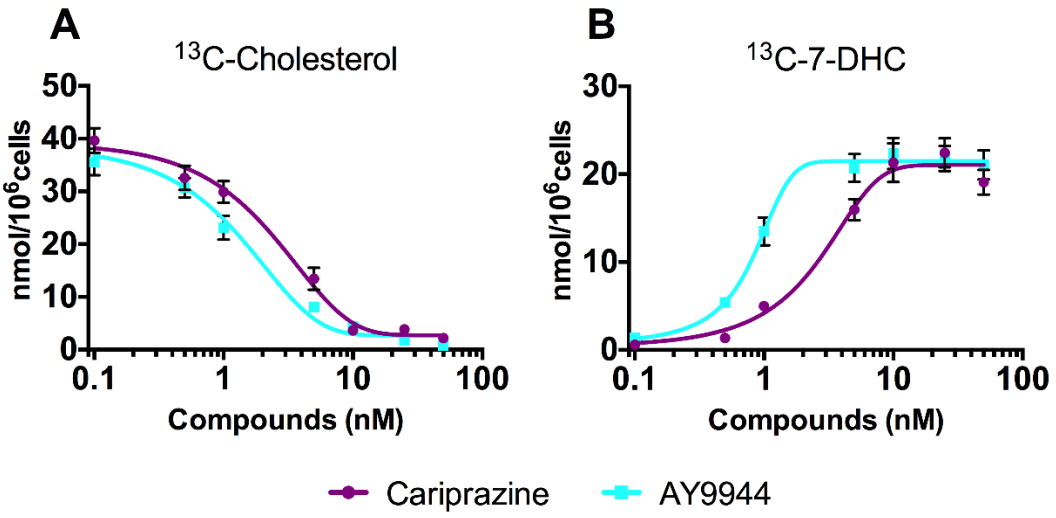
Supporting information for:

Dichlorophenyl piperazines, including a recently-approved atypical antipsychotic, are potent inhibitors of DHCR7, the last enzyme in cholesterol biosynthesis

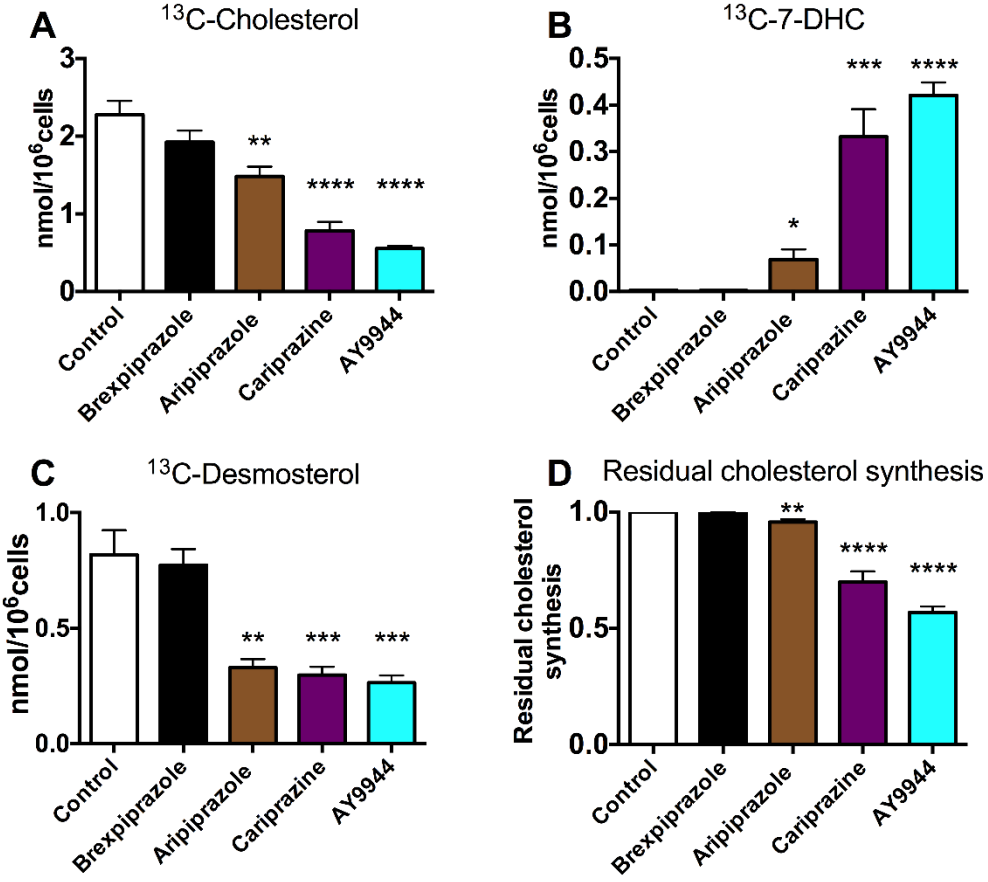
[a]Thiago C. Genaro-Mattos, [a]Keri A. Tallman, [c]Luke B. Allen, [d]Allison Anderson, [d]Karoly Mirnics, [c]Zeljka Korade and [a,b]Ned A. Porter*

Supporting Figure 1



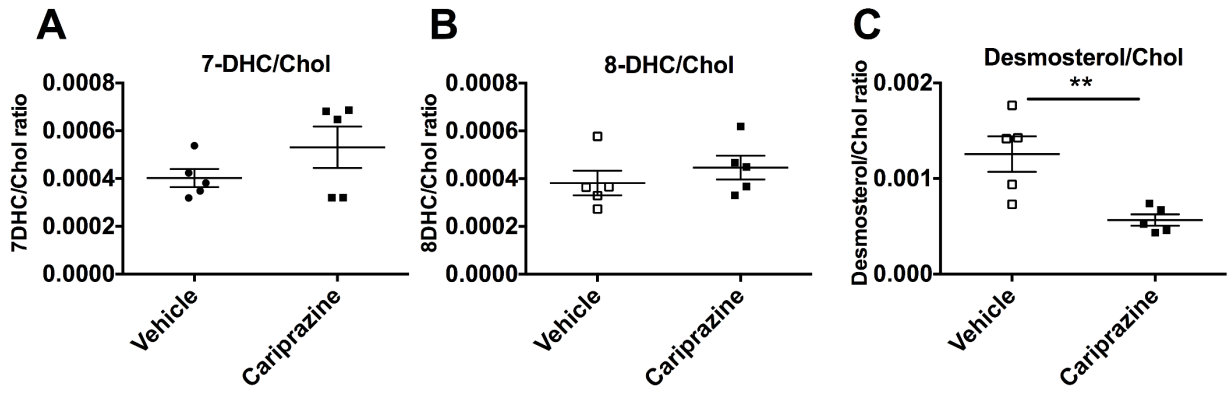
Supporting Figure 1. Effect of different concentrations of cariprazine and AY9944 on human fibroblasts ^{13}C-sterols. Three different control human fibroblasts were cultured in the presence of cariprazine and AY9944 for 7 days in lipid-deficient medium containing 10 mM ^{13}C-glucose as major energy source.

Supporting Figure 2



Supporting Figure 2. ^{13}C-sterols and Residual Cholesterol Synthesis in A549 cells lines in the presence and absence of the antipsychotics. Cells were incubated with 10 mM ^{13}C-glucose in lipid-deficient medium for 48 h. All four compounds were present at 100 nM. *p<0.05, **p<0.01, ***p<0.005 and ****p<0.001.

Supporting Figure 3



Supporting Figure 3. Cariprazine affects serum sterol levels of mice injected with the drug. Cholesterol levels correspond to: 556.5 ± 13.1 nmol/million cells (vehicle) and 672.8 ± 26.1 nmol/million cells (cariprazine).

RNA Preparation and Quantitative PCR

These methods have been described previously (1, 2) and we provide here detailed description. Total RNA was isolated from the cells using Trizol (Life Technologies, Rockville, MD). The concentration of total RNA was measured on a Nanodrop instrument (Thermo Scientific, Wilmington, DE). Total RNA (500 ng) from each sample was reverse transcribed to cDNA using a High Capacity cDNA Archive Kit (Applied Biosystems, Foster City, CA). Real time PCR was performed with an StepOnePlus Real Time PCR System (ThermoFisher) using cDNA (equivalent to 5 ng of input RNA) per 25 µl reaction volume, 2X SYBR green master mix, and gene-specific primers. All samples were run in triplicate. Differential expression was calculated as ΔΔCt against expression of two normalizers (Actb, Pgk1).

Statistical analyses of the qPCR data were performed using pairwise Student t-test in MS-Excel 2010, while false discovery for multiple testing was performed by calculating the individual q-value (3) for transcript using the Benjamini-Hochberg approach (4).

References for the RNA Preparation and Quantitative PCR

1. Korade Z, Xu L, Shelton R, Porter NA. Biological activities of 7-dehydrocholesterol-derived oxysterols: implications for Smith-Lemli-Opitz syndrome. J Lipid Res. 2010; 51:3259–3269. [PubMed: 20702862]

2. Korade Z, Kenworthy AK, Mirnics K. Molecular consequences of altered neuronal cholesterol biosynthesis. J Neurosci Res. 2009; 87:866–875. [PubMed: 18951487]

3. Storey JD. A direct approach to false discovery rates. Journal of Royal Statistical Society B. 2002; 64:479–498.

4. Benjamini Y, Hochberg Y. Controlling the false discovery rate: a practical and powerful approach to multiple testing. Journal of the Royal Statistical Society Series B (Methodological). 1995; 57:289–300.

mRNA levels of *hmgcr* and *dhcr7* in the cortex.

ΔΔCt values correspond to the difference between cariprazine group and vehicles groups. Values correspond to the mean ± SE of five animals. ΔΔCt for *hmgcr* was 0.048 ± 0.077 and for *dhcr7* was 0.063 ± 0.108. There is no statistical difference between cariprazine and vehicle groups.

Purity of tested compounds

All tested compounds have purity >95% as determined by NMR and/or HPLC analysis. The Certificate of Analysis or analytical evidence is included for each compound.

Cariprazine (ChemScene, 99.76%)

ChemScene

Bioactive Molecules, Building Blocks, Intermediates

www.ChemScene.com

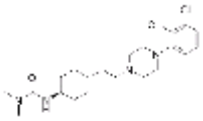
Certificate of Analysis

Product Name:	Cariprazine
Cat. No.:	CS-1569
CAS No.:	839712-12-8
Batch No.:	16216
Chemical Name:	Urea, N'-[trans-4-[2-[4-(2,3-dichlorophenyl)-1-piperazinyl]ethyl]cyclohexyl]-N,N-dimethyl-

PHYSICAL AND CHEMICAL PROPERTIES

Molecular Formula:	$C_{21}H_{32}Cl_2N_4O$		
Molecular Weight:	427.41		
Storage:	Powder	-20°C	3 years
		4°C	2 years
	In solvent	-80°C	6 months
		-20°C	1 month

Chemical Structure:



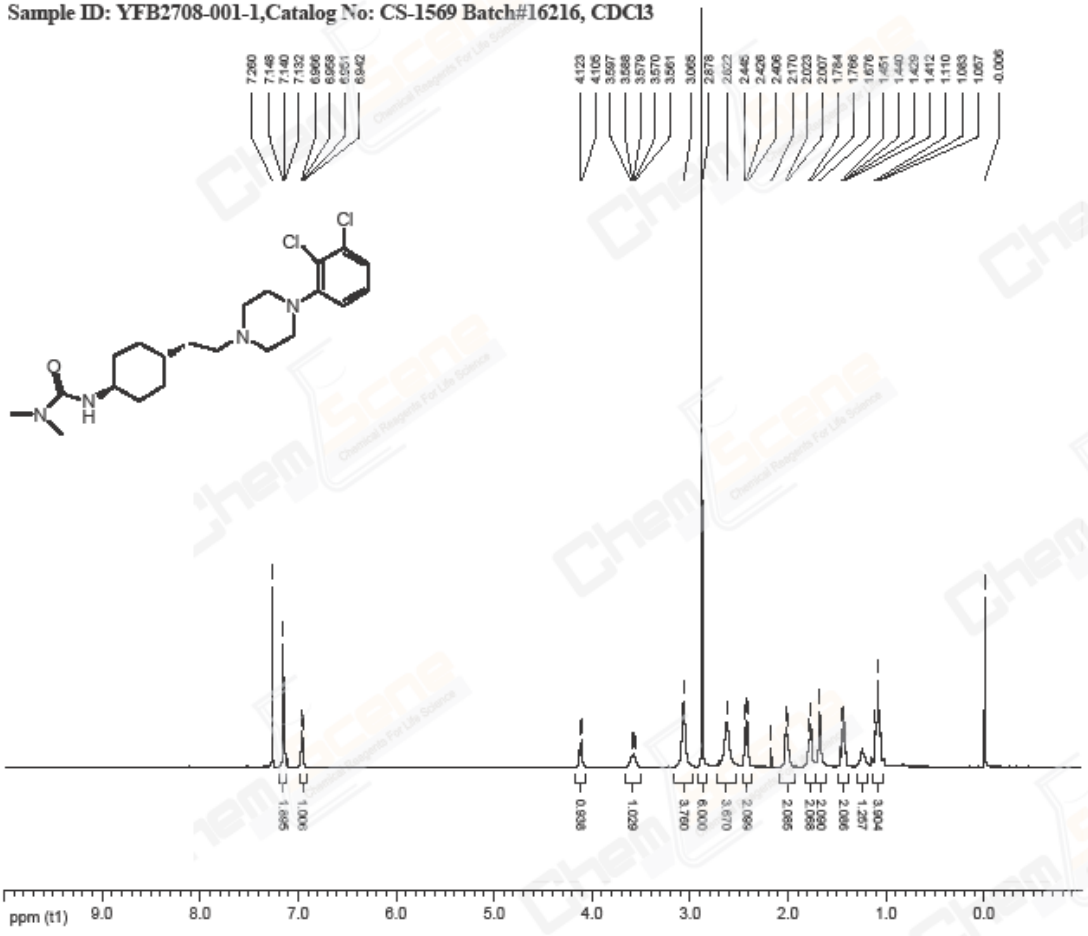
ANALYTICAL DATA

Appearance:	Light brown to brown (Solid)
^{1}H NMR Spectrum:	Consistent with structure
LCMS:	Consistent with structure
Purity (LCMS):	99.76%
Conclusion:	The product has been tested and complies with the given specifications.

Caution: Product has not been fully validated for medical applications. For research use only.

Tel: 732-484-9848 Fax: 888-484-5008 E-mail: sales@ChemScene.com
Address: 1 Deer Park Dr, Suite Q, Monmouth Junction, NJ 08852, USA

Sample ID: YFB2708-001-1,Catalog No: CS-1569 Batch#16216, CDCl3



ChemScene

Date:
25 May 2015
Document's Title:
Catalog No: CS-1569 Batch#16216

Spectrum Title:
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Frequency (MHz):
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Original Points Count:
(f1) 24999
Actual Points Count:
(f1) 65536
Acquisition Time (sec):
(f1) 2.4999
Spectral Width (ppm):
(f1) 24.992
Pulse Program:
ZG30
Temperature:
293.96
Number of Scans:
12
Acq. Date:
Mon May 25 11:11:04 AM

Brexpiprazole (ChemScene, 99.38%)

Bioactive Molecules, Building Blocks, Intermediates

www.ChemScene.com

Certificate of Analysis

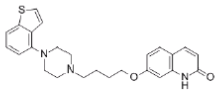
Product Name: Brexpiprazole
Cat. No.: CS-2108
CAS No.: 913611-97-9
Batch No.: 11421
Chemical Name: 2(1H)-Quinolinone, 7-[4-(4-benzo[b]thien-4-yl-1-piperazinyl)butoxy]-

PHYSICAL AND CHEMICAL PROPERTIES

Molecular Formula: $C_{25}H_{27}N_3O_2S$
Molecular Weight: 433.57
Storage:

Powder	-20°C	3 years
	4°C	2 years
In solvent	-80°C	6 months
	-20°C	1 month

Chemical Structure:

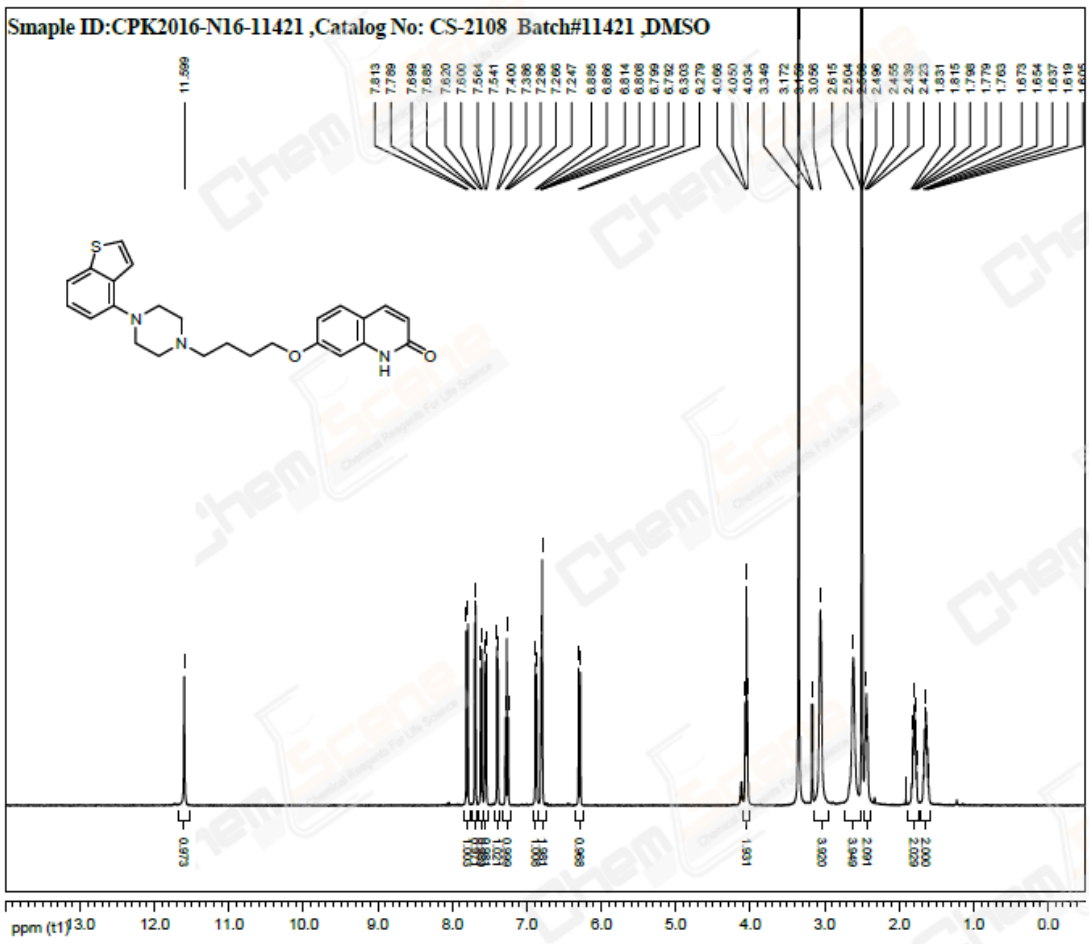


ANALYTICAL DATA

Appearance: White to off-white (Solid)
^{1}H NMR Spectrum: Consistent with structure
LCMS: Consistent with structure
Purity (LCMS): 99.38%
Conclusion: The product has been tested and complies with the given specifications.

Caution: Product has not been fully validated for medical applications. For research use only.

Tel: 732-484-9848 Fax: 888-484-5008 E-mail: sales@ChemScene.com
Address: 1 Deer Park Dr, Suite Q, Monmouth Junction, NJ 08852, USA



ChemScene

Date:
21 Nov 2016
Document's Title:
Catalog No: CS-2108 Batch#11421

Spectrum Title:
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(f1) 32768
Actual Points Count:
(f1) 32768
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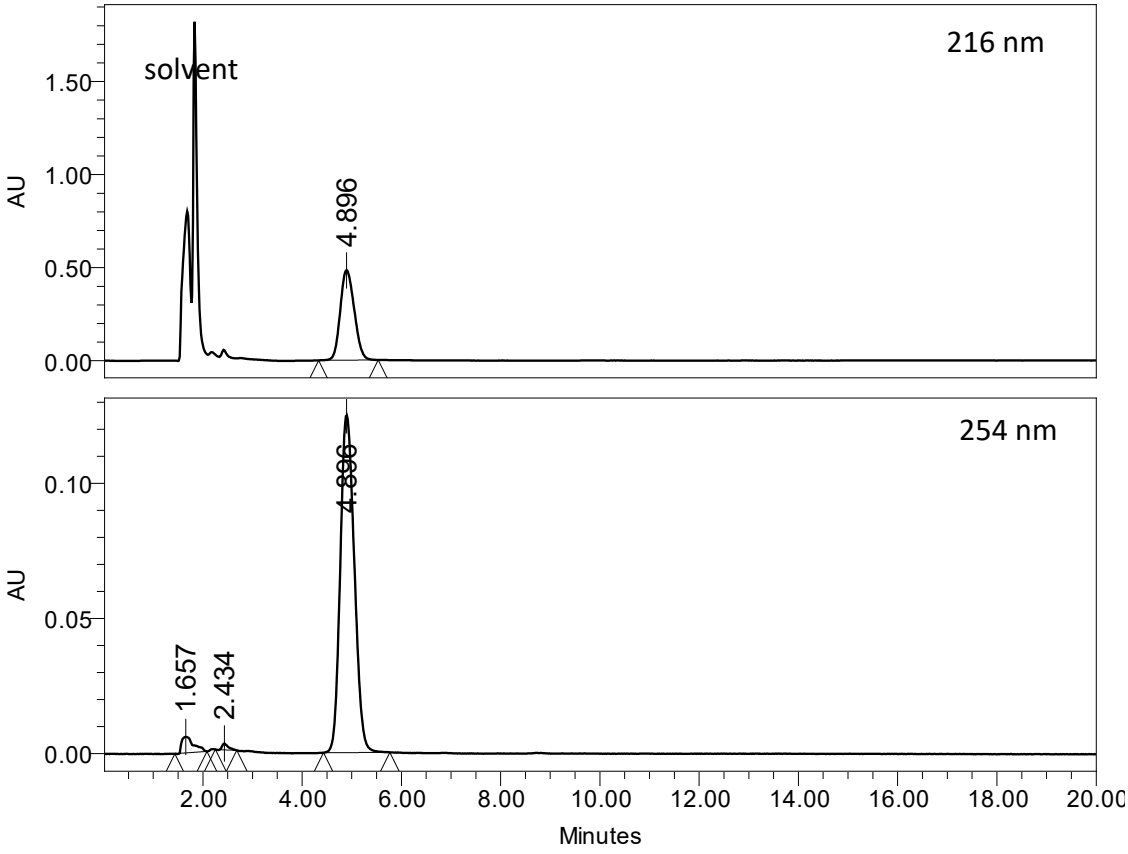
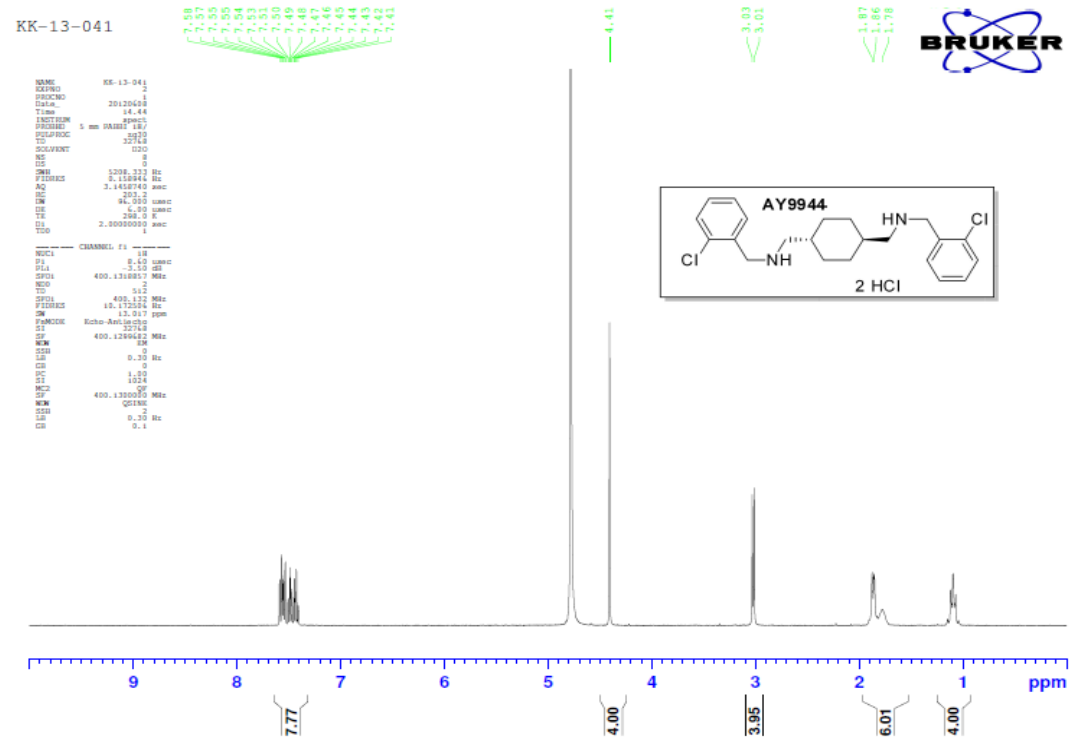
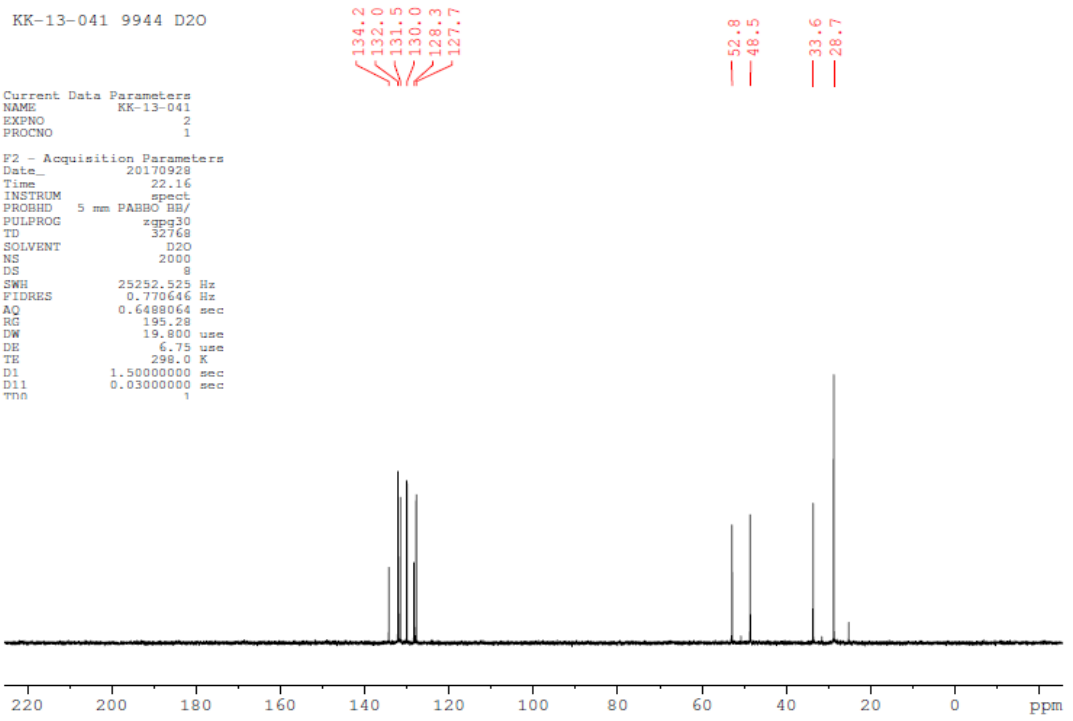
(f1) 3.2768
Spectral Width (ppm):
(f1) 24.992
Pulse Program:
Unknown

Aripiprazole (Bristol Myers Squibb, >95%)



AY9944 (Vanderbilt Synthesis Core, >95%)





VCNDD 12410 Agilent 1200 LCMS

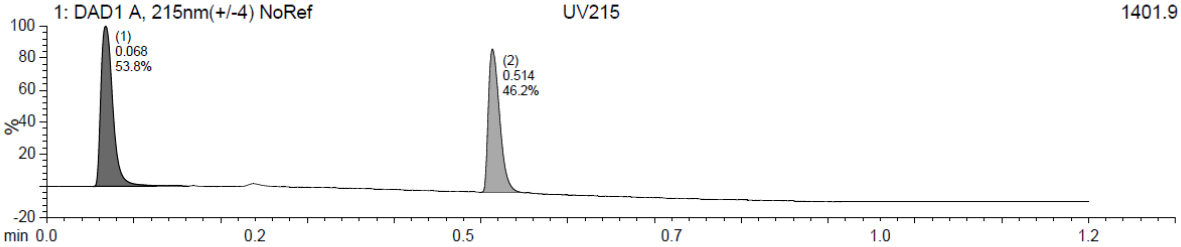
Sample Name: kk-13-043-pure
User: kimk3

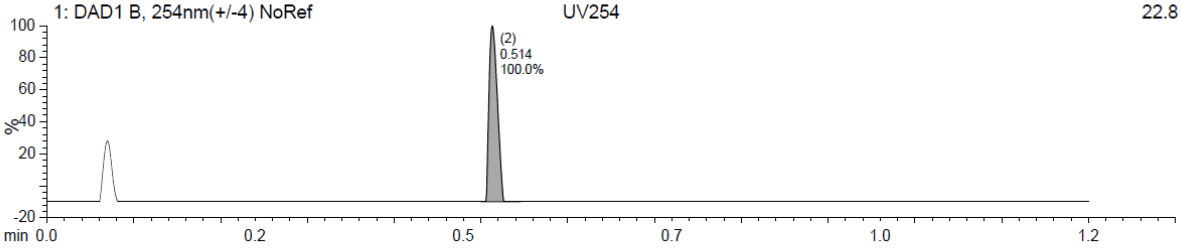
Acquired: 6/8/2012 11:47 AM
Filename: C:\LCMS\DATA\KWANGHO_KIM\06-12\KK-13-043-PURE11-12410 LCMS-23894.D

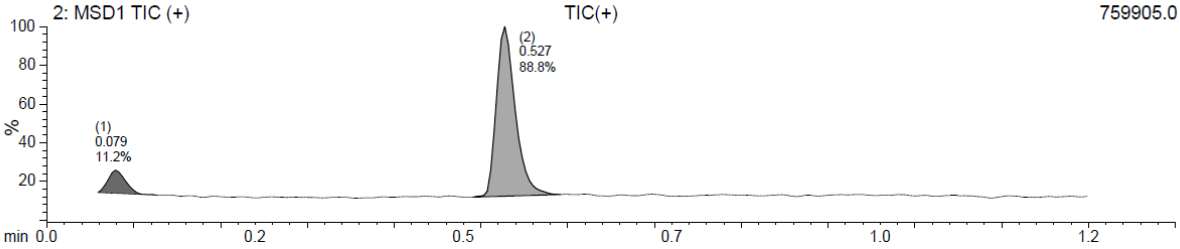
Method: KINETEX NEW1.M
Location: 1,1:E,4

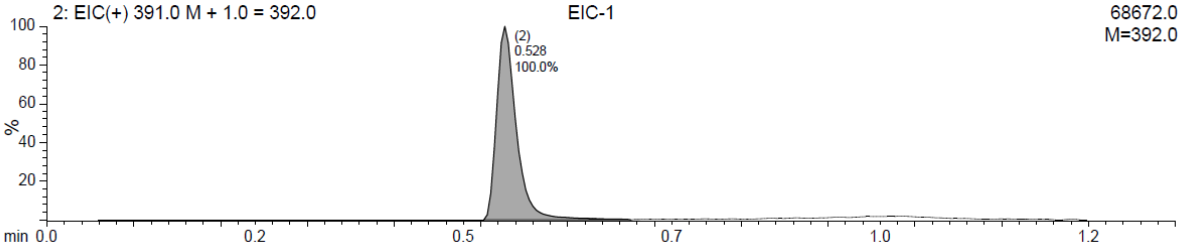
Description: Easy-Access Method: 'Kinetex 1 Minute' ,391.00, 141.00

Peak #	Time	Area % UV215	Area % UV254	Area % TIC(+)	BPM	Area Abs UV215	Area Abs UV254	Area Abs TIC(+)
1	0.074	53.8	0.0	11.2	157.2	1348.30	0	128110.30
1	0.074	53.8	0.0	11.2	157.2	1348.30	0	128110.30
2	0.521	46.2	100.0	88.8	391.2	1158.64	26.68	1010837.94
2	0.521	46.2	100.0	88.8	391.2	1158.64	26.68	1010837.94









1-(2,3-Dichlorophenyl)piperazine (Sigma-Aldrich, 97%)

SIGMA-ALDRICH®

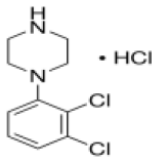
sigma-aldrich.com

3050 Spruce Street, Saint Louis, MO 63103, USA
Website: www.sigmaaldrich.com
Email USA: techserv@sial.com
Outside USA: eurtechserv@sial.com

Certificate of Analysis

Product Name:
1-(2,3-Dichlorophenyl)piperazine hydrochloride - 97%

Product Number:	**679135**
Batch Number:	**MKCC1665**
Brand:	ALDRICH
CAS Number:	119532-26-2
MDL Number:	MFCD00190238
Formula:	C10H13Cl3N2
Formula Weight:	267.58 g/mol
Quality Release Date:	27 DEC 2016



Test	Specification	Result
Appearance (Color)	White to Tan	White
Appearance (Form)	Powder or Crystals	Powder
Infrared Spectrum	Conforms to Structure	Conforms
Titration by AgNO3	96.5 - 103.5 %	100.4 %
Purity (HPLC)	≥ 96.5 %	100.0 %

Michael Grady, Manager
Quality Control
Milwaukee, WI US

1-(3-Chlorophenyl)piperazine (Sigma-Aldrich, 99%)

SIGMA-ALDRICH®

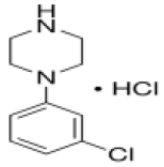
sigma-aldrich.com

3050 Spruce Street, Saint Louis, MO 63103, USA
Website: www.sigmaaldrich.com
Email USA: techserv@sial.com
Outside USA: eurtechserv@sial.com

Certificate of Analysis

Product Name:
1-(3-Chlorophenyl)piperazine hydrochloride – 99%

Product Number:	**125180**
Batch Number:	**MKBR2228V**
Brand:	ALDRICH
CAS Number:	65369-76-8
MDL Number:	MFCD00039032
Formula:	C10H13ClN2 · xHCl
Formula Weight:	196.68 g/mol
Quality Release Date:	14 MAR 2014



Test	Specification	Result
Appearance (Color)	Off-White to Brown	off-white
Appearance (Form)	Powder	Powder
Infrared Spectrum	Conforms to Structure	Conforms
Carbon (as 1 HCl)	50.8 - 52.8 %	51.2 %
Nitrogen (as 1 HCl)	11.8 - 12.3 %	12.2 %

Jamie Gleason, Manager
Quality Control
Milwaukee, Wisconsin US

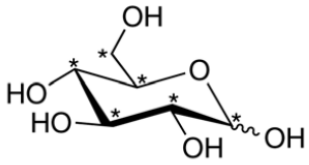
D-$^{13}C_6$-glucose (Cambridge Isotope Laboratories, 99%)

Cambridge Isotope Laboratories, Inc. **Certificate of Analysis**

Product Name: (Isotopic Label & Enrichment Specification)	D-GLUCOSE (U-13C6, 99%)
Lot Number:	PR-28579
Catalog Number:	CLM-1396-0

Product Information

Chemical Purity Specification:	≥ 98%
MW*: * *For isotopically labeled compounds, MW listed is for the fully enriched product.*	186.11
Labeled CAS Number:	110187-42-3
Unlabeled CAS Number:	50-99-7
Chemical Formula:	HO*CH2(*CHOH)4*CHO
Storage:	Store at room temperature away from light and moisture.
Stability:	Stable if stored under recommended conditions.



Certification

Cambridge Isotope Laboratories, Inc. guarantees that this material meets or exceeds the specifications stated. Absolute identity as well as chemical and isotopic purities are assured by the use of unambiguous synthetic routes and multiple chemical analyses whenever possible. Results are representative of QC testing at time of release from Quality Control unless otherwise stated. This COA references the bulk catalog number before packaging. The COA also applies to the CIL finished good catalog number which contains a different suffix such as -0.XX, -XXX, -10xX, -PK, etc. depending on the packaged size.

Approved by: Sashi Sivendran-Basak

Sashi Sivendran-Basak, Ph.D., Quality Review

Quality Control Tests and Results

13C NMR for Identification	Conforms
1H NMR for Chemical Purity	Pass
AgNO3 Test for the Presence of Salts	Pass
GC/MS for Methanol Content	2933 ppm
HPLC for Chemical Purity	99.0%
Karl Fischer Titration for Total Water Content	876 ppm

(continued on next page)

Cambridge Isotope Laboratories, Inc. **Certificate of Analysis**

Product Name: D-GLUCOSE
(Isotopic Label & Enrichment Specification) (U-13C6, 99%)

Lot Number: PR-28579

Catalog Number: CLM-1396-0

Quality Control Tests and Results (continued)

Melting Point Range Determination	140-150°C
Mass Spectrometry for Isotopic Enrichment	99.0%

Product Notes:
Enrichment analyses vary from 98%-100%, based on precision of measurement.