Combination Screen
Data Report

Neurophyxia BV

Study Completed: May 24, 2010

Report Printed: June 10, 2010

Ricerca PT#: 1127699

Alt. Code 1: 2-IB

Alt. Code 2: Batch: G01287

Alt. Code 3:

Sample(s): NRO-1

M.W.: 243.3

Objectives:

To evaluate, in Enzyme, Radioligand Binding assays, the activity of test compound 2-IB (PT# 1127699).

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Ricerca Biosciences, LLC Pharmacology Data Report On Compound 2-Iminobiotin For Neurophyxia BV

Work Order Number: [redacted]
Study Number: [redacted]
Quote No: [redacted]

Services Being Reported: Combination Screen
Alternative Work Order No:
Purchase Order Number:
Total # of Assays: 341

Compound Information:

Compound Code: NRO-1
Alternative Code 1: 2-IB
Alternative Code 2: Batch: G01287
Alternative Code 3:
Ricerca Internal #: 1127699
Molecular Weight: 243.3

Sponsor:
Neurophyxia BV
Onderwijsboulevard 219
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Netherlands

Undertaken at:
Ricerca Biosciences, LLC
Pharmacology Laboratories
158 Li-Teh Road, Peitou
Taipei, Taiwan 112
Taiwan R.O.C.

Date of Study: February 22, 2010 - May 24, 2010

Study Directors: [redacted]

Distribution: Neurophyxia BV

"This study was conducted according to the procedures described in this report. All data presented are authentic, accurate and correct to the best of our knowledge."

[redacted]
Study Director for Animal Assays

[redacted]
Study Director for Biochemical Assays

[redacted]
Quality Control and Data Reviewer

[redacted]
Technical Dire

Note: the below pages are an extract of the abovementioned report

SUMMARY

STUDY OBJECTIVE

To evaluate, in Enzyme, and Radioligand Binding assays, the activity of compound 2-iminobiotin (2-IB, NRO-1, PT#1127699).

METHODS

Methods employed in this study have been adapted from the scientific literature to maximize reliability and reproducibility. Reference standards were run as an integral part of each assay to ensure the validity of the results obtained. Assays were performed under conditions described in the accompanying "Methods" section of this report. The literature reference(s) for each assay are in the "Literature References" section. If either of these sections were not originally requested with the accompanying report, please contact us at the number below for a printout of either of these report sections.

Where presented, IC_{50} values were determined by a non-linear, least squares regression analysis using MathIQ™ (ID Business Solutions Ltd., UK). Where inhibition constants (K_I) are presented, the K_I values were calculated using the equation of Cheng and Prusoff (Cheng, Y., Prusoff, W.H., Biochem. Pharmacol. 22:3099-3108, 1973) using the observed IC_{50} of the tested compound, the concentration of radioligand employed in the assay, and the historical values for the KD of the ligand (obtained experimentally at **Ricerca Biosciences, LLC**). Where presented, the Hill coefficient (n_H), defining the slope of the competitive binding curve, was calculated using MathIQ™. Hill coefficients significantly different than 1.0, may suggest that the binding displacement does not follow the laws of mass action with a single binding site. Where IC_{50}, K_I, and/or n_H data are presented without Standard Error of the Mean (SEM), data are insufficient to be quantitative, and the values presented (K_I, IC_{50}, n_H) should be interpreted with caution.

RESULTS

A summary of results meeting the significance criteria is presented in the following sections. Complete results are presented under the section labeled "Experimental Results". Individual responses, if requested, are presented in the appendix to this report.

SUMMARY/CONCLUSION

Significant results are displayed in the following table(s) in rank order of potency for estimated IC_{50}, and/or K_I values.

SUMMARY OF SIGNIFICANT PRIMARY RESULTS

Biochemical assay results are presented as the percent inhibition of specific binding or activity throughout the report. All other results are expressed in terms of that assay's quantitation method (see Methods section).

- *For primary assays, only the lowest concentration with a significant response judged by the assays' criteria, is shown in this summary.*
- *Where applicable, either the secondary assay results with the lowest dose/concentration meeting the significance criteria or, if inactive, the highest dose/concentration that did not meet the significance criteria is shown.*
- *Unless otherwise requested, primary screening in duplicate with quantitative data (e.g., IC50 ± SEM, Ki ± SEM and nH) are shown where applicable for individual requested assays. In screening packages, primary screening in duplicate with semi-quantitative data (e.g., estimated IC50, Ki and nH) are shown where applicable (concentration range of 4 log units); available secondary functional assays are carried out (30 μM) and MEC or MIC determined only if active in primary assays >50% at 1 log unit below initial test concentration.*
- *Please see Experimental Results section for details of all responses.*

Significant responses (≥ 50% inhibition or stimulation for Biochemical assays) were noted in the primary assays listed below:

PRIMARY TESTS

CAT. #	PRIMARY BIOCHEMICAL ASSAY	SPECIES	CONC.	% INH.	IC_{50} *	K_I	n_H
142000	Nitric Oxide Synthase, Neuronal (nNOS)	rat	300 µM	68	142 µM		
143000	Nitric Oxide Synthase, Endothelial (eNOS)	bov	1000 µM	63	646 µM		
144000	Nitric Oxide Synthase, Inducible (iNOS)	mouse	300 µM	81	95.7 µM		

EXPERIMENTAL RESULTS - BIOCHEMICAL ASSAYS

Nitric Oxide Synthase, Inducible (iNOS) Nitric Oxide Synthase, Neuronal (nNOS)
Nitric Oxide Synthase, Endothelial (eNOS)

Cat. #	TARGET	BATCH*	SPP.	n=	CONC.	†% INHIBITION %	IC50	KI	nH	R
						-100 -50 0 50 100				
♦ 143000	Nitric Oxide Synthase, Endothelial (eNOS)	267565	bov	2	1000 µM	63	646 µM			
				2	300 µM	28				
				2	100 µM	6				
				2	30 µM	-2				
				2	10 µM	0				
♦ 144000	Nitric Oxide Synthase, Inducible (iNOS)	267564	mouse	2	1000 µM	98	95.7 µM			
♦				2	300 µM	81				
				2	100 µM	45				
				2	30 µM	24				
				2	10 µM	12				
♦ 142000	Nitric Oxide Synthase, Neuronal (nNOS)	267865	rat	2	1000 µM	85	142 µM			
♦				2	300 µM	68				
				2	100 µM	49				
				2	30 µM	3				
				2	10 µM	1				

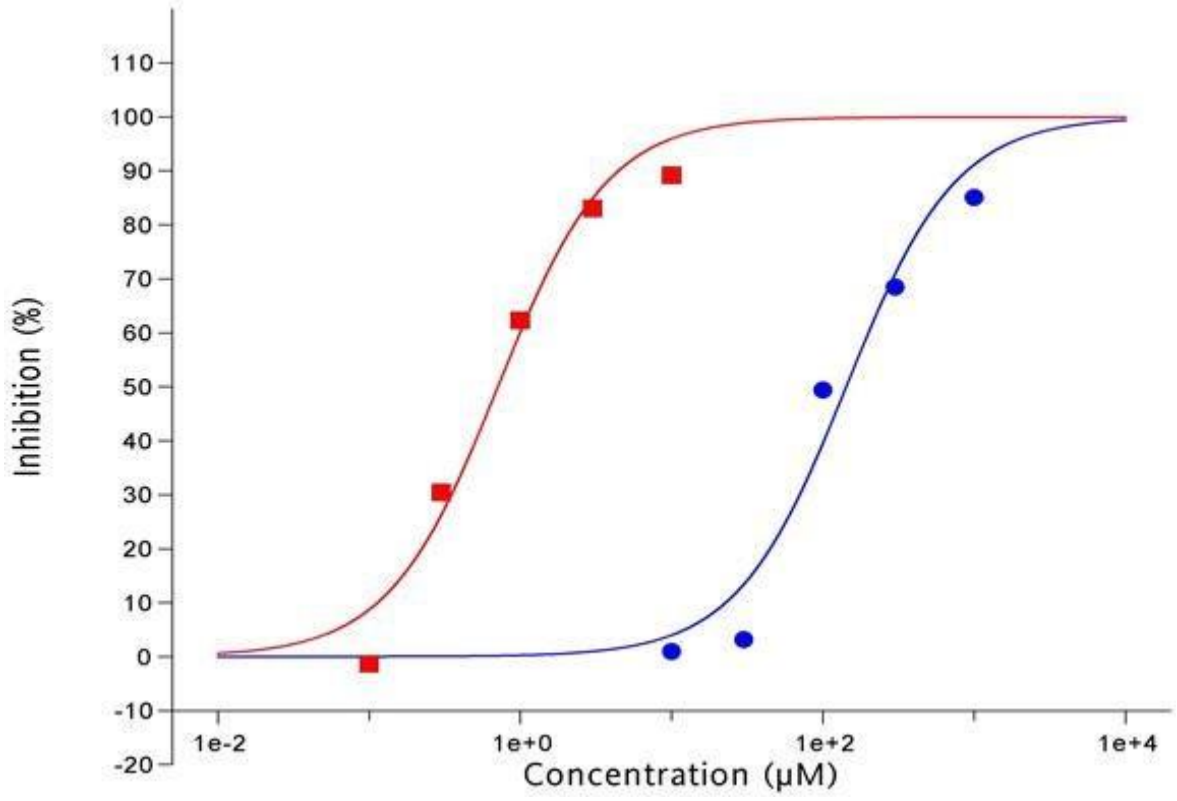
* Batch: Represents compounds tested concurrently in the same assay(s).
♦ Denotes item meeting criteria for significance
† Results with ≥ 50% stimulation or inhibition are highlighted.
R=See Remarks (if any) at end of this section.

PHARMACOLOGY REPORT

RESPONSE CURVES

RESPONSE CURVES

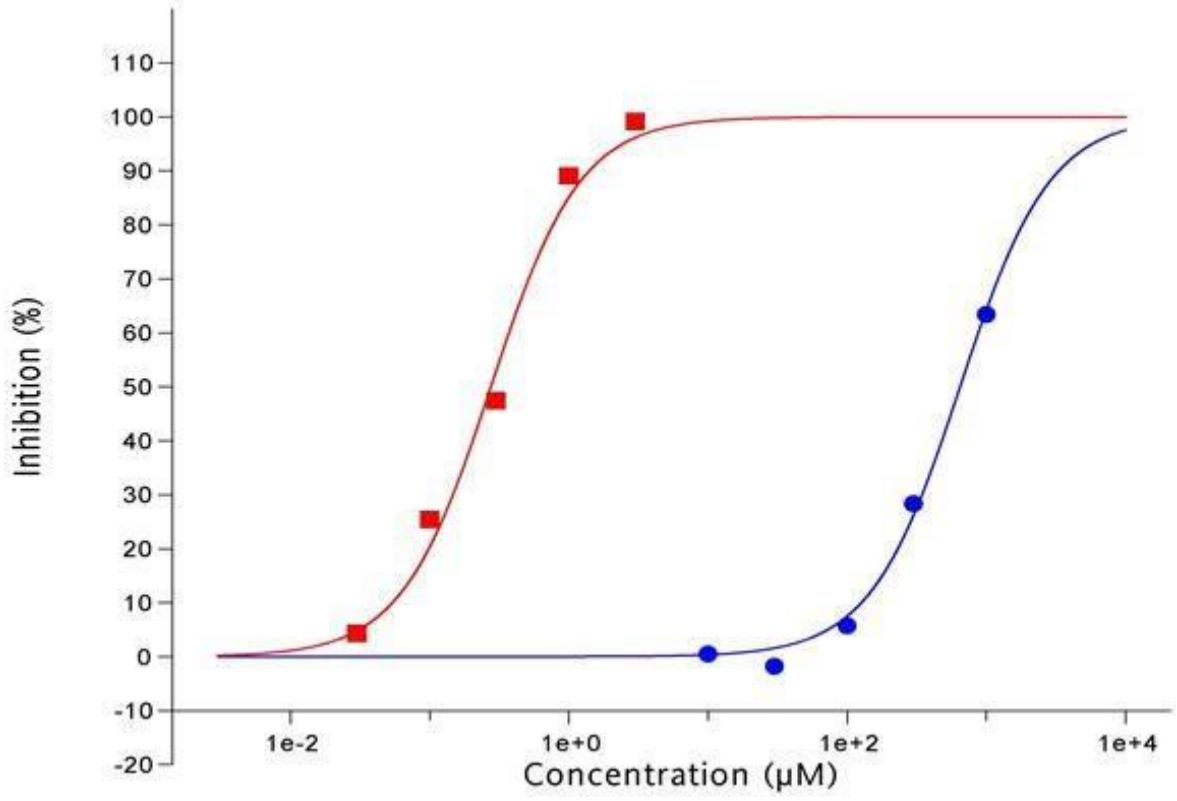
ASSAY: 142000 - 1 Nitric Oxide Synthase, Neuronal (nNOS)



Compound	IC_{50}
● 2-IB (1127699)	142 µM
■ S-Methylisothiourea	0.709 µM

RESPONSE CURVES

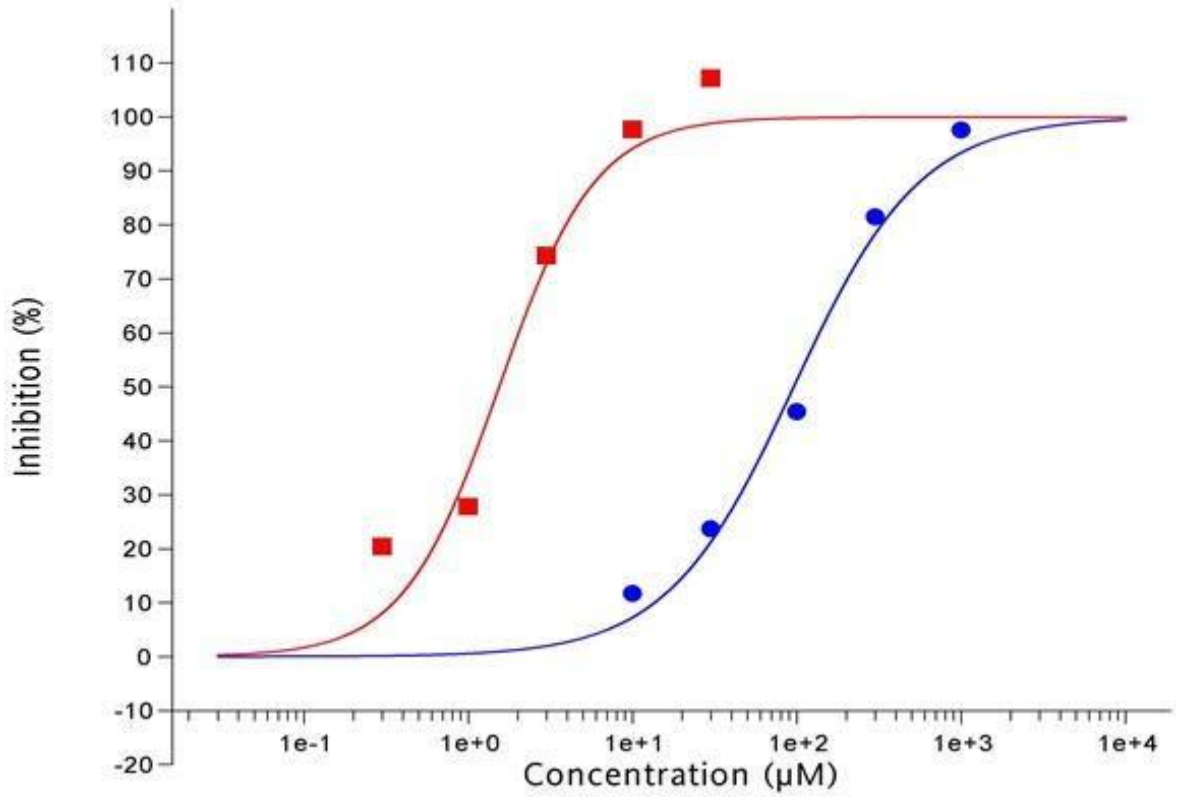
ASSAY: 143000 - 1 Nitric Oxide Synthase, Endothelial (eNOS)



Compound	IC,Œ
● 2-IB (1127699)	646 µM
■ S-Methylisothiourea	0.275 µM

RESPONSE CURVES

ASSAY: 144000 - 1 Nitric Oxide Synthase, Inducible (iNOS)



Compound	IC,Œ
2-IB (1127699)	95.7 µM
S-Methylisothiourea	1.54 µM

METHODS - ENZYME ASSAYS

143000 Nitric Oxide Synthase, Endothelial (eNOS)

Source:	Bovine recombinant insect Sf9 cells
Substrate:	0.08 µM [^{3}H]L-Arginine + L-Arginine
Vehicle:	1% DMSO
Pre-Incubation Time/Temp:	15 minutes @ 37°C
Incubation Time/Temp:	30 minutes @ 37°C
Incubation Buffer:	50 mM HEPES, pH 7.4, 1 mM DTT, 2 mM $CaCl_2$, 10 µg/ml CaM, 10 µM BH_4, 1 mM NADPH
Quantitation Method:	Quantitation of [^{3}H]Citrulline
Significance Criteria:	≥ 50% of max stimulation or inhibition

144000 Nitric Oxide Synthase, Inducible (iNOS)

Source:	Mouse recombinant E. coli
Substrate:	100 µM L-Arginine
Vehicle:	1% DMSO
Pre-Incubation Time/Temp:	15 minutes @ 37°C
Incubation Time/Temp:	2 hours @ 37°C
Incubation Buffer:	100 mM Tris-HCl, pH 7.9, 4 mM NADPH, 8 µM (6R)-5,6,7,8-Tetrahydro-L-Biopterin, 8 µM FAD, 6 mM DTT
Quantitation Method:	Spectrophotometric quantitation of NO_2^-
Significance Criteria:	≥ 50% of max stimulation or inhibition

METHODS - ENZYME ASSAYS

142000 Nitric Oxide Synthase, Neuronal (nNOS)

Source:	Wistar Rat cerebellum
Substrate:	0.02 µM [^{3}H]L-Arginine
Vehicle:	1% DMSO
Pre-Incubation Time/Temp:	15 minutes @ 25ºC
Incubation Time/Temp:	10 minutes @ 25ºC
Incubation Buffer:	50 mM HEPES, pH 7.4, 1 mM EDTA, 1 mM NADPH, 1.25 mM $CaCl_2 \cdot 2H_2O$, 1 mM DTT, 10 µg/ml Calmodulin
Quantitation Method:	Quantitation of [^{3}H]Citrulline
Significance Criteria:	≥ 50% of max stimulation or inhibition

LITERATURE REFERENCES

CAT. #	REFERENCE
142000	**Lowenstein CJ and Snyder SH (1992)** Nitric oxide, a novel biologic messenger. Cell. 70:705-707. **Nathan C (1992)** Nitric oxide as a secretory product of mammalian cells. FASEB J. 6:3051-3064.
143000	**Alderton WK, Cooper CE and Knowles RG (2001)** Nitric oxide synthases: structure, function and inhibition. Biochem J. 357: 593 - 615. **Southan GJ, Szabo C and Thiemermann C (1995)** Isothioureas: potent inhibitors of nitric oxide synthases with variable isoform selectivity. Br J Pharmacol. 114(2): 510 - 516.
144000	**Lowenstein CJ and Snyder SH (1992)** Nitric oxide, a novel biologic messenger. Cell. 70:705-707. **Nathan C (1992)** Nitric oxide as a secretory product of mammalian cells. FASEB J. 6:3051-3064.