EVALUATION OF TEST ARTICLES IN AN *IN VITRO* LPS-STIMULATED PBMC ASSAY.

FINAL REPORT

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In vitro phase initiation:	December 11, 2007
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1. SUMMARY

The potential anti-inflammatory effects of 11 Test Articles were assessed in an *in vitro* human peripheral blood mononuclear cell (PBMC) model. Human PBMC's were treated with Test Articles at 1 nM, 100 nM and 1000 nM followed by treatment with lipopolysaccharide (LPS; 0.1 µg/ml) for 24 hours. Cell culture supernatants were assayed for the presence of IL-1β, IL-6, IL-8 and IL-12 (p40/p70).

1.1. *Conclusions*

At high concentrations (100 nM and 1000 nM), triptolide and triptonide inhibited human PBMC cell proliferation. At a low concentration (1 nM), triptolide and triptonide inhibited LPS-stimulated IL-8 production without affecting cell growth.

The remaining 9 Test Articles did not reduce LPS-stimulated cytokine production from PBMC's.

2. OBJECTIVE

The objective of this study was to evaluate 11 Test Articles in an *in vitro* LPS-stimulated PBMC model.

3. REGULATORY GUIDELINES

This study does not follow any specific regulatory guidelines. This study follows standard operating procedures in place at MD Biosciences, Inc., St. Paul, Minnesota.

4. ARCHIVING

The following records are stored in the archives of MD Biosciences, Inc. in St. Paul, Minnesota for 2 years:

A copy of the final report, the study protocol, documentation of all raw data and specimens generated during the conduct of the study.

5. TEST MATERIALS

5.1. *Test Articles*

TA-ID	Sponsor ID	Batch/Lot No.	Physical State	Storage Cond.	Exp. Date
TA-070124	Triptolide	20480-107	white solid	4˚C	31-Jan-09
TA-070125	Triptonide	20482-202	white solid	4˚C	31-Jan-09
TA-070126	(QNZ) CAY10470	NA	crystalline solid	-30˚C	31-Jan-10
TA-070127	GB67A	NA	white solid	4˚C	31-Jan-09
TA-070128	GB67B	NA	white solid	4˚C	31-Jan-09
TA-070129	GB615	NA	white solid	4˚C	31-Jan-09
TA-070130	GB616	NA	white solid	4˚C	31-Jan-09
TA-070131	GB594	NA	white solid	4˚C	31-Jan-09
TA-070132	GB595	NA	white solid	4˚C	31-Jan-09
TA-070133	GB65B	NA	white solid	4˚C	31-Jan-09
TA-070134	GB117	NA	white solid	4˚C	31-Jan-09

5.2. *Experimental/Reference Articles*

Name	Vendor	Cat. No.	Lot No.	Exp. Date	Storage	Use
RPMI-1640	Invitrogen	61870-036	383190	30-Nov-08	4˚C	PBMC culture
Heat inactivated fetal bovine serum (FBS)	Invitrogen	10082-147	291539	30-Jun-12	-80˚C	PBMC culture
Penicillin/streptomycin stock solution	Cambrex	17-602E	1106064	27-Nov-08	-30˚C	PBMC culture
LPS from *Salmonella abortus equi*	Sigma	L1887	095K4041	04-Jun-09	4˚C	PBMC culture
Dexamethasone	Sigma	D4902	016K14521	NA	4˚C	Reference Article
IL-1β Ab Bead Kit, Human	Invitrogen	LHC0011	357561A	30-Apr-09	4˚C	Analyte Assay
IL-6 Ab Bead Kit, Human	Invitrogen	LHC0061	308512A	31-Jan-09	4˚C	Analyte Assay
IL-8 Ab Bead Kit, Human	Invitrogen	LHC0081	269643A	31-Oct-08	4˚C	Analyte Assay
IL-12 (p40/p70) Bead Kit, Human	Invitrogen	LHC0121	366548A	31-May-09	4˚C	Analyte Assay
Multiplex Buffer Kit	Invitrogen	LHB0001	299782A/ 299782D	31-Jul-09/ 31-Jul-09	4˚C	Analyte Assay
10 X Phosphate Buffered Saline (PBS)	EMD	6505	1376B024	NA	RT	Solution Prep
Ethanol	Sigma	362808-4L	02062DH	NA	RT	Solution Prep
DMSO	Sigma	D2650	067K2354	30-Jun-09	RT	Solution Prep
Cell Proliferation Kit with XTT	MD Biosciences	409005	703602	NA	-30˚C	Proliferation Assay
Trypan Blue	Cambrex	17-942E	01110005	NA	RT	PBMC culture

5.3. *Peripheral Blood Mononuclear Cells*

Donor	Vendor	Catalog Number	Lot Number	Storage
1	SeraCare	72001	051905	Liquid N_2

5.4. *Culture Media*

Complete Culture Media (CM): RPMI-1640 + 10% FBS + 100 U/ml penicillin + 100 µg/ml streptomycin.

5.5. *Preparation of Test Articles*

20 mM Test Article stock solutions were prepared in DMSO:

20 mM Stock Solutions				
Test Article	**MW**	**mg**	**DMSO (ml)**	**mg/ml**
TA-070124	360.4	2.8	0.388	7.208
TA-070125	358.4	3.6	0.502	7.168
TA-070126	356.4	5	0.701	7.128
TA-070127	208.3	8.4	2.016	4.166
TA-070128	208.3	11.2	2.688	4.166
TA-070129	224.3	10.5	2.341	4.486
TA-070130	224.3	5.4	1.204	4.486
TA-070131	242.3	9.3	1.919	4.846
TA-070132	222.3	7.4	1.664	4.446
TA-070133	194.2	5.1	1.313	3.884
TA-070134	210.2	4.5	1.070	4.204

2000 and 1000 X Test Article stock solutions were prepared in DMSO:

2000 X Stock Solutions				
	Volume	**Source**	**Diluent**	**Total Volume**
4000 µM	50 µl	20 mM	200 µl DMSO	250 µl
400 µM	25 µl	4000 µM	225 µl DMSO	250 µl
4 µM	2.5 µl	400 µM	247.5 µl DMSO	250 µl

1000 X Stock Solutions				
	Volume	**Source**	**Diluent**	**Total Volume**
2000 µM	125 µl	4000 µM	125 µl DMSO	250 µl
200 µM	125 µl	400 µM	125 µl DMSO	250 µl
2 µM	125 µl	4 µM	125 µl DMSO	250 µl

2 X Test Article working solutions were prepared in CM:

2 X Working Solutions				
	Volume	**Source**	**Diluent**	**Total Volume**
2000 nM	5 µl	2000 µM	5 ml CM	5 ml
200 nM	5 µl	200 µM	5 ml CM	5 ml
2 nM	5 µl	2 µM	5 ml CM	5 ml

5.6. *Preparation of Dexamethasone*

A dexamethasone stock solution of 1 mg/ml (2.55 mM) was prepared in ethanol. A dexamethosone working solution of 2 µM was prepared by diluting the stock solution in CM.

5.7. *Preparation of Vehicle Control*

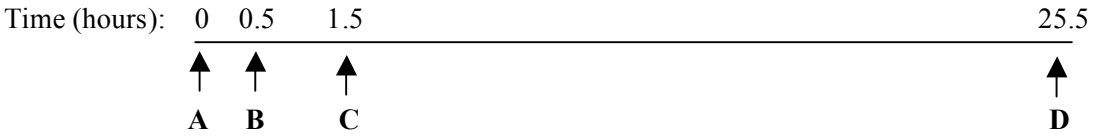
A vehicle control working solution was prepared by diluting DMSO to a final concentration of 0.1% in CM.

5.8. *Preparation of LPS*

An LPS stock solution of 1 mg/ml was prepared in 1 x PBS. An LPS working solution of 1 µg/ml was prepared by diluting the 1 mg/ml LPS stock solution in CM.

6. TEST PROCEDURE

6.1. *Schematic Depiction of in vitro Stimulation of PBMC's*



A: Plate PBMC's.
B: Add Test Articles and Reference Article.
C: Add LPS.
D: Harvest culture supernatants for cytokine analysis.

6.2. *Culture Setup*

Cryopreserved PBMC's were thawed, washed with CM and tested for viability using Trypan blue (PBMC viability = 92%). Cells were resuspended to 1 x 10^6 cells/ml in CM and 0.5 ml was plated into 24 well culture plates (5 x 10^5 cells/well). Cells were incubated for 30 minutes at 37˚C with 5% CO_2 prior to Test Article treatment.

6.3. *Test Article/Reference Article Treatment*

0.5 ml of the vehicle control working solution was added to the vehicle control wells, 0.5 ml of the 2 µM dexamethasone was added to the 1 µM dexamethasone wells and 0.5 ml of the 2 X Test Article working solutions was added to the appropriate wells. Plates were incubated for 1 hour at 37˚C with 5% CO_2 prior to LPS treatment.

6.4. *LPS Treatment*

110 µl of CM was added to the –LPS plates, 110 µl of 1 µg/ml LPS was added to the + 0.1 µg/ml LPS plates. Plates were incubated for 24 hours at 37˚C with 5% CO_2.

Cell Culture Plate Layout.

Plate 1	-LPS					
	1	2	3	4	5	6
A	Vehicle Control	Vehicle Control	Vehicle Control	TA-070124; 1000 nM	TA-070124; 1000 nM	TA-070124; 1000 nM
B	Dexamethasone	Dexamethasone	Dexamethasone	TA-070125; 1 nM	TA-070125; 1 nM	TA-070125; 1 nM
C	TA-070124; 1 nM	TA-070124; 1 nM	TA-070124; 1 nM	TA-070125; 100 nM	TA-070125; 100 nM	TA-070125; 100 nM
D	TA-070124; 100 nM	TA-070124; 100 nM	TA-070124; 100 nM	TA-070125; 1000 nM	TA-070125; 1000 nM	TA-070125; 1000 nM

Plate 2	-LPS					
	1	2	3	4	5	6
A	TA-070126; 1 nM	TA-070126; 1 nM	TA-070126; 1 nM	TA-070127; 100 nM	TA-070127; 100 nM	TA-070127; 100 nM
B	TA-070126; 100 nM	TA-070126; 100 nM	TA-070126; 100 nM	TA-070127; 1000 nM	TA-070127; 1000 nM	TA-070127; 1000 nM
C	TA-070126; 1000 nM	TA-070126; 1000 nM	TA-070126; 1000 nM	TA-070128; 1 nM	TA-070128; 1 nM	TA-070128; 1 nM
D	TA-070127; 1 nM	TA-070127; 1 nM	TA-070127; 1 nM	TA-070128; 100 nM	TA-070128; 100 nM	TA-070128; 100 nM

Plate 3	-LPS					
	1	2	3	4	5	6
A	TA-070128; 1000 nM	TA-070128; 1000 nM	TA-070128; 1000 nM	TA-070130; 1 nM	TA-070130; 1 nM	TA-070130; 1 nM
B	TA-070129; 1 nM	TA-070129; 1 nM	TA-070129; 1 nM	TA-070130; 100 nM	TA-070130; 100 nM	TA-070130; 100 nM
C	TA-070129; 100 nM	TA-070129; 100 nM	TA-070129; 100 nM	TA-070130; 1000 nM	TA-070130; 1000 nM	TA-070130; 1000 nM
D	TA-070129; 1000 nM	TA-070129; 1000 nM	TA-070129; 1000 nM	TA-070131; 1 nM	TA-070131; 1 nM	TA-070131; 1 nM

Plate 4	-LPS					
	1	2	3	4	5	6
A	TA-070131; 100 nM	TA-070131; 100 nM	TA-070131; 100 nM	TA-070132; 1000 nM	TA-070132; 1000 nM	TA-070132; 1000 nM
B	TA-070131; 1000 nM	TA-070131; 1000 nM	TA-070131; 1000 nM	TA-070133; 1 nM	TA-070133; 1 nM	TA-070133; 1 nM
C	TA-070132; 1 nM	TA-070132; 1 nM	TA-070132; 1 nM	TA-070133; 100 nM	TA-070133; 100 nM	TA-070133; 100 nM
D	TA-070132; 100 nM	TA-070132; 100 nM	TA-070132; 100 nM	TA-070133; 1000 nM	TA-070133; 1000 nM	TA-070133; 1000 nM

Plate 5	-LPS					
	1	2	3	4	5	6
A	TA-070134; 1 nM	TA-070134; 1 nM	TA-070134; 1 nM			
B	TA-070134; 100 nM	TA-070134; 100 nM	TA-070134; 100 nM			
C	TA-070134; 1000 nM	TA-070134; 1000 nM	TA-070134; 1000 nM			
D						

Plate 6	+ 0.1 µg/ml LPS					
	1	2	3	4	5	6
A	Vehicle Control	Vehicle Control	Vehicle Control	TA-070124; 1000 nM	TA-070124; 1000 nM	TA-070124; 1000 nM
B	Dexamethasone	Dexamethasone	Dexamethasone	TA-070125; 1 nM	TA-070125; 1 nM	TA-070125; 1 nM
C	TA-070124; 1 nM	TA-070124; 1 nM	TA-070124; 1 nM	TA-070125; 100 nM	TA-070125; 100 nM	TA-070125; 100 nM
D	TA-070124; 100 nM	TA-070124; 100 nM	TA-070124; 100 nM	TA-070125; 1000 nM	TA-070125; 1000 nM	TA-070125; 1000 nM

Plate 7	+ 0.1 µg/ml LPS					
	1	2	3	4	5	6
A	TA-070126; 1 nM	TA-070126; 1 nM	TA-070126; 1 nM	TA-070127; 100 nM	TA-070127; 100 nM	TA-070127; 100 nM
B	TA-070126; 100 nM	TA-070126; 100 nM	TA-070126; 100 nM	TA-070127; 1000 nM	TA-070127; 1000 nM	TA-070127; 1000 nM
C	TA-070126; 1000 nM	TA-070126; 1000 nM	TA-070126; 1000 nM	TA-070128; 1 nM	TA-070128; 1 nM	TA-070128; 1 nM
D	TA-070127; 1 nM	TA-070127; 1 nM	TA-070127; 1 nM	TA-070128; 100 nM	TA-070128; 100 nM	TA-070128; 100 nM

Plate 8	+ 0.1 µg/ml LPS					
	1	2	3	4	5	6
A	TA-070128; 1000 nM	TA-070128; 1000 nM	TA-070128; 1000 nM	TA-070130; 1 nM	TA-070130; 1 nM	TA-070130; 1 nM
B	TA-070129; 1 nM	TA-070129; 1 nM	TA-070129; 1 nM	TA-070130; 100 nM	TA-070130; 100 nM	TA-070130; 100 nM
C	TA-070129; 100 nM	TA-070129; 100 nM	TA-070129; 100 nM	TA-070130; 1000 nM	TA-070130; 1000 nM	TA-070130; 1000 nM
D	TA-070129; 1000 nM	TA-070129; 1000 nM	TA-070129; 1000 nM	TA-070131; 1 nM	TA-070131; 1 nM	TA-070131; 1 nM

Plate 9	+ 0.1 µg/ml LPS					
	1	2	3	4	5	6
A	TA-070131; 100 nM	TA-070131; 100 nM	TA-070131; 100 nM	TA-070132; 1000 nM	TA-070132; 1000 nM	TA-070132; 1000 nM
B	TA-070131; 1000 nM	TA-070131; 1000 nM	TA-070131; 1000 nM	TA-070133; 1 nM	TA-070133; 1 nM	TA-070133; 1 nM
C	TA-070132; 1 nM	TA-070132; 1 nM	TA-070132; 1 nM	TA-070133; 100 nM	TA-070133; 100 nM	TA-070133; 100 nM
D	TA-070132; 100 nM	TA-070132; 100 nM	TA-070132; 100 nM	TA-070133; 1000 nM	TA-070133; 1000 nM	TA-070133; 1000 nM

Plate 10	+ 0.1 µg/ml LPS					
	1	2	3	4	5	6
A	TA-070134; 1 nM	TA-070134; 1 nM	TA-070134; 1 nM			
B	TA-070134; 100 nM	TA-070134; 100 nM	TA-070134; 100 nM			
C	TA-070134; 1000 nM	TA-070134; 1000 nM	TA-070134; 1000 nM			
D						

6.5. *Supernatant Harvesting/XTT assay*

Cell culture supernatants were collected after 24 hours of LPS treatment and stored at -30˚C until assayed. 200 µl of media was left in each cell culture well for the XTT assay. 200 µl of media was added to a cell-free culture well for use as a blank in the XTT assay. 100 µl of activated XTT reagent was added to each well. Plates were incubated for 2 hours at 37˚C with 5% CO_2. 100 µl was removed from each well and read at 450 nm (630 nm correction) using a ThermoMax microplate reader (Molecular Devices, Sunnyvale, CA).

6.6. *Cytokine/chemokine assays*

Cell culture supernatants were assayed for IL-1β, IL-6, IL-8 and IL-12 (p40/p70) using a Luminex-based assay according the manufacturer's instructions. Data were collected using a Luminex 100 (Luminex Corporation, Austin, TX). Standard curves were generated using a 5-parameter logistic curve fitting equation weighted by 1/y (StarStation V 2.0; Applied Cytometry Systems, Sacramento, CA). Each sample reading

was interpolated from the appropriate standard curve. Calculated concentrations were multiplied by the appropriate dilution factor when necessary.

7. DATA EVALUATION

Values were analyzed using one-way ANOVA followed by Tukey's post test comparing sample values to the appropriate vehicle + LPS value (Prism V 4.0, GraphPad Software, San Diego, CA).

8. RESULTS

8.1. *Cell proliferation*

Triptolide and triptonide reduced cell proliferation at 100 nM and 1000 nM in the presence and absence of LPS (Tables 1 – 2, Figures 1 – 2). 1 nM triptolide and 1 nM triptonide did not affect cell proliferation. Therefore, care should be taken when evaluating the affect of these compounds on cytokine release at 100 nM and 1000 nM. The remaining Test Articles did not affect cell proliferation in the presence or absence of LPS.

8.2. *Inflammatory mediator production*

The Test Articles did not significantly induce cytokine production from PBMC's not treated with LPS (Tables 3 – 6). IL-1β, IL-6, IL-8 and IL-12 levels increased upon LPS treatment (Tables 3 – 6, Figures 3 – 6).

8.3. *Effect of dexamethasone on inflammatory mediator production*

The Reference Article, 1 µM dexamethasone, significantly decreased the LPS-stimulated induction of IL-1β, IL-8 and IL-12 (Tables 3, 5 and 6, Figures 3, 5 and 6). These effects are consistent with previous studies.

8.4. *Effect of Triptolide on inflammatory mediator production*

Triptolide significantly reduced the induction of IL-1β, IL-6, IL-8 and IL-12 from LPS-stimulated PBMC's at 100 nM and 1000 nM (Tables 3 – 6, Figures 3 – 6). However, the reduction could be due to a general inhibition of cell proliferation rather than a specific anti-inflammatory activity. 1 nM triptolide, which did not affect cell proliferation, significantly reduced IL-8 production from LPS-stimulated PBMC's. IL-12 was increased in the presence of 1 nM triptolide and LPS.

8.5. *Effect of Triptonide on inflammatory mediator production*

Triptonide significantly reduced the induction of IL-1β, IL-6, IL-8 and IL-12 from LPS-stimulated PBMC's at 100 nM and 1000 nM (Tables 3 – 6, Figures 3 – 6). However, the reduction could be due to a general inhibition of cell proliferation rather than a specific anti-inflammatory activity. 1 nM triptolide, which did not affect cell proliferation, significantly reduced IL-8 production from LPS-stimulated PBMC's.

8.6. ***Effect of (QNZ)CAY10470 on inflammatory mediator production***

(QNZ) CAY10470 significantly increased IL-1β production from LPS-stimulated PBMC's at 100 nM and 1000 nM (Tables 3 – 6, Figures 3 – 6). (QNZ) CAY10470 did not affect IL-6, IL-8 or IL-12 production.

8.7. ***Effect of GB67A on inflammatory mediator production***

GB67A did not affect IL-1β, IL-6, IL-8 or IL-12 production from LPS-stimulated PBMC's (Tables 3 – 6, Figures 3 – 6).

8.8. ***Effect of GB67B on inflammatory mediator production***

GB67B did not affect IL-1β, IL-6, IL-8 or IL-12 production from LPS-stimulated PBMC's (Tables 3 – 6, Figures 3 – 6).

8.9. ***Effect of GB615 on inflammatory mediator production***

GB615 did not affect IL-1β, IL-6, IL-8 or IL-12 production from LPS-stimulated PBMC's (Tables 3 – 6, Figures 3 – 6).

8.10. ***Effect of GB616 on inflammatory mediator production***

GB616 did not affect IL-1β, IL-6, IL-8 or IL-12 production from LPS-stimulated PBMC's (Tables 3 – 6, Figures 3 – 6).

8.11. ***Effect of GB594 on inflammatory mediator production***

GB594 did not affect IL-1β, IL-6, IL-8 or IL-12 production from LPS-stimulated PBMC's (Tables 3 – 6, Figures 3 – 6).

8.12. ***Effect of GB595 on inflammatory mediator production***

GB595 did not affect IL-1β, IL-6, IL-8 or IL-12 production from LPS-stimulated PBMC's (Tables 3 – 6, Figures 3 – 6).

8.13. ***Effect of GB65B on inflammatory mediator production***

GB65B significantly increased IL-12 production from LPS-stimulated PBMC's at 1000 nM (Tables 3 – 6, Figures 3 – 6). GB65B did not affect IL-1β, IL-6 or IL-8 production.

8.14. ***Effect of GB117 on inflammatory mediator production***

GB117 did not affect IL-1β, IL-6, IL-8 or IL-12 production from LPS-stimulated PBMC's (Tables 3 – 6, Figures 3 – 6).

9. CONCLUSIONS

Triptolide and triptonide reduced cell proliferation at 100 nM and 1000 nM suggesting that the compounds are either cytoxic or cytostatic at high concentrations. At 1 nM, a

concentration that did not affect cell proliferation, triptolide and triptonide significantly reduced IL-8 production. None of the other Test Articles analyzed in this study inhibited LPS-stimulated cytokine production.

Table 1. XTT assay (- LPS).

Test Article	Concentration	Stimulation	Mean XTT (OD 450)	Std. Dev.	Mean XTT (% Vehicle - LPS)
Vehicle		- LPS	0.111	0.026	100%
Dexamethasone	1 µM	- LPS	0.097	0.009	88%
Triptolide	1 nM	- LPS	0.128	0.012	115%
Triptolide	100 nM	- LPS	0.064	0.000	58%
Triptolide	1000 nM	- LPS	0.050	0.002	45%
Triptonide	1 nM	- LPS	0.142	0.039	128%
Triptonide	100 nM	- LPS	0.080	0.007	72%
Triptonide	1000 nM	- LPS	0.057	0.004	51%
(QNZ) CAY10470	1 nM	- LPS	0.141	0.019	127%
(QNZ) CAY10470	100 nM	- LPS	0.165	0.014	149%
(QNZ) CAY10470	1000 nM	- LPS	0.162	0.015	146%
GB67A	1 nM	- LPS	0.154	0.014	138%
GB67A	100 nM	- LPS	0.147	0.024	132%
GB67A	1000 nM	- LPS	0.153	0.023	138%
GB67B	1 nM	- LPS	0.143	0.018	129%
GB67B	100 nM	- LPS	0.159	0.015	144%
GB67B	1000 nM	- LPS	0.165	0.019	149%
GB615	1 nM	- LPS	0.144	0.012	130%
GB615	100 nM	- LPS	0.138	0.016	124%
GB615	1000 nM	- LPS	0.154	0.015	139%
GB616	1 nM	- LPS	0.149	0.017	135%
GB616	100 nM	- LPS	0.143	0.039	129%
GB616	1000 nM	- LPS	0.146	0.024	131%
GB594	1 nM	- LPS	0.151	0.021	136%
GB594	100 nM	- LPS	0.149	0.012	134%
GB594	1000 nM	- LPS	0.153	0.008	138%
GB595	1 nM	- LPS	0.156	0.017	140%
GB595	100 nM	- LPS	0.163	0.007	147%
GB595	1000 nM	- LPS	0.144	0.008	130%
GB65B	1 nM	- LPS	0.139	0.017	125%
GB65B	100 nM	- LPS	0.141	0.021	127%
GB65B	1000 nM	- LPS	0.160	0.019	144%
GB117	1 nM	- LPS	0.171	0.072	154%
GB117	100 nM	- LPS	0.127	0.006	115%
GB117	1000 nM	- LPS	0.135	0.011	122%

Table 2. XTT assay (+ LPS).

Test Article	Concentration	Stimulation	Mean XTT (OD 450)	Std. Dev.	Mean XTT (% Vehicle + LPS)
Vehicle		+ LPS	0.240	0.060	100%
Dexamethasone	1 µM	+ LPS	0.244	0.034	102%
Triptolide	1 nM	+ LPS	0.227	0.023	95%
Triptolide	100 nM	+ LPS	0.067	0.008	28%
Triptolide	1000 nM	+ LPS	0.055	0.007	23%
Triptonide	1 nM	+ LPS	0.223	0.042	93%
Triptonide	100 nM	+ LPS	0.070	0.001	29%
Triptonide	1000 nM	+ LPS	0.053	0.006	22%
(QNZ) CAY10470	1 nM	+ LPS	0.226	0.024	94%
(QNZ) CAY10470	100 nM	+ LPS	0.181	0.010	75%
(QNZ) CAY10470	1000 nM	+ LPS	0.169	0.009	71%
GB67A	1 nM	+ LPS	0.206	0.006	86%
GB67A	100 nM	+ LPS	0.211	0.033	88%
GB67A	1000 nM	+ LPS	0.168	0.039	70%
GB67B	1 nM	+ LPS	0.191	0.030	80%
GB67B	100 nM	+ LPS	0.200	0.037	83%
GB67B	1000 nM	+ LPS	0.194	0.009	81%
GB615	1 nM	+ LPS	0.191	0.012	80%
GB615	100 nM	+ LPS	0.190	0.002	79%
GB615	1000 nM	+ LPS	0.191	0.009	80%
GB616	1 nM	+ LPS	0.209	0.039	87%
GB616	100 nM	+ LPS	0.184	0.033	77%
GB616	1000 nM	+ LPS	0.173	0.025	72%
GB594	1 nM	+ LPS	0.184	0.021	77%
GB594	100 nM	+ LPS	0.209	0.016	87%
GB594	1000 nM	+ LPS	0.190	0.011	79%
GB595	1 nM	+ LPS	0.200	0.013	84%
GB595	100 nM	+ LPS	0.193	0.006	81%
GB595	1000 nM	+ LPS	0.214	0.045	89%
GB65B	1 nM	+ LPS	0.200	0.022	83%
GB65B	100 nM	+ LPS	0.193	0.033	81%
GB65B	1000 nM	+ LPS	0.165	0.023	69%
GB117	1 nM	+ LPS	0.227	0.021	95%
GB117	100 nM	+ LPS	0.215	0.017	90%
GB117	1000 nM	+ LPS	0.224	0.011	94%

Table 3. Mean IL-1β production.

Test Article	Concentration	Stimulation	Mean IL-1β (pg/ml)	Std. Dev.	Mean IL-1β (% Vehicle + LPS)	P Value[1]
Vehicle		- LPS	<LD	NA	NA	
Dexamethasone	1 μM	- LPS	<LD	NA	NA	
Triptolide	1 nM	- LPS	<LD	NA	NA	
Triptolide	100 nM	- LPS	<LD	NA	NA	
Triptolide	1000 nM	- LPS	<LD	NA	NA	
Triptonide	1 nM	- LPS	<LD	NA	NA	
Triptonide	100 nM	- LPS	<LD	NA	NA	
Triptonide	1000 nM	- LPS	<LD	NA	NA	
(QNZ) CAY10470	1 nM	- LPS	<LD	NA	NA	
(QNZ) CAY10470	100 nM	- LPS	<LD	NA	NA	
(QNZ) CAY10470	1000 nM	- LPS	<LD	NA	NA	
GB67A	1 nM	- LPS	<LD	NA	NA	
GB67A	100 nM	- LPS	<LD	NA	NA	
GB67A	1000 nM	- LPS	<LD	NA	NA	
GB67B	1 nM	- LPS	<LD	NA	NA	
GB67B	100 nM	- LPS	<LD	NA	NA	
GB67B	1000 nM	- LPS	<LD	NA	NA	
GB615	1 nM	- LPS	<LD	NA	NA	
GB615	100 nM	- LPS	<LD	NA	NA	
GB615	1000 nM	- LPS	<LD	NA	NA	
GB616	1 nM	- LPS	<LD	NA	NA	
GB616	100 nM	- LPS	<LD	NA	NA	
GB616	1000 nM	- LPS	<LD	NA	NA	
GB594	1 nM	- LPS	<LD	NA	NA	
GB594	100 nM	- LPS	<LD	NA	NA	
GB594	1000 nM	- LPS	<LD	NA	NA	
GB595	1 nM	- LPS	<LD	NA	NA	
GB595	100 nM	- LPS	<LD	NA	NA	
GB595	1000 nM	- LPS	<LD	NA	NA	
GB65B	1 nM	- LPS	<LD	NA	NA	
GB65B	100 nM	- LPS	<LD	NA	NA	
GB65B	1000 nM	- LPS	<LD	NA	NA	
GB117	1 nM	- LPS	<LD	NA	NA	
GB117	100 nM	- LPS	<LD	NA	NA	
GB117	1000 nM	- LPS	<LD	NA	NA	
Vehicle		+ LPS	581	97	100%	
Dexamethasone	1 μM	+ LPS	194	19	33%	**P < 0.001**
Triptolide	1 nM	+ LPS	346	5	60%	P > 0.05
Triptolide	100 nM	+ LPS	18	4	3%	**P < 0.001**
Triptolide	1000 nM	+ LPS	<LD	NA	NA	**P < 0.001**
Triptonide	1 nM	+ LPS	364	24	63%	P > 0.05
Triptonide	100 nM	+ LPS	85	3	15%	**P < 0.001**
Triptonide	1000 nM	+ LPS	<LD	NA	NA	**P < 0.001**
(QNZ) CAY10470	1 nM	+ LPS	668	110	115%	P > 0.05
(QNZ) CAY10470	100 nM	+ LPS	961	119	165%	**P < 0.01**
(QNZ) CAY10470	1000 nM	+ LPS	1005	77	173%	**P < 0.001**
GB67A	1 nM	+ LPS	749	197	129%	P > 0.05
GB67A	100 nM	+ LPS	703	15	121%	P > 0.05
GB67A	1000 nM	+ LPS	416	93	72%	P > 0.05
GB67B	1 nM	+ LPS	709	39	122%	P > 0.05
GB67B	100 nM	+ LPS	726	150	125%	P > 0.05
GB67B	1000 nM	+ LPS	395	72	68%	P > 0.05
GB615	1 nM	+ LPS	779	82	134%	P > 0.05
GB615	100 nM	+ LPS	697	52	120%	P > 0.05
GB615	1000 nM	+ LPS	625	42	108%	P > 0.05
GB616	1 nM	+ LPS	652	80	112%	P > 0.05
GB616	100 nM	+ LPS	670	28	115%	P > 0.05
GB616	1000 nM	+ LPS	749	79	129%	P > 0.05
GB594	1 nM	+ LPS	771	101	133%	P > 0.05
GB594	100 nM	+ LPS	634	120	109%	P > 0.05
GB594	1000 nM	+ LPS	769	98	132%	P > 0.05
GB595	1 nM	+ LPS	783	179	135%	P > 0.05

Table 3. Mean IL-1β production.

Test Article	Concentration	Stimulation	Mean IL-1β (pg/ml)	Std. Dev.	Mean IL-1β (% Vehicle + LPS)	P Value[1]
GB595	100 nM	+ LPS	742	96	128%	P > 0.05
GB595	1000 nM	+ LPS	715	103	123%	P > 0.05
GB65B	1 nM	+ LPS	707	128	122%	P > 0.05
GB65B	100 nM	+ LPS	707	79	122%	P > 0.05
GB65B	1000 nM	+ LPS	502	23	86%	P > 0.05
GB117	1 nM	+ LPS	594	39	102%	P > 0.05
GB117	100 nM	+ LPS	709	143	122%	P > 0.05
GB117	1000 nM	+ LPS	683	103	117%	P > 0.05

[1]P values from one-way ANOVA with Tukey's post test (sample vs. Vehicle + LPS). P values below 0.05 are bold.
<LD: Below level of detection.
Values in grey were extrapolated below the low standard.

Table 4. Mean IL-6 production.

Test Article	Concentration	Stimulation	Mean IL-6 (pg/ml)	Std. Dev.	Mean IL-6 (% Vehicle + LPS)	P Value[1]
Vehicle		- LPS	3	0	0%	
Dexamethasone	1 µM	- LPS	3	NA	0%	
Triptolide	1 nM	- LPS	3	0	0%	
Triptolide	100 nM	- LPS	<LD	NA	NA	
Triptolide	1000 nM	- LPS	<LD	NA	NA	
Triptonide	1 nM	- LPS	3	1	0%	
Triptonide	100 nM	- LPS	3	NA	0%	
Triptonide	1000 nM	- LPS	<LD	NA	NA	
(QNZ) CAY10470	1 nM	- LPS	3	NA	0%	
(QNZ) CAY10470	100 nM	- LPS	<LD	NA	NA	
(QNZ) CAY10470	1000 nM	- LPS	11	11	0%	
GB67A	1 nM	- LPS	3	NA	0%	
GB67A	100 nM	- LPS	3	0	0%	
GB67A	1000 nM	- LPS	3	0	0%	
GB67B	1 nM	- LPS	3	NA	0%	
GB67B	100 nM	- LPS	3	0	0%	
GB67B	1000 nM	- LPS	4	NA	0%	
GB615	1 nM	- LPS	<LD	NA	NA	
GB615	100 nM	- LPS	3	0	0%	
GB615	1000 nM	- LPS	3	NA	0%	
GB616	1 nM	- LPS	3	NA	0%	
GB616	100 nM	- LPS	3	NA	0%	
GB616	1000 nM	- LPS	3	0	0%	
GB594	1 nM	- LPS	<LD	NA	NA	
GB594	100 nM	- LPS	3	0	0%	
GB594	1000 nM	- LPS	3	0	0%	
GB595	1 nM	- LPS	3	0	0%	
GB595	100 nM	- LPS	<LD	NA	NA	
GB595	1000 nM	- LPS	<LD	NA	NA	
GB65B	1 nM	- LPS	<LD	NA	NA	
GB65B	100 nM	- LPS	<LD	NA	NA	
GB65B	1000 nM	- LPS	<LD	NA	NA	
GB117	1 nM	- LPS	101	NA	2%	
GB117	100 nM	- LPS	<LD	NA	NA	
GB117	1000 nM	- LPS	<LD	NA	NA	
Vehicle		+ LPS	4435	939	100%	
Dexamethasone	1 µM	+ LPS	2351	384	53%	P > 0.05
Triptolide	1 nM	+ LPS	4321	206	97%	P > 0.05
Triptolide	100 nM	+ LPS	216	4	5%	**P < 0.001**
Triptolide	1000 nM	+ LPS	<LD	NA	NA	**P < 0.001**
Triptonide	1 nM	+ LPS	4753	529	107%	P > 0.05
Triptonide	100 nM	+ LPS	879	53	20%	**P < 0.001**
Triptonide	1000 nM	+ LPS	<LD	NA	NA	**P < 0.001**
(QNZ) CAY10470	1 nM	+ LPS	5346	624	121%	P > 0.05
(QNZ) CAY10470	100 nM	+ LPS	4826	1051	109%	P > 0.05
(QNZ) CAY10470	1000 nM	+ LPS	4736	152	107%	P > 0.05
GB67A	1 nM	+ LPS	6412	2386	145%	P > 0.05
GB67A	100 nM	+ LPS	5932	107	134%	P > 0.05
GB67A	1000 nM	+ LPS	3497	765	79%	P > 0.05
GB67B	1 nM	+ LPS	5192	453	117%	P > 0.05
GB67B	100 nM	+ LPS	6208	1890	140%	P > 0.05
GB67B	1000 nM	+ LPS	4869	479	110%	P > 0.05
GB615	1 nM	+ LPS	6338	805	143%	P > 0.05
GB615	100 nM	+ LPS	5556	418	125%	P > 0.05
GB615	1000 nM	+ LPS	4430	277	100%	P > 0.05
GB616	1 nM	+ LPS	4555	388	103%	P > 0.05
GB616	100 nM	+ LPS	4681	163	106%	P > 0.05
GB616	1000 nM	+ LPS	5418	834	122%	P > 0.05
GB594	1 nM	+ LPS	5577	797	126%	P > 0.05
GB594	100 nM	+ LPS	4880	994	110%	P > 0.05
GB594	1000 nM	+ LPS	4956	461	112%	P > 0.05
GB595	1 nM	+ LPS	5535	1188	125%	P > 0.05

Table 4. Mean IL-6 production.

Test Article	Concentration	Stimulation	Mean IL-6 (pg/ml)	Std. Dev.	Mean IL-6 (% Vehicle + LPS)	P Value[1]
GB595	100 nM	+ LPS	5938	856	134%	P > 0.05
GB595	1000 nM	+ LPS	5489	939	124%	P > 0.05
GB65B	1 nM	+ LPS	5235	1395	118%	P > 0.05
GB65B	100 nM	+ LPS	4918	549	111%	P > 0.05
GB65B	1000 nM	+ LPS	4048	111	91%	P > 0.05
GB117	1 nM	+ LPS	4363	531	98%	P > 0.05
GB117	100 nM	+ LPS	4893	1278	110%	P > 0.05
GB117	1000 nM	+ LPS	4860	800	110%	P > 0.05

[1]P values from one-way ANOVA with Tukey's post test (sample vs. Vehicle + LPS). P values below 0.05 are bold.
<LD: Below level of detection.
Values in grey were extrapolated below the low standard.

Table 5. Mean IL-8 production.

Test Article	Concentration	Stimulation	Mean IL-8 (pg/ml)	Std. Dev.	Mean IL-8 (% Vehicle + LPS)	P Value[1]
Vehicle		- LPS	393	158	0%	
Dexamethasone	1 µM	- LPS	60	4	0%	
Triptolide	1 nM	- LPS	185	6	0%	
Triptolide	100 nM	- LPS	67	3	0%	
Triptolide	1000 nM	- LPS	47	4	0%	
Triptonide	1 nM	- LPS	228	92	0%	
Triptonide	100 nM	- LPS	91	12	0%	
Triptonide	1000 nM	- LPS	42	2	0%	
(QNZ) CAY10470	1 nM	- LPS	338	150	0%	
(QNZ) CAY10470	100 nM	- LPS	368	97	0%	
(QNZ) CAY10470	1000 nM	- LPS	872	706	1%	
GB67A	1 nM	- LPS	273	6	0%	
GB67A	100 nM	- LPS	310	103	0%	
GB67A	1000 nM	- LPS	628	225	0%	
GB67B	1 nM	- LPS	365	82	0%	
GB67B	100 nM	- LPS	373	84	0%	
GB67B	1000 nM	- LPS	992	401	1%	
GB615	1 nM	- LPS	289	7	0%	
GB615	100 nM	- LPS	294	82	0%	
GB615	1000 nM	- LPS	242	6	0%	
GB616	1 nM	- LPS	298	85	0%	
GB616	100 nM	- LPS	253	20	0%	
GB616	1000 nM	- LPS	305	25	0%	
GB594	1 nM	- LPS	262	20	0%	
GB594	100 nM	- LPS	258	70	0%	
GB594	1000 nM	- LPS	312	175	0%	
GB595	1 nM	- LPS	443	319	0%	
GB595	100 nM	- LPS	272	68	0%	
GB595	1000 nM	- LPS	463	293	0%	
GB65B	1 nM	- LPS	326	71	0%	
GB65B	100 nM	- LPS	313	39	0%	
GB65B	1000 nM	- LPS	1079	31	1%	
GB117	1 nM	- LPS	3873	5491	3%	
GB117	100 nM	- LPS	522	449	0%	
GB117	1000 nM	- LPS	386	153	0%	
Vehicle		+ LPS	150057	10341	100%	
Dexamethasone	1 µM	+ LPS	58003	12968	39%	**P < 0.05**
Triptolide	1 nM	+ LPS	40888	20258	27%	**P < 0.01**
Triptolide	100 nM	+ LPS	1961	588	1%	**P < 0.001**
Triptolide	1000 nM	+ LPS	81	9	0%	**P < 0.001**
Triptonide	1 nM	+ LPS	48314	17970	32%	**P < 0.01**
Triptonide	100 nM	+ LPS	3132	432	2%	**P < 0.001**
Triptonide	1000 nM	+ LPS	71	9	0%	**P < 0.001**
(QNZ) CAY10470	1 nM	+ LPS	136846	45510	91%	P > 0.05
(QNZ) CAY10470	100 nM	+ LPS	109615	32359	73%	P > 0.05
(QNZ) CAY10470	1000 nM	+ LPS	94002	35918	63%	P > 0.05
GB67A	1 nM	+ LPS	109832	24536	73%	P > 0.05
GB67A	100 nM	+ LPS	118329	44400	79%	P > 0.05
GB67A	1000 nM	+ LPS	100502	40668	67%	P > 0.05
GB67B	1 nM	+ LPS	89888	19671	60%	P > 0.05
GB67B	100 nM	+ LPS	102632	16626	68%	P > 0.05
GB67B	1000 nM	+ LPS	87818	35818	59%	P > 0.05
GB615	1 nM	+ LPS	159398	46684	106%	P > 0.05
GB615	100 nM	+ LPS	77249	12692	51%	P > 0.05
GB615	1000 nM	+ LPS	94514	22326	63%	P > 0.05
GB616	1 nM	+ LPS	129264	55185	86%	P > 0.05
GB616	100 nM	+ LPS	128329	23190	86%	P > 0.05
GB616	1000 nM	+ LPS	72190	17886	48%	P > 0.05
GB594	1 nM	+ LPS	120119	18763	80%	P > 0.05
GB594	100 nM	+ LPS	121795	28715	81%	P > 0.05
GB594	1000 nM	+ LPS	128511	14560	86%	P > 0.05
GB595	1 nM	+ LPS	159430	43037	106%	P > 0.05

Table 5. Mean IL-8 production.

Test Article	Concentration	Stimulation	Mean IL-8 (pg/ml)	Std. Dev.	Mean IL-8 (% Vehicle + LPS)	P Value[1]
GB595	100 nM	+ LPS	145731	21451	97%	P > 0.05
GB595	1000 nM	+ LPS	159939	24067	107%	P > 0.05
GB65B	1 nM	+ LPS	172193	21663	115%	P > 0.05
GB65B	100 nM	+ LPS	196901	37402	131%	P > 0.05
GB65B	1000 nM	+ LPS	142462	19167	95%	P > 0.05
GB117	1 nM	+ LPS	160004	6305	107%	P > 0.05
GB117	100 nM	+ LPS	189911	35004	127%	P > 0.05
GB117	1000 nM	+ LPS	167263	7306	111%	P > 0.05

[1]P values from one-way ANOVA with Tukey's post test (sample vs. Vehicle + LPS). P values below 0.05 are bold.
<LD: Below level of detection.
Values in grey were extrapolated below the low standard.

Table 6. Mean IL-12 (p40/p70) production.

Test Article	Concentration	Stimulation	Mean IL-12 p40/p70 (pg/ml)	Std. Dev.	Mean IL-12 p40-p70 (% Vehicle + LPS)	P Value[1]
Vehicle		- LPS	<LD	NA	NA	
Dexamethasone	1 µM	- LPS	<LD	NA	NA	
Triptolide	1 nM	- LPS	<LD	NA	NA	
Triptolide	100 nM	- LPS	<LD	NA	NA	
Triptolide	1000 nM	- LPS	<LD	NA	NA	
Triptonide	1 nM	- LPS	<LD	NA	NA	
Triptonide	100 nM	- LPS	<LD	NA	NA	
Triptonide	1000 nM	- LPS	<LD	NA	NA	
(QNZ) CAY10470	1 nM	- LPS	<LD	NA	NA	
(QNZ) CAY10470	100 nM	- LPS	<LD	NA	NA	
(QNZ) CAY10470	1000 nM	- LPS	<LD	NA	NA	
GB67A	1 nM	- LPS	<LD	NA	NA	
GB67A	100 nM	- LPS	<LD	NA	NA	
GB67A	1000 nM	- LPS	<LD	NA	NA	
GB67B	1 nM	- LPS	<LD	NA	NA	
GB67B	100 nM	- LPS	<LD	NA	NA	
GB67B	1000 nM	- LPS	<LD	NA	NA	
GB615	1 nM	- LPS	<LD	NA	NA	
GB615	100 nM	- LPS	<LD	NA	NA	
GB615	1000 nM	- LPS	<LD	NA	NA	
GB616	1 nM	- LPS	<LD	NA	NA	
GB616	100 nM	- LPS	<LD	NA	NA	
GB616	1000 nM	- LPS	<LD	NA	NA	
GB594	1 nM	- LPS	<LD	NA	NA	
GB594	100 nM	- LPS	<LD	NA	NA	
GB594	1000 nM	- LPS	<LD	NA	NA	
GB595	1 nM	- LPS	<LD	NA	NA	
GB595	100 nM	- LPS	<LD	NA	NA	
GB595	1000 nM	- LPS	<LD	NA	NA	
GB65B	1 nM	- LPS	<LD	NA	NA	
GB65B	100 nM	- LPS	<LD	NA	NA	
GB65B	1000 nM	- LPS	<LD	NA	NA	
GB117	1 nM	- LPS	<LD	NA	NA	
GB117	100 nM	- LPS	<LD	NA	NA	
GB117	1000 nM	- LPS	<LD	NA	NA	
Vehicle		+ LPS	502	106	100%	
Dexamethasone	1 µM	+ LPS	52	8	10%	**P < 0.001**
Triptolide	1 nM	+ LPS	829	14	165%	**P < 0.05**
Triptolide	100 nM	+ LPS	<LD	NA	NA	**P < 0.001**
Triptolide	1000 nM	+ LPS	<LD	NA	NA	**P < 0.001**
Triptonide	1 nM	+ LPS	798	96	159%	P > 0.05
Triptonide	100 nM	+ LPS	<LD	NA	NA	**P < 0.001**
Triptonide	1000 nM	+ LPS	<LD	NA	NA	**P < 0.001**
(QNZ) CAY10470	1 nM	+ LPS	505	90	101%	P > 0.05
(QNZ) CAY10470	100 nM	+ LPS	452	39	90%	P > 0.05
(QNZ) CAY10470	1000 nM	+ LPS	541	13	108%	P > 0.05
GB67A	1 nM	+ LPS	676	206	135%	P > 0.05
GB67A	100 nM	+ LPS	653	39	130%	P > 0.05
GB67A	1000 nM	+ LPS	671	172	134%	P > 0.05
GB67B	1 nM	+ LPS	477	115	95%	P > 0.05
GB67B	100 nM	+ LPS	694	76	138%	P > 0.05
GB67B	1000 nM	+ LPS	800	114	160%	P > 0.05
GB615	1 nM	+ LPS	562	97	112%	P > 0.05
GB615	100 nM	+ LPS	519	121	103%	P > 0.05
GB615	1000 nM	+ LPS	501	7	100%	P > 0.05
GB616	1 nM	+ LPS	506	91	101%	P > 0.05
GB616	100 nM	+ LPS	422	43	84%	P > 0.05
GB616	1000 nM	+ LPS	528	41	105%	P > 0.05
GB594	1 nM	+ LPS	654	49	130%	P > 0.05
GB594	100 nM	+ LPS	589	188	117%	P > 0.05
GB594	1000 nM	+ LPS	546	103	109%	P > 0.05

Table 6. Mean IL-12 (p40/p70) production.

Test Article	Concentration	Stimulation	Mean IL-12 p40/p70 (pg/ml)	Std. Dev.	Mean IL-12 p40-p70 (% Vehicle + LPS)	P Value[1]
GB595	1 nM	+ LPS	622	88	124%	P > 0.05
GB595	100 nM	+ LPS	714	47	142%	P > 0.05
GB595	1000 nM	+ LPS	638	69	127%	P > 0.05
GB65B	1 nM	+ LPS	531	118	106%	P > 0.05
GB65B	100 nM	+ LPS	601	90	120%	P > 0.05
GB65B	1000 nM	+ LPS	894	82	178%	**P < 0.001**
GB117	1 nM	+ LPS	539	35	107%	P > 0.05
GB117	100 nM	+ LPS	650	170	130%	P > 0.05
GB117	1000 nM	+ LPS	643	78	128%	P > 0.05

[1]P values from one-way ANOVA with Tukey's post test (sample vs. Vehicle + LPS). P values below 0.05 are bold.
<LD: Below level of detection.
Values in grey were extrapolated below the low standard.

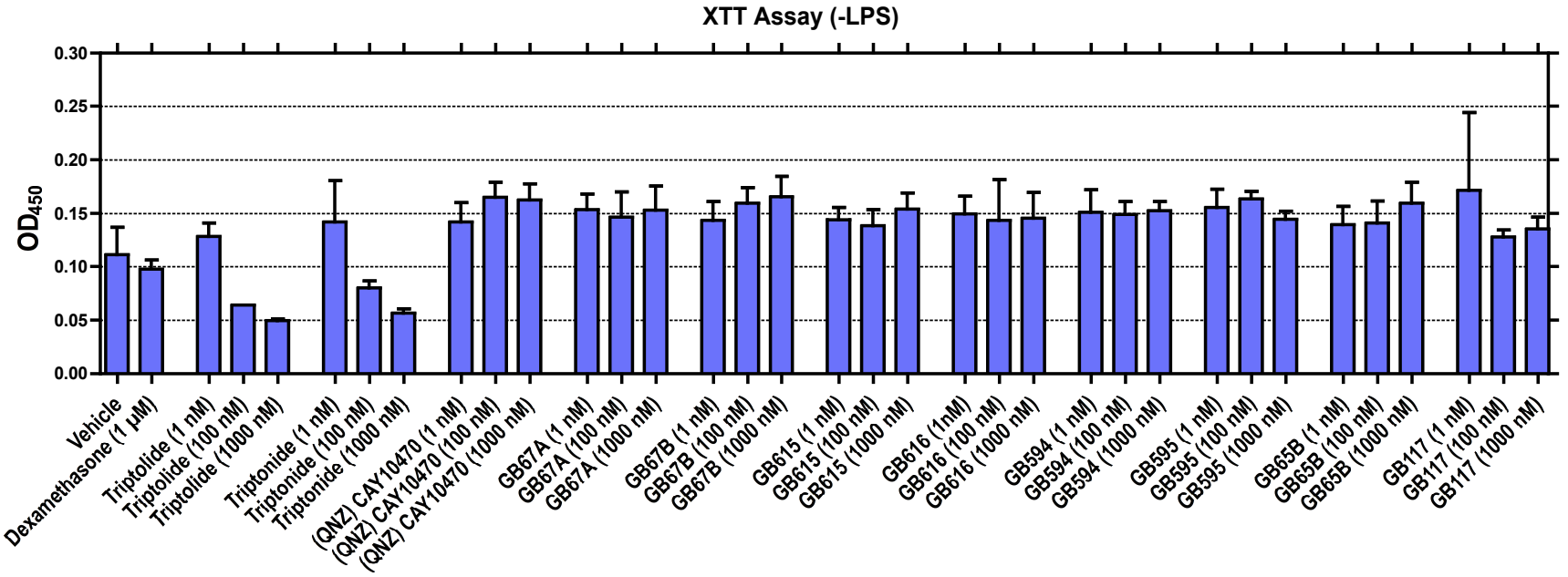


Figure 1. Cell proliferation assay (- LPS). PBMC's treated as described above were incubated with XTT. The amount of reduced XTT, a measure of metabolic activity, was measured at 450 nm. Mean values are shown. Error bars represent standard deviations.

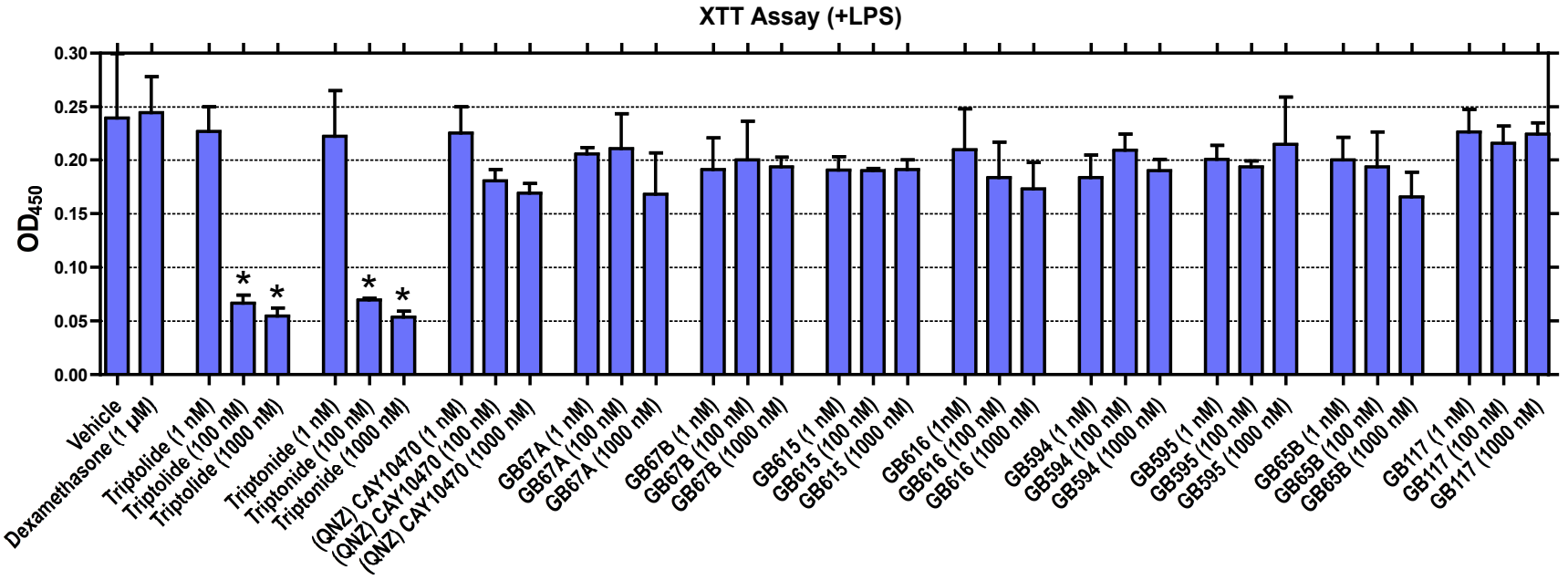


Figure 2. Cell proliferation assay (+ LPS). PBMC's treated as described above were incubated with XTT. The amount of reduced XTT, a measure of metabolic activity, was measured at 450 nm. Mean values are shown. Error bars represent standard deviations. Values were analyzed by one-way ANOVA with Tukey's post-test comparing sample values to the vehicle value.*$P < 0.05$.

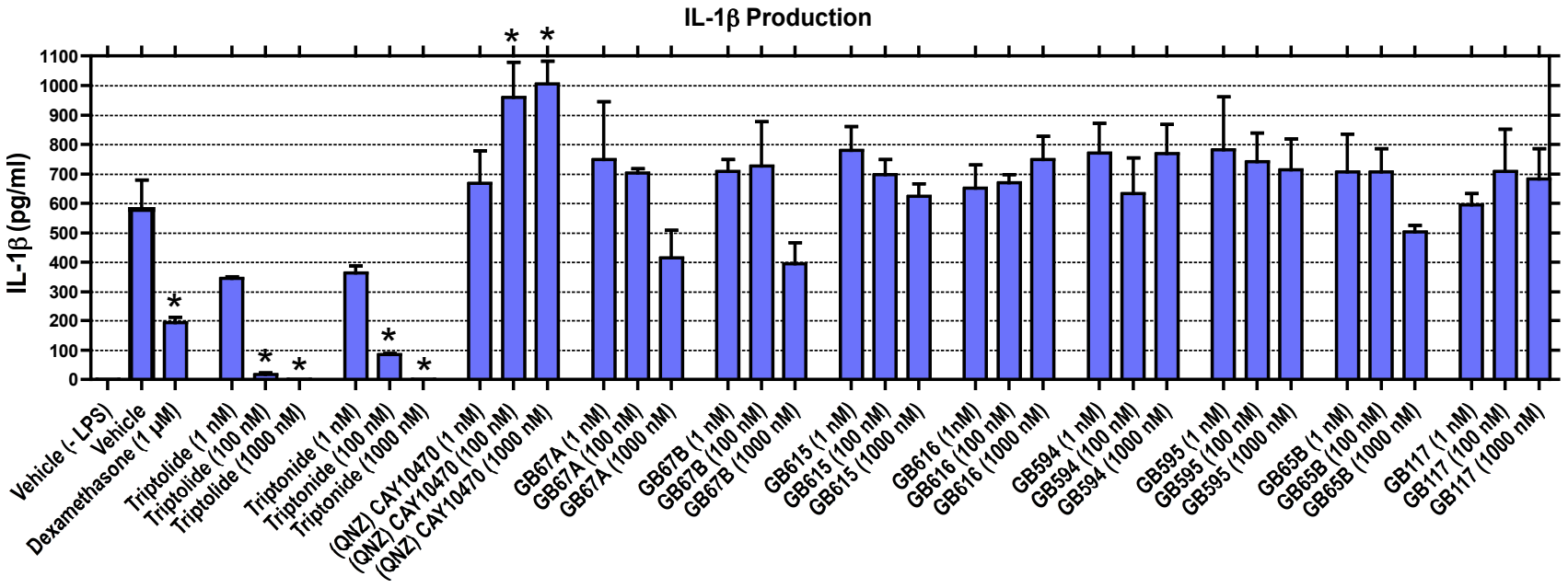


Figure 3. Mean LPS-stimulated IL-1β production. PBMC's were stimulated with LPS following a one hour incubation with vehicle, dexamethasone or test article. After 24 hours of LPS treatment, cell culture supernatants were assayed for IL-1β. Mean values are shown. Error bars represent standard deviations. Cytokine production was analyzed by one-way ANOVA with Tukey's post-test comparing sample values to the vehicle + LPS value. *P < 0.05.

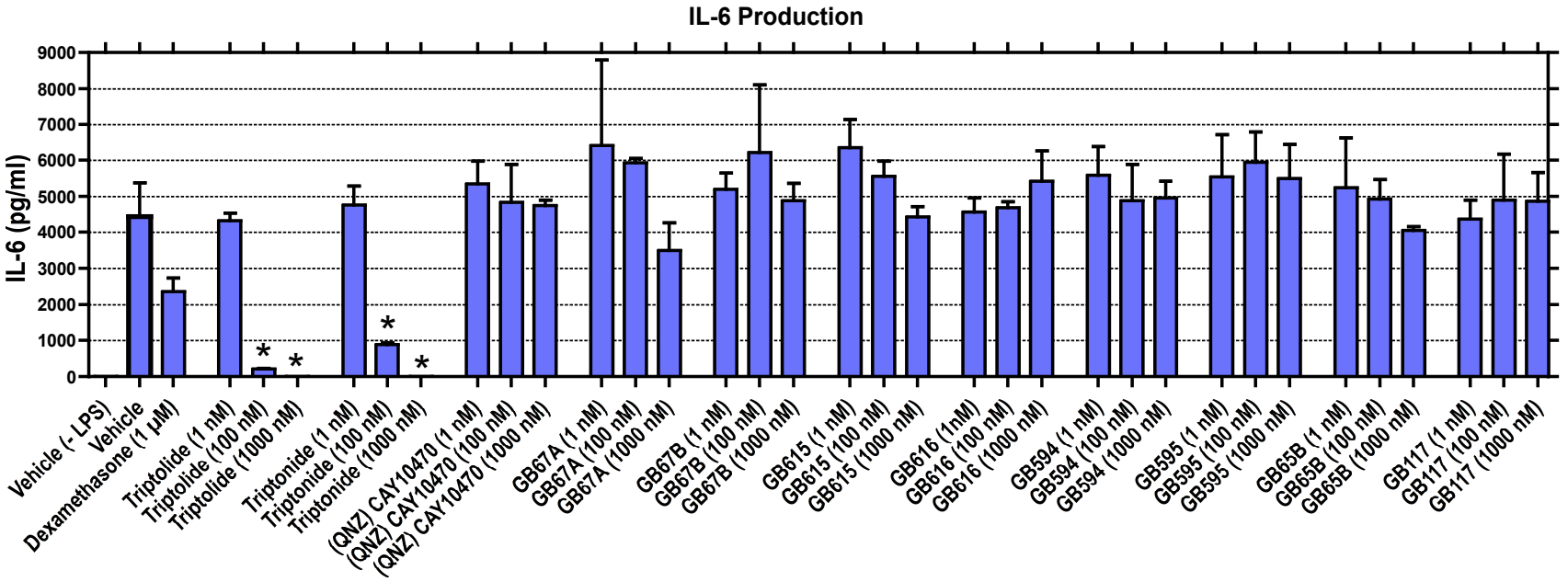


Figure 4. Mean LPS-stimulated IL-6 production. PBMC's were stimulated with LPS following a one hour incubation with vehicle, dexamethasone or test article. After 24 hours of LPS treatment, cell culture supernatants were assayed for IL-6. Mean values are shown. Error bars represent standard deviations. Cytokine production was analyzed by one-way ANOVA with Tukey's post-test comparing sample values to the vehicle + LPS value. *P < 0.05.

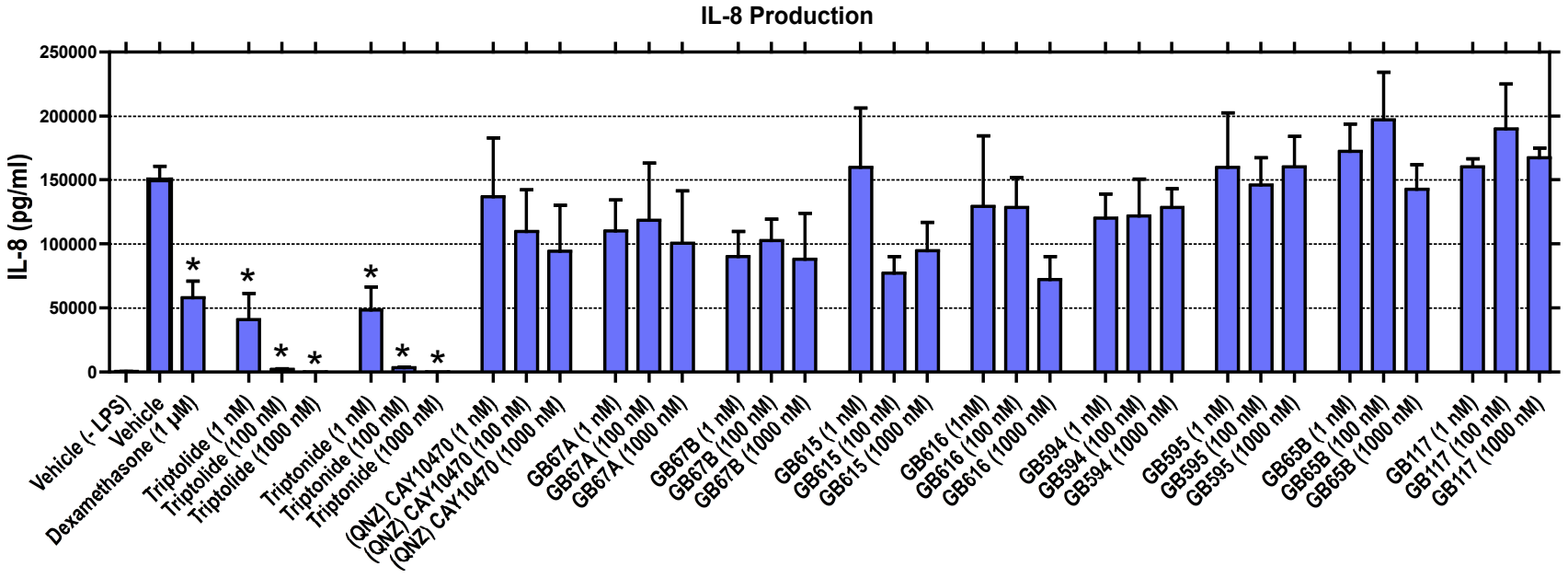


Figure 5. Mean LPS-stimulated IL-8 production. PBMC's were stimulated with LPS following a one hour incubation with vehicle, dexamethasone or test article. After 24 hours of LPS treatment, cell culture supernatants were assayed for IL-8. Mean values are shown. Error bars represent standard deviations. Cytokine production was analyzed by one-way ANOVA with Tukey's post-test comparing sample values to the vehicle + LPS value. *P < 0.05.

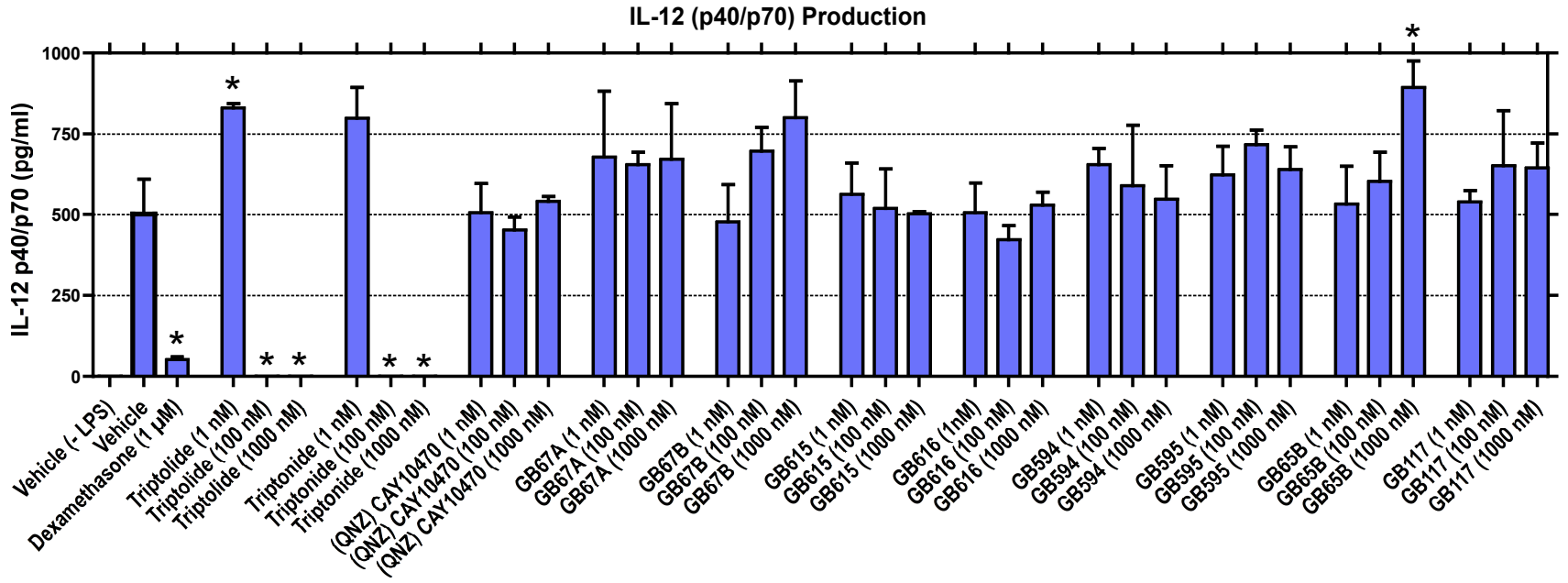


Figure 6. Mean LPS-stimulated IL-12 (p40/p70) production. PBMC's were stimulated with LPS following a one hour incubation with vehicle, dexamethasone or test article. After 24 hours of LPS treatment, cell culture supernatants were assayed for IL-12 (p40/p70). Mean values are shown. Error bars represent standard deviations. Cytokine production was analyzed by one-way ANOVA with Tukey's post-test comparing sample values to the vehicle + LPS value. *P < 0.05.

Table 7. Raw data.

Test Article	Concentration	Stimulation	XTT (OD 450)	IL-6 (pg/ml)	IL-1β (pg/ml)	IL-12 p40/p70 (pg/ml)	IL-8 (pg/ml)
Vehicle		- LPS	0.086	3	<LD	<LD	239
Vehicle		- LPS	0.110	3	<LD	<LD	387
Vehicle		- LPS	0.137	4	<LD	<LD	554
Dexamethasone	1 µM	- LPS	0.090	3	<LD	<LD	62
Dexamethasone	1 µM	- LPS	0.095	<LD	<LD	<LD	62
Dexamethasone	1 µM	- LPS	0.107	<LD	<LD	<LD	56
Triptolide	1 nM	- LPS	0.114	3	<LD	<LD	182
Triptolide	1 nM	- LPS	0.133	3	<LD	<LD	191
Triptolide	1 nM	- LPS	0.137	3	<LD	<LD	181
Triptolide	100 nM	- LPS	0.064	<LD	<LD	<LD	70
Triptolide	100 nM	- LPS	0.064	<LD	<LD	<LD	65
Triptolide	100 nM	- LPS	0.064	<LD	<LD	<LD	65
Triptolide	1000 nM	- LPS	0.048	<LD	<LD	<LD	51
Triptolide	1000 nM	- LPS	0.050	<LD	<LD	<LD	46
Triptolide	1000 nM	- LPS	0.051	<LD	<LD	<LD	43
Triptonide	1 nM	- LPS	0.108	3	<LD	<LD	181
Triptonide	1 nM	- LPS	0.133	3	<LD	<LD	170
Triptonide	1 nM	- LPS	0.184	4	<LD	<LD	334
Triptonide	100 nM	- LPS	0.073	<LD	<LD	<LD	78
Triptonide	100 nM	- LPS	0.081	<LD	<LD	<LD	93
Triptonide	100 nM	- LPS	0.086	3	<LD	<LD	103
Triptonide	1000 nM	- LPS	0.053	<LD	<LD	<LD	43
Triptonide	1000 nM	- LPS	0.056	<LD	<LD	<LD	40
Triptonide	1000 nM	- LPS	0.061	<LD	<LD	<LD	42
(QNZ) CAY10470	1 nM	- LPS	0.126	<LD	<LD	<LD	248
(QNZ) CAY10470	1 nM	- LPS	0.136	<LD	<LD	<LD	256
(QNZ) CAY10470	1 nM	- LPS	0.162	3	<LD	<LD	511
(QNZ) CAY10470	100 nM	- LPS	0.149	<LD	<LD	<LD	346
(QNZ) CAY10470	100 nM	- LPS	0.175	<LD	<LD	<LD	283
(QNZ) CAY10470	100 nM	- LPS	0.171	<LD	<LD	<LD	474
(QNZ) CAY10470	1000 nM	- LPS	0.145	<LD	<LD	<LD	330
(QNZ) CAY10470	1000 nM	- LPS	0.173	19	<LD	<LD	1671
(QNZ) CAY10470	1000 nM	- LPS	0.169	3	<LD	<LD	615
GB67A	1 nM	- LPS	0.141	3	<LD	<LD	276
GB67A	1 nM	- LPS	0.151	<LD	<LD	<LD	267
GB67A	1 nM	- LPS	0.169	<LD	<LD	<LD	278
GB67A	100 nM	- LPS	0.124	3	<LD	<LD	287
GB67A	100 nM	- LPS	0.145	<LD	<LD	<LD	221
GB67A	100 nM	- LPS	0.171	3	<LD	<LD	423
GB67A	1000 nM	- LPS	0.131	3	<LD	<LD	389
GB67A	1000 nM	- LPS	0.152	3	<LD	<LD	660
GB67A	1000 nM	- LPS	0.176	<LD	<LD	<LD	835
GB67B	1 nM	- LPS	0.123	<LD	<LD	<LD	439
GB67B	1 nM	- LPS	0.149	3	<LD	<LD	379
GB67B	1 nM	- LPS	0.157	<LD	<LD	<LD	276
GB67B	100 nM	- LPS	0.143	3	<LD	<LD	357
GB67B	100 nM	- LPS	0.163	3	<LD	<LD	464
GB67B	100 nM	- LPS	0.172	3	<LD	<LD	298
GB67B	1000 nM	- LPS	0.146	4	<LD	<LD	1451
GB67B	1000 nM	- LPS	0.166	<LD	<LD	<LD	704
GB67B	1000 nM	- LPS	0.184	<LD	<LD	<LD	823
GB615	1 nM	- LPS	0.133	<LD	<LD	<LD	281
GB615	1 nM	- LPS	0.143	<LD	<LD	<LD	291
GB615	1 nM	- LPS	0.156	<LD	<LD	<LD	294
GB615	100 nM	- LPS	0.128	<LD	<LD	<LD	245
GB615	100 nM	- LPS	0.130	3	<LD	<LD	248
GB615	100 nM	- LPS	0.156	3	<LD	<LD	389
GB615	1000 nM	- LPS	0.138	<LD	<LD	<LD	241
GB615	1000 nM	- LPS	0.156	3	<LD	<LD	248
GB615	1000 nM	- LPS	0.168	<LD	<LD	<LD	237
GB616	1 nM	- LPS	0.132	<LD	<LD	<LD	352
GB616	1 nM	- LPS	0.151	3	<LD	<LD	342

Table 7. Raw data.

Test Article	Concentration	Stimulation	XTT (OD 450)	IL-6 (pg/ml)	IL-1β (pg/ml)	IL-12 p40/p70 (pg/ml)	IL-8 (pg/ml)
GB616	1 nM	- LPS	0.165	<LD	<LD	<LD	200
GB616	100 nM	- LPS	0.111	3	<LD	<LD	265
GB616	100 nM	- LPS	0.132	<LD	<LD	<LD	230
GB616	100 nM	- LPS	0.186	<LD	<LD	<LD	265
GB616	1000 nM	- LPS	0.122	3	<LD	<LD	319
GB616	1000 nM	- LPS	0.145	<LD	<LD	<LD	319
GB616	1000 nM	- LPS	0.170	3	<LD	<LD	276
GB594	1 nM	- LPS	0.127	<LD	<LD	<LD	239
GB594	1 nM	- LPS	0.162	<LD	<LD	<LD	272
GB594	1 nM	- LPS	0.164	<LD	<LD	<LD	274
GB594	100 nM	- LPS	0.135	3	<LD	<LD	336
GB594	100 nM	- LPS	0.156	3	<LD	<LD	236
GB594	100 nM	- LPS	0.156	<LD	<LD	<LD	202
GB594	1000 nM	- LPS	0.147	4	<LD	<LD	514
GB594	1000 nM	- LPS	0.149	3	<LD	<LD	196
GB594	1000 nM	- LPS	0.162	3	<LD	<LD	227
GB595	1 nM	- LPS	0.147	3	<LD	<LD	310
GB595	1 nM	- LPS	0.145	3	<LD	<LD	212
GB595	1 nM	- LPS	0.175	<LD	<LD	<LD	806
GB595	100 nM	- LPS	0.155	<LD	<LD	<LD	194
GB595	100 nM	- LPS	0.167	<LD	<LD	<LD	312
GB595	100 nM	- LPS	0.168	<LD	<LD	<LD	310
GB595	1000 nM	- LPS	0.136	<LD	<LD	<LD	799
GB595	1000 nM	- LPS	0.146	<LD	<LD	<LD	329
GB595	1000 nM	- LPS	0.151	<LD	<LD	<LD	260
GB65B	1 nM	- LPS	0.119	<LD	<LD	<LD	244
GB65B	1 nM	- LPS	0.147	<LD	<LD	<LD	372
GB65B	1 nM	- LPS	0.151	<LD	<LD	<LD	362
GB65B	100 nM	- LPS	0.118	<LD	<LD	<LD	347
GB65B	100 nM	- LPS	0.145	<LD	<LD	<LD	270
GB65B	100 nM	- LPS	0.159	<LD	<LD	<LD	322
GB65B	1000 nM	- LPS	0.138	<LD	<LD	<LD	1061
GB65B	1000 nM	- LPS	0.167	<LD	<LD	<LD	1115
GB65B	1000 nM	- LPS	0.174	<LD	<LD	<LD	1061
GB117	1 nM	- LPS	0.141	<LD	<LD	<LD	1075
GB117	1 nM	- LPS	0.119	<LD	<LD	<LD	345
GB117	1 nM	- LPS	0.254	101	<LD	<LD	10199
GB117	100 nM	- LPS	0.120	<LD	<LD	<LD	238
GB117	100 nM	- LPS	0.132	<LD	<LD	<LD	1039
GB117	100 nM	- LPS	0.130	<LD	<LD	<LD	288
GB117	1000 nM	- LPS	0.129	<LD	<LD	<LD	563
GB117	1000 nM	- LPS	0.128	<LD	<LD	<LD	292
GB117	1000 nM	- LPS	0.148	<LD	<LD	<LD	304
Vehicle		+ LPS	0.171	3438	495	391	150772
Vehicle		+ LPS	0.268	5302	687	603	160022
Vehicle		+ LPS	0.280	4563	561	511	139377
Dexamethasone	1 µM	+ LPS	0.207	1954	172	56	56213
Dexamethasone	1 µM	+ LPS	0.253	2720	205	56	71772
Dexamethasone	1 µM	+ LPS	0.273	2380	204	43	46023
Triptolide	1 nM	+ LPS	0.202	4469	342	821	35871
Triptolide	1 nM	+ LPS	0.232	4085	351	846	63183
Triptolide	1 nM	+ LPS	0.247	4410	345	821	23611
Triptolide	100 nM	+ LPS	0.074	213	14	<LD	2641
Triptolide	100 nM	+ LPS	0.059	214	18	<LD	1622
Triptolide	100 nM	+ LPS	0.067	220	22	<LD	1622
Triptolide	1000 nM	+ LPS	0.047	<LD	<LD	<LD	91
Triptolide	1000 nM	+ LPS	0.056	<LD	<LD	<LD	76
Triptolide	1000 nM	+ LPS	0.061	<LD	<LD	<LD	76
Triptonide	1 nM	+ LPS	0.177	5069	357	715	58732
Triptonide	1 nM	+ LPS	0.231	5048	390	903	58646
Triptonide	1 nM	+ LPS	0.260	4142	344	775	27564
Triptonide	100 nM	+ LPS	0.069	829	84	<LD	3061

Table 7. Raw data.

Test Article	Concentration	Stimulation	XTT (OD 450)	IL-6 (pg/ml)	IL-1β (pg/ml)	IL-12 p40/p70 (pg/ml)	IL-8 (pg/ml)
Triptonide	100 nM	+ LPS	0.069	935	89	<LD	2740
Triptonide	100 nM	+ LPS	0.071	873	82	<LD	3596
Triptonide	1000 nM	+ LPS	0.047	<LD	<LD	<LD	76
Triptonide	1000 nM	+ LPS	0.056	<LD	<LD	<LD	76
Triptonide	1000 nM	+ LPS	0.057	<LD	<LD	<LD	60
(QNZ) CAY10470	1 nM	+ LPS	0.198	4650	543	402	86659
(QNZ) CAY10470	1 nM	+ LPS	0.236	5533	748	561	148447
(QNZ) CAY10470	1 nM	+ LPS	0.243	5855	714	554	175432
(QNZ) CAY10470	100 nM	+ LPS	0.169	3855	854	437	146923
(QNZ) CAY10470	100 nM	+ LPS	0.184	4679	939	423	92751
(QNZ) CAY10470	100 nM	+ LPS	0.189	5943	1088	497	89172
(QNZ) CAY10470	1000 nM	+ LPS	0.159	4908	928	547	74354
(QNZ) CAY10470	1000 nM	+ LPS	0.171	4683	1003	550	72196
(QNZ) CAY10470	1000 nM	+ LPS	0.177	4617	1083	525	135458
GB67A	1 nM	+ LPS	0.199	4959	596	547	115827
GB67A	1 nM	+ LPS	0.210	5110	680	568	130815
GB67A	1 nM	+ LPS	0.208	9165	971	914	82853
GB67A	100 nM	+ LPS	0.177	6054	719	610	69084
GB67A	100 nM	+ LPS	0.212	5886	702	666	130599
GB67A	100 nM	+ LPS	0.243	5855	689	683	155303
GB67A	1000 nM	+ LPS	0.131	2748	348	483	127416
GB67A	1000 nM	+ LPS	0.165	4277	522	821	120372
GB67A	1000 nM	+ LPS	0.208	3466	377	708	53719
GB67B	1 nM	+ LPS	0.161	5571	721	451	87971
GB67B	1 nM	+ LPS	0.191	4690	666	376	110448
GB67B	1 nM	+ LPS	0.221	5315	741	603	71247
GB67B	100 nM	+ LPS	0.164	8367	889	779	111147
GB67B	100 nM	+ LPS	0.199	5405	698	673	113277
GB67B	100 nM	+ LPS	0.237	4853	592	631	83474
GB67B	1000 nM	+ LPS	0.188	4815	366	807	54592
GB67B	1000 nM	+ LPS	0.189	4420	342	683	83101
GB67B	1000 nM	+ LPS	0.204	5373	477	910	125760
GB615	1 nM	+ LPS	0.184	5860	721	669	134109
GB615	1 nM	+ LPS	0.183	5886	744	536	130815
GB615	1 nM	+ LPS	0.205	7268	873	480	213271
GB615	100 nM	+ LPS	0.190	5938	677	645	76923
GB615	100 nM	+ LPS	0.188	5622	756	509	90101
GB615	100 nM	+ LPS	0.192	5109	657	403	64724
GB615	1000 nM	+ LPS	0.181	4186	581	493	107911
GB615	1000 nM	+ LPS	0.194	4732	664	505	106889
GB615	1000 nM	+ LPS	0.198	4372	630	505	68741
GB616	1 nM	+ LPS	0.173	4359	561	407	65680
GB616	1 nM	+ LPS	0.205	4305	711	525	164691
GB616	1 nM	+ LPS	0.250	5002	682	585	157421
GB616	100 nM	+ LPS	0.150	4505	663	374	149793
GB616	100 nM	+ LPS	0.185	4710	701	436	131461
GB616	100 nM	+ LPS	0.216	4827	646	457	103732
GB616	1000 nM	+ LPS	0.148	5561	795	481	92334
GB616	1000 nM	+ LPS	0.174	6172	794	549	58171
GB616	1000 nM	+ LPS	0.197	4522	658	553	66067
GB594	1 nM	+ LPS	0.162	6199	875	688	132349
GB594	1 nM	+ LPS	0.185	5853	765	676	129492
GB594	1 nM	+ LPS	0.204	4678	673	597	98517
GB594	100 nM	+ LPS	0.195	4872	687	774	141680
GB594	100 nM	+ LPS	0.206	5878	719	593	88874
GB594	100 nM	+ LPS	0.226	3890	496	399	134831
GB594	1000 nM	+ LPS	0.180	5203	752	641	130581
GB594	1000 nM	+ LPS	0.189	4425	681	436	113027
GB594	1000 nM	+ LPS	0.201	5241	875	561	141925
GB595	1 nM	+ LPS	0.187	4195	578	521	202618
GB595	1 nM	+ LPS	0.201	5949	858	684	116547
GB595	1 nM	+ LPS	0.213	6461	912	661	159124

Table 7. Raw data.

Test Article	Concentration	Stimulation	XTT (OD 450)	IL-6 (pg/ml)	IL-1β (pg/ml)	IL-12 p40/p70 (pg/ml)	IL-8 (pg/ml)
GB595	100 nM	+ LPS	0.187	4963	634	676	125254
GB595	100 nM	+ LPS	0.196	6282	770	767	143901
GB595	100 nM	+ LPS	0.197	6569	821	700	168038
GB595	1000 nM	+ LPS	0.166	5904	729	645	132796
GB595	1000 nM	+ LPS	0.223	6150	810	704	168347
GB595	1000 nM	+ LPS	0.254	4415	605	565	178673
GB65B	1 nM	+ LPS	0.180	4042	604	399	187737
GB65B	1 nM	+ LPS	0.197	6768	851	625	181394
GB65B	1 nM	+ LPS	0.223	4894	666	569	147449
GB65B	100 nM	+ LPS	0.160	4664	707	497	185944
GB65B	100 nM	+ LPS	0.194	5548	786	661	166201
GB65B	100 nM	+ LPS	0.226	4542	628	645	238558
GB65B	1000 nM	+ LPS	0.139	4122	513	852	145407
GB65B	1000 nM	+ LPS	0.175	4101	476	841	121993
GB65B	1000 nM	+ LPS	0.182	3921	518	988	159986
GB117	1 nM	+ LPS	0.205	4030	564	569	166810
GB117	1 nM	+ LPS	0.228	4975	639	545	158839
GB117	1 nM	+ LPS	0.247	4083	579	501	154362
GB117	100 nM	+ LPS	0.200	4674	608	581	224344
GB117	100 nM	+ LPS	0.213	6265	873	845	154362
GB117	100 nM	+ LPS	0.233	3739	646	525	191027
GB117	1000 nM	+ LPS	0.213	4569	669	585	165293
GB117	1000 nM	+ LPS	0.226	5765	792	731	175353
GB117	1000 nM	+ LPS	0.234	4245	587	613	161144

<LD: Below the level of detection.
Values in grey were extrapolated below the low standard.