Supplementary Information

Assaying specificity and cytotoxicity of BT362

To check specificity of our new molecule we used the following cell lines and measured the inhibition of cAMP 1) **Sertoli cell line TM4 -** These FSH receptor expressing cells were obtained from ATCC (CRL-1715) and cultured in DMEM: F12 medium with 2.5% FBS and 5% horse serum. 2) **LH/hCGR HEK cells -** The specificity against the LH/hCG receptor was tested using a stable line of rat LH/hCG receptor expressing HEK 293 cells that were kindly provided by Dr K.M.J Menon, University of Michigan, Ann Arbor, Michigan. These cells were cultured in DMEM medium with 10% FBS and 100IU/ml of penicillin, 100µg/ml streptomycin. 30000 cells of the above cells were seeded in clear bottom black 96 well plates in their respective mediums. As described above the cells were first preincubated with BT360 or K14 with increasing doses for 1hr at 37°C. Following this FSHR cells were stimulated with 20ng/ml of FSH and LH/hCGR cells were stimulated with 0.5Iu/ml of hCG for 1hr at 37°C. cAMP was measured in these cells using HTRF assay.

Cytotoxicity by Alamar Blue: Cytoxicity was measured using Alamar Blue HR , which monitors the reducing environment of the living cell by resazurin, which changes from the oxidized, non-fluorescent, blue state to the reduced, fluorescent, pink state. These studies were carried out on CHO cells grown on black clear bottom 96 well plates. Cells in the log phase were harvested by trypsin and seeded as $30x10^3$ cells/well and allowed

to adhere to the bottom of plate in complete HamF12 medium incubating the cells overnight at 37˚C. The cells were then exposed to BT362 and VA-K14 at different doses for 48 hr at 37°C Following 48 hrs of treatment, Alamar Blue was aseptically added to each well in an amount equal to 10% of the volume in the wells. Cells with Alamar Blue HR were further incubated at 37˚C for another 5hr prior to reading the plates. Cytotoxicity was assessed by measuring fluorescence intensity of the reduced dye at 540/580nm. Wells with medium only was used as the background control and results of treated cells was compared to untreated for analyzing the cytotoxicity of the small molecule. Log change between untreated over that of treated groups was deduced from the fluorescent intensities obtained after back

Supplementary Table 1. Helix-helix angle changes

	2		3		4		5		61		6.2		7.1		7.2		8	
1	-3.2	S	13.5	n	-7.1	S	16.2	n	-16.6	n	-3.4	S	32.0	n	-6.0	S	-15.1	S
2			-15.6	S	-2.5	S	-22.5	S	8.4	S	17.1	S	-25.6	S	11.6	S	13.6	S
3					5.1	S	7.6	S	3.3	S	-16.1	n	14.2	S	-10.9	S	-15.1	S
4							-5.7	S	3.4	S	18.4	S	-23.1	S	10.1	S	6.7	S
5									0.4	S	-14.1	S	-4.5	S	25.8	S	-21.8	S
6.1											13.2	S	-17.9	S	9.5	S	11.8	S
6.2													25.6	S	-29.7	S	33.4	S
7.1															11.3	S	-31.9	S
7.2																	-4.4	S

Positive number indicates an increase w.r.t. the starting structure. The character ‘S’ indicates that the reference value is outside the range of the representative structure values.

Supplementary Table 2. Proline kink results

Proline		Bend		Wobble		Face-shift		Pseudo-rotation	
232	Reference	35.2		-76.5		91.8	S	86.9	S
	Average	37.9		-59.2		88.9		--90.95	
	Minimum	24.9		-81.0		-159.2		-99.7	
	Maximum	56.4		-20.6		-134.2		86.5	
261	Reference	24.4		175.9	S	-0.8	S	-119.0	S
	Average	15.8		-71,4		71.5		-83.3	
	Minimum	4.7		-142..4		42.1		-105.0	
	Maximum	25.7		-41.7		86.0		-70.0	
268	Reference	37.2		138.4	S	-7.3	S	-117.8	S
	Average	39.8		-79.5		65.6		-108.3	
	Minimum	31.1		-88.4		-154.8		-115.8	
	Maximum	43.0		-71.7		-137.1		-98.8	

When the reference value is outside the range of the representative structures' value the difference is marked with 'S'.