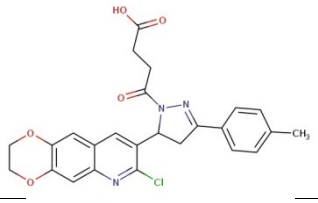
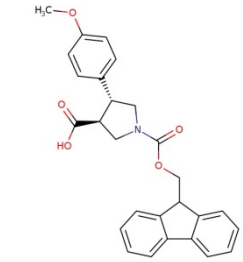
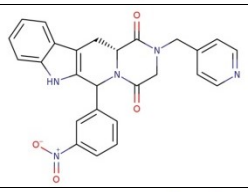
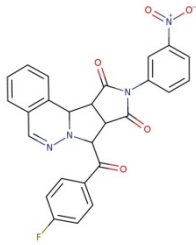
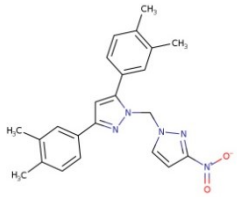
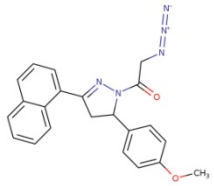
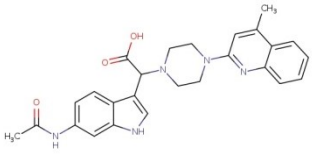
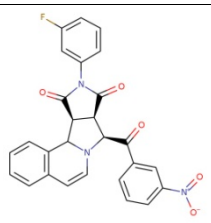
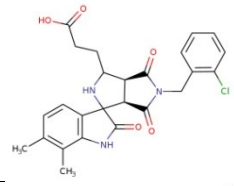
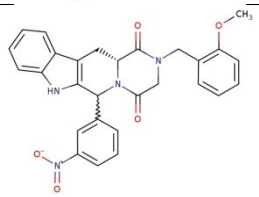
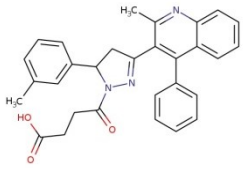
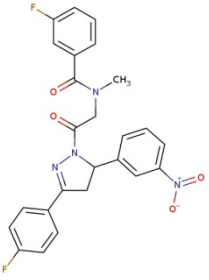
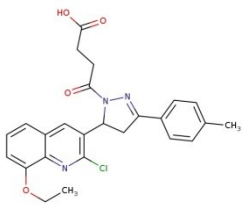
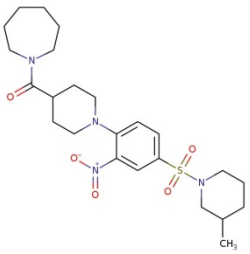
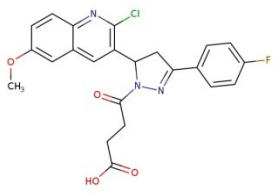
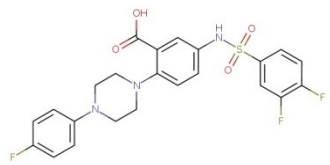
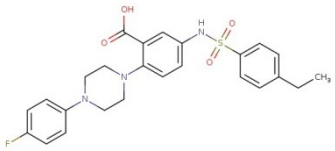
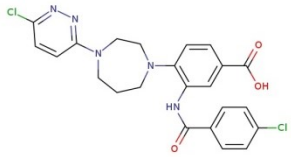
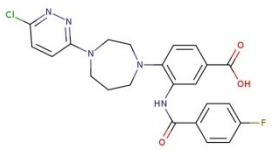
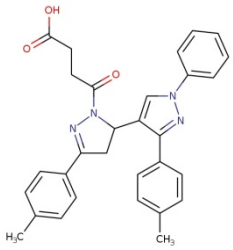
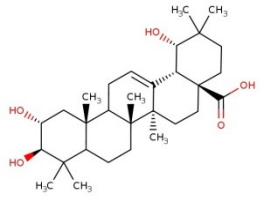
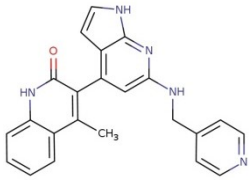
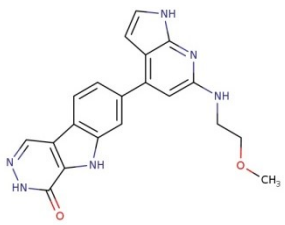
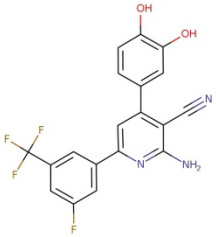
Supplementary Table 1. List of initially screened computationally identified lead compounds as PTHR ligands. Indicated are predicted computed binding scores, and area under the curve (A.U.C.) of cAMP time courses induced by 1 nM PTH in the presence of 10 μM of small molecules (mean ± S.D. of *N* independent experiments), normalized to the PTH response without compounds and expressed as % values. In brackets is alternative nomenclature used in Source Data Files.

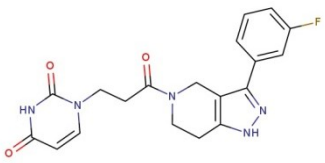
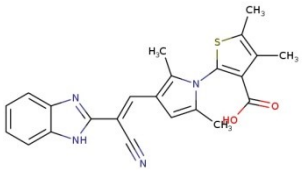
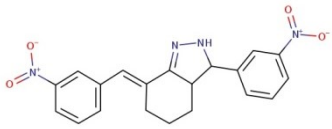
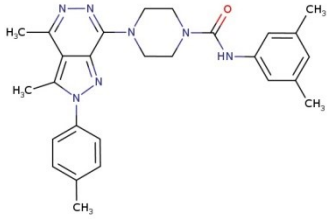
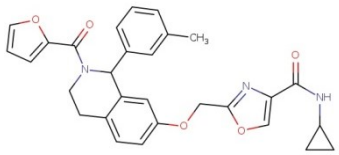
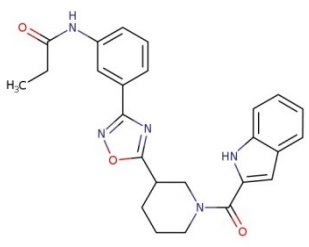
Compound	MolPort ID	score	Integrated cAMP (mean ± s.d.)			N
1 (Pitt8)	002-583-206	-8.41	32.3	±	9.5	3
2 (Pitt12)	039-313-655	-8.47	39.4	±	10.6	3
3 (Pitt1)	000-837-761	-8.16	82.7	±	10.0	2
4 (Pitt2)	000-749-199	-9.28	72.4	±	8.1	2
5 (Pitt3)	002-764-247	-8.24	50.6	±	10.2	2
6 (Pitt4)	019-692-354	-8.23	118.8	±	8.9	2
7 (Pitt5)	006-815-377	-8.62	79.6	±	8.9	2
8 (Pitt6)	002-582-026	-8.17	64.9	±	7.5	2
9 (Pitt7)	002-527-649	-8.31	70.3	±	6.3	2
10 (Pitt9)	000-829-806	-8.21	85.3	±	14.3	2
11 (Pitt10)	028-805-319	-8.33	92.5	±	12.0	2
12 (Pitt11)	016-916-811	-8.38	106.2	±	12.9	2
13 (Pitt13)	030-037-643	-8.42	96.1	±	10.9	2
14 (Pitt14)	003-269-448	-8.39	137.2	±	5.9	2
15 (Pitt15)	023-186-594	-8.33	41.9	±	18.9	2
16 (Pitt16)	010-715-401	-8.28	76.7	±	12.8	2
17 (Pitt17)	010-715-395	-8.33	99.9	±	9.2	2
18 (Pitt18)	010-807-325	-8.5	71.1	±	10.9	2
19 (Pitt19)	010-807-350	-8.5	75.0	±	7.7	2
20 (Pitt20)	002-369-638	-9.33	63.5	±	14.3	2
21 (Pitt21)	005-944-940	-8.54	62.0	±	5.5	2
22 (Pitt22)	021-757-743	-8.92	73.6	±	9.3	2
23 (Pitt23)	020-207-515	-8.61	111.2	±	10.7	2
24 (Pitt24)	020-198-951	-8.80	117.2	±	7.5	2
25 (Pitt25)	005-011-602	-8.40	119.1	±	11.6	2
26 (Pitt26)	002-284-377	-7.19	81.6	±	2.9	2
27 (Pitt27)	002-169-278	-7.71	100.9	±	11.2	2
28 (Pitt28)	007-831-482	-10.39	152.0	±	7.9	2
29 (Pitt29)	006-598-622	-9.74	135.2	±	4.8	2
30 (Pitt30)	010-693-446	-8.85	140.4	±	7.0	2

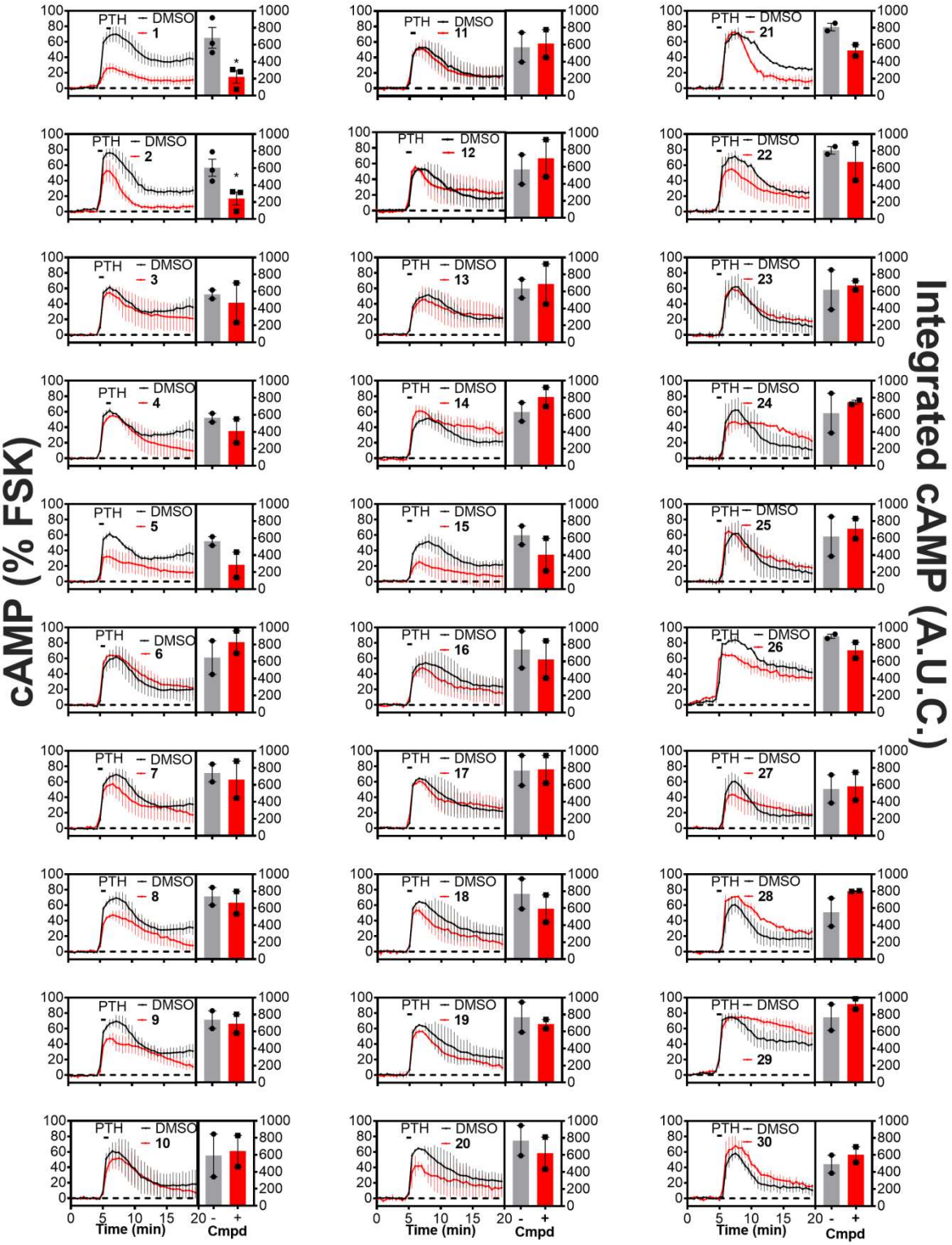
Supplementary Table 2. Detailed chemical properties of hit compounds.

Compound	Structure	SMILES	Molecular weight	LogP
1		Cc1ccc(cc1)C1=NN(C(C1)c1cc2cc3OCCOc3cc2nc1Cl)C(=O)CCC(O)=O	479.92	4.51
2		COc1ccc(cc1)[C@@H]1CN(C[C@H]1C(O)=O)C(=O)OCC1c2ccccc2-c2ccccc12	443.499	4.744
3		[O-][N+](=O)c1cccc(c1)C1N2[C@H](Cc3c1[nH]c1ccccc31)C(=O)N(Cc1ccncc1)CC2=O	467.485	3.356
4		[O-][N+](=O)c1cccc(c1)N1C(=O)C2C(C3N(N=Cc4ccccc34)C2C(=O)c2ccc(F)cc2)C1=O	484.443	3.495
5		Cc1ccc(cc1C)-c1cc(-c2ccc(C)c(C)c2)n(Cn2ccc(n2)[N+]([O-])=O)n1	401.47	5.061
6		COc1ccc(cc1)C1CC(=NN1C(=O)CN=[N+]=[N-])c1cccc2ccccc12	385.427	4.836
7		CC(=O)Nc1ccc2c(c[nH]c2c1)C(N1CCN(CC1)c1cc(C)c2ccccc2n1)C(O)=O	457.534	4.58

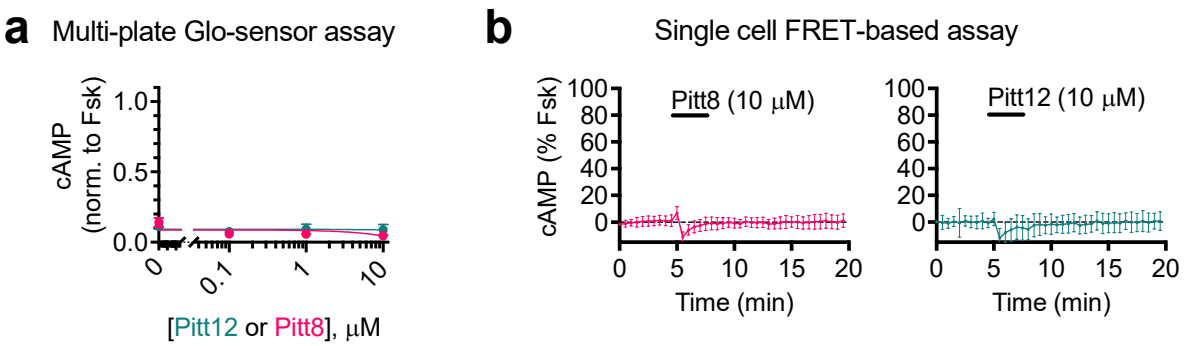
8		[O-][N+](=O)c1cccc(c1)C(=O)[C@@H]1[C@H]2[C@@H](C3N1C=Cc1ccccc31)C(=O)N(C2=O)c1cccc(F)c1	483.455	4.132
9		Cc1ccc2c(NC(=O)C22NC(CCC(O)=O)[C@@H]3[C@H]2C(=O)N(Cc2ccccc2Cl)C3=O)c1C	481.93	2.742
10		COc1ccccc1CN1CC(=O)N2[C@H](Cc3c([nH]c4ccccc34)C2c2cccc(c2)[N+]([O-])=O)C1=O	496.523	3.97
11		Cc1cccc(c1)C1CC(=NN1C(=O)CCC(O)=O)c1c(C)nc2ccccc2c1-c1ccccc1	477.564	6.061
12		CN(CC(=O)N1N=C(CC1c1cccc(c1)[N+]([O-])=O)c1ccc(F)cc1)C(=O)c1cccc(F)c1	478.456	4.323
13		CCOc1cccc2cc(C3CC(=NN3C(=O)CCC(O)=O)c3ccc(C)cc3)c(Cl)nc12	465.93	5.138
14		CC1CCCN(C1)S(=O)(=O)c1ccc(N2CCC(CC2)C(=O)N2CCCCCC2)c(c1)[N+]([O-])=O	492.64	3.634
15		COc1ccc2nc(Cl)c(cc2c1)C1CC(=NN1C(=O)CCC(O)=O)c1ccc(F)cc1	455.87	4.578

16		OC(=O)c1cc(NS(=O)(=O)c2ccc(F)c(F)c2)ccc1N1CCN(CC1)c1ccc(F)cc1	491.49	3.93
17		CCc1ccc(cc1)S(=O)(=O)Nc1ccc(N2CCN(CC2)c2ccc(F)cc2)c(c1)C(O)=O	483.56	4.214
18		OC(=O)c1ccc(N2CCCN(CC2)c2ccc(Cl)nn2)c(NC(=O)c2ccc(Cl)cc2)c1	486.35	4.451
19		OC(=O)c1ccc(N2CCCN(CC2)c2ccc(Cl)nn2)c(NC(=O)c2ccc(F)cc2)c1	469.9	3.936
20		Cc1ccc(cc1)C1=NN(C(C1)c1cn(nc1-c1ccc(C)cc1)-c1ccccc1)C(=O)CCC(O)=O	492.579	5.699
21		CC1(C)CC[C@@]2(CC[C@]3(C)C(=CCC4[C@@]5(C)C[C@@H](O)[C@H](O)C(C)(C)C5CC[C@@]34C)[C@@H]2[C@@H]1O)C(O)=O	488.709	5.175
22		Cc1c(-c2cc(NCc3ccncc3)nc3[nH]ccc23)c(=O)[nH]c2ccccc12	381.439	4.387
23		COCCNc1cc(-c2ccc3c(c2)[nH]c2c3cn[nH]c2=O)c2cc[nH]c2n1	374.404	3.006
24		Nc1nc(cc(-c2ccc(O)c(O)c2)c1C#N)-c1cc(F)cc(c1)C(F)(F)F	389.31	4.439

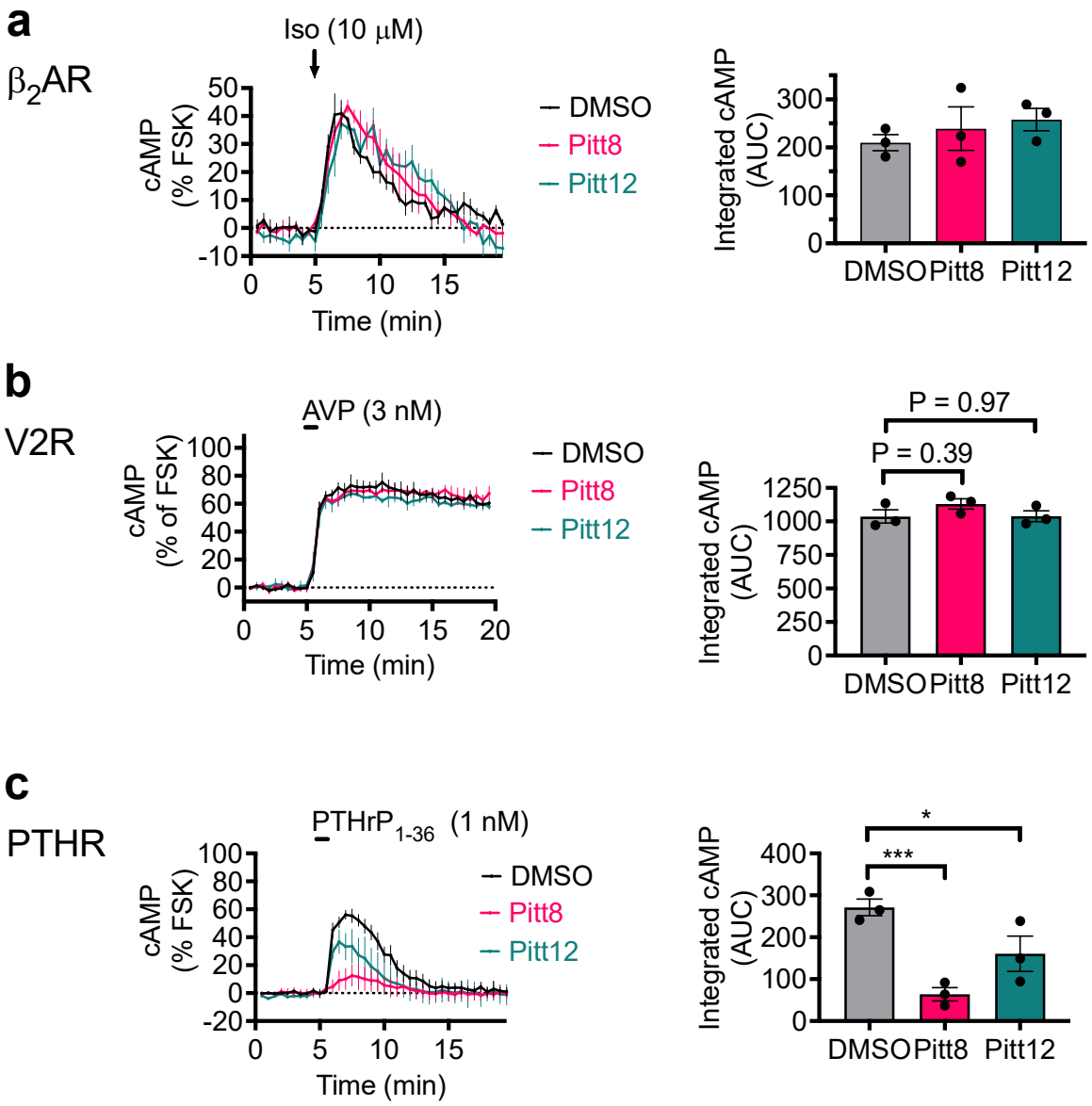
25		Fc1cccc(c1)-c1n[nH]c2CCN(Cc12)C(=O)CCn1ccc(=O)[nH]c1=O	383.383	1.041
26		Cc1cc(\C=C(/C#N)c2nc3ccccc3[nH]2)c(C)n1-c1sc(C)c(C)c1C(O)=O	416.5	5.411
27		[O-][N+](=O)c1cccc(\C=C2/CCCC3C(NN=C23)c2cccc(c2)[N+]([O-])=O)c1	378.388	4.387
28		Cc1n(nc2c(nnc(C)c12)N1CCN(CC1)C(=O)Nc1cc(C)cc(C)c1)-c1ccc(C)cc1	469.593	4.712
29		Cc1cccc(c1)C1N(CCc2ccc(OCc3nc(co3)C(=O)NC3CC3)cc12)C(=O)c1ccco1	497.551	4.835
30		CCC(=O)Nc1cccc(c1)-c1noc(n1)C1CCCN(C1)C(=O)c1cc2ccccc2[nH]1	443.507	4.586



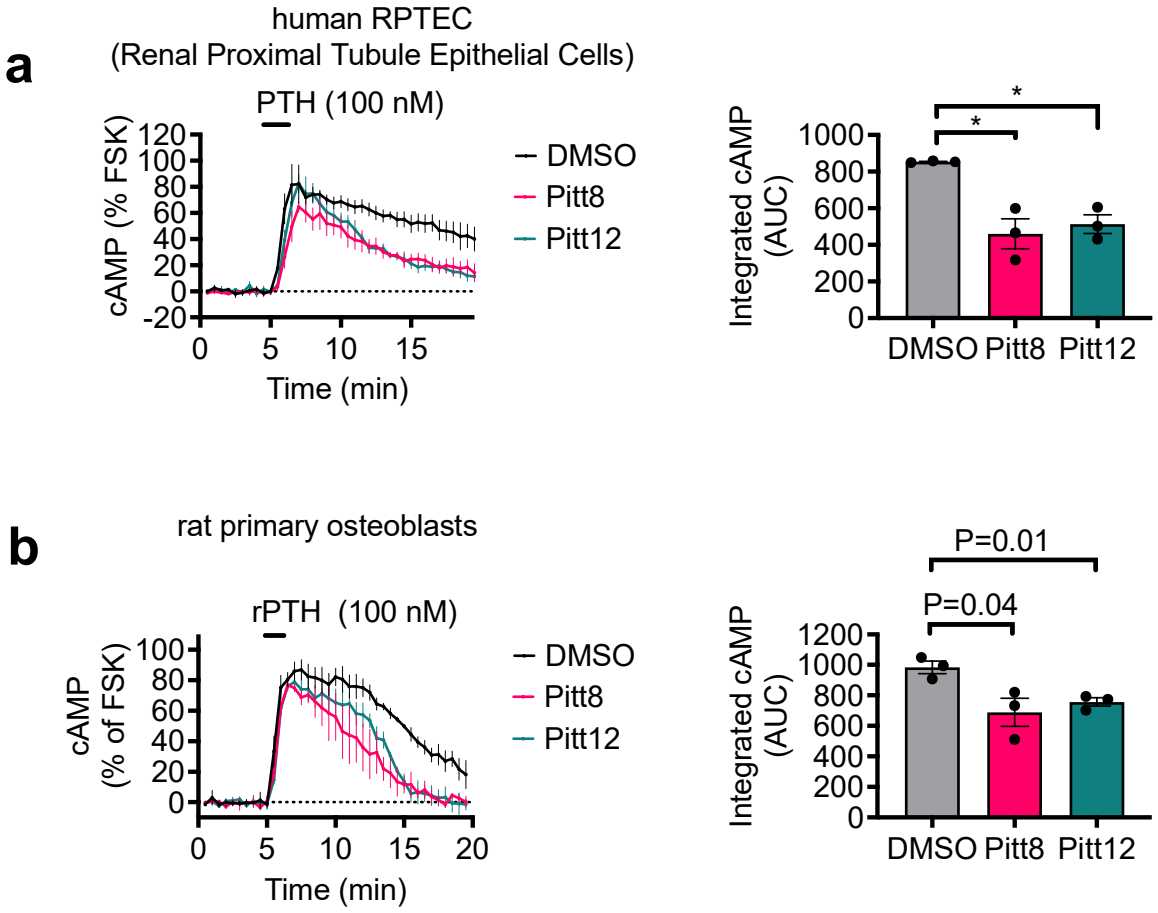
Supplementary Figure 1. Effect of computationally identified small molecules on PTH-induced cAMP production. Averaged cAMP time-courses following brief stimulation with 1 nM PTH_{1-34} without (*black*) or with (*red*) 10 μM corresponding compounds measured by FRET changes from HEK293 cells stably expressing the recombinant human PTHR. The bars graph represents the area under the curve (AUC) of cAMP time-courses. Data were normalized to the maximal forskolin induced cAMP response, which is set to 100%. Error bars represent the mean values ± s.d. of $N = 3$ (Pitt8 and Pitt12) or $N = 2$ (all the rest compounds) independent experiments.



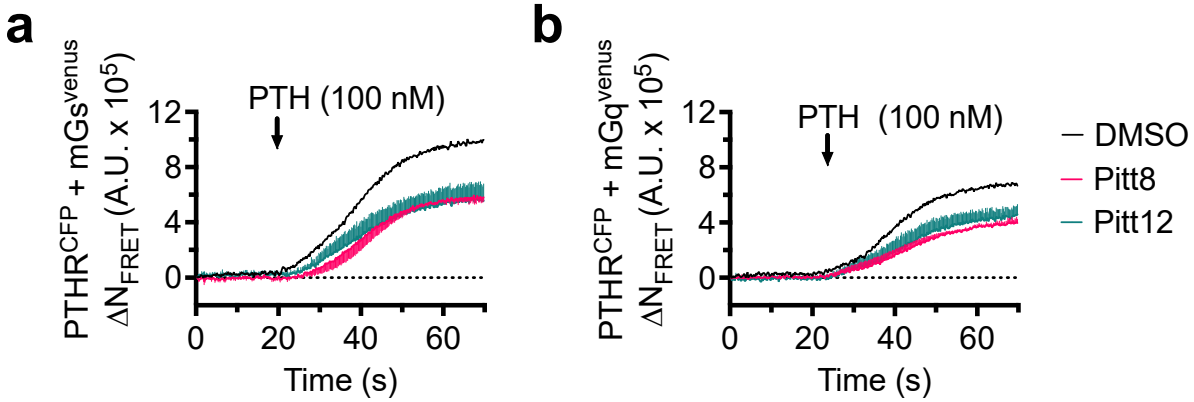
Supplementary Figure 2. Basal cAMP controls. (a, b) Effects of Pitt8 and Pitt12 on Fsk-mediated cAMP in HEK-293 cells recorded via either the Glo-sensor (a) or FRET (b) assays. Mean ± s.d. of N = 4 (Pitt12), and 3 (Pitt8) experiments carried out in duplicate for (a), and N = 3 experiments for (b).



Supplementary Figure 3. Selectivity of compounds. (**a–c**) Averaged cAMP time-courses following brief stimulation with either isoproterenol (Iso), vasopressin (AVP) or $PTHrP_{1–36}$ without (*black*) or with (*colored*) 10 μM compounds measured by FRET changes from HEK293 cells expressing the β_2-adrenergic receptor (β_2AR, panel a), a HA-tagged vasopressin type 2 receptor (V2R, panel b), or the PTHR (panel c) and a FRET-based cAMP sensor $Epac^{CFP/YFP}$. Bars represent the corresponding quantitation of cAMP responses by measuring the area under the curve (A.U.C.) from 0 to 20 min. Data are the mean ± s.e.m. of $N = 3$ independent experiments with $n = 16$ (DMSO), 21 (Pitt8), 16 (Pitt12) cells examined in panel a, $n = 41$ (DMSO), 29(Pitt8), 36 (Pitt12) cells examined in panel b, and $n = 54$ (DMSO), 49 (Pitt8), 48 (Pitt12) cells examined in panel c. *P* values were assessed by two-tailed Student's *t*-test and are **P*=0.037, ****P*=0.012.



Supplementary Figure 4. Effect of Selected Pitt molecules on native PTHR. (**a, b**) Averaged cAMP time-courses following brief stimulation with PTH_{1-34} without (*black*) or with (*colored*) 10 µM Pitt molecules measured by FRET changes from RPTEC (a) or osteoblasts (b) expressing the FRET-based cAMP sensor $Epac^{CFP/YFP}$. Bars represent the corresponding quantitation of cAMP responses by measuring the area under the curve (A.U.C.) from 0 to 20 min. Data are the mean ± s.e.m. of $N = 3$ independent experiments with $n = 29$ (DMSO), 44 (Pitt8) and 33 (Pitt12) cells examined in panel a, and $n = 23$ (DMSO), 26 (Pitt8) and 26 (Pitt12) cells examined in panel b. *P* values were assessed by Paired two-tailed Student's *t*-test and are **P*=0.041 (Pitt8) and **P*=0.023 (Pitt12).



Supplementary Figure 5. Effect of small molecules on G-protein coupling to PTHR. (**a, b**) Time-course recorded by single cell FRET assay in HEK-293 cells expressing PTHR-CFP and mini-G proteins mGs-YFP (a) or mGq-YFP (b) in response to PTH ± the indicated Pitt molecule. Mean ± s.e.m. of $N = 3$ experiments.