

RI内用療法を目指した シグマ受容体標的化合物の基礎的検討

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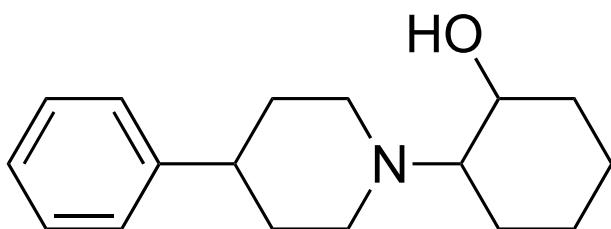
Introduction

- シグマ受容体は、シグマ-1とシグマ-2といった二つのサブタイプが同定されている⁽¹⁾
- シグマ受容体は、種々の癌細胞において過剰発現していることが報告されている⁽²⁾
- この癌における過剰発現により、シグマ受容体は、癌診断薬や癌治療薬開発のための標的分子と成り得る

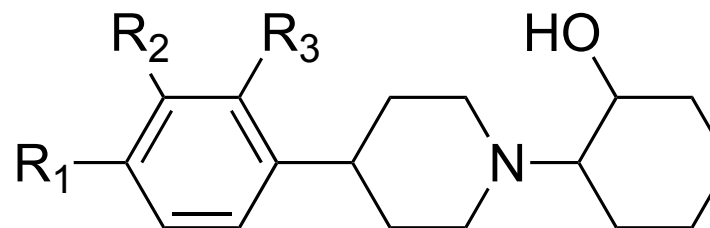
(1) Quirion R et al. *Trends Pharmacol Sci* (1992)

(2) Vilner BJ et al. *Cancer Res* (1995)

Chemical Structures of Vesamicol Analogs



Vesamicol



Iodovesamicol (IV)

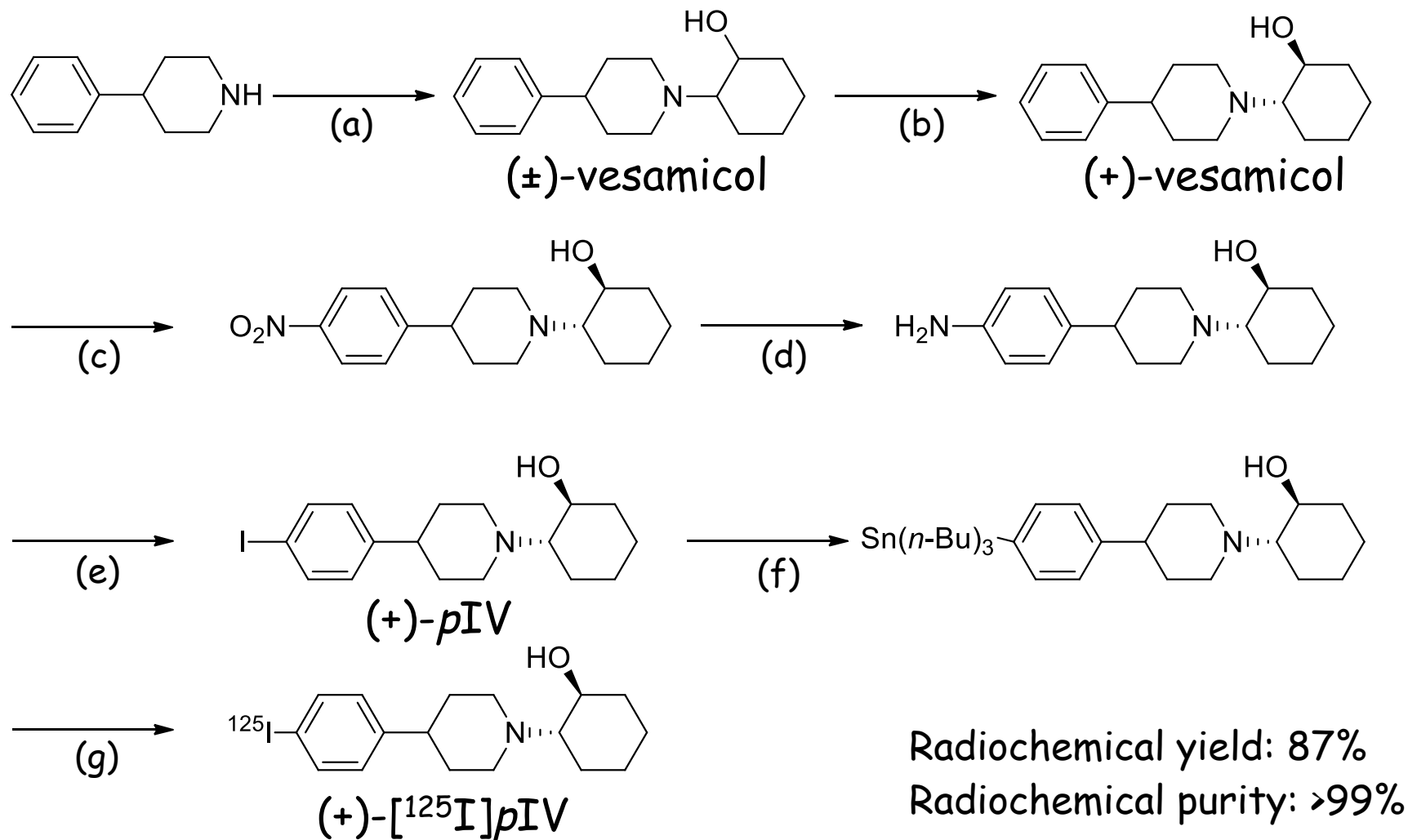
	R_1	R_2	R_3
<i>o</i> -Iodovesamicol (<i>o</i> IV)	I	H	H
<i>m</i> -Iodovesamicol (<i>m</i> IV)	H	I	H
<i>p</i> -Iodovesamicol (<i>p</i> IV)	H	H	I

Affinities (nM) of Vesamicol Analogs for Sigma Receptors

	Sigma-1	Sigma-2
(+)-Vesamicol	31.8 ± 11.4	359 ± 39
(-)-Vesamicol	74.9 ± 18.6	421 ± 69
(+)- <i>o</i> IV	14.6 ± 1.7	198 ± 93
(-)- <i>o</i> IV	62.2 ± 12.0	554 ± 137
(+)- <i>m</i> IV	2.5 ± 0.5	40.0 ± 14.7
(-)- <i>m</i> IV	4.5 ± 0.5	42.9 ± 15.0
(+)- <i>p</i> IV	1.3 ± 0.5	20.4 ± 2.0
(-)- <i>p</i> IV	3.4 ± 0.5	28.1 ± 3.9
(+)-Pentazocine	19.9 ± 3.5	2680 ± 162
Haloperidol	13.5 ± 2.0	110 ± 4

K_i values derived from IC_{50} values according to the equation,
 $K_i = IC_{50}(1+C/K_d)$. Data are expressed as K_i (nM) (mean ± SEM).

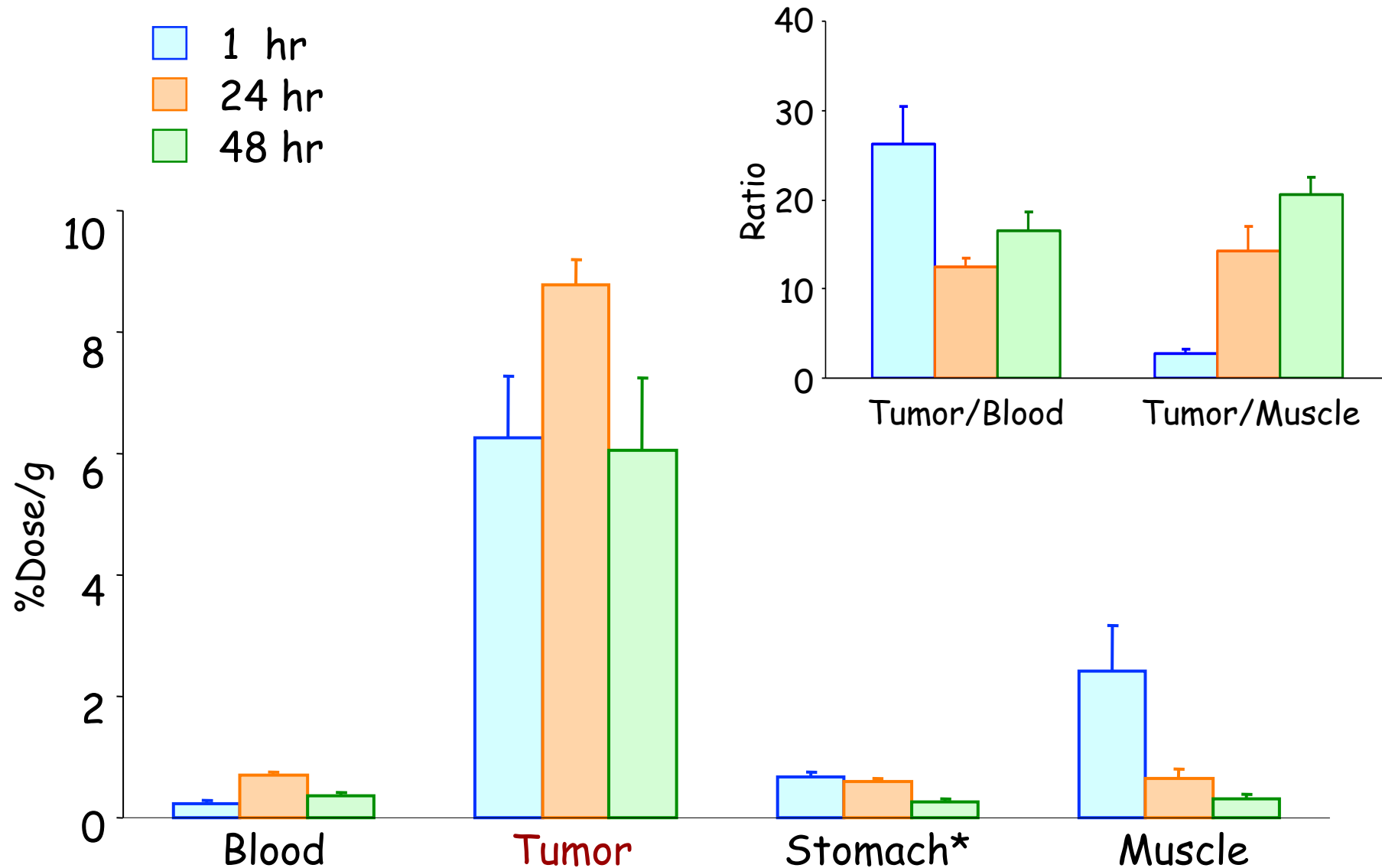
Preparation of (+)-[¹²⁵I]pIV



(a) cyclohexene oxide. (b) (+)-di-*p*-toluoyl-D-tartaric acid. (c) HNO₃, H₂SO₄.

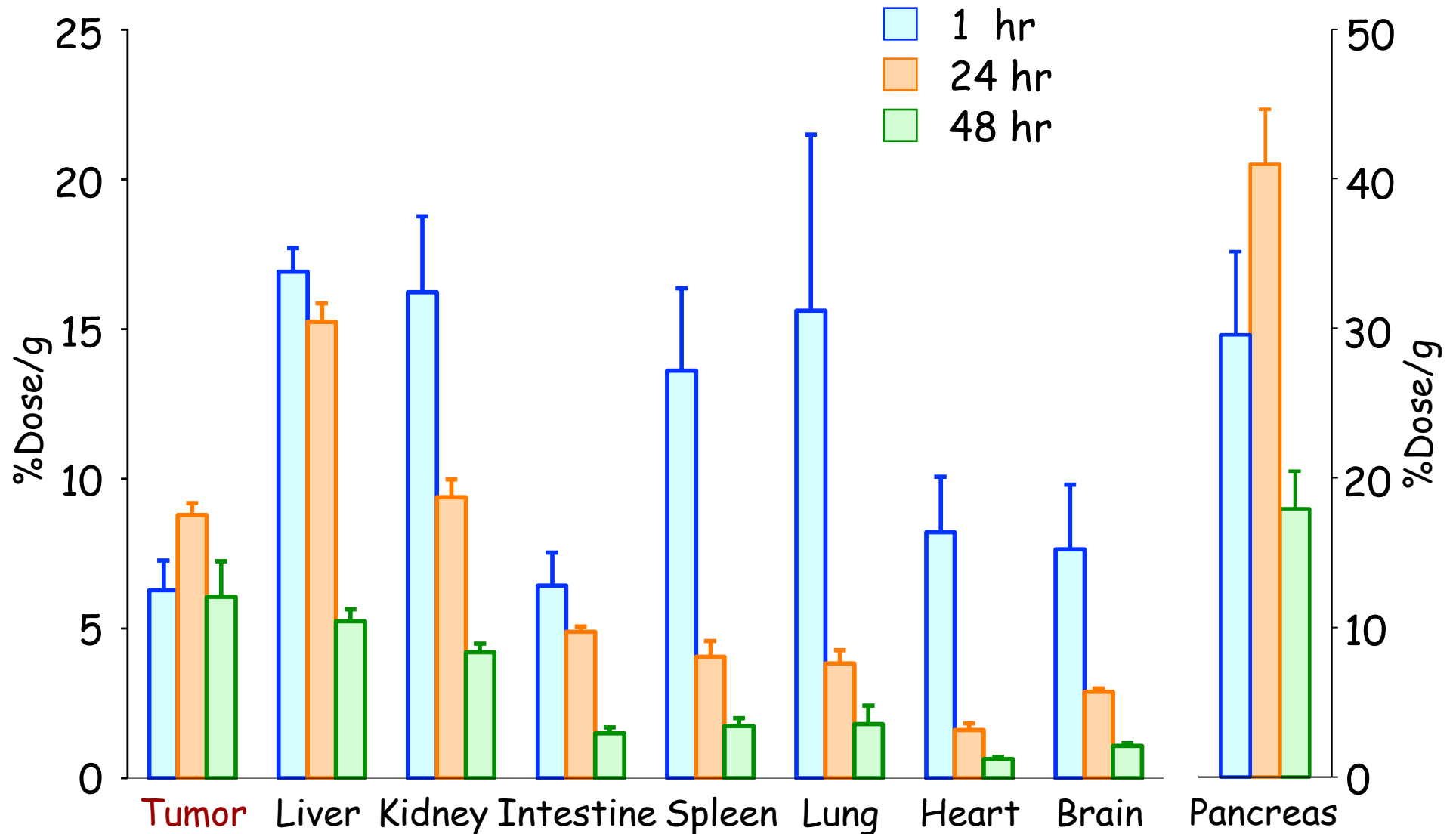
(d) Fe, HCl. (e) NaNO₂, NaI. (f) [(*n*-Bu)₃Sn]₂, (Ph₃P)₄Pd. (g) [¹²⁵I]NaI, H₂O₂

Biodistribution of (+)-[¹²⁵I]pIV in DU-145 Tumor-bearing Mice



*%Dose

Biodistribution of (+)-[¹²⁵I]pIV in DU-145 Tumor-bearing Mice



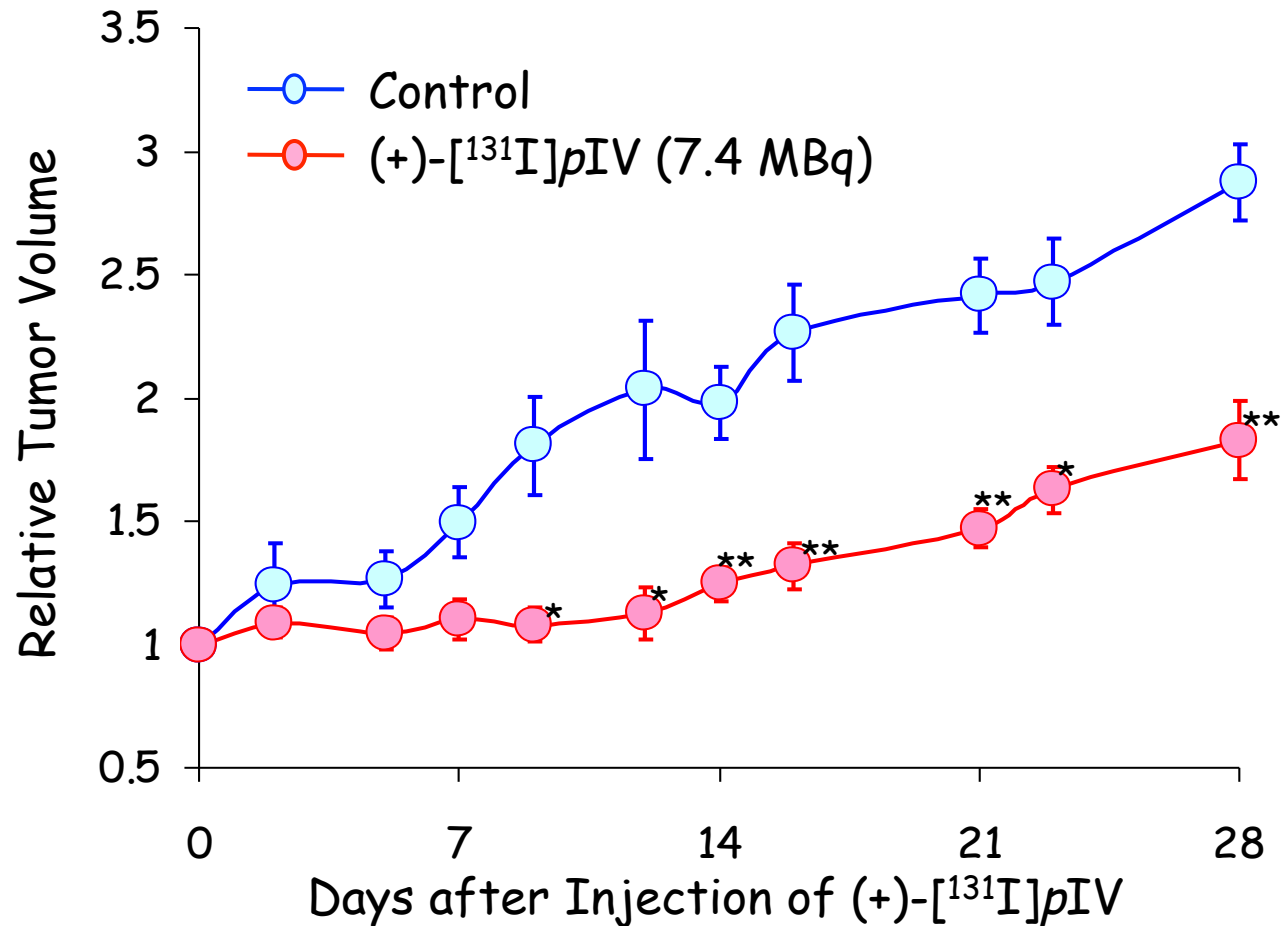
Analysis of Metabolites after Injection of (+)-[¹²⁵I]pIV in Tumor-bearing Mice

Tissue	Time after Injection	
	1 hour	24 hours
Blood	3.7 (2.6)	1.4 (1.5)
Tumor	83.1 (6.0)	40.7 (2.8)
Liver	76.0 (5.3)	10.4 (2.2)
Kidney	86.5 (5.5)	8.7 (2.0)
Lung	86.2 (4.0)	10.8 (0.8)
Brain	94.8 (2.6)	22.8 (1.3)

Data are expressed as % of intact (+)-[¹²⁵I]pIV.
Each value represents the mean (SD) for three samples.

Receptor Radionuclide Therapy

腫瘍の大きさをノギスにより測り、腫瘍の体積を $\text{Volume} = [\text{length} \times (\text{width})^2]/2$ により求め、(+)- ^{131}I pIV投与時に対する相対値で評価した



* $p < 0.05$, ** $p < 0.01$ vs control

K. Ogawa et al. *Cancer Sci*, (2009)

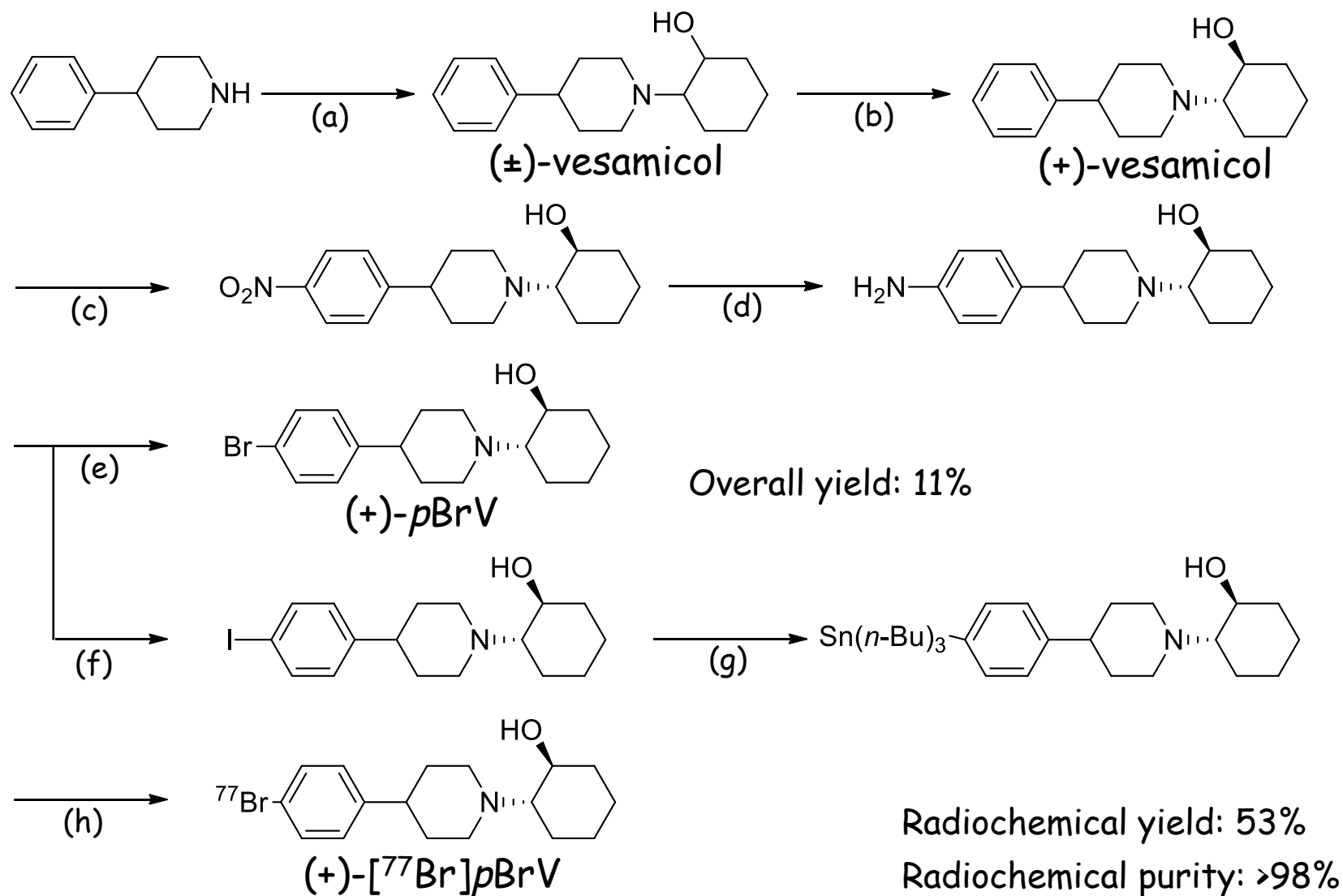
Bromine Radioisotopes

同位体	^{76}Br	^{77}Br
半減期	16時間	57時間
壊変形式	EC (43%) β^+ (57%)	EC (99%) β^+ (1%)
製造核反応	$^{76}\text{Se} (p, n) ^{76}\text{Br}$	$^{77}\text{Se} (p, n) ^{77}\text{Br}$

Periodic Table of the Elements
<http://chemistry.about.com>
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 About Chemistry

1A 1 H 1.00794	2A 4 He 4.002602																
3 Li 6.941	4 Be 9.012182											5 B 10.811	6 C 12.0107	7 N 14.0067	8 O 15.9994	9 F 18.9984032	10 Ne 20.1797
11 Na 22.989769	12 Mg 24.3050											13 Al 26.9815386	14 Si 28.0855	15 P 30.973762	16 S 32.065	17 Cl 35.453	18 Ar 39.948
19 K 39.0983	20 Ca 40.078	21 Sc 44.955912	22 Ti 47.867	23 V 50.9415	24 Cr 51.9961	25 Mn 54.938045	26 Fe 55.845	27 Co 58.933195	28 Ni 58.6934	29 Cu 63.546	30 Zn 65.38	31 Ga 69.723	32 Ge 72.64	33 As 74.92160	34 Se 78.96	35 Br 79.904	36 Kr 83.798
37 Rb 85.4678	38 Sr 87.62	39 Y 88.90585	40 Zr 91.224	41 Nb 92.90638	42 Mo 95.96	43 Tc [98]	44 Ru 101.07	45 Rh 102.90550	46 Pd 106.42	47 Ag 107.8682	48 Cd 112.411	49 In 114.818	50 Sn 118.710	51 Sb 121.760	52 Te 127.60	53 I 126.90447	54 Xe 131.293
55 Cs 132.9054519	56 Ba 137.327	57-71 Lanthanides	72 Hf 178.49	73 Ta 180.94788	74 W 183.84	75 Re 186.207	76 Os 190.23	77 Ir 192.217	78 Pt 195.084	79 Au 196.966569	80 Hg 200.59	81 Tl 204.3833	82 Pb 207.2	83 Bi 208.98040	84 Po [209]	85 At [210]	86 Rn [222]
87 Fr [223]	88 Ra [226]	89-103 Actinides	104 Rf [267]	105 Db [268]	106 Sg [271]	107 Bh [272]	108 Hs [270]	109 Mt [276]	110 Ds [281]	111 Rg [280]	112 Cn [285]	113 Uut [284]	114 Uuq [289]	115 Uup [288]	116 Uuh [293]	117 Uus [294]	118 Uuo [294]
Lanthanides		57 La 138.90547	58 Ce 140.116	59 Pr 140.90765	60 Nd 144.242	61 Pm [145]	62 Sm 150.36	63 Eu 151.964	64 Gd 157.25	65 Tb 158.92535	66 Dy 162.500	67 Ho 164.93032	68 Er 167.259	69 Tm 168.93421	70 Yb 173.054	71 Lu 174.9668	
Actinides		89 Ac [227]	90 Th 232.03806	91 Pa 231.03688	92 U 238.02891	93 Np [237]	94 Pu [244]	95 Am [243]	96 Cm [247]	97 Bk [247]	98 Cf [251]	99 Es [252]	100 Fm [257]	101 Md [258]	102 No [259]	103 Lr [262]	

Preparation of (+)-[⁷⁷Br]pBrV



(a) cyclohexene oxide. (b) (+)-di-*p*-toluoyl-D-tartric acid. (c) HNO₃, H₂SO₄. (d) Fe, HCl.
(e) HBr, NaNO₂, CuBr (f) NaNO₂, NaI. (g) [(*n*-Bu)₃Sn]₂, (Ph₃P)₄Pd. (h) [⁷⁷I]Br⁻, chloramine-T

Affinities (nM) of Vesamicol Analogs for Sigma Receptors

	Sigma 1 K_i (nM)	Sigma 2 K_i (nM)
(+)-Vesamicol	19.9 ± 2.7	164.7 ± 75.4
(+)- <i>p</i> BrV	1.0 ± 0.2	21.2 ± 1.1
(-)- <i>p</i> BrV	3.4 ± 0.3	48.9 ± 6.5
(+)- <i>p</i> IV	1.6 ± 0.8	14.3 ± 2.4
(+)-Pentazocine	10.2 ± 1.6	2541.8 ± 426.6
Haloperidol	6.1 ± 0.9	56.7 ± 4.8

K_i values derived from IC_{50} values according to the equation,
 $K_i = IC_{50}(1+C/K_d)$. Data are expressed as K_i (nM) (mean ± SEM).

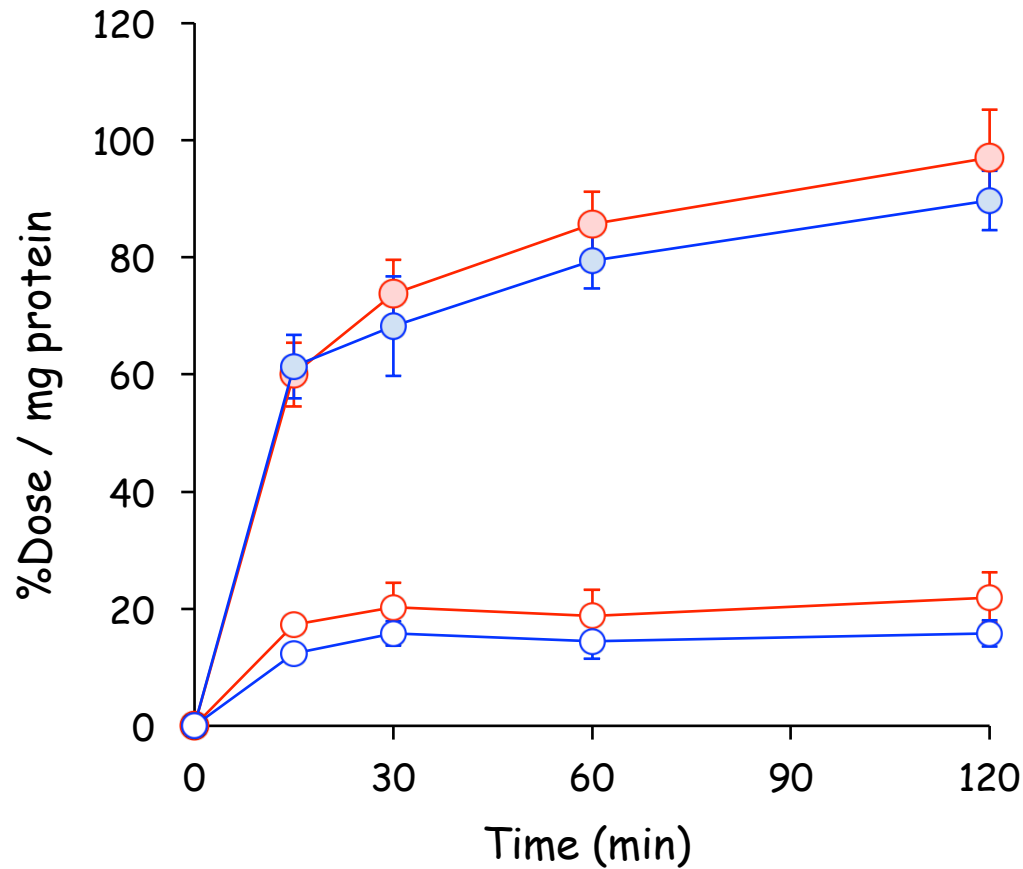
Partition Coefficient

Compound	Log P
(+)-[¹²⁵ I]pIV	2.08 (0.02)
(+)-[⁷⁷ Br]pBrV	1.58 (0.02)

Each value represents the mean (SD) for four samples.

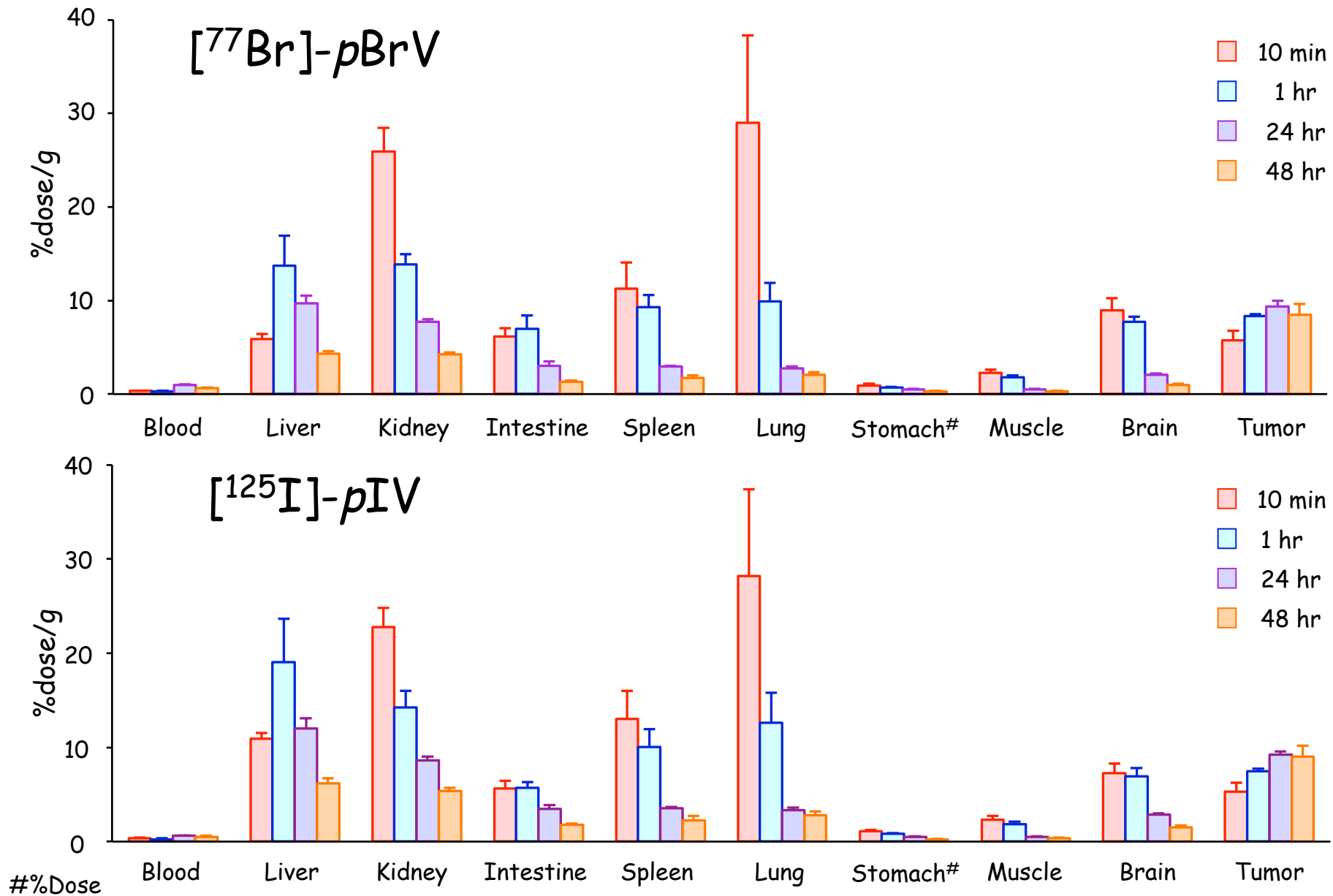
同量の1-オクタノールと0.02 Mリン酸緩衝液(pH 7.4)に(+)-[¹²⁵I]-IV-OHを加えボルテックス、遠心分離後、1-オクタノールのみをとり、新たに同量の緩衝液を加えた。同様にボルテックス、遠心分離後、有機相、水相の放射能を測定した
分配係数log P は $\log P = \log (\text{有機相の放射能濃度} / \text{水相の放射能濃度})$ で求めた

Cell (DU-145) Uptake Study of (+)-[⁷⁷Br]pBrV



- (+)-[⁷⁷Br]pBrV
- (+)-[⁷⁷Br]pBrV with haloperidol (10 μM)
- (+)-[¹²⁵I]pIV
- (+)-[¹²⁵I]pIV with haloperidol (10 μM)

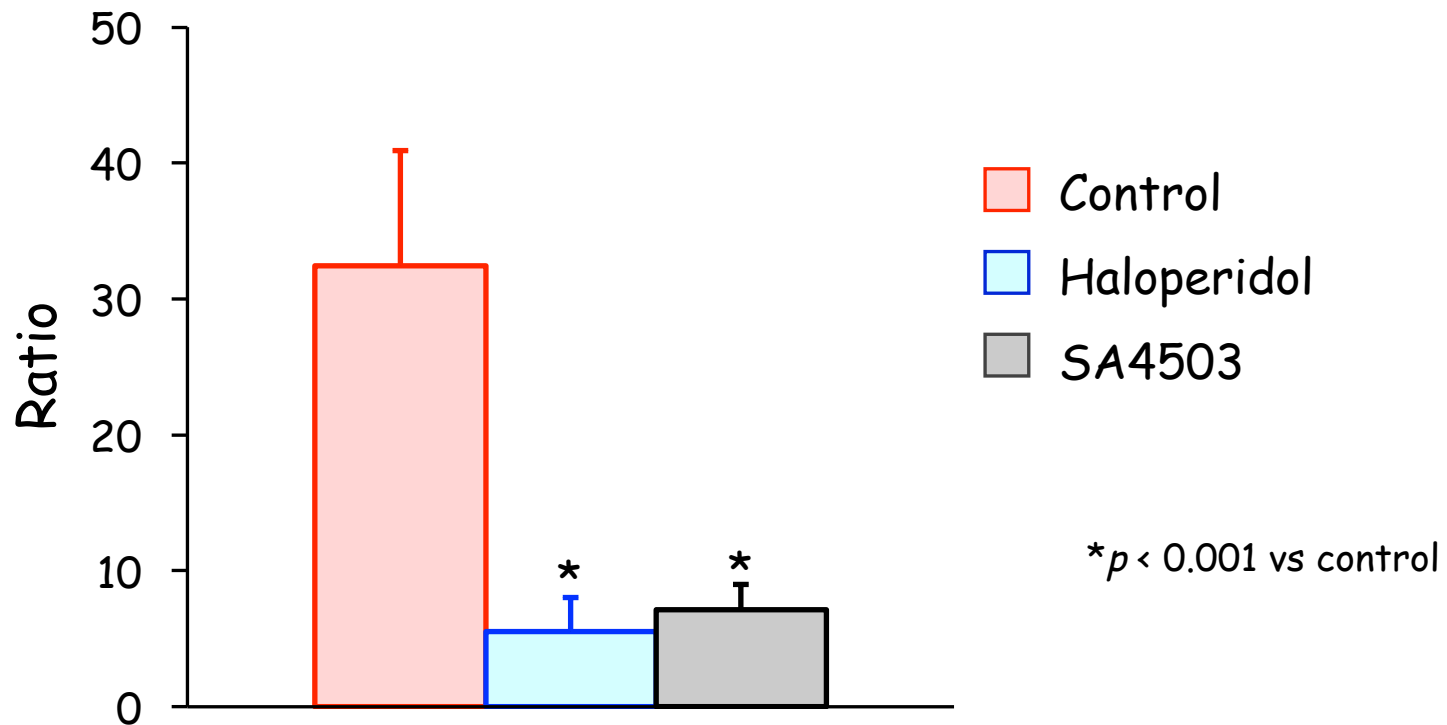
Biodistribution of (+)-[⁷⁷Br]pBrV and (+)-[¹²⁵I]pIV in DU-145 Tumor-bearing Mice



Blocking Study In Vivo

DU-145担癌マウスに $[^{77}\text{Br}]\text{-pBrV}$ とシグマリグンド[haloperidol (10 $\mu\text{mol}/\text{kg}$), SA4503 (10 $\mu\text{mol}/\text{kg}$)]を同時静脈内投与した
投与1時間後に屠殺し、腫瘍の重量と放射能を測定した

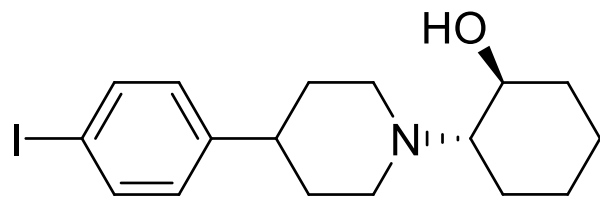
Tumor / Blood Ratio



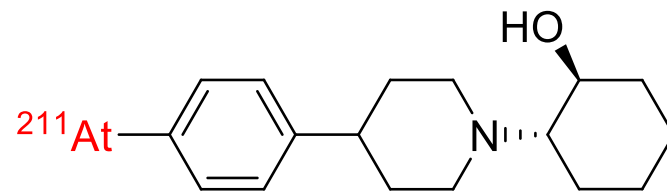
The Aim of This Study

(+)-*p*IVのIを ^{211}At に代替した(+)-[^{211}At]*pAtV*を作製し、RI内用療法に向けた基礎的検討を行った

- (+)-[^{211}At]*pAtV*の合成
- 分配係数測定
- DU145細胞取込実験
- DU145担癌マウスを用いた体内放射能分布実験

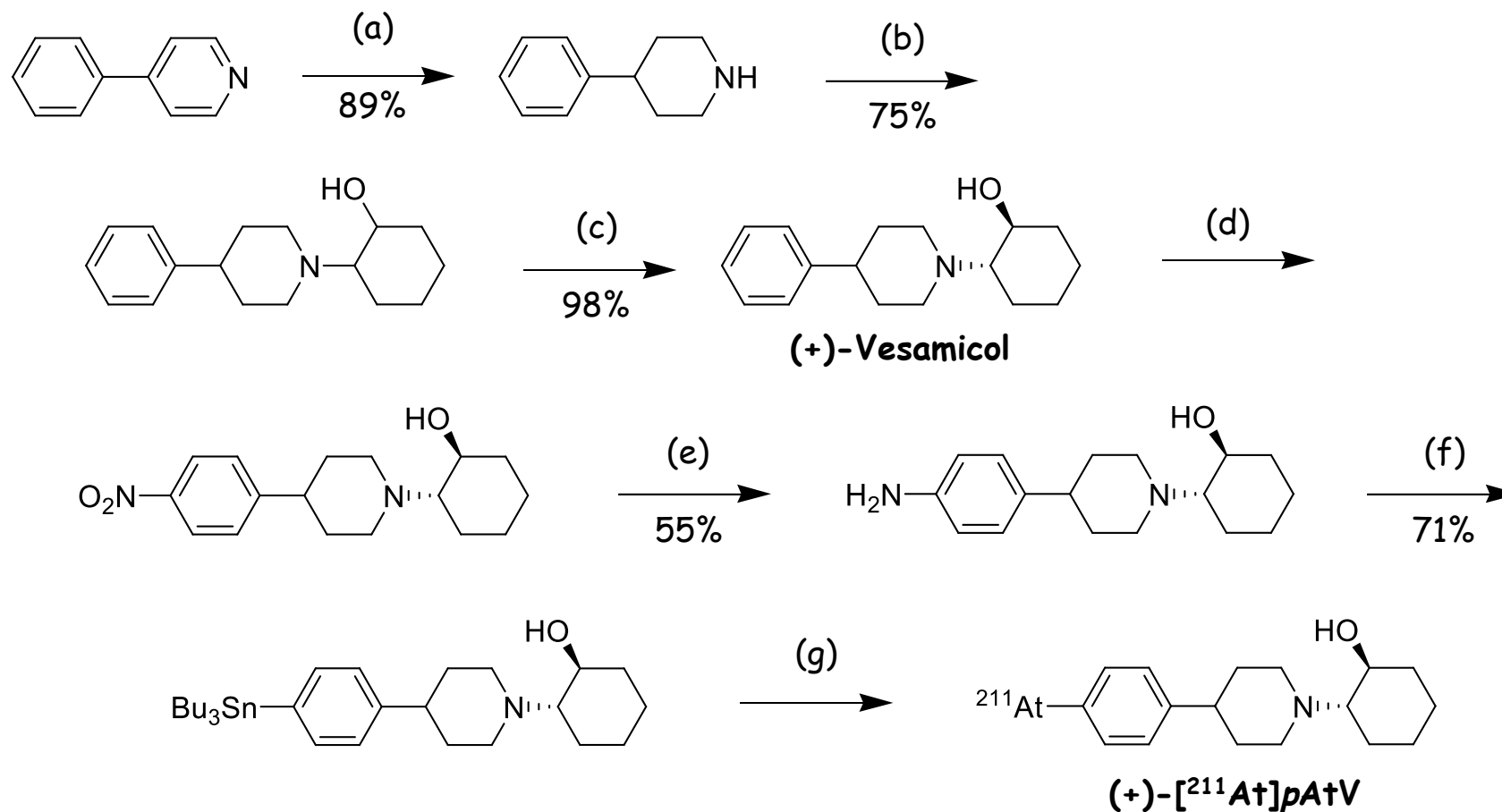


(+)-*p*-Iodovesamicol [(+)-*p*IV]



(+)-*p*-Astatovesamicol [(+)-*p*AtV]

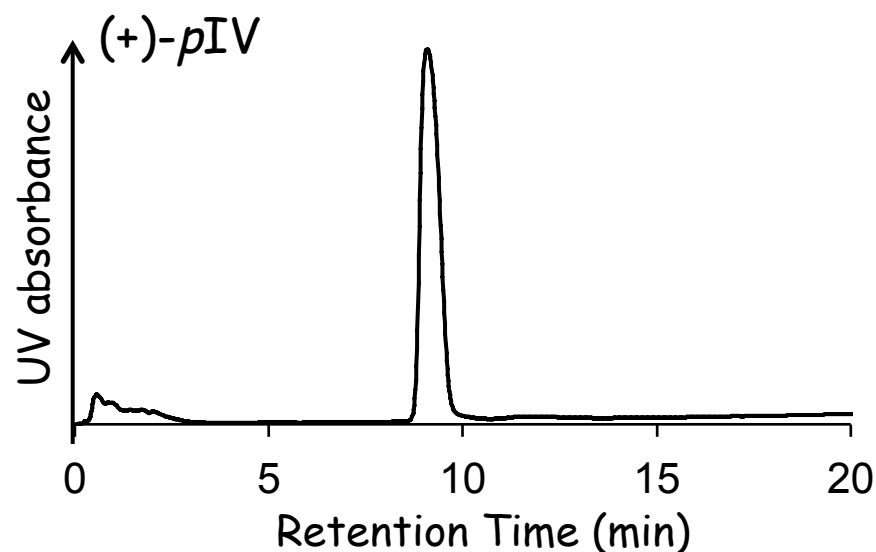
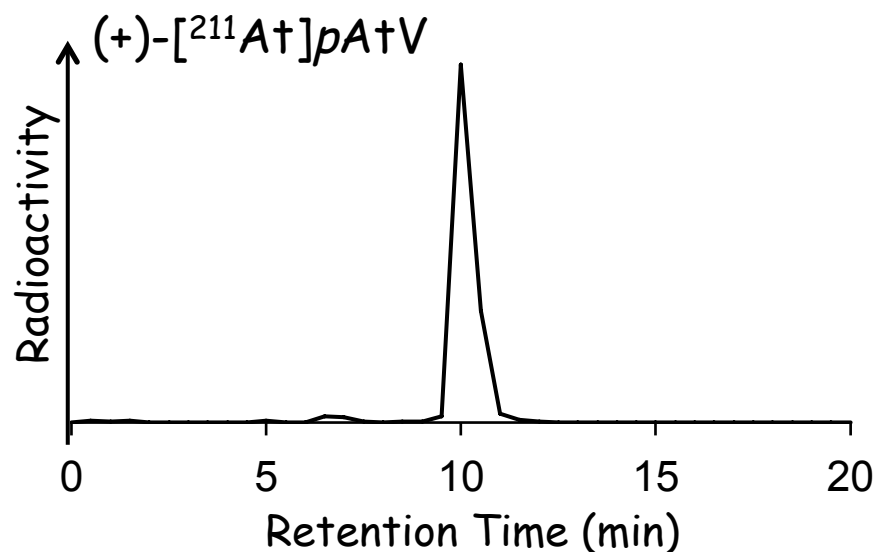
Preparation of (+)-[²¹¹At]pAtV



Radiochemical yield : 56%
Radiochemical purity : > 95%

(a) Na; (b) Cyclohexene oxide; (c) (+)-Di-*p*-toluoyl-D-tartaric acid; (d) HNO₃, H₂SO₄; (e) Fe, HCl;
(f) I₂, Hexabutyliditin; (g) ²¹¹At⁺, N-Chlorosuccinimide

Chromatogram and Partition Coefficient of (+)-[²¹¹At]pAtV



HPLC条件

カラム：5C₁₈ MS-II 4.6×150 mm

溶出溶媒：CH₃CN:H₂O(0.05% TEAを含む)
75:25→95:5(20 min)

波長：280 nm

流速：1 mL/min

	log <i>P</i> value
(+)-[²¹¹ At]pAtV	2.14 ± 0.02
(+)-[¹²⁵ I]pIV	2.08 ± 0.11

Each value represents the mean ± SD for four samples.

Summary

- 放射化学的純度95%以上で、(+)-[²¹¹At]pAtVの標識に成功した
- (+)-[²¹¹At]pAtVの癌細胞への取込はシグマ受容体特異的なものであった
- (+)-[²¹¹At]pAtVは投与後早期の腫瘍への高集積および滞留が観察された



(+)-[²¹¹At]pAtVのRI内用療法への応用の可能性が示された