Supporting Information

Assessment of NR4A Ligands that Directly Bind and Modulate the Orphan Nuclear Receptor Nurr1

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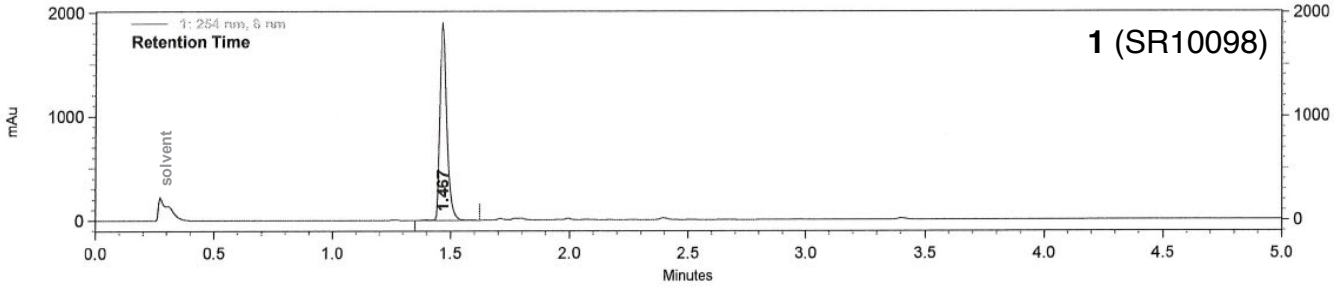
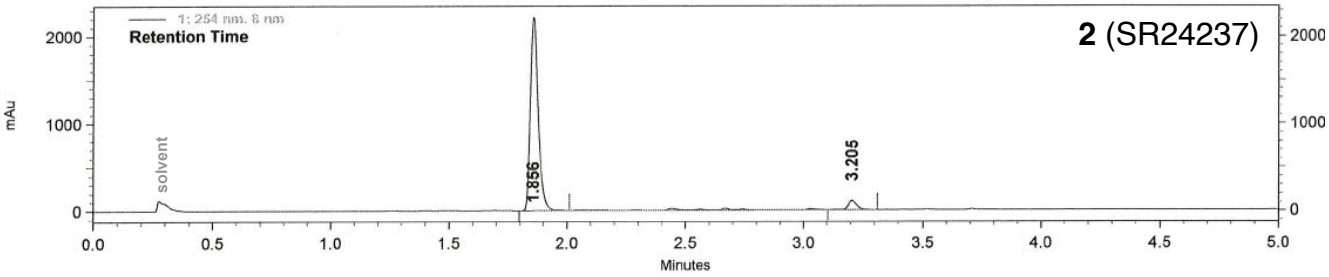
Pages



PDA-254 nm
Results

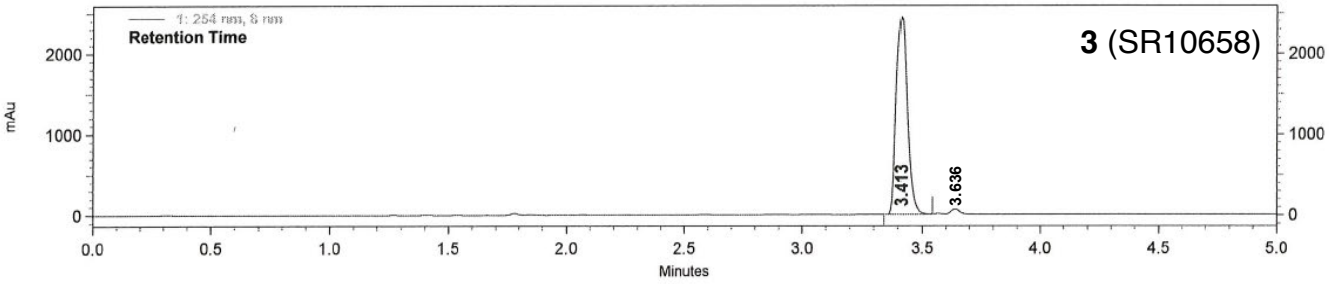
Retention Time	Area	Area %	Height	Height %
1.467	3923198	100.00	1900623	100.00
Totals	3923198	100.00	1900623	100.00



2 (SR24237)

PDA-254 nm
Results

Retention Time	Area	Area %	Height	Height %
1.856	5019377	95.81	2199525	95.30
3.205	219639	4.19	108449	4.70
Totals	5239016	100.00	2307974	100.00



PDA-254 nm
Results

Retention Time	Area	Area %	Height	Height %
3.413	7301453	98.42	2405941	97.62
3.637	117225	1.58	58633	2.38
Totals	7418678	100.00	2464574	100.00

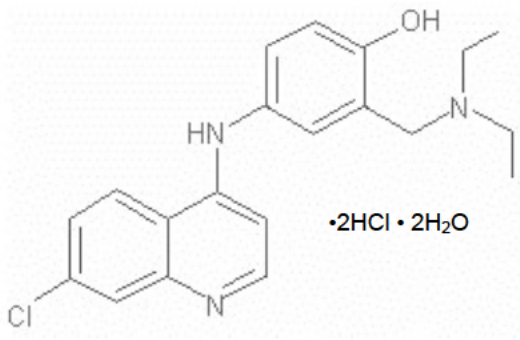
[redacted]

Product Information Sheet

Amodiaquine dihydrochloride dihydrate

Catalogue Number:	CYP526
Amount:	5 g
Molecular formula:	$C_{20}H_{22}ClN_3O \cdot 2HCl \cdot 2H_2O$
Molecular weight:	464.8
Storage:	Store at ambient temperature. Keep tightly closed.
Intended Use:	For laboratory (research) purposes only. Not for drug or human use.

Structure:



Batch Number:	6712012008
IR Spectrum:	Conforms
UV Spectrum:	Conforms
Purity:	99.65 %
Analysis date:	December 2006

Approved by _____ Date 12th November, 2007

Safety Data

The toxicological properties of this reagent have not been investigated. Exercise due care when handling.

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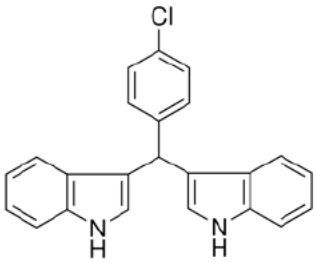
Certificate of Analysis

Product Name : **C-DIM12 ≥98% (HPLC)**
Product Number : **SML1508-25MG**
Batch Number : **0000043274**
Source Batch : 0000033598
CAS Number : 178946-89-9
Storage Temperature : Coo er/Refr gerated
Mo ecu ar Formu a : $C_{23}H_{17}N_2C$
Formu a We ght : 356.85



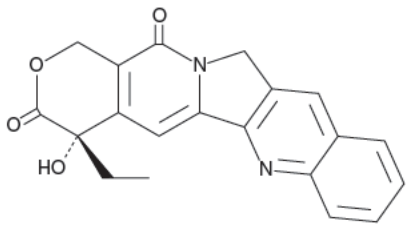
Test	Spec f cat on	Resu t
Appearance (Co or)	L ght Orange to Dark Red	Orange Red
Appearance (Form)	Powder	Powder
So ub ty (Turb d ty)	C ear	C ear
So ub ty (Co or)	Conforms to Requ rements	Dark Ye ow
Concentrat on DMSO	≥ 20.0 mg/m	40.0 mg/m
Pur ty (HPLC)	≥ 97.5 %	99.9 %
Ident ty	Conf rmed	Conf rmed
Water Content	0.0 - 2.0 mo	0.2 mo
E ementa Compos t on	Pass	Pass

PRODUCT INFORMATION

Camptothecin

Item No. 11694

CAS Registry No.: 7689-03-4
Formal Name: (4S)-4-ethy -4-hydroxy-1H-pyrano[3',4':6,7] ndo z no[1,2-b] qu no ne-3,14(4H,12H)-d one
Synonyms: MAG-CPT, NSC 94600
MF: $C_{20}H_{16}N_2O_4$
FW: 348.4
Purity: ≥98%
Stability: ≥2 years at -20°C
Supplied as: A crysta ne so d
UV/Vis.: λ_{max}: 218, 253, 289, 359 nm



Laboratory Procedures

For ong term storage, we suggest that camptothec n be stored as supp ed at -20°C. It shou d be stab e for at east two years.

Camptothec n s supp ed as a crysta ne so d. A stock so ut on may be made by d sso v ng the camptothec n n the so vent of cho ce. Camptothec n s so ub e n organ c so vents such as DMSO and d methy formam de, wh ch shou d be purged w th an nert gas. The so ub ty of camptothec n n these so vents s approx mate y 3 and 2 mg/m , repsect ve y.

Camptothec n s spar ng y so ub e n aqueous buffers. For max mum so ub ty n aqueous buffers, camptothec n shou d f rst be d sso ved n DMSO and then d uted w th the aqueous buffer of cho ce. Camptothec n has a so ub ty of approx mate y 0.25 mg/m n a 1:3 so ut on of DMSO:PBS (pH 7.2) us ng th s method. We do not recommend stor ng the aqueous so ut on for more than one day.

Description

DNA topo somerases re ax DNA tors ona stra n created dur ng rep cat on, transcr pt on, recomb nat on, repa r, and chromosome condensat on. The re axat on of DNA superco ng by topo somerase I at s ng e-strand breaks enab es ant cancer agents to revers b y trap the comp ex by nterca at ng between DNA base pa rs at the c eavage s te, thus nh b t ng re gat on, wh ch act vates apoptot c and ce cyc e arrest pathways.[1] Camptothec n s a cytotox c, qu no ne a ka o d, d scovered as the act ve pr nc p e of extracts from the Ch nese tree *C. acuminate*, that nh b ts the DNA enzyme topo somerase I (Top1). It b nds the Top1-DNA c eavage comp ex, nduc ng DNA-strand breaks.[2,3] Camptothec n has strong ant -tumor act v ty aga nst a w de range of exper menta tumors and nh b ts both DNA and RNA synthes s n mamma an ce s.[4] It d sp ays cytotox ty n HT-29 ce s w th an IC_{50} va ue of 10 nM and nduces DNA damage at concentrat ons as ow as 51 nM n who e ce s and 12 nM n so ated nuc e n *in vitro* assays.[5]

References

1. Drwa , M.N., Agama, K., Wake n, L.P.G., *et al. PLoS One* **6(9)**, 1-12 (2011).
2. Hs ang, Y.-H., Hertzberg, R., Hecht, S., *et al. J. Biol. Chem.* **260(27)**, 14873-14878 (1985).
3. Marchand, C., Antony, S., Kohn, K.W., *et al. Mol. Cancer Ther.* **5(2)**, 287-295 (2006).
4. Dancey, J. and E senhauer, E.A. *Br. J. Cancer* **74**, 327-338 (1996).
5. Rothenberg, M.L. *Ann. Oncol.* **8(9)**, 837-855 (1997).

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CERTIFICATE of ANALYSIS

[redacted]

Purity Specification: ≥98%
Molecular Formula : C20H16N2O4
CAS Number: 7689-03-4 **Formula Weight : 348.4** **Expiry date: 7/17/2021**

HPLC	Purity: 99.1 %
Mass spec	MH+: 349.1
TLC	Purity: 100 %
UV	λ max: 219, 253, 289, 362 nm

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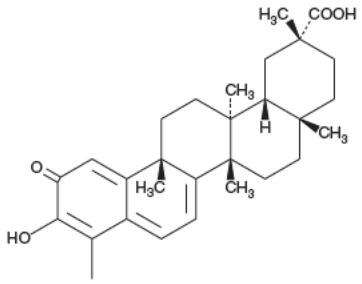
PRODUCT INFORMATION

Celastrol

Item No. 70950

CAS Registry No.: 34157-83-0
Formal Name: 3-hydroxy-9β,13α-d methy -2-oxo-24,25,26-tr noro eana-1(10),3,5,7-tetraen-29-o c ac d
MF: $C_{29}H_{38}O_4$
FW: 450.6
Purity: ≥98%
Stability: ≥2 years at -20°C
Supplied as: A crysta ne so d
UV/Vis.: λ_{max}: 253, 424 nm



Laboratory Procedures

For ong term storage, we suggest that ce astro be stored as supp ed at -20°C. It shou d be stab e for at east two years.

Ce astro s supp ed as a crysta ne so d. A stock so ut on may be made by d sso v ng the ce astro n an organ c so vent purged w th an nert gas. Ce astro s so ub e n organ c so vents such as ethano , DMSO, and d methy formam de (DMF). The so ub ty of ce astro n these so vents s approx mate y 10 mg/m n ethano and DMSO and 20 mg/m n DMF.

Ce astro s spar ng y so ub e n aqueous buffers. For max mum so ub ty n aqueous buffers, ce astro shou d f rst be d sso ved n DMF and then d uted w th the aqueous buffer of cho ce. Ce astro has a so ub ty of approx mate y 1 mg/m n a 1:10 so ut on of DMF:PBS (pH 7.2) us ng th s method. We do not recommend stor ng the aqueous so ut on for more than one day.

Description

A var ety of natura products from p ant sources, part cu ar y f avono ds, have ong been observed to have ant ox dant act v ty w th potent a benef ts for human hea th.[1 3] Ant ox dant tr terpenes are ess common. Ce astro s a tr terpeno d ant ox dant compound so ated from Ch nese thunder god v ne (*T. wilfordii*). In an so ated rat ver assay of p d perox dat on, ce astro had an IC_{50} va ue of 7 µM, equ va ent to about 15 t mes the ant ox dant potency of α-tocophero .[4]

References

1. Frémont, L., Be guendouz, L., and De pa , S. Ant ox dant act v ty of resveratro and a coho -free w ne po ypheno s re ated to LDL ox dat on and po yunsaturated fatty ac ds. *Life Sci.* **64**, 2511-2521 (1999).
2. Johnson, J.L. and Madd pat , K.R. Paradox ca effects of resveratro on the two prostag and n H synthases. *Prostaglandins and Other Lipid Mediators* **56**, 131-143 (1998).
3. M er, N.J. and R ce-Evans, C. Ant ox dant act v ty of resveratro n red w ne. *Clin. Chem.* **41**, 1789 (1995).
4. Sassa, H., Taka sh , Y., and Terada, H. The tr terpene ce astro as a very potent nh b tor of p d perox dat on n m tochondr a. *Biochem. Biophys. Res. Commun.* **172(2)**, 890-897 (1990).

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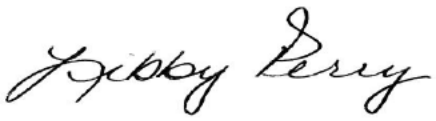
CERTIFICATE of ANALYSIS

[redacted]

Purity Specification: ≥98%
Molecular Formula : C29H38O4
CAS Number: 34157-83-0 **Formula Weight: 450.6** **Expiry date: 8/16/2018**

Mass spec	M-H+: 449.8
TLC	Purity: 100 %
UV	λ max: 247, 423 nm



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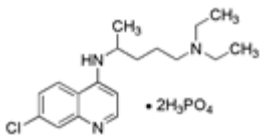
Product Specifications



Catalogue Number	22113
Product	**Chloroquine diphosphate salt**
CAS Number	50-63-5
Molecular Formula	$C_{18}H_{26}ClN_3 \bullet 2H_3PO_4$
Molecular Weight	515.86

Appearance	White or almost white crystalline powder
Appearance of Solution	≤ BY5 or GY5
Solubility	Comply with test
Melting Point	~ 195 °C
Heavy Metals	≤ 20 ppm
Water Content (Karl Fisher)	≤ 2%
pH	3.8 - 4.3
Infrared Spectrum	Conforms to structure
Related Substances	One spot : ≤ 1% Any other spot : ≤ 0.5%
Assay by titration	98.5 - 101.0%
Purity by UV	Conforms
Grade	BP
Storage	Store at 0 - 8 °C
Additional Information	Identification : Picric acid precipitate, test for phosphates should comply the standards.

For Laboratory Use Only

Chem-Impex International, Inc.

Tel: (630) 766-2112
Fax: (630) 766-2218
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935 Dillon Drive
Wood Dale, IL 60191
Manufacturing site:
825 Dillon Drive
Wood Dale, IL 60191

Certificate of Analysis

Catalogue Number	22113
Lot Number	000052-20-027
Product	**Chloroquine diphosphate salt**
	N^4-(7-Chloro-4-quinolinyl)-N^1,N^1-dimethyl-1,4-pentanediamine diphosphate salt
CAS Number	50-63-5
Molecular Formula	$C_{18}H_{26}ClN_3 \cdot 2H_3PO_4$

Appearance	Solid
Assay by titration	99.0% (neutralization)
Purity by HPLC	98.6%
Storage	Store at 0-8°C
Remarks	See material safety data sheet for additional information For laboratory use only

The foregoing is a copy of the Certificate of Analysis as provided by our supplier

Bala Kumar
Quality Control Manager

[redacted]

Print Date: Jan 27th 2016

Certificate of Analysis

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Product Name: **Cytosporone B** **Catalog No.: 5459** **Batch No.: 1**

CAS Number: 321661-62-5

IUPAC Name: Ethyl 3,5-dihydroxy-2-(1-oxooctyl)-benzeneacetate

1. PHYSICAL AND CHEMICAL PROPERTIES

Batch Molecular Formula: $C_{18}H_{26}O_5$

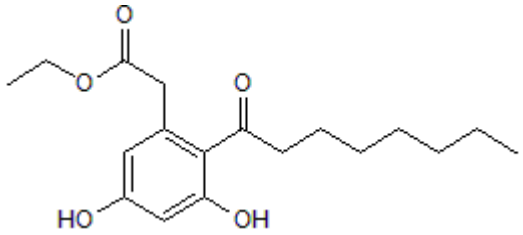
Batch Molecular Weight: 322.4

Physical Appearance: White solid

Solubility: DMSO to 100 mM
ethanol to 100 mM

Storage: Store at -20°C

Batch Molecular Structure:



2. ANALYTICAL DATA

TLC: R_f = 0.2 (Dichloromethane:Methanol [19:1])

HPLC: Shows 99.5% purity

^{1}H NMR: Consistent with structure

Mass Spectrum: Consistent with structure

Microanalysis:

	Carbon	Hydrogen	Nitrogen
Theoretical	67.06	8.13	0
Found	67.12	8.32	0.1

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Print Date: Jan 27th 2016

Product Information

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Product Name: **Cytosporone B** **Catalog No.: 5459** **Batch No.: 1**

CAS Number: 321661-62-5

IUPAC Name: Ethyl 3,5-dihydroxy-2-(1-oxooctyl)-benzeneacetate

Description:

Naturally occurring Nur77 (NR4A1) agonist (K_d = 8.52 x 10^{-7} M). Induces apoptosis in gastric cancer cells in vitro. Increases blood glucose levels in fasting C57 mice and suppresses xenograft tumor growth. Also exhibits a potent antifibrotic effect in bleomycin-induced skin fibrosis in vivo.

Physical and Chemical Properties:

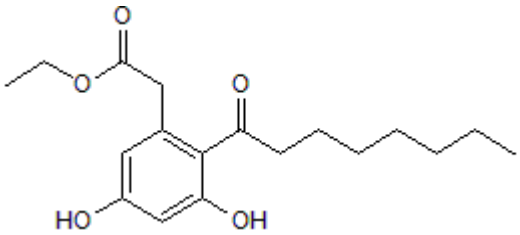
Batch Molecular Formula: $C_{18}H_{26}O_5$

Batch Molecular Weight: 322.4

Physical Appearance: White solid

Minimum Purity: >98%

Batch Molecular Structure:



Storage: Store at -20°C

Solubility & Usage Info:

DMSO to 100 mM
ethanol to 100 mM

Stability and Solubility Advice:

Some solutions can be difficult to obtain and can be encouraged by rapid stirring, sonication or gentle warming (in a 45-60°C water bath).

Information concerning product stability, particularly in solution, has rarely been reported and in most cases we can only offer a general guide. Our standard recommendations are:

SOLIDS: Provided storage is as stated on the product label and the vial is kept tightly sealed, the product can be stored for up to 6 months from date of receipt.

SOLUTIONS: We recommend that stock solutions, once prepared, are stored aliquoted in tightly sealed vials at -20°C or below and used within 1 month. Wherever possible solutions should be made up and used on the same day.

References:

Zhan *et al* (2008) Cytosporone B is an agonist for nuclear orphan receptor Nur77. Nat.Chem.Biol. ***4*** 548. PMID: 18690216.

Palumbo-Zerr *et al* (2015) Orphan nuclear receptor NR4A1 regulates transforming growth factor-β signaling and fibrosis. Nat.Med. ***21*** 150. PMID: 25581517.

[redacted]

Print Date: May 27th 2016

Certificate of Analysis

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Product Name: **IP7e** **Catalog No.: 5699** **Batch No.: 1**

CAS Number: 500164-74-9

IUPAC Name: 6-[4-[(2-Methoxyethoxy)methyl]phenyl]-5-methyl-3-phenyl-isoxazolo[4,5-*c*]pyridin-4(5*H*)-one

1. PHYSICAL AND CHEMICAL PROPERTIES

Batch Molecular Formula: $C_{23}H_{22}N_2O_4$

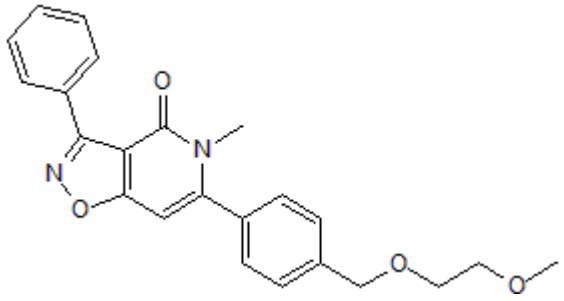
Batch Molecular Weight: 390.43

Physical Appearance: White solid

Solubility: DMSO to 100 mM
ethanol to 20 mM with gentle warming

Storage: Store at +4°C

Batch Molecular Structure:



2. ANALYTICAL DATA

TLC: R_f = 0.37 (Ethyl acetate:Petroleum ether [1:1])

HPLC: Shows 98.7% purity

^{1}H NMR: Consistent with structure

Mass Spectrum: Consistent with structure

Microanalysis:

	Carbon	Hydrogen	Nitrogen
Theoretical	70.75	5.68	7.17
Found	71.07	5.67	7.14

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Print Date: May 27th 2016

Product Information

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Product Name: **IP7e** **Catalog No.: 5699** **Batch No.: 1**

CAS Number: 500164-74-9

IUPAC Name: 6-[4-[(2-Methoxyethoxy)methyl]phenyl]-5-methyl-3-phenyl-isoxazolo[4,5-*c*]pyridin-4(5*H*)-one

Description:

Potent Nurr1 activator (EC_{50} = 3.9 nM). Delays onset and reduces severity of symptoms in mice with experimental autoimmune encephalomyelitis (EAE). Also suppresses NF-κB signaling. Brain penetrant and orally bioavailable.

Physical and Chemical Properties:

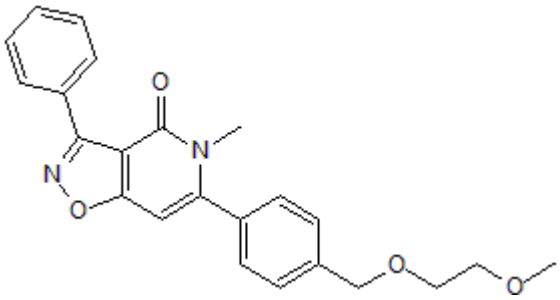
Batch Molecular Formula: $C_{23}H_{22}N_2O_4$

Batch Molecular Weight: 390.43

Physical Appearance: White solid

Minimum Purity: >98%

Batch Molecular Structure:



Storage: Store at +4°C

Solubility & Usage Info:

DMSO to 100 mM
ethanol to 20 mM with gentle warming

Stability and Solubility Advice:

Some solutions can be difficult to obtain and can be encouraged by rapid stirring, sonication or gentle warming (in a 45-60°C water bath).

Information concerning product stability, particularly in solution, has rarely been reported and in most cases we can only offer a general guide. Our standard recommendations are:

SOLIDS: Provided storage is as stated on the product label and the vial is kept tightly sealed, the product can be stored for up to 6 months from date of receipt.

SOLUTIONS: We recommend that stock solutions, once prepared, are stored aliquoted in tightly sealed vials at -20°C or below and used within 1 month. Wherever possible solutions should be made up and used on the same day.

References:

Montarolo *et al* (2014) Effects of isoxazolo-pyridinone 7e, a potent activator of the Nurr1 signaling pathway, on experimental autoimmune encephalomyelitis in mice. PLoS One ***9*** e108791. PMID: 25265488.

Hintermann *et al* (2007) Identification of a series of highly potent activators of the Nurr1 signaling pathway. Bioorg.Med.Chem.Lett. ***17*** 193. PMID: 17035009.

[redacted]
INDOFINE Chemical Company, Inc.
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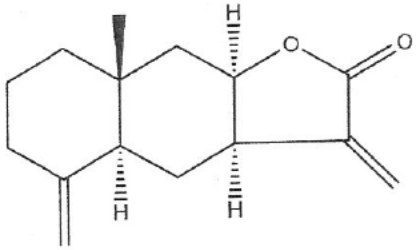
CERTIFICATE OF ANALYSIS

Catalog Number:	06-725
Product Name:	**ISOALANTOLACTONE** (Isohelenin)
CAS Number:	[470-17-7]
Lot Number:	1901182
Chemical Formula:	$C_{15}H_{20}O_2$
Molecular Weight:	232.32
Appearance:	Off-white powder
Loss on Drying:	0.87%
Storage:	Store in a cool, dry place
Purity(HPLC):	99.20%



Prepared by:
Ramesh Mandadi
Ramesh Mandadi
Director of Operations

Reviewed and approved by:
Sujata Moton
S. Moton
VP

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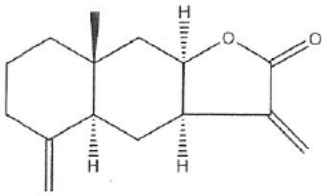
HPLC ANALYSIS

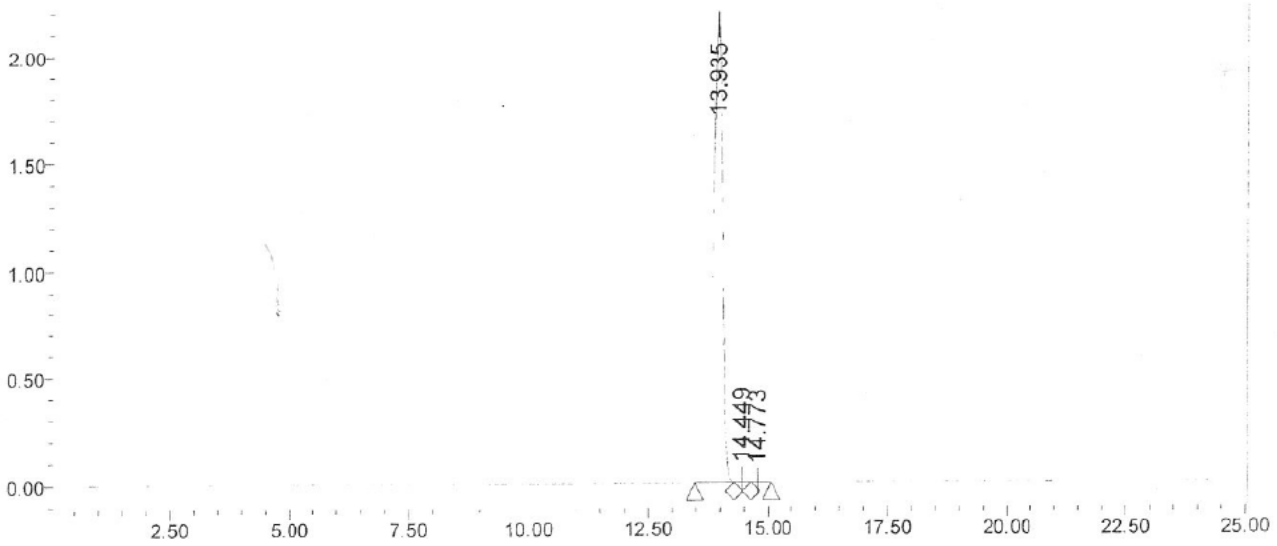


Catalog No.: 06-725
Product Name: Isoalantolactone
Lot No.: 1901182

SAMPLE INFORMATION

Sample Name:	Isoalantolactone	Acquired By:	System
Sample Type:	Standard	Sample Set Name:	
Vial:	73	Acq. Method Set:	Isoalantolactone
Injection #:	1	Processing Method:	Samples
Injection Volume:	7.00 ul	Channel Name:	205.0nm
Run Time:	25.0 Minutes	Proc. Chnl. Descr.:	PDA 205.0 nm

Auto-Scaled Chromatogram



Peak Results

	RT	Area	% Area	USP Plate Count	USP Resolution
1	13.935	27947035	99.20	27614.77	
2	14.449	172180	0.61	21003.53	1.29
3	14.773	51796	0.18	20460.48	

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Certificate of Analysis

Nur77 LBD Antagonist, TMPA - Calbiochem

Batch Number:	3013545
Material Number:	492910-10MG
Molecular Formula:	$C_{21}H_{32}O_6$
Molecular Weight:	380.5

Quality Release Date: 17-JAN-2018
Recommended Retest Date: 22-APR-2023

Analytical Data

Test	Tolerance	Result
Solubility:		DMSO (100 mg/ml). Use only fresh DMSO for reconstitution.
Color:		Off-white
Form:		Solid
Purity by HPLC:	≥97.00 %	97.62 %
NMR:		Conforms
LC-MS:		Conforms
Carbon:	≥0.00 %	66.01 %
Hydrogen:	≥0.00 %	8.87 %
Water by CHN (mol):	≥0.0 %	0.0 %

Storage and Handling: +2°C to +8°C

This lot conforms to specifications established by EMD Millipore Corporation for this product.

Suzanne Lee

Quality Control/ Assurance Signature

10 December 2018

Date

EMD Millipore Corporation | 28820 Single Oak Dr., Temecula, CA 92590

Technical Support NA +1-800-221-1975 | email: www.millipore.com/techservices | www.calbiochem.com

Technical Support All Other Countries - Contact Your Local Office

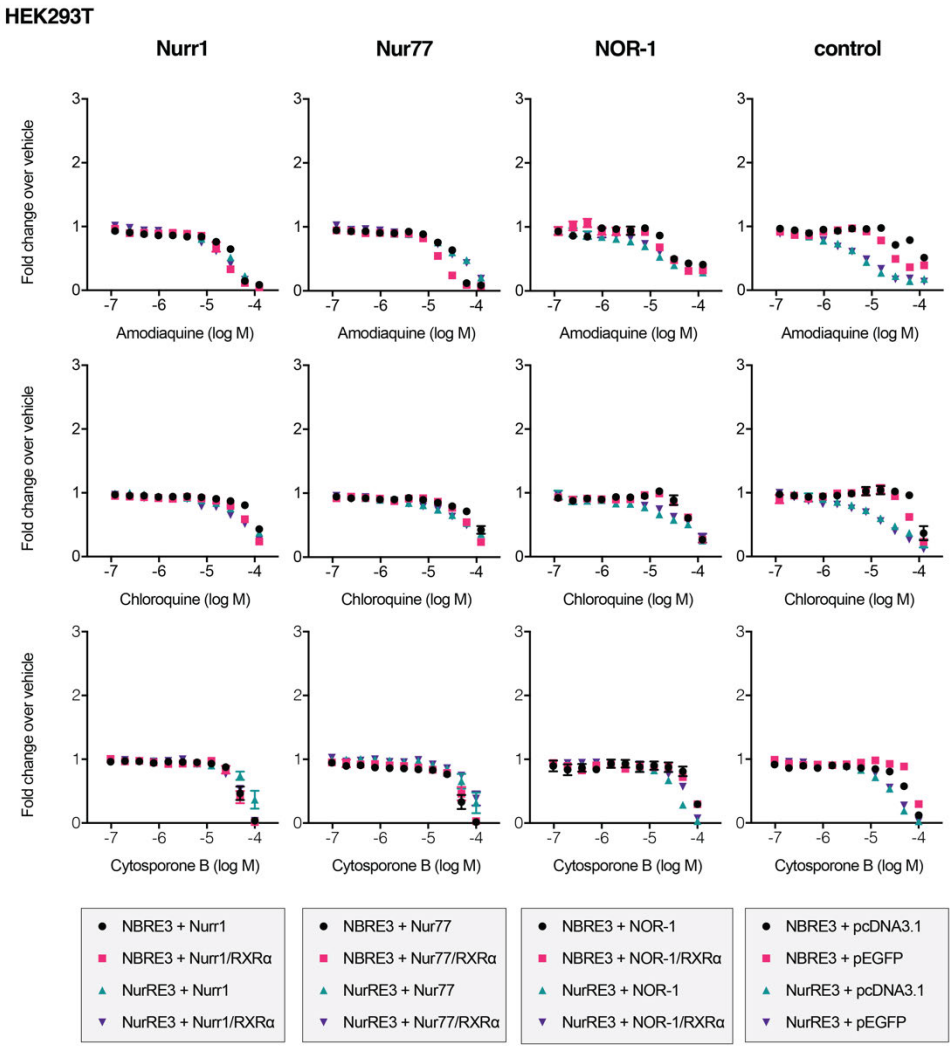

Figure S1. Effect of the Nurr1-binding ligands on transcription in HEK293T cells transfected with full-length NR4As (Nurr1, Nur77, or NOR-1) or empty control plasmid without a NR4A insert using using NBRE3-luc or NurRE3-luc reporters with or without cotransfection of full-length RXRα. Data represent mean ± s.e.m. (n=4).

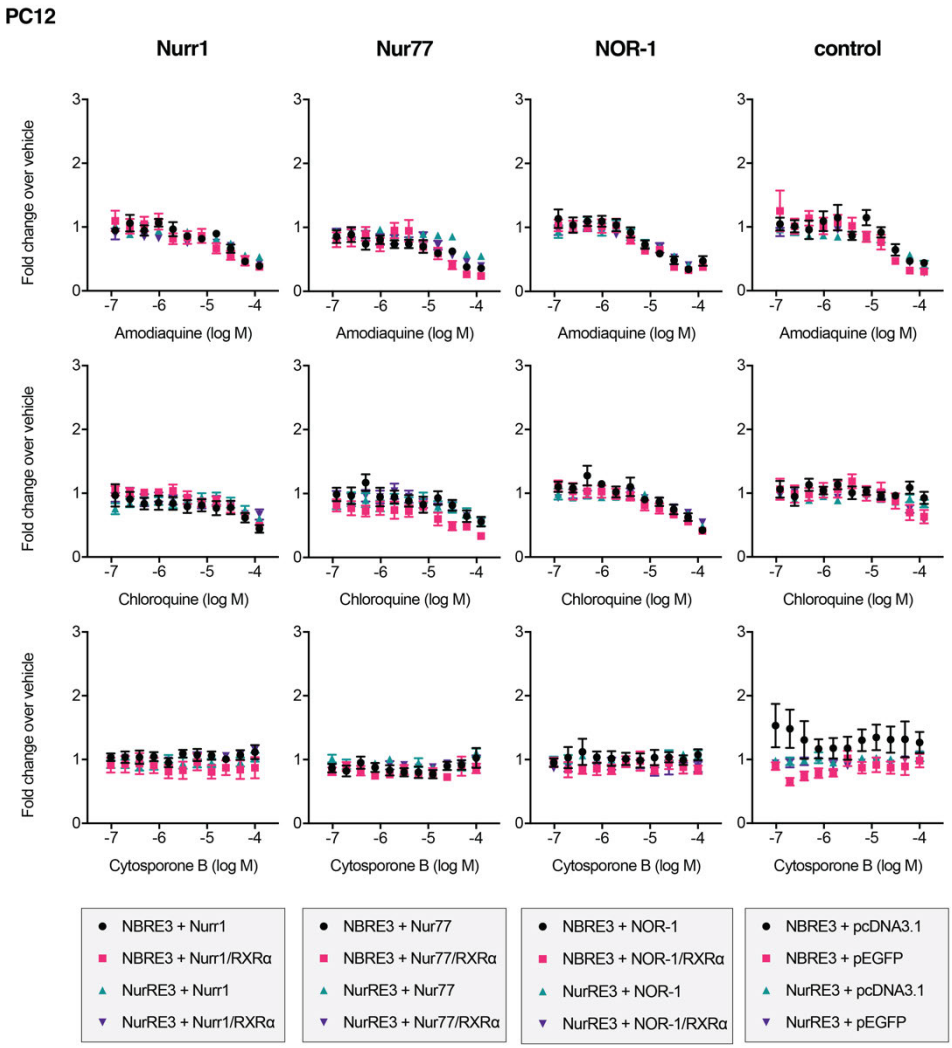

Figure S2. Effect of the Nurr1-binding ligands on transcription in PC12 cells transfected with full-length NR4As (Nurr1, Nur77, or NOR-1) or empty control plasmid without a NR4A insert using using NBRE3-luc or NurRE3-luc reporters with or without cotransfection of full-length RXRα. Data represent mean ± s.e.m. (n=4).

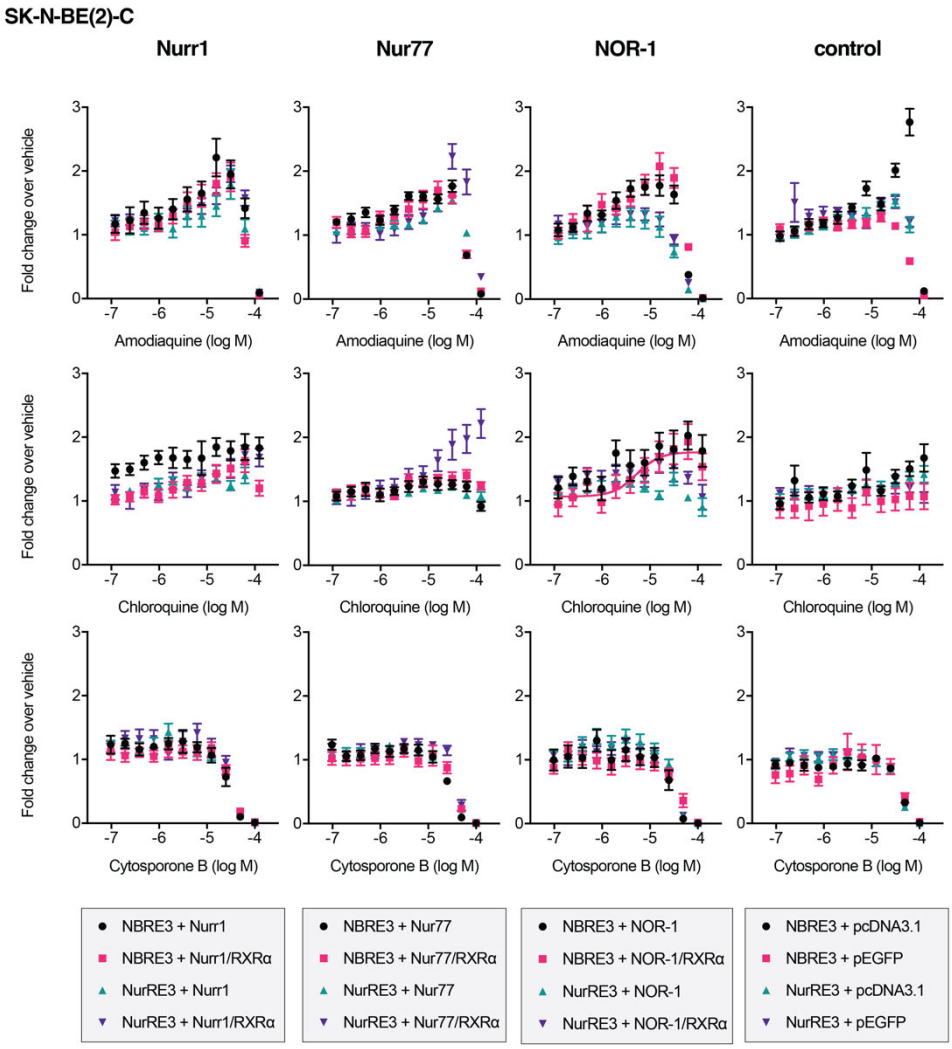

Figure S3. Effect of the Nurr1-binding ligands on transcription in SK-N-BE(2)-C cells transfected with full-length NR4As (Nurr1, Nur77, or NOR-1) or empty control plasmid without a NR4A insert using using NBRE3-luc or NurRE3-luc reporters with or without cotransfection of full-length RXRα. Data represent mean ± s.e.m. (n=4).