**SUPPORTING INFORMATION**

# Dose-Response Effects of 7-Dehydrocholesterol Reductase Inhibitors on Sterol Profiles and Vesicular Stomatitis Virus Replication

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**Table S1. MS parameters for sterol analysis.**

| Metabolite | SRM parent (M+H) | SRM fragment | CE (eV) | rt (min) |
|---|---|---|---|---|
| 7-DHD | 468.30 | 365.30 | 15 | 5.6 |
| 14d-Zym | 468.31 | 104.10 | 21 | 5.1 |
| 8-DHD | 468.32 | 365.30 | 20 | 5.8 |
| 7-DHC | 470.30 | 367.30 | 13 | 8.4 |
| 8-DHC | 470.31 | 367.31 | 20 | 8.8 |
| Des | 470.31 | 367.31 | 20 | 7.6 |
| DHL | 470.32 | 104.10 | 21 | 7.4 |
| Zym | 470.32 | 104.10 | 21 | 6.9 |
| 14d-Zyme | 470.32 | 104.10 | 21 | 7.1 |
| Chol | 472.30 | 369.30 | 20 | 11.3 |
| Lath | 472.31 | 104.10 | 21 | 10.5 |
| Zyme | 472.31 | 104.10 | 21 | 10.8 |
| Lan | 512.31 | 104.10 | 21 | 11.3 |
| diHLan | 514.31 | 104.10 | 21 | 15.6 |
| $d_6$-7-DHD | 474.30 | 371.30 | 15 | 5.4 |
| $d_6$-14d-Zym | 474.31 | 104.10 | 21 | 4.9 |
| $d_6$-8-DHD | 474.32 | 371.30 | 20 | 5.6 |
| $d_7$-7-DHC | 477.30 | 374.30 | 13 | 8.2 |
| $d_7$-8-DHC | 477.31 | 374.31 | 20 | 8.6 |
| $d_7$-14d-Zyme | 477.32 | 104.10 | 21 | 7.4 |
| $d_6$-Des | 476.30 | 373.30 | 20 | 7.2 |
| $d_6$-DHL | 476.31 | 104.10 | 21 | 6.7 |
| $d_6$-Zym | 476.31 | 104.10 | 21 | 6.9 |
| $d_7$-Chol | 479.30 | 376.30 | 20 | 11.1 |
| $d_7$-Lath | 479.31 | 104.10 | 21 | 10.3 |
| $d_7$-Zyme | 479.31 | 104.10 | 21 | 10.6 |
| $d_6$-Lan | 518.31 | 104.10 | 21 | 11.1 |
| $d_7$-diHLan | 521.31 | 104.10 | 21 | 15.4 |

**Figure S1**. Protocol for drug treatment and VSV-eGFP infection in Neuro2a cells.

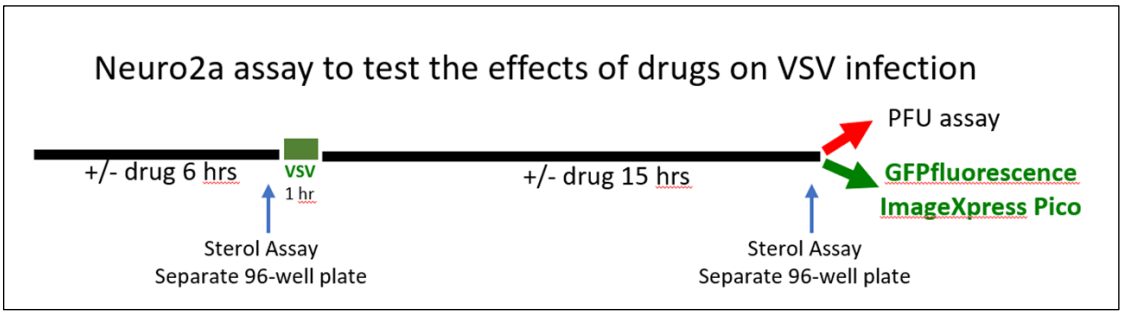



**Figure S2. Comparison of Viability Assay and Cell counts.** Neuro2a were plated and treated with three different concentrations of five compounds: cariprazine (0.5, 5, 50 nM), trazodone (25, 250, 2500 nM), metoprolol (25, 250, 2500 nM), ifenprodil (10, 100, 1000 nM) and AY9944 (10, 100, 1000 nM) for 24 hrs and cell viability measured using Alamar Blue. After fluorescence reading, medium was removed and fresh medium with Hoechst added to cells and the cells were counted on ImageXpress Pico.

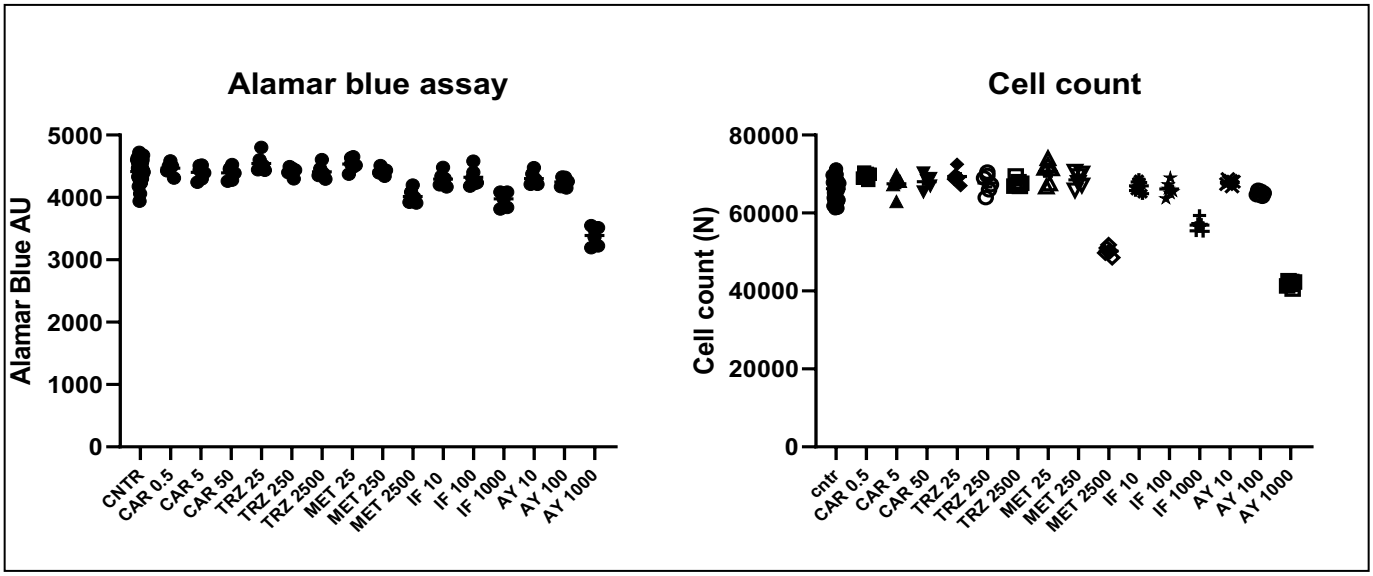


**Figure S3. Alamar-Blue Assay after Drug Treatment and VSV infection**. Neuro2a were plated and pre-treated for 8 hrs with three different concentrations of five compounds: cariprazine (0.5, 5, 50 nM), trazodone (25, 250, 2500 nM), metoprolol (25, 250, 2500 nM), ifenprodil (10, 100, 1000 nM) and AY9944 (10, 100, 1000 nM). Following infection of Neuro2a with 0.1 MOI VSV for 15 hrs, cell viability was measured using Alamar-Blue. First bar shows cell viability without VSV and the rest of bars show viability in presence of VSV only or VSV plus drugs. Different shades of bars show different drugs.

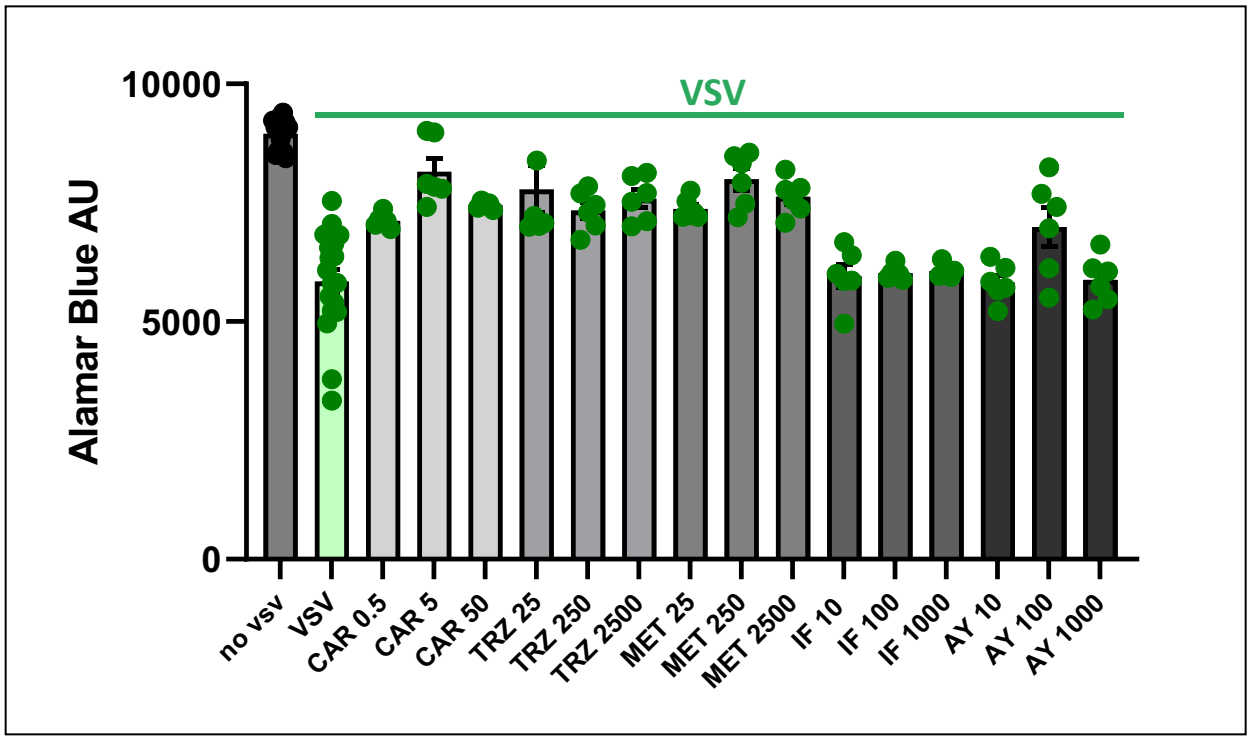



**Figure S4**. **Comparison of Viability Assay and Cell Counts of VSV Infections.** Neuro2a cells were infected with the indicated MOI of VSVeGFP and cells were assayed by Alamar Blue or cell count at 24 hrs after the infection.

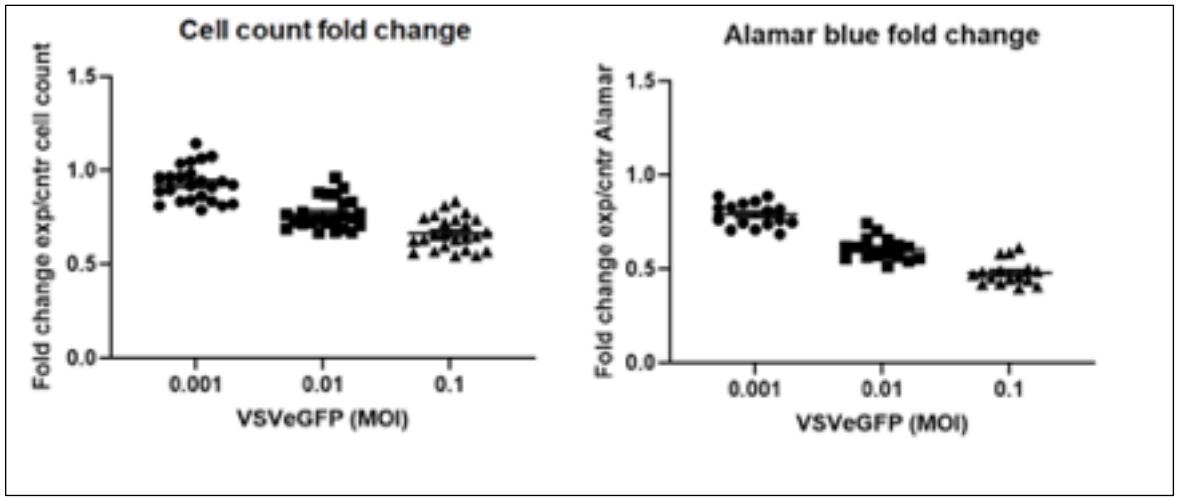


**Figure S5. Effects of VSV-eGFP on Sterol Levels in Neuro2a Cells.** Cells were treated with VSV-eGFP for 1 hr followed by a 24 hr incubation. Sterols were assayed by LC/MS in nmol/million cells and values presented here are normalized vs. cholesterol's value for no drug as 100%. Only those sterols that undergo significant change as a result of VSV-eGFP treatment are shown. n>6, * p<0.05, ** p<0.01, *** p<0.001, **** p<0.0001

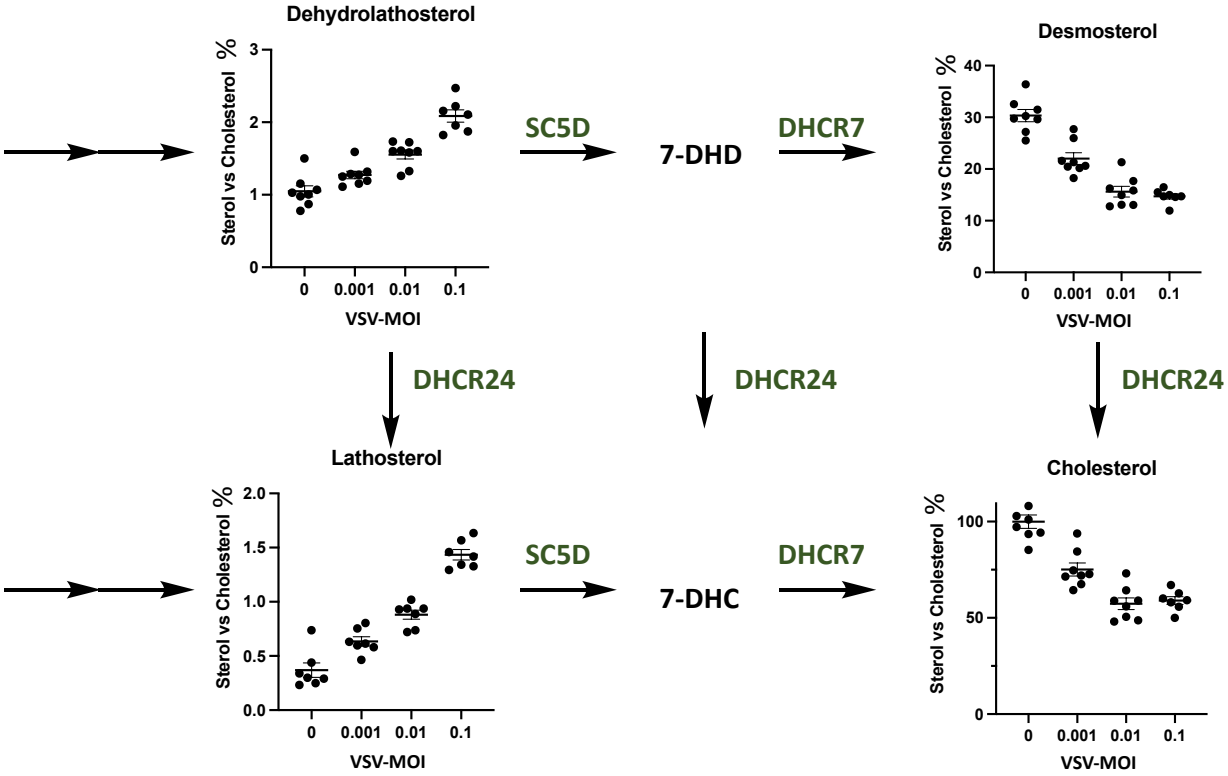


**Figure S6**. **Time Course for Sterols During Neuro2a Infection with VSVe-GFP.** Cells were infected with 0.1 MOI VSV-eGFP for 1 hr and samples taken at the indicated time points. Sterols were assayed by LC/MS and expressed in nmol/million cells.The values are presented as the ratio of the sterols before and after 7-DHD and 7-DHC on the biosynthetic path, dehydro-lathosterol/desmosterol on the Bloch path and lathosterol/cholesterol on the Kandutsch-Russell path. n=8, * =p<0.05, **** = p<0.0001

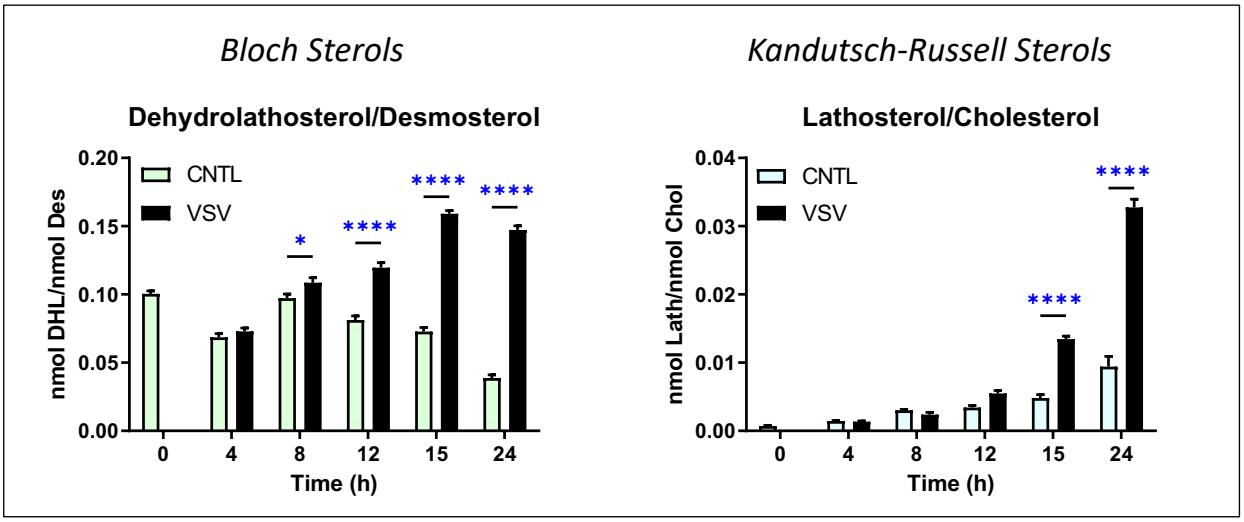


**Figure S7. Effects of Tamoxifen on Sterols and VSV in Neuro2a. A.** Sterol profile of cells treated as described in Figure 3. Levels of zymosterol and zymostenol increased but 7-DHC and 7-DHD were unaffected by treatment. **B**. Fluorescence intensity of cells treated with tamoxifen and 0.1 MOI VSV-eGFP, fluorescence determined and cell counts were measured as described in Figures 5. **C**. Normalized cell count. n>6, ** p<0.01, **** p<0.0001

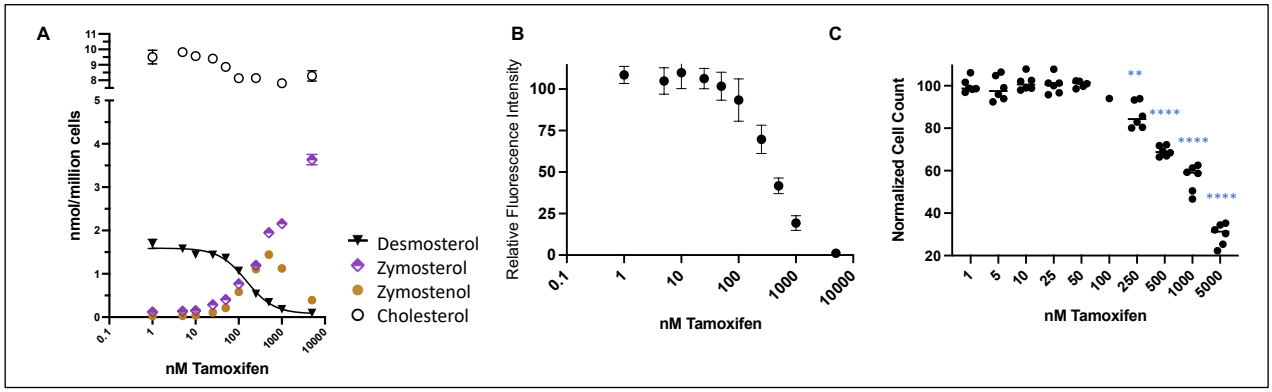
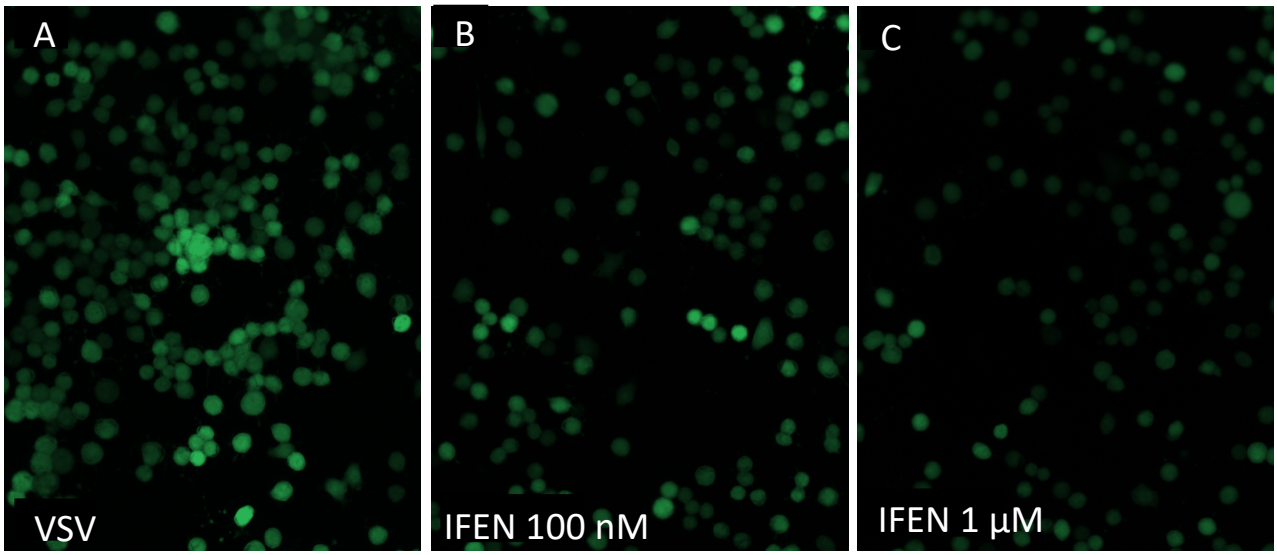


**Figure S8**. **Fluorescent Images of Neuro2a Cells Infected with VSV-eGFP in the Presence of Ifenprodil.** Images (10X) of Neuro2a cells treated with 0.1 MOI VSV-eGFP, control vs VSV + 100 nM and 1 μM ifenprodil.

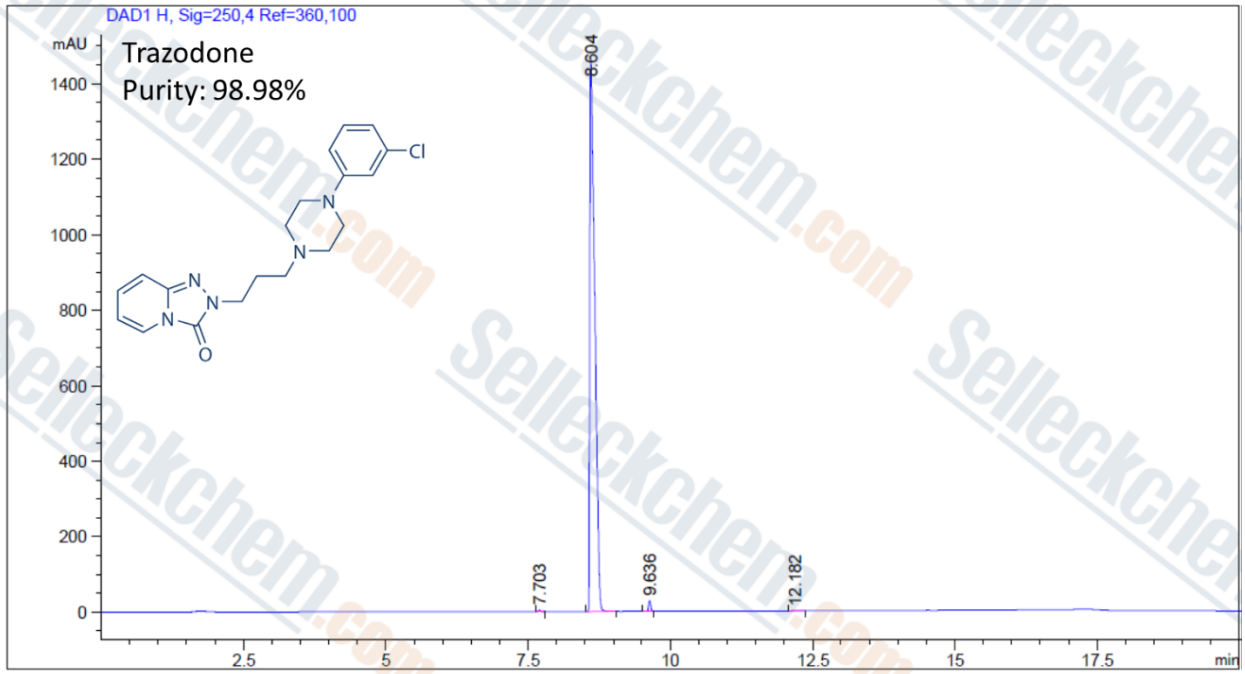


## COMPOUND PURITY

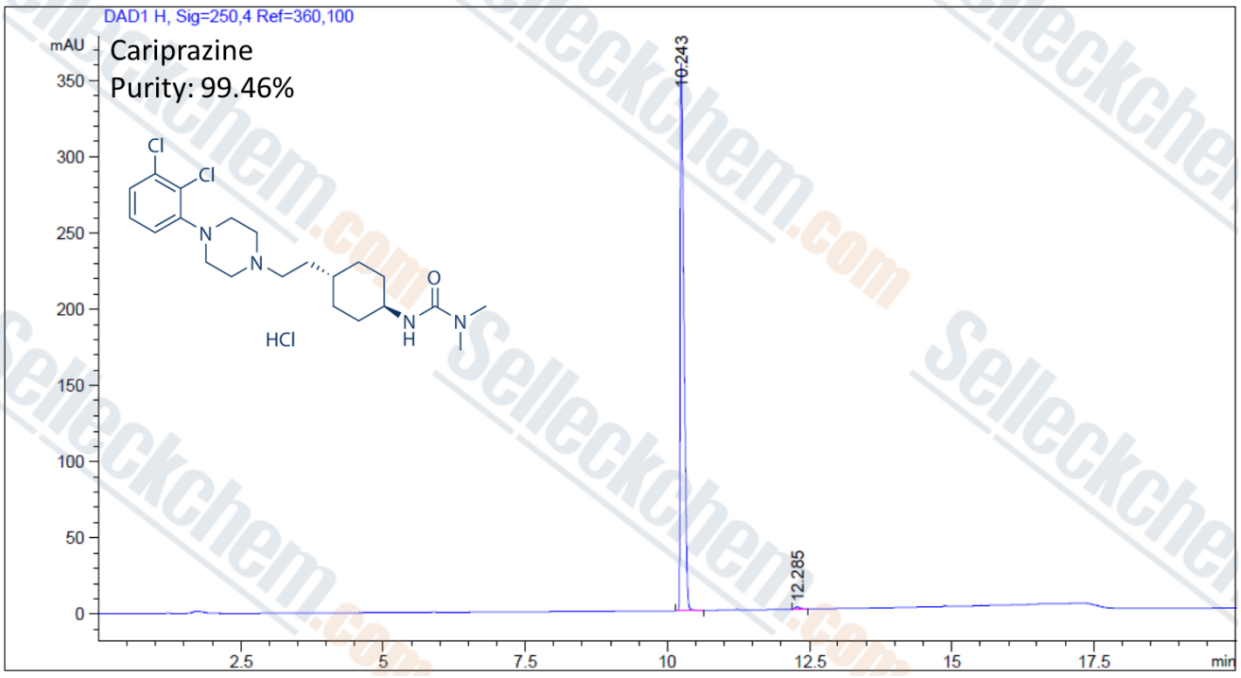
**Figure S9. Compound Purity Spectra and Chromatograms**. Compound purity provided by Selleckchem for A. Trazodone, B. Cariprazine, C. Metoprolol, D. Ifenprodil, and E. Tamoxifen. F-H. Compound characterization and purity for AY9944 provided by Vanderbilt University Synthesis Core.

**(provided by Selleckchem)**

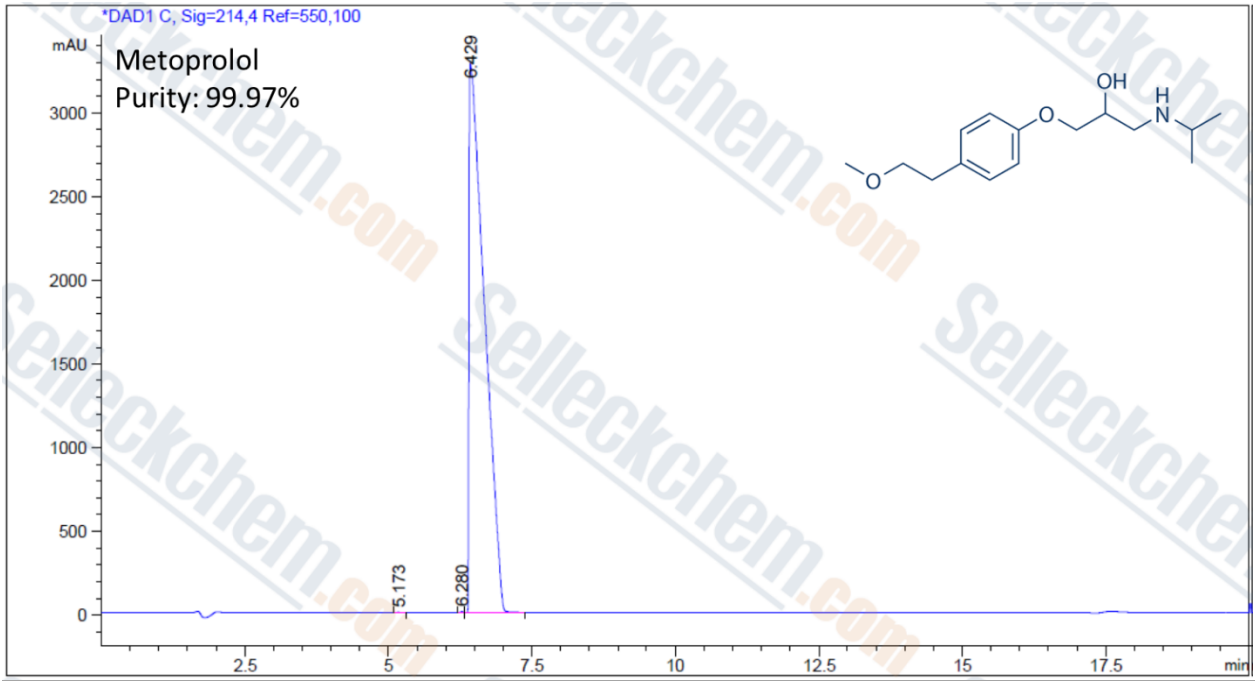
**A.**



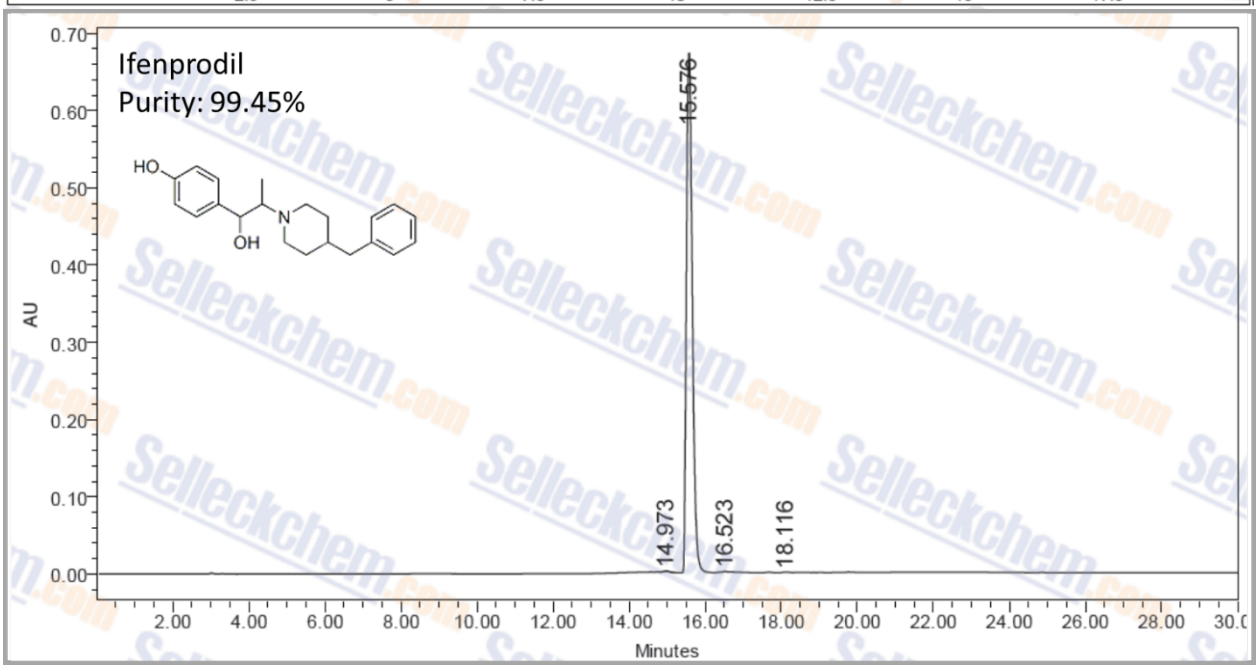

**B.**



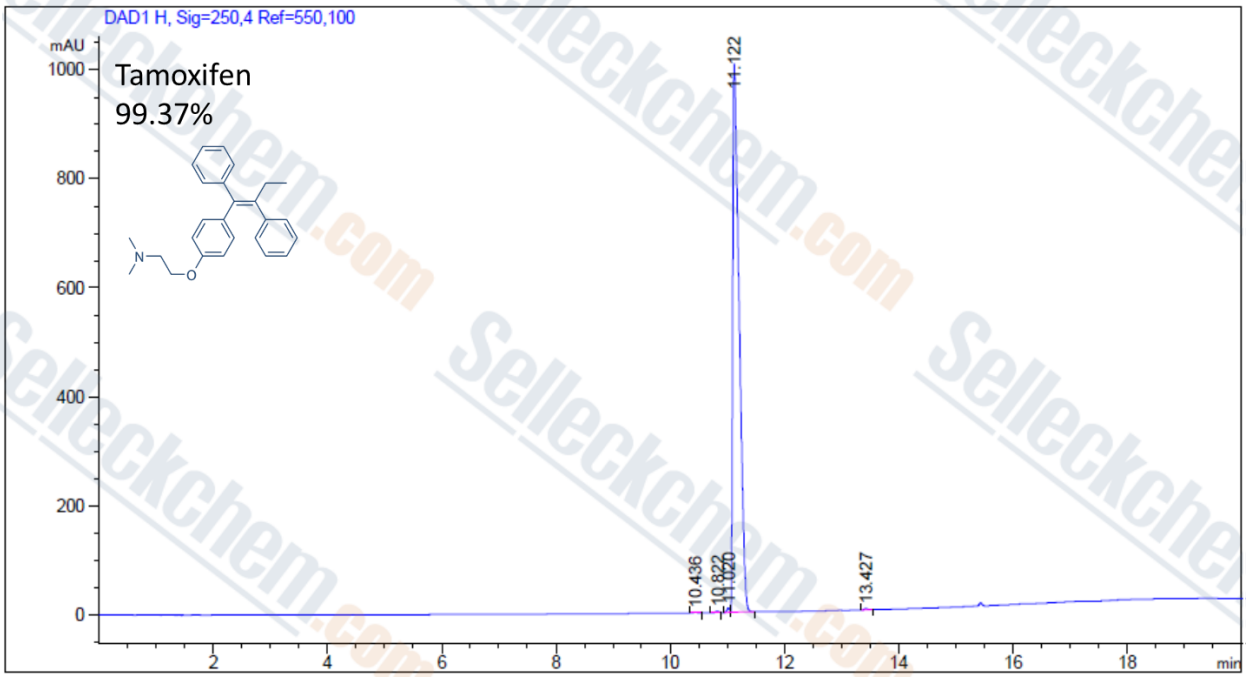
**C.**


*DAD1 C, Sig=214,4 Ref=550,100
mAU
Metoprolol
Purity: 99.97%
6.429
5.173
6.280
OH
H
N
O
O
3000
2500
2000
1500
1000
500
0
2.5
5
7.5
10
12.5
15
17.5
min

**D.**

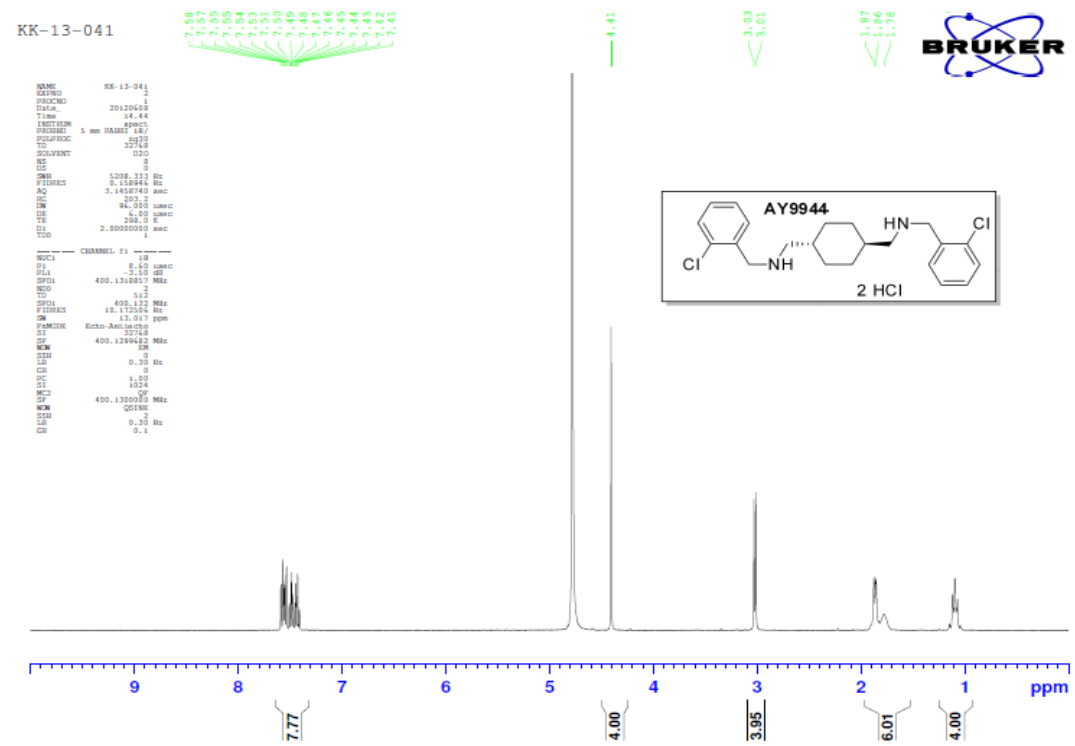

Ifenprodil
Purity: 99.45%
HO
OH
N
15.576
14.973
16.523
18.116
AU
0.70
0.60
0.50
0.40
0.30
0.20
0.10
0.00
2.00
4.00
6.00
8.00
10.00
12.00
14.00
16.00
18.00
20.00
22.00
24.00
26.00
28.00
Minutes

**E.**

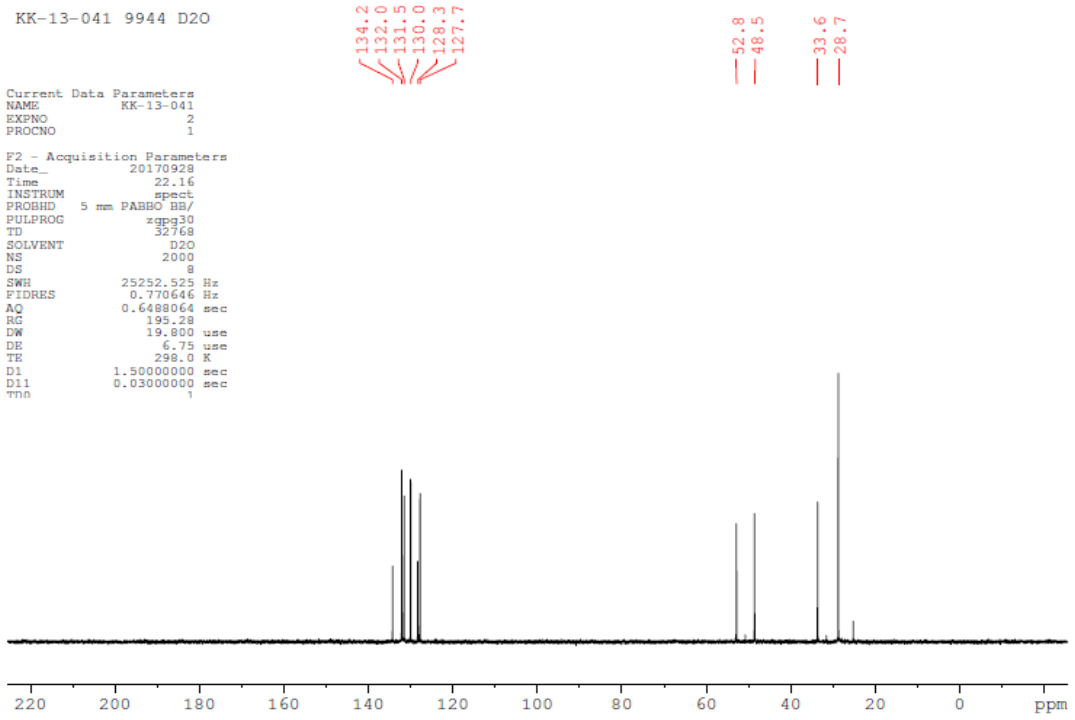

DAD1 H, Sig=250,4 Ref=550,100
mAU
1000
800
600
400
200
0
Tamoxifen
99.37%
11.122
10.436
10.822
11.020
13.427
2
4
6
8
10
12
14
16
18
min

**AY9944, Provided by (The Vanderbilt Synthesis Core, >95%)**

**F.**




**G.**



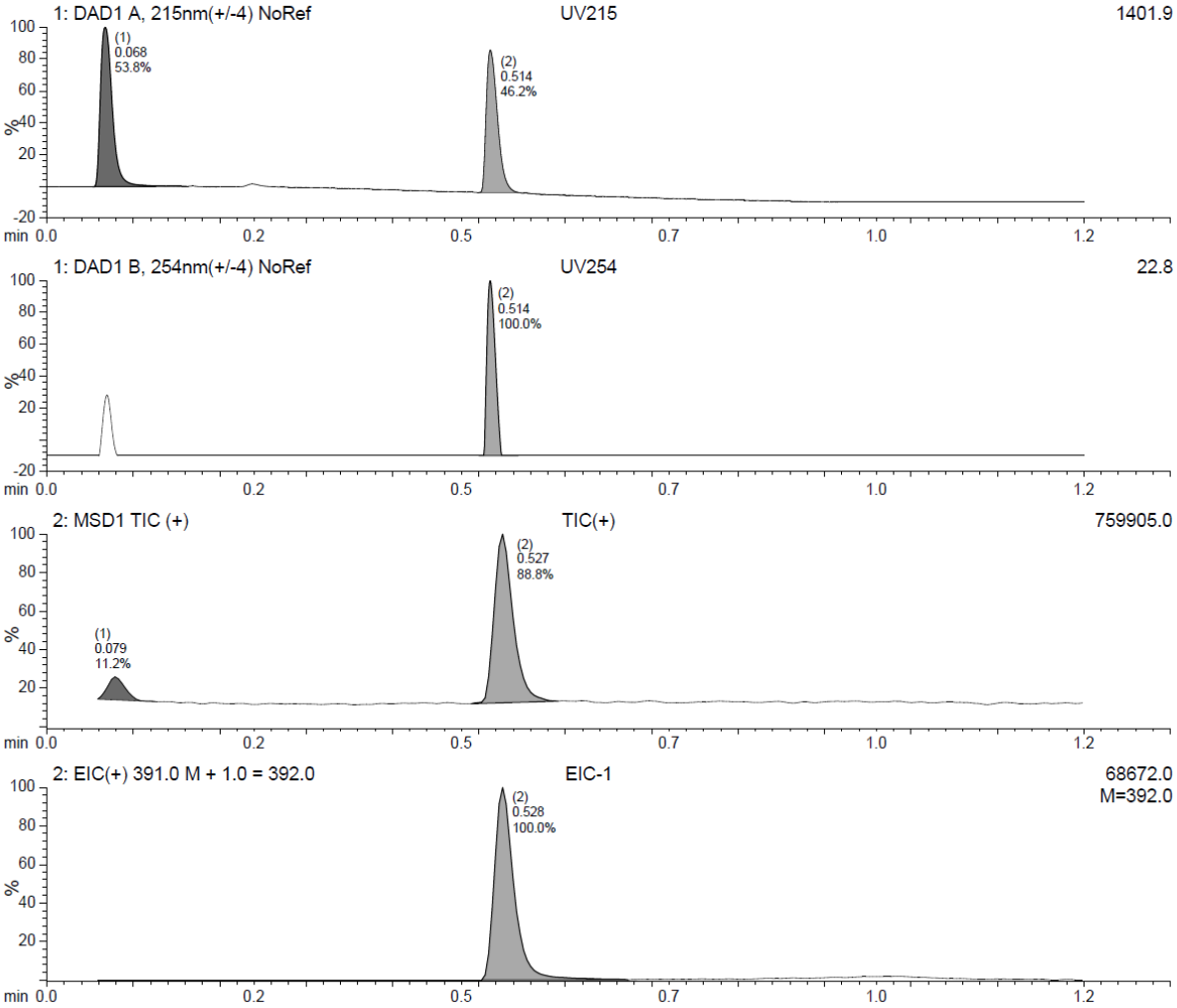
**H.**

**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 |