EVALUATION OF COMPOUNDS IN AN NF-κB REPORTER ASSAY.

FINAL REPORT

STUDY NUMBER

MD-3-3-093-1100

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DATA PAGE

In vitro phase initiation:	July 22, 2008
Completion of *in vitro* phase:	July 25, 2008
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1. SUMMARY

Four compounds were tested for their ability to inhibit NF-κB in an immortalized T lymphocyte cell line (Jurkat) transfected with an NF-κB reporter plasmid.

At high concentrations (50 nM), triptolide inhibited Jurkat cell proliferation suggesting that it may be cytotoxic/cytostatic at this concentration.

QNZ-CAY10470 significantly inhibited NF-κB activity in Jurkat cells treated with PMA and PHA for 6 hours. Significant inhibition was not detected after 12, 24 or 36 hours of stimulation.

Triptolide, GB67B and GB594 did not significantly affect NF-κB activity in PMA/PHA-stimulated Jurkat cells.

2. OBJECTIVE

The objective of this study was to evaluate 4 Test Articles in an NF-κB reporter 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
Jurkat E6.1 cell line	ATCC	TIB-152	7681669	NA	Liquid N_2	Cell culture
RPMI 1640	Invitrogen	61870-036	438108	30-Apr-09	4˚C	Cell culture
Heat inactivated FBS	Invitrogen	10082-147	1412361	31-May-12	-80˚C	Cell culture
100X penicillin streptomycin solution	Invitrogen	15140	430302	31-May-09	-30˚C	Cell culture
SuperFect transfection reagent	Qiagen	301305	130169029	14-Jul-09	4˚C	Cell transfection
PathDetect NF-κB cis-reporter plasmid	Stratagene	219078	0280573	NA	-30˚C	Cell transfection
DMSO	Sigma	D2650	058K2311	31-May-10	RT	Solution prep.
PMA	Sigma	P1585	086K2064	NA	-30˚C	Cell treatment
PHA	Sigma	61764	1344947	NA	4˚C	Cell treatment
XTT cell proliferation kit	MD Biosciences	409005	717789	NA	-30˚C	Cell proliferation
ONE-Glo luciferase assay system	Promega	E6110	262211	30-Jun10	-30˚C	Reporter assay

5.4. *Culture Media*

RPMI-1640 + 10% FBS + 100 units/ml + 100 µg/ml streptomycin (Jurkat Complete Media; JCM)

6. TEST METHOD

6.1. *Schematic Depiction of NF-κB Activity Assay*

A: Add Test Articles and Reference Articles to transiently transfected Jurkat cells.
B: Add PMA/PHA.
C: Perform luciferase assay (6 hour time point).
D: Perform luciferase assay (12 hour time point).
E: Perform luciferase and XTT assays (24 hour time point).
F: Perform luciferase 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 JCM:

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 JCM:

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 in JCM.

6.4. *Vehicle Preparation*

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

6.5. *PMA and PHA Preparation*

A 100 µg/ml PMA stock solution was prepared in DMSO.

A 5 mg/ml PHA stock solution was prepared in PBS.

A 10X working PMA/PHA solution (100 ng/ml PMA/1000 µg/ml PHA) was prepared by diluting the 100 µg/ml PMA and 5 mg/ml PHA solutions with JCM.

6.6. *Transient Transfection*

Jurkat cells were suspended to 1 x 10^6 cells/ml and added to each well of 7, 6 well plates (2 x 10^6 cells/well). For each 6 well plate, 1 tube of transfection mix was prepared.

Transfection mix: 6 µl of pNF-κB-luc was added to 600 µl of serum-free media (RPMI 1640). 48 µl of SuperFect reagent was added to the DNA mixture and incubated for 10 minutes. 2.4 ml of JCM was added to the solution.

0.5 ml of the transfection mix was added dropwise to each well. Plates were incubated for 24 hours at 37˚C with 5% CO_2.

6.7. *Cell Treatment*

Transfected cells were collected, pooled and suspended to 4 x 10^6 cells/ml. 50 µl per well was added to 4, 96 well white walled plates (2 x 10^5 cells/well). 50 µl per well was added to a clear 96 well plate for the XTT assay.

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 JCM was added to each –PMA/PHA well. 11 µl of the 10X PMA/PHA solution was added to each +PMA/PHA well (final concentrations: 10 ng/ml PMA and 100 µg/ml PHA).

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

Cell Culture Plate Layout (5 plates: 6, 12, 24, and 36 hour plates for luciferase assay. 24 hour plate for XTT assay).

	- PMA/PHA						+ 10 ng/ml PMA/+ 100 µg/ml PHA					
	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			

6.8. *Luciferase Assay*

After the appropriate incubation time, 111 µl of ONE-Glo luciferase assay reagent was added to each well and plates were incubated for 10 minutes at room temperature. Luminescence was detected using a FLUOstarOmega (BMG Labtech, Durham NC, USA) plate reader.

6.9. *XTT Assay*

After 24 hours ± PMA/PHA, 50 µl of activated XTT reagent was added to each well of cells in the 96 well clear plate and incubated at 37˚C with 5% CO_2 for 1 hour. Reduced XTT was detected at 450 nm (630 nm correction) using a ThermoMax microplate reader (Molecular Devices, Sunnyvale, CA).

7. DEVIATIONS

The Experimental Protocol stated that the XTT assay was to be run on non-transfected Jurkat cells in the absence of PMA/PHA. After transfection, there were sufficient cells to run the XTT on transfected cells ± PMA/PHA.

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. 50 nM triptolide reduced cell proliferation in the presence and absence of PMA/PHA (Table 1, Figure 1). Cell proliferation was not reduced by 1 nM or 10 nM triptolide. Therefore, care should be taken when evaluating the affect of 50 nM triptolide on NF-κB activity. The remaining Test Articles did not reduce cell proliferation in the presence or absence of PMA/PHA.

9.2. *NF-κB Activation*

The Test Articles did not significantly induce NF-κB activation in the absence of PMA/PHA (Table 2, Figure 3). Incubation with 10 ng/ml PMA and 100 µg/ml PHA induced NF-κB activity in Jurkat cells. Activity decreased over time (Table 2, Figure 2).

9.3. *Effect of Dexamethasone on NF-κB Activity*

The Reference Article, dexamethasone, did not significantly affect PMA/PHA-stimulated NK-κB activation (Tables 2 and 3, Figure 3). Dexamethasone should not be used as the Reference Article in this system.

9.4. *Effect of Triptolide on NF-κB Activity*

Triptolide did not significantly reduce NF-κB activity at 1 nM or 10 nM (Tables 2 and 3, Figure 3). The NF-κB activity reduction observed in the presence of 50 nM triptolide is likely due to reduced cell viability.

9.5. *Effect of QNZ-CAY10470 on NF-κB Activity*

QNZ-CAY10470 significantly reduced NF-κB activity at 1 nM, 100 nM and 1000 nM after 6 hours of PMA/PHA stimulation (Tables 2 and 3, Figure 3). NF-κB activity was not significantly reduced by QNZ-CAY10470 after 12, 24 or 36 hours of PMA/PHA stimulation.

9.6. *Effect of GB67B on NF-κB Activity*

GB67B did not significantly reduce NF-κB activity (Tables 2 and 3, Figure 3).

9.7. *Effect of GB594 on NF-κB Activity*

GB594 did not significantly reduce NF-κB activity (Tables 2 and 3, Figure 3).

10. CONCLUSIONS

Triptolide reduced cell proliferation at 50 nM suggesting that it is cytotoxic to transfected Jurkat cells at this concentration.

QNZ-CAY10470 reduced NF-κB activity after 6 hours of PMA/PHA stimulation. This inhibition was not seen after 12, 24 or 36 hours of stimulation with PMA/PHA.

Triptolide, GB67B and GB594 did not affect PMA/PHA-sitmulated NF-κB activation in Jurkat cells.

Table 1. XTT cell proliferation assay.

Treatment	Concentration	PMA/PHA	Mean XTT (OD_{450})	Std.Dev.
Vehicle		-	0.771	0.059
Dexamethasone	1 µM	-	0.836	0.033
Triptolide	1 nM	-	0.867	0.02
Triptolide	10 nM	-	0.859	0.088
Triptolide	50 nM	-	0.389	0.014
QNZ (CAY10470)	1 nM	-	0.716	0.034
QNZ (CAY10470)	100 nM	-	0.678	0.023
QNZ (CAY10470)	1000 nM	-	0.721	0.015
GB67B	1 nM	-	0.771	0.075
GB67B	100 nM	-	0.738	0.028
GB67B	1000 nM	-	0.764	0.024
GB594	1 nM	-	0.775	0.091
GB594	100 nM	-	0.794	0.017
GB594	1000 nM	-	0.867	0.062
Vehicle		+	0.354	0.004
Dexamethasone	1 µM	+	0.343	0.009
Triptolide	1 nM	+	0.397	0.006
Triptolide	10 nM	+	0.401	0.009
Triptolide	50 nM	+	0.113	0.006
QNZ (CAY10470)	1 nM	+	0.373	0.025
QNZ (CAY10470)	100 nM	+	0.39	0.017
QNZ (CAY10470)	1000 nM	+	0.364	0.013
GB67B	1 nM	+	0.32	0.021
GB67B	100 nM	+	0.338	0.025
GB67B	1000 nM	+	0.336	0.018
GB594	1 nM	+	0.355	0.015
GB594	100 nM	+	0.37	0.031
GB594	1000 nM	+	0.377	0.031

Table 2. Mean luciferase activity, RLU.

			Mean Luciferase Activity (Relative Luminescence Units; RLU)							
Treatment	Concentration	PMA/PHA	6 hours	Std. Dev.	12 hours	Std. Dev.	24 hours	Std. Dev.	36 hours	Std. Dev.
Vehicle		-	67	13.3	68	14.4	67	4.7	60	4.7
Dexamethasone	1 µM	-	64	11.1	56	8.2	58	3.1	55	5.1
Triptolide	1 nM	-	71	23.4	62	4.5	58	5.5	52	2.3
Triptolide	10 nM	-	57	10	63	3.6	57	3.2	53	6.7
Triptolide	50 nM	-	52	4.2	58	4.5	57	5.2	49	2.3
QNZ (CAY10470)	1 nM	-	56	6.7	61	2.6	59	8	55	5.9
QNZ (CAY10470)	100 nM	-	63	10.3	65	8.5	57	3.6	49	1
QNZ (CAY10470)	1000 nM	-	62	17.2	58	8	56	6.2	52	1
GB67B	1 nM	-	65	11.1	64	4.7	67	7.4	60	2.1
GB67B	100 nM	-	65	13.3	70	20.4	70	10.1	52	2.1
GB67B	1000 nM	-	62	7	64	8.4	68	7.5	58	6.4
GB594	1 nM	-	69	11.5	61	17.6	67	10.5	54	6
GB594	100 nM	-	63	6.5	60	4.9	66	7.2	53	1.2
GB594	1000 nM	-	58	7.5	57	1	62	3.2	54	8.6
Vehicle		+	1393	300.5	737	95.6	342	121.5	239	99.5
Dexamethasone	1 µM	+	1377	265.8	547	39.2	354	38.6	192	66
Triptolide	1 nM	+	1181	158.6	873	28.8	373	54.4	203	59
Triptolide	10 nM	+	1110	73.1	797	206.4	378	48.9	335	118.2
Triptolide	50 nM	+	548	65.8	224	20.2	82	23.4	62	12.3
QNZ (CAY10470)	1 nM	+	738	107.7	548	84.9	417	53.6	239	25.6
QNZ (CAY10470)	100 nM	+	679	105.4	506	140.9	402	195.5	293	176.6
QNZ (CAY10470)	1000 nM	+	510	31.1	498	65.5	280	62.2	212	40.2
GB67B	1 nM	+	1302	137.8	716	24.1	372	53.7	242	156.9
GB67B	100 nM	+	1247	62.9	588	35	392	150.6	251	61.6
GB67B	1000 nM	+	1387	228.9	606	52.1	345	156.3	239	55.1
GB594	1 nM	+	1167	124.4	654	136	299	84.8	219	106.7
GB594	100 nM	+	1168	26.6	960	36.1	383	163.7	224	95.7
GB594	1000 nM	+	1269	260	816	196.9	406	108.9	246	188.8

Table 3. Mean luciferase activity, % vehicle.

			Mean Luciferase Activity (% Vehicle)							
Treatment	**Concentration**	**PMA/PHA**	**6 hours**	**Std. Dev.**	**12 hours**	**Std. Dev.**	**24 hours**	**Std. Dev.**	**36 hours**	**Std. Dev.**
Vehicle		+	100%	22%	100%	13%	100%	36%	100%	42%
Dexamethasone	1 µM	+	99%	19%	74%	5%	104%	11%	80%	28%
Triptolide	1 nM	+	85%	11%	119%	4%	109%	16%	85%	25%
Triptolide	10 nM	+	80%	5%	108%	28%	111%	14%	140%	49%
Triptolide	50 nM	+	39%	5%	30%	3%	24%	7%	26%	5%
QNZ (CAY10470)	1 nM	+	53%	8%	74%	12%	122%	16%	100%	11%
QNZ (CAY10470)	100 nM	+	49%	8%	69%	19%	117%	57%	123%	74%
QNZ (CAY10470)	1000 nM	+	37%	2%	68%	9%	82%	18%	89%	17%
GB67B	1 nM	+	93%	10%	97%	3%	109%	16%	101%	66%
GB67B	100 nM	+	90%	5%	80%	5%	115%	44%	105%	26%
GB67B	1000 nM	+	100%	16%	82%	7%	101%	46%	100%	23%
GB594	1 nM	+	84%	9%	89%	18%	88%	25%	92%	45%
GB594	100 nM	+	84%	2%	130%	5%	112%	48%	94%	40%
GB594	1000 nM	+	91%	19%	111%	27%	119%	32%	103%	79%

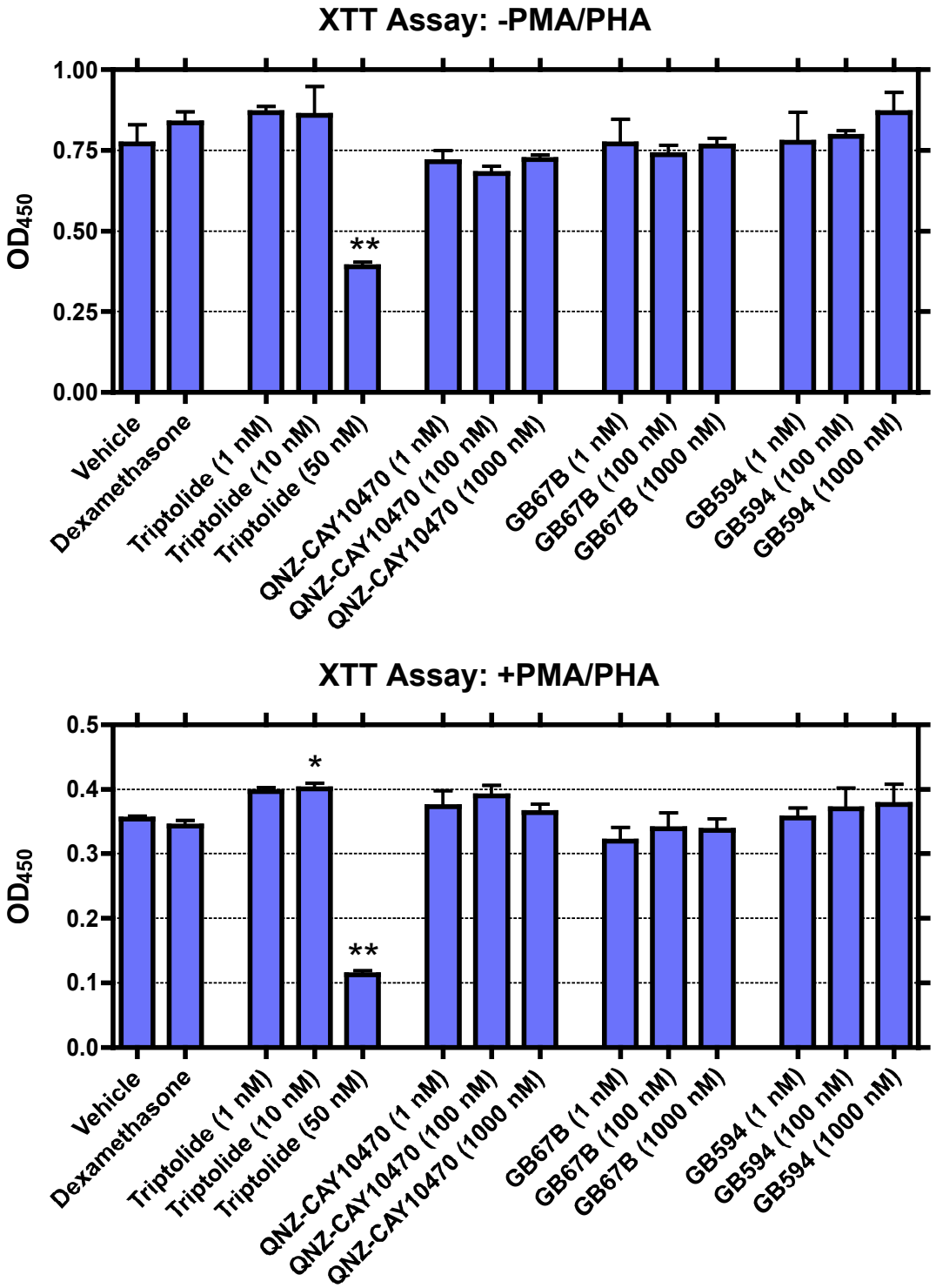

Figure 1. Cell proliferation assay. pNF-κB-luc transfected Jurkat cells treated as described above for 24 hours were incubated with XTT. The amount of reduced XTT, a measure of metabolic activity, was measured at 450 nm. Mean values are shown. Error bars represent standard deviations. Values were analyzed by one-way ANOVA with Dunnett's post-test comparing sample values to the vehicle + PMA/PHA value. *P < 0.05, **P < 0.01.

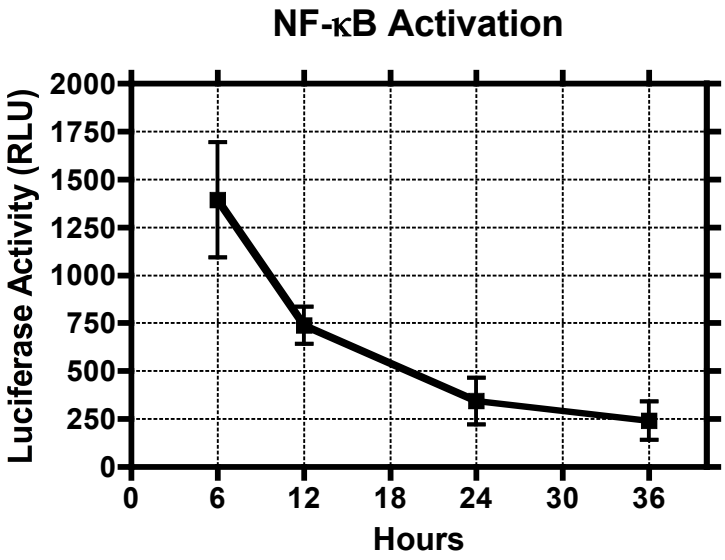

Figure 2. NF-κB activity time course. pNF-κB–luc transfected Jurkat cells were treated with PMA and PHA. Luciferase activity was determined after 6, 12, 24 and 36 hours of PMA/PHA stimulation. Mean values are shown. Error bars represent standard deviations.

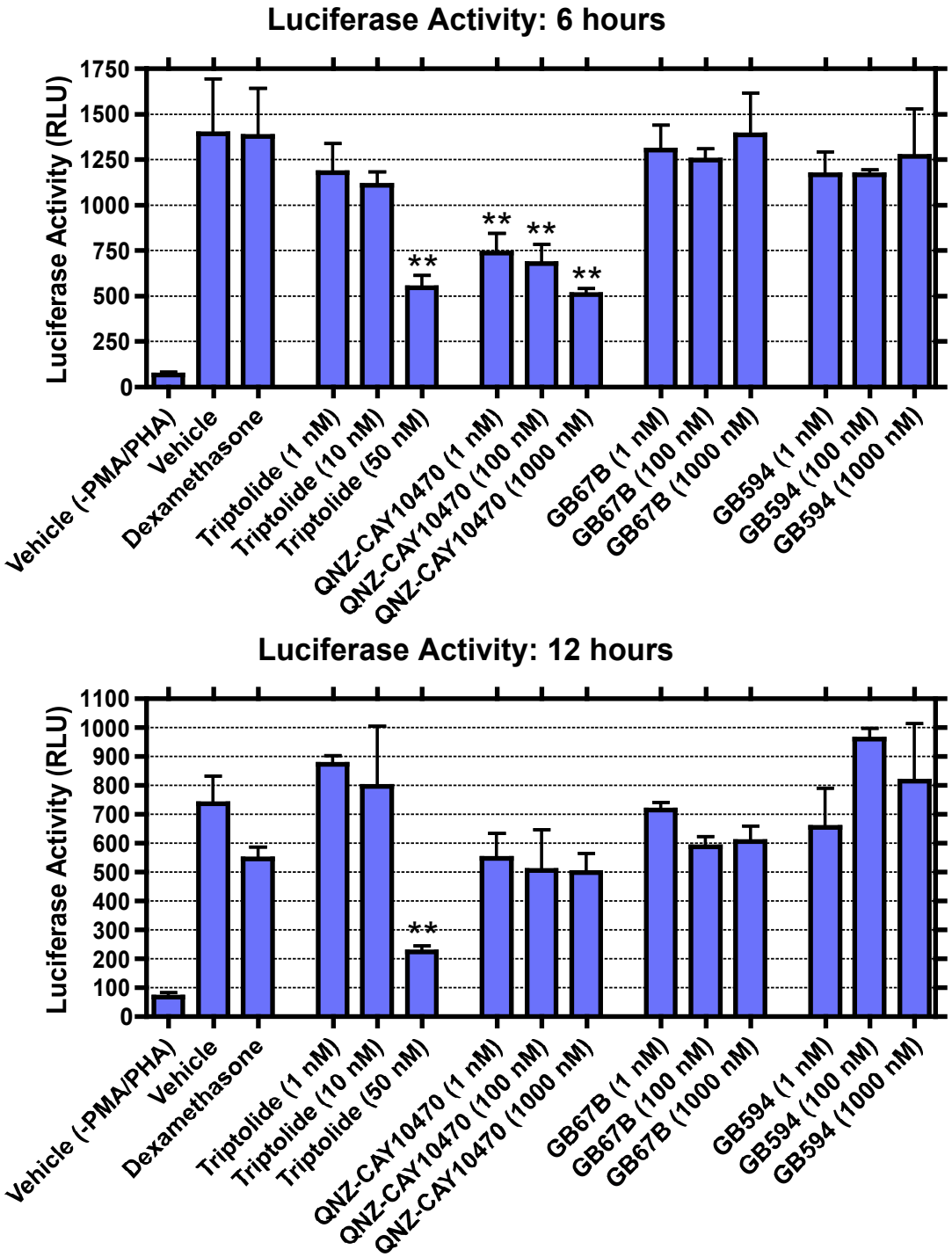

Figure 3. Luciferase activity. pNF-κB-luc transfected Jurkat cells were treated with PMA and PHA following a one hour incubation with vehicle, dexamethasone or test article. Luciferase activity was determined after 6, 12, 24 and 36 hours of PMA/PHA stimulation. Mean relative luminescence units (RLU) 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 + PMA/PHA value. **P < 0.01.

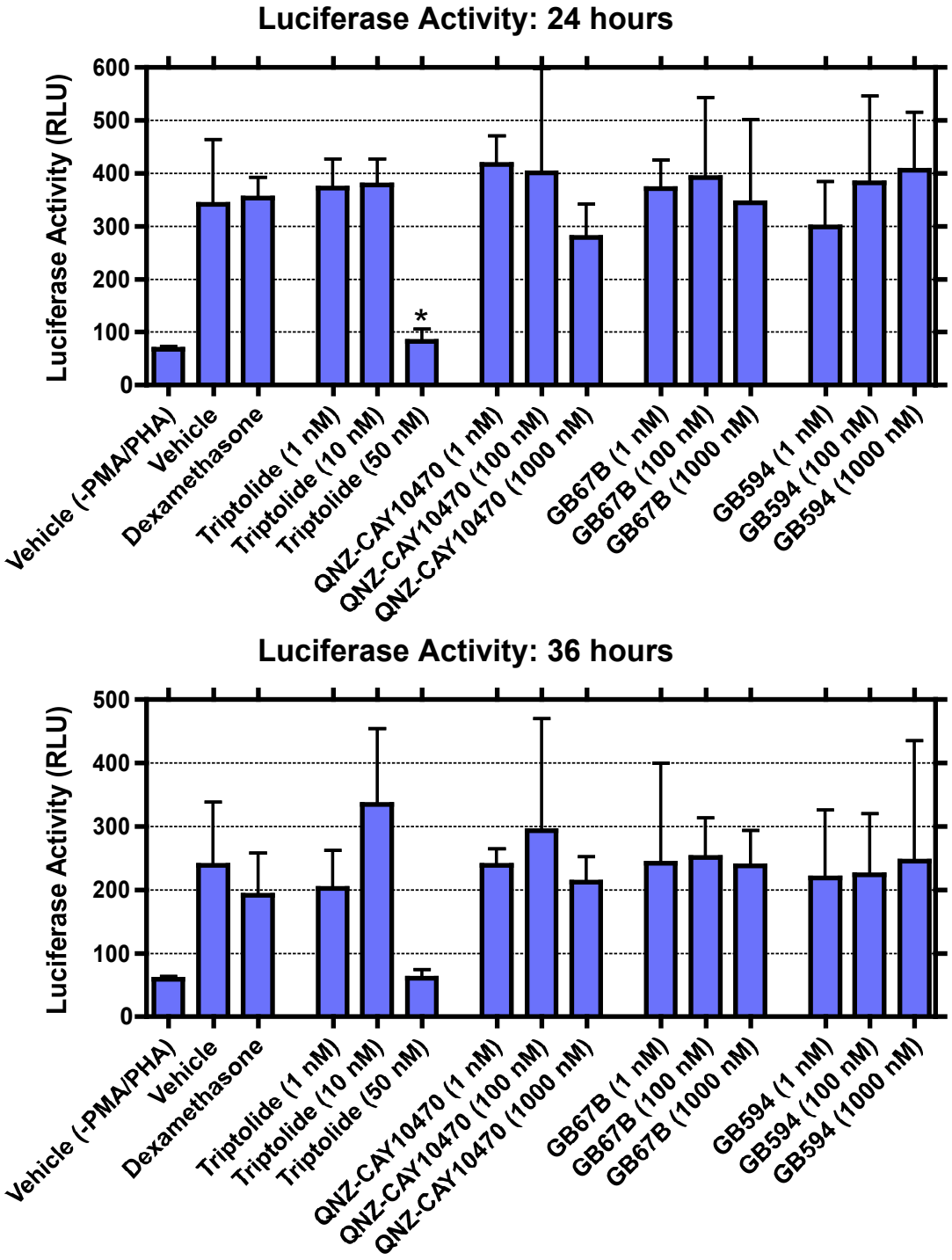

Figure 3 (continued). Luciferase actvity. pNF-κB-luc transfected Jurkat cells were treated with PMA and PHA following a one hour incubation with vehicle, dexamethasone or test article. Luciferase activity was determined after 6, 12, 24 and 36 hours of PMA/PHA stimulation. Mean relative luminescence units (RLU) 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 + PMA/PHA value. *P < 0.05.

Table 4. Raw data, XTT assay.

Treatment	Concentration	PMA/PHA	Blank Corrected OD_{450}
Vehicle		-	0.706
Vehicle		-	0.789
Vehicle		-	0.819
Dexamethasone	1 µM	-	0.873
Dexamethasone	1 µM	-	0.808
Dexamethasone	1 µM	-	0.828
Triptolide	1 nM	-	0.887
Triptolide	1 nM	-	0.848
Triptolide	1 nM	-	0.865
Triptolide	10 nM	-	0.892
Triptolide	10 nM	-	0.926
Triptolide	10 nM	-	0.759
Triptolide	50 nM	-	0.393
Triptolide	50 nM	-	0.401
Triptolide	50 nM	-	0.374
QNZ (CAY10470)	1 nM	-	0.73
QNZ (CAY10470)	1 nM	-	0.677
QNZ (CAY10470)	1 nM	-	0.74
QNZ (CAY10470)	100 nM	-	0.652
QNZ (CAY10470)	100 nM	-	0.694
QNZ (CAY10470)	100 nM	-	0.688
QNZ (CAY10470)	1000 nM	-	0.736
QNZ (CAY10470)	1000 nM	-	0.707
QNZ (CAY10470)	1000 nM	-	0.72
GB67B	1 nM	-	0.858
GB67B	1 nM	-	0.728
GB67B	1 nM	-	0.728
GB67B	100 nM	-	0.715
GB67B	100 nM	-	0.769
GB67B	100 nM	-	0.729
GB67B	1000 nM	-	0.75
GB67B	1000 nM	-	0.792
GB67B	1000 nM	-	0.751
GB594	1 nM	-	0.878
GB594	1 nM	-	0.745
GB594	1 nM	-	0.703
GB594	100 nM	-	0.782
GB594	100 nM	-	0.813
GB594	100 nM	-	0.786
GB594	1000 nM	-	0.878
GB594	1000 nM	-	0.923
GB594	1000 nM	-	0.8
Vehicle		+	0.349
Vehicle		+	0.355
Vehicle		+	0.357
Dexamethasone	1 µM	+	0.334
Dexamethasone	1 µM	+	0.351
Dexamethasone	1 µM	+	0.344
Triptolide	1 nM	+	0.396
Triptolide	1 nM	+	0.403
Triptolide	1 nM	+	0.391
Triptolide	10 nM	+	0.408
Triptolide	10 nM	+	0.403
Triptolide	10 nM	+	0.391
Triptolide	50 nM	+	0.115
Triptolide	50 nM	+	0.106
Triptolide	50 nM	+	0.117
QNZ (CAY10470)	1 nM	+	0.398
QNZ (CAY10470)	1 nM	+	0.373
QNZ (CAY10470)	1 nM	+	0.349
QNZ (CAY10470)	100 nM	+	0.388

Table 4. Raw data, XTT assay.

Treatment	Concentration	PMA/PHA	Blank Corrected OD_{450}
QNZ (CAY10470)	100 nM	+	0.407
QNZ (CAY10470)	100 nM	+	0.374
QNZ (CAY10470)	1000 nM	+	0.372
QNZ (CAY10470)	1000 nM	+	0.349
QNZ (CAY10470)	1000 nM	+	0.371
GB67B	1 nM	+	0.318
GB67B	1 nM	+	0.3
GB67B	1 nM	+	0.341
GB67B	100 nM	+	0.366
GB67B	100 nM	+	0.331
GB67B	100 nM	+	0.318
GB67B	1000 nM	+	0.354
GB67B	1000 nM	+	0.336
GB67B	1000 nM	+	0.318
GB594	1 nM	+	0.362
GB594	1 nM	+	0.366
GB594	1 nM	+	0.338
GB594	100 nM	+	0.399
GB594	100 nM	+	0.375
GB594	100 nM	+	0.337
GB594	1000 nM	+	0.412
GB594	1000 nM	+	0.36
GB594	1000 nM	+	0.358

Table 4. Raw data, luciferase assay.

			Luciferase Activity (Relative Luminescence Units; RLU)			
Treatment	Concentration	PMA/PHA	6 hours	12 hours	24 hours	36 hours
Vehicle		-	74	62	71	65
Vehicle		-	76	57	62	56
Vehicle		-	52	84	69	58
Dexamethasone	1 µM	-	52	47	61	54
Dexamethasone	1 µM	-	74	63	57	61
Dexamethasone	1 µM	-	66	58	55	51
Triptolide	1 nM	-	58	58	62	55
Triptolide	1 nM	-	57	67	61	51
Triptolide	1 nM	-	98	62	52	51
Triptolide	10 nM	-	67	66	53	59
Triptolide	10 nM	-	47	59	59	55
Triptolide	10 nM	-	57	64	58	46
Triptolide	50 nM	-	47	53	54	48
Triptolide	50 nM	-	53	62	54	48
Triptolide	50 nM	-	55	58	63	52
QNZ (CAY10470)	1 nM	-	50	59	67	51
QNZ (CAY10470)	1 nM	-	63	64	58	53
QNZ (CAY10470)	1 nM	-	54	60	51	62
QNZ (CAY10470)	100 nM	-	66	75	60	49
QNZ (CAY10470)	100 nM	-	72	59	58	50
QNZ (CAY10470)	100 nM	-	52	62	53	48
QNZ (CAY10470)	1000 nM	-	56	50	49	52
QNZ (CAY10470)	1000 nM	-	48	57	58	51
QNZ (CAY10470)	1000 nM	-	81	66	61	53
GB67B	1 nM	-	53	59	61	58
GB67B	1 nM	-	66	66	64	62
GB67B	1 nM	-	75	68	75	59
GB67B	100 nM	-	56	61	61	53
GB67B	100 nM	-	58	55	68	54
GB67B	100 nM	-	80	93	81	50
GB67B	1000 nM	-	59	59	76	55
GB67B	1000 nM	-	70	74	67	53
GB67B	1000 nM	-	57	60	61	65
GB594	1 nM	-	82	47	68	60
GB594	1 nM	-	62	81	56	48

Table 4. Raw data, luciferase assay.

			Luciferase Activity (Relative Luminescence Units; RLU)			
Treatment	**Concentration**	**PMA/PHA**	**6 hours**	**12 hours**	**24 hours**	**36 hours**
GB594	1 nM	-	62	56	77	53
GB594	100 nM	-	63	58	71	54
GB594	100 nM	-	57	66	58	54
GB594	100 nM	-	70	57	70	52
GB594	1000 nM	-	51	57	61	52
GB594	1000 nM	-	66	56	66	46
GB594	1000 nM	-	58	58	60	63
Vehicle		+	1092	628	280	308
Vehicle		+	1693	808	264	125
Vehicle		+	1395	774	482	284
Dexamethasone	1 µM	+	1353	560	388	162
Dexamethasone	1 µM	+	1654	578	312	147
Dexamethasone	1 µM	+	1124	503	362	268
Triptolide	1 nM	+	1034	849	423	200
Triptolide	1 nM	+	1159	865	315	145
Triptolide	1 nM	+	1349	905	380	263
Triptolide	10 nM	+	1026	968	421	204
Triptolide	10 nM	+	1145	568	325	369
Triptolide	10 nM	+	1159	856	389	433
Triptolide	50 nM	+	489	235	109	53
Triptolide	50 nM	+	619	201	67	76
Triptolide	50 nM	+	536	237	70	57
QNZ (CAY10470)	1 nM	+	672	451	413	212
QNZ (CAY10470)	1 nM	+	862	587	365	263
QNZ (CAY10470)	1 nM	+	679	607	472	242
QNZ (CAY10470)	100 nM	+	738	473	249	337
QNZ (CAY10470)	100 nM	+	557	660	334	444
QNZ (CAY10470)	100 nM	+	741	384	622	99
QNZ (CAY10470)	1000 nM	+	489	442	208	166
QNZ (CAY10470)	1000 nM	+	496	482	320	234
QNZ (CAY10470)	1000 nM	+	546	570	311	237
GB67B	1 nM	+	1431	732	354	418
GB67B	1 nM	+	1319	688	432	116
GB67B	1 nM	+	1157	727	329	193
GB67B	100 nM	+	1306	619	515	181
GB67B	100 nM	+	1181	595	224	277
GB67B	100 nM	+	1255	550	437	296
GB67B	1000 nM	+	1254	603	216	242
GB67B	1000 nM	+	1255	555	301	292
GB67B	1000 nM	+	1651	659	519	182
GB594	1 nM	+	1057	631	219	175
GB594	1 nM	+	1302	800	291	341
GB594	1 nM	+	1142	531	388	142
GB594	100 nM	+	1184	990	262	265
GB594	100 nM	+	1137	920	569	115
GB594	100 nM	+	1182	970	317	293
GB594	1000 nM	+	1172	626	330	136
GB594	1000 nM	+	1072	802	358	464
GB594	1000 nM	+	1564	1019	531	138