

## SUPPORTING INFORMATION

### A Survey of Stapling Methods to Increase Affinity, Activity, and Stability of Ghrelin Analogues

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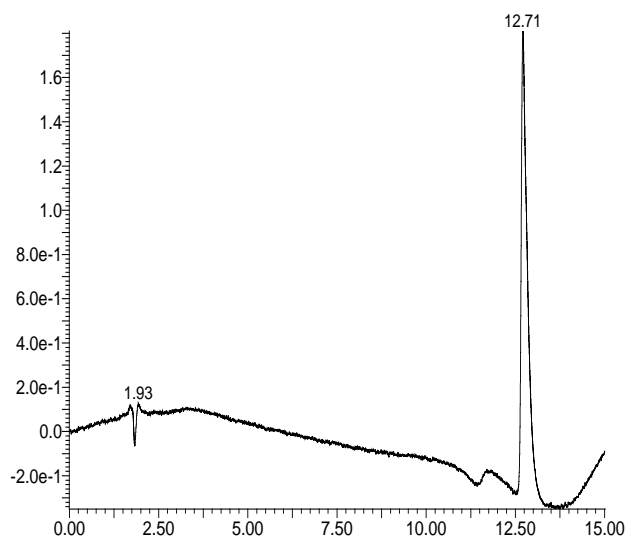
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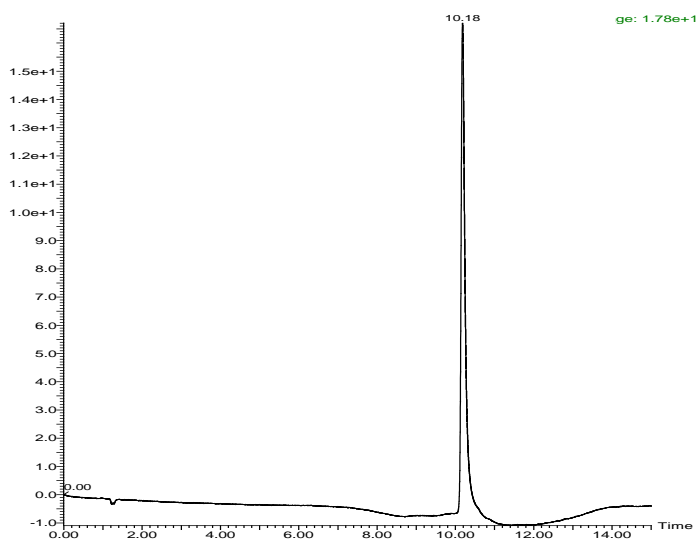
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Analytical HPLC Results for Compounds <b>1-5</b> .....	S2
Mass Spectra for Compounds <b>1-5</b> , [ <b>Dpr<sup>3</sup></b> ]Ghrelin.....	S4
IC <sub>50</sub> Displacement Curves for Compounds <b>1-5</b> , [ <b>Dpr<sup>3</sup></b> ]Ghrelin, and <b>Human Ghrelin</b> .....	S8
Circular Dichroism Spectra for Compounds <b>1-5</b> , <b>Human Ghrelin</b> .....	S11
Statistical Analysis of $\beta$ -arrestin Recruitment BRET Assays.....	S15

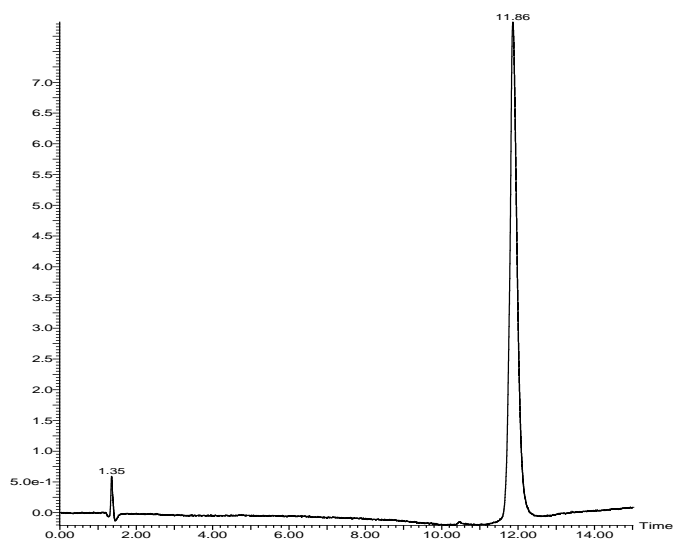
## Analytical HPLC Results for Compounds 1-5



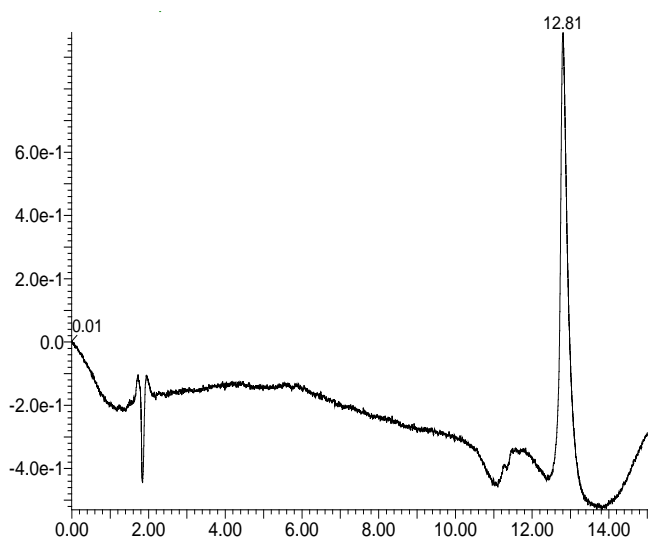
**Figure S1.** Analytical UV chromatogram from HPLC of purified compound **1** (20-80% acetonitrile/water)



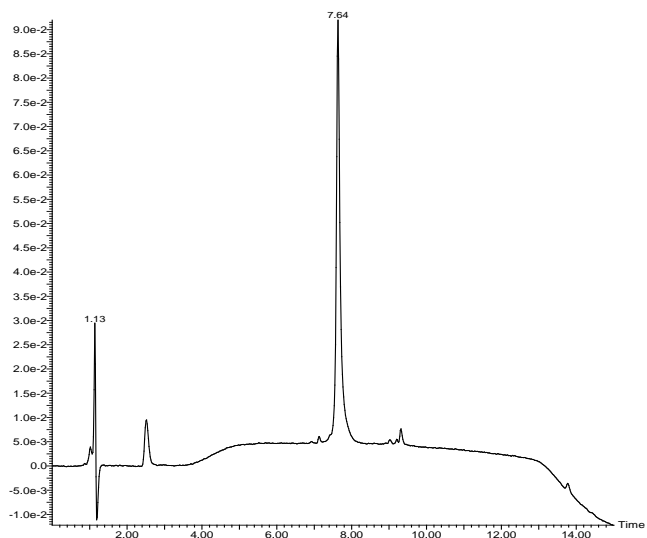
**Figure S2.** Analytical UV chromatogram from HPLC of purified compound **2** (20-80% acetonitrile/water)



**Figure S3.** Analytical UV chromatogram from HPLC of purified compound **3** (20-50% acetonitrile/water)

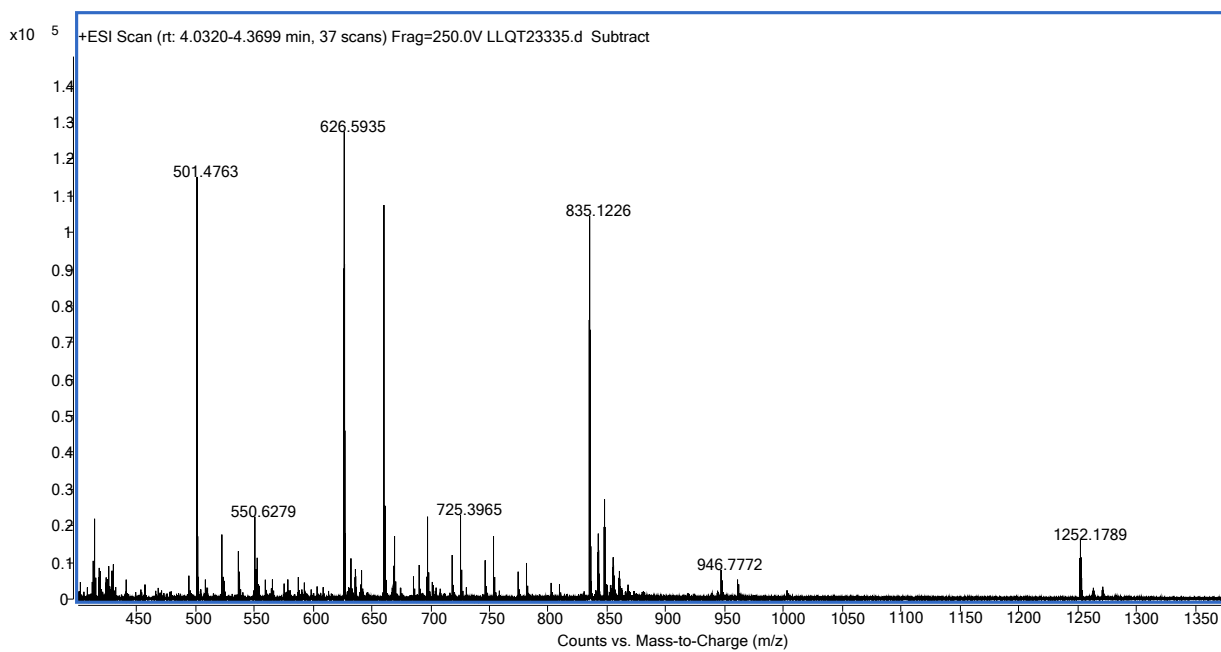


**Figure S4.** Analytical UV chromatogram from HPLC of purified compound **4** (20-80% acetonitrile/water)

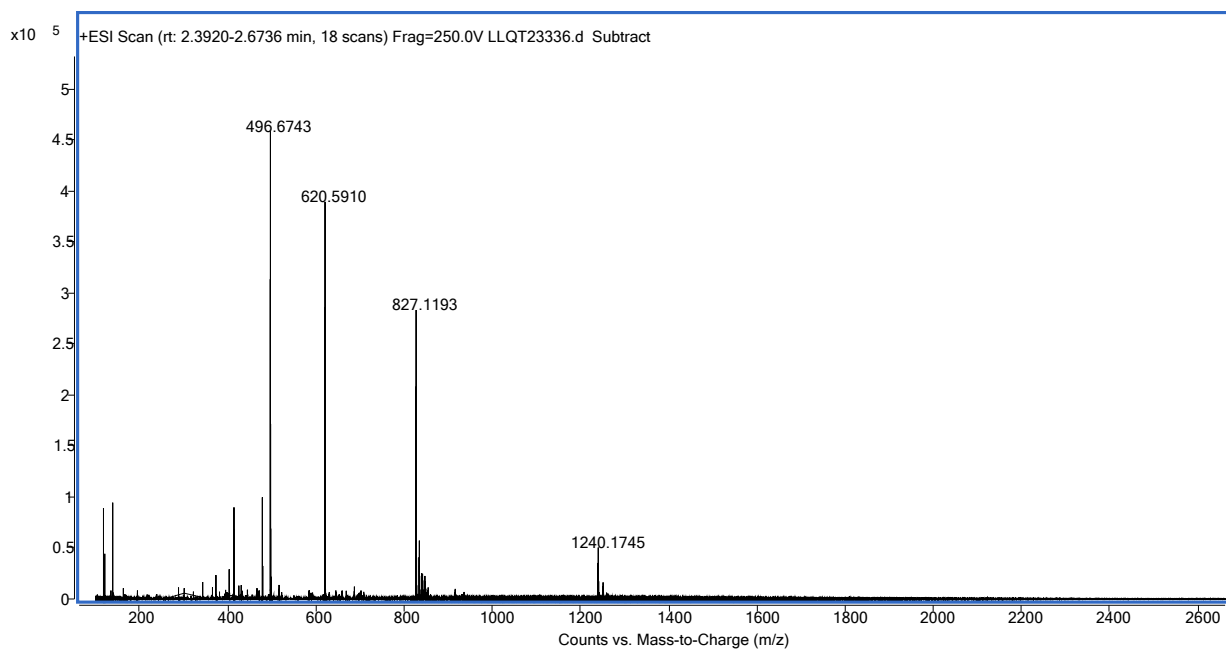


**Figure S5.** Analytical UV chromatogram from HPLC of purified compound **5** (10-70% acetonitrile/water).

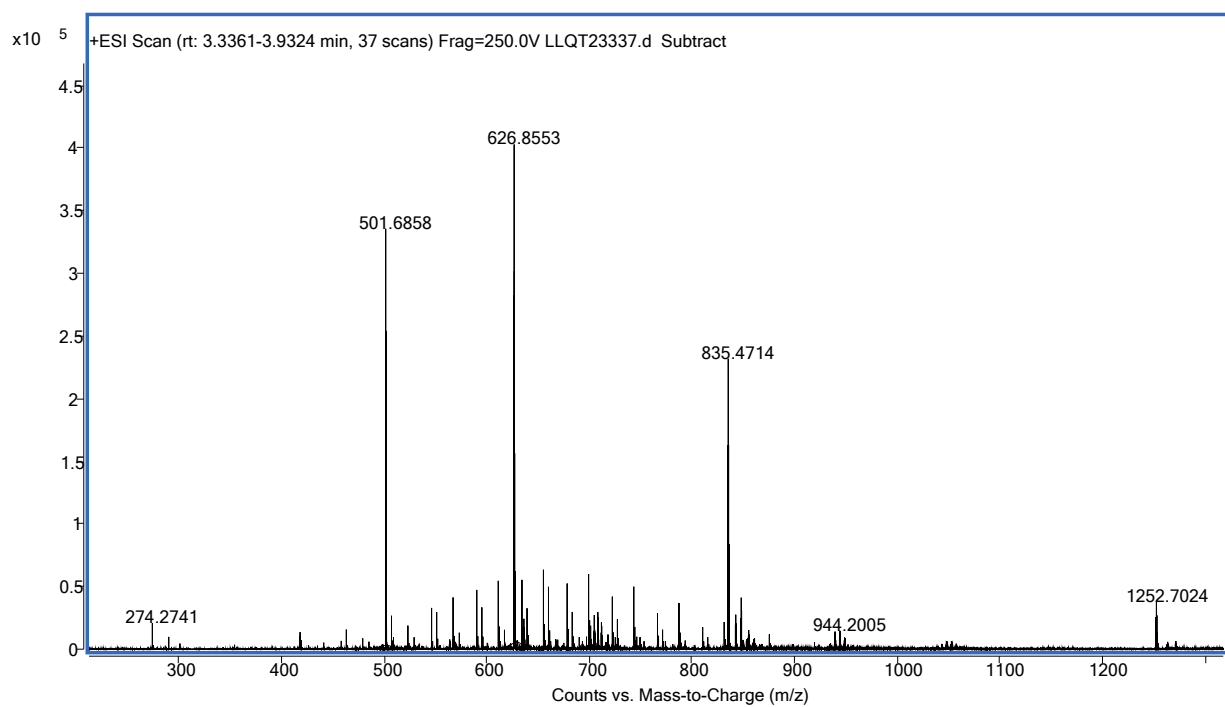
## Mass Spectra for Compounds **1-5**, [Dpr<sup>3</sup>]Ghrelin



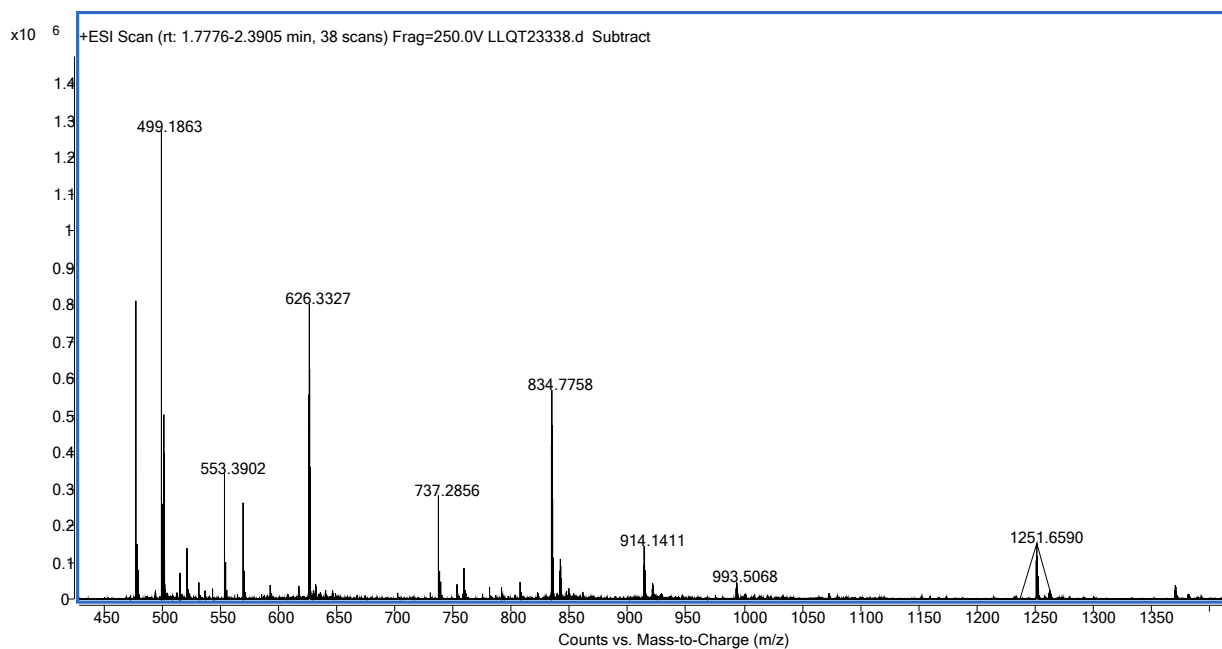
**Figure S6.** ESI+ Mass Spectrum of compound **1**.



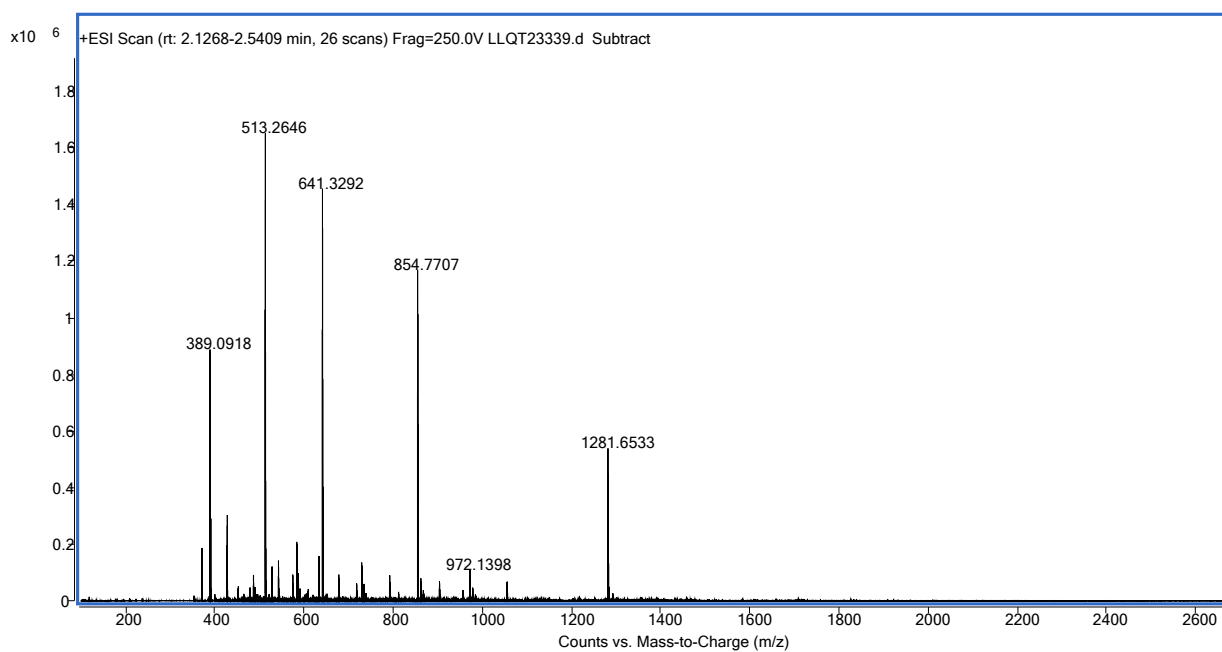
**Figure S7.** ESI+ Mass Spectrum of compound **2**.



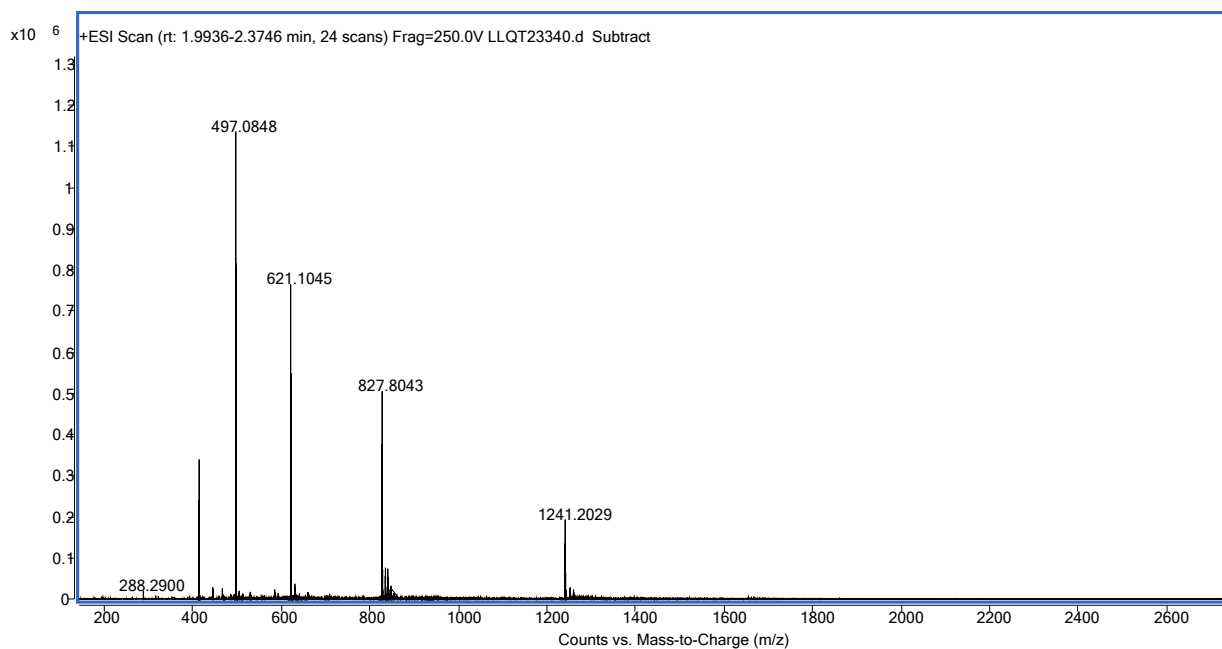
**Figure S8.** ESI+ Mass Spectrum of compound **3**.



**Figure S9.** ESI+ Mass Spectrum of compound **4**.



**Figure S10.** ESI+ Mass Spectrum of compound **5**.

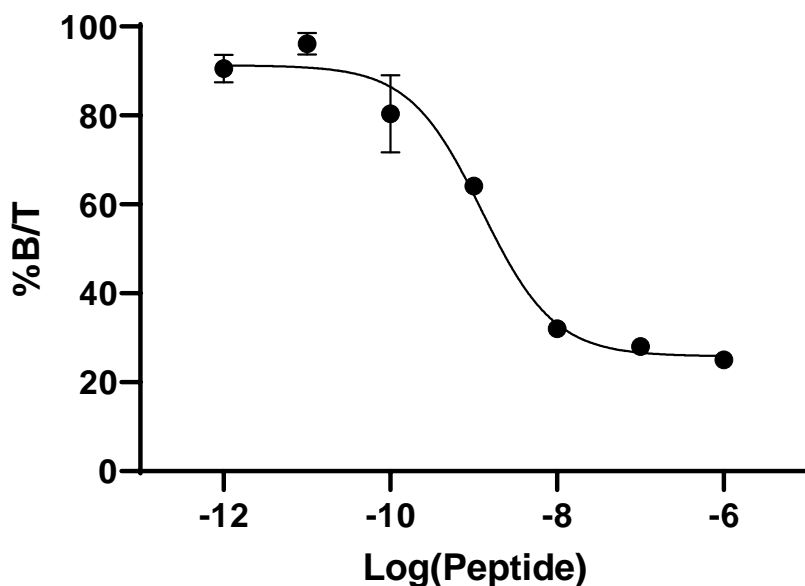


**Figure S11.** ESI+ Mass Spectrum of **[Dpr<sup>3</sup>]Ghrelin(1-20)**.

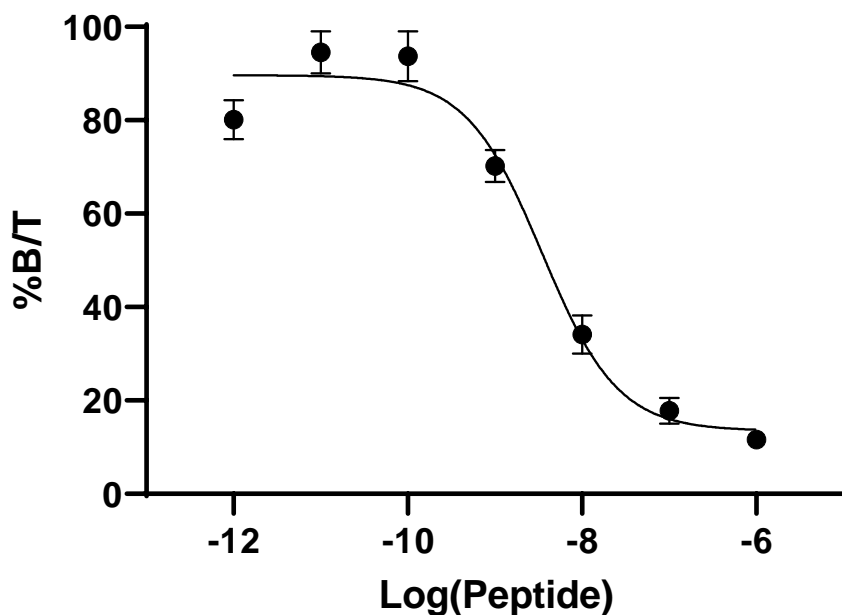
**Table S1.** HRMS for compounds **1-5** and **[Dpr<sup>3</sup>]Ghrelin(1-20)**.

Compound	Sequence	[M+3H] <sup>3+</sup> Calculated	[M+3H] <sup>3+</sup> Observed
<b>[Dpr<sup>3</sup>]Ghrelin(1-20)</b>	H-GS-Dpr(octanoyl)- FLSPEHQRVQQRKESKK-OH	827.4663	827.4700
<b>1 - Triazole Staple</b>	H-GS-Dpr(octanoyl)- FLSPEHQR[XQQRY]ESKK-OH	834.7866	834.7880
<b>2 - Lactam Staple</b>	H-GS-Dpr(octanoyl)- FLSPEHQR[KQQRD]ESKK-OH	826.7829	826.7851
<b>3 - Hydrocarbon Staple</b>	H-GS-Dpr(octanoyl)- FLSPEHQR[OQQRO]ESKK-OH	835.1351	835.1368
<b>4 - Glaser Staple</b>	H-GS-Dpr(octanoyl)- FLSPEHQR[ZQQRZ]ESKK-OH	834.4390	834.4411
<b>5 - Xylene-Thioether Staple</b>	H-GS-Dpr(octanoyl)- FLSPEHQR[CQQRC]ESKK-OH	854.4342	854.4370

## IC<sub>50</sub> Displacement Curves for Compounds 1-5, [Dpr<sup>3</sup>]Ghrelin, and Human Ghrelin

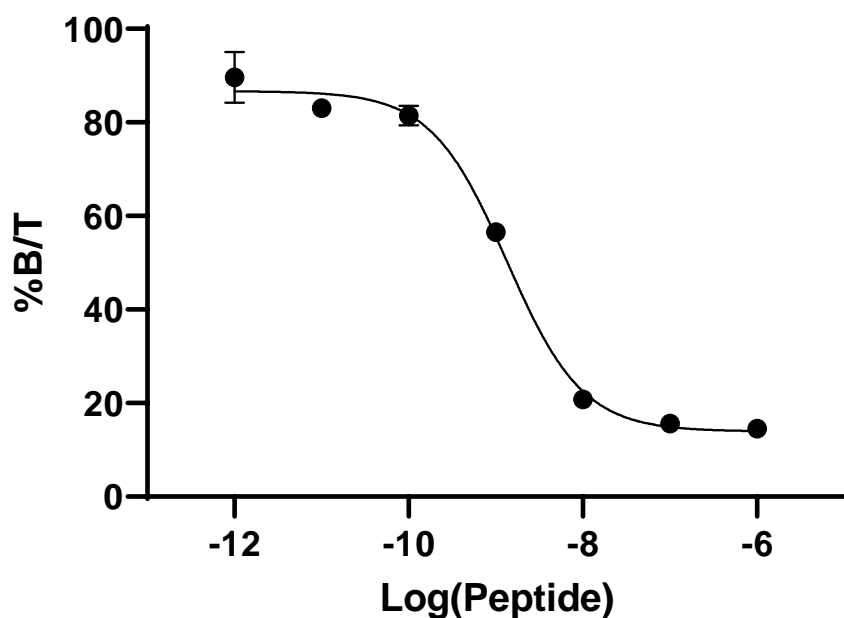


**Figure S12.** IC<sub>50</sub> curve for compound 1. Log concentration of compound (M) plotted against %B/T, denoting the percentage of bound [<sup>125</sup>I]-ghrelin to the GHSR-1a transfected HEK293 cells.

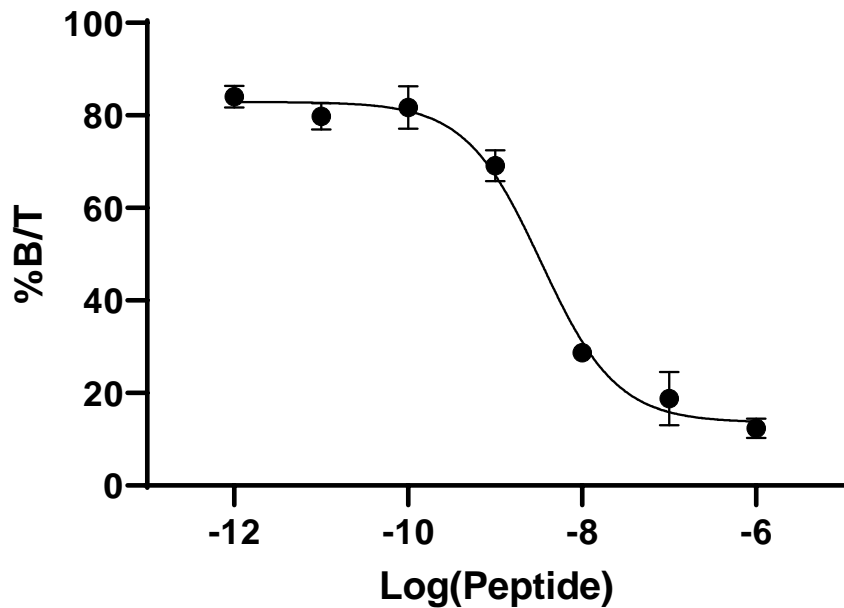


**Figure S13.** IC<sub>50</sub> curve for compound 2. Log concentration of compound (M) plotted against %B/T, denoting the percentage of bound [<sup>125</sup>I]-ghrelin to the GHSR-1a transfected HEK293 cells.

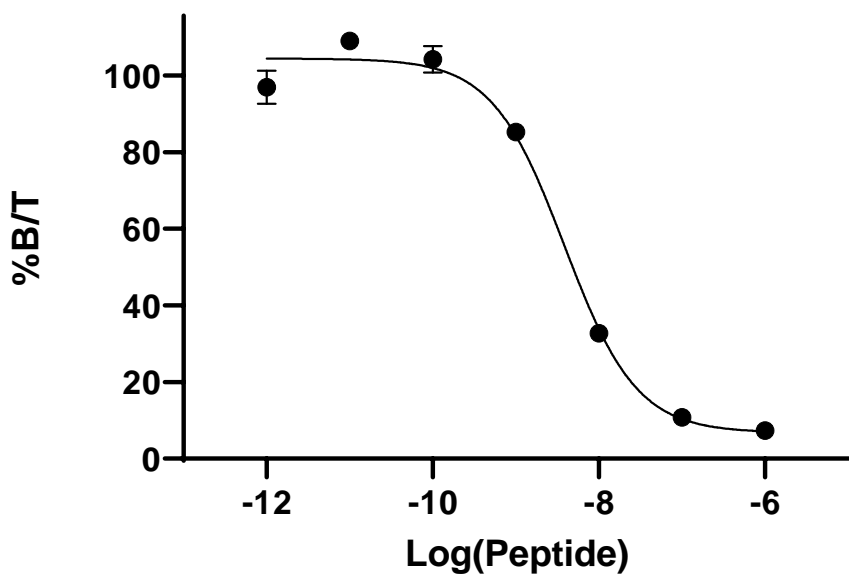




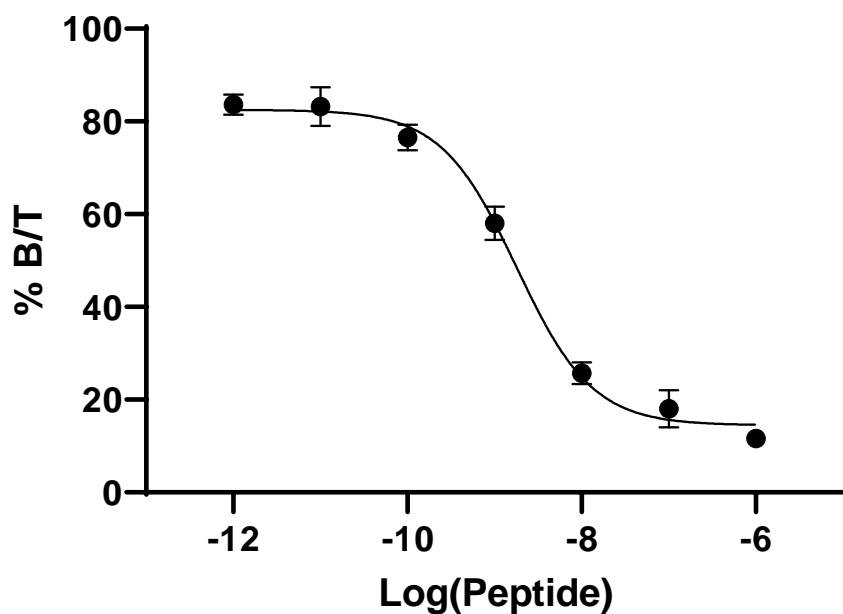
**Figure S14.**  $IC_{50}$  curve for compound 3. Log concentration of compound (M) plotted against %B/T, denoting the percentage of bound  $[^{125}\text{I}]$ -ghrelin to the GHSR-1a transfected HEK293 cells.



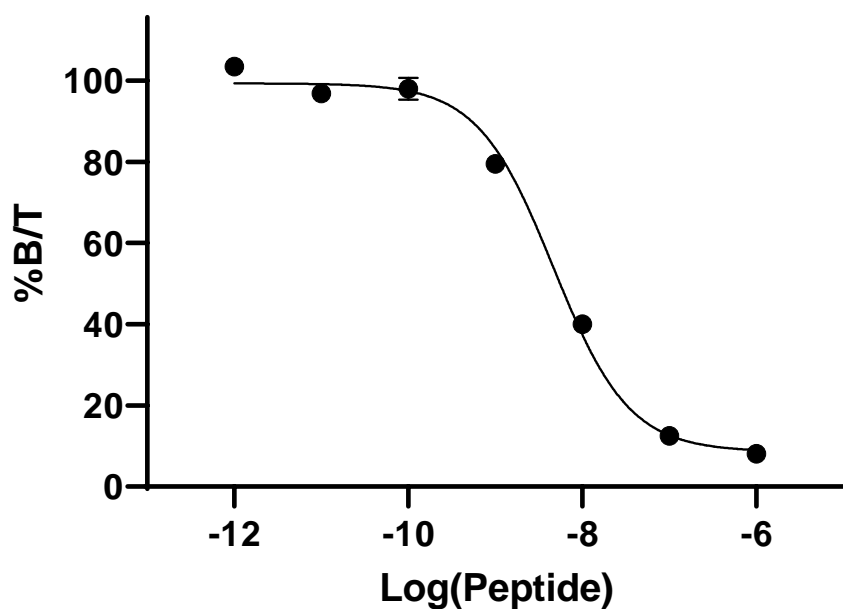
**Figure S15.**  $IC_{50}$  curve for compound 4. Log concentration of compound (M) plotted against %B/T, denoting the percentage of bound  $[^{125}\text{I}]$ -ghrelin to the GHSR-1a transfected HEK293 cells.



**Figure S16.** IC<sub>50</sub> curve for compound 5. Log concentration of compound (M) plotted against %B/T, denoting the percentage of bound [<sup>125</sup>I]-ghrelin to the GHSR-1a transfected HEK293 cells.

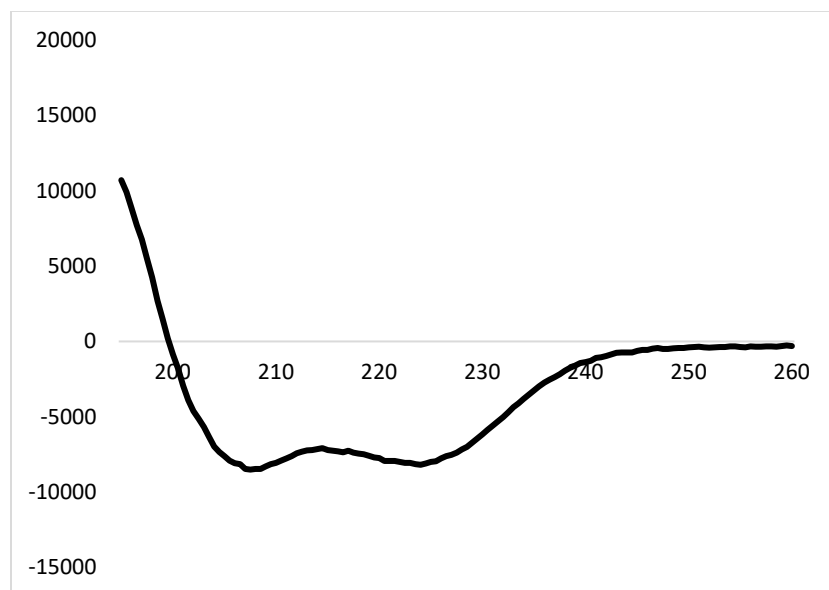


**Figure S17.** IC<sub>50</sub> curve for [Dpr<sup>3</sup>]Ghrelin(1-20). Log concentration of compound (M) plotted against %B/T, denoting the percentage of bound [<sup>125</sup>I]-ghrelin to the GHSR-1a transfected HEK293 cells.

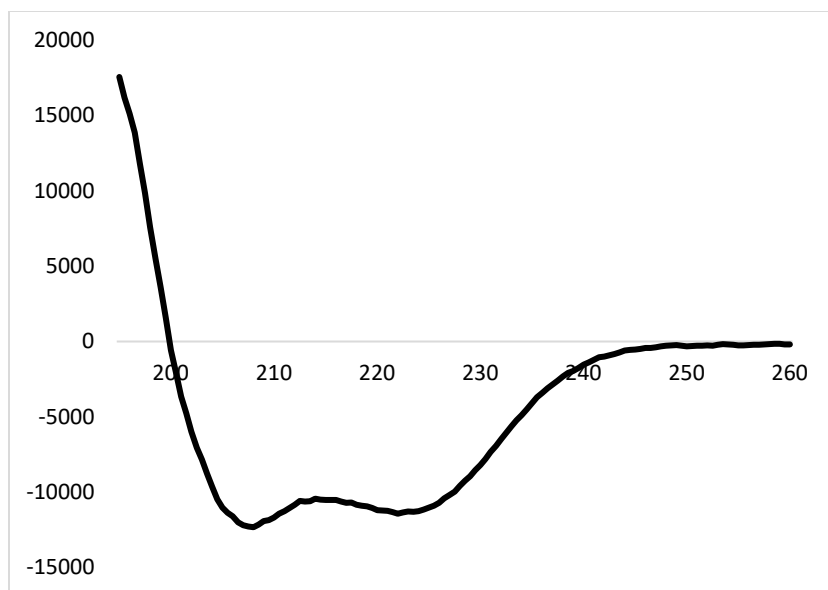


**Figure S18.** IC<sub>50</sub> curve for Human ghrelin. Log concentration of compound (M) plotted against %B/T, denoting the percentage of bound [<sup>125</sup>I]-ghrelin to the GHSR-1a transfected HEK293 cells.

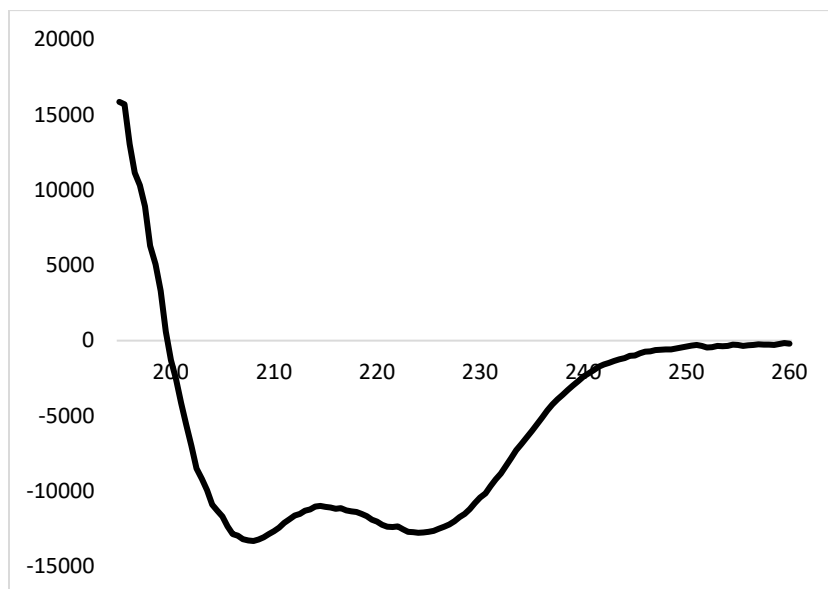
### Circular Dichroism Spectra for Compounds **1-5**, **Human Ghrelin**



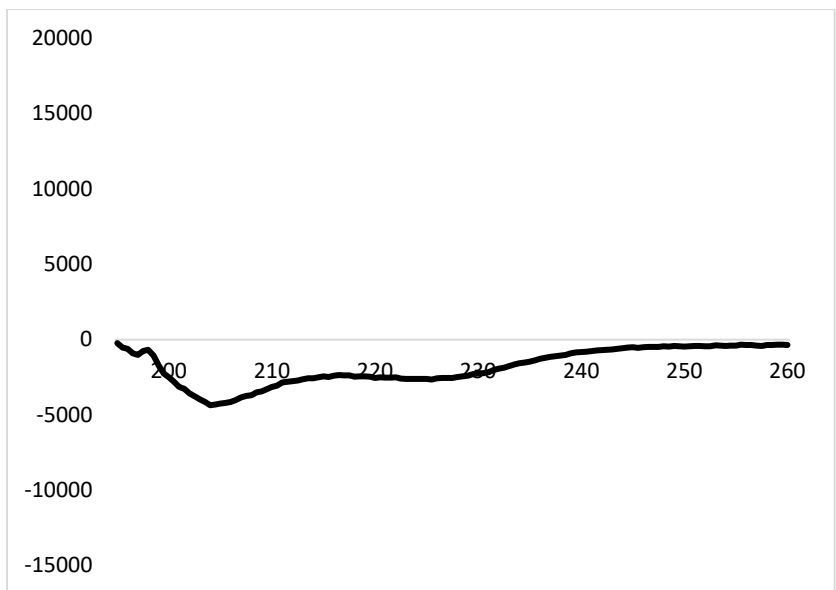
**Figure S19.** CD spectra for compound **1**



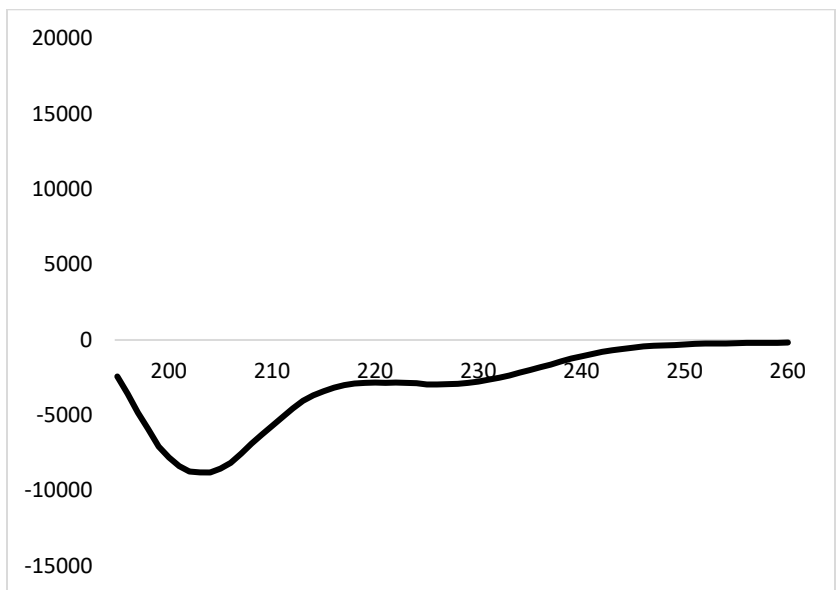
**Figure S20.** CD spectra for compound 2



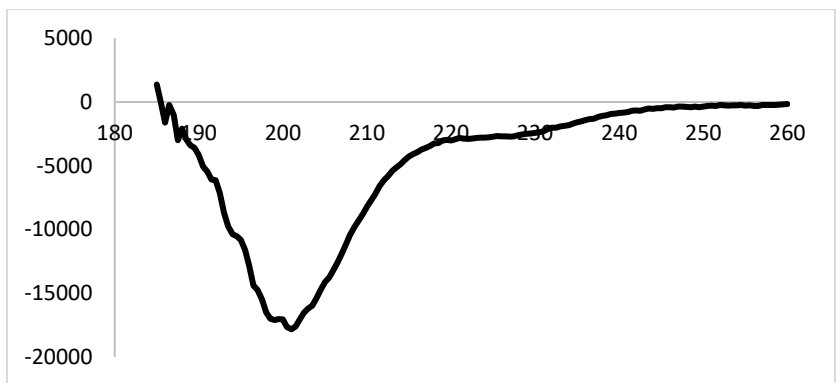
**Figure S21.** CD spectra for compound 3



**Figure S22.** CD spectra for compound **4**



**Figure S23.** CD spectra for compound **5**



**Figure S24.** CD spectra for Human Ghrelin

## Statistical Analysis of $\beta$ -arrestin Recruitment BRET Assays

**Table S2.** Statistical analysis *t*-test and *p* values of  $\beta$ -arrestin 1 Recruitment BRET Assays.

$\beta$ -arrestin 1					
	<b>E<sub>max</sub></b>	<b>SEM</b>	<b>N</b>	<b>P value</b>	<b>P value summary</b>
<b>[Dpr<sup>3</sup>]Ghrelin(1-20)</b>	0.331	0.027	24		
<b>1 - Triazole Staple</b>	0.259	0.016	18	0.0394	*
<b>2 - Lactam Staple</b>	0.220	0.016	18	0.002	**
<b>3 - Hydrocarbon Staple</b>	0.383	0.030	9	0.2828	ns
<b>4 - Glaser Staple</b>	0.435	0.028	9	0.0347	*
<b>5 - Xylene-Thioether Staple</b>	0.455	0.029	12	0.0068	**

**Table S3.** Statistical analysis *t*-test and *p* values of  $\beta$ -arrestin 2 Recruitment BRET Assays.

$\beta$ -arrestin 2					
	<b>E<sub>max</sub></b>	<b>SEM</b>	<b>N</b>	<b>P value</b>	<b>P value summary</b>
<b>[Dpr<sup>3</sup>]Ghrelin(1-20)</b>	0.550	0.028	23		
<b>1 - Triazole Staple</b>	0.394	0.022	18	0.0002	***
<b>2 - Lactam Staple</b>	0.388	0.020	18	<0.0001	****
<b>3 - Hydrocarbon Staple</b>	0.547	0.044	9	0.9529	ns
<b>4 - Glaser Staple</b>	0.544	0.042	8	0.9043	ns
<b>5 - Xylene-Thioether Staple</b>	0.609	0.028	12	0.192	ns