INDIGO Biosciences, Inc.
The Nuclear Receptor Company™

Indigo Biosciences, Inc
1981 Pine Hall Road
State College, PA 16801

T:(814) 234-1919
F:(814) 272-0152
www.indigobiosciences.com

FINAL REPORT # R081202

Evaluation of USP Test Compounds 1 & 2 for Antagonist Activity against the Human Glucocorticoid Receptor (GR)

AUTHOR

Bruce A. Sherf, Ph.D.
Chief Technology Officer, Indigo Biosciences, Inc

STUDY REPORT COMPLETED ON

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PERFORMING LABORATORY

Indigo Biosciences, Inc
1981 Pine Hall Road, State College, PA 16801, USA

LABORATORY PROJECT IDENTIFICATION

Invoices 081114-2

SPONSOR

USP Labs, LLC; Attn. Cy Willson
3941 Waterford Way
Denton, TX, 76210

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Signature Page

Study Director	*(signature on file)*
CTO	Bruce A. Sherf, Ph.D.
Laboratory Personnel	*(signature on file)*
	Ewa Maddox
Management	*(signature on file)*
CSO	John P.Vanden Heuvel, Ph.D

Statement of Quality Assurance

The Indigo Biosciences, Inc. officials listed above have inspected the study and reported any quality issues to the Client.

This final report reflects Indigo Biosciences' interpretation of the primary data generated during this study.

Phase of study	Date of inspection	Reported to Study Director and to Client
Study Plan: Quote		
Conduct of Study	11/17/08 – 11/21/08	
Report	12/02/08	

Approved *(signature on file)*
Date 10/22/08 Dr. John P.Vanden Heuvel

SUMMARY OF FINDINGS

The data presented herein support the following overview of this study's findings.

		Test Compounds, USP Labs Study	
		Compound 1 (Tar)	**Compound 2 (Tea)**
Human Glucocorticoid Receptor (GR) *section 3.1*	**Agonist Activity**	*not tested*	*not tested*
	Antagonist Activity	None	None
	Observed Cyto-toxicity	None	None

1. INTRODUCTION

1.1 AIM OF STUDY

The aim of this study was to evaluate two test compounds for possible antagonist activity to human Glucocorticoid Receptor. Test compounds were supplied by the study sponsor, USP Labs, LLC.

1.2 SELECTION OF DOSES

The Sponsor selected a six point dilution series of each test compound for evaluation, as described in Section 2.1.

1.3 RETENTION OF RECORDS

Unless otherwise requested, Indigo Biosciences will retain electronic versions of all quotes, reports to, and communications with, the study sponsor. All client information and study data is confidential, and will at no time be released to a third party without prior written consent from the client.

2. MATERIAL AND METHODS

2.1 TEST SUBSTANCES

USP Labs provided Indigo Biosciences with two test compounds for evaluation:

USP Test Compound 1 was provided as a solid mass, and had the appearance of hardened tar. This compound is henceforth designated **"Compound 1 (Tar)"**, or "Cmpd1 (Tar)". Compound 1 (Tar) was added to 95% methanol at a concentration equivalent to 1 gram (Gm) per 100 ml. This preparation was allowed to swirl overnight, at room temperature, in a tightly capped & foil-wrapped glass bottle. The sponsor advised us that the maximum solubility of cmpd 1 (Tar) is 89.07%. Immediately prior to assay, the solution was clarified *via* filtration. Based on the provided solubility information, the neat filtrate contained 890.7 µGm of compound 1 (Tar) per ml of solution. Serial dilutions were prepared using cell culture treatment media, as follows: 1/100 > 1/200 > 1/400 > 1/800 > 1/1600 > 1/3200. 500 µl of these treatment media dilutions were added to respective assay wells. Each dilution of test Compound 1 (Tar) was assayed in quadruplicate.

USP Test Compound 2 was provided as a finely flaked material, and had the appearance of crushed tea leaves. This compound is henceforth designated **"Compound 2 (Tea)"**, or "Cmpd 2 (Tea)". Compound 2 (Tea) was added to 95% methanol at a concentration equivalent to 1 gram (Gm) per 100 ml. This preparation was allowed to swirl overnight, at room temperature, in a tightly capped & foil-wrapped glass bottle. The sponsor advised us that the maximum solubility of cmpd 1 (Tar) is 89.02%. Immediately prior to assay, the solution was clarified via filtration. Based on the provided solubility information, the neat filtrate contained 890.2 µGm of compound 2 (Tea) per ml of solution. Serial dilutions were prepared using cell culture treatment media, as follows: 1/100 > 1/200 > 1/400 > 1/800 > 1/1600 > 1/3200. 500 µl of treatment media dilutions were added to respective assay wells. Each dilution of test Compound 1 (Tea) was assayed in quadruplicate.

In addition to evaluating the two test compounds, a dose-response assay was performed using the known GR agonist dexamethasone ("Dex"). Dexamethasone was initially prepared as a 10 mM stock in DMSO. Treatment doses were prepared *via* serial dilution using cell culture treatment media to obtain the following picoMolar (pM; 10^{-12} Molar) concentrations: 7.81, 15.6, 31.3, 62.5, 125, 250, 500 and 1000 pM. The "0 Dex" (i.e., media only) was performed using 8 assay replicates. The "0" control value was used to calculate the ratio of signal-to-noise for all assays. The "250 pM Dex" value corresponds to the level of GR activity expressed in the *absence* of added antagonist; therefore, it is the value to which all determined values for each test compound are compared in the antagonist assays.

2.2 ASSAYS PERFORMED

Three different variations of GR assays were performed in this study:

a. A "positive-control" Dexamethasone agonist dose-response assay was performed to validate the functionality and responsiveness of the GR reporter cells used at the specific time of this study.

b. A solution of Compound 1 (Tar) was prepared as previously described and used in a limiting dilution assay to assess potential GR antagonist activity of this test material.

c. A solution of Compound 2 (Tea) was prepared as previously described and used in a limiting dilution assay to assess potential GR antagonist activity of this test material.

2.3 EXPERIMENTAL PROCEDURES

These assays were conducted using reporter cells specific to the human GR nuclear receptor, the composition and preparation of which are proprietary to Indigo Biosciences. In general these assays employ a mammalian host cell expressing GR-responsive firefly (FF) luciferase as the experimental reporter gene, and sea pansy (*Renilla*) luciferase as an internal-control reporter gene. The expression of *Renilla* luciferase provides a quantitative measure of adverse cytological effects that may arise from exposure to the test compounds, and provides a convenient means of normalizing sets of experimental data within independent GR assays. Luminescence intensities from respective firefly and *Renilla* luciferase reactions are quantified using a plate-reading luminometer, and are reported in subjective terms of Relative Light Units (RLU).

All graphical representations of GR functional activities are presented as normalized reporter data, calculated by dividing FF luciferase RLU values by *Renilla* luciferase RLU values. Primary Luminometry data corresponding to the independent FF luciferase and *Renilla* luciferase measurements are provided in the Appendix, Section 3.1.5.

3. ASSAY RESULTS & ASSESSMENT OF FINDINGS

Averaged and Normalized (FF luc/*Renilla* luc) RLU values, as well as respective Standard Deviations (StDev), percent Coefficients of Variation (%CV), "Ratio to Media" and "- Fold Reduction" relative to the control treatment, were calculated for USP Test Compounds #1 (Tar) and #2 (Tea), and dexamethasone positive-control agonist. These calculations were performed using Microsoft Excel software

Non-linear curve-fitting of transformed reporter data, and EC_{50} calculations were performed. Additionally, normalized RLU values for each individual measurement within a set of minimally four replicates were analyzed by 1-way ANOVA followed by Dunnett's post-test to determine statistical significance, if any, of differences observed between test compound(s) and the control values. These analyses and graphing manipulations were performed using GraphPad Prism, v.5.0.

Figures embedded in each section provide graphical representations of normalized average RLU values, and their respective standard deviation values, for each treatment dose.

3.1 GLUCOCORTICOID RECEPTOR (GR) ANTAGONIST ASSAYS

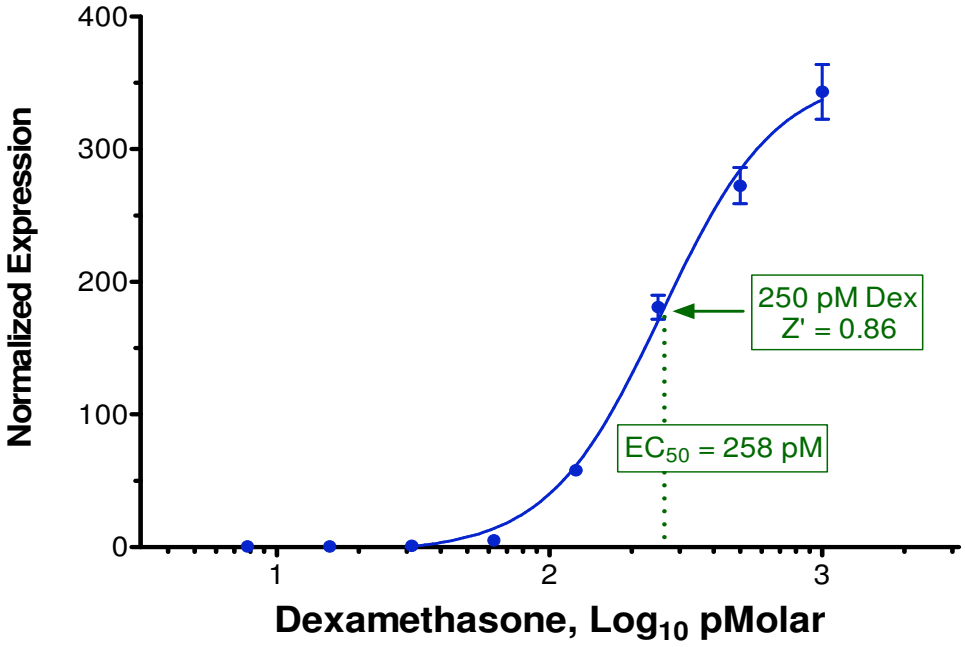
3.1.1 GR Validation Assay: Dexamethasone Dose-Response

Dexamethasone (Dex), a potent agonist of GR, was used to validate the functionality of the GR reporter cells used in this study. Averaged then normalized RLU values were calculated from the primary Firefly and *Renilla* luciferase data, as were values for %CV and "Ratio to Media" (**Table 3.1.2A**). Normalized RLU values were curve-fit against Log_{10}-transformed picoMolar concentrations of dexamethasone. For the reporter cells used in this assay group, the EC_{50} value for dexamethasone was determined to be **258 pM** (**Figure 3.1.2A**).

Table 3.1.1A

GR Assay, Normalized (Fire fly / Renilla Luciferase) Dexamethasone Dose-Response									Ratio to Media Control
[Dex] pM	#1	#2	#3	#4	**Norm Ave**	**StDev**	**%CV**	**Z'**	
0.000	0.480	0.457	0.448	0.529	**0.569**	**0.109**	**19.2**		**1.00**
	0.559	0.687	0.706	0.687					
7.81	0.606	0.591	0.720	0.659	**0.644**	**0.0584**	**9.07**		**1.13**
15.6	0.596	0.682	0.641	0.724	**0.661**	**0.0551**	**8.34**		**1.16**
31.3	1.067	0.990	0.911	0.881	**0.962**	**0.0836**	**8.68**		**1.69**
62.5	4.85	5.14	5.32	5.17	**5.12**	**0.198**	**3.86**	**0.798**	**9.00**
125	55.3	60.8	55.2	60.8	**58.0**	**3.17**	**5.47**	**0.829**	**102**
200	128	138	138	132	**132**	**6.46**	**4.89**	**0.850**	**232**
	131	136	133	119					
250	173	184	176	191	**181**	**8.25**	**4.56**	**0.861**	**318**
500	270	255	278	287	**272**	**13.8**	**5.05**	**0.847**	**479**
1000	323	348	333	369	**343**	**19.8**	**5.78**	**0.825**	**603**

Figure 3.1.2A
GR Validation Assay
Dexamethasone Dose-Response
(Normalized Data)



Best-fit values	
Bottom	-3.884
Top	357.6
LogEC50	2.412
HillSlope	2.08
EC50	258.3
Span	361.5

Std. Error	
Bottom	6.127
Top	17.35
LogEC50	0.032
HillSlope	0.271
Span	20.06

95% Confidence Intervals	
Bottom	-20.89 to 13.12
Top	309.4 to 405.8
LogEC50	2.322 to 2.502
HillSlope	1.329 to 2.831
EC50	210.0 to 317.7
Span	305.8 to 417.2

Goodness of Fit	
R^2	0.997

3.1.2 USP Test Compound #1 (Tar): GR Antagonist Assay

Treatment media were prepared to contain six concentrations of USP Test Compound #1 (Tar) AND the agonist dexamethasone at a concentration of 250 pM. Each treatment media was applied to four wells of an assay plate containing adherent GR reporter cells. Plates were processed as described above.

Averaged and Normalized RLU values were calculated from the primary Firefly and *Renilla* luciferase antagonist assay data, as were values for %CV, "Ratio to Media", and "Fold Reduction" for each concentration of test compound #1 (Tar), (**Table 3.1.2A**).

Normalized RLU values are depicted in **Figure 3.1.2A**.

Analysis of Variance (ANOVA) and Dunnett's multiple comparison post-test were performed to determine statistical significance, if any, between the "250 pM Dexamethasone" control value and the value for respective concentrations of test compound #1 (Tar), (**Table 3.1.2B**).

Table 3.1.2A

	Normalized Data (FF / Renilla Luc): 250 pM Dex + Test Cmpd #1 (Tar) ~ GR Antagonism Assay ~									Ratio to Media Control	- Fold Reduction from Dex Control
	Dilution	µGm per 500 ul	#1	#2	#3	#4	Ave	Std Dev	%CV		
Media Control	Media Only	0	0.480 0.559	0.457 0.687	0.448 0.706	0.529 0.687	0.569	0.109	19.2	1.00	*na*
Media + 250 Dex + Cmpd 1	1/3200	0.114	175	167	174	171	172	3.87	2.25	302	1.05
	1/1600	0.228	150	157	177	159	161	11.5	7.13	283	1.12
	1/800	0.556	156	161	164	156	159	3.86	2.42	280	1.14
	1/400	1.11	148	144	153	163	152	8.14	5.37	267	1.19
	1/200	2.23	141	151	144	155	148	6.09	4.13	260	1.23
	1/100	4.45	188	161	148	170	167	16.9	10.2	293	1.08
	Neat 89.07%	445									
250 pM Dex Control	Media + Dex	0	173	184	176	191	181	8.25	4.56	318	1.00

Figure 3.1.2A

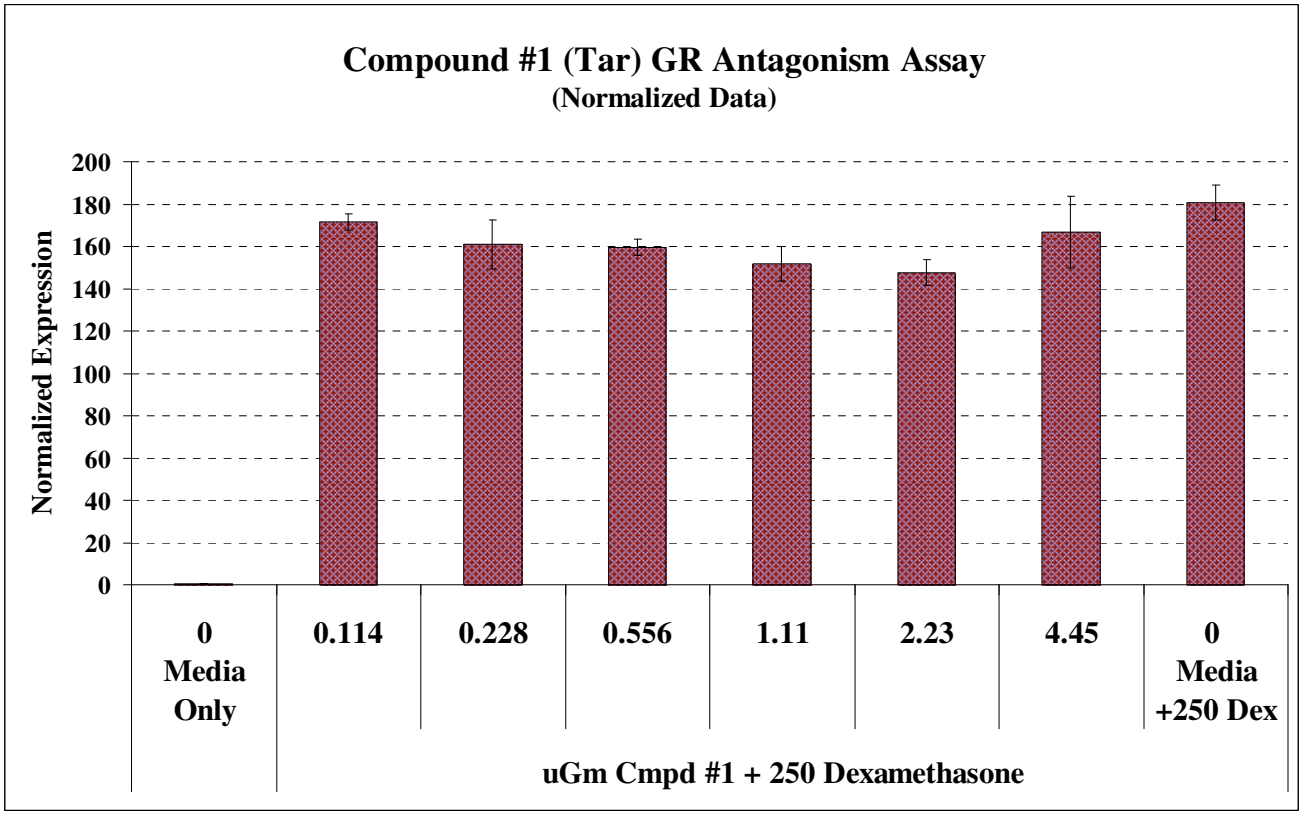


Table 3.1.2B

One-way Analysis of Variance: GR Antagonism Assay Data, USP Compound #1 (Tar)	
P value	< 0.0001
P value summary	***
Are means signif. different? (P < 0.05)	Yes
Number of groups	8
F	360
R squared	0.989

Dunnett's Multiple Comparison Test: GR Antagonism Assay Data, USP Compound #1 (Tar)					
Comparison	**Mean Diff.**	**q**	**P < 0.05?**	**Significant?**	**95% CI of diff**
250 pM Dex *vs* 0.114 uGm #1 + Dex	9.25	1.61	No	ns	-6.77 to 25.3
250 pM Dex *vs* 0.228 uGm #1 + Dex	20.3	3.52	**Yes**	**	4.23 to 36.3
250 pM Dex *vs* 0.556 uGm #1 + Dex	21.8	3.78	**Yes**	**	5.73 to 37.8
250 pM Dex *vs* 1.11 uGm #1 + Dex	29	5.04	**Yes**	***	13.0 to 45.0
250 pM Dex *vs* 2.23 uGm #1 + Dex	33.3	5.78	**Yes**	***	17.2 to 49.3
250 pM Dex *vs* 4.45 uGm #1 + Dex	14.3	2.48	No	ns	-1.77 to 30.3
250 pM Dex *vs* Media (No Dex)	180	36.2	**Yes**	***	167 to 194

3.1.3 USP Test Compound #2 (Tea): GR Antagonist Assay

Treatment media were prepared to contain six concentrations of USP Test Compound #2 (Tea) AND the agonist dexamethasone at a concentration of 250 pM. Each treatment media was applied to four wells of an assay plate containing adherent GR reporter cells. Plates were processed as described above.

Averaged and Normalized RLU values were calculated from the primary Firefly and *Renilla* luciferase antagonist assay data, as were values for %CV, "Ratio to Media", and "Fold Reduction" for each concentration of test compound #2, (**Table 3.1.3A**).

Normalized RLU values are depicted in **Figure 3.1.3A**.

Analysis of Variance (ANOVA) and Dunnett's multiple comparison post-test were performed to determine statistical significance, if any, between the "250 pM Dexamethasone" control value and the value for respective concentrations of test compound #2 (Tea), (**Table 3.1.3B**).

Table 3.1.3A

	Normalized Data (FF / Renilla Luc): 250 pM Dex + Test Cmpd #2 (Tea) ~ GR Antagonism Assay ~									Ratio to Media Control	- Fold Reduction from Dex Control
	Dilution	µGm per 500 ul	#1	#2	#3	#4	Ave	Std Dev	%CV		
Media Control	Media Only	0	0.480 0.559	0.457 0.687	0.448 0.706	0.529 0.687	0.569	0.109	19.2	1.00	*na*
Media + 250 Dex + Cmpd 2	1/3200	0.114	166	166	179	168	169	6.23	3.68	298	1.07
	1/1600	0.228	165	170	171	168	169	2.53	1.50	297	1.07
	1/800	0.556	161	141	152	150	151	8.24	5.46	265	1.20
	1/400	1.11	150	162	152	159	156	5.78	3.71	274	1.16
	1/200	2.23	150	151	149	160	152	5.35	3.51	268	1.19
	1/100	4.45	182	172	176	180	177	4.23	2.38	312	1.02
	Neat 89.02%	445									
250 pM Dex Control	Media + Dex	0	173	184	176	191	181	8.25	4.56	318	1.00

Figure 3.1.3A

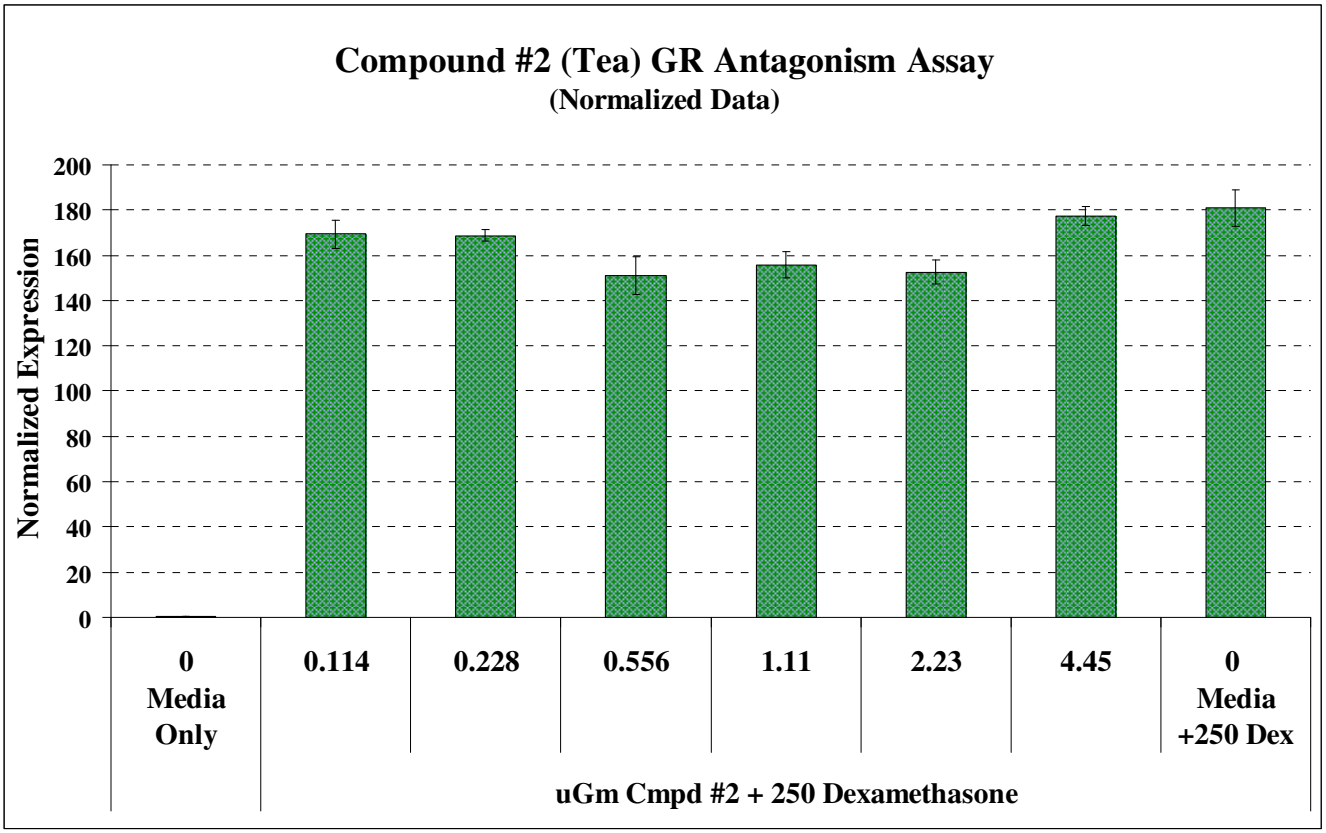


Table 3.1.3B

One-way Analysis of Variance: GR Antagonism Assay Data, USP Compound #2 (Tea)	
P value	< 0.0001
P value summary	***
Are means signif. different? (P < 0.05)	Yes
Number of groups	8
F	893
R squared	0.996

Dunnett's Multiple Comparison Test: GR Antagonism Assay Data, USP Compound #2 (Tea)					
Comparison	Mean Diff.	q	P < 0.05?	Significant?	95% CI of diff
250 pM Dex *vs* 0.114 uGm #2 + Dex	11.3	3.03	**Yes**	*	0.914 to 21.6
250 pM Dex *vs* 0.228 uGm #2 + Dex	12.5	3.37	**Yes**	*	2.16 to 22.8
250 pM Dex *vs* 0.556 uGm #2 + Dex	30.0	8.09	**Yes**	***	19.7 to 40.3
250 pM Dex *vs* 1.11 uGm #2 + Dex	25.3	6.81	**Yes**	***	14.9 to 35.6
250 pM Dex *vs* 2.23 uGm #2 + Dex	28.5	7.68	**Yes**	***	18.2 to 38.8
250 pM Dex *vs* 4.45 uGm #2 + Dex	3.50	0.944	No	ns	-6.84 to 13.8
250 pM Dex *vs* Media (No Dex)	180	56.2	**Yes**	***	171 to 189

3.1.4 GR Antagonism Assays: Conclusions & Discussion

The GR agonist reporter assay, as determined from analysis of the dexamethasone dose-response control assay, performed very well during the course of this study. The "Ratio to Media" values for the "internal control" *Renilla* luciferase reactions (Table 3.1.6B, below) show no consistent pattern of change with increasing dexamethasone concentration, hence, treatments of Dex and/or 250 pM Dex + Test Cmpd did not adversely impact reporter cell health. Further, the determined EC_{50} of 258 pM, as well as the dexamethasone "Ratio to Media" values of the "reporter" Firefly luciferase reactions are closely similar to historical values for this assay. The low %CV and high Z' values attest to the high precision of replicate measurements, and overall robust performance of these GR assays.

The data contained herein support the following conclusions:

1. Table 3.1.2B presents ANOVA and Dunnetts post-test statistical analyses of data presented in Figure 3.1.2A. This analyses confirms that many, but not all, of the reductions in GR activity observed between the Cmpd #1 (Tar)-treated and non-treated samples are, indeed, statistically significant. However, the Cmpd #1 (Tar) -treated samples show only minor reductions in GR activities, and reveal no dose-dependent trend. **Therefore, we conclude that USP Cmpd #1 (Tar) dose not demonstrate a significant level of antagonist activity against the human Glucocorticoid receptor.**

2. Table 3.1.3B presents ANOVA and Dunnetts post-test statistical analyses of data presented in Figure 3.1.3A. This analyses confirms that many, but not all, of the reductions in GR activity observed between the Cmpd #2 (Tea)-treated and non-treated samples are, indeed, statistically significant. However, the Cmpd #2 (Tea) -treated samples show only minor reductions in GR activities, and reveal no dose-dependent trend. **Therefore, we conclude that USP Cmpd #2 (Tea) does not demonstrate a significant level of antagonist activity against the human Glucocorticoid receptor.**

3.1.5 Appendix: Primary Luminometry Data, GR Antagonism Assays

Tables 3.1.5A & B Firefly and *Renilla* luciferase primary data: Dexamethasone dose-response data for GR assay validation.

A. GR Assay, Dexamethasone Dose-Response: Fire fly Luciferase								Ratio to Media Control
[Dex] pM	#1	#2	#3	#4	**Ave**	**StDev**	**%CV**	
0.000	1,112	3,352	3,147	3,793	**3,514**	**1,085**	**30.9**	**1.00**
	4,409	4,068	3,670	4,562				
7.81	4,354	3,541	4,373	4,448	**4,179**	**427**	**10.2**	**1.19**
15.6	4,230	3,816	4,165	5,216	**4,357**	**601**	**13.8**	**1.24**
31.3	7,106	6,047	6,234	5,810	**6,299**	**565**	**8.97**	**1.79**
62.5	37,652	35,772	33,315	41,179	**36,980**	**3,315**	**8.97**	**10.5**
125	493,575	463,367	439,172	464,330	**465,111**	**22,261**	**4.79**	**132**
200	951,486	903,200	907,115	1,020,060	**945,465**	**54,338**	**5.75**	**269**
200	936,011	901,247	931,526	794,120	**890,726**	**66,229**	**7.44**	**253**
250	1,378,043	1,228,959	1,314,509	1,462,931	**1,346,111**	**98,978**	**7.35**	**383**
500	2,132,719	1,690,503	1,862,292	2,038,370	**1,930,971**	**195,600**	**10.1**	**549**
1000	2,392,854	2,059,339	2,183,178	2,550,691	**2,296,516**	**218,315**	**9.51**	**654**

B. GR Assay, *Renilla* Luciferase								Ratio to Media Control
[Dex] pM	#1	#2	#3	#4	**Ave**	**StDev**	**%CV**	
0.000	2,318	7,330	7,028	7,170	**6,188**	**1,777**	**28.7**	**1.00**
	7,893	5,921	5,200	6,645				
7.81	7,184	5,995	6,076	6,752	**6,502**	**567**	**8.73**	**1.05**
15.6	7,098	5,593	6,500	7,201	**6,598**	**738**	**11.2**	**1.07**
31.3	6,661	6,106	6,844	6,594	**6,551**	**315**	**4.81**	**1.06**
62.5	7,767	6,962	6,262	7,959	**7,238**	**781**	**10.8**	**1.17**
125	8,919	7,627	7,951	7,636	**8,033**	**609**	**7.59**	**1.30**
200	7,428	6,526	6,551	7,713	**7,055**	**607**	**8.61**	**1.14**
200	7,128	6,624	6,994	6,688	**6,859**	**242**	**3.52**	**1.11**
250	7,976	6,695	7,470	7,650	**7,448**	**544**	**7.30**	**1.20**
500	7,900	6,634	6,704	7,093	**7,083**	**581**	**8.20**	**1.14**
1000	7,409	5,915	6,548	6,918	**6,698**	**630**	**9.40**	**1.08**

Tables 3.1.5C & D Firefly and *Renilla* luciferase primary data: USP Test compound #1 (Tar) in GR antagonist assay:

	C. Fire Fly Luc: 250 pM Dex + Test Cmpd #1 (Tar)							Ratio to Media Control
	Dilution	**µGm per 500 ul**	#1	#2	#3	#4	**Ave**	
Media Control	**Media Only**	**0**	1,112	3,352	3,147	3,793	**3,514**	**1.00**
			4,409	4,068	3,670	4,562		
Media + 250 Dex + Cmpd 1	**1/3200**	**0.114**	1,297,905	1,203,409	1,192,174	1,299,853	**1,248,335**	**355**
	1/1600	**0.228**	1,065,725	997,012	1,034,709	1,089,098	**1,046,636**	**298**
	1/800	**0.556**	1,097,343	1,004,208	936,992	1,029,078	**1,016,905**	**289**
	1/400	**1.11**	988,115	890,401	865,037	1,050,432	**948,496**	**270**
	1/200	**2.23**	869,650	808,442	774,064	887,360	**834,879**	**238**
	1/100	**4.45**	1,033,343	850,407	782,741	786,710	**863,300**	**246**
	Neat 89.07%	**445**						
250 pM Dex Control	**Media + Dex**	**0**	1,378,043	1,228,959	1,314,509	1,462,931	**1,346,111**	**383**

	D. Renilla Luc Internal Control 250 pM Dex + Test Cmpd #1 (Tar)							Ratio to Media Control
	Dilution	**µGm per 500 ul**	#1	#2	#3	#4	**Ave**	
Media Control	**Media Only**	**0**	2,318	7,330	7,028	7,170	**6,188**	**1.00**
			7,893	5,921	5,200	6,645		
Media + 250 Dex + Cmpd 1	**1/3200**	**0.114**	7,412	7,227	6,846	7,602	**7,272**	**1.18**
	1/1600	**0.228**	7,087	6,361	5,841	6,835	**6,531**	**1.06**
	1/800	**0.556**	7,034	6,232	5,716	6,584	**6,392**	**1.03**
	1/400	**1.11**	6,696	6,189	5,669	6,459	**6,253**	**1.01**
	1/200	**2.23**	6,149	5,356	5,381	5,742	**5,657**	**0.91**
	1/100	**4.45**	5,492	5,287	5,293	4,621	**5,173**	**0.84**
	Neat 89.07%	**445**						
250 pM Dex Control	**Media + Dex**	**0**	7,976	6,695	7,470	7,650	**7,448**	**1.20**

Tables 3.1.5E & F Firefly and *Renilla* luciferase primary data: USP Test compound #2 (Tea) in GR antagonist assay:

	E. Fire Fly Luc: 250 pM Dex + Test Cmpd #2 (Tea)							Ratio to Media Control
	Dilution	**µGm per 500 ul**	#1	#2	#3	#4	**Ave**	
Media Control	**Media Only**	**0**	1,112	3,352	3,147	3,793	**3,514**	**1.00**
			4,409	4,068	3,670	4,562		
Media + 250 Dex + Cmpd 2	**1/3200**	**0.114**	1,396,442	1,192,731	1,265,816	1,274,972	**1,282,490**	**365**
	1/1600	**0.228**	1,226,900	1,033,034	1,080,277	1,086,179	**1,106,598**	**315**
	1/800	**0.556**	1,069,283	812,100	879,390	997,364	**939,534**	**267**
	1/400	**1.11**	976,187	849,471	850,510	974,029	**912,549**	**260**
	1/200	**2.23**	912,093	767,049	783,170	902,889	**841,300**	**239**
	1/100	**4.45**	917,990	843,579	861,062	971,865	**898,624**	**256**
	Neat 89.02%	**445**						
250 pM Dex Control	**Media + Dex**	**0**	1,378,043	1,228,959	1,314,509	1,462,931	**1,346,111**	**383**

	F. Renilla Luc Internal Control 250 pM Dex + Test Cmpd #2 (Tea)							Ratio to Media Control
	Dilution	**µGm per 500 ul**	#1	#2	#3	#4	**Ave**	
Media Control	**Media Only**	**0**	2,318	7,330	7,028	7,170	**6,188**	**1.00**
			7,893	5,921	5,200	6,645		
Media + 250 Dex + Cmpd 2	**1/3200**	**0.114**	8,436	7,203	7,088	7,597	**7,581**	**1.23**
	1/1600	**0.228**	7,414	6,069	6,309	6,454	**6,562**	**1.06**
	1/800	**0.556**	6,655	5,776	5,780	6,636	**6,212**	**1.00**
	1/400	**1.11**	6,518	5,239	5,592	6,129	**5,870**	**0.95**
	1/200	**2.23**	6,065	5,094	5,271	5,629	**5,515**	**0.89**
	1/100	**4.45**	5,052	4,903	4,887	5,410	**5,063**	**0.82**
	Neat 89.02%	**445**						
250 pM Dex Control	**Media + Dex**	**0**	7,976	6,695	7,470	7,650	**7,448**	**1.20**

4. REFERENCES

None

(~ End of Report ~)