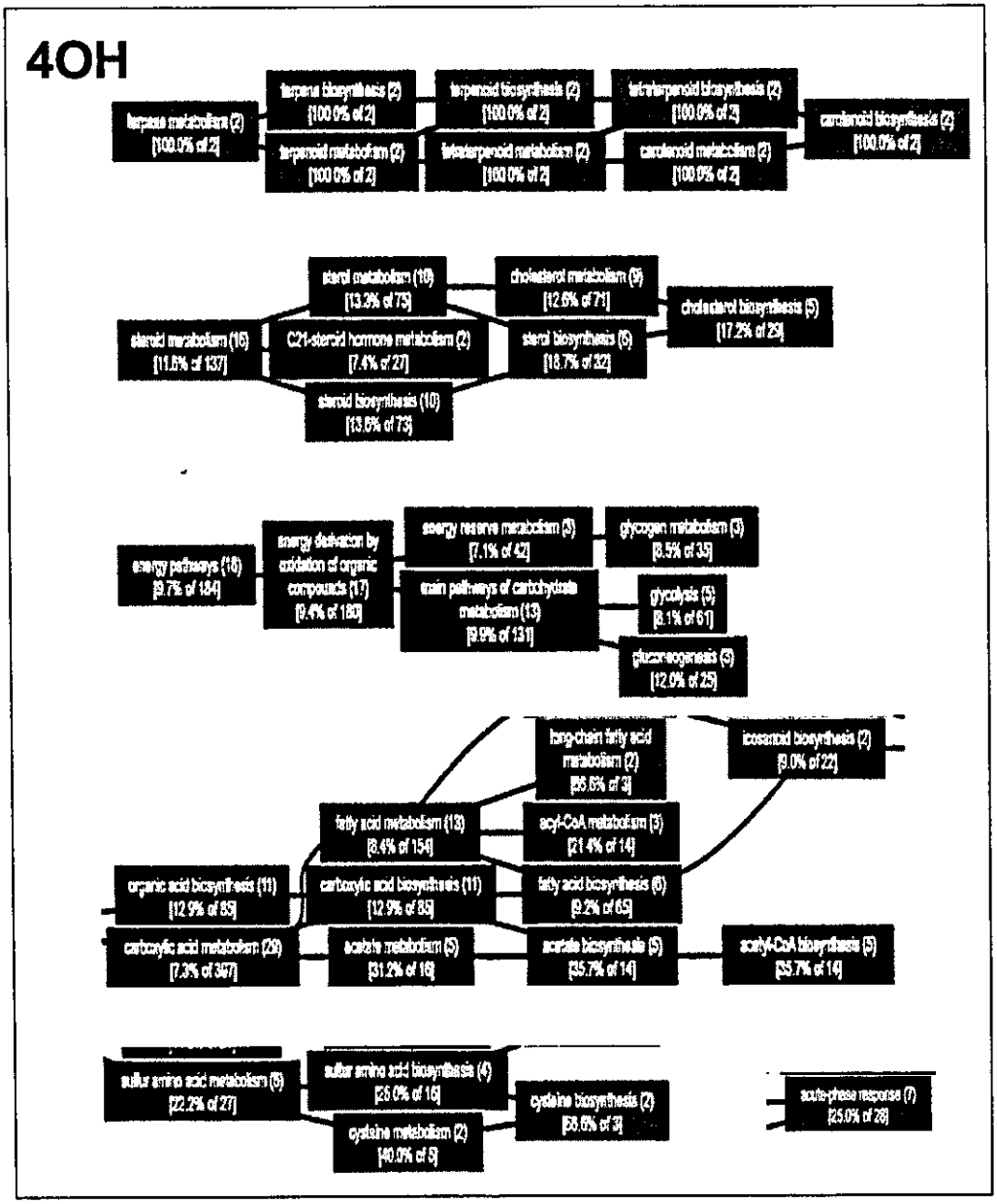
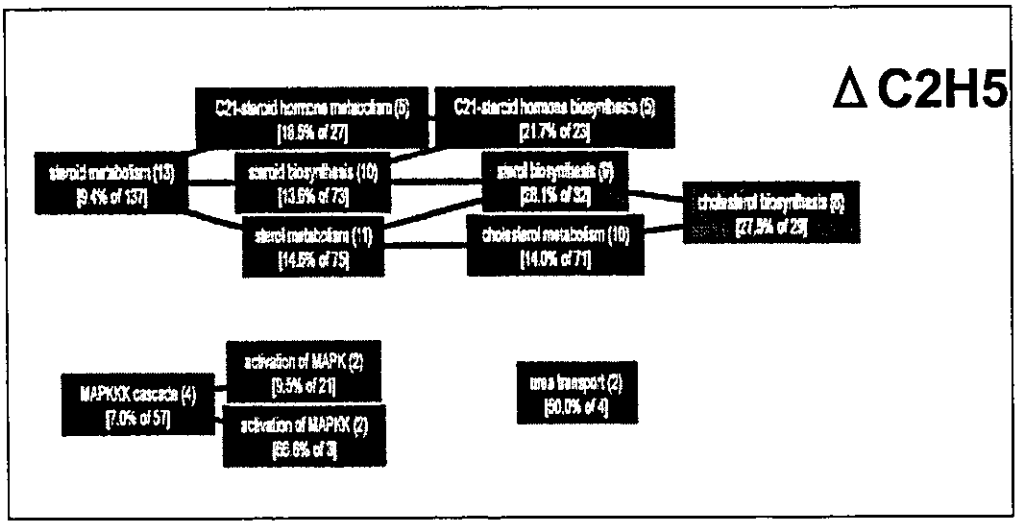
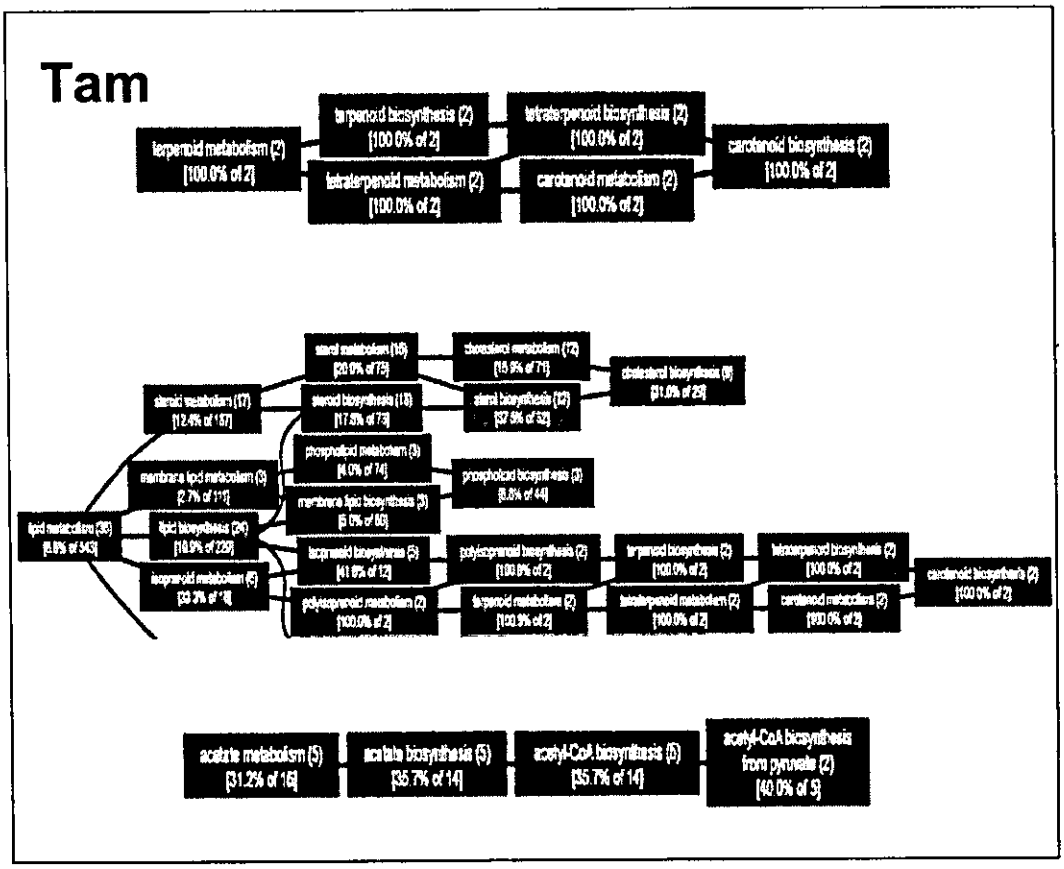

4OH
terpene metabolism (2) [100.0% of 2]
terpene biosynthesis (2) [100.0% of 2]
terpenoid biosynthesis (2) [100.0% of 2]
tetraterpenoid biosynthesis (2) [100.0% of 2]
carotenoid biosynthesis (2) [100.0% of 2]
terpenoid metabolism (2) [100.0% of 2]
tetraterpenoid metabolism (2) [100.0% of 2]
carotenoid metabolism (2) [100.0% of 2]
steroid metabolism (16) [11.6% of 137]
sterol metabolism (10) [13.3% of 75]
C21-steroid hormone metabolism (2) [7.4% of 27]
cholesterol metabolism (9) [12.6% of 71]
sterol biosynthesis (6) [18.7% of 32]
cholesterol biosynthesis (5) [17.2% of 29]
steroid biosynthesis (10) [13.6% of 73]
energy pathways (18) [9.7% of 184]
energy derivation by oxidation of organic compounds (17) [9.4% of 180]
energy reserve metabolism (3) [7.1% of 42]
glycogen metabolism (3) [8.5% of 35]
main pathways of carbohydrate metabolism (13) [9.9% of 131]
glycolysis (5) [8.1% of 61]
gluconeogenesis (3) [12.0% of 25]
long-chain fatty acid metabolism (2) [66.6% of 3]
icosanoid biosynthesis (2) [9.0% of 22]
fatty acid metabolism (13) [8.4% of 154]
acyl-CoA metabolism (3) [21.4% of 14]
organic acid biosynthesis (11) [12.9% of 85]
carboxylic acid biosynthesis (11) [12.9% of 85]
fatty acid biosynthesis (6) [9.2% of 65]
carboxylic acid metabolism (20) [7.3% of 367]
acetate metabolism (5) [31.2% of 16]
acetate biosynthesis (5) [35.7% of 14]
acetyl-CoA biosynthesis (5) [35.7% of 14]
sulfur amino acid metabolism (5) [22.2% of 27]
sulfur amino acid biosynthesis (4) [36.0% of 16]
cysteine biosynthesis (2) [66.6% of 3]
cysteine metabolism (2) [40.0% of 5]
acute-phase response (7) [25.0% of 28]


Δ C2H5
C21-steroid hormone metabolism (5) [18.5% of 27]
C21-steroid hormone biosynthesis (5) [21.7% of 23]
steroid metabolism (13) [9.4% of 137]
steroid biosynthesis (10) [13.5% of 73]
sterol biosynthesis (9) [28.1% of 32]
cholesterol biosynthesis (8) [27.5% of 29]
sterol metabolism (11) [14.5% of 75]
cholesterol metabolism (10) [14.0% of 71]
activation of MAPK (2) [9.5% of 21]
MAPKKK cascade (4) [7.0% of 57]
activation of MAPKK (2) [66.6% of 3]
urea transport (2) [50.0% of 4]


Tam
terpenoid biosynthesis (2) [100.0% of 2]
tetraterpenoid biosynthesis (2) [100.0% of 2]
terpenoid metabolism (2) [100.0% of 2]
tetraterpenoid metabolism (2) [100.0% of 2]
carotenoid metabolism (2) [100.0% of 2]
carotenoid biosynthesis (2) [100.0% of 2]
steroid metabolism (18) [20.0% of 75]
cholesterol metabolism (12) [16.9% of 71]
cholesterol biosynthesis (6) [21.0% of 29]
steroid biosynthesis (13) [17.3% of 75]
sterol biosynthesis (12) [37.5% of 32]
steroid metabolism (17) [12.4% of 137]
phospholipid metabolism (3) [4.0% of 74]
membrane lipid metabolism (3) [2.7% of 111]
phospholipid biosynthesis (3) [6.8% of 44]
membrane lipid biosynthesis (3) [5.0% of 60]
lipid metabolism (36) [6.8% of 543]
lipid biosynthesis (24) [10.9% of 220]
terpenoid biosynthesis (5) [41.6% of 12]
polyprenoid biosynthesis (2) [100.0% of 2]
terpenoid biosynthesis (2) [100.0% of 2]
tetraterpenoid biosynthesis (2) [100.0% of 2]
isoprenoid metabolism (6) [33.3% of 18]
polyprenoid metabolism (2) [100.0% of 2]
terpenoid metabolism (2) [100.0% of 2]
tetraterpenoid metabolism (2) [100.0% of 2]
carotenoid metabolism (2) [100.0% of 2]
acetate metabolism (5) [31.2% of 16]
acetate biosynthesis (5) [35.7% of 14]
acetyl-CoA biosynthesis (5) [35.7% of 14]
acetyl-CoA biosynthesis from pyruvate (2) [40.0% of 5]

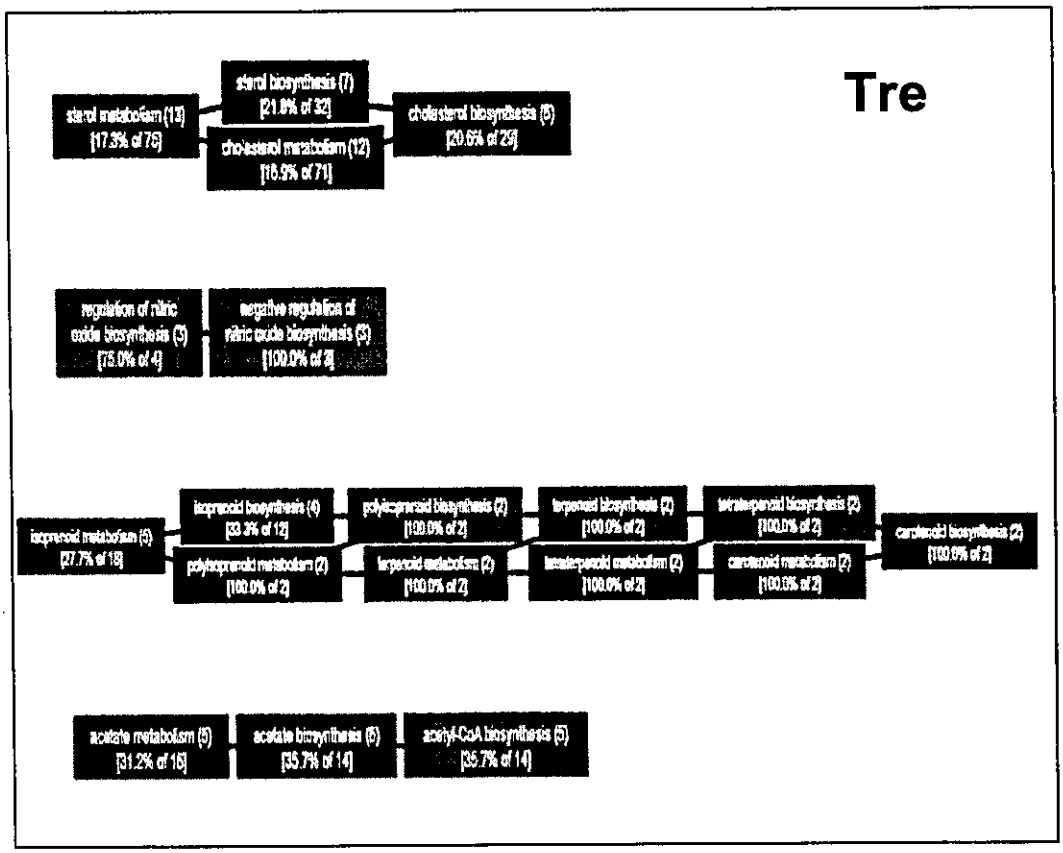

Tre
sterol metabolism (13) [17.3% of 75]
sterol biosynthesis (7) [21.8% of 32]
cholesterol metabolism (12) [16.9% of 71]
cholesterol biosynthesis (6) [20.6% of 29]
regulation of nitric oxide biosynthesis (3) [75.0% of 4]
negative regulation of nitric oxide biosynthesis (2) [100.0% of 2]
isoprenoid metabolism (5) [27.7% of 18]
isoprenoid biosynthesis (4) [33.3% of 12]
polyprenoid biosynthesis (2) [100.0% of 2]
terpenoid biosynthesis (2) [100.0% of 2]
tetraterpenoid biosynthesis (2) [100.0% of 2]
carotenoid biosynthesis (2) [100.0% of 2]
polyprenoid metabolism (2) [100.0% of 2]
terpenoid metabolism (2) [100.0% of 2]
tetraterpenoid metabolism (2) [100.0% of 2]
carotenoid metabolism (2) [100.0% of 2]
acetate metabolism (5) [31.2% of 16]
acetate biosynthesis (5) [35.7% of 14]
acetyl-CoA biosynthesis (5) [35.7% of 14]

表 12　タモキシフェン誘導体処理により子宮において共通性をもって発現上昇した遺伝子

control				4OH				Tam				Tre				ΔC2Hs				4OH				Tam				Tre				ΔC2Hs				Gene Name
4h		24h		4h																24h																
F	Raw	c	F	Raw	OH4hr	F	Raw	TAM4h.t	F	Raw	Tore4h.t	F	Raw	chem4h.t	F	Raw	OH24hr	F	Raw	TAM24h.	F	Raw	Tore24h.	F	Raw	chem24h	F	Raw	sc	Description
A	20	M	24	46.52	P	897	50.34	P	914	3.59	0	P	72	16.54	P	303	144.03	P	3388	107.30	P	2716	104.59	P	2543	3.37	P	82	8	kallikrein 9
A	13	P	25	13.99	P	176	22.29	P	287	3.25	P	43	4.72	P	59	8.83	P	216	10.99	P	290	9.85	P	249	1.22	P	31	7	interleukin 1 receptor, type II
P	44	P	54	9.29	P	385	11.96	P	487	2.28	P	99	9.85	P	392	5.72	P	295	7.10	P	394	5.25	P	280	2.64	P	141	7	MAD2 (mitotic arrest deficient, homolog)-li
A	17	A	26	95.81	P	1534	126.00	P	1969	27.40	0	P	461	79.65	P	1250	66.93	P	1671	25.50	P	685	29.86	P	770	20.77	A	538	7	kallikrein 8
A	1	A	1	16.61	P	19	13.84	P	15	6.44	0	A	8	9.59	P	11	68.22	P	59	47.45	P	44	62.15	P	74	19.33	P	17	7	S100 calcium binding protein A14
P	40	P	60	4.20	P	161	6.01	P	223	1.32	A	52	4.37	P	183	4.91	P	279	6.27	P	387	5.49	P	325	0.94	P	56	6	desmocollin 2
P	27	P	31	5.10	P	127	6.71	P	165	1.47	P	39	6.41	P	159	9.03	P	266	7.78	P	247	6.87	P	261	1.09	P	33	6	desmocollin 2
P	92	P	86	8.91	P	765	7.15	P	603	1.62	P	146	7.05	P	600	2.83	P	235	3.95	P	344	4.36	P	373	0.89	P	76	6	GADD 45 gamma
P	38	P	73	3.24	P	116	5.15	P	181	1.08	P	41	3.79	P	133	3.57	P	251	2.45	P	186	3.25	P	237	0.66	P	48	6	galactose-4-epimerase, UDP
P	64	P	50	3.95	P	238	5.19	P	308	1.08	P	66	3.19	P	191	5.46	P	263	6.39	P	332	6.73	P	335	0.92	P	46	6	Jun dimerization protein 2
P	21	P	31	23.48	P	463	32.23	P	623	2.74	P	57	7.65	P	147	52.00	P	1549	59.09	P	1894	42.37	P	1304	1.06	P	33	6	kallikrein 24
P	25	P	28	3.74	P	87	5.33	P	122	1.05	P	26	5.76	P	133	4.53	P	121	6.84	P	159	14.03	P	387	1.48	P	41	6	similar to Isopentenyl-diphosphate delta-is
P	248	P	372	3.87	P	854	8.07	P	1845	1.30	P	318	3.73	P	862	9.86	P	2107	5.95	P	2294	9.74	P	2124	0.84	P	311	6	peptidyl arginine deiminase, type II
P	158	P	269	11.85	P	1758	8.00	P	1166	1.60	P	249	7.56	P	1112	4.57	P	1134	5.73	P	1600	5.59	P	1498	0.87	P	233	6	purine-nucleoside phosphorylase
P	41	P	207	5.32	P	203	11.03	P	413	1.02	P	41	5.87	P	214	3.84	P	766	3.04	P	652	3.37	P	894	0.40	1	P	50	6	urate oxidase
A	16	P	45	6.82	P	104	13.07	P	195	0.51	0	A	8	4.32	P	85	8.70	P	379	8.21	P	385	7.04	P	317	0.37	1	A	12	6	apolipoprotein B editing complex 2
P	44	P	37	9.68	P	403	7.17	P	293	1.62	0	P	71	4.85	P	200	2.96	P	140	4.56	P	174	7.00	P	256	0.55	P	20	6	adult male diencephalon cDNAprotein
A	22	P	78	3.19	P	66	13.46	P	273	1.49	0	P	32	7.56	P	155	4.44	P	334	5.90	P	478	4.32	P	336	0.66	P	51	6	RIKEN cDNA A930031D07 gene
A	42	A	58	17.48	P	881	16.17	P	695	2.38	0	A	97	8.53	P	253	31.92	P	1769	24.86	P	1483	15.86	P	908	0.63	A	37	6	kallikrein 21
P	262	P	491	3.08	P	759	6.90	P	1427	1.07	0	P	277	3.20	P	781	3.22	P	1524	3.25	P	1657	3.90	P	1906	0.83	P	409	6	keratin complex 2, basic, gene 7
A	3	P	19	25.77	P	80	51.36	P	156	1.30	0	A	4	20.89	P	84	4.91	P	88	7.65	P	148	12.92	P	237	0.83	P	16	6	stratifin
P	32	P	23	4.91	P	149	4.78	P	142	1.12	0	P	36	3.64	P	107	3.69	P	80	5.82	P	136	4.40	P	99	0.99	P	22	6	paired related homeobox 2
P	215	P	156	2.93	P	592	3.96	P	785	1.12	0	P	237	3.89	P	778	5.93	P	892	7.42	P	1201	7.55	P	1172	1.43	P	223	6	aldehyde dehydrogenase family 1, subfamily
P	27	P	31	4.06	P	104	3.94	P	99	1.77	P	47	4.50	P	114	3.32	P	98	2.13	P	88	3.91	P	119	1.73	P	53	5	RIKEN cDNA 2610307C23 gene
P	22	P	30	3.48	P	70	4.78	P	95	1.24	P	26	3.74	P	75	2.38	P	69	2.88	P	114	7.34	P	220	1.07	P	32	5	cytochrome P450, 51
P	86	P	91	1.72	P	139	3.63	P	288	0.99	P	84	3.62	P	289	2.76	P	241	5.70	P	536	5.79	P	621	2.15	P	195	5	farnesyl diphosphate synthetase
P	118	P	81	2.17	P	241	1.91	P	208	1.24	P	144	2.95	P	325	4.23	P	331	3.52	P	296	3.82	P	308	2.79	P	226	5	karyopherin (importin) alpha 2
P	53	P	78	3.96	P	197	5.08	P	248	1.30	P	68	4.68	P	231	2.36	P	178	3.01	P	317	4.63	P	360	1.01	P	79	5	low density lipoprotein receptor
P	430	P	704	5.83	P	2352	6.55	P	2594	1.39	P	588	5.38	P	2151	5.86	P	2488	2.61	P	1835	2.55	P	1788	0.92	P	649	5	purine-nucleoside phosphorylase
P	22	P	46	9.77	P	200	3.89	P	74	2.18	P	68	8.78	P	138	3.85	P	162	0.40	P	19	0.54	P	25	1.04	P	48	5	ribosomal protein L37a
P	21	P	44	4.00	P	77	5.41	P	102	1.11	P	22	5.30	P	101	1.62	P	69	2.71	P	124	6.81	P	389	0.65	P	29	5	sterol-C4-methyl oxidase-like
P	202	P	223	5.41	P	1025	3.34	P	620	2.01	P	399	4.66	P	875	0.75	P	160	2.60	P	578	2.70	P	599	1.10	P	245	5	splicing factor, arginine/serine-rich 2
P	28	P	28	3.63	P	96	3.32	P	86	0.95	P	26	4.47	P	117	3.55	P	95	2.51	P	73	3.20	P	89	2.05	P	57	5	splicing factor, arginine/serine-rich 2
P	236	P	311	2.87	P	637	3.82	P	832	1.02	P	236	3.00	P	659	2.69	P	775	2.33	P	750	3.92	P	902	0.81	P	251	5	testis expressed gene 2
P	114	P	156	4.43	P	473	6.69	P	686	1.29	P	144	2.97	P	314	2.86	P	397	2.33	P	376	2.91	P	450	0.94	P	146	5	neurturin
P	32	P	37	3.35	P	100	3.45	P	101	1.20	P	38	2.41	P	71	3.03	P	109	3.22	P	124	4.50	P	167	0.99	P	37	5	Nedd4 WW binding protein 4
P	59	P	23	3.65	P	202	4.21	P	229	1.30	P	75	2.19	P	120	6.59	P	123	8.72	P	206	8.19	P	141	2.69	P	61	5	crystallin, alpha B
P	61	P	61	1.46	P	83	5.00	P	336	0.81	A	48	3.71	P	209	10.93	P	639	10.24	P	844	12.63	P	762	1.04	P	83	5	RIKEN cDNA A930031D07 gene
M	11	A	8	0.43	M	4	0.60	A	6	1.55	P	17	3.09	P	37	9.05	P	69	9.28	P	76	5.67	P	45	7.41	P	59	5	protein regulator of cytokinesis 1-like
A	10	A	22	10.00	P	90	8.75	P	77	2.49	A	24	12.45	P	111	2.09	A	44	2.93	P	67	3.00	P	65	0.37	A	8	5	a disintegrin-like and metalloprotease
A	13	A	8	18.49	P	226	13.12	P	157	1.94	A	25	14.32	P	173	2.53	A	19	20.75	P	168	19.45	P	151	0.44	A	3	5	TNF receptor superfamily, member 12a
P	24	P	45	9.91	P	227	12.76	P	287	2.24	P	54	7.55	P	171	2.72	P	118	4.65	P	217	4.87	P	218	0.89	P	40	5	Nedd4 WW binding protein 4
P	536	P	565	2.99	P	1505	3.50	P	1728	1.18	P	624	3.03	P	1510	2.11	P	1150	2.96	P	1734	3.61	P	2030	1.16	P	655	5	receptor (calcitonin) activity modifying prot
P	116	A	130	5.23	P	587	3.60	P	383	1.10	M	125	6.37	P	899	1.31	P	183	2.79	P	375	3.46	P	446	1.10	P	142	5	serum-inducible kinase
P	41	P	56	4.73	P	184	5.36	P	205	1.40	P	57	4.41	P	170	2.27	P	123	3.24	P	189	3.08	P	173	1.03	P	58	5	melanophilin
A	24	A	27	3.91	P	80	3.29	P	74	1.83	A	44	3.23	P	73	2.80	P	74	3.16	P	108	3.23	P	91	0.92	A	25	5	rat proto-oncogene
P	67	P	68	7.15	P	450	4.56	P	282	2.16	P	143	5.62	P	350	1.91	P	126	3.35	P	237	3.71	P	252	1.20	P	82	5	chemokine orphan receptor 1
P	13	A	12	3.54	P	67	5.56	P	86	1.22	A	15	5.19	P	62	3.76	A	43	3.81	P	47	5.35	P	63	0.87	A	10	5	histamine receptor H 1
A	22	P	21	7.07	P	148	7.08	P	146	2.25	P	49	10.65	P	221	2.74	P	55	5.83	P	128	4.81	P	93	1.03	A	22	5	a disintegrin-like and metalloprotease
P	21	P	19	8.39	P	127	4.83	P	94	1.14	P	24	4.46	P	88	3.32	M	60	6.80	P	134	7.48	P	139	1.02	A	19	5	parathyroid hormone-like peptide
P	8	P	8	9.00	P	62	7.02	P	54	1.78	P	15	4.13	P	32	6.00	M	47	8.47	P	54	6.42	P	43	0.86	P	7	5	parathyroid hormone-like peptide
A	22	A	7	13.83	P	286	10.45	P	212	0.56	A	12	12.52	P	256	9.11	A	61	30.16	P	216	31.93	P	219	0.93	A	8	5	TNF receptor superfamily, member 12a

表 13　タモキシフェン誘導体処理により子宮において共通性をもって発現低下した遺伝子

control				4OH				Tam				Tre				ΔC2Hs				4OH				Tam				Tre				ΔC2Hs				Gene Name
4h		24h		4h																24h																
F	Raw	c	F	Raw	OH4hr	F	Raw	TAM4h.t	F	Raw	Tore4h.t	F	Raw	chem4h.t	F	Raw	OH24	F	Raw	TAM24h.	F	Raw	Tore24h.	F	Raw	chem24h	F	Raw	score	Description
P	173	P	91	0.23	P	37	0.14	P	23	0.94	P	159	0.35	P	57	0.30	A	25	0.21	P	20	0.16	P	15	1.20	P	109	6	N-sulfotransferase
P	350	P	489	0.35	P	114	0.31	P	101	0.93	P	321	0.24	P	79	0.24	P	112	0.36	P	192	0.24	P	118	1.26	P	815	6	D site albumin promoter binding protein
P	534	P	326	0.32	P	160	0.29	P	142	0.72	P	378	0.39	P	195	0.41	P	128	0.37	P	126	0.31	P	101	0.94	P	306	6	glucocorticoid-induced leucine zipper
P	186	P	93	0.40	A	70	0.19	A	33	0.87	P	159	0.36	A	62	0.13	A	12	0.25	A	24	0.09	A	8	1.15	P	106	6	flavin containing monooxygenase 2
P	820	P	781	0.25	P	190	0.24	P	182	0.98	P	791	0.37	P	284	0.32	P	240	0.23	P	183	0.26	P	204	1.14	P	891	6	REV3-like
P	49	P	37	0.36	P	16	0.26	P	12	1.24	P	59	0.28	P	13	0.03	A	1	0.09	P	4	0.25	A	9	1.75	P	85	6	carbonic anhydrase-like sequence 1
P	53	P	35	0.36	A	21	0.15	A	9	0.99	P	61	0.20	A	12	0.18	A	5	0.05	A	2	0.18	A	6	1.06	P	37	6	claudin 1
P	80	P	79	0.29	P	22	0.20	P	15	0.35	P	28	0.46	P	35	0.23	P	18	0.29	P	24	0.35	P	27	1.20	P	94	6	RIKEN cDNA 2610002J02 gene
P	370	P	604	0.25	P	85	0.20	P	68	1.00	P	364	0.23	P	78	0.24	P	142	0.25	P	156	0.24	P	144	1.31	P	794	6	D site albumin promoter binding protein
P	493	P	461	0.32	P	150	0.22	P	98	0.89	P	432	0.31	P	142	0.24	P	107	0.23	P	110	0.17	P	76	0.99	P	457	6	secreted frizzled-related sequence protein
P	53	P	36	0.24	A	14	0.13	A	8	0.81	P	61	0.24	A	20	0.05	A	2	0.09	A	3	0.15	A	5	1.16	P	42	6	prostaglandin E receptor 2 (subtype EP2)
P	264	P	313	0.19	P	46	0.35	P	85	0.78	P	204	0.36	P	89	0.35	P	110	0.35	P	114	0.32	P	100	0.83	P	260	6	Max interacting protein 1
P	282	P	307	0.14	P	38	0.29	P	75	1.04	P	289	0.21	P	54	0.21	P	63	0.36	P	115	0.28	P	87	1.07	P	328	6	Cbp/p300-interacting transactivator
P	938	P	1221	0.28	P	243	0.40	P	347	0.91	P	838	0.25	P	221	0.23	P	271	0.39	P	490	0.11	P	133	1.03	P	1260	5	thioredoxin interacting protein
P	40	P	83	0.46	A	17	0.07	A	3	0.35	P	14	1.03	P	38	0.19	A	15	0.22	P	19	0.29	P	24	0.40	P	26	5	neutral sphingomyelinase activation associ
P	110	P	116	0.29	P	30	0.15	P	15	0.83	P	90	0.64	P	66	0.31	P	35	0.18	P	22	0.12	P	13	0.59	P	68	5	RIKEN cDNA E330010H22 gene
P	61	P	59	0.09	A	5	0.17	A	9	1.00	P	80	0.37	A	21	0.18	A	10	0.44	P	27	0.20	A	12	1.05	P	62	5	RIKEN cDNA 4632432J16 gene
P	61	P	47	0.33	A	19	0.32	A	18	1.03	P	81	0.32	A	16	0.33	A	15	0.39	A	19	0.30	A	14	2.14	P	101	5	growth factor receptor bound protein 14
P	193	P	284	0.67	P	122	0.42	P	76	1.03	P	195	0.36	P	64	0.17	A	45	0.17	P	49	0.13	P	37	1.63	P	462	5	RIKEN cDNA 1200011D03 gene
P	203	P	152	0.26	P	49	0.32	P	59	0.86	P	171	0.30	P	57	0.58	P	85	0.28	P	44	0.36	P	54	1.29	P	196	5	hydroxyprostaglandin dehydrogenase 15 (N
P	146	P	97	0.34	P	47	0.31	P	41	1.14	P	163	0.35	P	47	0.06	A	6	0.62	P	62	0.28	P	27	1.40	P	137	5	regulator of G-protein signaling 5
P	602	P	956	0.54	P	302	0.28	P	157	1.08	P	640	0.37	P	208	0.23	P	210	0.26	P	256	0.21	P	197	0.66	P	631	5	ATP-binding cassette, sub-family A (ABC1
P	301	P	324	0.35	P	100	0.51	P	141	0.74	P	219	0.33	P	92	0.18	P	56	0.28	P	95	0.23	P	76	0.85	P	276	5	insulin-like growth factor binding protein 3
P	105	P	147	0.19	A	19	0.01	P	20	0.85	P	88	0.28	P	27	0.39	P	55	0.34	P	51	0.28	P	41	0.85	P	125	5	RIKEN cDNA 2310014B08 gene
P	81	P	269	0.29	A	22	1.21	P	90	0.68	P	54	0.32	A	24	0.10	A	25	0.10	P	28	0.13	P	36	0.37	P	99	5	immunoglobulin lambda chain, variable 1
P	397	P	192	0.24	P	88	0.22	P	80	0.72	P	280	0.24	P	87	0.38	P	71	0.49	P	98	0.29	P	55	1.12	P	215	5	glucocorticoid-induced leucine zipper
P	81	P	91	0.55	P	42	0.21	P	16	0.82	P	65	0.36	P	27	0.03	A	2	0.13	P	13	0.11	P	10	1.18	P	108	5	leptin receptor
P	522	P	356	0.21	P	105	0.01	P	64	0.94	P	482	0.18	P	88	0.14	P	47	0.29	P	106	0.12	P	43	1.10	P	390	5	odd-skipped related 2 (Drosophila)
P	379	P	254	0.09	A	32	0.01	A	18	1.00	P	373	0.18	P	83	0.26	A	65	0.09	A	24	0.15	P	39	1.04	P	264	5	RIKEN cDNA 9930023K05 gene
P	157	P	321	0.56	P	82	0.42	P	61	0.96	P	147	0.38	P	55	0.28	P	87	0.20	P	67	0.26	P	84	0.84	P	204	5	viral hemorrhagic septicemia virus(VHSV) i
P	272	P	260	0.55	P	140	0.32	P	81	1.00	P	269	0.42	P	107	0.06	A	16	0.19	P	52	0.09	A	22	1.43	P	372	5	ectonucleotide pyrophosphatase/phosphod
P	314	P	344	0.09	A	26	0.01	P	28	0.97	P	298	0.12	P	34	0.22	P	73	0.30	P	106	0.17	P	58	0.84	P	288	5	F-box only protein 32
P	84	P	47	0.23	A	18	0.17	P	13	1.01	P	83	0.28	P	22	0.62	A	28	0.26	P	13	0.29	P	14	1.12	P	53	5	Norrie disease homolog
P	132	P	139	0.35	P	43	0.01	P	27	0.84	P	108	0.26	P	32	0.26	P	35	0.27	P	38	0.27	P	38	0.98	P	135	5	caspase 12
P	630	P	810	0.27	P	159	0.01	P	155	0.87	P	538	0.36	P	209	0.38	P	224	0.26	P	168	0.18	P	109	1.04	P	634	5	RIKEN cDNA 4933425F03 gene
P	263	P	137	0.26	P	62	0.10	P	23	0.77	P	199	0.21	P	51	0.16	A	22	0.55	P	78	0.66	P	90	0.40	P	49	5	receptor (calcitonin) activity modifying prot
P	529	P	293	0.38	P	189	0.07	P	32	0.94	P	487	0.06	P	37	0.13	A	38	1.07	P	326	0.53	P	155	0.40	P	104	5	receptor (calcitonin) activity modifying prot

表14　タモキシフェン誘導体処理したマウス子宮でのエストロジェンレセプター関連遺伝子群の発現変化

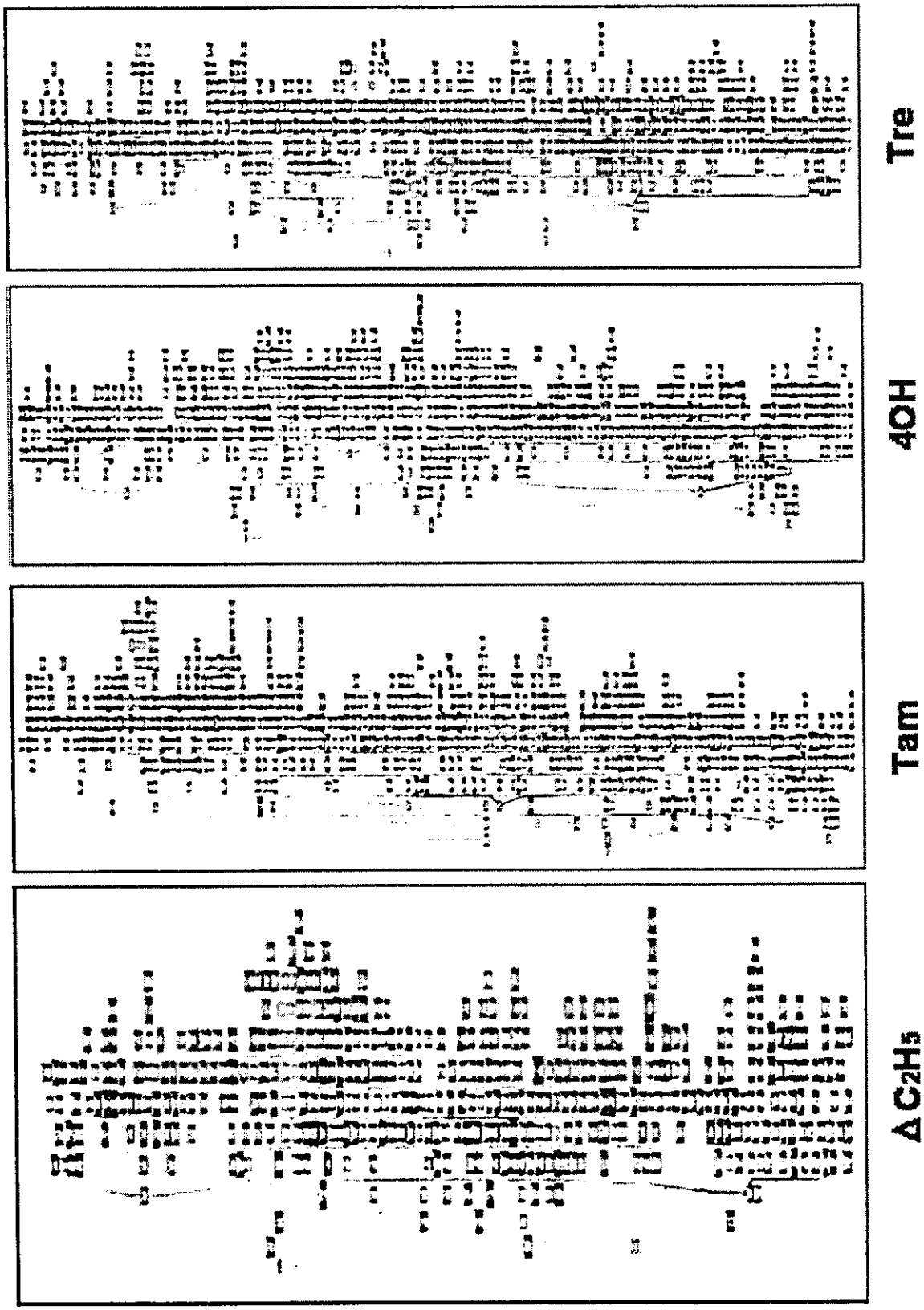
4OH				Tam				Tre				ΔC2H5				Gene
4hr	F/Raw	24hr	F/Raw	4hr	F Raw	24hr	F Raw	4hr	F Raw	24hr	F Raw	4hr	F Raw	24hr	F Raw	Common	Description
0.33 P	118	0.71 P	351	0.73 P	251	0.60 P	317	0.88 P	317	0.76 P	390	0.51 P	179	0.69 P	354	Trim25	tripartite motif protein 25(efp)
2.20 P	1939	0.87 P	4438	2.77 P	2390	0.47 P	2614	0.99 P	916	0.30 P	1599	0.97 P	847	0.33 P	1697	Ltf	lactotransferrin
1.84 P	578.4	1.95 P	594.4	1.45 P	502.4	0.8 P	280.7	1.34 P	494.2	0.72 P	228.2	1.74 P	607.9	1.04 P	328.7	TFF1	trefoil factor 1(breast cancer estrogen-inducible sequ
1.18 P	884.7	2.24 P	2424	2.17 P	1591	1.8 P	2128	0.9 P	702	2.09 P	2337	1.44 P	1067	1.14 P	1278	KRT1-	keratin19
0.41 A	7	0.71 A	15	0.43 A	7	0.57 P	13	1.19 A	21	0.28 A	6	0.57 A	10	0.85 P	19	Hgf	hepatocyte growth factor
3.02 P	62	3.67 A	67	2.00 A	40	2.34 P	46	1.19 A	25	1.41 M	27	2.71 A	55	1.30 A	25	Oxtr	Mus sp. mRNA for oxytocin receptor, complete cds.
1.21 P	1599	1.39 P	6788	3.15 P	4100	0.88 P	3567	0.96 P	1332	0.50 P	2543	1.29 P	1692	0.40 P	2028	C3	complement component 3
5.28 P	1979	2.45 P	1327	7.94 P	4427	0.76 P	4442	1.00 P	602	0.70 P	3889	3.54 P	2004	0.40 P	1367	Lcn2	lipocalin 2
0.36 P	798	0.85 P	967	0.46 P	994	0.88 P	835	1.00 P	2335	0.78 P	894	0.78 P	1715	0.81 P	952	Ctsd	cathepsin D
0.56 A	49	0.79 A	53	0.43 A	37	0.84 M	61	1.11 M	102	0.44 A	31	0.40 A	35	1.13 P	79	Vegfb	vascular endothelial growth factor B
0.07 A	2	0.39 A	8	0.01 A	6	0.56 A	12	0.85 A	26	0.70 P	15	0.31 A	9	1.36 P	29	Vegfc	vascular endothelial growth factor C
2.7 P	83.9	0.87 P	45.7	1.73 P	52.8	0.9 P	49.7	1.49 P	48.6	1 P	54.1	1.5 P	46	1.02 P	55.2	Tgfa	transforming growth factor, alpha
0.81 P	94	0.30 A	34	0.68 P	77	0.52 P	63	0.60 P	73	0.38 P	44	0.53 P	61	1.41 P	185	Tgfb3	transforming growth factor, beta 3
2.00 P	32	0.34 A	1	2.58 P	41	1.28 P	22	1.17 P	20	3.04 P	50	2.87 P	48	0.96 P	16	Hmgcr	3-hydroxy-3-methylglutaryl-Coenzyme A reductase
2.19 P	107.1	2.04 P	59.2	1.61 P	77.1	2.3 P	72.2	1.07 P	54.8	1.34 P	40	1.88 P	81.3	1.53 P	46.1	PGR	progesterone receptor
2.20 A	47	0.83 A	36	0.01 A	7	1.07 P	50	2.40 A	54	0.26 A	11	1.04 A	22	1.13 P	51	Esr1	estrogen receptor 1 (alpha)
0.85 P	110.5	0.95 P	110.5	0.7 P	90.3	1 P	129.7	1 P	136.1	1.13 P	135.7	0.74 P	95.3	0.88 P	118.7	EBAG9	estrogen receptor-binding site-associated antigen 9
0.57 P	188	0.68 A	160	0.43 M	139	1.07 P	271	0.91 P	312	0.55 P	134	0.55 P	177	1.58 P	385	Igfbp4	insulin-like growth factor binding protein 4
1.10 P	310	4.51 P	582	1.39 P	384	3.43 P	753	1.08 P	321	3.69 P	491	2.14 P	599	1.95 P	261	Igf1	insulin-like growth factor 1
1.16 P	184	3.16 P	220	1.24 P	204	3.26 P	391	1.06 P	186	3.13 P	223	2.25 P	372	2.49 P	178	Igf1	insulin-like growth factor 1
0.50 A	63	0.38 A	50	0.01 P	45	0.46 P	66	1.02 P	135	0.23 P	32	0.43 P	53	0.76 P	105	Igf1r	insulin-like growth factor 1 receptor
2.16 A	30	5.21 P	75	1.03 A	14	2.13 P	33	0.39 A	6	2.75 P	41	1.52 A	21	1.02 P	15	E2f1	E2F transcription factor 1
3.96 P	197	2.36 P	178	5.28 P	248	3.91 P	317	1.30 P	68	4.63 P	380	4.86 P	231	1.01 P	79	Ldlr	low density lipoprotein receptor
3.96 P	197	2.36 P	178	5.28 P	248	3.91 P	317	1.30 P	68	4.63 P	380	4.86 P	231	1.01 P	79	Ldlr	low density lipoprotein receptor
5.51 P	443	1.01 P	276	5.19 P	417	0.89 P	262	1.42 P	121	1.25 P	354	6.08 P	492	0.43 P	122	Fos	FBJ osteosarcoma oncogene
0.88 A	68	0.67 A	33	0.55 A	42	0.93 P	49	0.92 P	75	0.22 A	12	0.69 A	53	1.36 P	69	Bcl2	B-cell leukemia/lymphoma 2
1.84 P	584.4	1.82 P	476.5	1.75 P	526.6	1.7 P	480	0.98 P	313.9	2.13 P	575	1.91 P	576	1.06 P	287.8	ccnd1	
1.84 P	279.4	1.61 P	195.1	2.05 P	306.5	2.3 P	322.7	1.48 P	236.3	2.01 P	267.5	2.24 P	338	0.92 P	122.4	ccnd1	
1.29 P	60.3	1.3 A	52.1	1.79 P	82.2	1.6 P	70.5	1.17 P	57.4	2.01 P	83.4	1.61 P	84.1	0.93 P	38.6	ccnd1	
1.43 P	317.8	1.74 P	344	1.18 P	257.3	1.7 P	360.9	1.1 P	254.4	1.39 P	283.4	1.44 P	315.8	1.04 P	212.1	ccnd3	
0.44 P	17	1.48 A	32	0.88 P	33	3.96 P	91	0.93 P	37	3.76 P	83	1.00 P	38	2.42 P	53	Serpinb1a	ovalbumin
1.8 P	10.8	0.39 A	2.6	1.53 P	9	0.4 A	3	1.34 P	8.4	0.22 A	1.5	1.33 A	7.9	0.41 A	2.8	Il6	
0.90 M	14	4.24 P	40	0.83 A	12	4.04 P	41	1.39 P	22	5.16 P	50	1.83 P	28	2.77 P	27	Brca1	breast canbreast cancer 1
0.59 P	136	0.45 A	79	0.43 P	99	0.54 P	102	1.02 P	247	0.27 P	64	0.79 P	182	0.76 P	138	Myc	myelocytomatosis oncogene
1.57 P	78	1.74 P	273	5.26 P	257	1.73 P	292	1.43 P	75	2.28 P	370	2.70 P	134	0.86 P	139	Ckmt1	creatine kinase, mitochondrial 1, ubiquitous
1.82 P	121.8	1.69 P	67.6	1.5 P	98.9	1.7 P	72.8	0.8 P	56.1	1.37 P	56.6	1.27 P	84.2	0.95 P	39.5	NOS3	eNOS
0.83 P	44	0.30 P	112	0.48 P	25	0.36 P	144	1.11 P	62	0.33 P	128	0.52 P	27	0.67 P	260	Mmp11	matrix metalloproteinase 11
0.88 P	101	0.37 P	57	0.68 P	76	0.93 P	153	0.89 P	107	0.46 P	74	0.79 P	90	0.94 P	149	Mmp14	matrix metalloproteinase 14 (membrane-inserted)
0.60 A	17	1.78 A	31	0.26 A	7	3.15 P	58	0.76 A	22	4.14 P	84	0.15 A	4	1.28 P	23	Mmp15	matrix metalloproteinase 15
1.58 P	57	3.81 P	134	1.91 P	68	1.59 P	63	1.43 P	54	1.33 P	51	1.54 P	55	1.12 P	43	Mmp17	matrix metalloproteinase 17
2.91 M	74	3.90 A	60	0.79 P	20	2.93 P	48	1.31 M	35	1.91 P	30	1.51 P	38	2.33 P	37	Mmp19	matrix metalloproteinase 19
0.72 A	19	0.16 A	7	1.01 A	26	0.18 A	8	0.90 A	24	0.11 A	5	0.83 A	21	0.50 A	23	Mmp3	matrix metalloproteinase 3
4.81 P	198	1.04 A	184	2.77 A	127	0.66 P	133	0.89 A	43	0.60 P	154	1.30 A	60	0.23 A	45	Mmp7	matrix metalloproteinase 7
0.69 A	33	0.34 P	153	3.24 P	152	0.72 P	321	0.99 A	50	0.27 P	147	1.92 P	91	0.40 P	121	Ceacam1	CEA-related cell adhesion molecule 1
1.12 A	37	0.34 A	37	1.62 P	59	0.45 P	63	1.35 A	47	0.27 P	29	2.37 A	78	0.40 P	42	Ceacam1	CEA-related cell adhesion molecule 1
1.08 P	269	0.69 P	820	2.36 P	574	0.66 P	854	1.02 P	264	0.53 P	651	1.50 P	368	0.40 P	366	Ceacam2	CEA-related cell adhesion molecule 2
0.91 P	157	0.63 P	541	2.21 P	376	0.56 P	523	0.87 P	157	0.44 P	388	1.46 P	251	0.40 P	278	Ceacam2	CEA-related cell adhesion molecule 2
0.40 A	70	0.34 A	12	0.18 A	33	0.25 A	24	0.87 P	159	0.27 A	8	0.36 A	62	1.15 P	106	Fmo2	flavin cont flavin-containing monooxygenase 2
0.43 A	63	0.41 A	27	0.26 M	34	0.24 M	17	0.78 P	100	0.27 A	2	0.17 A	21	0.73 P	50	Fmo2	flavin cont flavin-containing monooxygenase 2
0.93 P	116	0.24 A	25	0.59 P	72	0.74 P	110	0.89 P	90	0.27 A	15	0.56 P	69	1.38 P	197	Il6st	interleukin 6 signal transducer
0.55 A	44	1.00 A	134	0.53 P	42	0.51 P	74	1.57 P	132	0.27 A	32	0.65 M	52	0.40 P	48	Tiarp-pendin	Tnfa-induced adipose-related protein
2.43 P	40	0.34 A	19	2.05 P	33	0.49 P	33	1.09 P	19	0.42 P	27	2.17 P	36	0.40 P	15	Tnfaip2	tumor necrosis factor, alpha-induced protein 2
0.95 A	3	0.34 A	0	0.12 A	0	0.66 A	3	1.40 P	5	0.45 A	2	1.37 A	5	1.16 M	4	Tnfrsf11b	tumor necrosis factor receptor superfamily, member 1
0.27 P	34	0.48 P	57	0.01 P	33	0.22 P	28	1.03 P	134	0.35 P	43	0.24 P	29	1.34 P	185	Tnfrsf1b	tumor necrosis factor receptor superfamily, member 1
0.15 A	5	0.26 A	7	0.52 A	16	0.24 A	6	0.80 A	26	0.41 A	10	0.08 A	3	1.29 P	32	Tnfrsf19	tumor necrosis factor receptor superfamily, member 1
0.31 P	46	0.53 A	73	0.01 P	63	0.64 P	95	0.95 P	149	0.66 P	94	0.38 P	56	0.66 P	96	Tnfrsf21	expressed sequence AA859878
1.33 P	163	1.61 P	199	1.32 P	159	1.91 P	254	1.02 P	131	2.84 P	363	1.29 P	157	0.89 P	127	Ap1s1	adaptor protein complex AP-1, sigma 1
0.93 P	91	0.61 P	98	1.29 P	123	0.52 P	89	1.32 P	134	0.27 P	60	1.04 P	100	0.74 P	123	Stat1	signal transducer and activator of transcription 1
0.90 P	35	0.24 P	21	0.86 P	33	0.45 P	32	0.76 P	31	0.27 P	18	0.67 P	26	0.83 P	43	Stat2	signal transducer and activator of transcription 2
1.49 P	57	0.34 A	8	1.54 P	58	1.75 P	55	0.80 P	32	0.72 P	22	1.26 P	49	1.32 P	40	Stat5a	signal transducer and activator of transcription 5A

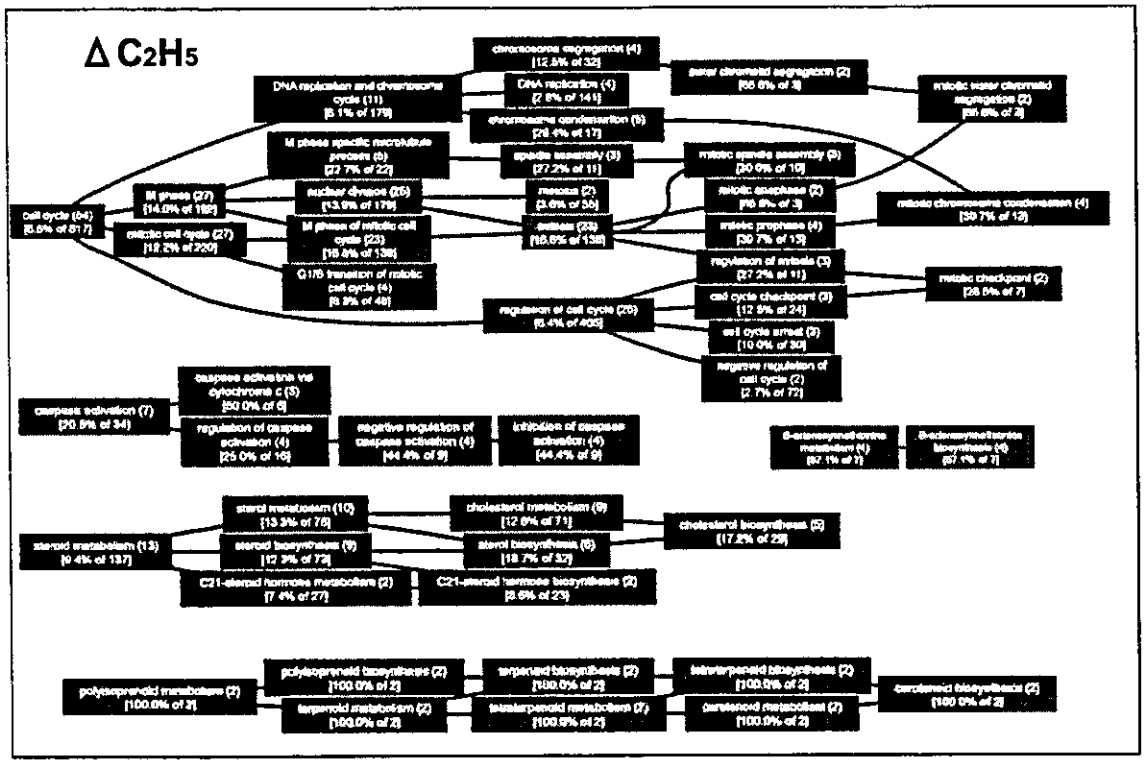
ERE-dependent genomic action　　ERE-independent genomic action　　non-genomic action

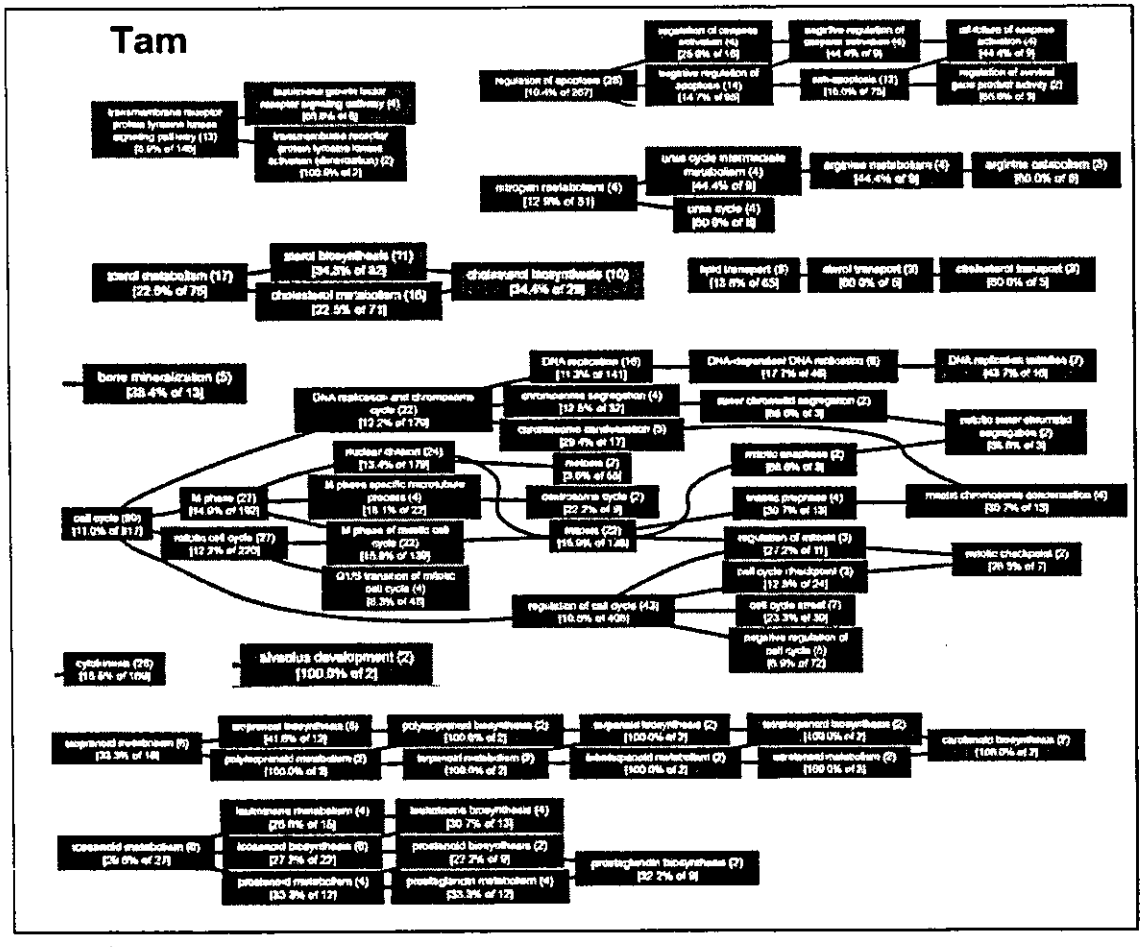
表15　タモキシフェン誘導体処理したマウス子宮での遺伝子傷害関連遺伝子群の発現変化

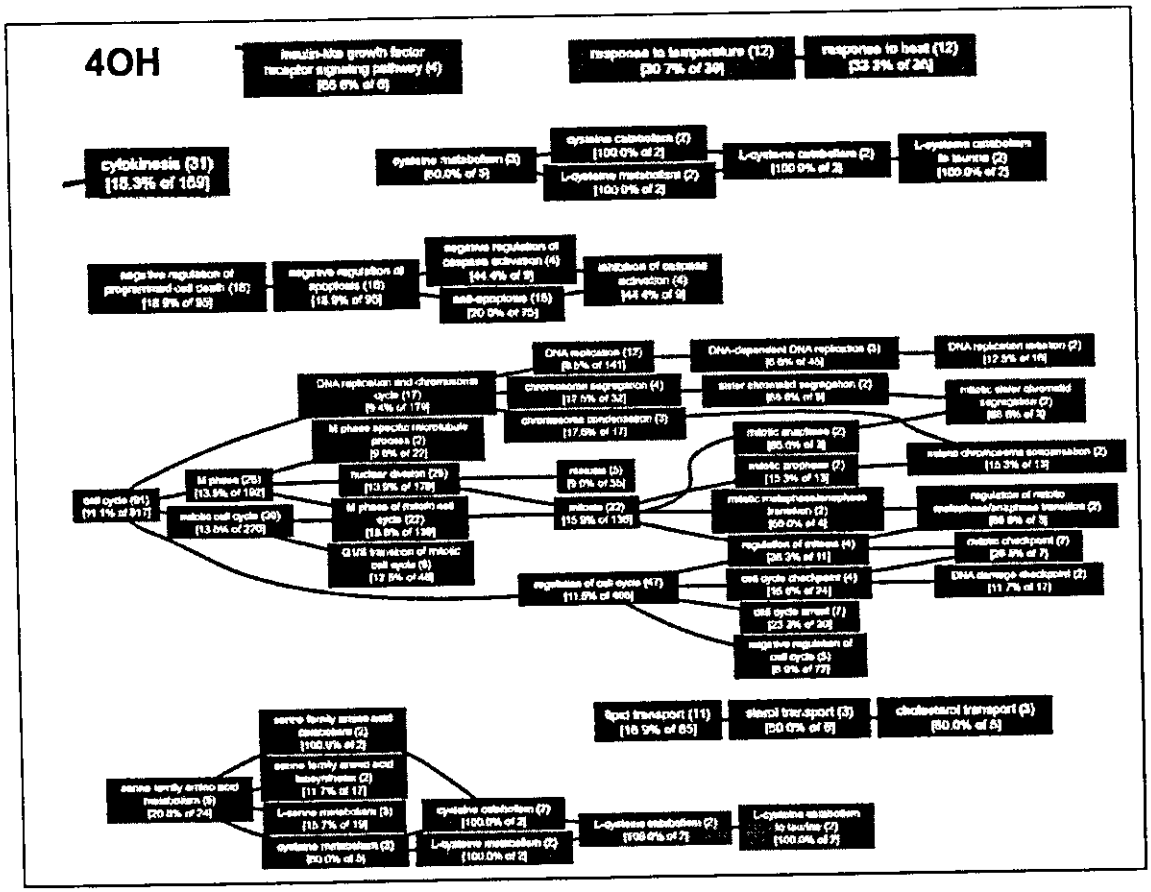
4OH				Tam				Tre				ΔC2H5				Gene
4hr	F/Raw	24hr	F/Raw	4hr	F/Raw	24hr	F/Raw	4hr	F/Raw	24hr	F/Raw	4hr	F/Raw	24hr	F/Raw	Description
1.13 P	78	4.63 P	401	1.70 P	116	2.80 P	270	1.00 P	73	3.62 P	324	1.59 P	110	0.41 P	37	fatty acid binding protein 5, epidermal
3.02 P	155	1.85 P	143	3.82 P	198	0.92 P	86	1.37 A	74	1.38 P	123	2.89 P	147	0.67 M	60	cyclin-dependent kinase inhibitor 1A (P21)
1.87 P	136	2.36 P	192	3.09 P	220	2.43 P	212	1.38 P	105	2.65 P	223	2.62 P	188	0.69 P	58	pleckstrin homology-like domain, family A, member 1
1.59 P	261	2.62 P	312	1.93 P	312	2.64 P	338	1.18 P	203	2.63 P	323	1.53 P	249	1.08 P	133	pleckstrin homology-like domain, family A, member 3
2.42 P	815	2.33 P	563	2.04 P	675	2.72 P	708	0.91 P	320	4.98 P	1243	1.37 P	457	1.06 P	266	RIKEN cDNA 5830413E08 gene
1.14 P	259	1.51 P	322	1.43 P	318	2.60 P	598	1.10 P	261	2.58 P	569	1.50 P	337	1.12 P	249	RIKEN cDNA 1700037B15 gene
0.88 A	36	3.83 P	223	1.35 P	70	2.97 P	181	0.91 P	50	2.47 P	145	0.61 A	32	1.19 P	70	growth arrest and DNA-damage-inducible 45 gamma
0.53 P	205	0.81 P	145	0.62 P	236	2.57 P	496	0.89 P	364	0.14 A	26	0.50 P	193	0.54 P	101	carbonic anhydrase 3
2.89 P	226	1.71 P	125	2.06 P	158	1.03 P	81	1.18 P	97	1.25 P	94	2.21 P	171	0.66 P	50	RIKEN cDNA 2700083E06 gene
2.26 P	141	0.91 P	87	2.23 P	137	2.07 P	214	1.01 P	66	2.71 P	268	1.57 P	97	0.73 P	73	sterol-C5-desaturase (fungal ERG3, delta-5-desaturase) homolo
3.70 P	78	0.25 A	8	2.90 A	60	0.73 A	25	1.55 A	35	0.68 A	22	2.65 A	56	0.72 A	23	growth differentiation factor 15
2.73 P	1659	1.43 P	710	1.71 P	1019	1.44 P	767	1.22 P	777	1.49 P	759	2.33 P	1401	1.14 P	586	heat shock protein, 1
0.65 P	55	2.95 P	70	0.61 P	50	1.56 P	40	1.11 P	98	0.35 P	8	0.57 P	48	1.02 P	25	ESTs, Highly similar to 2118318A promyelocyte leukemia Zn fing
0.21 A	10	2.12 A	14	0.04 A	2	4.08 P	28	1.25 P	64	0.88 A	6	0.62 A	30	0.77 A	5	ESTs, Highly similar to 2118318A promyelocyte leukemia Zn fing
1.06 P	183	0.69 P	231	1.02 P	173	0.39 P	139	1.28 P	231	0.58 P	200	1.32 P	225	0.56 P	195	B-cell translocation gene 2, anti-proliferative
1.10 P	235	0.22 A	71	1.89 P	398	1.83 P	617	1.15 P	258	0.22 P	70	1.34 P	286	0.87 P	283	RIKEN cDNA 1300007C21 gene
0.47 P	75	0.53 P	44	0.34 P	52	0.47 P	42	1.31 P	217	0.33 P	29	0.48 P	75	0.85 P	73	RIKEN cDNA 1110002J03 gene
0.27 P	159	0.38 P	224	0.27 P	155	0.28 P	168	0.67 P	538	0.18 P	109	0.36 P	209	1.04 P	634	RIKEN cDNA 4933425F03 gene
0.67 A	20	0.57 A	32	0.79 A	23	0.57 P	34	1.10 P	35	0.16 A	9	0.57 A	17	0.91 P	54	stress induced protein
0.55 P	70	1.09 P	123	0.69 P	85	0.92 P	111	0.92 P	121	0.24 P	27	0.76 P	94	2.21 P	257	insulin-like growth factor binding protein 5

図6　タモキシフェン誘導体処理した子宮において選択した遺伝子の Ontology tree 解析




Δ C2H5


Tam


4OH
insulin-like growth factor receptor signaling pathway (4) [66.6% of 6]
response to temperature (12) [80.7% of 36]
response to heat (12) [92.3% of 26]
cytokinesis (31) [18.9% of 169]
cysteine catabolism (2) [100.0% of 2]
cysteine metabolism (3) [60.0% of 5]
L-cysteine metabolism (2) [100.0% of 2]
L-cysteine catabolism to taurine (2) [100.0% of 2]
negative regulation of programmed cell death (18) [18.9% of 95]
negative regulation of apoptosis (18) [18.9% of 95]
negative regulation of caspase activation (4) [44.4% of 9]
anti-apoptosis (18) [20.5% of 75]
inhibition of caspase activation (4) [44.4% of 9]
DNA metabolism (12) [8.5% of 141]
DNA-dependent DNA replication (5) [5.6% of 45]
DNA replication initiation (2) [12.5% of 16]
DNA replication and chromosome cycle (17) [9.4% of 179]
chromosome segregation (4) [17.1% of 32]
sister chromatid segregation (2) [66.6% of 3]
mitotic sister chromatid segregation (2) [66.6% of 3]
chromosome condensation (3) [17.6% of 17]
M phase specific microtubule process (2) [9.0% of 22]
mitotic anaphase (2) [66.6% of 3]
mitotic chromosome condensation (2) [15.3% of 13]
cell cycle (61) [11.1% of 547]
M phase (29) [13.9% of 192]
nuclear division (29) [13.9% of 179]
mitosis (5) [9.0% of 55]
mitotic prophase (2) [15.3% of 13]
mitotic cell cycle (29) [13.6% of 209]
M phase of mitotic cell cycle (22) [16.8% of 139]
mitosis (22) [15.9% of 139]
mitotic metaphase/anaphase transition (2) [50.0% of 4]
regulation of mitotic metaphase/anaphase transition (2) [66.6% of 3]
G1/S transition of mitotic cell cycle (5) [12.5% of 48]
regulation of mitosis (4) [36.3% of 11]
mitotic checkpoint (7) [28.5% of 7]
regulation of cell cycle (47) [11.6% of 406]
cell cycle checkpoint (4) [16.0% of 24]
DNA damage checkpoint (2) [11.7% of 17]
cell cycle arrest (7) [23.3% of 30]
negative regulation of cell cycle (5) [6.9% of 72]
lipid transport (11) [16.9% of 65]
sterol transport (3) [50.0% of 6]
cholesterol transport (3) [60.0% of 5]
serine family amino acid catabolism (2) [100.0% of 2]
serine family amino acid metabolism (5) [20.8% of 24]
serine family amino acid biosynthesis (2) [11.7% of 17]
L-serine metabolism (3) [15.7% of 19]
cysteine catabolism (2) [100.0% of 2]
cysteine metabolism (3) [60.0% of 5]
L-cysteine metabolism (2) [100.0% of 2]
L-cysteine catabolism to taurine (2) [100.0% of 2]

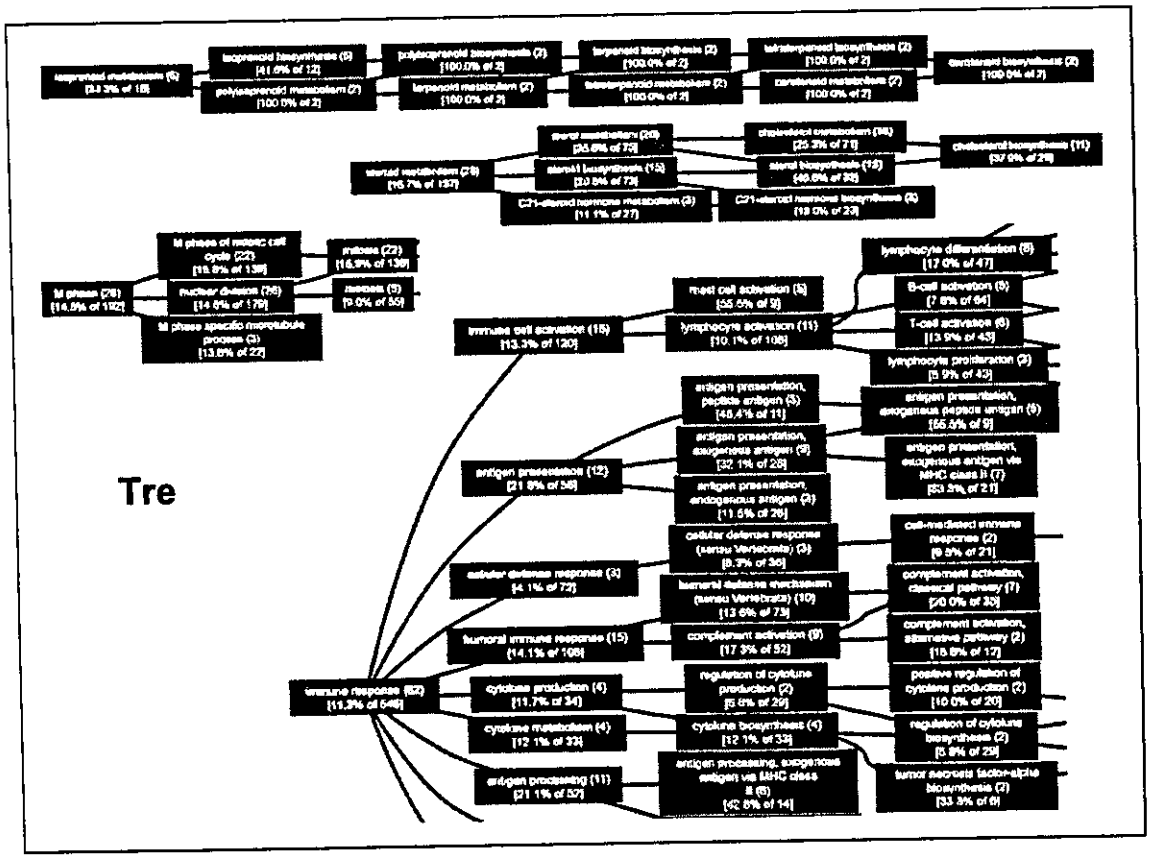

Tre
isoprenoid biosynthesis (5) [41.6% of 12]
polyisoprenoid biosynthesis (2) [100.0% of 2]
terpenoid biosynthesis (2) [100.0% of 2]
tetraterpenoid biosynthesis (2) [100.0% of 2]
carotenoid biosynthesis (2) [100.0% of 2]
polyisoprenoid metabolism (2) [100.0% of 2]
terpenoid metabolism (2) [100.0% of 2]
tetraterpenoid metabolism (2) [100.0% of 2]
carotenoid metabolism (2) [100.0% of 2]
sterol metabolism (20) [20.8% of 72]
cholesterol metabolism (16) [25.3% of 71]
cholesterol biosynthesis (11) [57.8% of 23]
steroid metabolism (29) [16.7% of 157]
steroid biosynthesis (15) [20.8% of 72]
steroid biosynthesis (15) [60.0% of 32]
C21-steroid hormone metabolism (3) [11.1% of 27]
C21-steroid hormone biosynthesis (3) [18.0% of 23]
M phase of mitotic cell cycle (22) [16.8% of 139]
mitosis (22) [16.8% of 139]
M phase (29) [14.8% of 192]
nuclear division (29) [14.8% of 179]
mitosis (5) [9.0% of 55]
M phase specific microtubule process (2) [13.6% of 22]
immune cell activation (15) [13.3% of 120]
mast cell activation (5) [55.5% of 9]
lymphocyte activation (11) [10.1% of 108]
lymphocyte differentiation (8) [17.0% of 47]
B-cell activation (5) [7.8% of 64]
T-cell activation (6) [13.9% of 43]
lymphocyte proliferation (3) [5.9% of 43]
antigen presentation, peptide antigen (3) [46.4% of 11]
antigen presentation, exogenous peptide antigen (5) [55.5% of 9]
antigen presentation, exogenous antigen (9) [32.1% of 28]
antigen presentation, exogenous antigen via MHC class II (7) [53.3% of 21]
antigen presentation (12) [21.9% of 58]
antigen presentation, endogenous antigen (3) [11.5% of 26]
cellular defense response (sensu Vertebrata) (3) [8.3% of 36]
cell-mediated immune response (2) [9.5% of 21]
cellular defense response (3) [4.1% of 72]
humoral defense mechanism (sensu Vertebrata) (10) [13.6% of 73]
complement activation, classical pathway (7) [20.0% of 35]
humoral immune response (15) [14.1% of 106]
complement activation (9) [17.3% of 52]
complement activation, alternative pathway (2) [18.0% of 17]
immune response (62) [11.3% of 548]
cytokine production (4) [11.7% of 34]
regulation of cytokine production (2) [5.6% of 29]
positive regulation of cytokine production (2) [10.0% of 20]
cytokine metabolism (4) [12.1% of 33]
cytokine biosynthesis (4) [12.1% of 33]
regulation of cytokine biosynthesis (2) [5.9% of 29]
antigen processing (11) [21.1% of 57]
antigen processing, exogenous antigen via MHC class II (6) [42.8% of 14]
tumor necrosis factor-alpha biosynthesis (2) [33.3% of 6]

図７　遺伝子発現データベースの構造

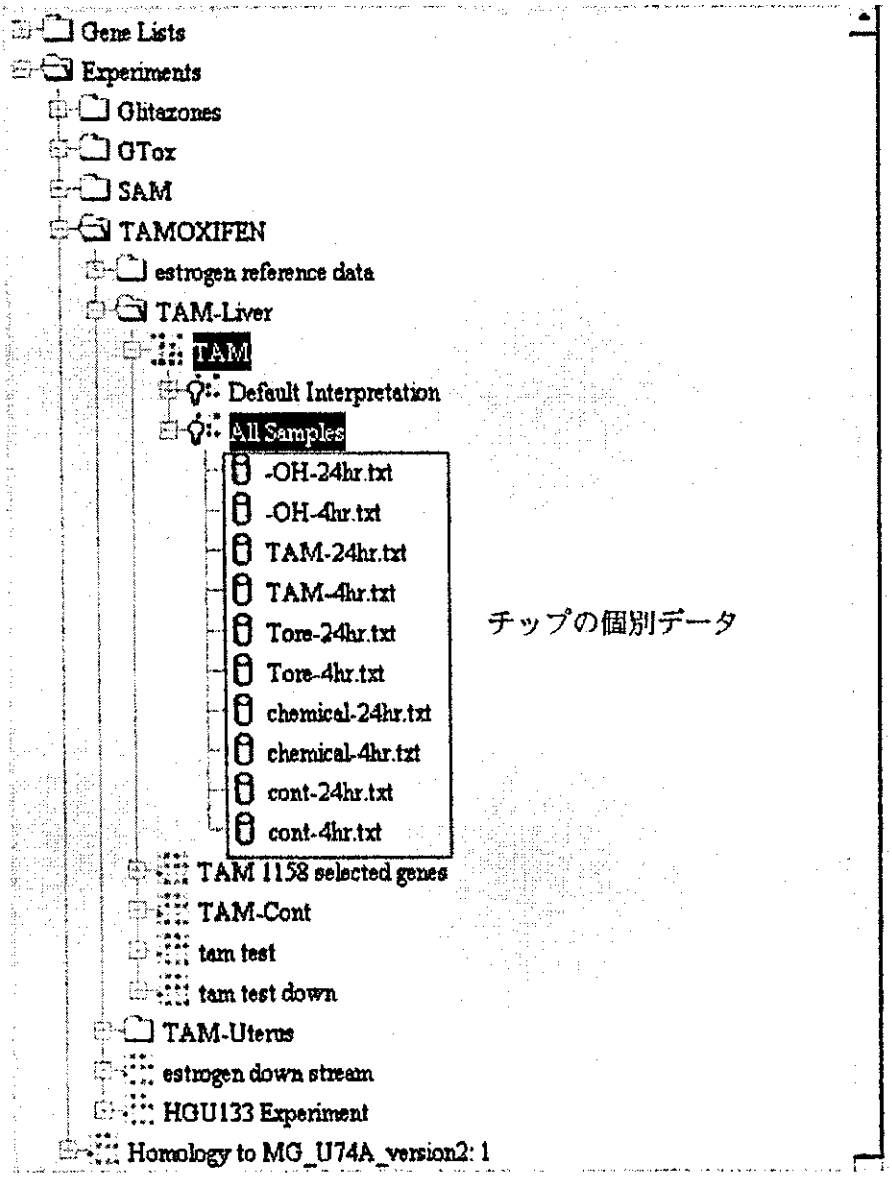
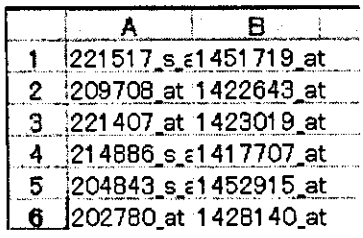
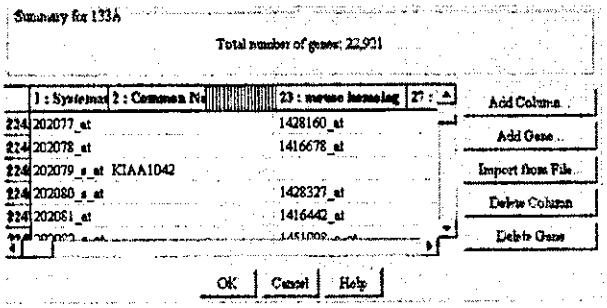


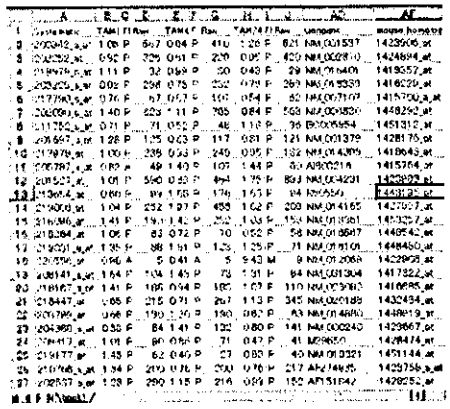
図8　ヒトデータとマウスデータの直接比較法（HU133A から MOE430 への変換の例）

1. Excel にて HU133A MOE430 アレイのプローブ ID をリストアップした表を作成。
2. このファイルを MOE430.homology という名前で、GeneSpring の HU133 チップフォルダー内にある "Homology Tables" というフォルダーへ保存する。
3. これにより、自動的に HU133A チップでの遺伝子リストをマウスのゲノムへ変換するための準備が整うが、GeneSpring 上で変換操作を行うと、両者のデータは横並びには表示されず、対応するマウスのチップ上のプローブ ID のみが右のように表示される。
4. 次に mouse homolog ID を含む HU133A データを表形式で Excel 上へ書き出すと右の表の左側の赤色で示した部分に mouse homolog（青色）が追加される。
5. 次に、この mouse homolog を用いて MOE430 用の遺伝子リストを作成し、GeneSpring 上にて対応するマウスチップのデータから対応するデータをリストアップし、同様に Excel 上へ転送する。
6. Mouse homolog の項をキーとして HU133A および MOE430 のデータを並び替え、一部重複する項目を手作業にて整理することにより両者を一つの Excel ファイル上で横並びの表として結合させる。これにより、両者のデータを用いてスキャタープロットを書かせるなどの直接比較が可能となる。



	A	B
1	221517_s_a	1451719_at
2	209708_at	1422643_at
3	221407_at	1423019_at
4	214886_s_a	1417707_at
5	204843_s_a	1452915_at
6	202780_at	1428140_at





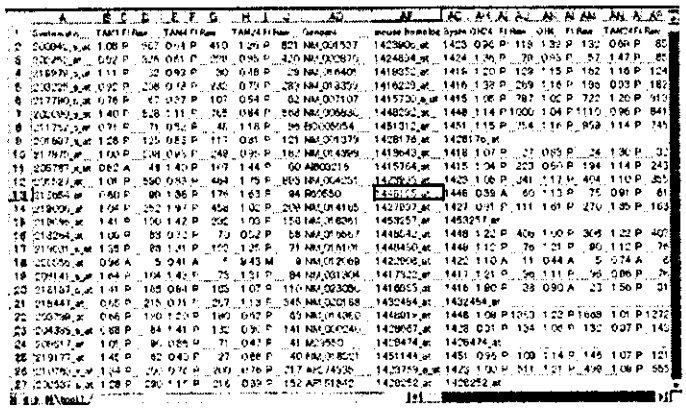


図9　タモキシフェン処理ヒト肝初代培養細胞でのデータとマウス肝臓でのデータの直接比較

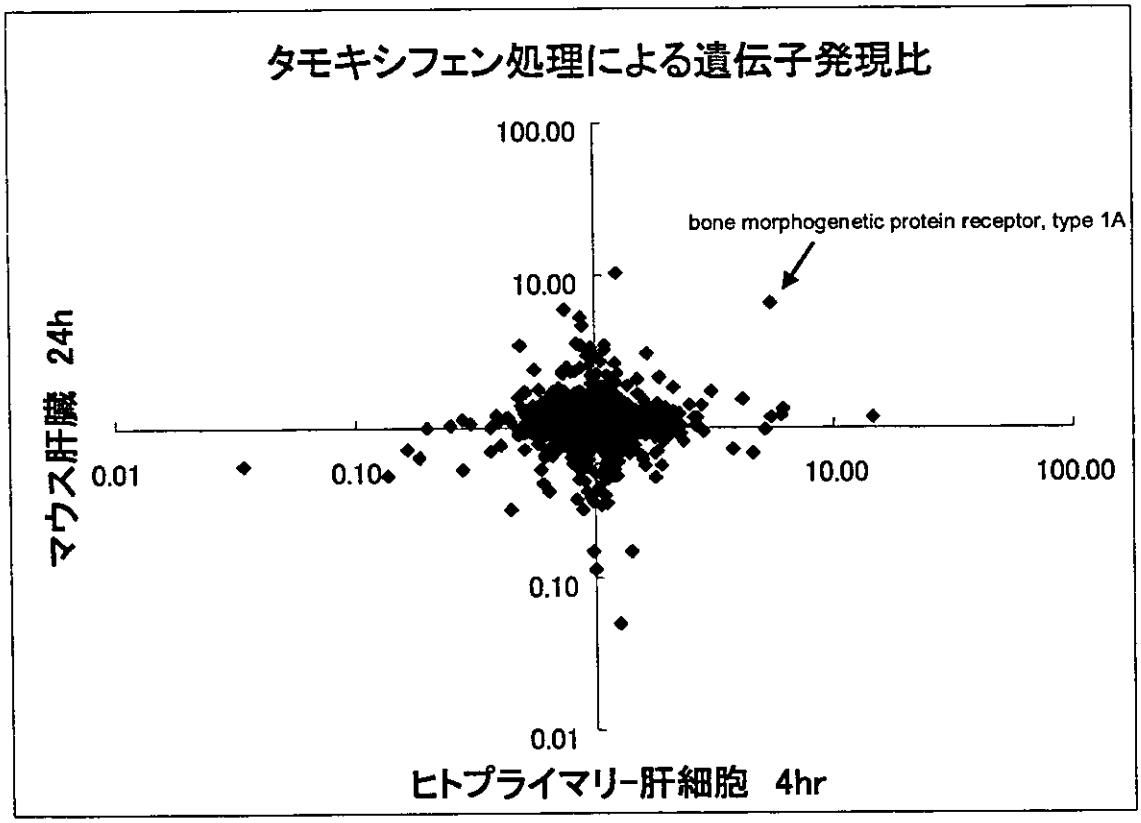


表16　トログリタゾン処理による in vivo(マウス)と in vitro(ヒト)データの共通性の比較

Mouse in vivo																										Human in vitro												Description
1F2D	1R2D	1T2D	4F2D	4R2D	4T2D	24F2D	24R2D	24T2D	72F2D	72R2D	72T2D	1F2H	1R2H	1T2H	4F2H	4R2H	4T2H	24F2H	24R2H	24T2H	72F2H	72R2H	72T2H	1F	1R	1T	4F	4R	4T	24F	24R	24T	72F	72R	72T	
1.76	1.76	1.52	1.78	1.12	1.49	1.31	1.88	3.03	0.95	0.79	1.14	6.48	1.58	0.74	5.72	4.14	1.57	2.57	1.68	1.01	1.73	3.28	1.50	0.92	0.90	0.84	1.10	1.14	1.04	1.36	1.57	2.93	1.91	1.40	1.58	lipin 1
2.05	3.03	1.50	2.59	2.82	3.50	4.41	4.18	2.35	2.34	4.38	1.68	1.14	1.55	0.80	1.73	1.23	1.31	1.55	2.74	0.98	1.19	2.93	1.01	5.83	4.11	4.31	1.03	0.98	1.34	1.31	1.41	0.79	1.53	1.34	1.07	CD36 antigen
1.74	1.87	1.88	1.81	1.85	1.46	2.31	2.32	2.00	1.90	2.04	1.92	1.08	0.87	1.00	0.97	0.84	1.04	1.39	1.18	1.24	1.13	1.27	1.03	0.86	0.78	0.67	1.40	1.58	1.91	0.83	0.55	1.52	4.98	3.02	3.47	expressed sequence AI315365
49.35	85.24	76.36	51.04	92.18	45.47	1.46	2.77	3.33	0.42	2.64	1.46	1.78	1.42	0.81	1.85	0.30	1.20	1.27	0.93	1.40	0.85	1.69	0.74	0.96	0.88	0.95	1.14	1.02	1.15	1.58	1.41	2.83	0.97	0.79	1.33	farnesyl diphosphate synthetase
3.85	3.03	3.03	3.42	3.52	2.59	1.30	1.11	1.02	0.43	1.00	1.00	0.98	1.13	1.09	0.86	0.31	1.00	1.40	1.10	1.14	0.99	1.07	1.01	2.83	0.93	0.99	1.21	0.16	0.61	7.93	1.94	7.95	0.51	5.39	2.68	cathepsin Z
1.34	1.16	2.16	2.35	1.85	1.86	1.08	0.81	1.54	0.83	1.04	1.33	0.88	0.47	0.88	0.88	1.43	0.84	0.97	0.77	0.94	0.60	0.88	1.43	0.82	1.30	0.24	0.84	1.22	0.83	4.75	6.39	10.82	21.99	32.43	15.54	fatty acid binding protein 4, adipocyte
3.49	2.67	2.02	2.38	2.30	2.67	1.73	1.61	1.30	1.08	1.24	1.08	1.51	1.48	1.25	1.43	0.88	1.16	1.53	1.55	1.25	1.32	1.40	1.17	1.24	1.35	1.32	1.38	1.41	1.54	3.52	3.79	4.25	2.33	2.35	2.60	P450 (cytochrome) oxidoreductase
1.24	1.41	1.15	1.25	1.33	1.17	1.05	1.41	1.24	1.38	1.25	0.99	0.70	0.95	0.93	0.98	0.99	1.12	1.17	1.19	1.23	0.84	1.22	1.18	0.80	1.09	0.90	1.00	0.97	0.97	3.35	4.63	5.97	6.17	8.08	10.09	fatty acid binding protein 1, liver
0.55	0.37	0.31	0.33	0.68	0.56	1.52	0.45	1.39	1.21	1.51	1.13	0.71	0.98	0.83	0.84	1.17	0.57	0.65	0.84	0.94	1.26	0.71	1.45	0.91	0.67	0.73	0.41	1.61	0.93	0.82	0.13	0.14	0.47	0.25	0.14	solute carrier family 24, member 1
0.76	0.46	0.78	0.62	0.48	0.64	0.41	0.84	0.74	0.78	1.28	1.30	0.88	0.89	0.88	0.55	1.61	0.98	0.80	0.99	1.43	1.20	1.16	1.43	0.65	0.61	0.72	0.39	0.91	0.98	0.32	0.38	0.28	1.27	0.87	0.84	cardiac responsive adriamycin protein
1.19	1.48	1.80	1.28	1.55	1.48	1.24	1.52	1.26	1.49	1.67	1.27	0.88	0.89	1.08	0.90	0.79	1.08	0.77	0.98	1.01	1.05	1.10	0.99	0.52	0.45	0.38	0.53	1.41	0.94	0.17	0.37	0.39	2.00	0.80	0.54	RIKEN cDNA 1210001E11 gene

＊　各処理において共通性の高かった遺伝子に関して異種データの相同遺伝子のデータを比較した。

ピンクは発現上昇、水色は発現低下

表 17　グリタゾン化合物各処理間でのデータの相関性の比較

Pioglitazone		Rosiglitazone		Troglitazone	
処理	相関係数	処理	相関係数	処理	相関係数
1P20,1P200	0.87	1R20,1R200	0.91	1T20,1T200	0.85
24P20,24P200	0.52	72R20,72R200	0.53	4T20,4T200	0.45
1P20,4P20	0.41	1R20,4R20	0.47	1T20,4T20	0.38
1P200,4P20	0.39	4R20,4R200	0.45	24T20,24T200	0.36
4P20,72P20	0.37	1R200,4R20	0.39	72T20,72T200	0.28
1P20,24P20	0.31	4R20,72R20	0.32	4T20,72T20	0.26
24P20,72P20	0.30	4R20,72R200	0.30	1T200,4T20	0.18
1P20,72P20	0.29	24R20,72R20	0.29	24T,72T	0.16
1P200,24P200	0.23	24R20,24R200	0.26	1T20,4T200	0.14
72P20,72P200	0.23	1R20,72R200	0.22	4T,72T	0.13
1P20,24P200	0.22	1R200,72R200	0.19	4T20,24T20	0.12
4P20,24P20	0.21	4R200,72R200	0.15	1T,4T	0.12
4P200,72P200	0.19	4R20,24R20	0.15	24T20,72T200	0.11
1P200,24P20	0.19	1R20,72R20	0.15	24T20,72T20	0.10
1P200,72P20	0.17	4R200,72R20	0.09	1T20,24T200	0.10
4P20,24P200	0.13	1R200,72R20	0.08	24T200,72T20	0.09
4P20,4P200	0.12	1R20,24R	0.08	1T200,24T200	0.09
1P,24P	0.12	72R200,24R	0.07	1T20,72T20	0.09
24P200,72P20	0.10	4R200,24R	0.07	24T200,72T200	0.06
1P,72P	0.09	1R20,24R20	0.07	4T200,72T20	0.06
4P,24P	0.09	4R20,24R	0.06	1T200,4T200	0.06
72P20,24P	0.08	4R,72R	0.06	1T20,72T	0.06
72P200,1P	0.06	1R,72R	0.06	1T200,72T	0.05
4P,72P	0.05	72R20,24R	0.06	1T200,1T	0.05
1P,4P	0.05	1R200,24R	0.05	4T,24T	0.05
24P20,1P	0.05	1R20,4R200	0.05	1T,72T	0.04
1P200,24P	0.05	24R200,1R	0.05	24T200,72T	0.04
72P200,4P	0.05	24R,72R	0.05	24T200,4T	0.04
24P20,24P	0.04	4R200,24R200	0.04	72T20,24T	0.04
1P20,24P	0.04	1R200,72R	0.04	72T200,24T	0.03
4P20,24P	0.04	4R200,24R20	0.03	4T200,24T200	0.03
72P20,4P	0.03	1R20,72R	0.03	1T200,4T	0.03
4P200,4P	0.02	24R200,4R	0.03	24T20,4T	0.02
72P200,24P	0.02	4R200,4R	0.03	72T200,4T	0.01
72P20,72P	0.02	24R20,1R	0.02	1T20,1T	0.01
72P20,1P	0.02	24R20,72R200	0.02	4T20,72T	0.01
1P20,72P	0.01	1R200,4R200	0.02	24T200,24T	0.01
1P20,1P	0.01	72R20,1R	0.02	1T20,4T	0.01
1P200,72P	0.01	1R,24R	0.02	4T200,72T	0.01
72P200,72P	0.01	1R200,24R20	0.01	24T200,1T	0.01
24P,72P	0.01	24R20,4R	0.01		

	time point (hr)	dose
in vivo	1,4,24,72	20 mg/kg, 200 mg/kg
in vitro	1,4,24,72	30 uM

別添5

III. 研究成果の刊行に関する一覧表

平成16年度

別添5

研究成果の刊行に関する一覧表(平成16年度)

雑誌

	発表者氏名	論文タイトル名	発表誌名	巻号	ページ	出版年
1)	Takayoshi Suzuki, Yuki Nagano, Akiyasu Kouketsu, Azusa Matsuura, Sakiko Maruyama, Mineko Kurotaki, Hidehiko Nakagawa, Naoki Miyata	Novel inhibitors of human histone deacetylases: design, synthesis, enzyme inhibition, and cancer cell growth inhibition of SAHA-based non- hydroxamates	*J. Med. Chem.*	48	1019-1032	2005
2)	Shinya Usui, Takayoshi Suzuki, Yoshifumi Hattori, Kazuma Etoh, Hiroki Fujieda, Makoto Nishizuka, Masayoshi Imagawa, Hidehiko Nakagawa, Kohfuku Kohda, Naoki Miyata	Design, synthesis and biological activity of novel PPARg ligands based on rosiglitazone and 15d-PGJ2	*Bioorganic & Medicinal Chemistry Letters*	15	1547-1551	2005
3)	Takayoshi Suzuki, Azusa Matsuura, Akiyoshi Kouketsu, Hidehiko Nakagawa, Naoki Miyata	Identification of a potent non-hydroxamate histone deacetylase inhibitor by mechanism-based drug design	*Bioorganic & Medicinal Chemistry Letters*	15	331-335	2005
4)	Takayoshi Suzuki, Akiyasu Kouketsu, Azusa Matsuura, Arihiro Kohara, Shin-ichi Ninomiya, Kohfuku Kohda, Naoki Miyata	Thiol-based SAHA analogues as potent histone deacetylase inhibitors,	*Bioorganic & Medicinal Chemistry Letters*	14(12)	3313-3317	2004
5)	Masaaki Kurihara, Abu Shara Shamsur Rouf, Hisao Kansui, Hiroyuki Kagechika, Haruhiro Okuda, Naoki Miyata	Design and synthesis of cyclic urea compounds: a pharmacological study for retinoidal acty	*Bioorganic & Medicinal Chemistry Letters*	14(16)	4131-4134	2004
6)	Eiji Okada, Yuka Komazawa, Masaaki Kurihara, Hideshi Inoue, Naoki Miyata, Haruhiro Okuda, Toshie Tsuchiya, Yoko Yamakoshi	Synthesis of C60 derivatives for photoaffinity labeling	*Tetrahedron Letters*	45(3)	527-529	2004
7)	Nozomi Saito, Yoshitomo Suhara, Masaaki Kurihara, Toshie Fujishima, Shinobu Honzawa, Hitoshi Takayanagi, Toshiro Kozono, Masahiko Matsumoto, Masayuki Ohmori, Naoki Miyata, Hiroaki Takayama, Atsushi Kittaka,	Design and efficient synthesis of 2alpha-(omega-hydroxy)-1alpha,25-dihydroxyvitamin D3 analogues, includeing 2-epi-ED-71 and their 20-epimers with HL-60 cell differentiation activity	*J. Org. Chem.*	69(22)	7463-7471	2004
8)	Mariko Murata, Shiho Ohnishi, Kazuharu Seike, Kiyoshi Fukuhara, Naoki Miyata, Shosuke Kawanishi	Oxidative DNA damage induced by carcinogenic dinitropyrenes in the presence of P450 reductase	*Chem. Res. Toxicol.*	17(12)	1750-1756	2004
9)	Yasuhiro Noda, Tomoe Sumino, Yuki Fujisawa, Naoki Miyata, Toyo Kaiya, Kohfuku Kohda	1-Amino-4-phenyl-1,2,3,6-tetrahydro- pyridine and 1-amino-4-phenylpyridinium salt, the 1-amino analogues of neurotoxins, MPTP and MPP+, induce apoptosis in PC12 cells: Detection of apoptotic cells by comet assay and flow cytometric analysis	*In vivo*	18	561-569	2004

	発表者氏名	論文タイトル名	発表誌名	巻名	ページ	出版年
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12)	I. Nakanishi, T. Kawashima, K. Ohkubo, H. Kanazawa, K. Inami, M. Mochizuki, K. Fukuhara, H. Okuda, T. Ozawa, S. Itoh, S. Fukuzumi, and N. Ikota	Electron-Tansfer Mechanism in Radical-Scavenging Reactions by a Vitamin E Model in a Protic Medium	*Org. Biomol. Chem*	3	626-629	2005
13)	I. Nakanishi, K. Ohkubo, K. Miyazaki, W. Hakamata, S. Urano, T, Ozawa, H. Okuda, S, Fukuzumi, N. Ikota, and K. Fukuhara	A planar catechin analogue having a more negative oxidation potential than (+)-catechin as an electron-transfer antioxidant against a peroxyl Rradical	*Chem. Res. Toxicol.*	17	26-31	2004
14)	Yamada K, Suzuki T, Kohara A, Hayashi M, Mizutani T, Saeki K	In vivo mutagenicity of benzo[f]quinoline, benzo[h]quinoline, and 1,7-phenanthroline using the lac Z transgenic mice	*Mutation Research*	559	83-95	2004
15)	Arlt VM, Zhan L, Schmeiser HH, Honma M, Hayashi M, Phillips DH, Suzuki T	DNA adducts and mutagenic specificity of the ubiquitous environmental pollutant 3-nitrobenzanthrone in Muta Mouse	*Environ. Mol. Mutagen.*	43	186-195	2004
16)	鈴木孝禎、宮田直樹	ヒストン脱アセチル化酵素阻害剤開発の最前線	*ファルマシア*	41	*in press*	2005
17)	鈴木孝禎、中川秀彦、宮田直樹	癌の分子標的治療薬の開発：非ヒドロキサム酸系ヒストン脱アセチル化酵素阻害薬の設計、合成と生物活性評価	*有機合成化学協会誌*	63	*in press*	2005

Ⅳ. 研究成果の刊行物・別刷

（平成16年度）

Novel Inhibitors of Human Histone Deacetylases: Design, Synthesis, Enzyme Inhibition, and Cancer Cell Growth Inhibition of SAHA-Based Non-hydroxamates

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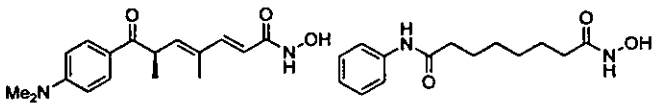
To find novel non-hydroxamate histone deacetylase (HDAC) inhibitors, a series of compounds modeled after suberoylanilide hydroxamic acid (SAHA) was designed and synthesized. In this series, compound **7**, in which the hydroxamic acid of SAHA is replaced by a thiol, was found to be as potent as SAHA, and optimization of this series led to the identification of HDAC inhibitors more potent than SAHA. In cancer cell growth inhibition assay, *S*-isobutyryl derivative **51** showed strong activity, and its potency was comparable to that of SAHA. The cancer cell growth inhibitory activity was verified to be the result of histone hyperacetylation and subsequent induction of $p21^{WAF1/CIP1}$ by Western blot analysis. Kinetical enzyme assay and molecular modeling suggest the thiol formed by enzymatic hydrolysis within the cell interacts with the zinc ion in the active site of HDACs.

Introduction

The reversible acetylation of the ϵ-amino groups of specific histone lysine residues by histone deacetylases (HDACs) and histone acetyl transferases is an important regulatory mechanism of gene expression.[1] When HDACs are inhibited, histone hyperacetylation occurs. The disruption of the chromatin structure by histone hyperacetylation leads to the transcriptional activation of a number of genes.[2] One important outcome of the activation is induction of the cyclin-dependent kinase inhibitory protein $p21^{WAF1/CIP1}$, which causes cell cycle arrest.[3] Indeed, HDAC inhibitors such as trichostatin A (TSA) and suberoylanilide hydroxamic acid (SAHA) (Chart 1) have been reported to inhibit cell growth, induce terminal differentiation in tumor cells,[4] and prevent the formation of malignant tumors in mice.[5] Therefore, HDACs have emerged as attractive targets in anticancer drug development, and HDAC inhibitors have also been viewed as useful tools to study the function of these enzymes.

Many groups have ongoing research programs to find nonpeptide small-molecule inhibitors of HDACs, and these efforts have led to the identification of several classes of inhibitors.[6] Most previously reported HDAC inhibitors belong to hydroxamic acid derivatives, typified by TSA and SAHA, which are thought to chelate the zinc ion in the active site in a bidentate fashion through its CO and OH groups.[7] However, hydroxamic acids occasionally have been associated with problems such as poor pharmacokinetics and severe toxicity.[8] Thus, it has become increasingly desirable to find

Chart 1



replacements that possess strong inhibitory action against HDACs. In addition, in terms of biological research, the discovery of novel zinc-binding groups (ZBGs) may lead to a new type of HDAC isozyme-selective inhibitors which are useful as tools for probing the biology of the enzyme.[9] Thus far, *o*-aminoanilide,[9,10] electrophilic ketones,[11] and *N*-formyl hydroxylamine[12] have been reported as ZBGs in small-molecule HDAC inhibitors. However, most of them have reduced potency as compared to hydroxamic acid, and unfortunately, HDAC inhibitors bearing electrophilic ketones[11] have a metabolic disadvantage in that they are readily reduced to inactive alcohols in vivo, even within cells. We therefore initiated a search for replacement groups for hydroxamic acid with the goal of drug discovery as well as finding new tools for biological research, and found some potent non-hydroxamate small-molecule HDAC inhibitors.[13] We now present a full account of our study reporting the design, synthesis, HDAC inhibition, cancer cell growth inhibition, and binding mode analysis of non-hydroxamates based on the structure of SAHA.

Chemistry

The compounds prepared for this study are shown in Tables 1−5. The routes used for synthesis of the compounds are shown in Schemes 1−4. Scheme 1 shows the preparation of compounds **4**, **5**, **10**, **12**−**17**, and **18**. Compounds **4** and **5** were synthesized from pimelic acid **56**. The condensation of pimelic acid **56** with an equiva-

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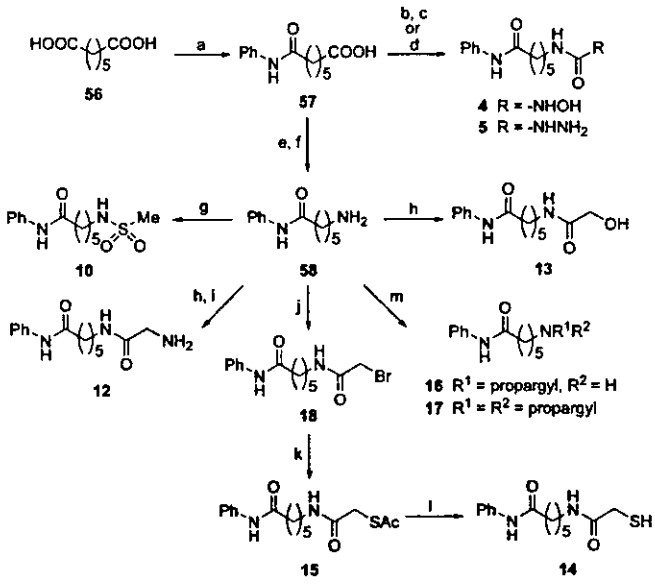
Scheme 1[a]



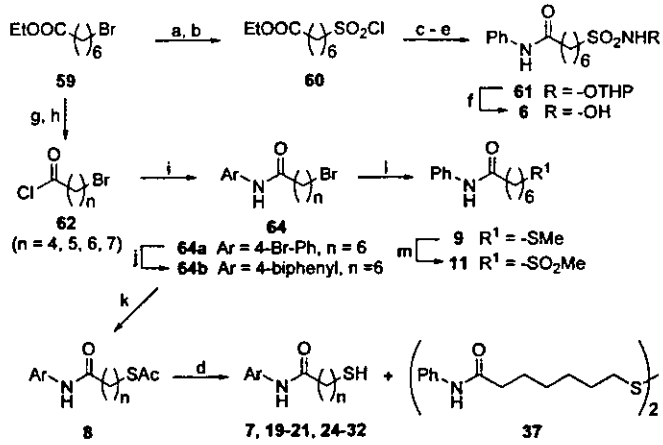
[a] Reagents and conditions: (a) aniline, 180 °C; (b) diphenylphosphoryl azide (DPPA), Et_3N, toluene, reflux, and then *O*-(2-tetrahydropyranyl)hydroxylamine, reflux; (c) TsOH, MeOH, rt; (d) DPPA, Et_3N, benzene, reflux, and then hydrazine monohydrate, reflux; (e) DPPA, Et_3N, benzene, reflux, and then BnOH, reflux; (f) H_2, 5%Pd−C, MeOH, rt; (g) MsCl, pyridine, rt; (h) $HOCH_2COOH$ or $BocNHCH_2COOH$, EDCI, HOBt, DMF, rt; (i) TFA, $CHCl_3$, rt; (j) $BrCH_2COBr$, Et_3N, THF, rt; (k) AcSK, EtOH, rt; (l) K_2CO_3, MeOH, rt; (m) propargyl bromide, K_2CO_3, MeOH, rt.

lent amount of aniline gave mono-anilide **57**. Curtius rearrangement of the acyl azide prepared from carboxylic acid **57** using diphenylphosphoryl azide provided the isocanates, which on treatment with *O*-tetrahydropyranyl (THP) hydroxylamine or hydrazine gave *O*-THP hydroxyurea and semicarbazide **5**. Deprotection of the THP group of the *O*-THP hydroxyurea under acidic conditions afforded hydroxyurea **4**.

Compounds **10**, **12**−**17**, and **18** were prepared from carboxylic acid **57** obtained above via amine **58** by the procedure outlined in Scheme 1. Carboxylic acid **57** was converted to amine **58** with a three-step sequence: Curtius rearrangement of the acyl azide prepared from carboxylic acid **57**, treatment of the resulting isocyanates with benzyl alcohol, and removal of the Z group by hydrogenation. Coupling between amine **58** and methanesulfonyl chloride afforded sulfonamide **10**. The reaction of amine **58** with *N*-Boc glycine in the presence of EDCI and HOBt in DMF was followed by treatment with trifluoroacetic acid to give aminoacetamide **12**. Hydroxyacetamide **13** was obtained in one step using the procedure described for **12**. The amino group of **58** was acylated with bromoacetyl bromide to yield bromoacetamide **18**. Bromide **18** was treated with potassium thioacetate to give thioacetate **15**, after which deacetylation of the thioacetate in the presence of K_2CO_3 in MeOH gave mercaptoacetamide **14**. The amine **58** was allowed to react with propargyl bromide in the presence of K_2CO_3 to give mono- and di-alkylated compounds **16** and **17**.

Compounds **6**−**9**, **11**, **19**−**21**, **24**−**32**, and **37** were prepared from another starting material, **59** (Scheme 2). The preparation of hydroxysulfonamide **6** was achieved via sulfonyl chloride **60**. Bromide **59** was converted to sulfonyl chloride **60** by sulfation and by a

Scheme 2[a]



[a] Reagents and conditions: (a) Na_2SO_3, EtOH, H_2O, reflux; (b) $SOCl_2$, DMF, toluene, reflux; (c) *O*-(2-tetrahydropyranyl)hydroxylamine, 4-(dimethylamino)pyridine, pyridine, CH_2Cl_2, rt; (d) 2N aq NaOH, EtOH, rt; (e) aniline, EDCI, HOBt, DMF, rt; (f) TFA, CH_2Cl_2, 60 °C; (g) LiOH·H_2O, EtOH, THF, H_2O, rt; (h) $(COCl)_2$, DMF, CH_2Cl_2, rt; (i) $ArNH_2$ (**63**), Et_3N, CH_2Cl_2, rt; (j) $PhB(OH)_2$, $Pd(PPh_3)_4$, $NaHCO_3$, 1-methyl-2-pyrrolidinone, H_2O, 80 °C; (k) AcSK, EtOH, rt; (l) 15% aq NaSMe, EtOH, rt; (m) *m*-chloroperoxybenzoic acid, CH_2Cl_2, rt.

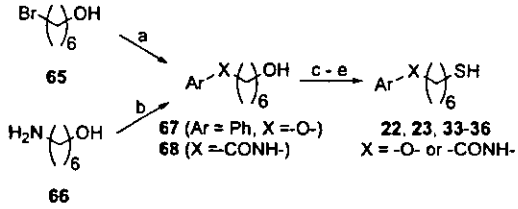
subsequent reaction with thionyl chloride. The sulfonyl chloride **60** was treated with *O*-THP hydroxylamine to give *O*-THP hydroxysulfonamide, after which hydrolysis of the ester under alkaline conditions and subsequent amide formation with aniline gave compound **61**. Removal of the THP group of compound **61** by treatment with trifluoroacetic acid gave hydroxysulfonamide **6**.

Compounds **7**−**9**, **11**, **19**−**21**, **24**−**32**, and **37** were synthesized from the corresponding acid chlorides **62** (**62a** ($n = 4$) and **62b** ($n = 5$) are commercially available) by the route shown in Scheme 2. **62c** ($n = 6$) was prepared from ester **59** by hydrolysis of the ethyl ester and a subsequent reaction with oxalyl chloride, and **62d** ($n = 7$) was obtained in the same way as **62c**. The amino group of aromatic amines **63** was acylated with an appropriate acid chloride **62** to give the amides **64**. Suzuki coupling[14] of bromobenzene **64a** with phenylboronic acid provided the biphenyl **64b**. Bromides **64** were treated with potassium thioacetate to give compound **8**, after which hydrolysis of the thioacetates under alkaline conditions gave the desired compounds **7**, **19**−**21**, **24**−**31**, and **32**, and disulfide **37** was obtained as a byproduct when thiol **7** was synthesized. Sulfide **9** was prepared by the alkylation of methylmercaptan with bromide **64c** (Ar = Ph, $n = 6$). Oxidation of **9** with 2 equiv of *m*-chloroperoxybenzoic acid provided the sulfone **11**.

Thiols **22**, **23**, **33**−**35**, and **36** were prepared from alcohol **65** or **66** by the procedure outlined in Scheme 3. Treatment of bromide **65** with phenol in the presence of K_2CO_3 gave ether **67**, and condensation of amine **66** with an appropriate aromatic carboxylic acid **69** afforded amides **68**. Alcohols **67** and **68** were converted to thiols **22**, **23**, **33**−**35**, and **36** in three steps by conversion of the alcohols to bromides, treatment of the bromides with potassium thioacetate, and hydrolysis of the resulting thioacetates.

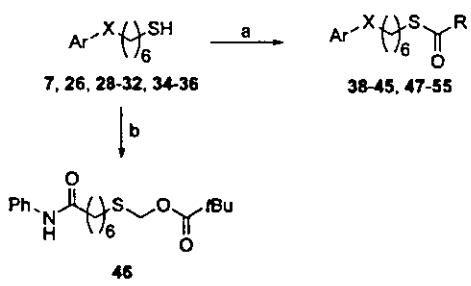
The preparation of *S*-chemically modified compounds **38**−**54**, and **55** is shown in Scheme 4. Thiols **7**, **26**, **28**−**32**, **34**, **35**, and **36** were coupled with the corresponding

Scheme 3[a]



[a] Reagents and conditions: (a) Phenol, K_2CO_3, DMF, 80 °C; (b) ArCOOH (**69**), EDCI, HOBt, DMF, rt; (c) CBr_4, PPh_3, CH_2Cl_2, 0 °C; (d) AcSK, EtOH, rt; (e) 2N aq NaOH, EtOH, THF, rt.

Scheme 4[a]



[a] Reagents and conditions: (a) RCOCl (**70**), 4-(dimethylamino)pyridine, pyridine, CH_2Cl_2, rt; (b) NaH, chloromethyl pivalate, DMF, 0 °C to room temperature.

acyl chloride **70** to give thioesters **38**–**45**, **47**–**54**, and **55**. Alkylation of thiol **7** with chloromethyl pivalate in the presence of sodium hydride in DMF afforded compound **46**.

Results and Discussion

Enzyme Assays. The compounds synthesized in this study were tested with an in vitro assay using a HeLa nuclear extract rich in HDAC activity. The results are summarized in Tables 1–3.

The IC_{50} values of SAHA and *o*-aminoanilide **1** were 0.28 μM and 120 μM, respectively (entries 1 and 2). α-Ketoamide **2** and *N*-formyl hydroxylamine **3** were reported previously to inhibit HDACs with an IC_{50} of 0.34 μM and 2.8 μM, respectively (entries 3 and 4).[11b,12]

The crystal structures of an archaebacterial HDAC homologue (HDAC-like protein, HDLP)/hydroxamates and HDAC8/hydroxamates complexes made it clear that the hydroxamic acid group coordinates the zinc ion in the active site through its CO and OH groups and also forms three hydrogen bonds between its CO, NH, and OH groups and Tyr 306, His 143, and His 142 (HDAC8 numbering), respectively.[7] From these data, hydroxyurea **4**, semicarbazide **5**, and hydroxysulfonamide **6** were synthesized and tested as HDAC inhibitors because it is possible for them to chelate zinc ion and form hydrogen bonds with Tyr and His like SAHA. Among these three compounds, hydroxyurea **4** and semicarbazide **5** showed anti-HDAC activity and the IC_{50} values were comparable to that of *o*-aminoanilide **1** (entries 5, 6, and 7). However, they were much less effective than SAHA.

Thiols seemed to be reasonable targets for hydroxamic acid replacements, because zinc ion is highly thiophilic and thiol derivatives have been reported to inhibit zinc-dependent enzymes such as angiotensin converting enzyme[15] and matrix metalloproteinases.[16] Furthermore, macrocyclic compounds bearing a disulfide group such as FK228 have been reported recently to inhibit

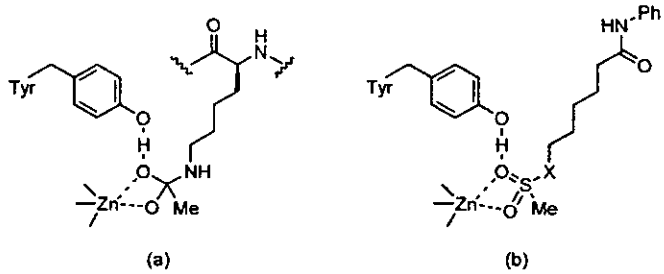


Figure 1. The transition state proposed for HDACs (a), and models for the binding of sulfone derivatives (b).

HDACs under reductive conditions.[17] Surprisingly, although the inhibitory ability of monodentate ZBGs such as thiol was thought to be less than that of bidentate ZBGs such as hydroxamate, *N*-formyl hydroxylamine, and hydrated electrophilic ketones,[16] the activity of thiol **7** was far greater than expected. A pronounced inhibitory effect (IC_{50} = 0.21 μM) was observed with thiol **7**, which was much more active than previously reported non-hydroxamates such as *o*-aminoanilide, *N*-formyl hydroxylamine, and trifluoromethyl ketone,[11a] and as potent as α-ketoamide **2** and SAHA (entry 8). To confirm that the thiol group plays an important role in anti-HDAC activity, thioacetate **8a** and methyl sulfide **9** were tested. As expected, thiol transformation into thioacetate and methyl sulfide led to an inhibitor that was about 30-fold less potent and a compound devoid of anti-HDAC activity, respectively (entries 9 and 10). These results suggest that thiolate anion generated under physiological conditions has an intimate involvement in the interaction with the zinc ion in the active site.

The crystal structures of the HDLP/hydroxamates and HDAC8/hydroxamates complexes have led to a solid understanding of not only the three-dimensional structure of the active site of HDACs but also the catalytic mechanism for the deacetylation of acetylated lysine substrate.[7] It has been proposed that the carbonyl oxygen of this substrate could bind the zinc, and the carbonyl could be attacked by a zinc-chelating water molecule (Figure 2a), which would result in the production of deacetylated lysine via a tetrahedral carbon-containing transition state (Figure 1a). On the basis of the proposed catalytic mechanism, we attempted to design non-hydroxamate HDAC inhibitors. First, we designed transition-state (TS) analogues. The TS of HDAC deacetylation was estimated to include a tetrahedral carbon (Figure 1a) as with other zinc proteases.[18] We focused attention on sulfone derivative TS analogues because it has been suggested that the sulfonamide moiety has strong similarity with the TS of amide bond hydrolysis, both from a steric and an electronic point of view.[19] Compounds **10** and **11**, in which a hydroxamic acid of SAHA is replaced by a sulfonamide and a sulfone, respectively, were designed and synthesized as TS analogues (Figure 1b). Of these two TS analogues, sulfone **11** showed anti-HDAC activity and the IC_{50} value was 230 μM (entries 11 and 12). However, sulfone **11** was approximately 820-fold less effective than SAHA.

Our next approach was based on the proposed deacetylation mechanism whereby a zinc-chelating water molecule activated by His142 and His 143 (HDAC8 numbering) makes a nucleophilic attack on the carbonyl carbon of an acetylated lysine substrate (Figure 2a).

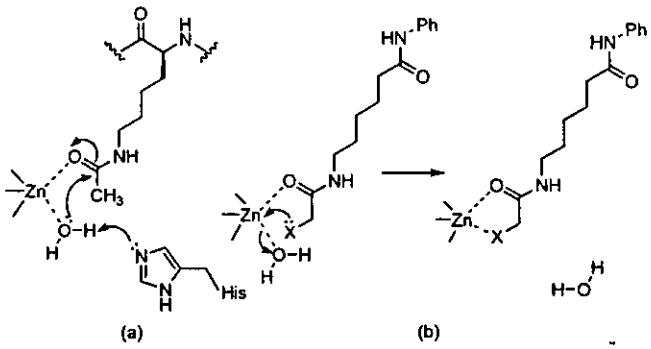
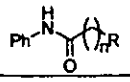
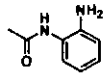
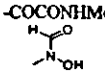


Figure 2. The mechanism proposed for the deacetylation of acetylated lysine substrate (a), and a model for the binding of heteroatom-containing substrate analogues to zinc ion (b).

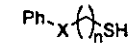
Table 1. HDAC Inhibition Data for SAHA and SAHA-based Non-hydroxamtes[a]



entry	compd	R	*n*	% inhbtn at 100 μM	IC_{50} (μM)
1	SAHA[b]	-CONHOH	6	100	0.28
2	1[c]		6	48	120
3	2	-COCONHMe	6	ND	0.34[d]
4	3		7	ND	2.8[e]
5	4	-NHCONHOH	5	58	80
6	5	$-NHCONHNH_2$	5	35	150
7	6	$-SO_2NHOH$	6	14	>100
8	7	-SH	6	100	0.21
9	8a	-SAc	6	85	7.1
10	9	-SMe	6	11	>100
11	10	$-NHSO_2Me$	5	10	7500
12	11	$-SO_2Me$	6	33	230
13	12[f]	$-NHCOCH_2NH_2$	5	6	>100
14	13	$-NHCOCH_2OH$	5	0	>100
15	14	$-NHCOCH_2SH$	5	99	0.39
16	15	$-NHCOCH_2SAc$	5	72	22
17	16		5	14	>100
18	17		5	0	>100
19	18	$-NHCOCH_2Br$	5	78	17

[a] Values are means of at least three experiments. [b] Prepared as described in ref 26. [c] Prepared as described in ref 9a. [d] Data taken from the literature (ref 11b). [e] Data taken from the literature (ref 12). [f] Trifluoroacetic acid salt. ND = No data.

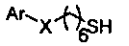
Table 2. Effect of Linker Variation on HDAC Inhibitory Activity of Thiols[a]

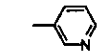
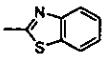
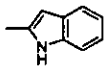


entry	compd	X	*n*	IC_{50} (μM)
1	7	-NHCO-	6	0.21
2	19	-NHCO-	7	1.5
3	20	-NHCO-	5	0.37
4	21	-NHCO-	4	6.2
5	22	-O-	6	11
6	23	-CONH-	6	0.36

[a] Values are means of at least three experiments.

Table 3. Effect of Aromatic Group Variation on HDAC Inhibitory Activity of Thiols[a]



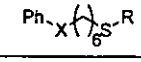
entry	compd	Ar	X	IC_{50} (μM)
1	7	-Ph	-NHCO-	0.21
2	24		-NHCO-	1.2
3	25		-NHCO-	1.1
4	26		-NHCO-	0.075
5	27		-NHCO-	0.62
6	28		-NHCO-	0.21
7	29		-NHCO-	0.11
8	30		-NHCO-	0.072
9	31		-NHCO-	0.17
10	32		-NHCO-	0.34
11	23	-Ph	-CONH-	0.36
12	33		-CONH-	0.61
13	34		-CONH-	0.085
14	35		-CONH-	0.079
15	36		-CONH-	0.10

[a] Values are means of at least three experiments.

With this mechanism, if the water molecule is forcibly removed from the zinc ion, the HDACs would supposedly be inhibited. We then designed and synthesized heteroatom-containing substrate analogues **12**, **13**, and **14**. These analogues would be recognized as substrates by HDACs and would be easily taken into the active site where they could force the water molecule off the zinc ion and the reactive site for the deacetylation by chelation of the heteroatom to the zinc ion, and might behave as HDAC inhibitors (Figure 2b). As shown in Table 1 (entries 13, 14, and 15), potent inhibition was observed with mercaptoacetamide **14**, while **12** and **13** did not possess HDAC inhibitory activities. Mercaptoacetamide **14** exhibited an IC_{50} of 0.39 μM, and its activity largely surpassed those of *o*-aminoanilide **1** and *N*-formyl hydroxylamine **3** and was comparable to those of α-ketoamide **2** and SAHA. As expected, thiol transformation into thioacetate (**15**) led to a 55-fold less potent inhibitor. This result suggests the ease of ionization of thiol is an important factor for HDAC inhibition like the case of thiol **7**.

We turned our attention to irreversible HDAC inhibitors. TPX B is an irreversible HDAC inhibitor,[20] and finding more specific and simpler irreversible HDAC inhibitors is useful for the isolation and cloning of an HDAC.[2] As described above, the crystal structures of the HDLP/hydroxamates and HDAC8/hydroxamates complexes revealed that the hydroxamic acid group forms three hydrogen bonds with Tyr 306, His 143, and His 142, and furthermore, zinc ion is coordinated by His 180, Asp 178, and Asp 267 (HDAC8 numbering). Since the phenol group of Tyr, the imidazole group of His, and the carboxyl group of Asp are able to react with electrophiles, we prepared analogues bearing propargyl

Table 4. Cell Growth Inhibition Data on NCI-H460 Cells for Compound **7** and Its *S*-Modified Prodrugs[a]



entry	compd	R	EC_{50} (μM)
1	7	-H	>50[b]
2	37		>50[c]
3	8a	-Ac	36
4	38	-COEt	28
5	39	-CO*n*-Pr	22
6	40	-CO*i*-Pr	20
7	41	-CO*t*-Bu	>50[d]
8	42		27
9	43		21
10	44	-Bz	25
11	45		24
12	46	-CH_2OCO*t*-Bu	25

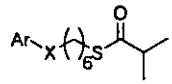
[a] Values are means of at least two experiments. [b] 34% inhibition at 50 μM. [c] 10% inhibition at 50 μM. [d] 42% inhibition at 50 μM.

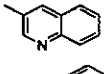
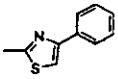
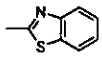
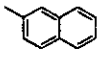
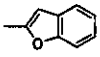
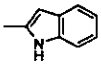
amino (**16**, **17**) and bromoacetamide (**18**) which could form covalent bonds with Tyr, His, and Asp of the enzyme, and evaluated their anti-HDAC activities. While propargyl amino compounds **16** and **17** did not possess HDAC inhibitory activities, more potent inhibition was observed with bromoacetamide **18** (entries 17, 18, and 19). Bromoacetamide **18** exhibited an IC_{50} of 17 μM and its activity was about 9-fold as strong as that of *o*-aminoanilide **1**, but much weaker than that of SAHA.

With the results shown in Table 1, we were encouraged to study further the structure–activity relationship (SAR) and structural optimization. We selected thiol **7** for further study.[21] First, we examined the effect of linker parts of thiol **7**. The results are summarized in Table 2. HDAC inhibition was distinctly dependent on chain length, with $n = 7$ (**19**) and $n = 4$ (**21**) resulting in less potent inhibitors. However, compound **20**, in which $n = 5$, showed essentially the same potency as compound **7**, in which $n = 6$ (entries 1–4). The similar SAR between thiols and hydroxamates, with $n = 6$ optimal,[22] indicates that thiols inhibit HDACs in a binding mode similar to that of hydroxamates. As for the group attaching the phenyl moiety, ether **22** displayed moderate activity, whereas the activity of the reversed amide **23** was maintained (entries 5 and 6).

Next, the aromatic group was examined (Table 3). In the amide-linked series (entries 1–10), 4-substituted phenyl compounds tended to decrease the potency. Specifically, compounds **24** (Ar = 4-NMe_2-Ph), **25** (Ar = 4-biphenyl), and **27** (Ar = 4-PhO-Ph) showed about a 3- to 6-fold decrease in potency when compared to the parent thiol **7** (entries 2, 3, and 5). On the other hand, compound **26**, in which a phenyl group was introduced at the 3-position of the phenyl group of **7**, showed 3-fold increased inhibitory activity (IC_{50} of 0.075 μM, entry 4). In addition, 3-phenoxy compound **28** was equipotent with compound **7** (entry 6). We investigated the effect of the replacement of the phenyl group of compound **7** with heteroaryl rings (entries 7, 8, 9, and 10). Changing the benzene ring to a 3-pyridine ring (**29**), 4-phenyl-2-thiazole ring (**31**), and 2-benzothiazole ring (**32**) sustained or slightly reduced the activity, whereas quinoline **30** had improved activity (IC_{50} of 0.072 μM), and turned out to be the most potent compound in this series. The reverse amide-linked series (entries 11–15) exhibited potencies similar to or greater than the parent thiol **23**, with the exception of **33** (Ar = 4-NMe_2-Ph), which resulted in a slightly less potent inhibitor. In particular, the reversed amides **34** with a naphthalene substituent and **35** with a benzofuran substituent exhibited about 3-fold increases in potency (IC_{50}s of 0.085 μM and 0.079 μM, respectively). As a result, IC_{50}s in the double-digit nanomolar range were observed with 3-biphenyl **26**, quinoline **30**, naphthalene **34**, and benzofuran **35**, which were approximately 3- to 4-fold more potent than SAHA.

Table 5. Cell Growth Inhibition Data on NCI-H460 Cells for Compound **40** and Its Derivatives[a]



entry	compd	Ar	X	EC_{50} (μM)
1	40	-Ph	-NHCO-	20
2	47		-NHCO-	2.8
3	48		-NHCO-	25
4	49		-NHCO-	2.9
5	50		-NHCO-	8.0
6	51		-NHCO-	2.1
7	52		-NHCO-	9.5
8	53		-CONH-	12
9	54		-CONH-	4.1
10	55		-CONH-	12

[a] Values are means of at least two experiments.

Table 6. Growth Inhibition of Various Cancer Cells Using SAHA and Compound **51**[a]

	cell	SAHA, EC_{50} (μM)	**51**, EC_{50} (μM)
MDA-MB-231	breast cancer	1.5	2.3
SNB-78	central nervous system	16	9.1
HCT116	colon cancer	0.58	3.0
NCI–H226	lung cancer	2.6	2.6
LOX-IMVI	melanoma	1.3	1.1
SK-OV-3	ovarian cancer	2.5	4.5
RXF-631L	renal cancer	2.0	2.4
St-4	stomach cancer	5.2	5.0
DU-145	prostate cancer	1.6	4.5
	mean	3.7	3.8

[a] Values are means of at least two experiments.

Cancer Cell Growth Inhibition Assay. To confirm the effectiveness of thiol-based HDAC inhibitors as anticancer drugs and tools for biological research, thiol **7** was initially tested in a cancer cell growth inhibition