

Fig. 1

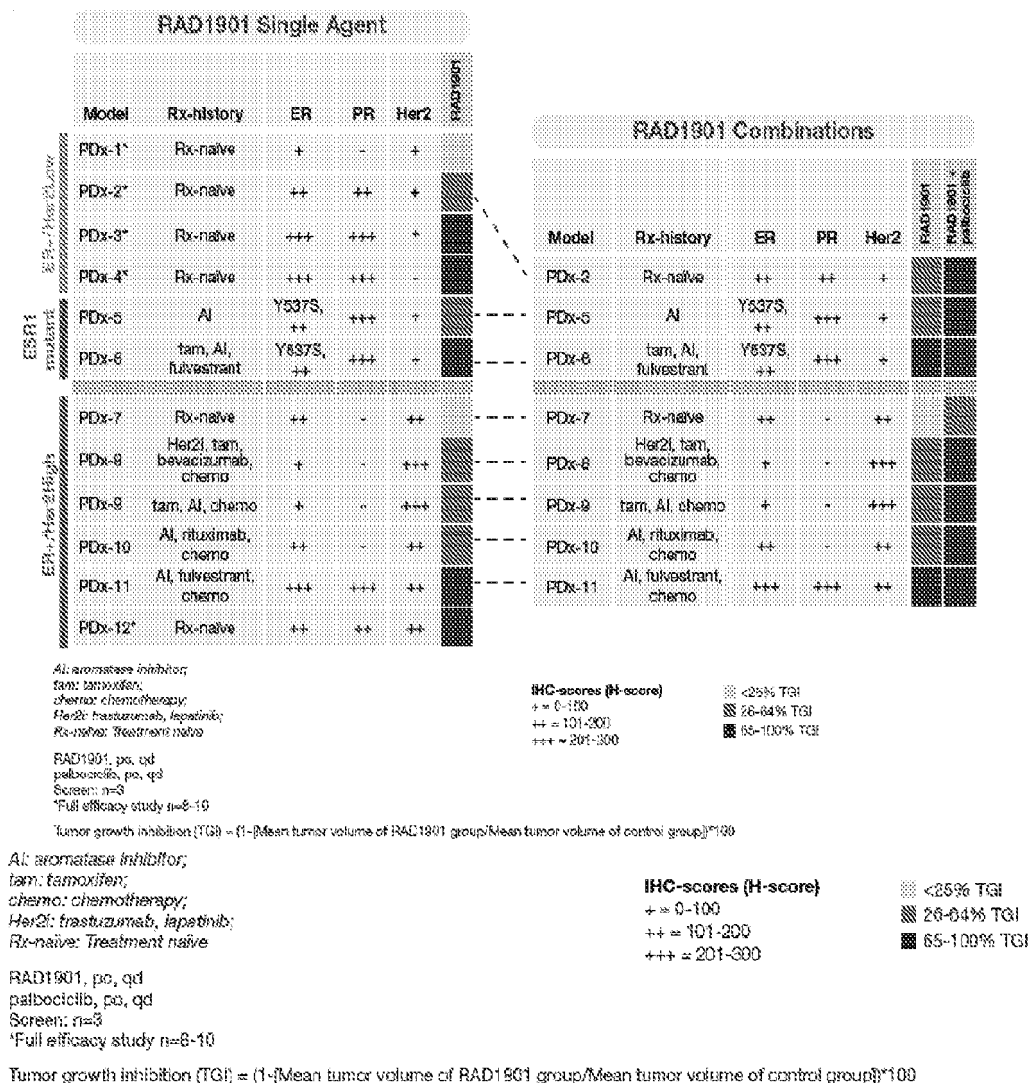


Fig. 2A

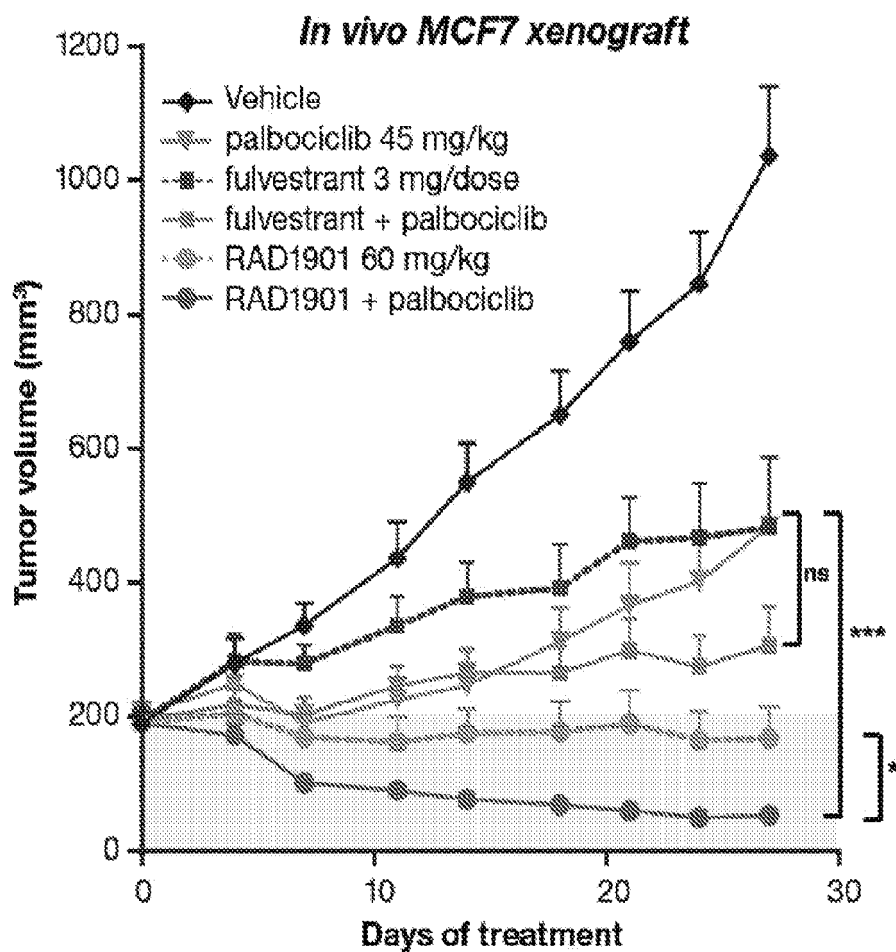


Fig. 2B

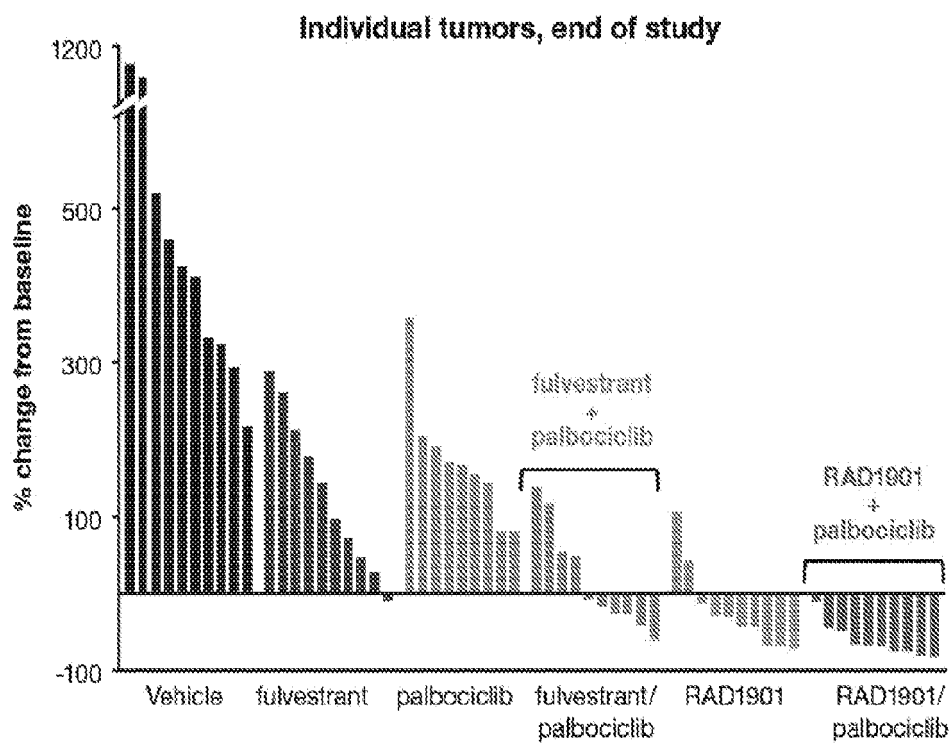


Fig. 2C

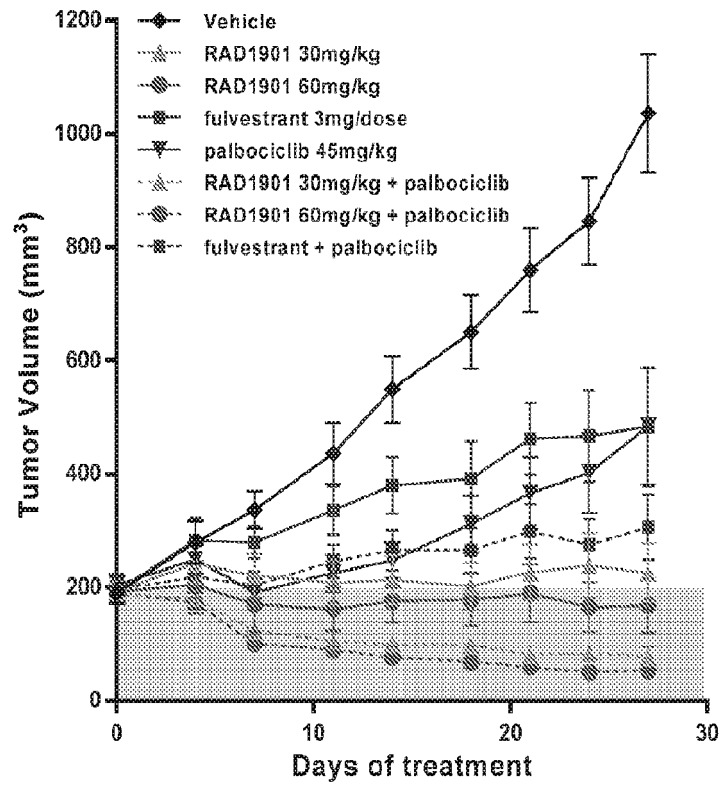


Fig. 3A

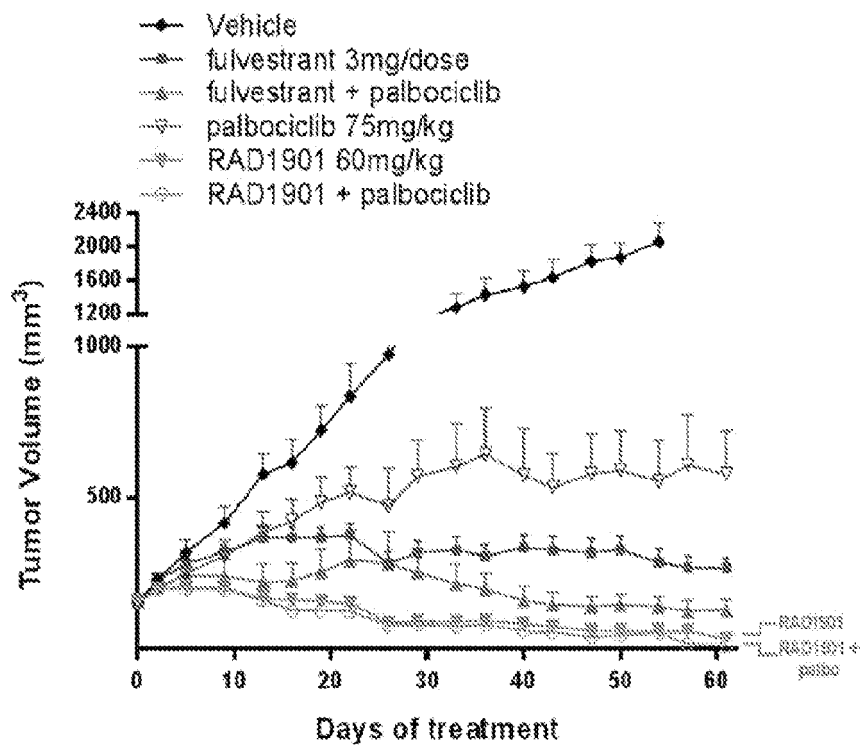


Fig. 3B

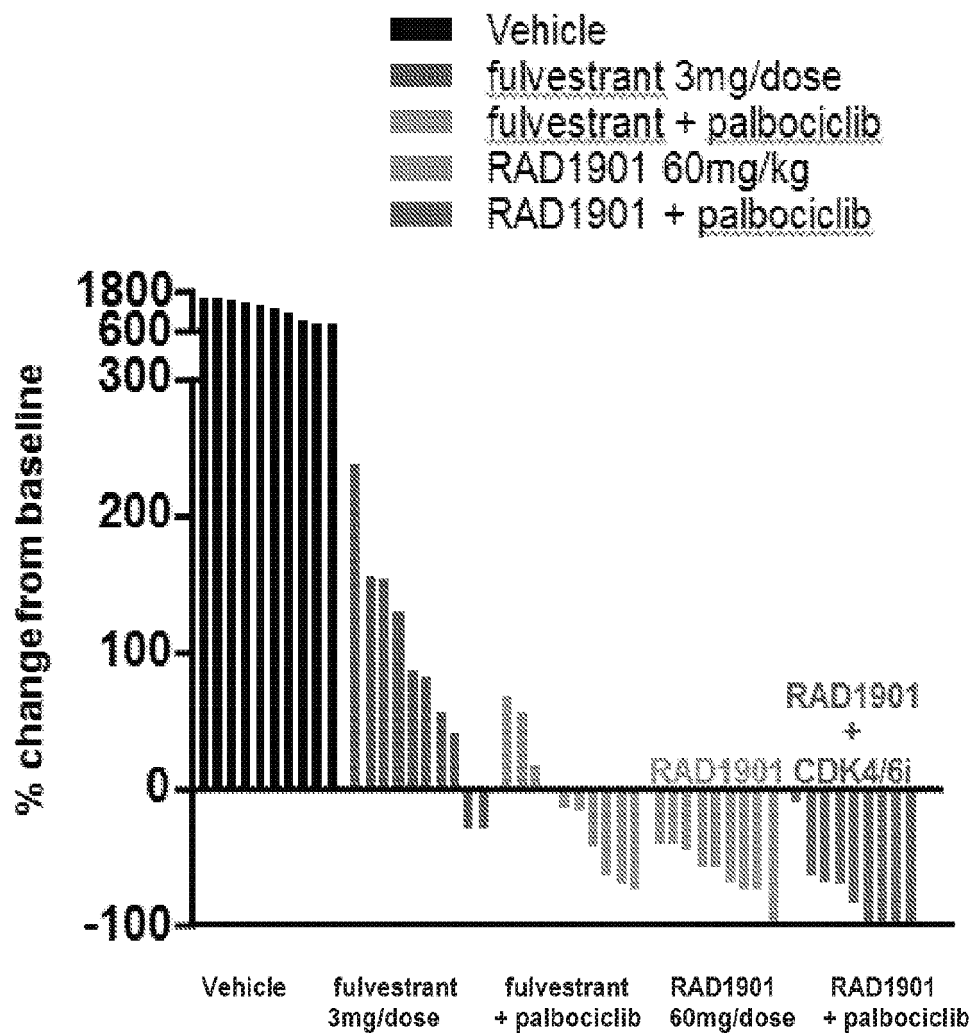


Fig. 4A

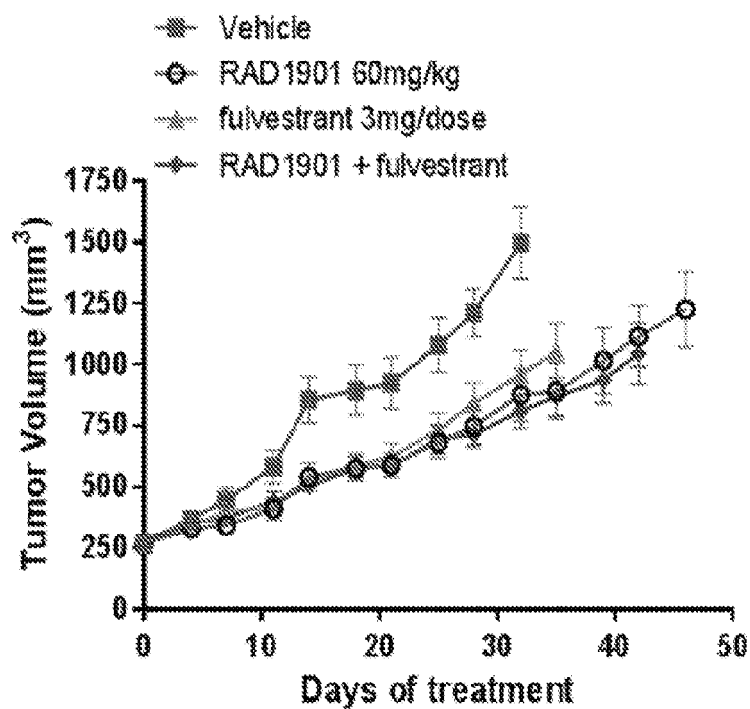


Fig. 4B

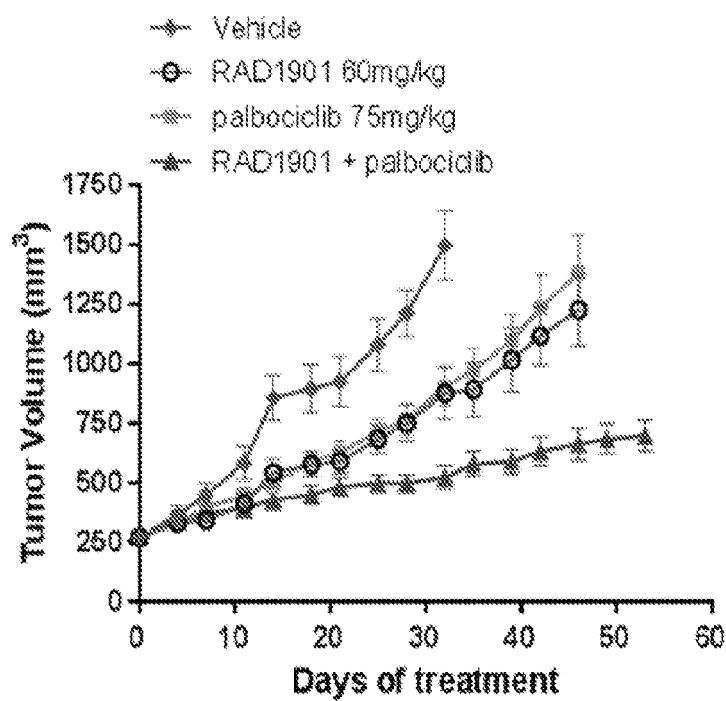


Fig. 5

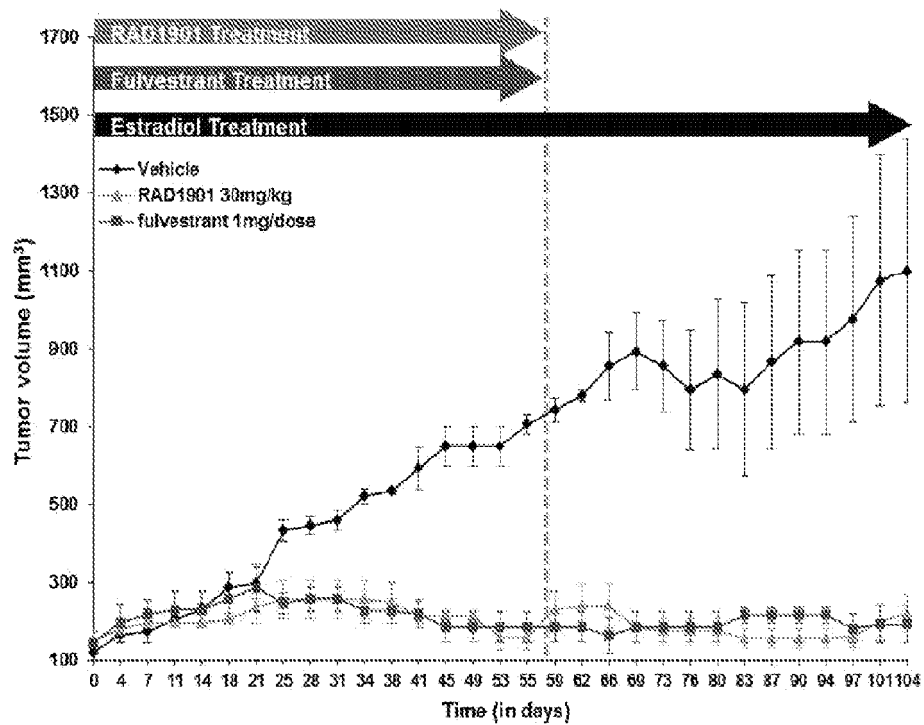


Fig. 6A

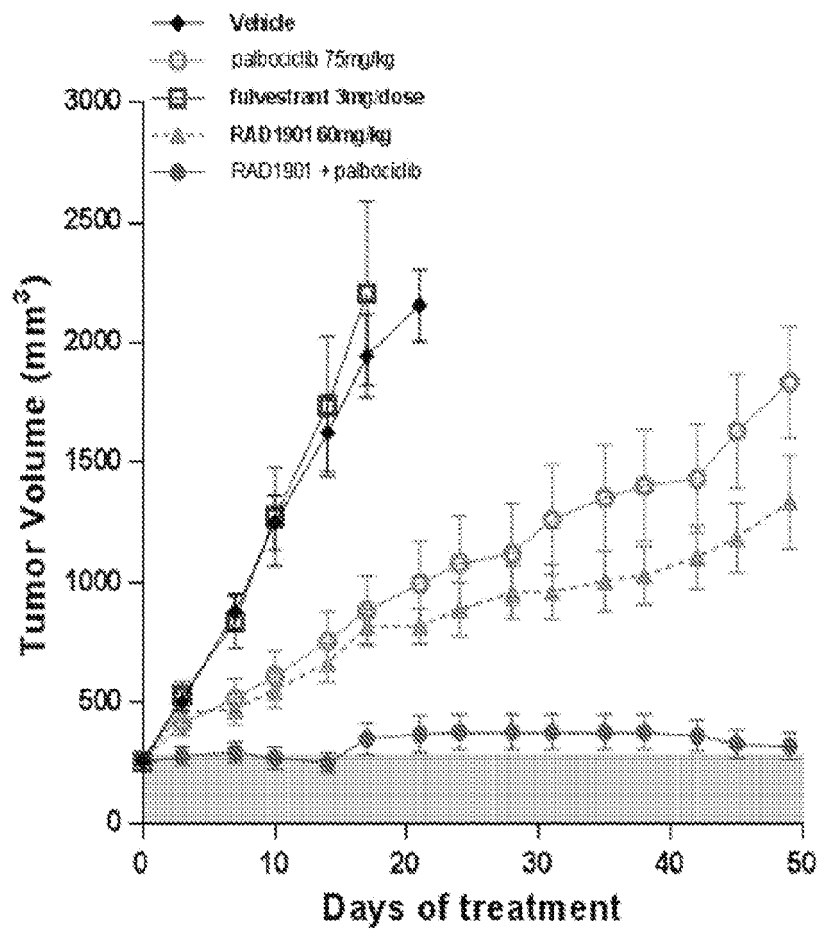


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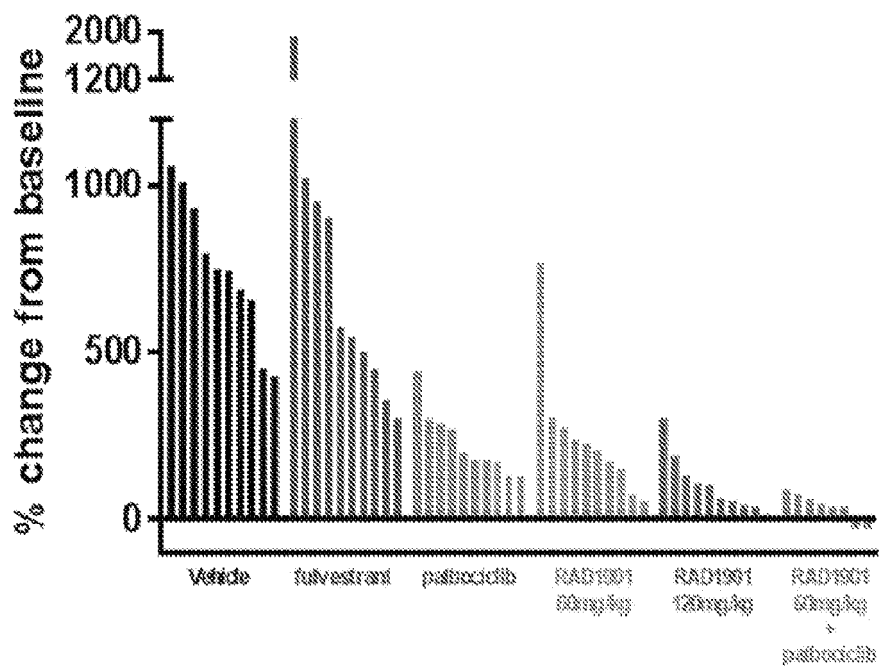


Fig. 6C

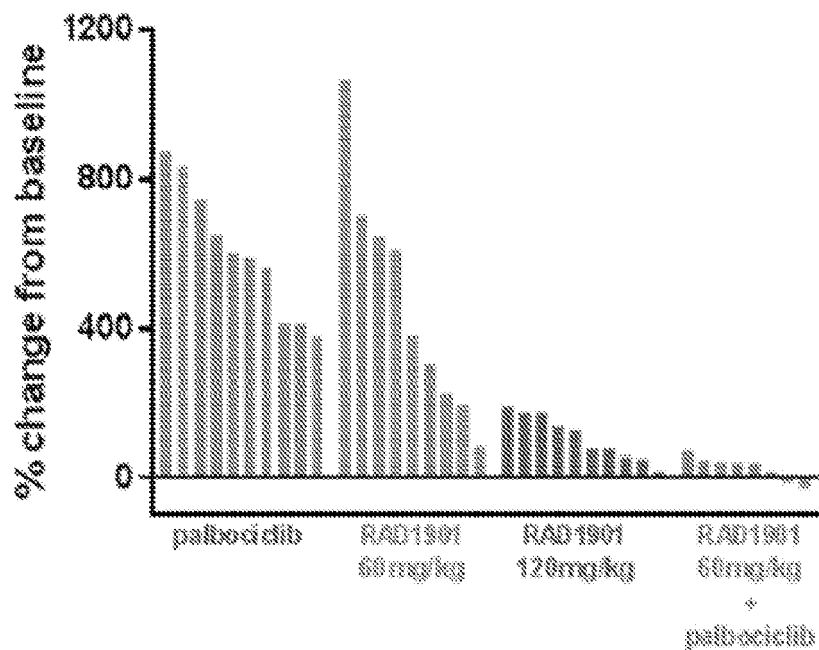


Fig. 7A

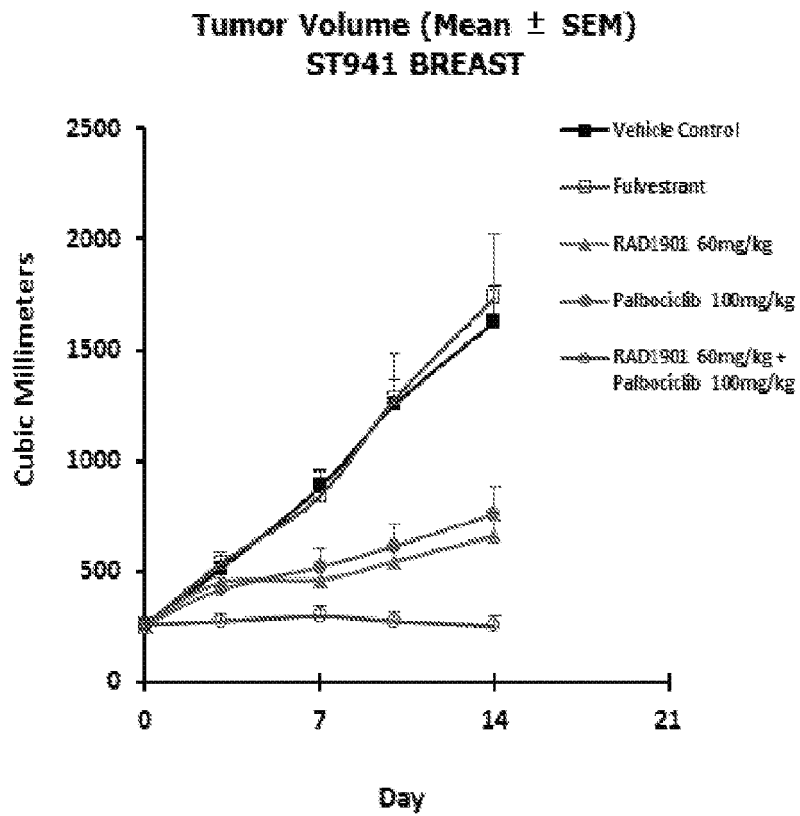


Fig. 7B

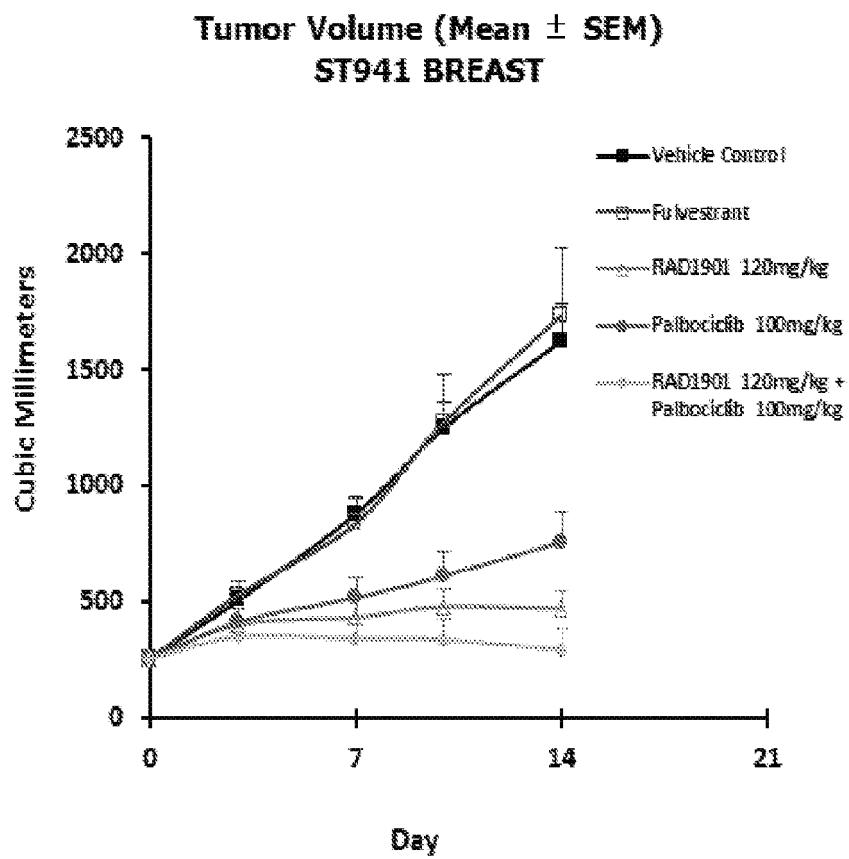


Fig. 8

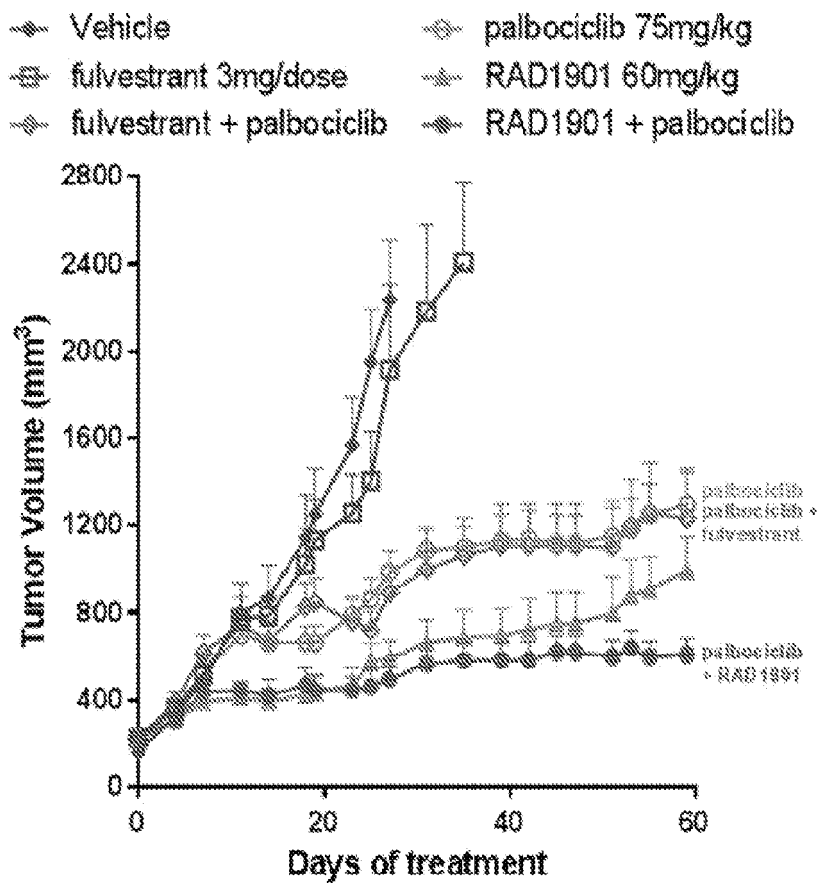


Fig. 9

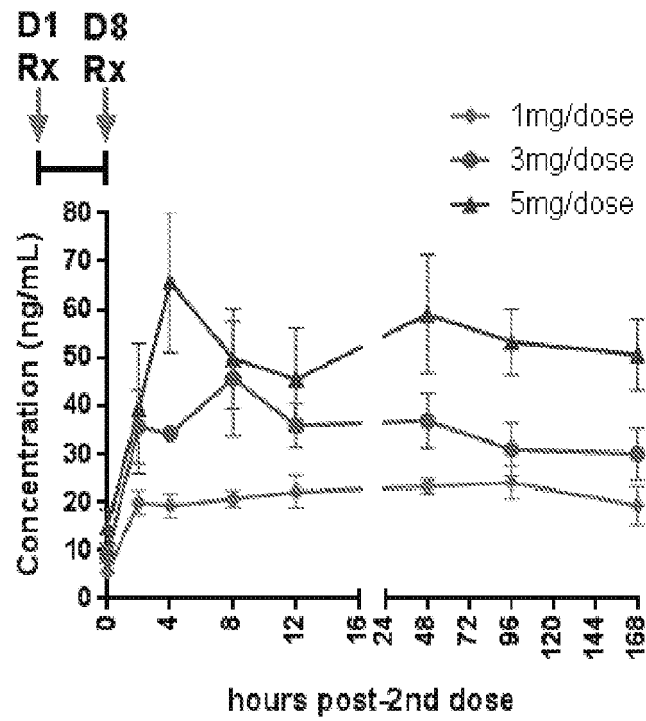


Fig. 10

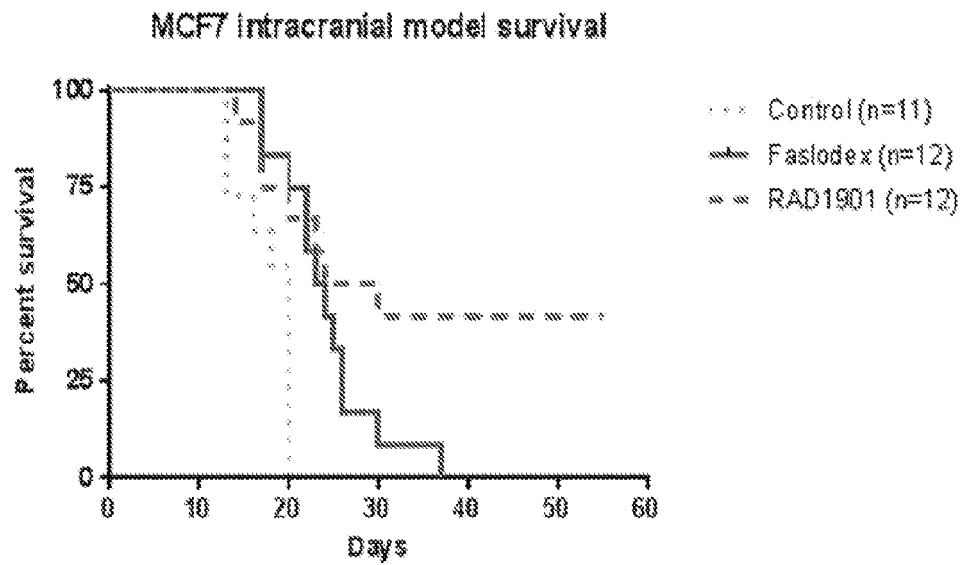


Fig. 11A

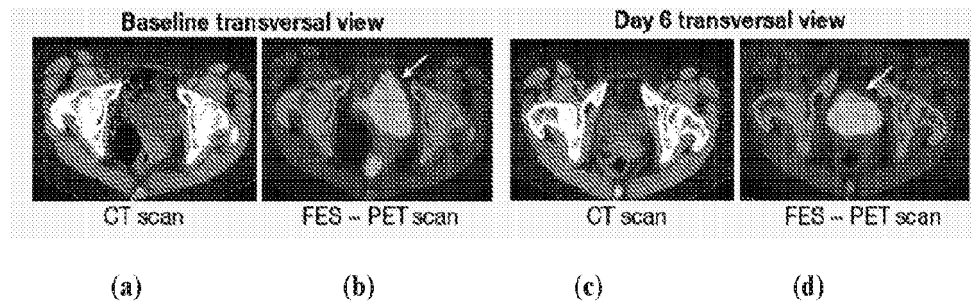


Fig. 11B

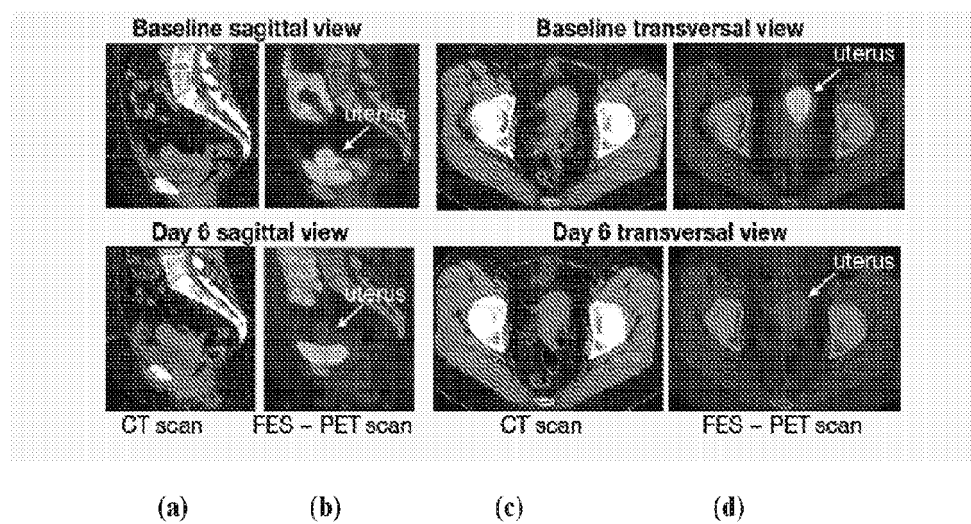


Fig. 11C

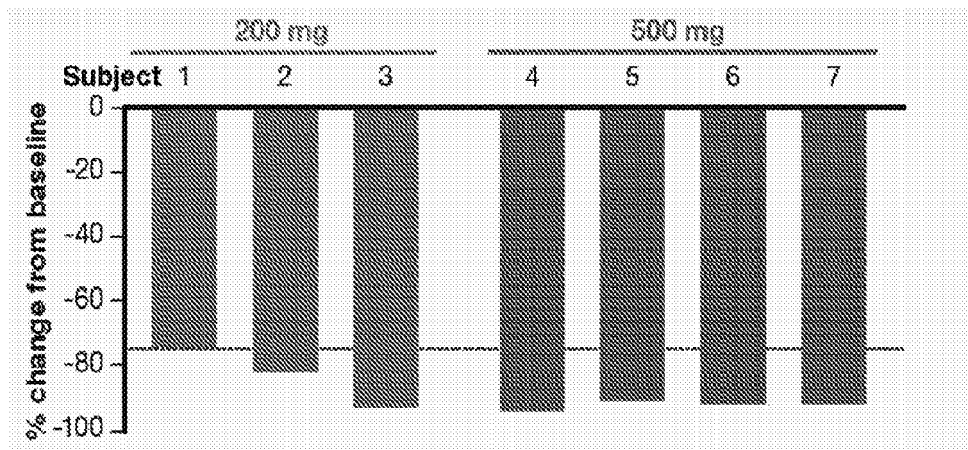


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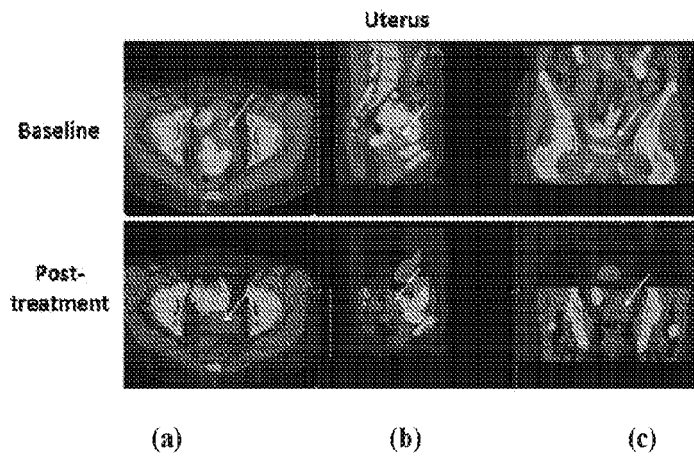


Fig. 12B



Fig. 13

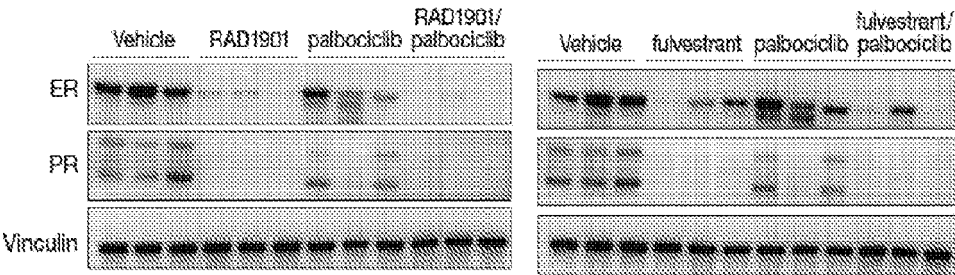


Fig. 14A

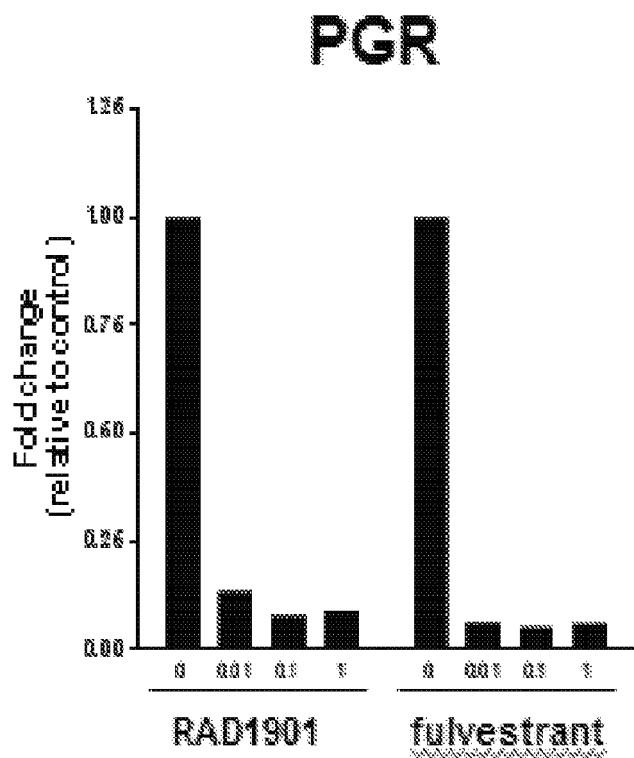
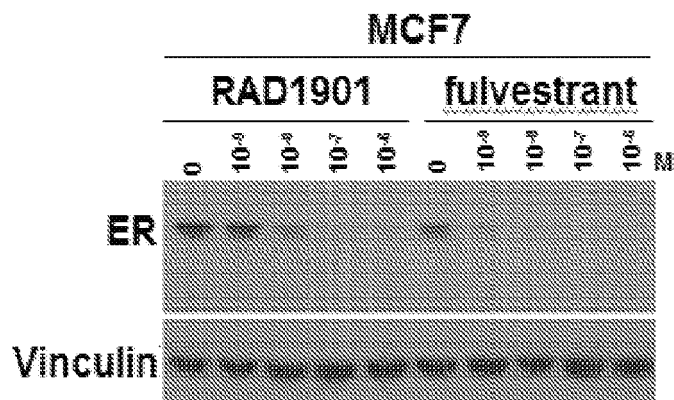


Fig. 14A (continued)

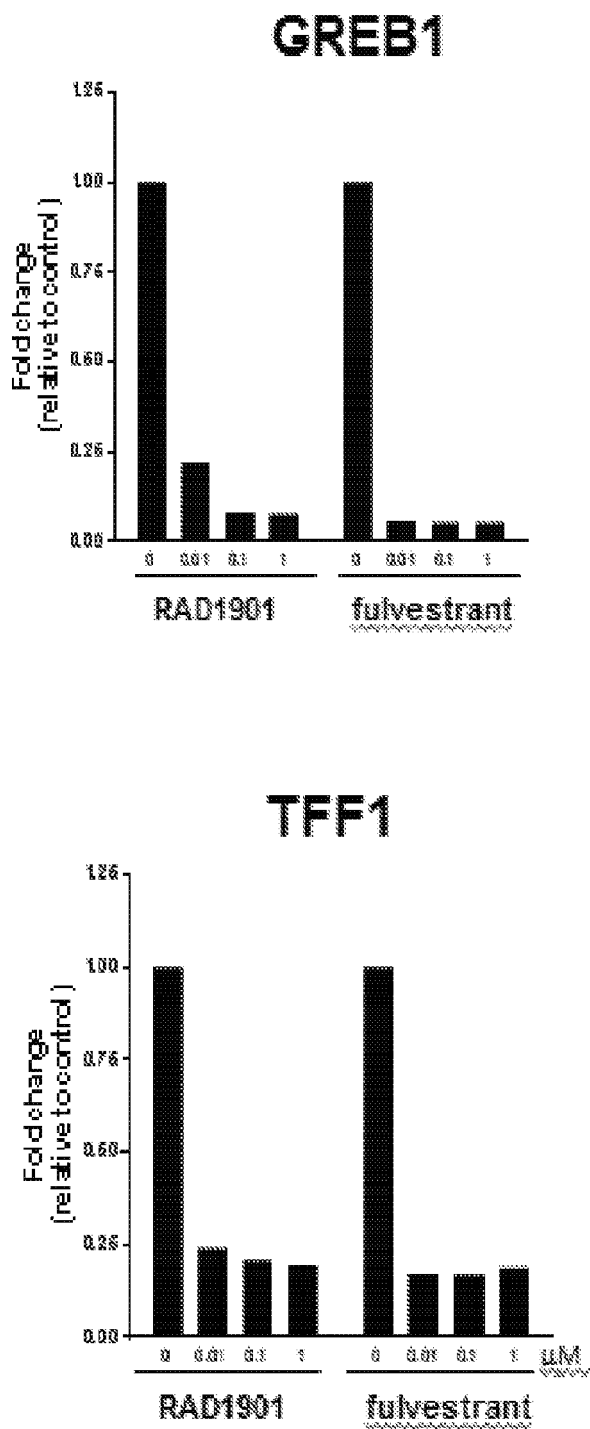


Fig. 14B

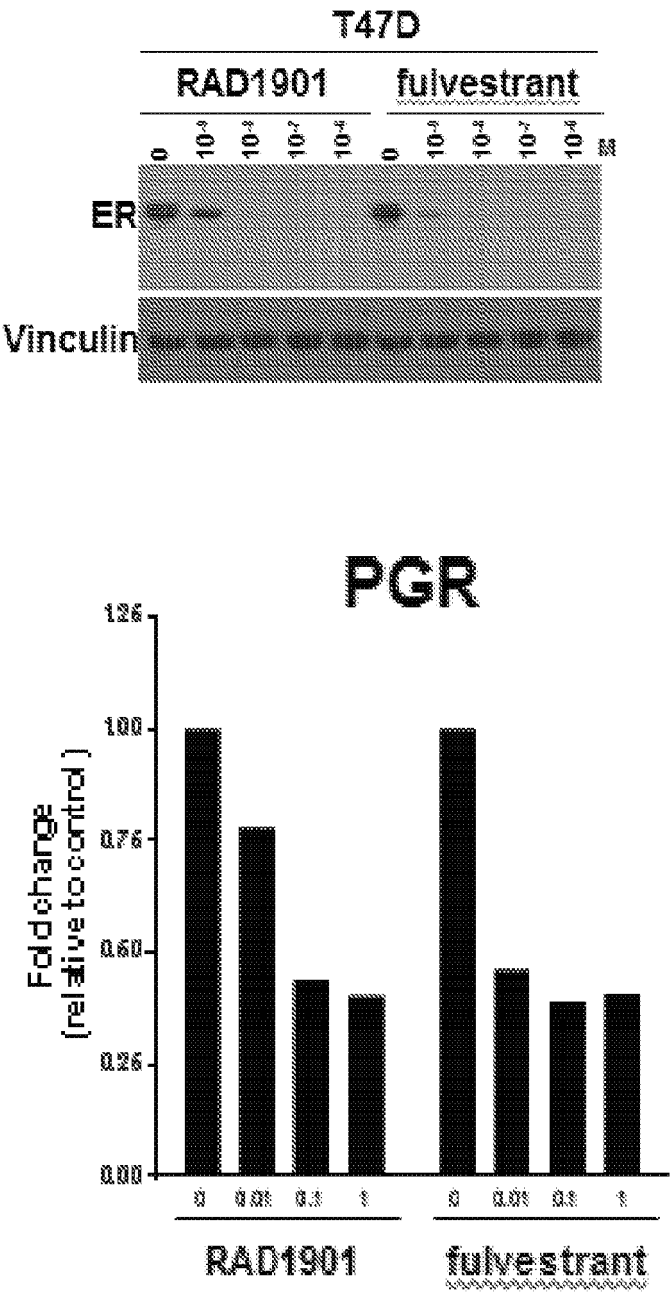


Fig. 14B (continued)

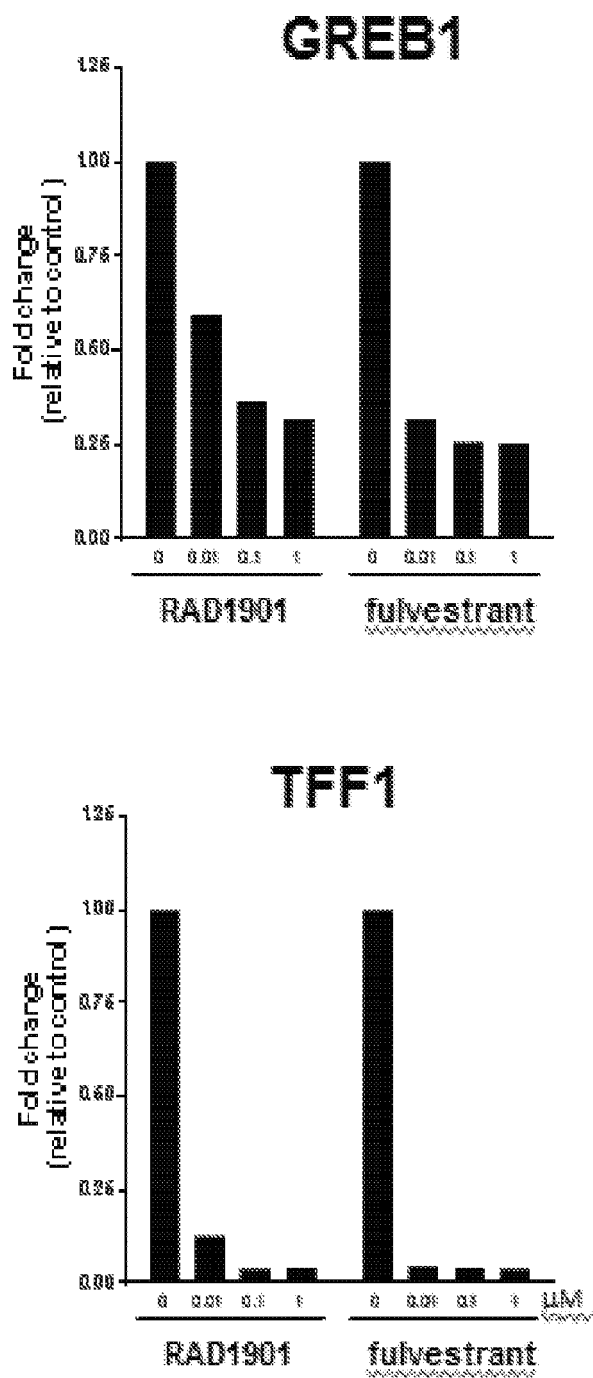


Fig. 15A

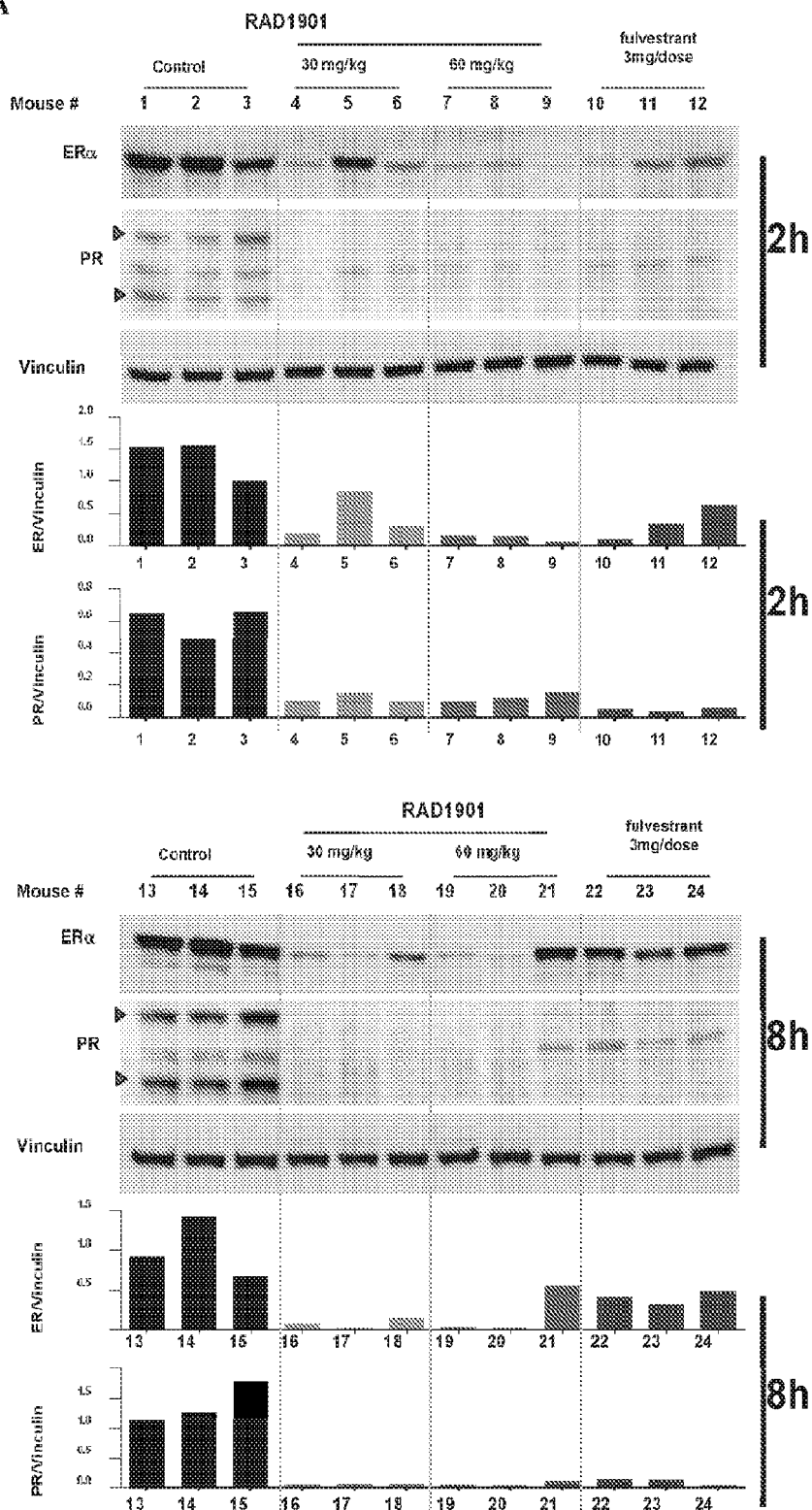


Fig. 15B

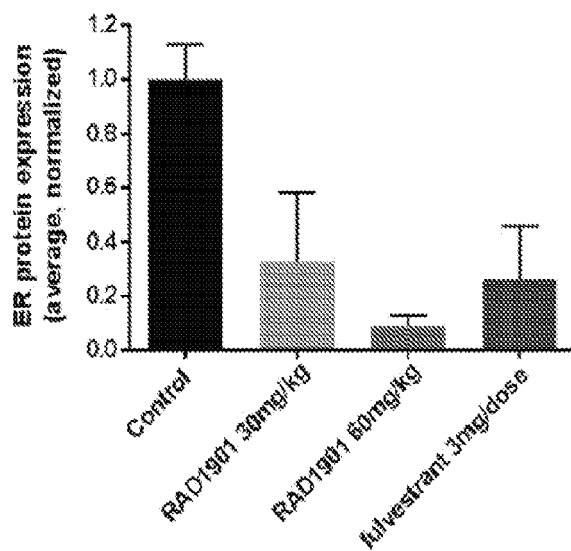


Fig. 15C

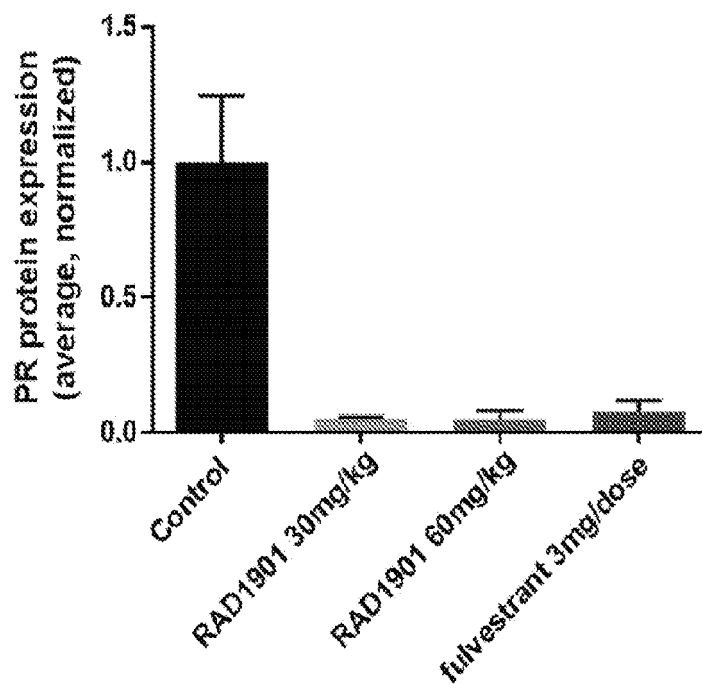


Fig. 16A

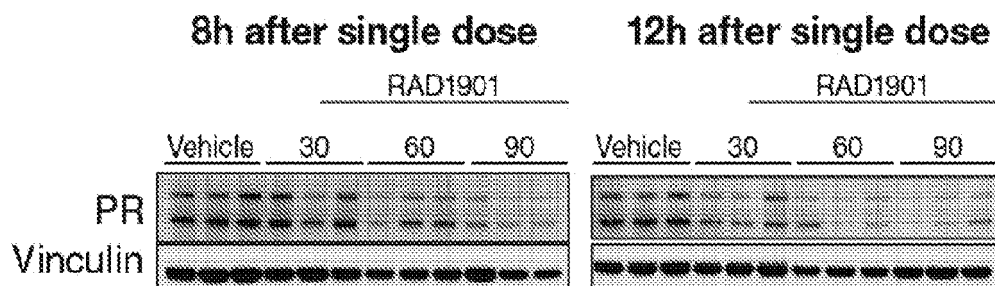


Fig. 16B

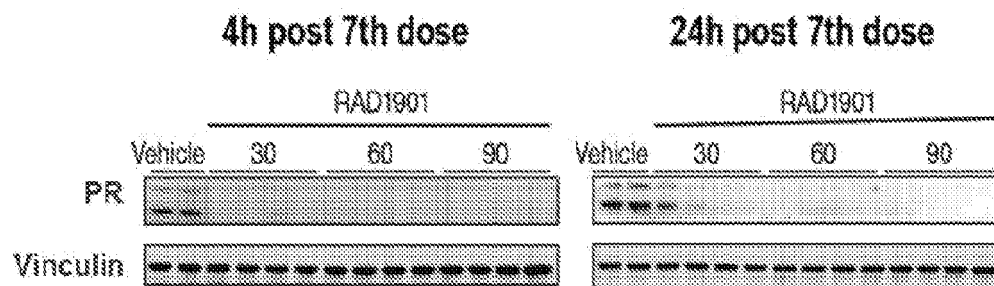


Fig. 16C

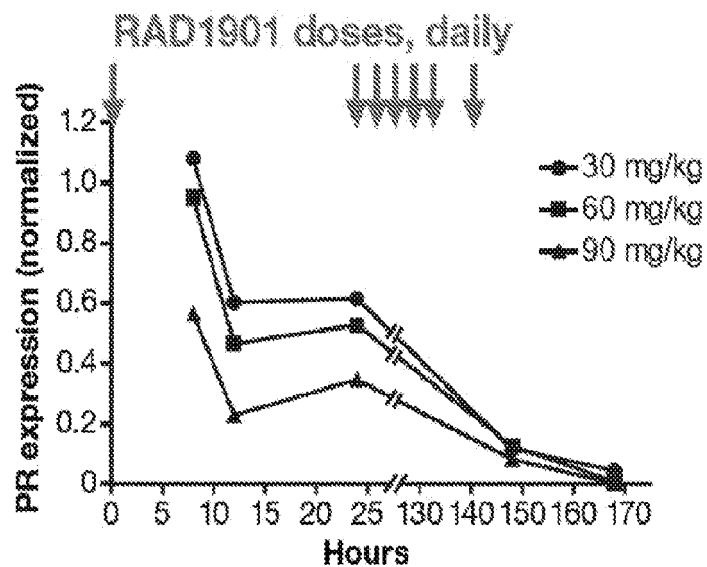


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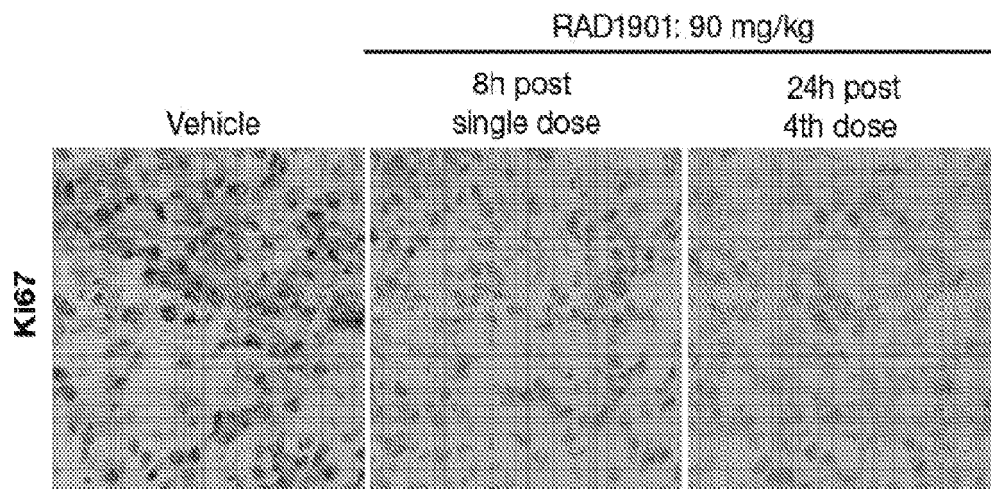


Fig. 17B

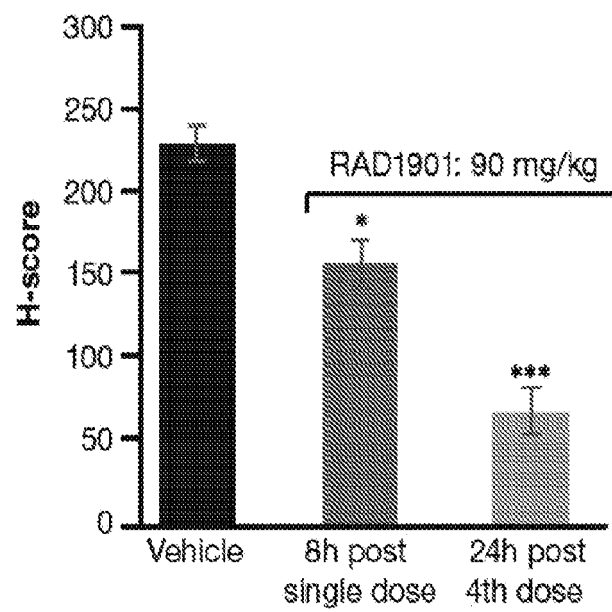


Fig. 18

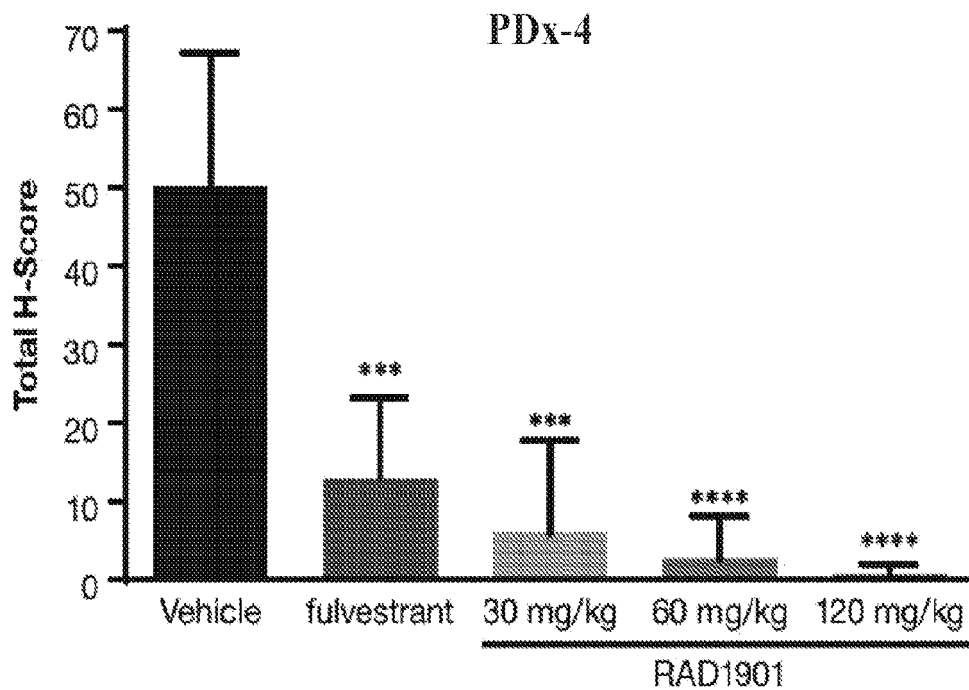


Fig. 19

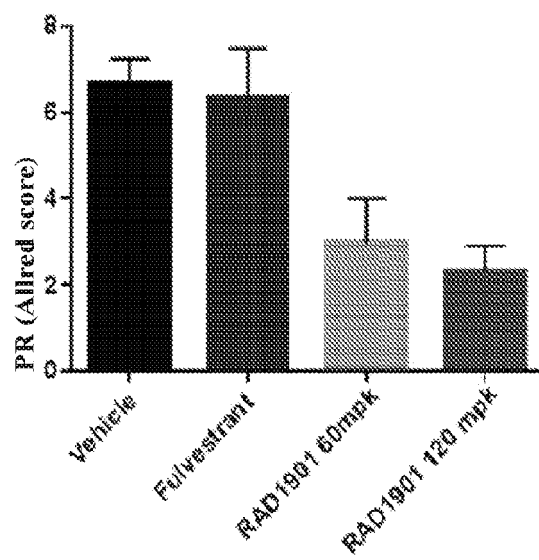


Fig. 20A

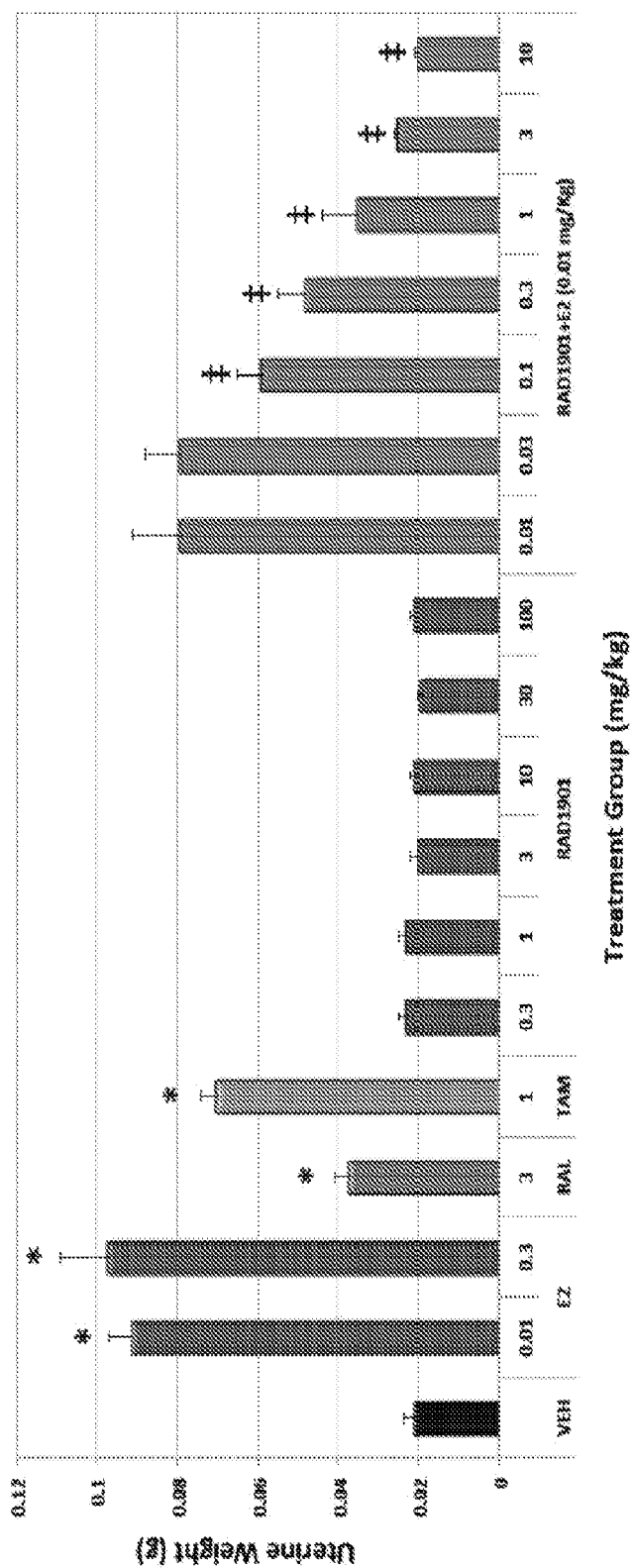


Fig. 20B

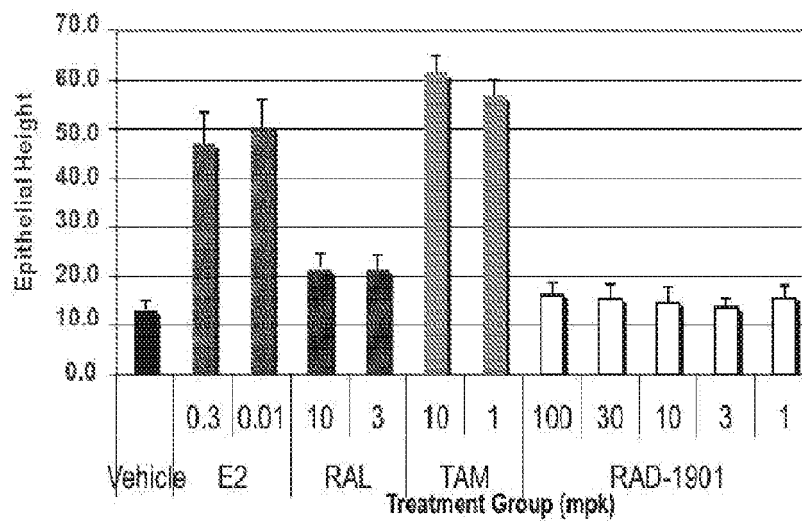


Fig. 20C

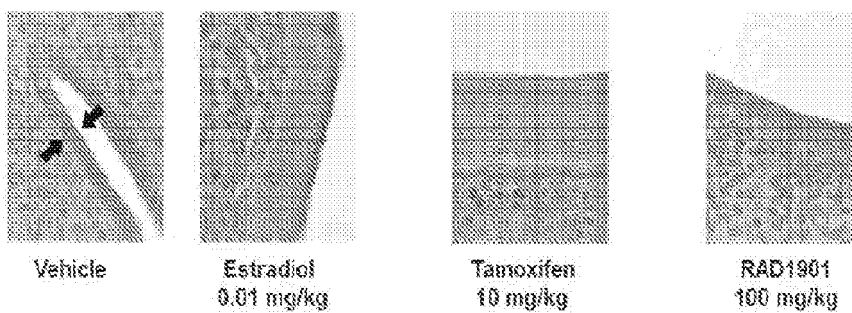


Fig. 20D

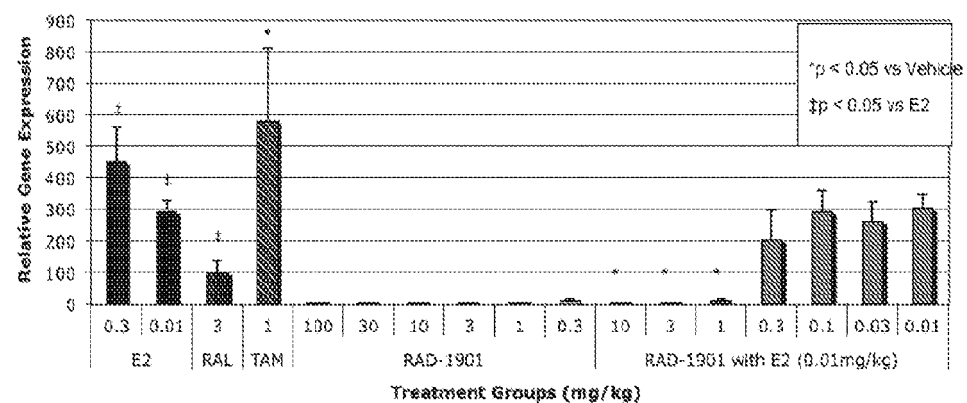


Fig. 21

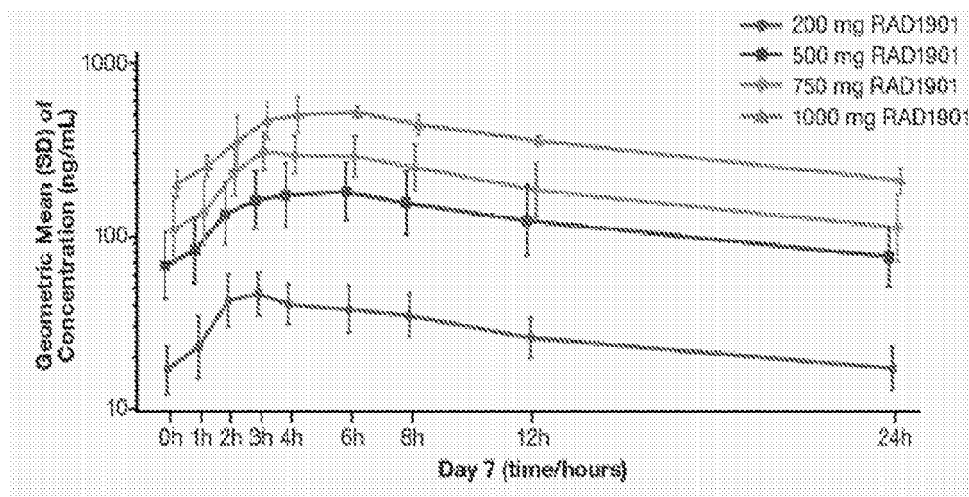


Fig. 22

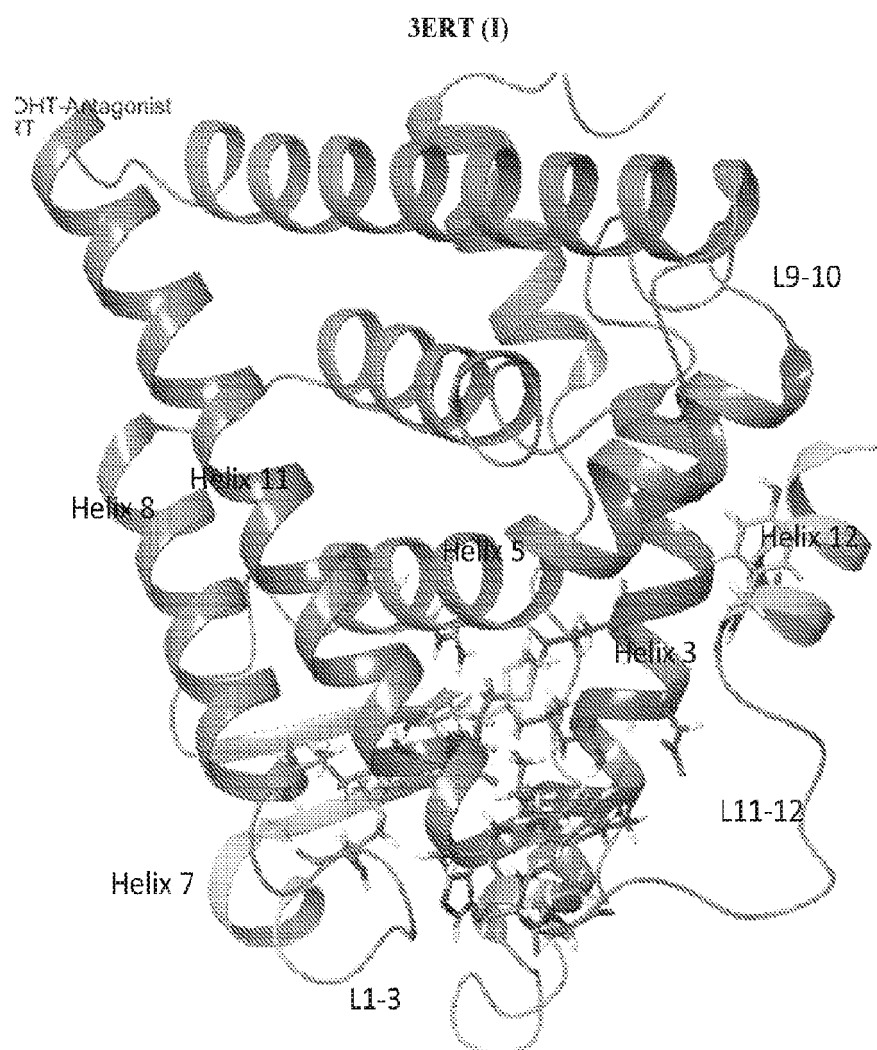


Fig. 23

3ERT (II)

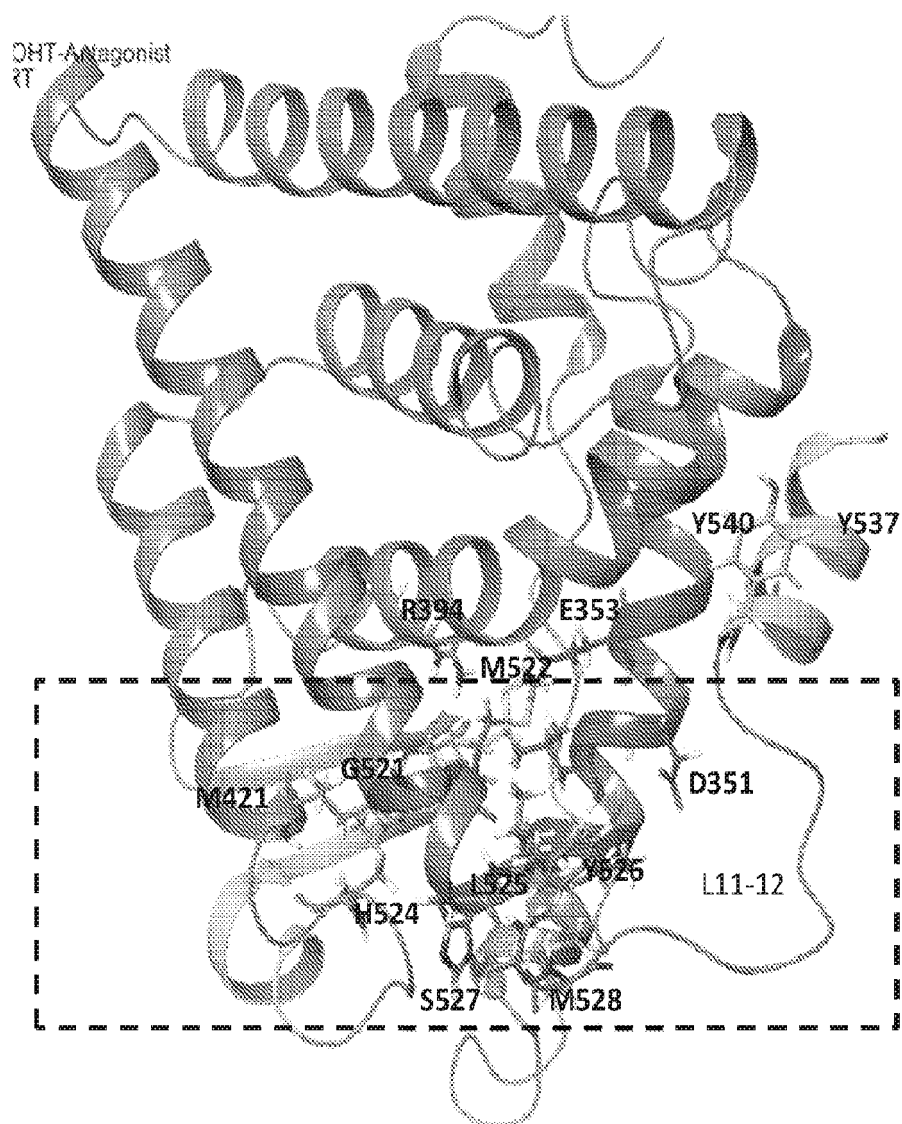


Fig. 24

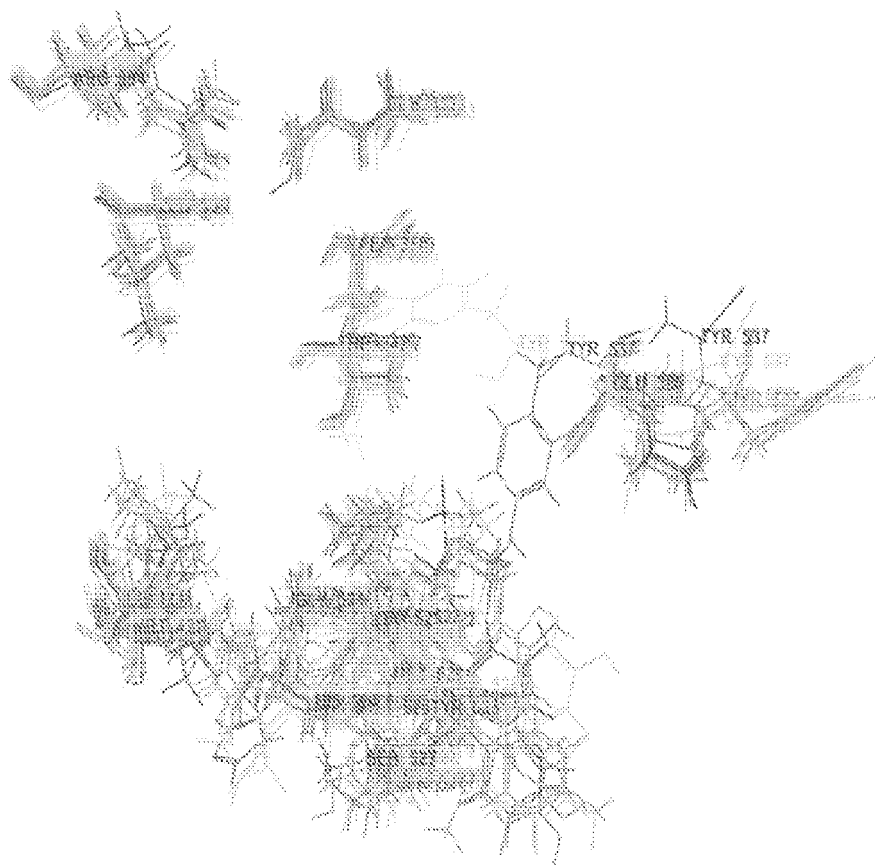


Fig. 25A

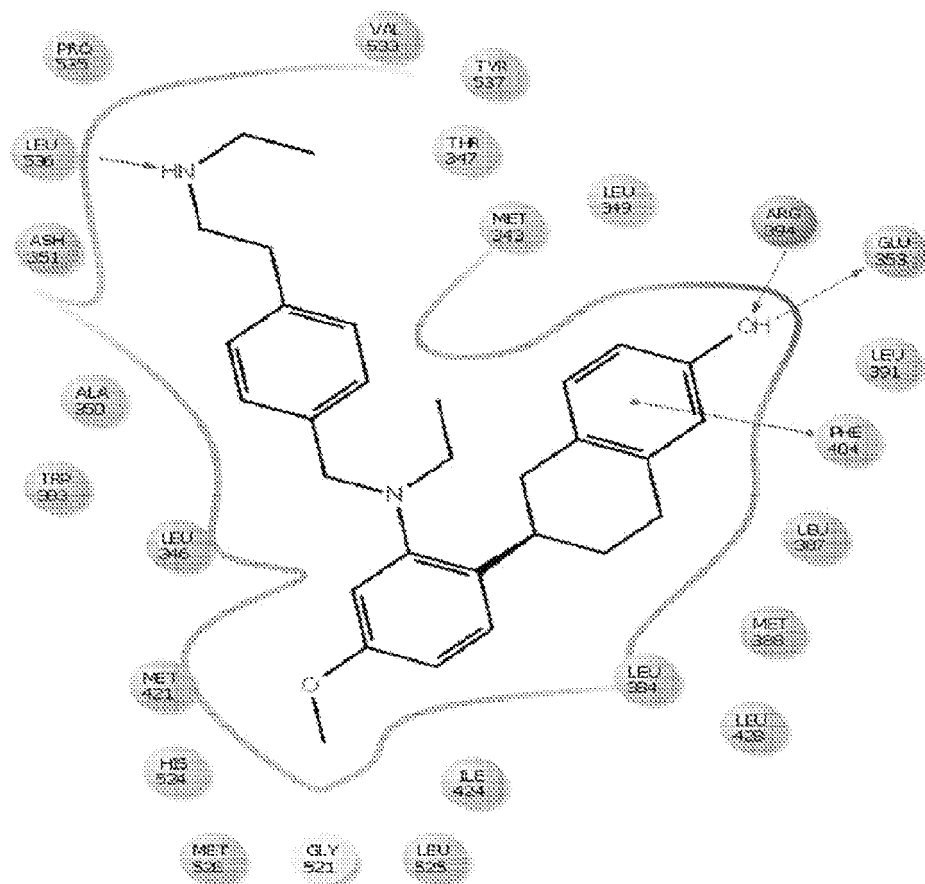


Fig. 25B

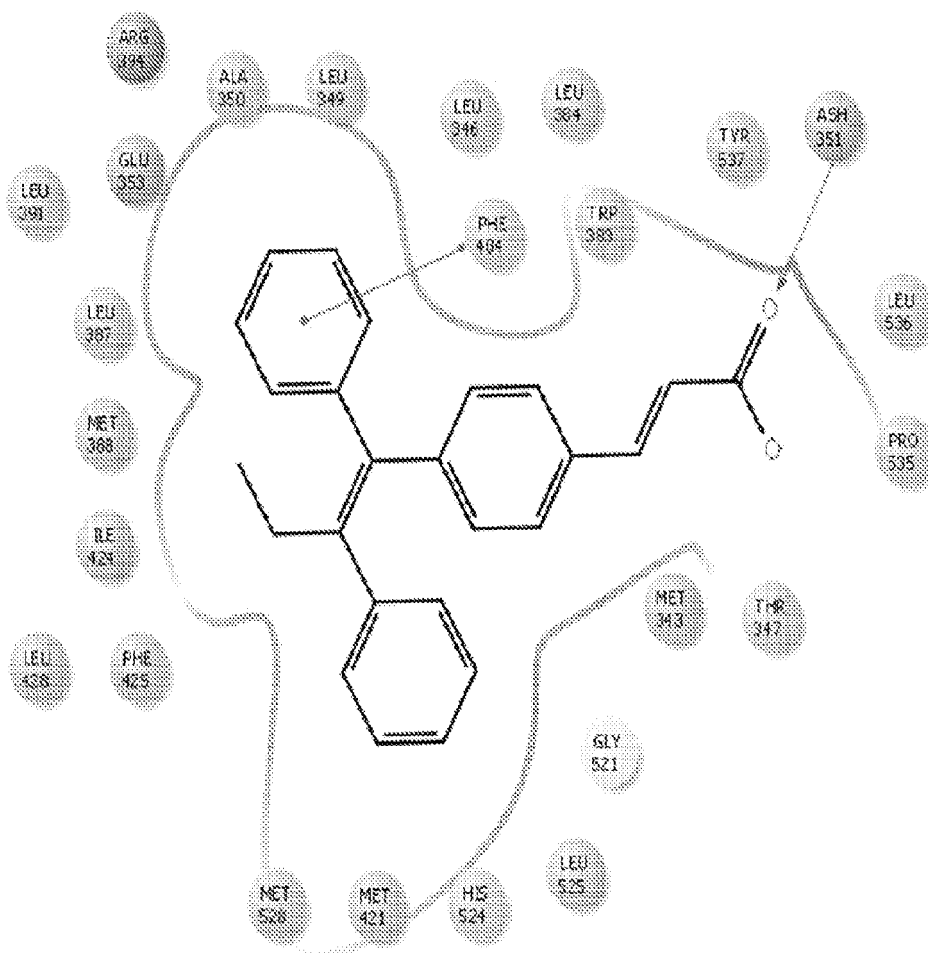


Fig. 26A

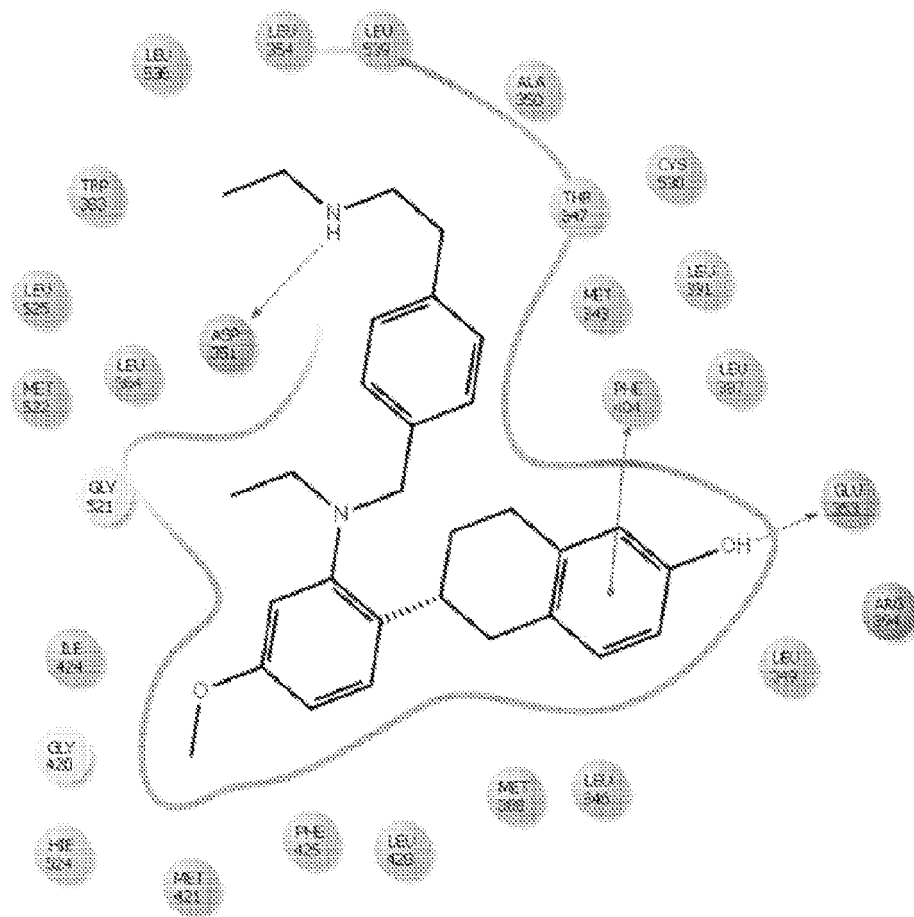


Fig. 26B

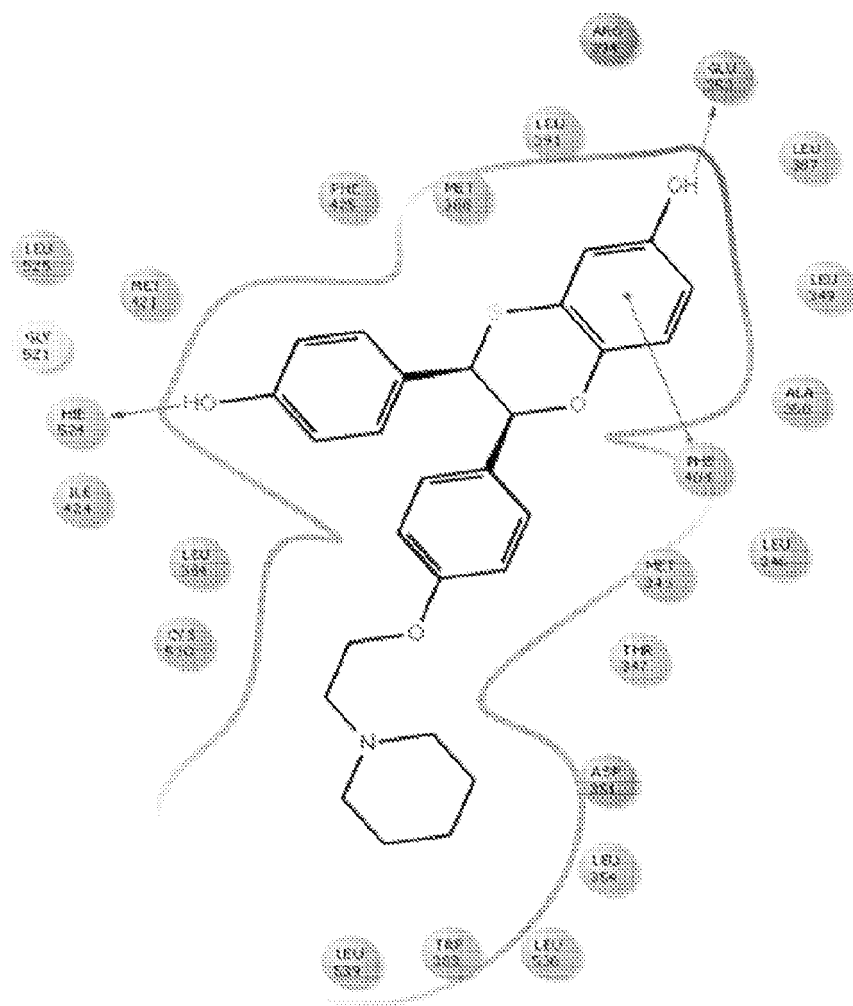


Fig. 27A

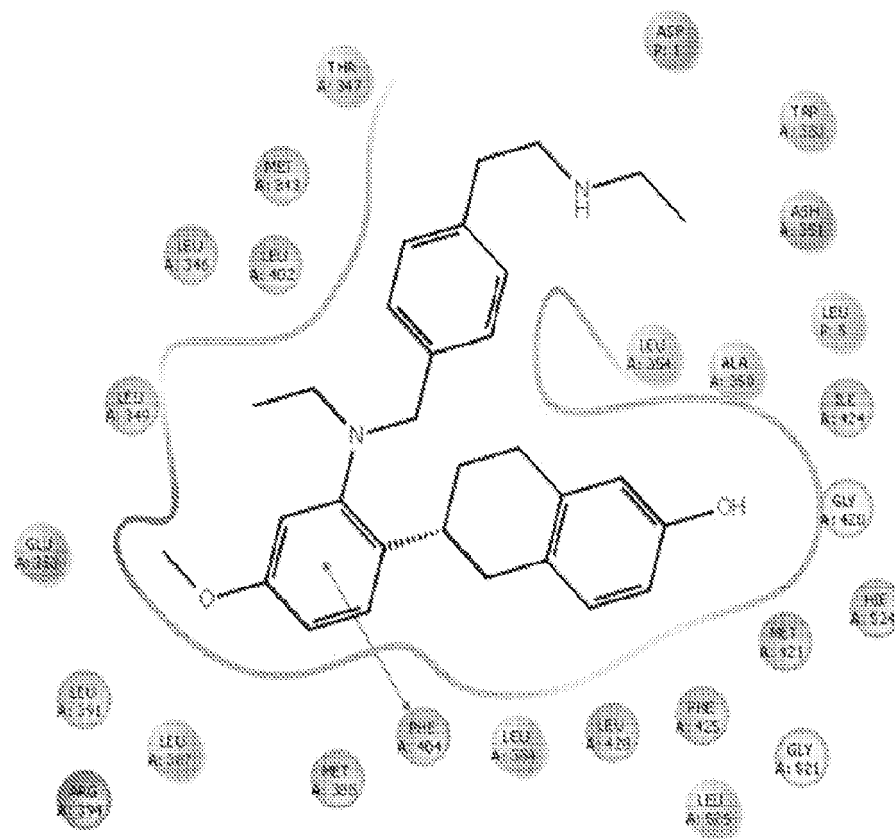


Fig. 27B

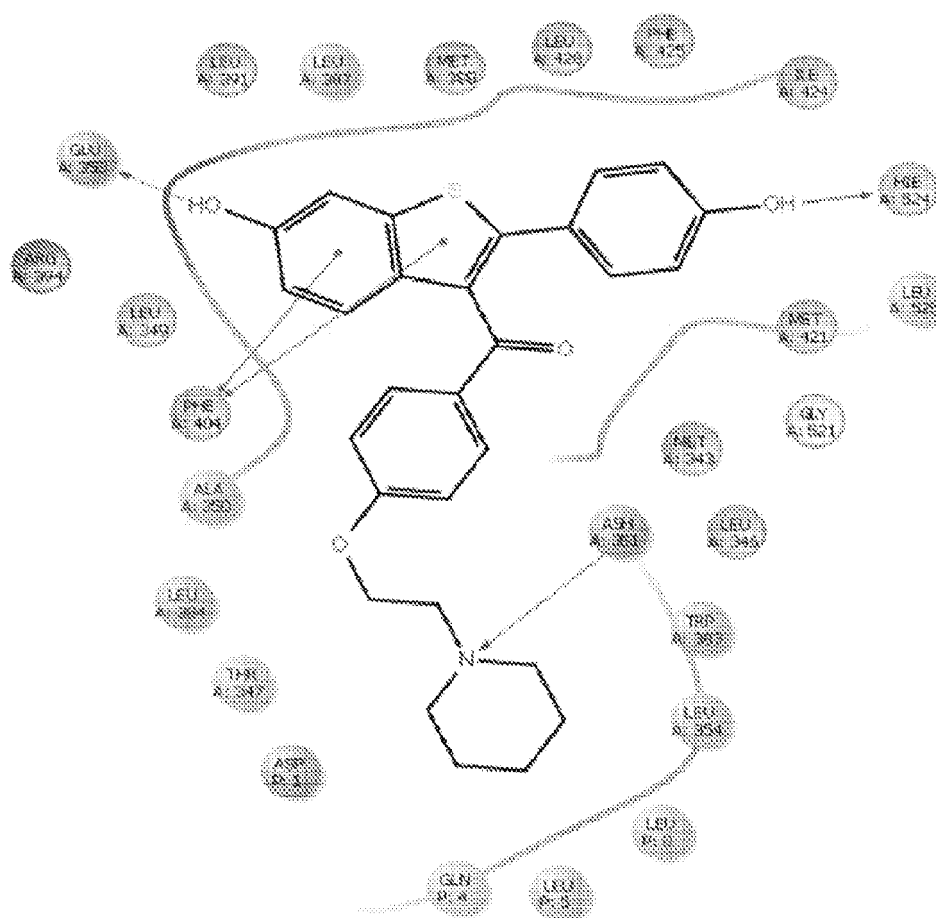


Fig. 28A

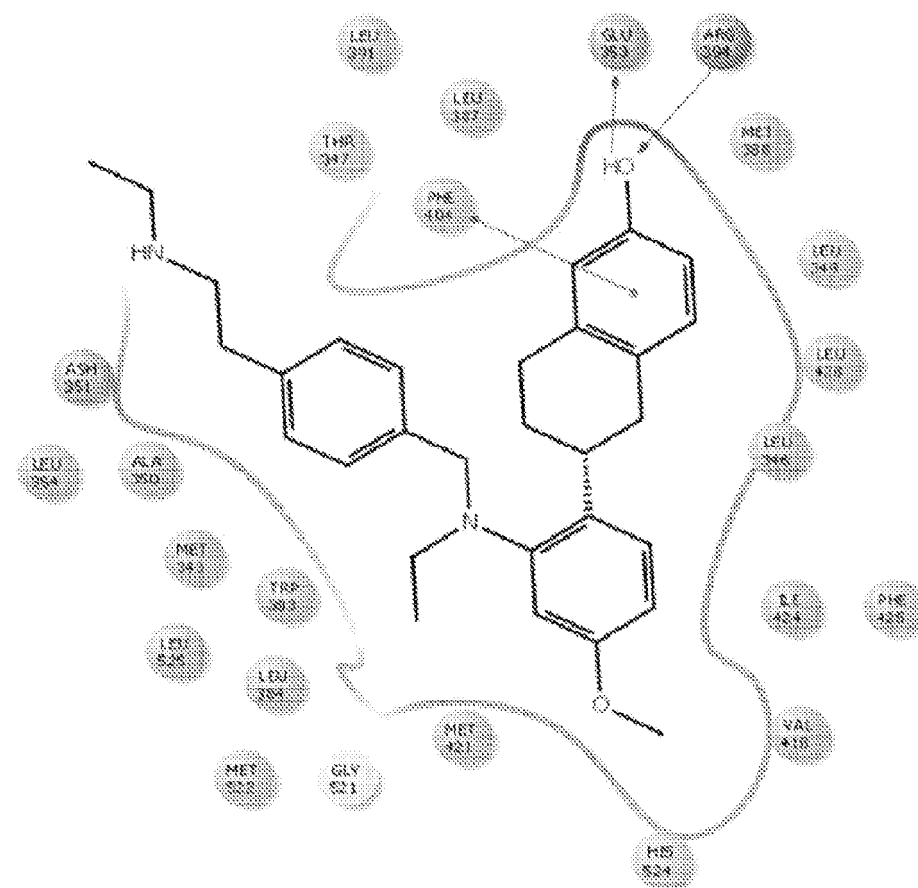


Fig. 28B

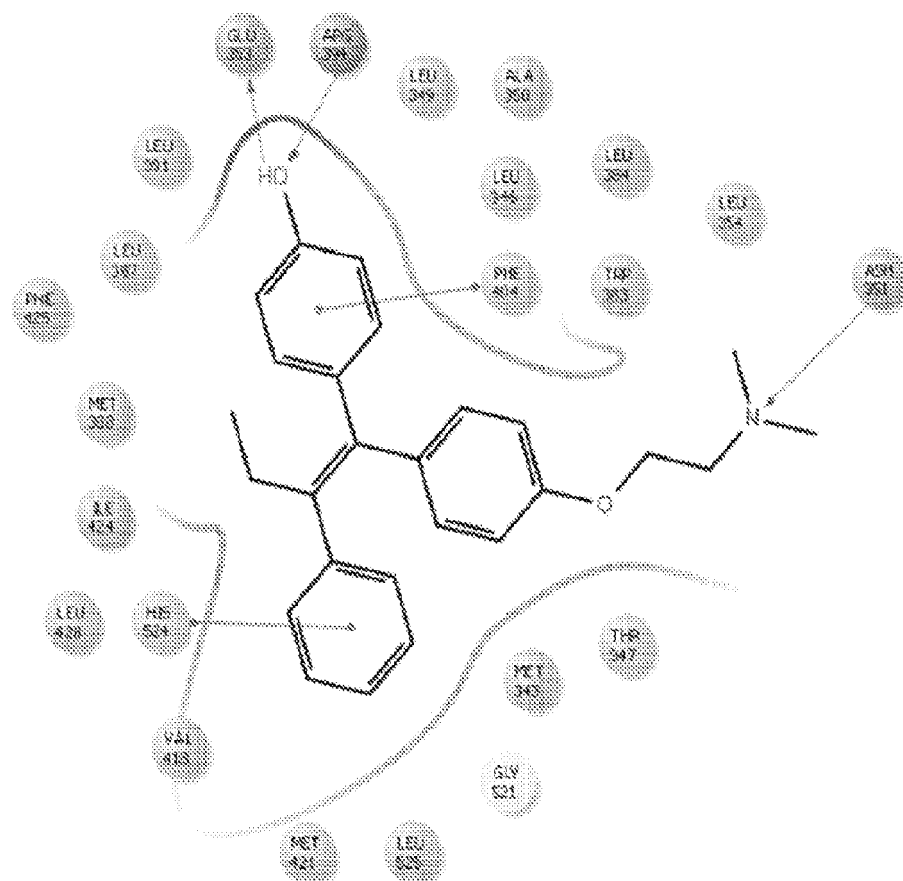


Fig. 29A

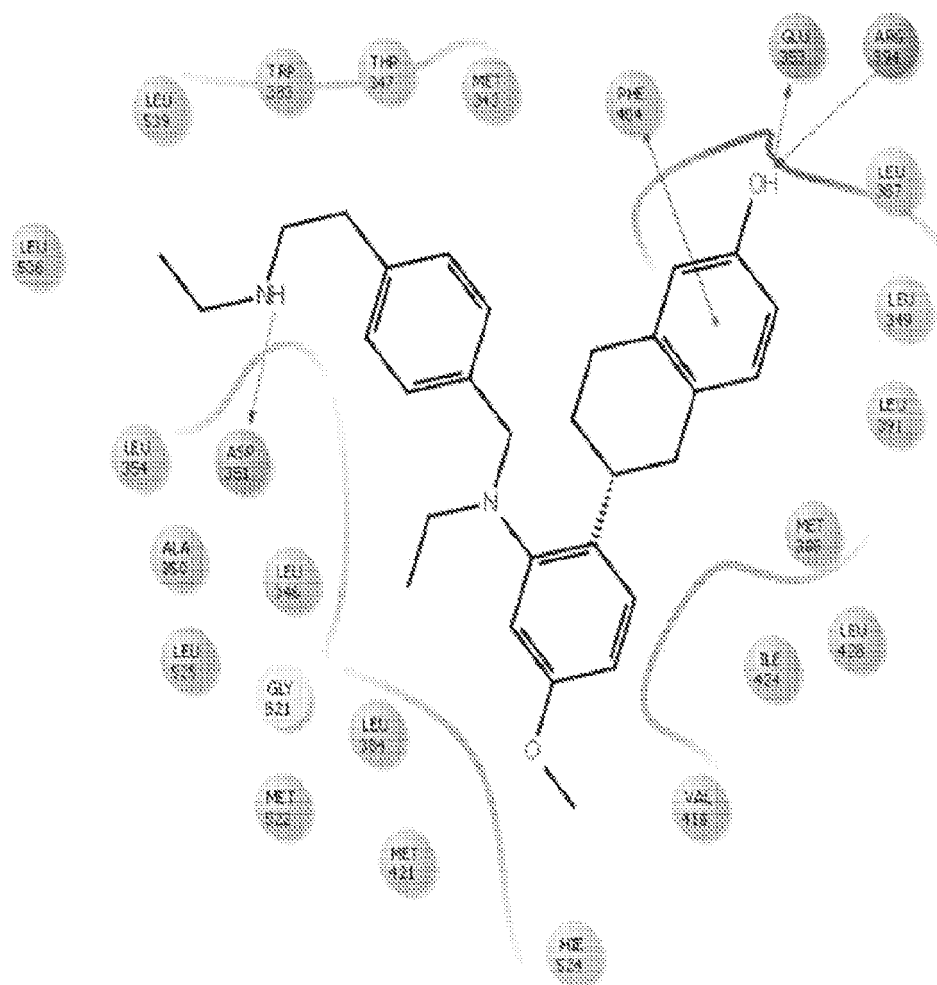


Fig. 29B

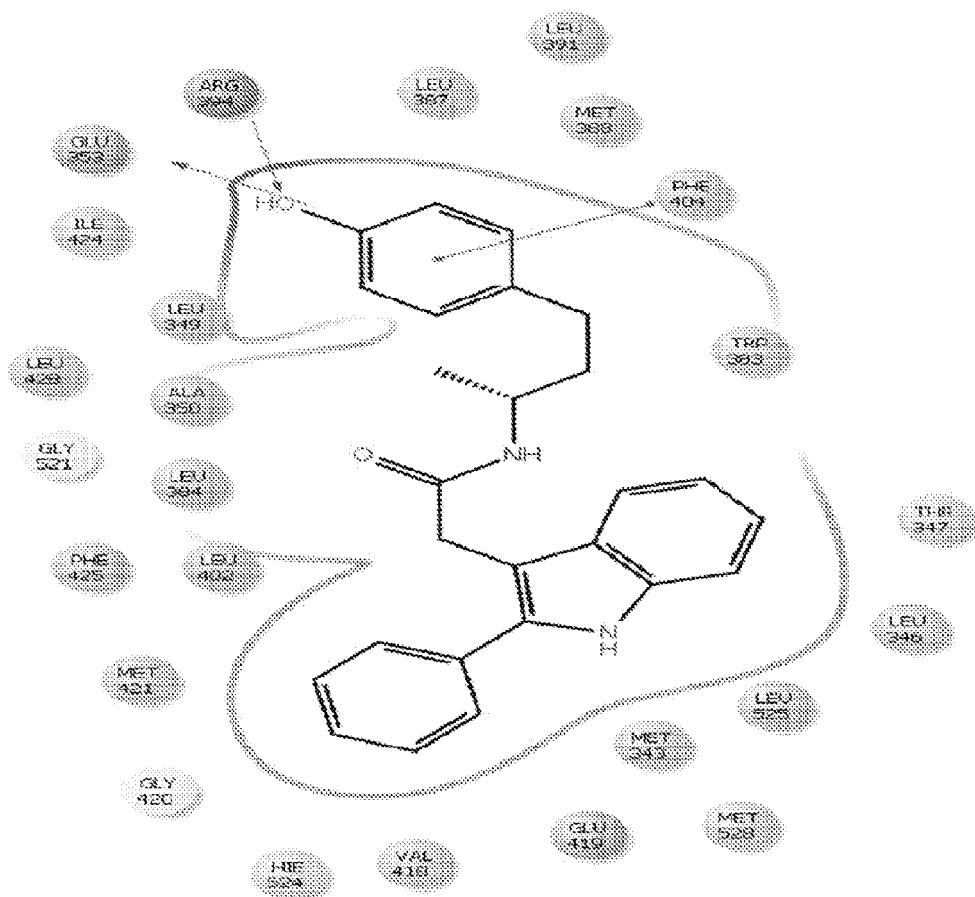


Fig. 30

N-benzyl-*N*-ethylaniline is on the front

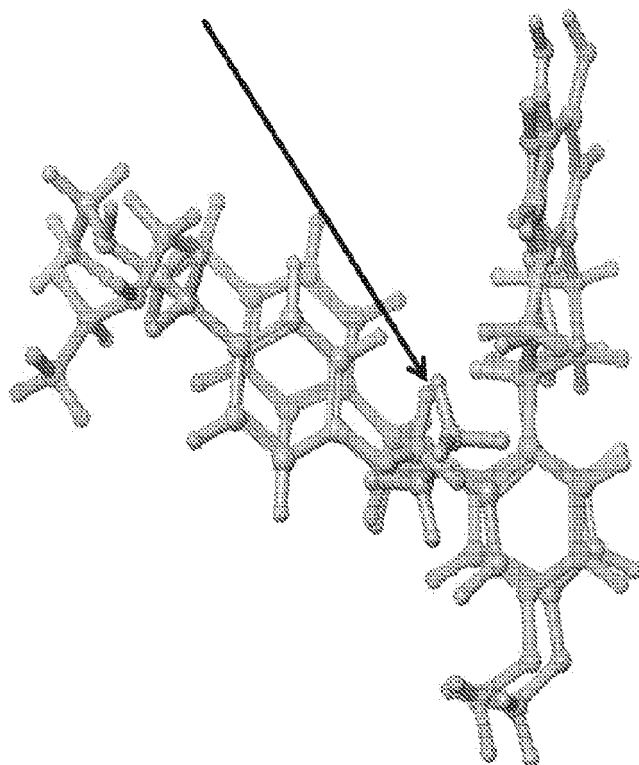


Fig. 31

ethylamino-ethyl

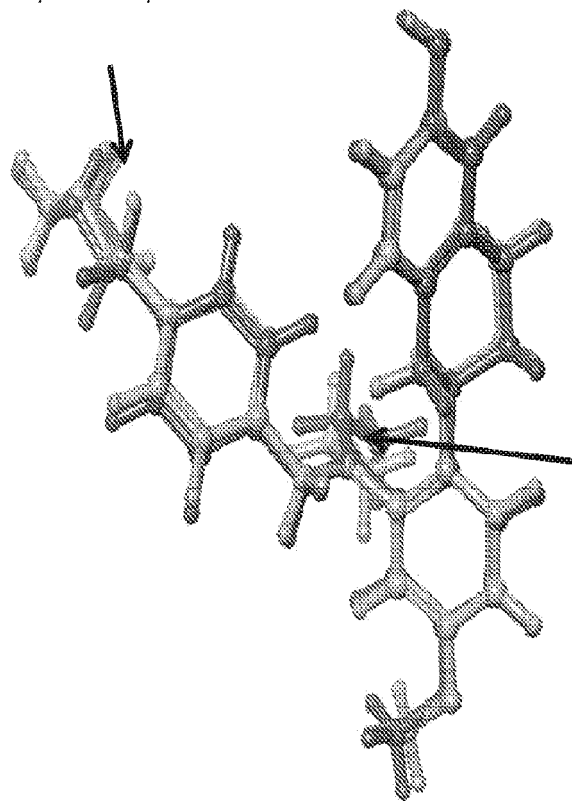
*N*-benzyl-*N*-ethylaniline is on the back

Fig. 32A

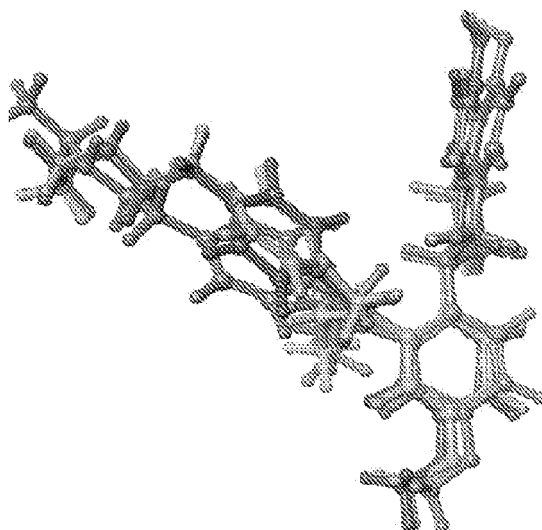


Fig. 32B

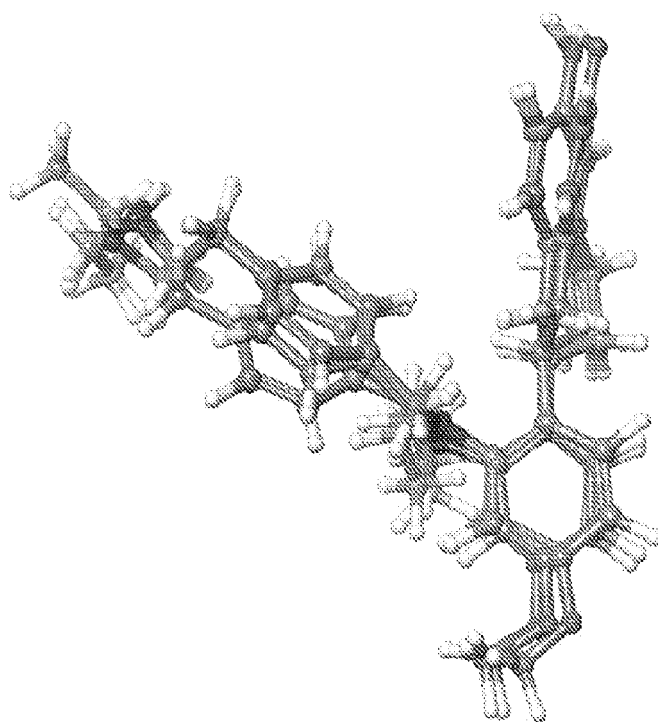


Fig. 33A

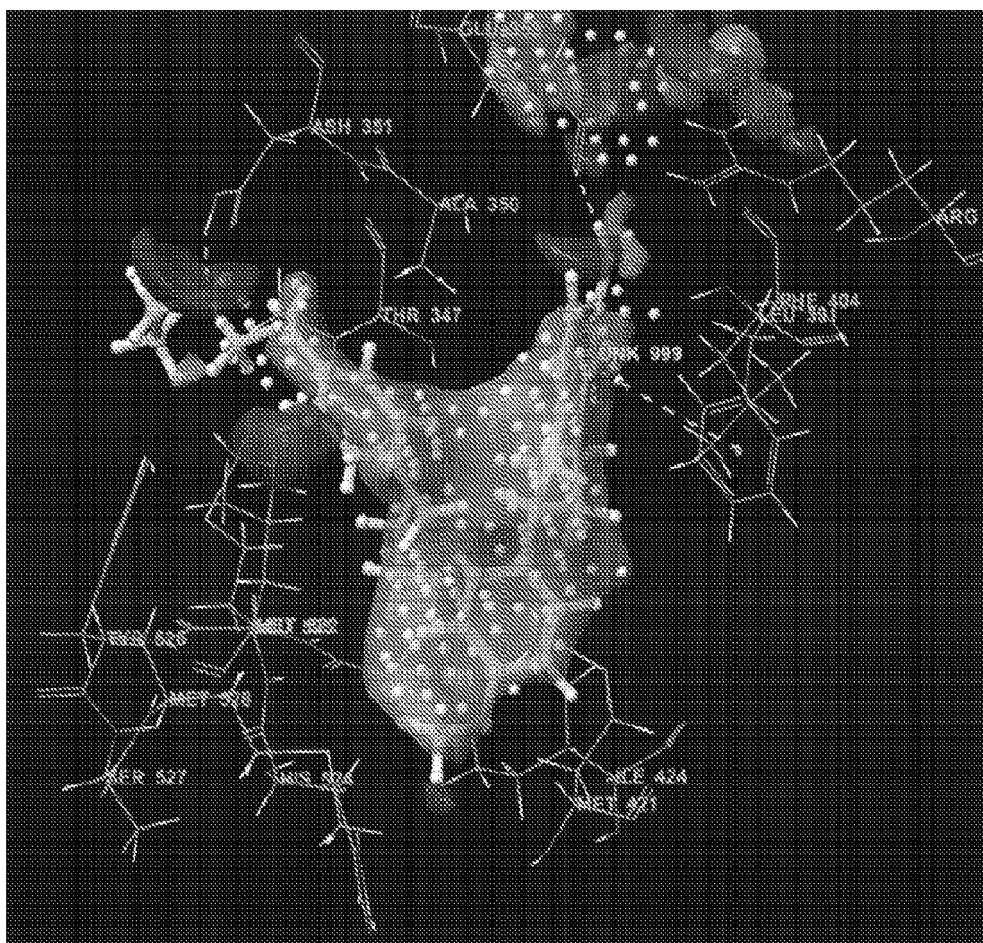


Fig. 33B

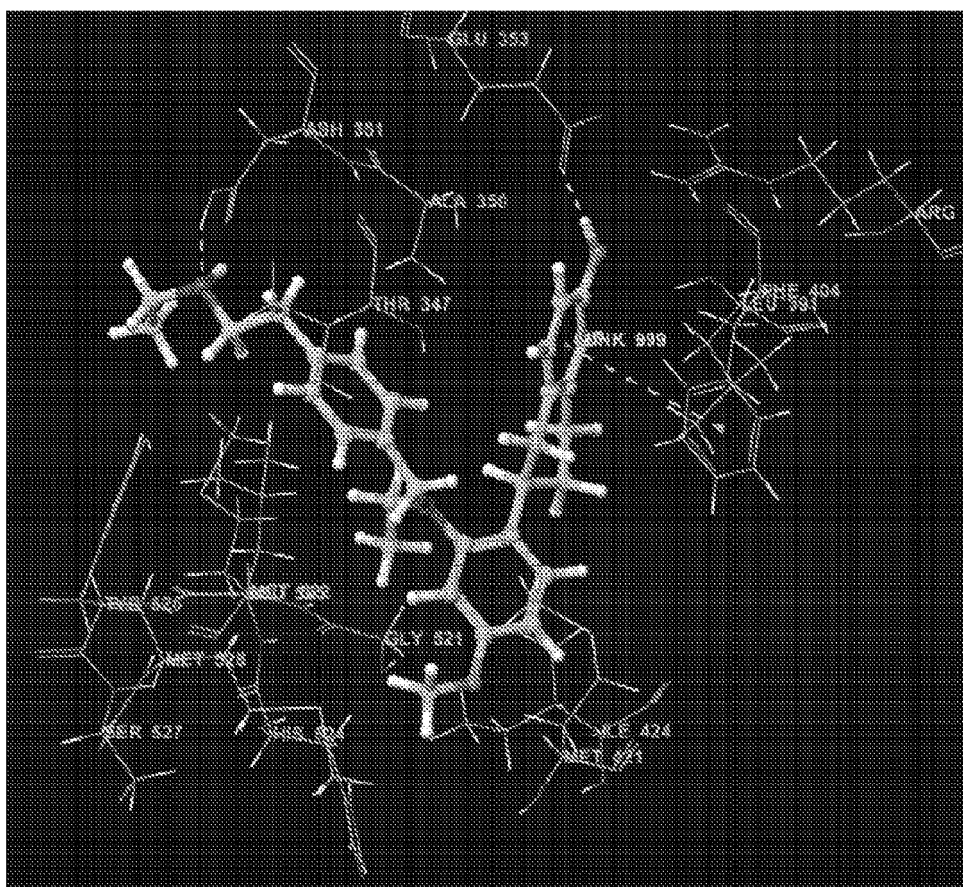


Fig. 33C

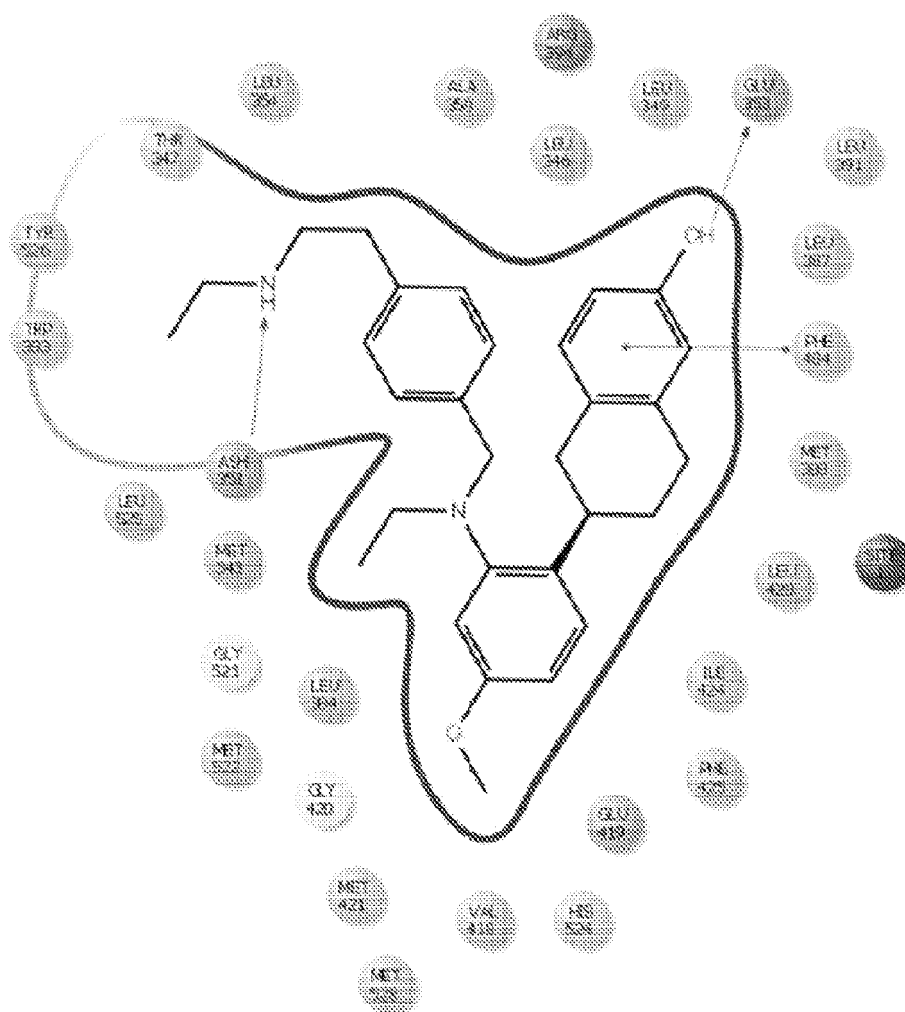


Fig. 34A

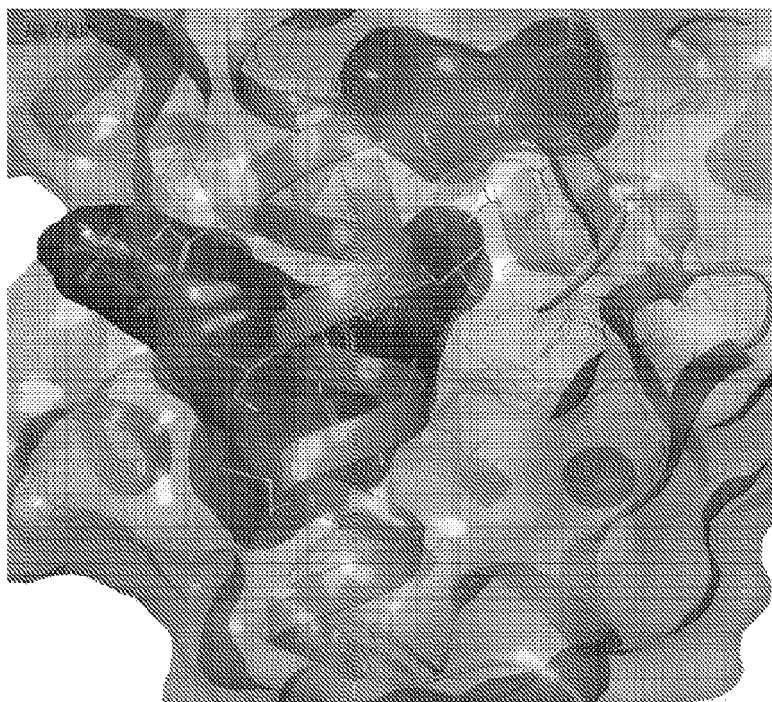


Fig. 34B

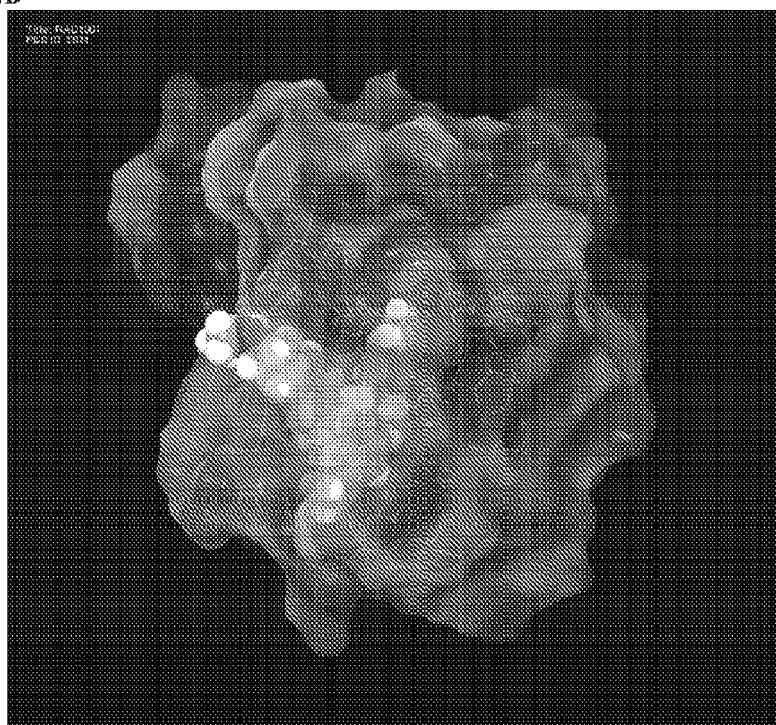


Fig. 34C

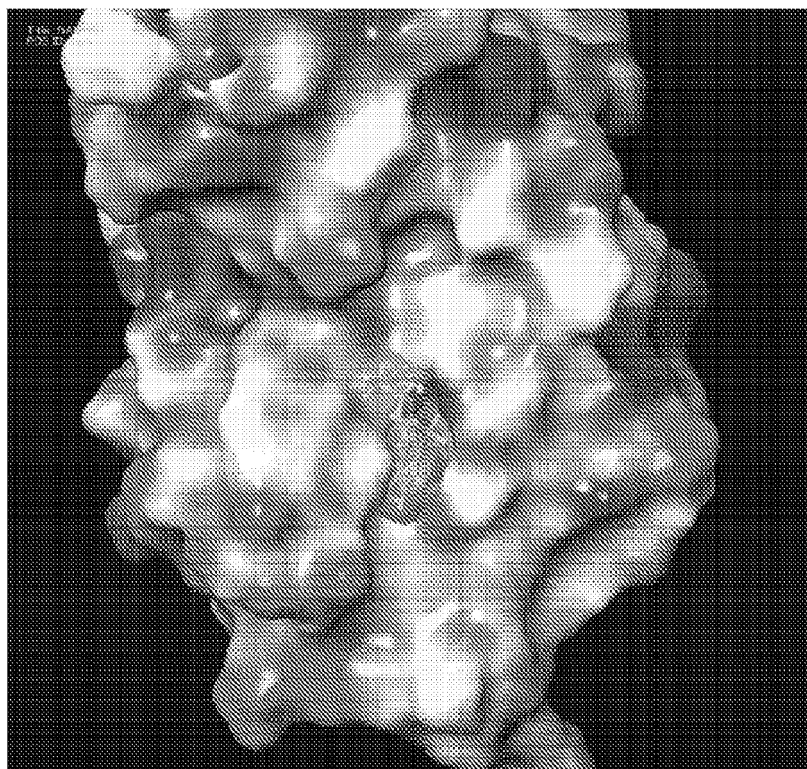


Fig. 35A

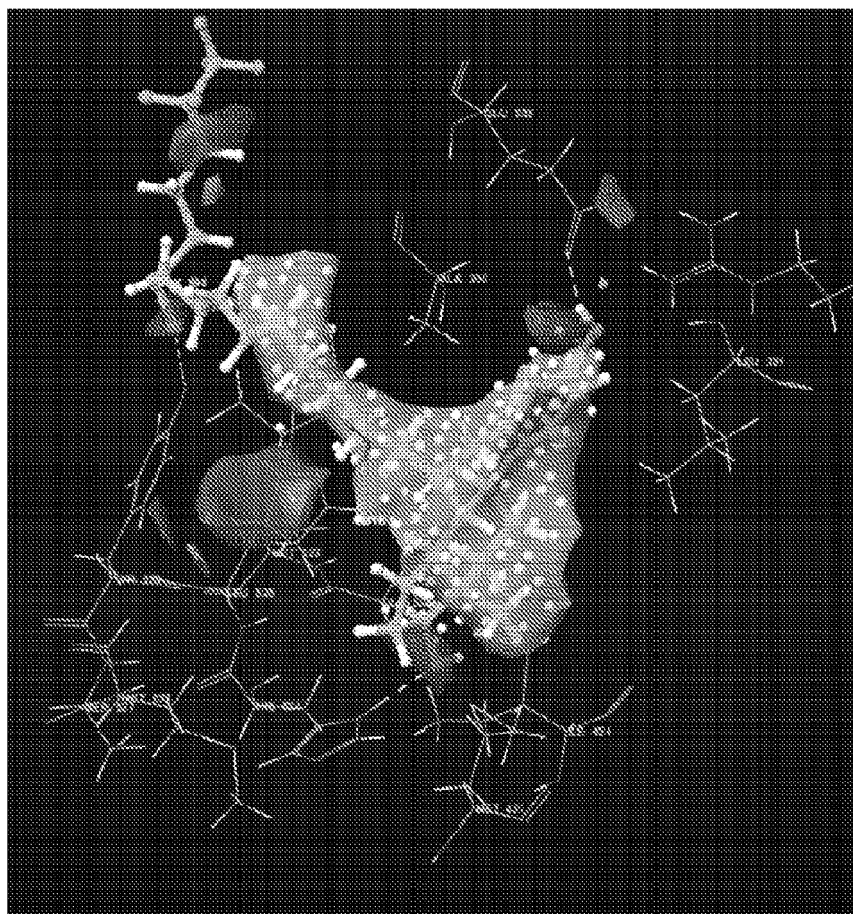


Fig. 35B

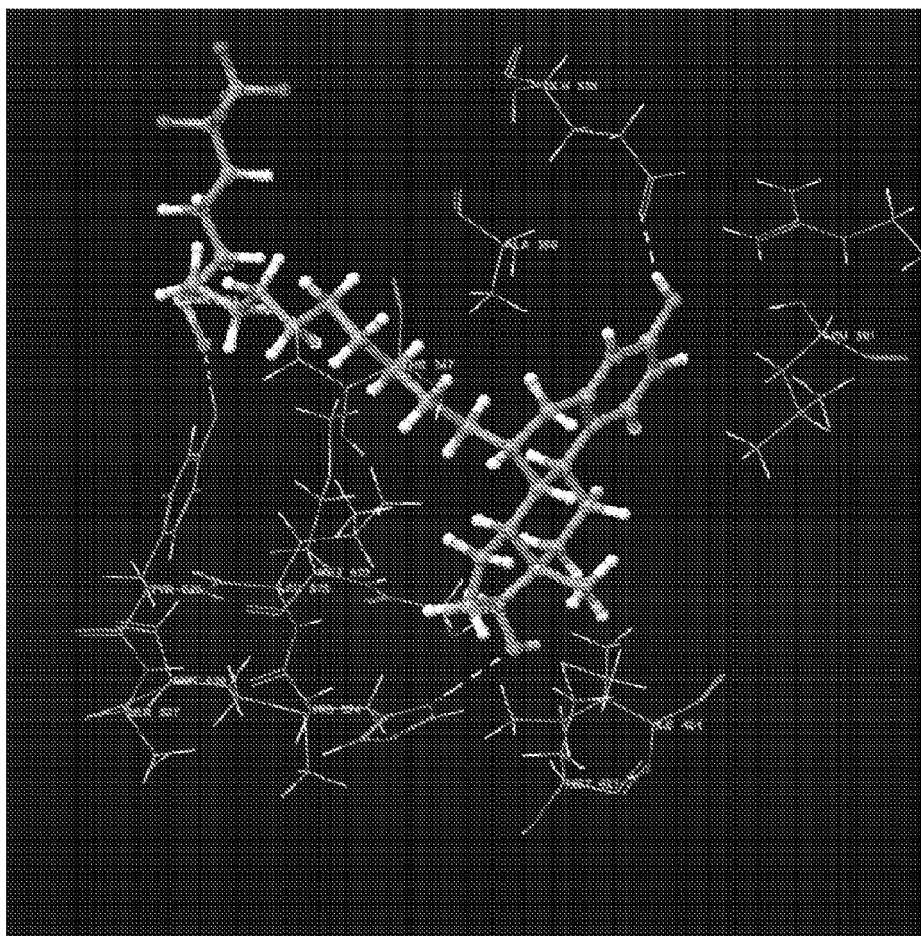


Fig. 35C

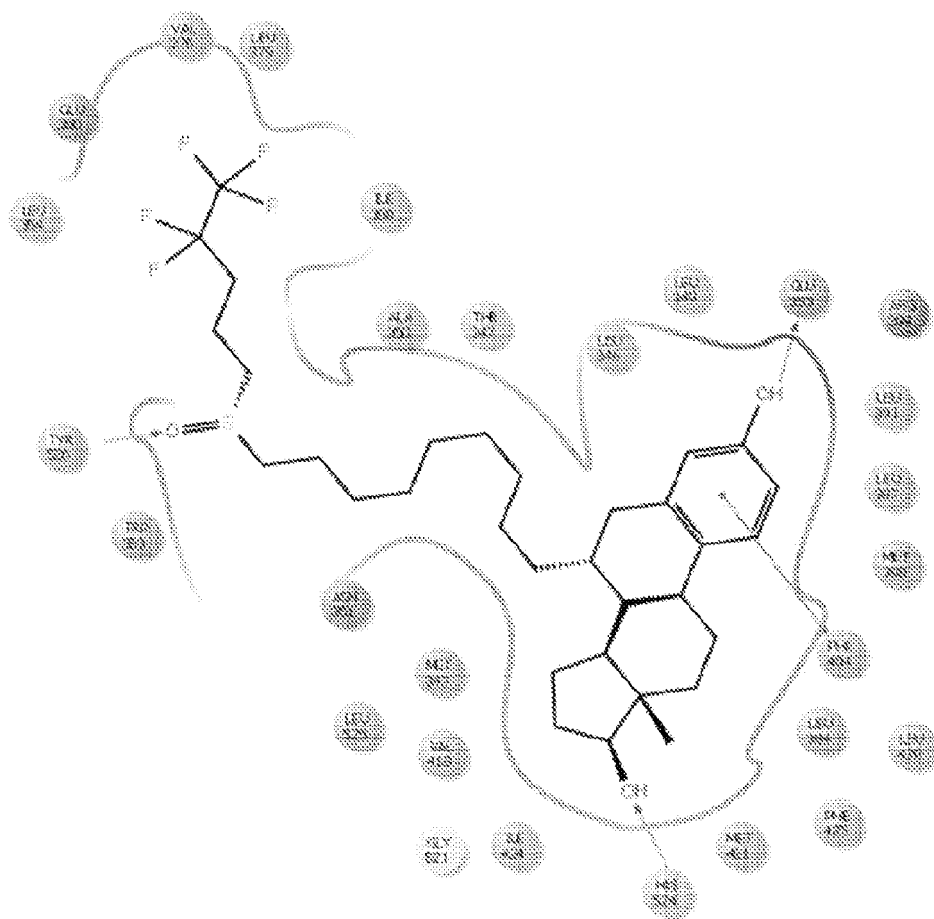


Fig. 36A

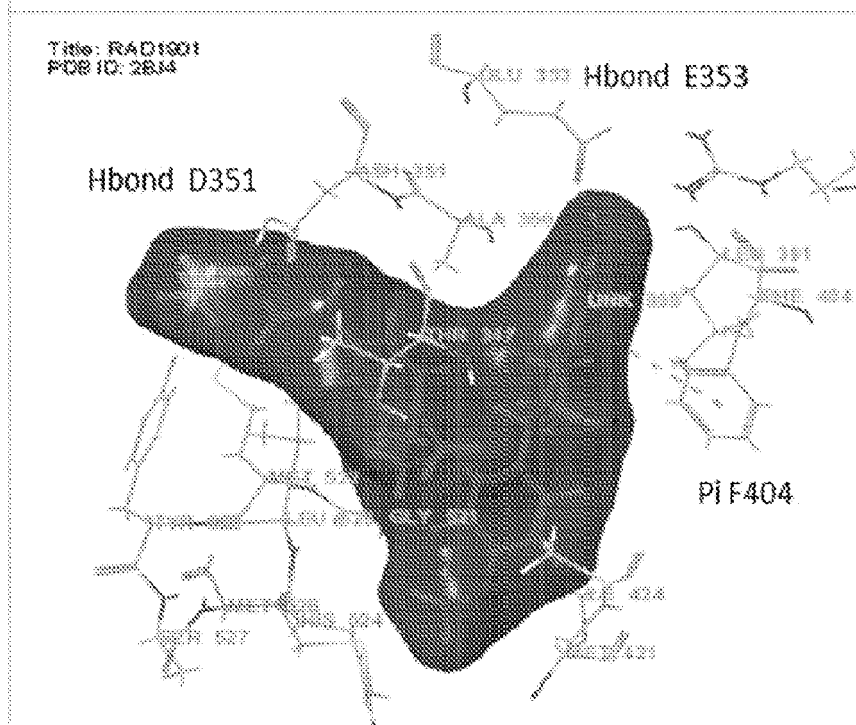
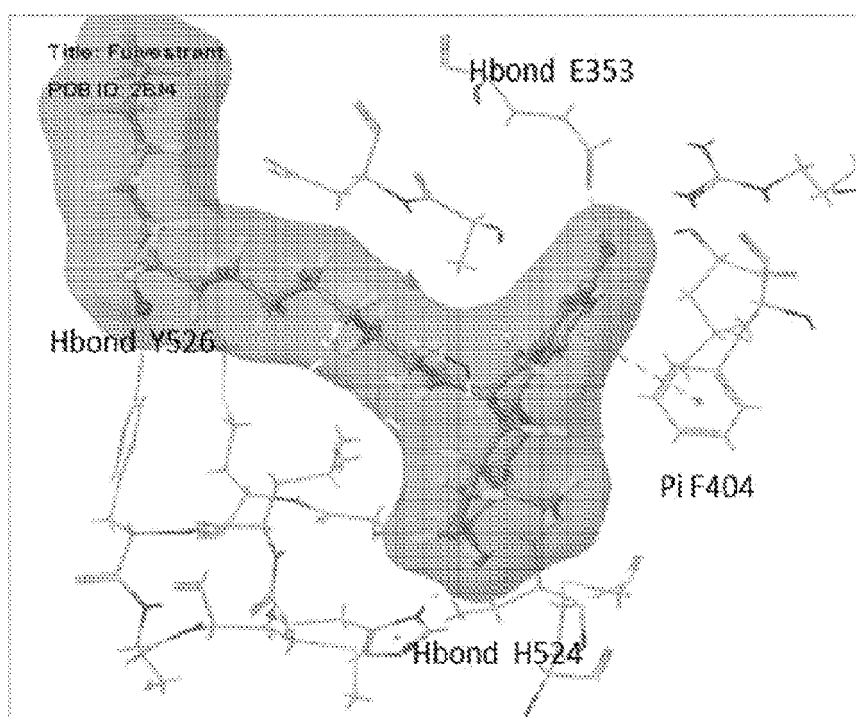


Fig. 36B

Fig. 37A

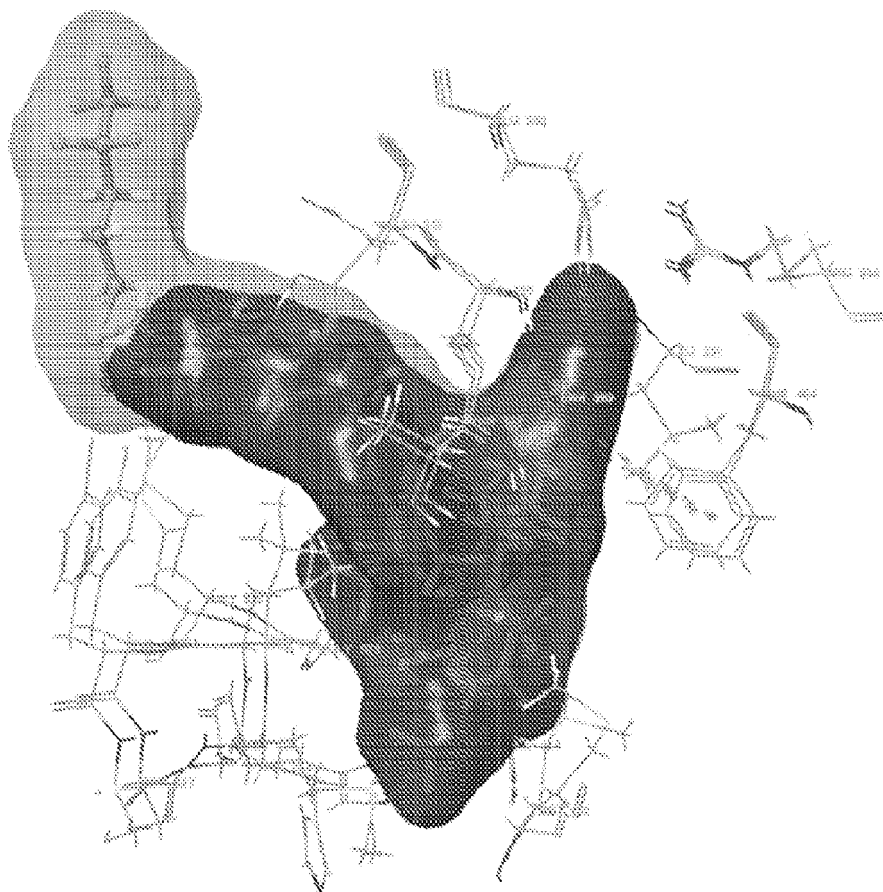


Fig. 37B

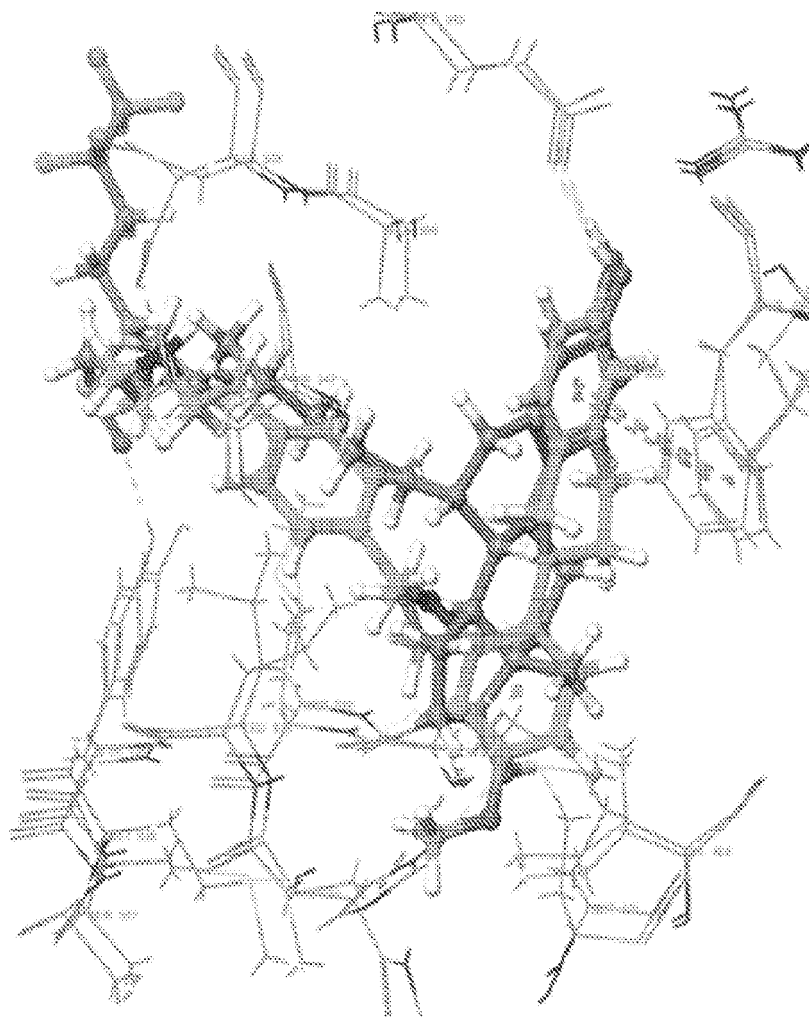
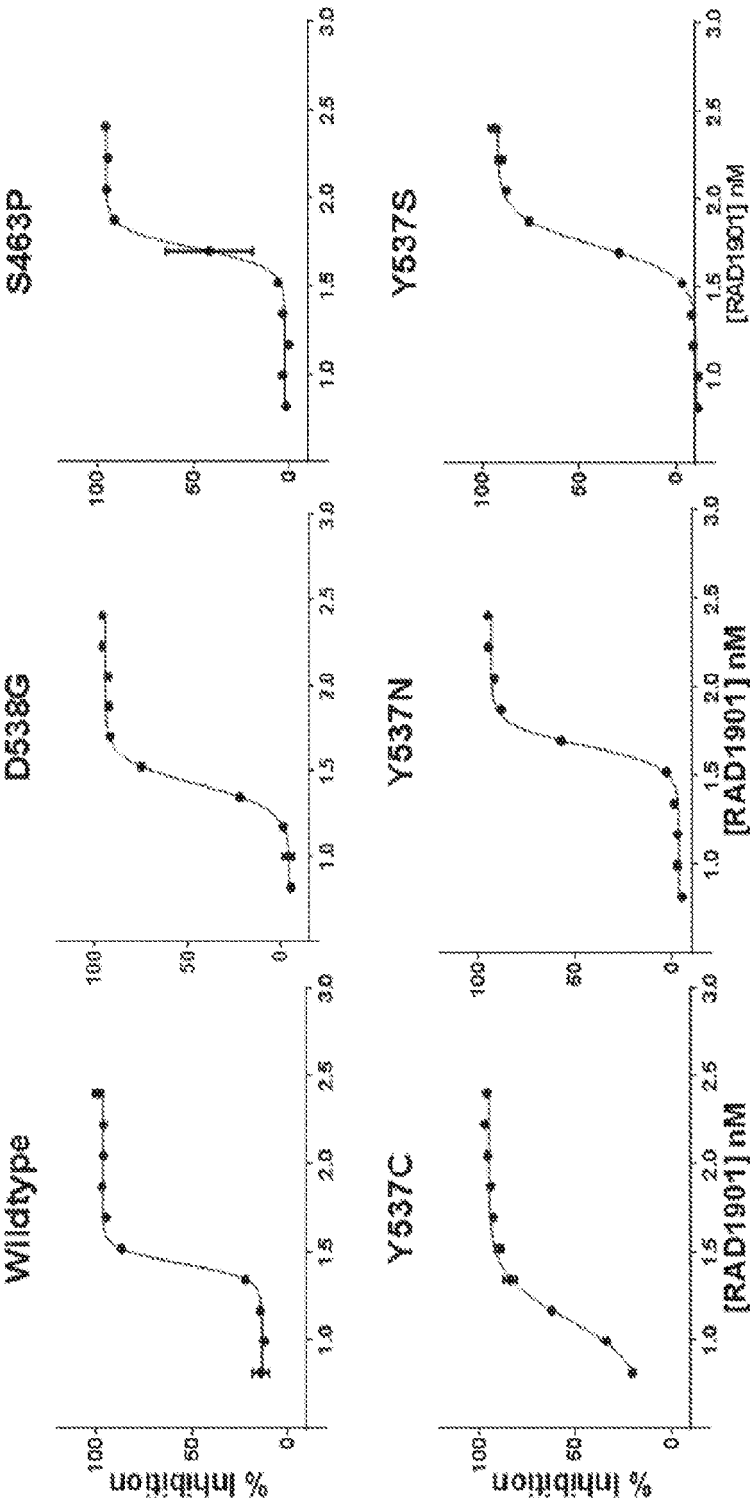


Fig. 38



In vitro binding assay, 2nM fluoromone

Fig. 39

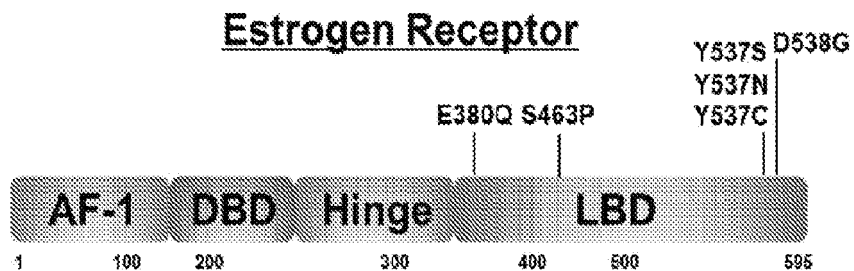


Table 1

RAD1901 levels in plasma, tumor and brain of mice implanted with MCF7 cells after treated for 40 days.

	Dose (mg/kg)	Plasma (ng/mL)	Tumor (ng/mL)	Brain (ng/mL)	B/P Ratio	T/P Ratio
Vehicle		BLQ*	BLQ	BLQ	-	-
RAD1901	0.3	2	11	BLQ	-	
RAD1901	1	3	45	BLQ	-	
RAD1901	3	9	169	7	0.78	18.78
RAD1901	10	39	757	14	0.36	19.41
RAD1901	30	137	3875	72	0.53	28.28
RAD1901	60	334	11117	201	0.60	33.28

*BLQ: below the limit of quantitation

Table 2

SUV for uterus, muscle, and bone for a human subject treated with 200 mg dose p.o.,
q.d., for six days

Dose	Uterus SUV % Change	Bone SUV % Change	Muscle SUV % Change
200 mg	-85%	16%	0%

Table 3

SUV for uterus, muscle, and bone for human subjects (n=4) treated with 500 mg dose p.o., q.d., for six days.

Subject #	Scan	Mean Uterus SUV	Uterus SUV Change (%)	Mean Muscle SUV	Muscle SUV Change (%)	Mean Bone SUV	Bone SUV Change (%)
1	Baseline	3.88		0.33		0.36	
	Day 6	0.58	-85	0.31	-6	0.48	33
2	Baseline	6.47		0.25		0.49	
	Day 6	0.33	-86	0.42	68	0.55	12
3	Baseline	3.66		0.50		0.41	
	Day 6	0.58	-84	0.31	-38	0.47	-23
4	Baseline	3.35		0.30		0.40	
	Day 6	0.41	-88	0.24	-20	0.52	30
Mean			-86		1		13

Table 4

Effect of RAD1901 on BMD in ovariectomized rats.^a

Treatment	Femur BMD (% change)	Lumbar Spine BMD (% change)
Sham	3.1 ± 2.4*	2.7 ± 5.0*
OVX+veh	-5.4 ± 5.1	-10.2 ± 12.8
OVX+E2	-0.5 ± 2.6*	-2.1 ± 12.2*
OVX+RAD1901	0.4 ± 2.8*	-1.1 ± 7.9*

^aAdult female rats underwent either sham or ovariectomy surgery before treatment initiation with vehicle, E2 (0.01 mg/kg) or RAD1901 (3 mg/kg) q.d. (n=20 per treatment group). BMD was measured by dual emission x-ray absorptiometry at baseline and after 4 weeks of treatment. Data are expressed as mean ± SD. *P < 0.05 versus the corresponding OVX+Veh control. BMD, bone mineral density; E2, beta estradiol; OVX, ovariectomized; Veh, vehicle.

Table 5

Effect of RAD1901 on femur microarchitecture in ovariectomized rats^a

Treatment	BV/TV (%)	ConnD (1/mm ³)	TbN (1/mm)	TbTh (mm)	TbSp (mm)	ABD (mgHA/ccm)
Sham	0.394 ± 0.069*	138 ± 21*	5.2 ± 0.6*	0.095 ± 0.008*	0.175 ± 0.029*	456 ± 61*
OVX+Veh	0.234 ± 0.065	91 ± 32	3.5 ± 0.9	0.085 ± 0.011	0.307 ± 0.086	301 ± 69
OVX+E2	0.309 ± 0.079*	125 ± 25*	4.8 ± 0.8*	0.086 ± 0.008	0.204 ± 0.054*	379 ± 75*
OVX+RAD1901	0.300 ± 0.066*	113 ± 22*	4.5 ± 0.8*	0.088 ± 0.008	0.218 ± 0.057*	370 ± 66*

^aAdult female rats underwent either sham or ovariectomy surgery before treatment initiation with vehicle, E2 (0.01 mg/kg) or RAD1901 (3 mg/kg) q.d. (n=20 per treatment group). After 4 weeks, Bone microarchitecture was evaluated using microcomputed tomography. Data are expressed as mean ± SD. *P < 0.05 versus the corresponding OVX+Veh control. ABD, apparent bone density; BV/TV, bone volume density; ConnD, connectivity density; E2, beta estradiol; OVX, ovariectomized; TbN, trabecular number; TbTh, trabecular thickness; TbSp, trabecular spacing; Veh, vehicle.

Table 6

Key baseline demographics of Phase 1 dose escalation study of RAD1901

	Placebo (N=8)	RAD1901 200 mg (N=15)	RAD1901 500 mg (N=14)	RAD1901 750 mg (N=8)	RAD1901 1,000 mg (N=7)
Race white (% of the cohort)	8(100)	14(93)	10(71)	8(100)	7(100)
Mean age, years	64	62	59	64	64
Mean BMI, kg/m ²	26.1	25	24.4	24.9	26.7

Table 7

Most frequent (>10%) treatment related AEs in a Phase 1 dose escalation study of RAD1901

	Placebo N=8 n(%)			200 mg N=15 n(%)			500 mg N=14 n(%)			750 mg N=8 n(%)			1000 mg N=7 n(%)			Total Active N=44 n(%)			Total TEAE N=44
	Gr1	Gr2	Gr3	Gr1	Gr2	Gr3	Gr1	Gr2	Gr3	Gr1	Gr2	Gr3	Gr1	Gr2	Gr3	Gr1	Gr2	Gr3	
Nausea	2 (25)	0	0	5 (33)	0	0	3 (21)	2 (14)	0	2 (25)	1 (13)	0	4 (57)	2 (29)	0	14 (32)	5 (11)	0	19 (43)
Dyspepsia	1 (13)	0	0	3 (20)	0	0	5 (33)	2 (14)	0	4 (50)	0	0	1 (14)	1 (14)	0	13 (30)	3 (7)	0	16 (36)
Vomiting	0	0	0	2 (13)	0	0	1 (7)	5 (36)	1 (7)	0	2 (25)	0	0	3 (43)	0	3 (7)	10 (23)	1 (2)	14 (32)
Hot flush	1 (13)	0	0	2 (13)	0	0	6 (43)	0	0	2 (25)	0	0	1 (14)	0	0	11 (25)	0	0	11 (25)
Abdominal pain	1 (13)	0	0	2 (13)	2 (13)	0	3 (21)	0	0	1 (13)	0	0	1 (14)	1 (14)	0	7 (16)	3 (7)	0	10 (23)
Oesophageal pain	0	0	0	0	2 (13)	0	1 (7)	3 (21)	0	1 (13)	0	0	1 (14)	1 (14)	1 (14)	3 (7)	6 (14)	1 (2)	10 (23)
Headache	0	0	0	3 (20)	0	0	1 (7)	1 (7)	0	3 (38)	0	0	2 (29)	0	0	9 (20)	1 (2)	0	10 (23)
Hiccups	0	0	0	1 (7)	0	0	4 (29)	0	0	2 (25)	0	0	2 (29)	0	0	9 (20)	0	0	9 (20)
Salivary hypersecretion	0	0	0	2 (13)	0	0	2 (14)	0	0	2 (25)	0	0	2 (29)	0	0	8 (18)	0	0	8 (18)
Diarrhoea	1 (13)	0	0	0	0	0	3 (21)	0	0	0	0	0	3 (43)	1 (14)	0	6 (14)	1 (2)	0	7 (16)
Dysphagia	0	0	0	0	0	0	1 (7)	2 (14)	0	3 (38)	1 (13)	0	0	0	0	4 (9)	3 (7)	0	7 (16)
Sensation of a foreign body	0	0	0	2 (13)	0	0	1 (7)	0	0	0	0	0	4 (57)	0	0	7 (16)	0	0	7 (16)
Abdominal distension	0	0	0	1 (7)	1 (7)	0	1 (7)	0	0	1 (13)	0	0	2 (29)	0	0	5 (11)	1 (2)	0	6 (14)
Odynophagia	0	0	0	2 (13)	0	0	1 (7)	1 (7)	0	0	0	0	1 (14)	1 (14)	0	4 (9)	2 (5)	0	6 (14)
Dizziness	2 (25)	0	0	1 (7)	0	0	2 (14)	0	0	1 (13)	0	0	1 (14)	0	0	5 (11)	0	0	5 (11)
Abdominal discomfort	0	0	0	3 (20)	0	0	0	0	0	1 (13)	1 (13)	0	0	0	0	4 (9)	1 (2)	0	5 (11)
Flatulence	0	0	0	2 (13)	0	0	2 (14)	0	0	1 (13)	0	0	0	0	0	5 (11)	0	0	5 (11)
Myalgia	1 (13)	0	0	2 (13)	1 (7)	0	0	1 (7)	0	1 (13)	0	0	0	0	0	3 (7)	2 (5)	0	5 (11)

Table 8

Pharmacokinetic parameters in a Phase 1 dose escalation study of RAD1901 (Day 7)

Parameter	Statistic	200 mg N=15	500 mg N=11	750 mg N=6	1000 mg N=3
$C_{\max}$ (ng/mL)	Geo-Mean	49.8	197	322	540
	Min, Max	30.6, 85.5	105, 316	248, 420	481, 602
$t_{\max}$ (h)	Median	3.00	4.00	3.00	4.00
	Min, Max	2.00, 6.00	2.00-6.02	3.00, 4.00	3.00, 6.00
$AUC_{0-\tau}$ (h*ng/mL)	Geo-Mean	670	2927	4614	8292
	Min, Max	418, 1181	1562, 5460	3209, 7183	7281, 8947
$t_{1/2}$ (h)	Geo-Mean	38.3	37.5	38.4	42.3
	Min, Max	27.7, 51.4	33.8, 41.3	34.6, 46.4	38.7, 49.4

Table 9

Frequency of LBD mutations

	Frequency (%)
D538G	29.5
Y537S	25.0
Y537N	13.6
Y537C	9.1
E380Q	6.8
S463P	4.5
L536R	2.3
L536Q	2.3
P535H	2.3
V392I	2.3
V534E	2.3

Table 10

Differences of ER- α LBD-antagonist complexes in residue poses versus 3ERT

Residue #/ PDB	L1-3/Helix 8		Helix 11							Helix 5	Helix 12	
	M421	I424	E521	M522	H524	L525	Y526	S527	M528	E380	Y537	L540
2BJ4	x	x		x	x		x		x	x	NA	
2JFA	x	x		x	x		x	x	x		NA	
1SJ0	x	x	x	x	x		x	x	x			
2JF9		x		x	x	x	x	x	x		NA	
1YIM	x	x		x	x		x		x			
1R5K	x	x		x	x		x	x	X		x	x
1UMO	x	x		x	x							
1ERR	x	x			x	x	x	x				
2IOK	x	x			x		x	x	x		x	x
3UUC	x	x			x		x	x	x	x	x	
1YIN	x	x	x	X	x	x	x	x	x			
2AYR	x			X	x				x			
2OUZ	x	x				x			x			

Table 11

Evaluation of structure overlap of ER- α LBD-antagonist complexes by RMSD calculations:

	3ERT	2BJ4	2JFA	1SJ0	2JF9	1Y1M	1R5K	1UOM	1ERR	2IOK	3UUC	1Y1N	2AYR
RMSD													
3ERT													
2BJ4	0.804												
2JFA	1.196	0.554											
1SJ0	0.786	0.637	1.115										
2JF9	1.177	0.411	0.415	1.186									
1Y1M	0.978	0.687	1.118	0.276	1.072								
1R5K	1.483	0.759	0.52	1.307	0.892	1.342							
1UOM	0.739	0.761	0.723	0.489	0.909	0.499	1.115						
1ERR	1.12	0.483	0.595	1.016	0.851	1.112	1.208	0.918					
2IOK	0.824	0.689	0.787	0.899	0.897	0.854	1.208	0.736	0.838				
3UUC	1.024	0.915	0.896	1.03	0.888	1.036	1.228	1.012	0.873	0.929			
1Y1N	0.749	0.683	1.105	0.432	1.061	0.318	1.293	0.557	1.076	0.744	1.015		
2AYR	0.659	0.682	0.95	0.792	1.124	0.777	1.391	0.491	1.118	0.071	1.031	0.581	

Table 12

Analysis of ligand binding in ER- α LBD-antagonist complexes

	Ligand: Binding to	EC ₅₀ (μ M)	Comments
3ERT	OHT: E353, R394	0.010	Flipped amine, F404 was too far from the phenol thus there were no pi-interactions
2BJ4	OHT: E353, R394, pi F404	0.010	
2JF9	OHT: E353, D351, H524, pi F404	0.010	
2JFA	RAL: E353, D351, H524 and pi F404 x2	0.002	
1ERR	RAL: E353, D351, R394 and pi F404 x2	0.002	Phenol flipped for H524
1YIM	CM3: E353, H524, D351 pi F404	0.0015(IC ₅₀)	D351-carboxyle oriented well with pyrrolidine
1YIN	CM3: E353, H524 pi F404	0.001	
1SJ0	E4D: E353, H524, pi F404 x 2	0.0008(IC ₅₀)	
1R5K	GW5: D351 pi F404	0.039(IC ₅₀)	No anchor bond with E353
1UOM	PTI: E353, H524 pi F404	NA	
2IOK	IOK: E353 pi F404	0.001	
3UUC	OD1: E353, R394, T347	NA	Very small compound
2OUZ	C3D :E353, pi F404	0.003	
2AYR	L4G: E353, pi F404 x2	0.0107	

Table 13

Model evaluation for RAD1901 docking

	EC ₅₀ (μ M)	EF ₅₀ (=predictive power)	Can model predict crystal structure?	Ligand docking score	RAD1901 docking score
1ERR	0.001		No	-11.452	-7.912
3ERT	0.002		No	-12.175	-8.151
3UCC	NA	8474	Yes	-9.278	NA
2IOK	0.001		Yes	-11.952	-10.478
1R5K	0.039	6100	Yes	-11.518	-12.102
1SJ0	0.001	7511	Yes	-12.507	-9.816
2JFA	0.001	6780	Yes	-11.480	-11.055
2BJ4	0.002	5642	Yes	-9.727	-11.971
2OUZ	0.003	--	Yes	-11.789	-9.611

Table 14

Induced Fit Docking Score of RAD1901 with 1R5K, 1SJ0, 2JFA, 2BJ4 and 2OUZ

ER- α Crystal Structure	RAD1901 IFD Docking Score
1R5K	-14.1
1SJ0	-13.1
2JFA	-13.9
2BJ4	-13.8
2OUZ	-13.4