649%	±	0.0038%	
Kidney	1.1177%	±	0.1473%	
Adipose	0.1607%	±	0.0323%	
Stomach	0.3653%	±	0.0245%	
CO Stomach	0.0596%	±	0.0162%	
Small Int	0.5997%	±	0.0634%	
CO Small Int	0.3892%	±	0.0121%	
Caecum	0.3575%	±	0.0325%	
CO Caecum	0.1692%	±	0.0253%	
Lg Int	0.3879%	±	0.0384%	
CO Lg Int	0.2024%	±	0.0430%	
Ovaries	0.3050%	±	0.0043%	
Uterus	0.3715%	±	0.0738%	
Testes	0.0964%	±	0.0008%	
Muscles	0.3269%	±	0.0439%	
Bone	0.3713%	±	0.0270%	
Blood	0.2647%	±	0.0118%	
Total	0.3614%	±	0.0343%	
Carcass	0.5322%	±	0.0371%	

	1	hrs		
Organ	%ID/g	±	S.E.	
Brain	0.0139%	±	0.0010%	
Eyes	0.0908%	±	0.0073%	
Heart	0.1370%	±	0.0027%	
Lungs	0.1176%	±	0.0021%	
Liver	0.1958%	±	0.0197%	
Pancreas	0.1603%	±	0.0007%	
Spleen	0.0997%	±	0.0018%	
Adrenals	0.1291%	±	0.0032%	
Kidney	0.6052%	±	0.1086%	
Adipose	0.0933%	±	0.0126%	
Stomach	0.2168%	±	0.0353%	

CO Stomach	0.2976%	±	0.0343%
Small Int	0.4855%	±	0.0581%
CO Small Int	1.5261%	±	0.1747%
Caecum	0.2338%	±	0.0243%
CO Caecum	0.3415%	±	0.0639%
Lg Int	0.2250%	±	0.0155%
CO Lg Int	0.3062%	±	0.0532%
Ovaries	0.1411%	±	0.0096%
Uterus	0.1758%	±	0.0006%
Testes	0.1006%	±	0.0001%
Muscles	0.1709%	±	0.0045%
Bone	0.1914%	±	0.0106%
Blood	0.1210%	±	0.0058%
Total	0.3460%	±	0.0139%
Carcass	0.3066%	±	0.0186%

2 hrs

Organ	%ID/g	±	S.E.
Brain	0.0077%	±	0.0007%
Eyes	0.0546%	±	0.0102%
Heart	0.0836%	±	0.0075%
Lungs	0.0713%	±	0.0054%
Liver	0.1088%	±	0.0079%
Pancreas	0.0904%	±	0.0081%
Spleen	0.0576%	±	0.0067%
Adrenals	0.0772%	±	0.0065%
Kidney	0.4346%	±	0.1225%
Adipose	0.0692%	±	0.0126%
Stomach	0.1450%	±	0.0236%
CO Stomach	0.2630%	±	0.1404%
Small Int	0.4632%	±	0.0516%
CO Small Int	2.1286%	±	0.6614%
Caecum	0.1925%	±	0.0115%
CO Caecum	0.3539%	±	0.0449%
Lg Int	0.1733%	±	0.0174%
CO Lg Int	0.3408%	±	0.0195%
Ovaries	0.0997%	±	0.0125%
Uterus	0.1131%	±	0.0104%
Testes	0.0690%	±	0.0084%
Muscles	0.1039%	±	0.0088%
Bone	0.1055%	±	0.0119%
Blood	0.0753%	±	0.0050%
Total	0.3529%	±	0.0349%

Carcass 0.2467% ± 0.0186%

Figure S17. Biodistribution data of [^{18}F]FluoFAPI 1 in Sprague-Dawley rats

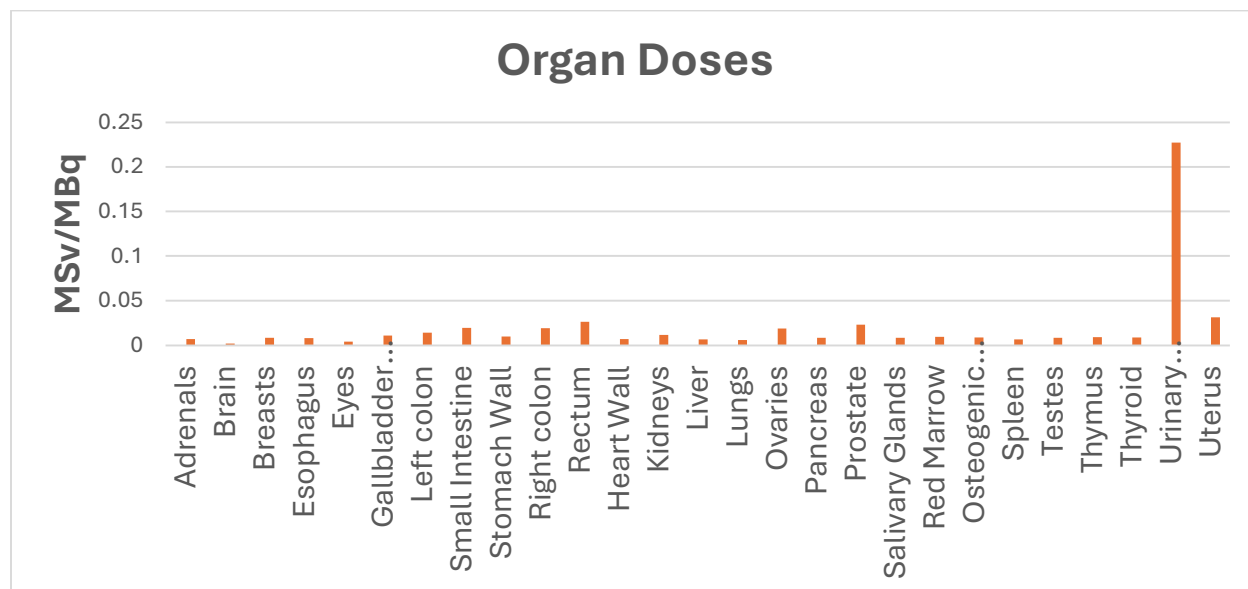
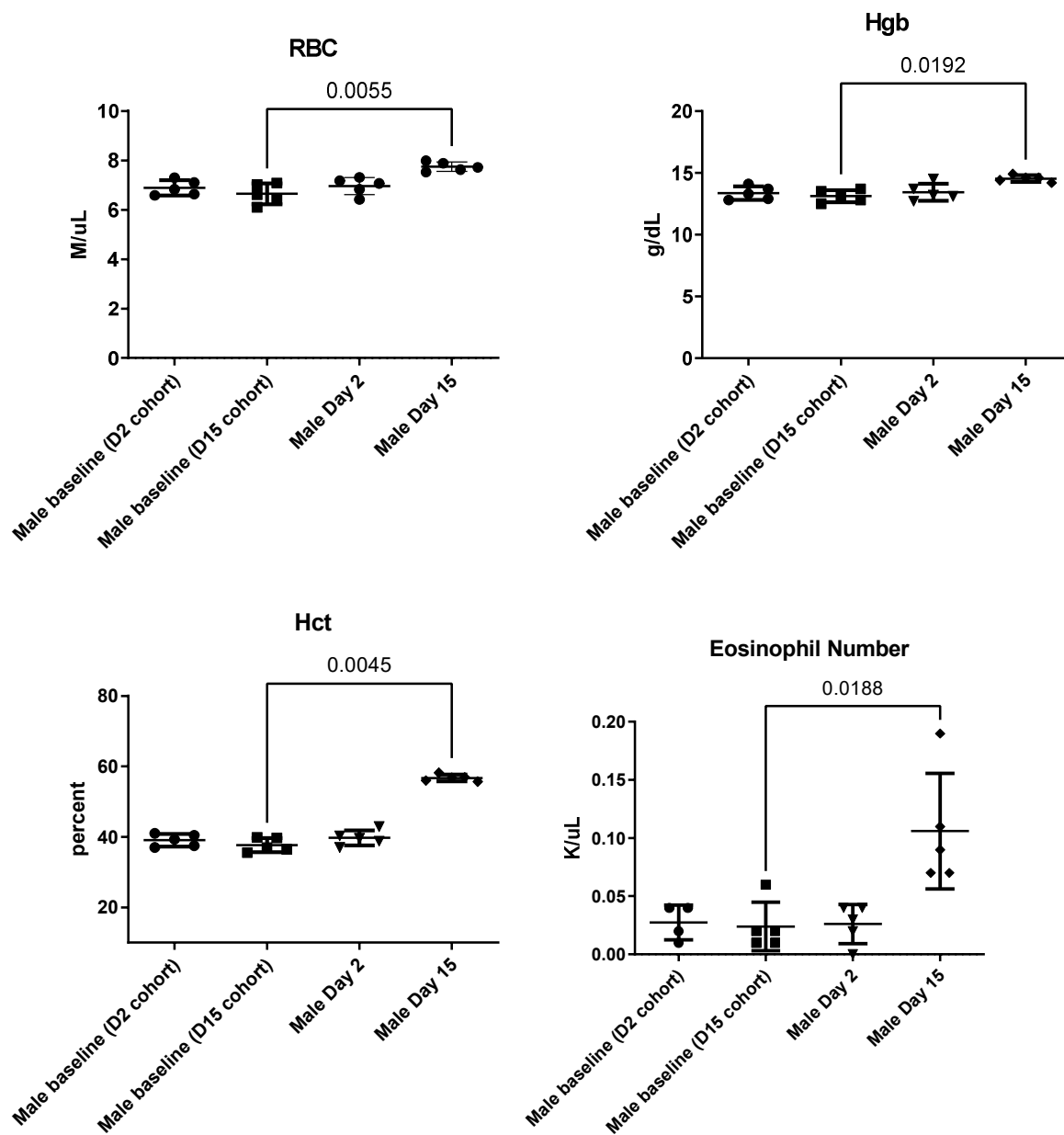


Figure S18. Bar graph of estimated organ doses for [^{18}F]FluoFAPI 1.

Single Acute Dose Toxicology Data

Figure S19. Sprague Dawley Rat Hematology Changes



Group	Animal No.	RBC M/uL	Hemoglobin g/dL	Hematocrit %	MCV fL	MCH pg	MCHC g/dL	RDW fL	RDW %
1 Male Day 2 cohort	101	7.31	14.5	42.9	58.7	19.8	33.8	30.7	17.9
	102	6.42	12.7	37.0	57.6	19.8	34.3	30.7	17.1
	103	7.07	13.1	39.6	56.0	18.5	33.1	30.9	19.8
	104	6.83	13.2	38.8	56.8	19.3	34.0	29.5	18.1
	105	7.18	13.7	40.3	56.1	19.1	34.0	30.3	19.3
2 Female Day 2 cohort	201	7.16	13.0	37.4	52.2	18.2	34.8	24.7	17.0
	202	6.89	13.0	37.9	55.0	18.9	34.3	25.8	18.5
	203	7.35	14.1	40.0	54.4	19.2	35.3	25.4	16.5
	204	7.22	13.3	38.7	53.6	18.4	34.4	25.4	17.1
	205	7.53	13.2	37.6	49.9	17.5	35.1	24.9	19.1
3 Male Day 15 cohort	301	7.99	14.6	55.7	53.6	18.3	34.1	27.3	18.7
	302	7.53	14.6	56.9	56.6	19.4	34.3	29.1	17.4
	303	7.89	14.4	56.0	54.0	18.3	33.8	27.8	18.3
	304	7.72	14.9	58.2	56.9	19.3	33.9	27.7	16.4
	305	7.63	14.2	56.8	55.0	18.6	33.6	26.9	16.6
4 Female Day 15 cohort	401	7.42	12.9	36.8	49.6	17.4	35.1	26.2	18.6
	402	7.88	14.4	41.1	52.2	18.3	35.0	25.9	18.0
	403	7.59	14.2	41.4	54.5	18.7	34.3	28.4	18.2
	404	7.61	14.2	40.9	53.7	18.7	34.7	29.1	19.3
	405	7.45	13.5	39.3	52.8	18.1	34.4	26.6	17.9

Group	Animal No.	Reticulocytes K/uL	Reticulocytes %	Platelets K/uL	PDW fL	MPV fL	WBC K/uL	Neutrophils K/uL	Lymphocytes K/uL
1 Male Day 2 cohort	101	570.2	7.80	912	8.0	7.2	10.94	2.92	7.49
	102	582.9	9.08	545	9.3	7.8	9.88	2.09	7.46
	103	581.9	8.23	998	7.5	6.7	8.19	2.54	5.38
	104	532.7	7.80	1061	7.5	6.8	11.84	2.29	9.05
	105	637.6	8.88	1214	8.0	7.1	8.66	1.98	6.38
2 Female Day 2 cohort	201	490.5	6.85	1128	8.8	7.3	7.94	1.74	5.89
	202	478.9	6.95	1049	7.6	6.8	6.59	2.45	3.79
	203	353.5	4.81	1063	8.4	7.1	8.19	1.63	6.27
	204	419.5	5.81	942	7.8	6.8	6.80	1.65	4.78
	205	320.0	4.26	1061	7.5	6.5	8.86	1.54	6.99
3 Male Day 15 cohort	301	239.7	3.00	706	9.7	8.1	6.69	0.91	5.29
	302	279.4	3.71	838	9.1	7.5	6.08	0.81	4.90
	303	288.0	3.65	843	8.1	7.1	5.70	0.87	4.46
	304	271.7	3.52	705	7.8	7.1	11.26	1.60	9.17
	305	288.4	3.78	931	7.4	6.5	6.86	0.93	5.59
4 Female Day 15 cohort	401	238.2	3.21	976	7.2	6.3	4.10	0.86	3.02
	402	302.6	3.84	860	7.8	6.8	8.90	1.45	6.97
	403	266.4	3.51	860	9.3	7.7	4.27	0.56	3.59
	404	265.6	3.49	883	8.3	7.2	4.63	0.86	3.56
	405	248.1	3.33	825	8.6	7.4	4.53	0.53	3.87

Group	Animal No.	Monocytes K/uL	Eosinophils K/uL	Basophils K/uL	Neutrophils %	Lymphocytes %	Monocytes %	Eosinophils %	Basophils %
1 Male Day 2 cohort	101	0.41	0.04	0.08	26.7	68.5	3.7	0.4	0.7
	102	0.27	0.02	0.04	21.2	75.5	2.7	0.2	0.4
	103	0.20	0.03	0.04	31.0	65.7	2.4	0.4	0.5
	104	0.43	0.04	0.03	19.4	76.4	3.6	0.3	0.3
	105	0.27	0.00	0.03	22.9	73.7	3.1	0.0	0.3
2 Female Day 2 cohort	201	0.25	0.05	0.01	22.0	74.2	3.1	0.6	0.1
	202	0.23	0.01	0.02	37.2	57.5	3.5	1.5	0.3
	203	0.20	0.05	0.04	19.9	76.6	2.4	0.6	0.5
	204	0.26	0.05	0.06	24.3	70.3	3.8	0.7	0.9
	205	0.18	0.10	0.05	17.4	78.9	2.0	1.1	0.6
3 Male Day 15 cohort	301	0.38	0.09	0.02	13.6	79.1	5.7	1.3	0.3
	302	0.28	0.07	0.02	13.3	80.6	4.6	1.2	0.3
	303	0.28	0.07	0.02	15.3	78.2	4.9	1.2	0.4
	304	0.27	0.19	0.03	14.2	81.4	2.4	1.7	0.3
	305	0.21	0.11	0.02	13.5	81.5	3.1	1.6	0.3
4 Female Day 15 cohort	401	0.14	0.07	0.01	21.0	73.7	3.4	1.7	0.2
	402	0.35	0.07	0.06	16.3	78.3	3.9	0.8	0.7
	403	0.09	0.02	0.01	13.1	84.1	2.1	0.5	0.2
	404	0.10	0.09	0.02	18.6	76.9	2.2	1.9	0.4
	405	0.05	0.05	0.03	11.7	85.4	1.1	1.1	0.7

Organ Weight (g)						
Group	Animal No.	Brain	Heart	Liver	Spleen	Kidneys (L&R combined)
Males, Day 2	101	1.917	1.104	9.864	0.860	1.751
	102	1.893	1.070	10.642	0.874	1.924
	103	1.962	1.158	9.298	0.715	1.925
	104	1.930	1.091	9.175	0.736	1.856
	105	1.866	1.042	8.860	0.496	1.914
Females, Day 2	201	1.881	0.869	6.719	0.551	1.266
	202	1.853	0.911	6.833	0.752	1.475
	203	1.784	0.841	6.977	0.586	1.341
	204	1.920	1.062	7.413	0.611	1.462
	205	1.810	0.836	6.127	0.598	1.349
Males, Day 15	301	1.936	1.002	9.742	0.558	1.886
	302	1.916	0.998	11.367	0.601	1.841
	303	2.004	1.136	11.373	0.622	2.356
	304	1.833	1.113	11.034	0.753	2.083
	305	1.850	1.204	10.599	0.677	2.178
Females, Day 15	401	1.951	0.921	7.882	0.536	1.428
	402	1.640	0.894	8.595	0.572	1.461
	403	2.011	0.863	7.790	0.610	1.588
	404	1.902	0.947	7.852	0.675	1.569
	405	2.050	0.838	6.481	0.660	1.384