EVALUATION OF COMPOUNDS IN AN LPS-STIMULATED SPLENOCYTE ASSAY.

FINAL REPORT

STUDY NUMBER

MD-3-3-093-1100

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REPORT DATE

September 18, 2008

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DATA PAGE

In vitro phase initiation:	July 30, 2008
Completion of *in vitro* phase:	July 31, 2008
MD Biosciences Study Reference Number:	MD-3-3-093-1100
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1. SUMMARY

Four compounds were tested for their ability to inhibit LPS-stimulated TNF-α production from mouse splenocytes.

Triptolide and QNZ-CAY10470 reduced TNF-α production from mouse splenocytes stimulated with LPS for 6, 12, 24 and 36 hours.

GB67B and GB594 reduced TNF-α production from mouse splenocytes stimulated with LPS for 12 hours.

2. OBJECTIVE

The objective of this study was to evaluate 4 Test Articles in an LPS-stimulated splenocyte assay.

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*

Test Article ID	Sponsor ID	Lot Number	Physical State	Exp	Storage
TA-080056	QNZ-CAY10470	128676-176822	White powder	31-Jul-09	4˚C
TA-080057	Triptolide	NA	White crystals	31-Jul-09	4˚C
TA-080058	GB594	NA	White powder	31-Jul-09	4˚C
TA-080059	GB67B	NA	White powder	31-Jul-09	4˚C

5.2. *Reference Article*

Name	Vendor	Catalog Number	Lot Number	Exp. Date	Storage
Dexamethasone	Sigma	D4902	016K1421	NA	4˚C

5.3. *Experimental Articles*

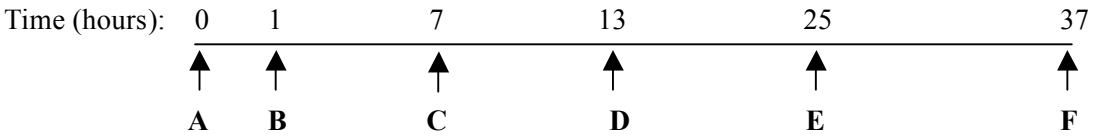
Name	Vendor	Catalog Number	Lot Number	Exp. Date	Storage	Use
Cryopreserved mouse splenocytes	3H Biomedical	3H-2000-50	MPS080615	NA	Liquid N_2	Cell culture
Splenocyte culture media	3H Biomedical	800-2-50	SPM080724	31-Aug-08	4˚C	Cell culture
Cell thawing medium	3H Biomedical	3H610-10-20	CTM080724	24-Jan-09	-30˚C	Cell culture
LPS (*E.coli* 0111:B4)	Sigma	L3012	046K4098	NA	4˚C	Cell treatment
TNF-α Ab bead kit, mouse	Invitrogen	LMC3011	311330A/ 416370A	28-Feb-09/ 31-Oct-09	4˚C	TNF-α assay
Buffer kit, mouse	Invitrogen	LMB0001	368453F	31-Oct-09	4˚C	TNF-α assay
XTT Cell proliferation kit	MD Biosciences	409005	717789	NA	-30˚C	Cell proliferation
DMSO	Sigma	D2650	058K2311	31-May-10	RT	Solution prep.

5.4. *Culture Media*

Splenocyte culture media (Splenocyte Complete Media; SCM)

6. TEST METHOD

6.1. *Schematic Depiction of LPS-stimulated Mouse Splenocyte Assay*



A: Add Test Articles and Reference Articles to mouse splenocytes.
B: Add LPS.
C: Harvest culture supernatants for TNF-α assay (6 hour time point).
D: Harvest culture supernatants for TNF-α assay (12 hour time point).
E: Harvest culture supernatants for TNF-α assay, perform XTT assay (24 hour time point).
F: Harvest culture supernatants for TNF-α assay (36 hour time point).

6.2. *Test Article Preparation*

20 mM Test Article stock solutions were prepared in DMSO:

			20 mM Stock Solution		
Test Article	**Name**	**MW**	**mg**	**DMSO (ml)**	**mg/ml**
TA-080056	QNZ-CAY10470	356.4	5	0.701	7.128
TA-080057	Triptolide	360.4	9.3	1.290	7.208
TA-080058	GB594	242.3	6.6	1.362	4.846
TA-080059	GB67B	208.3	6	1.440	4.166

2000X triptolide solutions were prepared in DMSO:

2000X Stock Solutions				
	Volume	**Source**	**Diluent**	**Total Volume**
100 µM	25 µl	1 mM	225 µl DMSO	250 µl
20 µM	50 µl	100 µM	200 µl DMSO	250 µl
2 µM	25 µl	20 µM	225 µl DMSO	250 µl

2X triptolide solutions were prepared in SCM:

2X Working Solutions				
	Volume	**Source**	**Diluent**	**Total Volume**
100 nM	5 µl	100 µM	5 ml CM	5 ml
20 nM	5 µl	20 µM	5 ml CM	5 ml
2 nM	5 µl	2 µM	5 ml CM	5 ml

2000X QNZ-CAY10470, GB67B and GB594 solutions were prepared in DMSO:

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

2X QNZ-CAY10470, GB67B and GB594 solutions were prepared in SCM:

2X 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

6.3. *Dexamethasone Preparation*

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

6.4. *Vehicle Preparation*

A 2X vehicle control working solution was prepared by diluting DMSO to a final concentration of 0.1% in SCM.

6.5. *LPS Preparation*

A 1 mg/ml LPS stock solution was prepared in 1X PBS. A 10X LPS working solution of 30 µg/ml was prepared by diluting the 1 mg/ml solution with SCM.

6.6. *Culture Setup*

Cells were thawed, washed with cell thawing media and resuspended in SCM to 2 x 10^7 cells/ml. 50 µl of cells was added to 4, 96-well plates (1 x 10^6 cells/well). 50 µl of cells was added to one additional 96-well plate for the XTT assay.

Cell Culture Plate Layout (4 plates: 6, 12, 24 and 36 hour plates for TNF-α assay).

	- LPS						**+ 3 µg/ml LPS**					
	1	**2**	**3**	**4**	**5**	**6**	**7**	**8**	**9**	**10**	**11**	**12**
A	Vehicle	Vehicle	Vehicle	GB67B 1 nM	GB67B 1 nM	GB67B 1 nM	Vehicle	Vehicle	Vehicle	GB67B 1 nM	GB67B 1 nM	GB67B 1 nM
B	Dex 1 µM	Dex 1 µM	Dex 1 µM	GB67B 100 nM	GB67B 100 nM	GB67B 100 nM	Dex 1 µM	Dex 1 µM	Dex 1 µM	GB67B 100 nM	GB67B 100 nM	GB67B 100 nM
C	Triptolide 1 nM	Triptolide 1 nM	Triptolide 1 nM	GB67B 1000 nM	GB67B 1000 nM	GB67B 1000 nM	Triptolide 1 nM	Triptolide 1 nM	Triptolide 1 nM	GB67B 1000 nM	GB67B 1000 nM	GB67B 1000 nM
D	Triptolide 10 nM	Triptolide 10 nM	Triptolide 10 nM	GB594 1 nM	GB594 1 nM	GB594 1 nM	Triptolide 10 nM	Triptolide 10 nM	Triptolide 10 nM	GB594 1 nM	GB594 1 nM	GB594 1 nM
E	Triptolide 50 nM	Triptolide 50 nM	Triptolide 50 nM	GB594 100 nM	GB594 100 nM	GB594 100 nM	Triptolide 50 nM	Triptolide 50 nM	Triptolide 50 nM	GB594 100 nM	GB594 100 nM	GB594 100 nM
F	QNZ 1 nM	QNZ 1 nM	QNZ 1 nM	GB594 1000 nM	GB594 1000 nM	GB594 1000 nM	QNZ 1 nM	QNZ 1 nM	QNZ 1 nM	GB594 1000 nM	GB594 1000 nM	GB594 1000 nM
G	QNZ 100 nM	QNZ 100 nM	QNZ 100 nM				QNZ 100 nM	QNZ 100 nM	QNZ 100 nM			
H	QNZ 1000 nM	QNZ 1000 nM	QNZ 1000 nM				QNZ 1000 nM	QNZ 1000 nM	QNZ 1000 nM			

Cell Culture Plate Layout (1 plate: 24 hour plate for XTT assay).

	- LPS											
	1	2	3	4	5	6	7	8	9	10	11	12
A	Vehicle	Vehicle	Vehicle	GB67B 1 nM	GB67B 1 nM	GB67B 1 nM						
B	Dex 1 µM	Dex 1 µM	Dex 1 µM	GB67B 100 nM	GB67B 100 nM	GB67B 100 nM						
C	Triptolide 1 nM	Triptolide 1 nM	Triptolide 1 nM	GB67B 1000 nM	GB67B 1000 nM	GB67B 1000 nM						
D	Triptolide 10 nM	Triptolide 10 nM	Triptolide 10 nM	GB594 1 nM	GB594 1 nM	GB594 1 nM						
E	Triptolide 50 nM	Triptolide 50 nM	Triptolide 50 nM	GB594 100 nM	GB594 100 nM	GB594 100 nM						
F	QNZ 1 nM	QNZ 1 nM	QNZ 1 nM	GB594 1000 nM	GB594 1000 nM	GB594 1000 nM						
G	QNZ 100 nM	QNZ 100 nM	QNZ 100 nM									
H	QNZ 1000 nM	QNZ 1000 nM	QNZ 1000 nM									

6.7. *Cell Treatment*

50 µl of the 2X vehicle, dexamethasone and Test Article solutions was added to the appropriate wells. Cells were incubated for 1 hour at 37˚C with 5% CO_2.

11 µl of SCM was added to each – LPS well. 11 µl of the 10X LPS solution was added to each + LPS well (final concentration: 3 µg/ml LPS).

Cells were incubated at 37˚C with 5% CO_2 for 6, 12, 24 and 36 hours.

6.8. *Supernatant Harvesting*

Cell culture supernatants were collected after 6, 12, 24 and 36 hours of LPS treatment and stored at -30˚C until assayed.

6.9. *XTT Assay*

After 24 hours, 50 µl of activated XTT reagent was added to each well of cells in the 96 well XTT plate and incubated at 37˚C with 5% CO_2 for 11 hours. Reduced XTT was detected at 450 nm (630 nm correction) using a ThermoMax microplate reader (Molecular Devices, Sunnyvale, CA).

6.10. *TNF-α Assay*

Cell culture supernatants were assayed for TNF-α using a Luminex-based assay according to 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. DEVIATIONS

There were no experimental deviations.

8. DATA EVALUATION

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

9. RESULTS

9.1. *Effect of Test Articles on Cell Proliferation*

To measure cell proliferation, the ability of cells to reduce XTT was determined. The amount of reduced XTT, as measured by the sample absorbance at 450 nm, is proportional to the metabolic activity of the cells. Since splenocytes grow slowly, the XTT assay was carried out for 11 hours. This extended incubation decreased the signal to noise ratio (compare raw OD_{450} values with blank corrected OD_{450} values in Table 4). Therefore, the cell proliferation data should be used with caution.

None of the compounds significantly reduced splenocyte viability after 24 hours (Table 1, Figure 1). These data indicate that the Test Articles are not cytotoxic to mouse splenocytes at the concentrations tested.

Triptolide, GB67B and GB594 increased the amount of reduced XTT at some of the concentrations tested (Table 1, Figure 1).

9.2. *TNF-α Production*

The Test Articles did not significantly induce TNF-α production in the absence of LPS (Table 2). Incubation with 3 µg/ml LPS induced TNF-α production from mouse splenocytes. TNF-α production peaked at 24 hours (Table 2, Figure 2).

9.3. *Effect of Dexamethasone on TNF-α Production*

The Reference Article, dexamethasone, significantly reduced LPS-stimulated TNF-α production at each time point (Tables 2 and 3, Figure 3).

9.4. *Effect of Triptolide on TNF-α Production*

Triptolide significantly reduced LPS-stimulated TNF-α production at each time point (Tables 2 and 3, Figure 3).

9.5. *Effect of QNZ-CAY10470 on TNF-α Production*

QNZ-CAY10470 significantly reduced LPS-stimulated TNF-α production at each time point (Tables 2 and 3, Figure 3).

9.6. *Effect of GB67B on TNF-α Production*

GB67B significantly reduced LPS-stimulated TNF-α production after 12 hours of LPS stimulation (Tables 2 and 3, Figure 3). This reduction was not observed at 6, 24 or 36 hours.

9.7. *Effect of GB594 on TNF-α Production*

GB594 significantly reduced LPS-stimulated TNF-α production after 12 hours of LPS stimulation (Tables 2 and 3, Figure 3). This reduction was not observed at 6, 24 or 36 hours.

10. CONCLUSIONS

Triptolide and QNZ-CAY10470 reduced TNF-α production from LPS-stimulated mouse splenocytes at 6, 12, 24 and 36 hours. GB67B and GB594 reduced TNF-α production after 12 hours but not after 6, 24 or 36 hours of LPS stimulation.

Table 1. XTT cell proliferation assay.

Treatment	Concentration	LPS	Mean XTT (OD_{450})	Std.Dev.
Vehicle		-	0.113	0.021
Dexamethasone	1 µM	-	0.08	0.009
Triptolide	1 nM	-	0.183	0.021
Triptolide	10 nM	-	0.16	0.013
Triptolide	50 nM	-	0.101	0.018
QNZ (CAY10470)	1 nM	-	0.153	0.017
QNZ (CAY10470)	100 nM	-	0.106	0.023
QNZ (CAY10470)	1000 nM	-	0.11	0.006
GB67B	1 nM	-	0.114	0.054
GB67B	100 nM	-	0.179	0.01
GB67B	1000 nM	-	0.178	0.014
GB594	1 nM	-	0.177	0.019
GB594	100 nM	-	0.18	0.024
GB594	1000 nM	-	0.168	0.014

Table 2. Mean TNF-α production.

			6 hours		12 hours		24 hours		36 hours	
Treatment	Concentration	LPS	Mean TNF-α (pg/ml)	Std. Dev.	Mean TNF-α (pg/ml)	Std. Dev.	Mean TNF-α (pg/ml)	Std. Dev.	Mean TNF-α (pg/ml)	Std. Dev.
Vehicle		-	<LD	NA	<LD	NA	<LD	NA	10.6	0.0
Dexamethasone	1 µM	-	<LD	NA	<LD	NA	<LD	NA	10.6	0.0
Triptolide	1 nM	-	<LD	NA	9.4	0	<LD	NA	10.6	0.0
Triptolide	10 nM	-	<LD	NA	9.4	0	<LD	NA	10.6	0.0
Triptolide	50 nM	-	<LD	NA	<LD	NA	<LD	NA	10.6	0.0
QNZ (CAY10470)	1 nM	-	<LD	NA	<LD	NA	<LD	NA	10.6	0.0
QNZ (CAY10470)	100 nM	-	<LD	NA	<LD	NA	<LD	NA	10.6	0.0
QNZ (CAY10470)	1000 nM	-	<LD	NA	<LD	NA	<LD	NA	10.6	0.0
GB67B	1 nM	-	<LD	NA	9.4	0	<LD	NA	9.3	1.1
GB67B	100 nM	-	<LD	NA	9.4	0	<LD	NA	9.3	1.1
GB67B	1000 nM	-	<LD	NA	<LD	NA	<LD	NA	9.3	1.1
GB594	1 nM	-	<LD	NA	<LD	NA	<LD	NA	9.3	1.1
GB594	100 nM	-	<LD	NA	<LD	NA	<LD	NA	9.5	1.0
GB594	1000 nM	-	<LD	NA	<LD	NA	<LD	NA	9.5	1.0
Vehicle		+	43.0	6.9	77.1	7.2	113.4	7.9	84.5	15.0
Dexamethasone	1 µM	+	<LD	NA	14.3	1.5	15.8	0.6	12.6	0.6
Triptolide	1 nM	+	29.5	4.8	57.3	3.1	70.9	0.9	65.8	9.2
Triptolide	10 nM	+	30.3	7.7	43.7	5.8	47.3	0.9	40.1	6.4
Triptolide	50 nM	+	25.6	7.3	28.1	0.5	25.9	2.3	24.3	2.0
QNZ (CAY10470)	1 nM	+	26.8	3.6	41.5	9.5	45.2	5.8	39.4	6.7
QNZ (CAY10470)	100 nM	+	13.2	5.5	26.0	4.1	30.1	1.6	28.2	0.8
QNZ (CAY10470)	1000 nM	+	9.4	7.1	27.9	1.6	29.8	0.5	28.5	2.5
GB67B	1 nM	+	50.1	6.8	61.0	4.5	120.7	29.6	116.5	18.7
GB67B	100 nM	+	40.4	7.4	56.2	7.1	106.7	22.7	95.9	30.4
GB67B	1000 nM	+	31.1	4.4	39.5	1.0	77.9	23.9	75.1	14.5
GB594	1 nM	+	43.5	9.4	56.5	3.5	91.3	22.0	97.1	26.5
GB594	100 nM	+	40.9	2.6	51.0	5.5	103.8	28.2	105.3	8.0
GB594	1000 nM	+	40.3	6.6	56.7	2.9	97.5	18.6	107.2	33.5

Means in grey were calculated using values extrapolated below the lowest assay standard.
<LD: Below level of detection.
NA: Not applicable

Table 3. Mean TNF-α production, % activity.

Treatment	Concentration	LPS	6 hours		12 hours		24 hours		36 hours	
			Mean TNF-α (% Vehicle)	Std. Dev.	Mean TNF-α (% Vehicle)	Std. Dev.	Mean TNF-α (% Vehicle)	Std. Dev.	Mean TNF-α (% Vehicle)	Std. Dev.
Vehicle		+	100%	16%	100%	9%	100%	7%	100%	18%
Dexamethasone	1 µM	+	NA	NA	19%	2%	14%	1%	15%	1%
Triptolide	1 nM	+	69%	11%	74%	4%	62%	1%	78%	11%
Triptolide	10 nM	+	71%	18%	57%	8%	42%	1%	47%	8%
Triptolide	50 nM	+	59%	17%	36%	1%	23%	2%	29%	2%
QNZ (CAY10470)	1 nM	+	62%	8%	54%	12%	40%	5%	47%	8%
QNZ (CAY10470)	100 nM	+	31%	13%	34%	5%	27%	1%	33%	1%
QNZ (CAY10470)	1000 nM	+	22%	16%	36%	2%	26%	0%	34%	3%
GB67B	1 nM	+	116%	16%	79%	6%	106%	26%	138%	22%
GB67B	100 nM	+	94%	17%	73%	9%	94%	20%	113%	36%
GB67B	1000 nM	+	72%	10%	51%	1%	69%	21%	89%	17%
GB594	1 nM	+	101%	22%	73%	5%	80%	19%	115%	31%
GB594	100 nM	+	95%	6%	66%	7%	92%	25%	125%	10%
GB594	1000 nM	+	94%	15%	74%	4%	86%	16%	127%	40%

NA: Not applicable

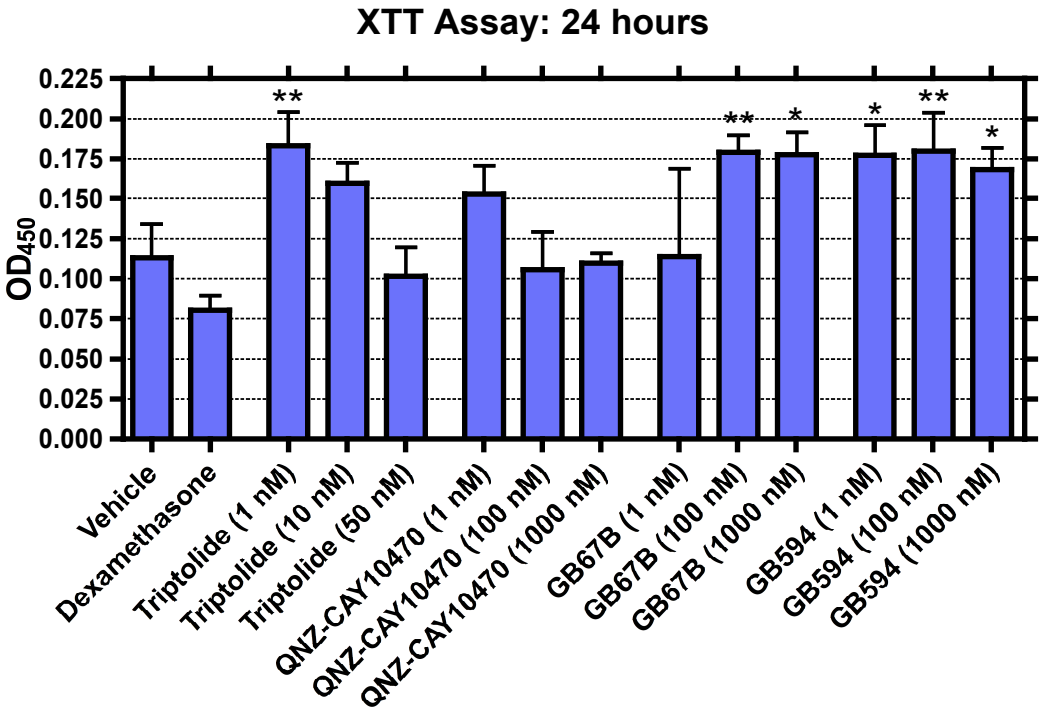


Figure 1. Cell proliferation assay. Mouse splenocytes were incubated with XTT after a 24 hour incubation with the compound indicated above. The amount of reduced XTT, a measure of metabolic activity, was measured at 450 nm. Mean values are shown. Error bars represent standard deviations. Values were analyzed by one-way ANOVA with Dunnett's post-test comparing sample values to the vehicle value. *P < 0.05, **P < 0.01.

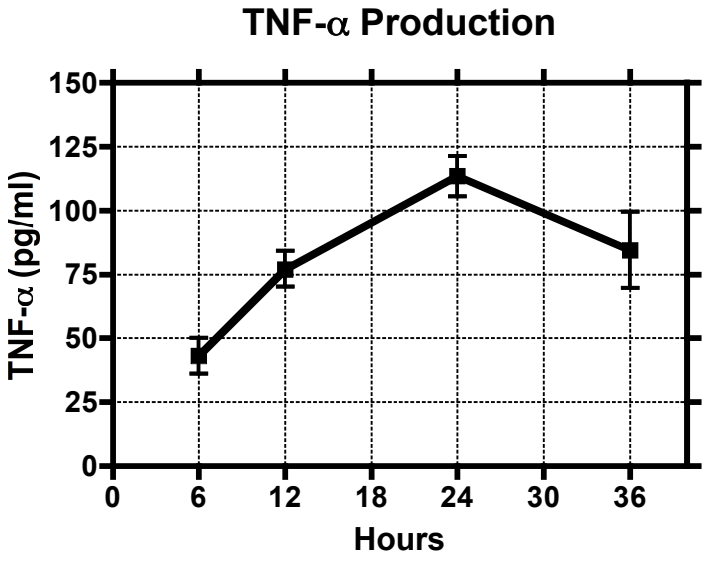


Figure 2. TNF-α time course. Mouse splenocytes were treated with LPS. TNF-α production was determined after 6, 12, 24 and 36 hours of LPS stimulation. Mean values are shown. Error bars represent standard deviations.

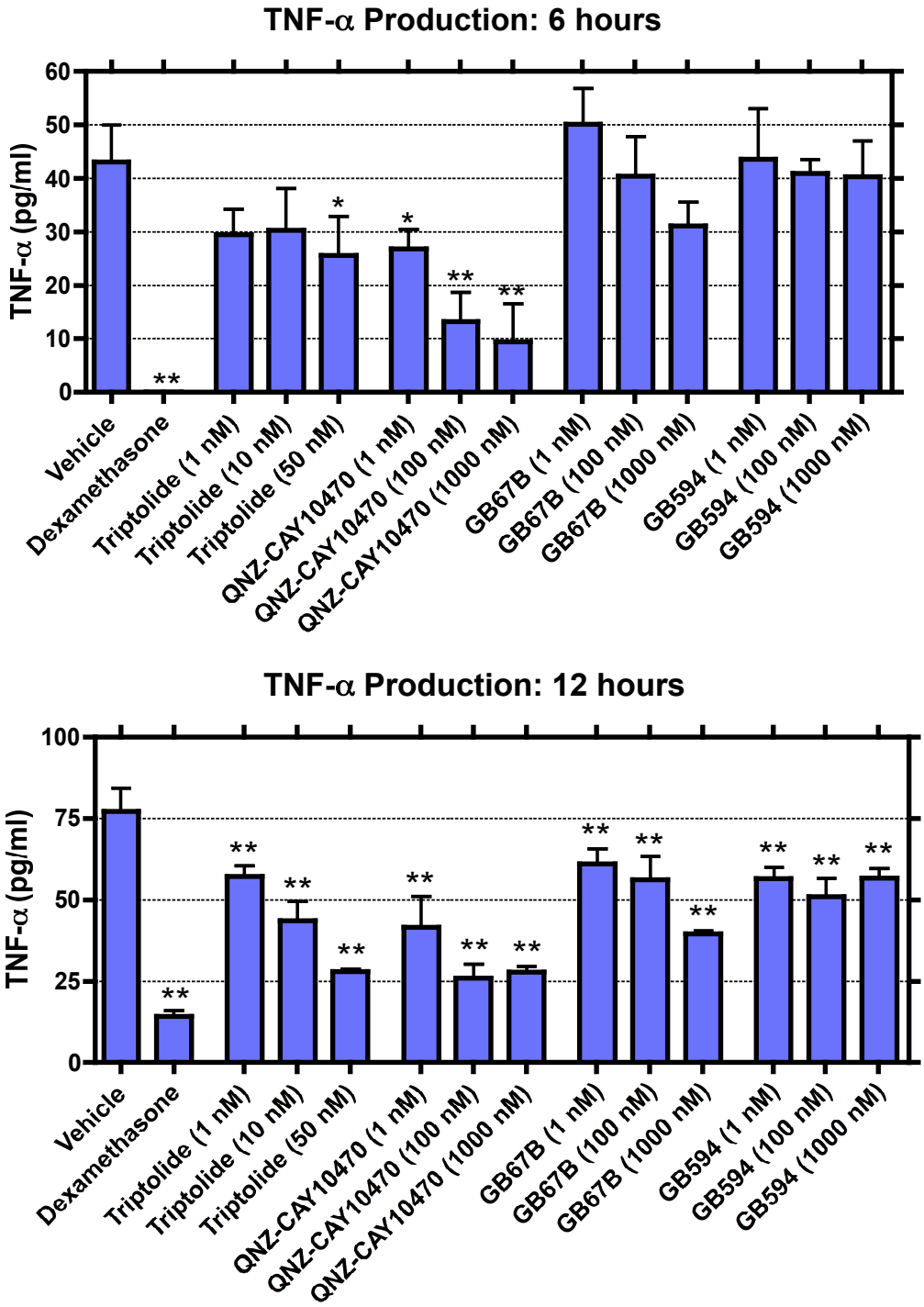


Figure 3. Mean TNF-α production. Mouse splenocytes were treated with LPS following a one hour incubation with vehicle, dexamethasone or test article. TNF-α production was determined after 6, 12, 24 and 36 hours of LPS stimulation. Mean values are shown. Error bars represent standard deviations. Values were analyzed by one-way ANOVA with Dunnett's post-test comparing sample values to the vehicle + LPS value. *P < 0.05, **P < 0.01.

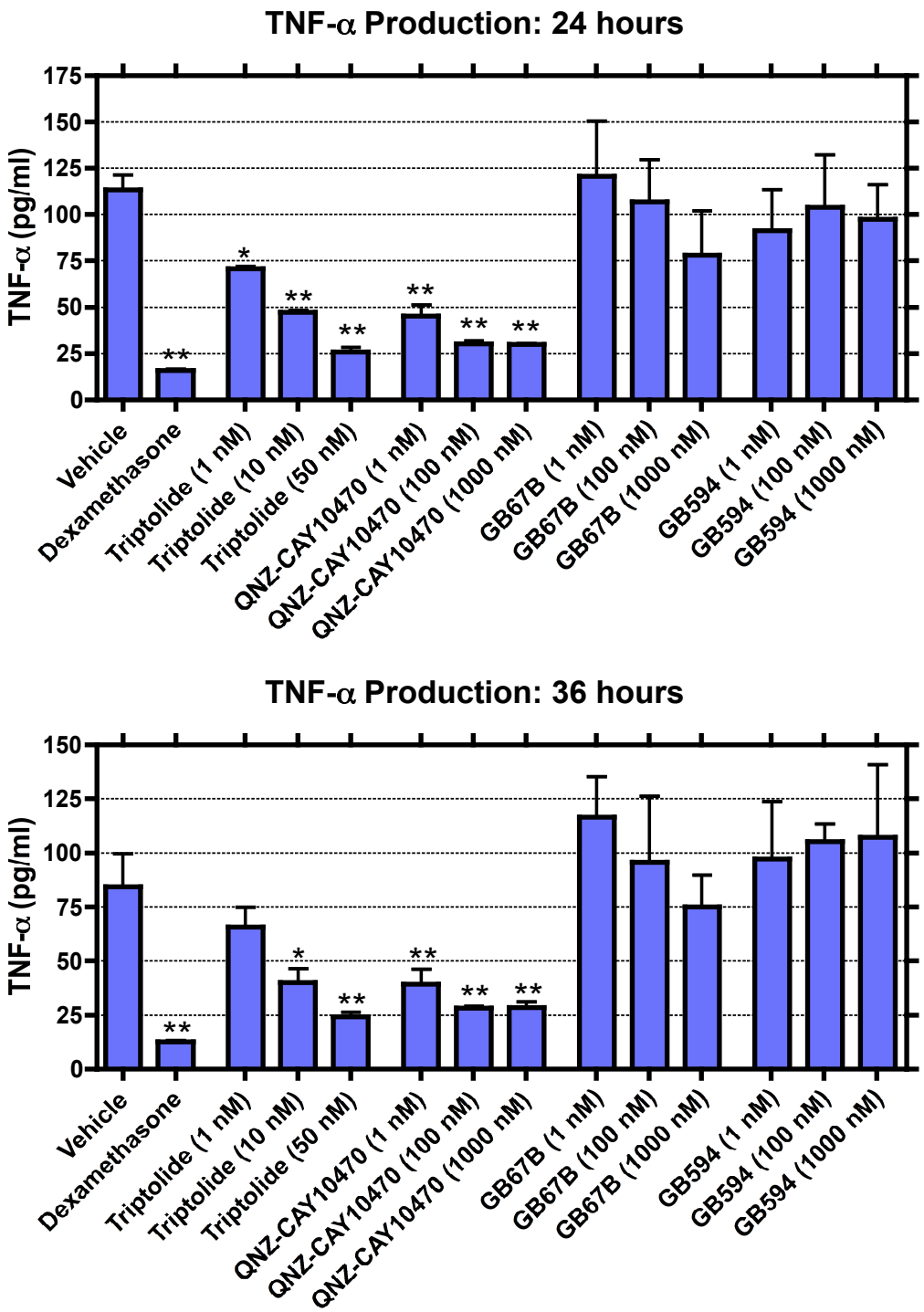


Figure 3 (continued). Mean TNF-α production. Mouse splenocytes were treated with LPS following a one hour incubation with vehicle, dexamethasone or test article. TNF-α production was determined after 6, 12, 24 and 36 hours of LPS stimulation. Mean values are shown. Error bars represent standard deviations. Values were analyzed by one-way ANOVA with Dunnett's post-test comparing sample values to the vehicle + LPS value. *P < 0.05, **P < 0.01.

Table 4. Raw data, XTT assay.

Treatment	Concentration	LPS	OD_{450}	Blank Corrected OD_{450}
Vehicle		-	0.876	0.134
Vehicle		-	0.855	0.113
Vehicle		-	0.834	0.092
Dexamethasone	1 μM	-	0.832	0.090
Dexamethasone	1 μM	-	0.820	0.078
Dexamethasone	1 μM	-	0.815	0.073
Triptolide	1 nM	-	0.949	0.207
Triptolide	1 nM	-	0.917	0.175
Triptolide	1 nM	-	0.910	0.168
Triptolide	10 nM	-	0.913	0.171
Triptolide	10 nM	-	0.904	0.162
Triptolide	10 nM	-	0.888	0.146
Triptolide	50 nM	-	0.857	0.115
Triptolide	50 nM	-	0.850	0.108
Triptolide	50 nM	-	0.823	0.081
QNZ (CAY10470)	1 nM	-	0.883	0.141
QNZ (CAY10470)	1 nM	-	0.915	0.173
QNZ (CAY10470)	1 nM	-	0.887	0.145
QNZ (CAY10470)	100 nM	-	0.822	0.080
QNZ (CAY10470)	100 nM	-	0.867	0.125
QNZ (CAY10470)	100 nM	-	0.854	0.112
QNZ (CAY10470)	1000 nM	-	0.854	0.112
QNZ (CAY10470)	1000 nM	-	0.845	0.103
QNZ (CAY10470)	1000 nM	-	0.856	0.114
GB67B	1 nM	-	0.794	0.052
GB67B	1 nM	-	0.896	0.154
GB67B	1 nM	-	0.878	0.136
GB67B	100 nM	-	0.918	0.176
GB67B	100 nM	-	0.913	0.171
GB67B	100 nM	-	0.933	0.191
GB67B	1000 nM	-	0.904	0.162
GB67B	1000 nM	-	0.925	0.183
GB67B	1000 nM	-	0.930	0.188
GB594	1 nM	-	0.917	0.175
GB594	1 nM	-	0.902	0.160
GB594	1 nM	-	0.939	0.197
GB594	100 nM	-	0.895	0.153
GB594	100 nM	-	0.938	0.196
GB594	100 nM	-	0.933	0.191
GB594	1000 nM	-	0.898	0.156
GB594	1000 nM	-	0.925	0.183
GB594	1000 nM	-	0.907	0.165
Blank[1]			0.742	0.000

[1]Blank: Cell free well with SCM and XTT reagent.

Table 5. Raw data, TNFα assay.

			TNF-α (pg/ml)			
Treatment	**Concentration**	**LPS**	**6 hours**	**12 hours**	**24 hours**	**36 hours**
Vehicle		-	<LD	<LD	<LD	<LD
Vehicle		-	<LD	<LD	<LD	10.6
Vehicle		-	<LD	<LD	<LD	10.6
Dexamethasone	1 µM	-	<LD	<LD	<LD	10.6
Dexamethasone	1 µM	-	<LD	<LD	<LD	10.6
Dexamethasone	1 µM	-	<LD	<LD	<LD	<LD
Triptolide	1 nM	-	<LD	9.4	<LD	10.6
Triptolide	1 nM	-	<LD	<LD	<LD	<LD
Triptolide	1 nM	-	<LD	<LD	<LD	10.6
Triptolide	10 nM	-	<LD	9.4	<LD	10.6
Triptolide	10 nM	-	<LD	<LD	<LD	10.6
Triptolide	10 nM	-	<LD	<LD	<LD	10.6
Triptolide	50 nM	-	<LD	<LD	<LD	10.6
Triptolide	50 nM	-	<LD	<LD	<LD	10.6
Triptolide	50 nM	-	<LD	<LD	<LD	10.6
QNZ (CAY10470)	1 nM	-	<LD	<LD	<LD	10.6
QNZ (CAY10470)	1 nM	-	<LD	<LD	<LD	10.6
QNZ (CAY10470)	1 nM	-	<LD	<LD	<LD	10.6
QNZ (CAY10470)	100 nM	-	<LD	<LD	<LD	10.6
QNZ (CAY10470)	100 nM	-	<LD	<LD	<LD	10.6
QNZ (CAY10470)	100 nM	-	<LD	<LD	<LD	10.6
QNZ (CAY10470)	1000 nM	-	<LD	<LD	<LD	10.6
QNZ (CAY10470)	1000 nM	-	<LD	<LD	<LD	<LD
QNZ (CAY10470)	1000 nM	-	<LD	<LD	<LD	10.6
GB67B	1 nM	-	<LD	9.4	<LD	10.6
GB67B	1 nM	-	<LD	9.4	<LD	8.6
GB67B	1 nM	-	<LD	<LD	<LD	8.6
GB67B	100 nM	-	<LD	<LD	<LD	10.6
GB67B	100 nM	-	<LD	<LD	<LD	8.6
GB67B	100 nM	-	<LD	9.4	<LD	8.6
GB67B	1000 nM	-	<LD	<LD	<LD	10.6
GB67B	1000 nM	-	<LD	<LD	<LD	8.6
GB67B	1000 nM	-	<LD	<LD	<LD	8.6
GB594	1 nM	-	<LD	<LD	<LD	10.6
GB594	1 nM	-	<LD	<LD	<LD	8.6
GB594	1 nM	-	<LD	<LD	<LD	8.6
GB594	100 nM	-	<LD	<LD	<LD	10.6
GB594	100 nM	-	<LD	<LD	<LD	9.3
GB594	100 nM	-	<LD	<LD	<LD	8.6
GB594	1000 nM	-	<LD	<LD	<LD	10.6
GB594	1000 nM	-	<LD	<LD	<LD	9.3
GB594	1000 nM	-	<LD	<LD	<LD	8.6
Vehicle		+	47.4	84.8	108.5	68.5
Vehicle		+	46.6	75.9	122.5	98.2
Vehicle		+	35.0	70.6	109.2	86.7
Dexamethasone	1 µM	+	<LD	15.2	15.1	12.0
Dexamethasone	1 µM	+	<LD	15.2	16.1	12.6
Dexamethasone	1 µM	+	<LD	12.6	16.1	13.2
Triptolide	1 nM	+	33.1	54.1	71.4	56.3
Triptolide	1 nM	+	31.2	57.5	71.4	66.7
Triptolide	1 nM	+	24.1	60.3	69.8	74.5
Triptolide	10 nM	+	36.9	48.6	48.1	33.7
Triptolide	10 nM	+	32.2	45.1	47.3	46.5
Triptolide	10 nM	+	21.9	37.3	46.4	40.0
Triptolide	50 nM	+	28.2	27.9	23.3	22.2
Triptolide	50 nM	+	31.2	28.7	27.2	26.2
Triptolide	50 nM	+	17.3	27.6	27.2	24.5
QNZ (CAY10470)	1 nM	+	27.2	39.3	40.2	31.6
QNZ (CAY10470)	1 nM	+	30.2	33.3	51.6	43.5
QNZ (CAY10470)	1 nM	+	23.0	51.9	43.8	43.0
QNZ (CAY10470)	100 nM	+	18.5	25.5	29.2	27.3

Table 5. Raw data, TNFα assay.

			TNF-α (pg/ml)			
Treatment	**Concentration**	**LPS**	**6 hours**	**12 hours**	**24 hours**	**36 hours**
QNZ (CAY10470)	100 nM	+	13.5	22.2	29.2	28.4
QNZ (CAY10470)	100 nM	+	7.6	30.4	32.0	28.9
QNZ (CAY10470)	1000 nM	+	13.5	27.9	30.1	25.6
QNZ (CAY10470)	1000 nM	+	13.5	26.3	29.2	30.0
QNZ (CAY10470)	1000 nM	+	1.2	29.5	30.1	30.0
GB67B	1 nM	+	48.3	58.0	97.9	103.4
GB67B	1 nM	+	44.4	66.2	110.0	108.2
GB67B	1 nM	+	57.5	58.8	154.1	138.0
GB67B	100 nM	+	41.4	50.3	91.0	71.8
GB67B	100 nM	+	32.6	64.0	96.4	85.8
GB67B	100 nM	+	47.2	54.2	132.7	130.0
GB67B	1000 nM	+	32.2	39.0	63.3	62.9
GB67B	1000 nM	+	26.3	39.0	64.9	71.3
GB67B	1000 nM	+	34.8	40.6	105.5	91.2
GB594	1 nM	+	42.3	53.4	77.0	69.5
GB594	1 nM	+	34.8	55.7	80.2	99.5
GB594	1 nM	+	53.5	60.3	116.6	122.4
GB594	100 nM	+	40.5	55.0	81.7	103.0
GB594	100 nM	+	38.6	44.7	94.1	98.6
GB594	100 nM	+	43.6	53.4	135.6	114.2
GB594	1000 nM	+	36.0	58.0	84.9	83.6
GB594	1000 nM	+	37.1	53.4	88.7	92.5
GB594	1000 nM	+	47.9	58.8	118.8	145.5

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