Supplementary Fig. 1

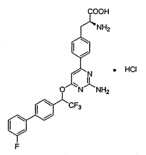
a

DALTON Pharma Services
Accelerated Drug Discovery And Development

Analytical Data Sheet

Name: LP-533401 Hydrochloride

Structure:



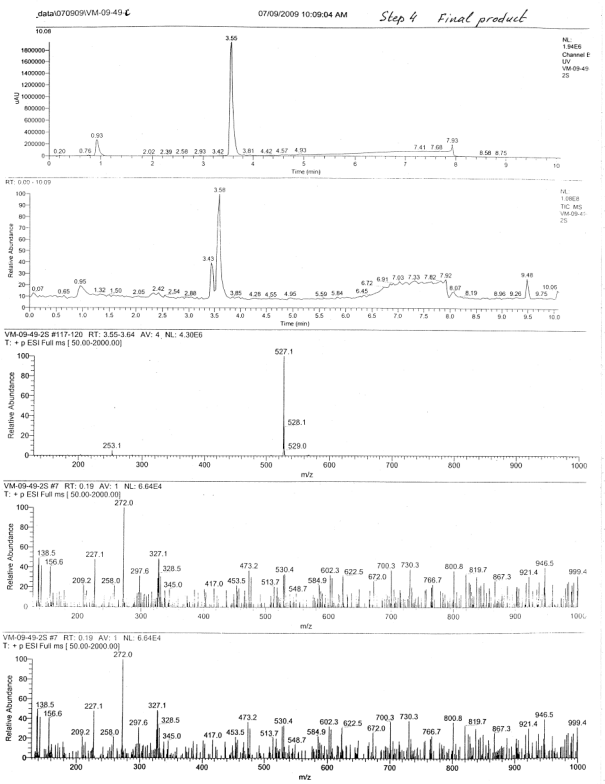
Lot No.: VM-09-49C
Quantity: 11.0 g
Appearance: Off-white powder
M. F.: $C_{27}H_{23}ClF_4N_4O_3$
M. W.: 526.48(Free Base)
^{1}H NMR: Conforms to structure
HPLC purity: 98.5%
LCMS: Conforms to structure. m/z 527.1 $[M+H]^+$
Pd Analysis: 891 ppm
OR: -11.75° (DMSO, c = 0.5)

Dalton Pharma Services Certifies that the above data is true and correct

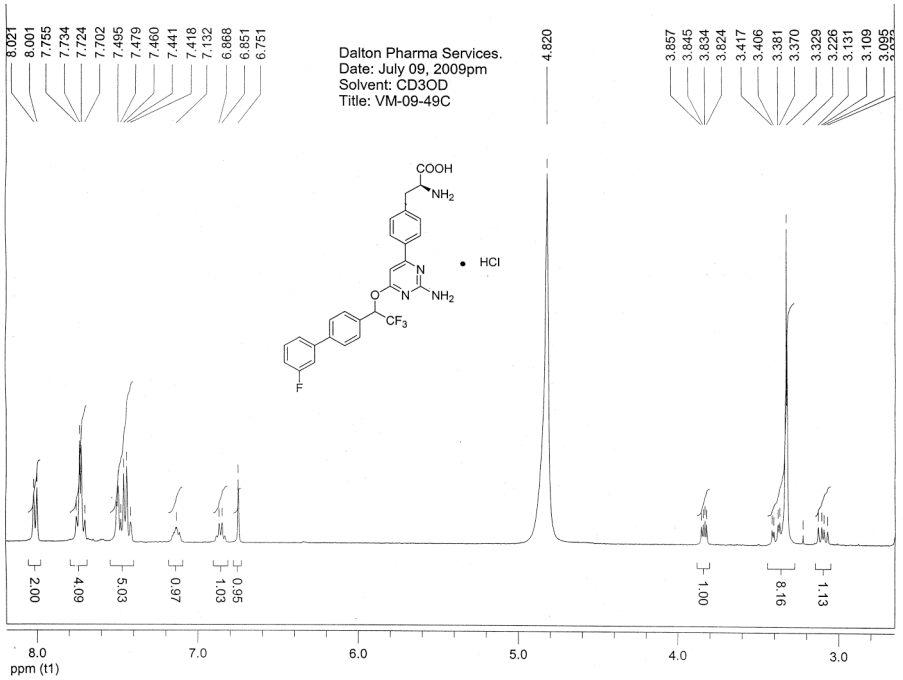
Martyn A Brown PhD AQPIC.
Chemistry Services Manager

07/16/09
Date

b



c



d

Project Name HPLCLAB2
User Name System
Softw are Version 3.05.01

Report Method Name BI_Lab2 Report
Current Date 7/13/09

Sample Information

SampleName VM-09-49C
Vial 1
Injection 1
Injection Volume 10.00 ul
Channel 2487Channel 1
Channel 2487Channel 2
Run Time 16.0 Minutes

Sample Type Unknow n
Date Acquired 7/13/09 10:20:22 AM
Acq Method Set BI_Lab2 Method Set
Processing Method BI_Lab2 processing
Date Processed 7/13/09 12:24:28 PM
Date Processed 7/13/09 12:25:00 PM

Instrument Method: BI_lab2

Stored: 4/21/09 11:32:08 AM

Comments
Modified User System
Locked No
Method Id 26928
Method Version 16
Edit User

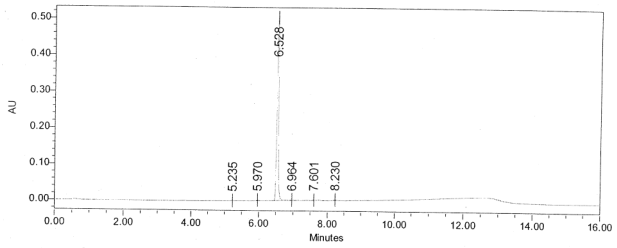
W600 Gradient Table

	Time	Flow	%A	%B	%C	%D	Curve
1		3.00	95.0	5.0	0.0	0.0	
2	0.50	3.00	95.0	5.0	0.0	0.0	6
3	10.50	3.00	0.0	100.0	0.0	0.0	6
4	12.50	3.00	95.0	5.0	0.0	0.0	6
5	16.00	3.00	95.0	5.0	0.0	0.0	6

Mobile Phase: A: 0.1% TFA/Water, B: 0.1% TFA/ACN

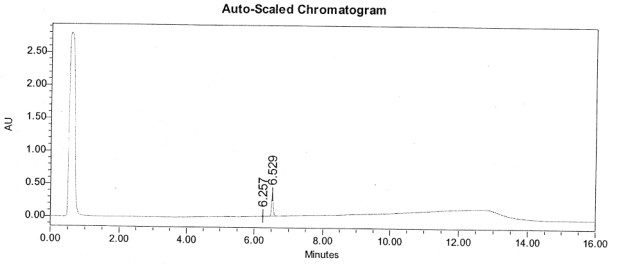
Wavelength: Channel 1 = 254 nm, Channel 2 = 220 nm

Auto-Scaled Chromatogram



SampleName VM-09-49C Vial 1 Injection 1 Channel Description 254 nm Channel 2487Channel 1

Auto-Scaled Chromatogram



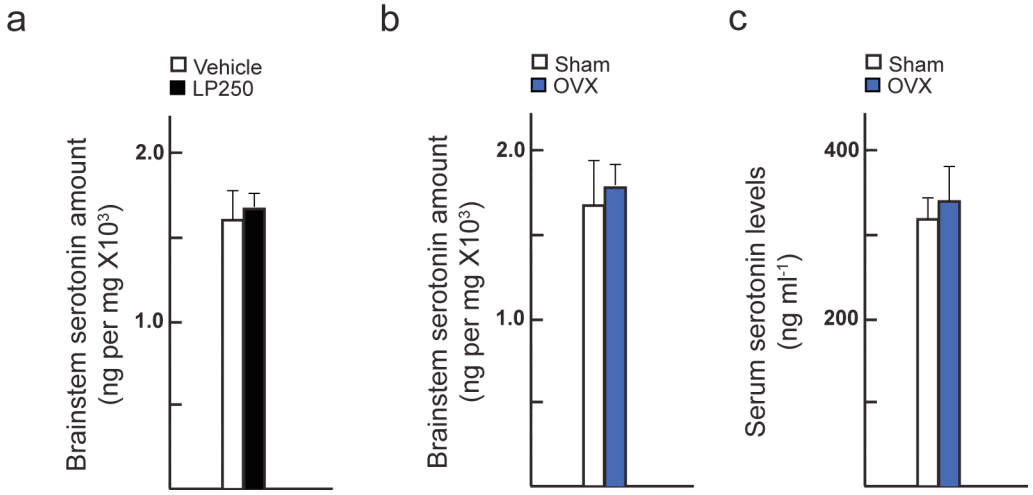
SampleName VM-09-49C Vial 1 Injection 1 Channel Description 220 nm Channel 2487Channel 2

Peak Results

	RT	Area	% Area	Height	Channel	Channel Description
1	5.235	1364	0.08	476	2487Channel 1	254 nm
2	5.970	5179	0.32	1783	2487Channel 1	254 nm
3	6.257	19513	1.68	6224	2487Channel 2	220 nm
4	6.528	1615371	98.50	481065	2487Channel 1	254 nm
5	6.529	1144295	98.32	325247	2487Channel 2	220 nm
6	6.964	4541	0.28	1146	2487Channel 1	254 nm
7	7.601	3502	0.21	1012	2487Channel 1	254 nm
8	8.230	10063	0.61	2103	2487Channel 1	254 nm

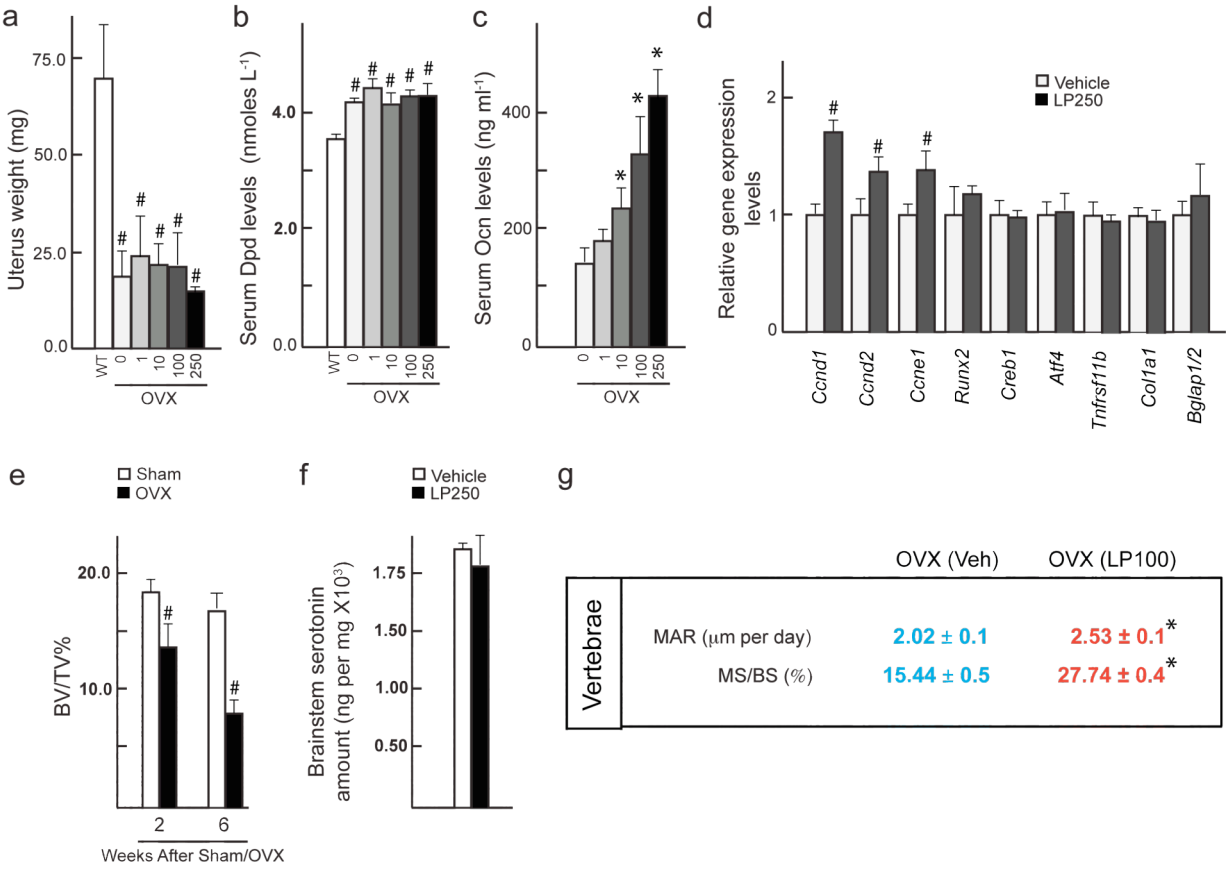
Supplementary Fig. 1. Structural analyses of LP533401 used in this study. (**a**) Analytical data sheet and compound structure. (**b**) LC/MS spectra (**c**) ^{1}H NMR spectra. Values are in ppm relative to tetramethylsilane. (**d**) Analytical HPLC. All analyses were performed by Dalton Pharma Services (Toronto, Canada).

Supplementary Fig. 2



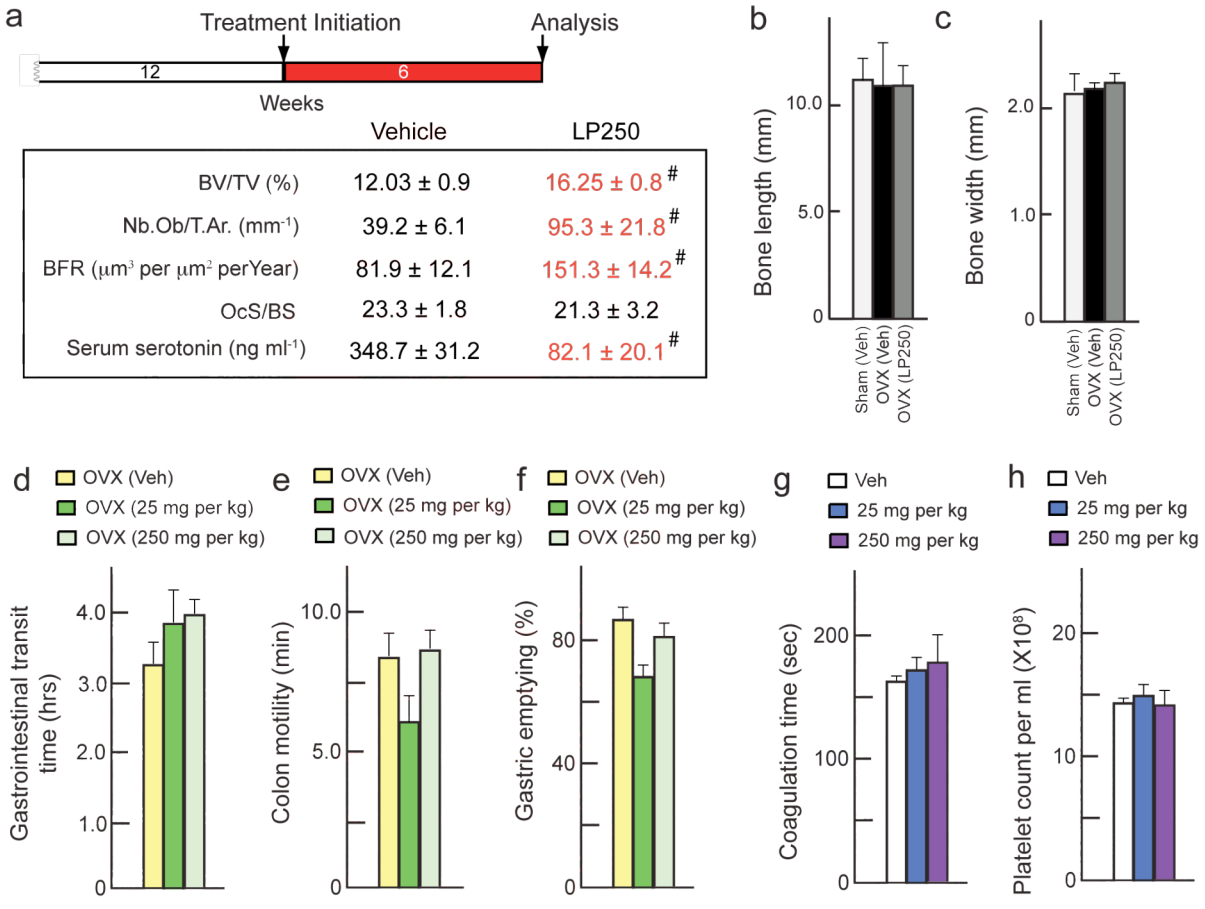
Supplementary Fig. 2. Serotonin measurement in brain and serum in mice. (**a**) HPLC analysis of serotonin content in the brain of wild–type mice that received vehicle or LP533401 (250 mg per kg body weight per day, orally) for 1 day. Brainstem serotonin content (**b**) and serum serotonin levels (**c**) in mice that were either Sham–operated or ovariectomized at 6 weeks and left untreated till 10 weeks of age.

Supplementary Fig. 3



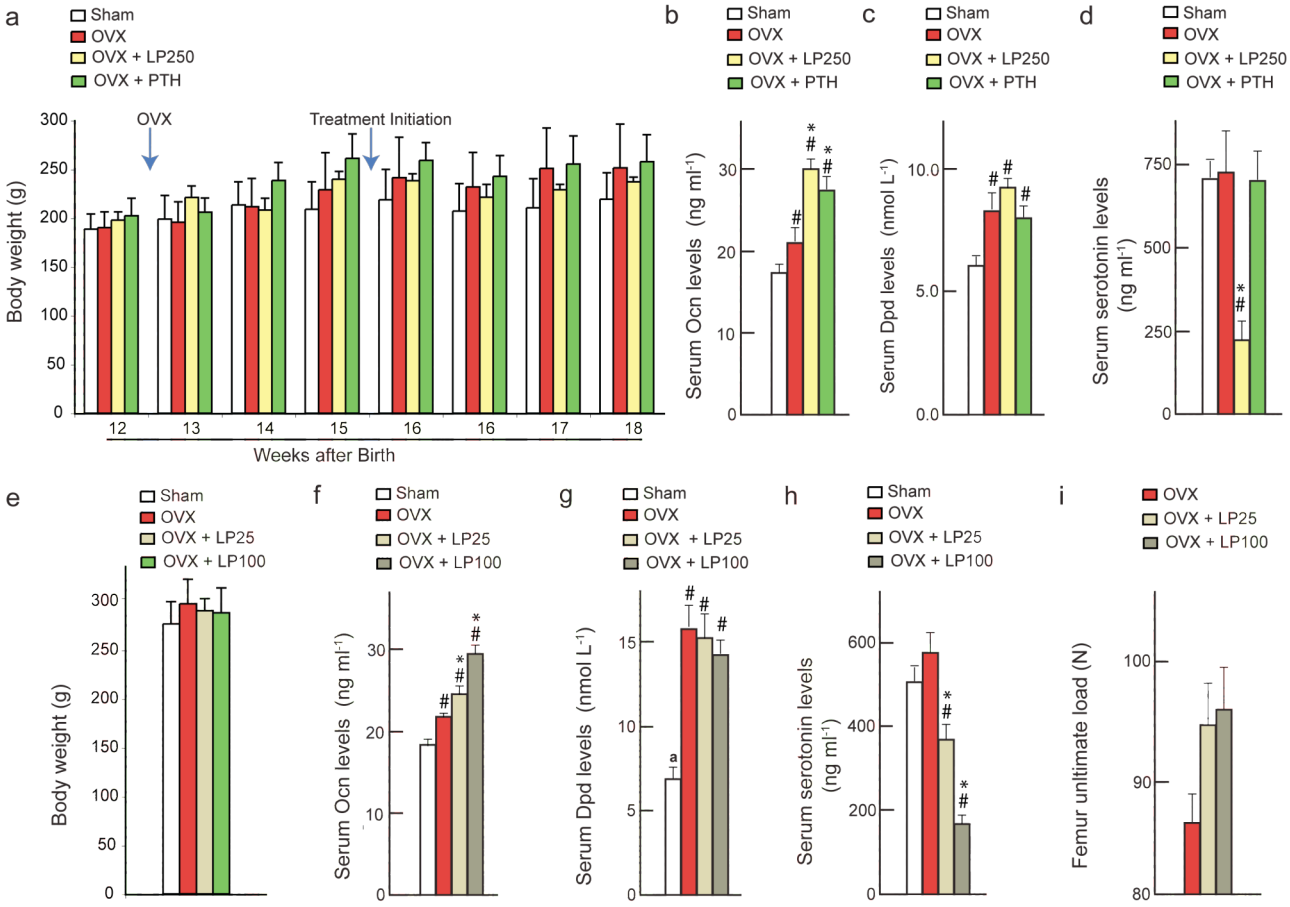
Supplementary Fig. 3. Assessment of ovariectomy–induced and LP533401–induced changes in sham or ovariectomized mice. (**a-d**) Uterus weight (**a**), serum Dpd (**b**) and serum osteocalcin levels (**c**) of sham–operated (sham) or ovariectomized wild–type mice that received different doses of LP533401 (0, 1, 10, 100 or 250 mg per kg body weight per day, orally) from day 1 to 28 post–ovariectomy. (**d**) Gene expression changes in the long bones assessed by real time PCR in long bones of wild–type mice that received either vehicle or LP533401 (250 mg per kg body weight per day). (**e**) Histomorphometric analysis of vertebrae of mice 2– and 6–weeks after sham or bilateral ovariectomy (OVX) to determine bone loss before the onset of treatment. (**f**) HPLC analysis of serotonin content in the brain of wild–type mice that received vehicle or LP533401 (250 mg per kg body weight per day, orally) treatment. (**g**) Mineral apposition rate (MAR) and mineralizing surface per bone surface (MS/BS) in vertebrae in ovariectomized mice treated with vehicle or LP533401 (100 mg per kg body weight per day, orally). # $p < 0.05$ vs sham and * $p < 0.05$ vs OVX (Veh).

Supplementary Fig. 4



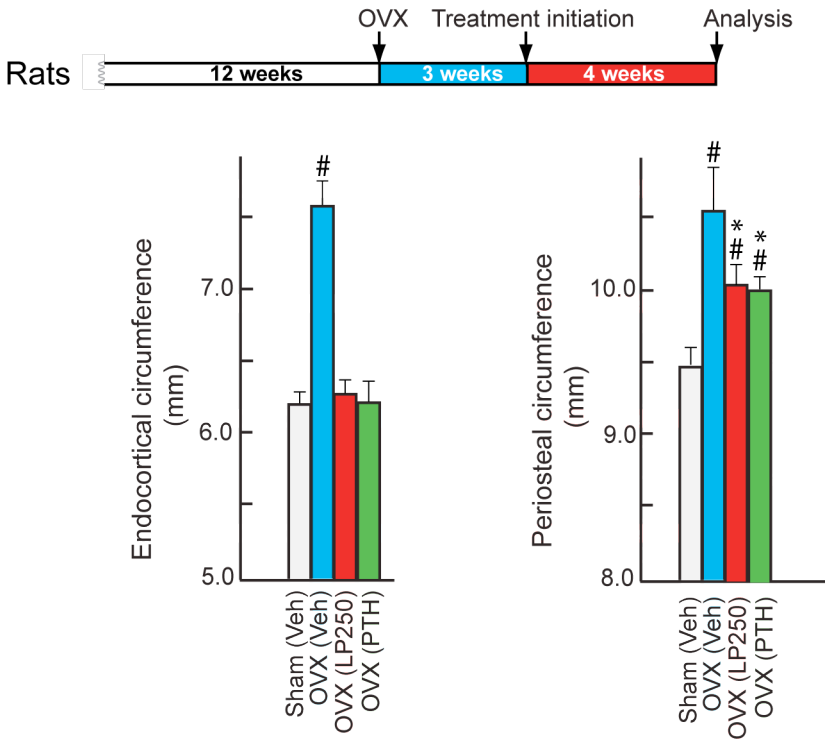
Supplementary Fig. 4. Assessment of bone mass, length and width, humoral and gastrointestinal parameters in mice. (**a**) Histomorphometric analysis of vertebrae and serum serotonin levels of 12 week–old wild–type mice treated for 6 weeks with vehicle or LP533401 (250 mg per kg body weight per day, orally). BV/TV, Bone volume over trabecular volume; Nb.Ob/T.Ar, osteoblast number over trabecular area; BFR, bone formation rate; OcS/BS, osteoclast surface over bone surface. (**b-c**) Bone length and width in sham, and OVX mice treated with vehicle or LP533401 (250 mg per kg body weight per day). (**d-f**) Changes in gastrointestinal transit time (**d**), colon motility (**e**) and gastric emptying (**f**) in ovariectomized mice that, 6–weeks after ovariectomy, were treated for 6–weeks with vehicle or LP533401 (25 or 250 mg per kg body weight per day, orally). (**g-h**) Changes in coagulation time (**g**) and platelet numbers (**h**) in wild–type mice that received either vehicle or LP533401 (25 or 250mg per kg body weight per day, orally) for 2 days. n=6-8 for each group. # $p < 0.05$ vs vehicle treated control.

Supplementary Fig. 5



Supplementary Fig. 5. Assessment of ovariectomy–induced and LP533401–induced changes in body weight, humoral and biomechanical parameters in sham or ovariectomized rats. (**a–d**) Body weight changes (**a**), serum osteocalcin (**b**), serum Dpd (**c**), and serum serotonin levels (**d**) in wilt–type sham–operated and ovariectomized rats that received either vehicle, LP533401 (250 mg per kg body weight per day, orally) or PTH (80 μg per kg body weight per day, subcutaneous) from week 3 to 7 post–ovariectomy. (**e-h**) body weight at sacrifice (**e**), serum osteocalcin (**f**), serum Dpd (**g**), serum serotonin levels (**h**) and femur ultimate load analysis (**i**) in wild–type sham–operated and ovariectomized rats that received either vehicle or LP533401 (25 or 100 mg per kg body weight per day, orally) from week 12 to 16 post–ovariectomy. *n*=5-10 for each group of rats. All values are expressed as means ± SEM. # $p < 0.05$ vs sham and * $p < 0.05$ vs OVX (Veh).

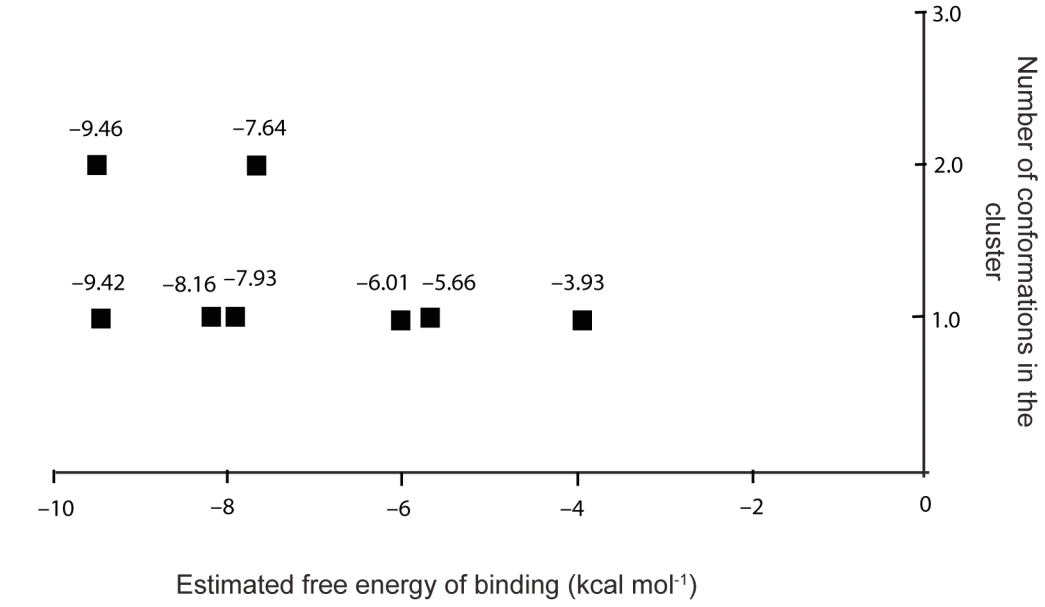
Supplementary Fig. 6



Supplementary Fig. 6. Assessment of endocortical and periosteal parameters in sham and ovariectomized rats treated with vehicle, LP533401 and PTH. Micro–computed tomography analysis of endocortical and periosteal circumference in wild–type sham–operated and ovariectomized rats that received either vehicle, LP533401 (250 mg per kg body weight per day, orally) or PTH (80 μg per kg body weight per day, subcutaneous) from week 3 to 7 post–ovariectomy. n=5–7 for each group of rats. All values are expressed as means ± SEM. # $p < 0.05$ vs sham and * $p < 0.05$ vs OVX (Veh).

Supplementary Fig. 7

a



b

Receptor Name	Tph1 (1mlwA)
Ligands' Names	LP533401 and HBI
Charge added	0.503 Å (tried on 0.403, 0.603, 0.703 and 0.753)
Method	Blind Docking
Algorithm	Genetic Algorithm
Other Parameters	Default

Supplementary Fig. 7. Bioinformatic analysis of Tph1 interaction with LP533401 and HBI. (**a**) Plot of lowest estimated free energies of binding and no of conformations in that cluster for Tph1 and LP533401 docking. (**b**) Summary of files and parameters for docking studies using Autodock.