**Supplementary Information**

**for**

# Synthesis and *In Vitro* Evaluation of Novel Amino-Phenylmethylene-Imidazolone 5-$HT_{2A}$ Receptor Antagonists

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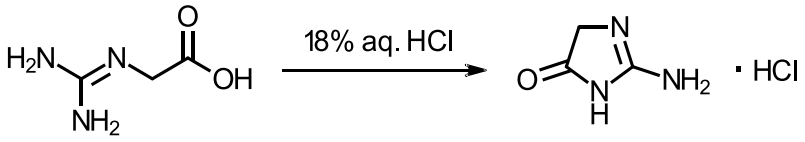
## I. Materials and General Procedures

All chemicals and solvents were analytical or reagent grade and purchased from either Sigma-Aldrich or Fisher Scientific and used as-received without further purification. All manipulations were performed under air in standard laboratory glassware. $^{1}H$ and $^{13}C$ NMR spectra were obtained using a JNM-ECZ400S (400 MHz for $^{1}H$) or Magitrek Spinsolve™ benchtop NMR (43 MHz for $^{1}H$) spectrometer. Chemical shifts are reported in ppm relative to residual nondeuterated solvent. High-resolution mass spectral analysis was performed using a Bruker Maxis 3G Ultra-High Resolution Time of Flight (UHR-TOF) tandem mass spectrometer, and ESI spectra were recorded on an AB Sciex Triple Quad™ 5500 instrument with a capillary voltage of 5.5 kV and an ESI mode positive ion trap detector. All MS data are reported in the form of *m/z*. High-performance liquid chromatography was conducted using an Agilent 1260 HPLC system with a HALO® C18 reverse phase column (2.1 x 50 mm, 2.7 μm); the column temperature was 40 °C, flow rate 1 mL/min, injection volume 0.5 μL, solvent system gradient of 90:10 to 5:95 A:B (A: 0.05% trifluoroacetic acid in water; B: 0.05% trifluoracetic acid in acetonitrile). All compounds were >95% pure by HPLC.

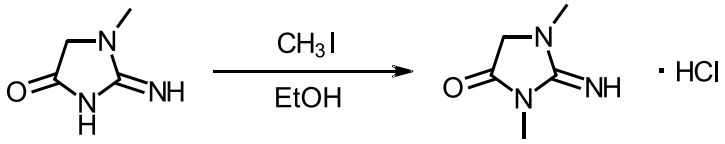
## II. Synthesis and Characterization of APMIs

*Glycocyamidine*



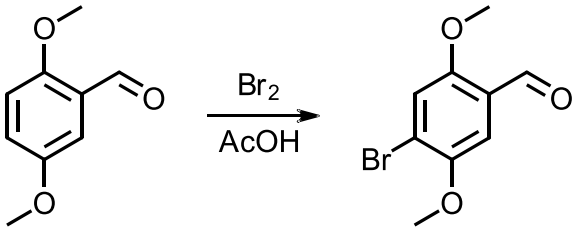

Glycocyamine (4.99 g, 42.6 mmol) was cyclized according to literature procedures[1] to give glycocyamidine hydrochloride (3.62 g, 26.7 mmol, 63% yield). The spectral data agreed with those previously reported in the literature.[1]

*2-Imino-1,3-dimethylimidazolidin-4-one*



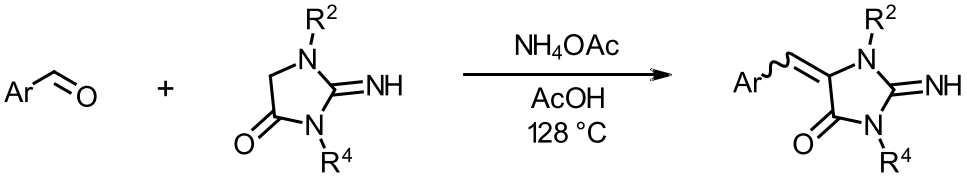

Creatinine (1.28 g, 11.3 mmol) was methylated according to literature procedures[2,3] to give 2-imino-1,3-dimethylimidazolidin-4-one hydrochloride (1.55 g, 6.1 mmol, 54% yield). The spectral data agreed with those previously reported in the literature.[2]

*4-Bromo-2,5-dimethoxybenzaldehyde*




2,5-Dimethoxybenzaldehyde (1.12 g, 6.74 mmol) was brominated according to literature procedures[4] to give 4-bromo-2,5-dimethoxybenzaldehyde (0.73 g, 2.97 mmol, 44% yield). The spectral data agreed with those previously reported in the literature.[4]

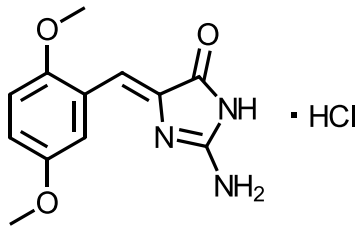
*General synthetic procedure for APMIs*




The aryl aldehyde (1 equiv.), imidalozone (1.1 equiv.), and $NH_4OAc$ (1.1 equiv) were dissolved in glacial acetic acid (5 mL per mmol aldehyde) in a 68 mL round-bottom pressure flask with a

PTFE cap. Upon dissolution, the resulting solutions were generally clear and light yellow in color. The flask was sealed and placed into a 128 °C oil bath. As the reaction proceeded, the solution darkened to a clear, dark orange/brown color. After 12-20 h, the flask was removed from the oil bath and the reaction was allowed to cool to room temperature. Deionized water (10 mL per mmol aldehyde) was added, and the mixture was slowly basified to pH > 8 with saturated aqueous $NaHCO_3$, resulting in the formation of a precipitate. The precipitate was collected by vacuum filtration, washed with excess ice-cold deionized water, and dried with suction to give the crude freebase product, which was typically a fluffy yellow-to-orange solid. The solid was dissolved or suspended in MeOH (5 mL per mmol crude product) and 1.25-1.5 equiv. of HCl was added as a 0.82 M solution of HCl in MeOH. The resulting solution was stirred for 30 min at room temperature. Solvents and volatiles (e.g., excess HCl) were removed by rotary evaporation to yield the crude product as the hydrochloride salt, which was purified by trituration with excess $CH_2Cl_2$ or recrystallization from boiling EtOH/DMF (7:3). Structural characterization of the APMIs was conducted by NMR spectroscopy and UHR-TOF high-resolution mass spectrometry (HRMS). Unfortunately, the solubility of the APMIs in common solvents (e.g., $H_2O$, MeOH, EtOH, iPrOH, acetone, DMSO, THF, EtOAc, $CH_2Cl_2$, $CHCl_3$, and mixtures thereof) was too low to obtain $^{13}C$ NMR spectra of **2b**, **2d**, **3b**, and **3d**.

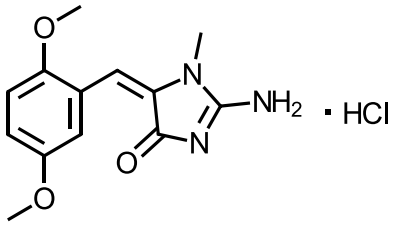
*(Z)-2-amino-4-[(2,5-dimethoxyphenyl)methylene]-2-imidazolin-5-one* (**2a**)




Yield: 85%, pale yellow solid. $^1H$ NMR (400 MHz, DMSO-$d_6$): $\delta$ 9.19 (s, 2H), 7.20 (s, 1H), 7.04 (m, 3H), 6.86 (s, 1H), 3.83 (s, 3H), 3.79 (s, 3H). $^{13}C$ NMR (15 MHz, DMSO-$d_6$): $\delta$ 174.4, 163.4, 153.3, 151.7, 138.9, 125.0, 116.0, 113.8, 112.0, 103.5, 56.3, 55.7.

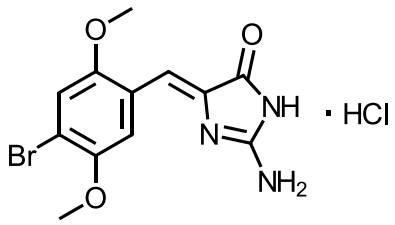
HRMS (ESI-TOF): *m/z* calculated for $C_{12}H_{14}N_3O_3$ $[M+H]^+$ 248.1035; found 248.1033

*(E)-2-amino-5-[(2,5-dimethoxyphenyl)methylene]-1-methyl-2-imidazolin-4-one* (**2b**)



Yield: 60%, orange solid. $^1$H NMR (400 MHz, DMSO-$d_6$): $\delta$ 7.80 (br s, 2H), 6.91 (m, 2H), 6.83 (dd, 1H, $J$ = 9 Hz, 3 Hz), 3.77 (s, 3H), 3.70 (s, 3H), 3.15 (s, 3H). HRMS (ESI-TOF): *m/z* calculated for $C_{13}H_{15}N_3O_3Br$ $[M+H]^+$ 340.0291; found 340.02923.

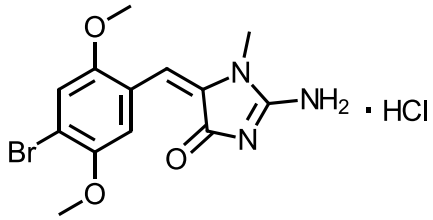
*(Z)-2-amino-4-[(4-bromo-2,5-dimethoxyphenyl)methylene]-2-imidazolin-5-one* (**2c**)




Yield: 71%, yellow solid. $^1$H NMR (400 MHz, DMSO-$d_6$): $\delta$ 9.44 (br s, 1H), 7.36 (s, 1H), 7.27 (s, 1H), 6.80 (s, 1H), 3.94 (s, 3H), 3.86 (s, 3H). $^{13}$C NMR (15 MHz, DMSO-$d_6$): $\delta$ 172.7, 161.1, 151.7, 149.5, 140.6, 124.7, 115.7, 114.5, 110.0, 102.8, 56.8, 56.4.

HRMS (ESI-TOF): *m/z* calculated for $C_{12}H_{13}N_3O_3Br$ $[M+H]^+$ 326.0135; found 326.0137.

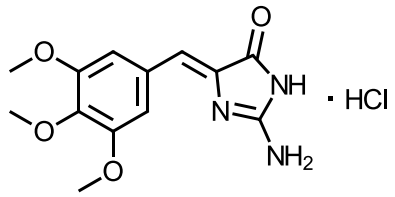
*(E)-2-amino-5-[(4-bromo-2,5-dimethoxyphenyl)methylene]-1-methyl-2-imidazolin-4-one* (**2d**)




Yield: 45%, orange solid. $^1$H NMR (400 MHz, DMSO-$d_6$): $\delta$ 9.59 (br s, 2H), 7.94 (s, 1H), 7.32 (s, 1H), 6.80 (s, 1H), 3.83 (s, 3H), 3.78 (s, 3H).

HRMS (ESI-TOF): *m/z* calculated for $C_{12}H_{13}N_3O_3Br$ $[M+H]^+$ 326.0135; found 326.0137.

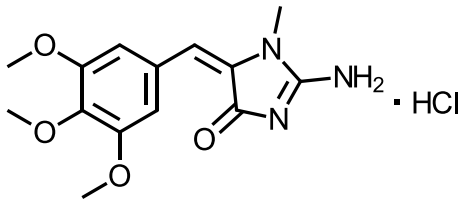
*(Z)-2-amino-4-[(3,4,5-trimethoxyphenyl)methylene]-2-imidazolin-5-one HCl* (**3a**)




Yield: 49%, yellow solid. $^1$H NMR (400 MHz, DMSO-$d_6$): $\delta$ 7.48 (s, 2H), 7.23 (s, 1H), 4.44 (s, 6H), 4.23 (s, 3H). $^{13}$C NMR (15 MHz, DMSO-d$_6$): $\delta$ 173.1, 162.9, 152.2, 139.1, 136.7, 130.4, 109.4, 106.6, 59.49, 55.31.

HRMS (ESI-TOF): *m/z* calculated for $C_{13}H_{16}N_3O_4$ $[M+H]^+$ 278.1141; found 278.1137

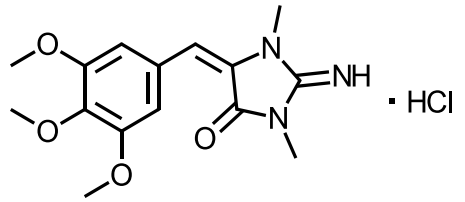
*(E)-2-amino-1-methyl-5-[(3,4,5-trimethoxyphenyl)methylene]-2-imidazolin-4-one* (**3b**)



Yield: 68%, orange solid. $^1$H NMR (400 MHz, DMSO-$d_6$): $\delta$ 8.11 (s, 2H), 7.41 (s, 1H), 4.30 (s, 6H), 4.23 (s, 3H), 4.00 (s, 3H).

HRMS (ESI-TOF): *m/z* calculated for $C_{14}H_{18}N_3O_4$ $[M+H]^+$ 292.1292; found 292.1292

*(E)-2-imino-1,3-dimethyl-5-[(3,4,5-trimethoxyphenyl)methylene]imidazolidin-4-one* (**3c**)

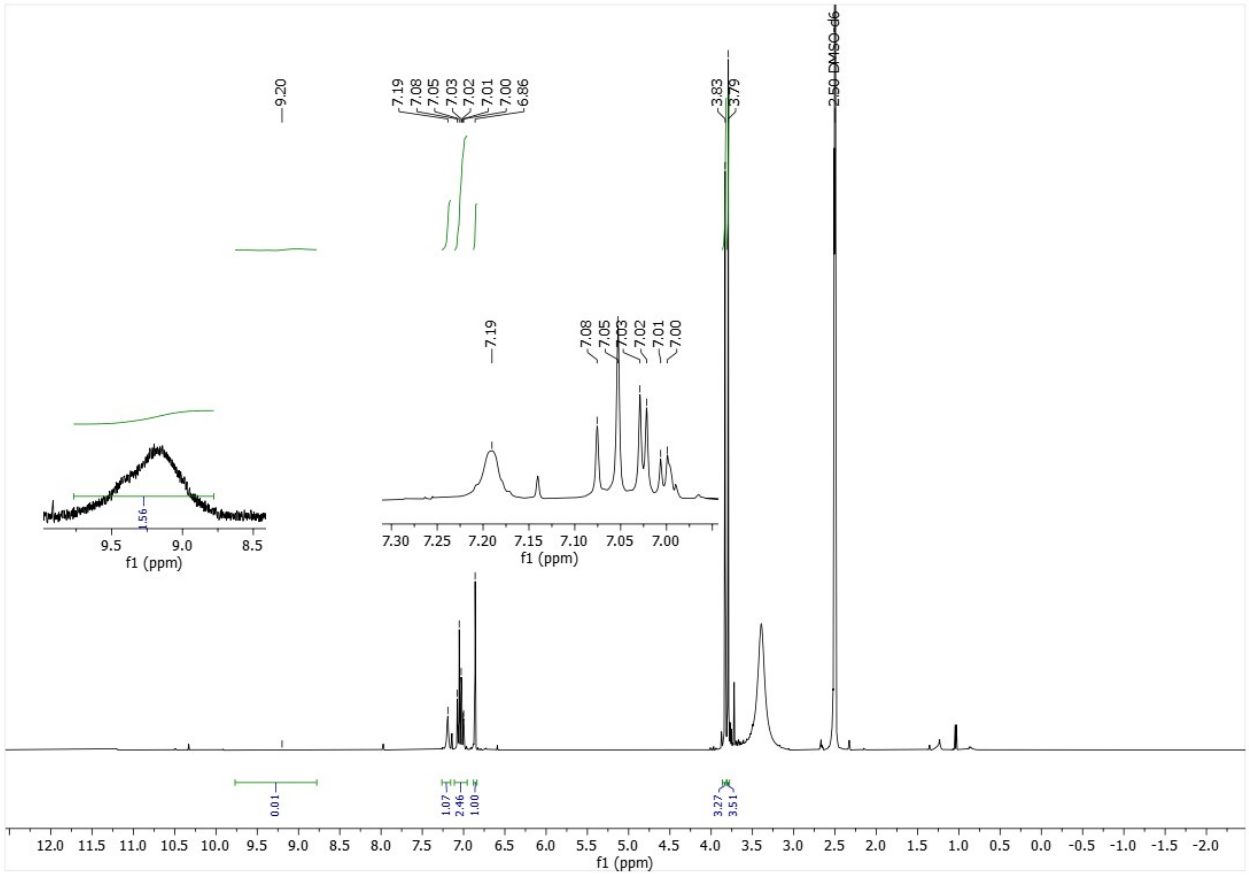


Yield: 83%, yellow solid. $^1$H NMR (43 MHz, $D_2O$): $\delta$ 7.43 (s, 2H), 6.82 (s, 1H), 3.91 (s, 6H), 3.86 (s, 3H), 3.38 (s, 3H), 3.27 (s, 3H).

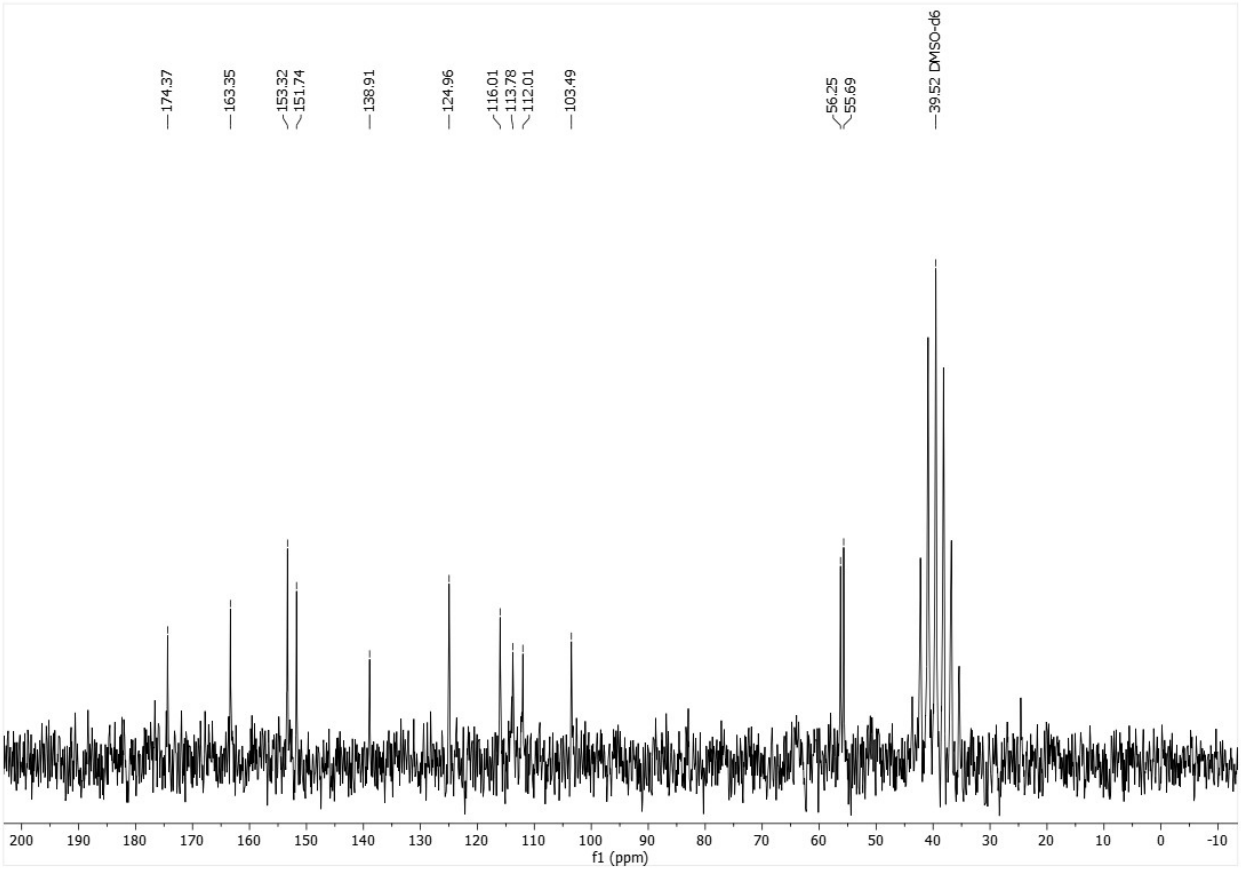
HRMS (ESI-TOF): *m/z* calculated for $C_{15}H_{20}N_3O_4$ $[M+H]^+$ 306.1448; found 306.1445.

## III. Characterization Data for Key Compounds

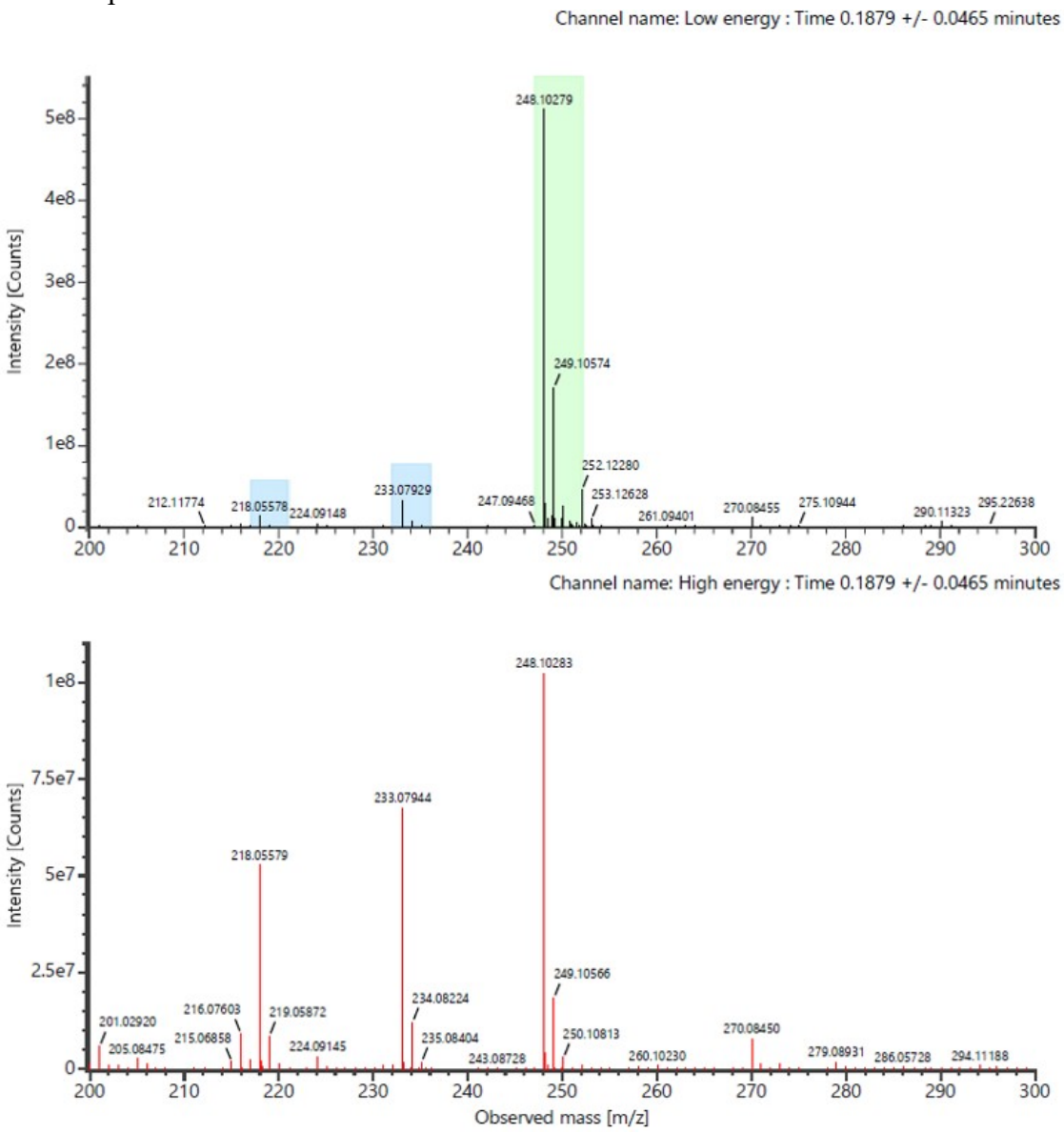
$^1$H NMR spectrum of **2a** in DMSO-$d_6$



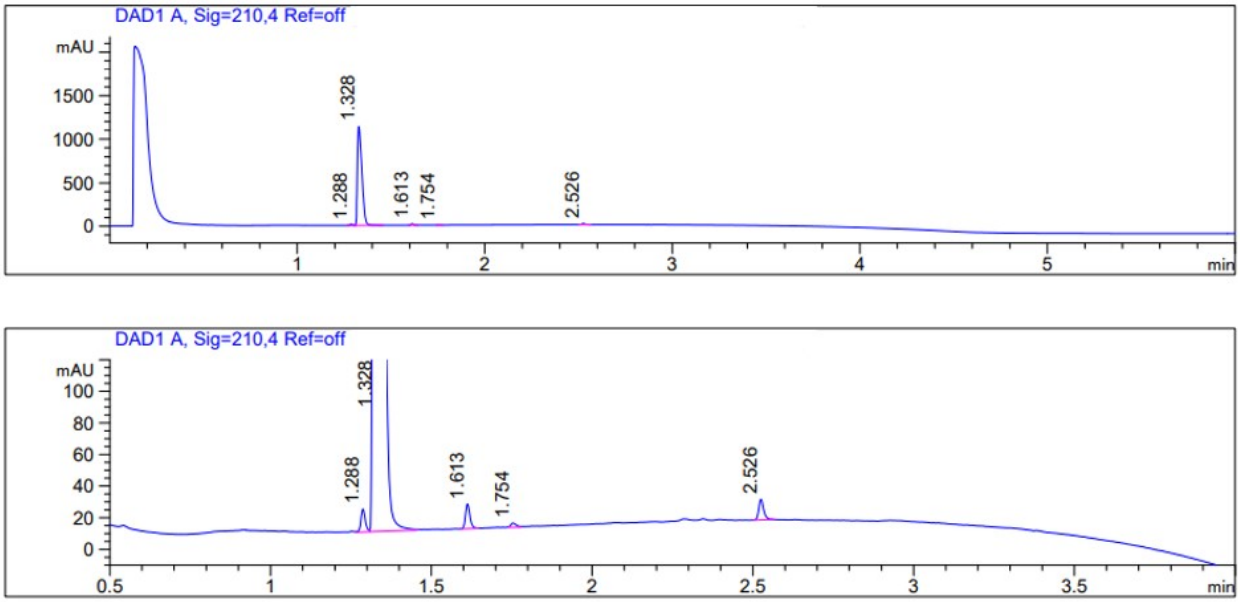
$^{13}$C NMR spectrum of **2a** in DMSO-$d_6$



HRMS Spectra for **2a**



HPLC Chromatogram of **2a** (purity: 97.6%; $t_R$ = 1.328 min)

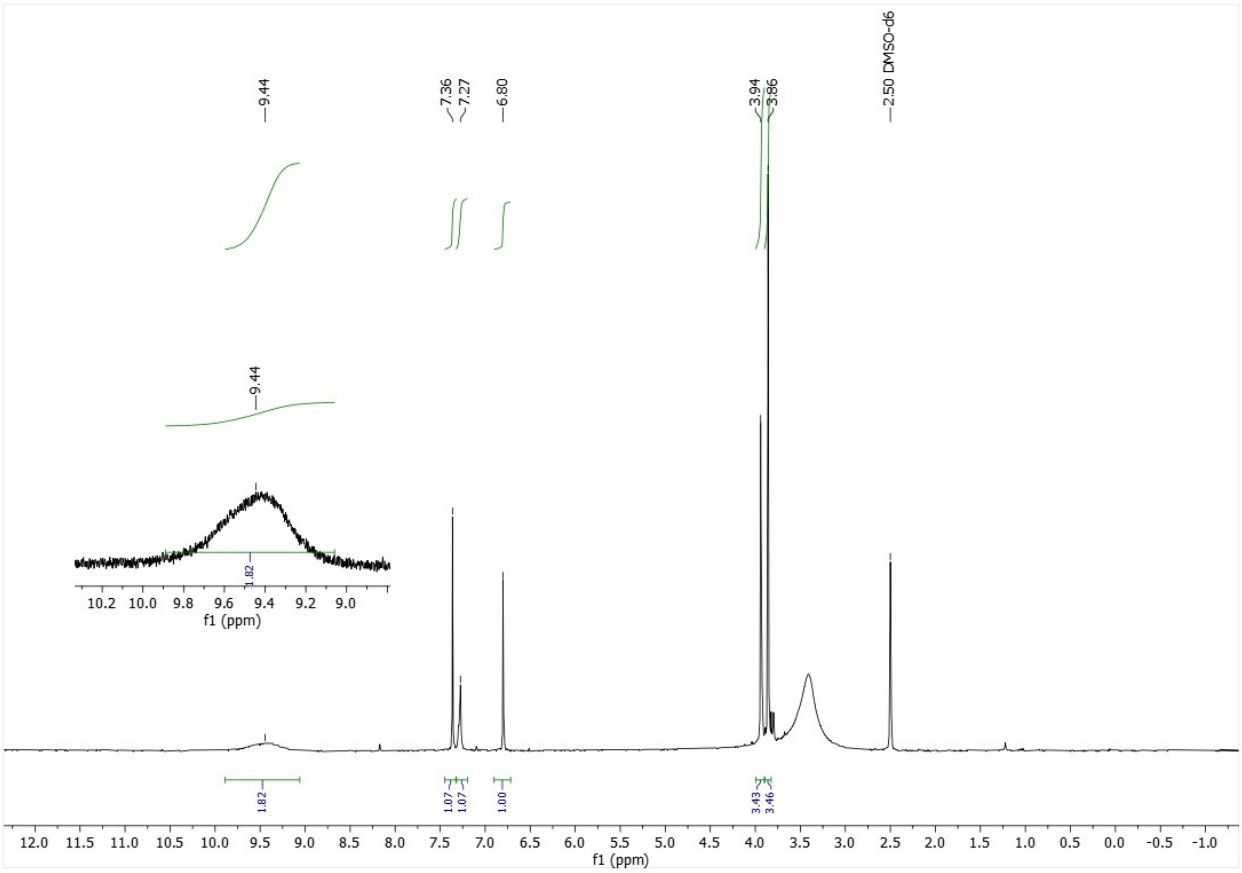




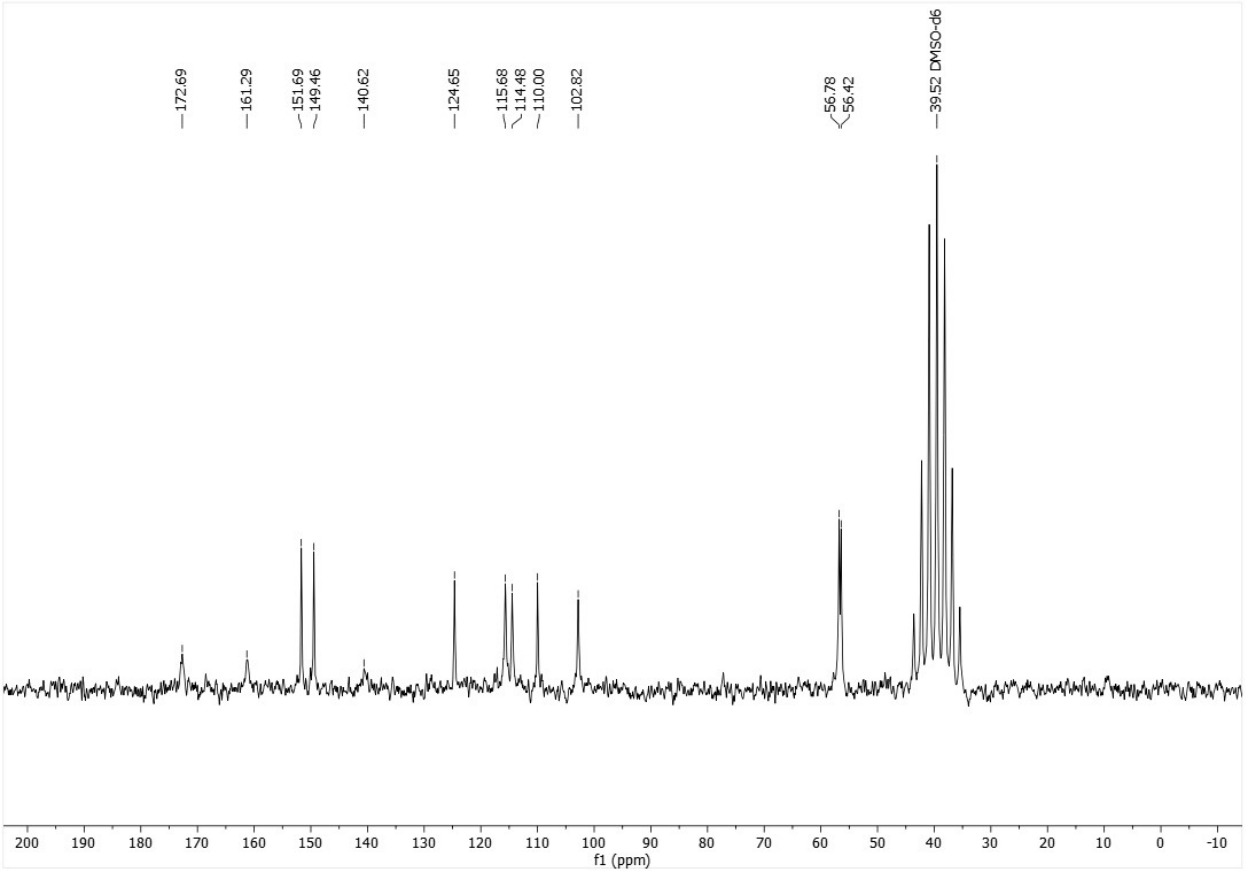


| # | RT | Symmetry | Width | Height | Height % | Area | Area % |
|---|---|---|---|---|---|---|---|
| 1 | 1.29 | 0.736 | 0.016 | 14.59 | 1.24 | 13.6261 | 0.7040 |
| 2 | 1.33 | 0.468 | 0.028 | 1132.78 | 96.08 | 1889.6178 | 97.6266 |
| 3 | 1.61 | 0.713 | 0.016 | 15.80 | 1.34 | 15.2396 | 0.7874 |
| 4 | 1.75 | 0.652 | 0.021 | 2.70 | 0.23 | 3.3301 | 0.1720 |
| 5 | 2.53 | 0.775 | 0.017 | 13.11 | 1.11 | 13.7428 | 0.7100 |

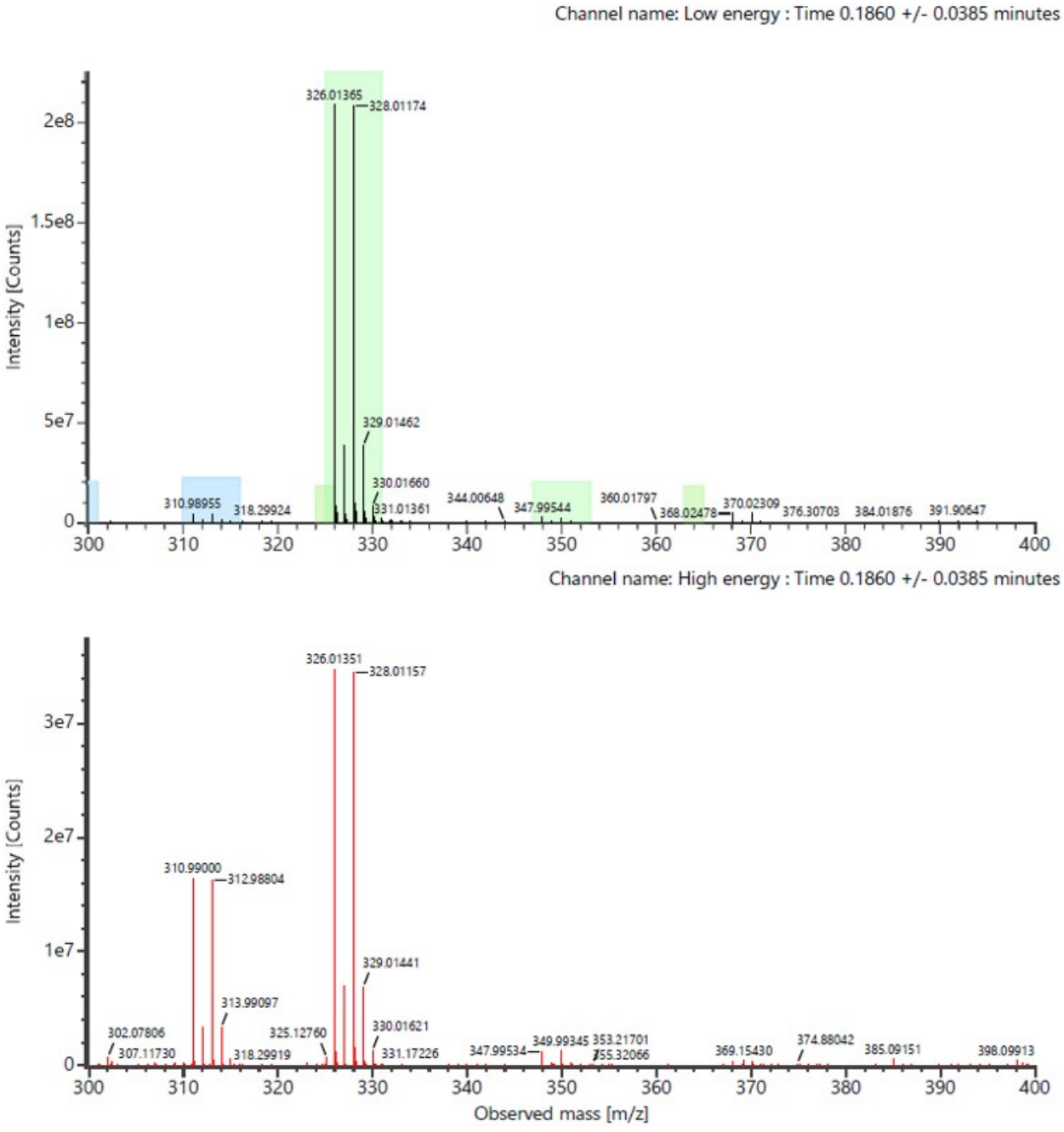
$^{1}$H NMR spectrum of **2c** in DMSO-$d_6$



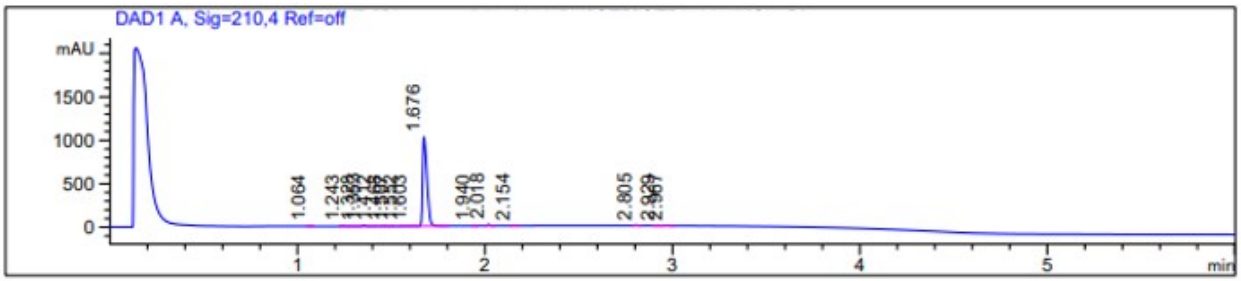
$^{13}$C NMR spectrum of **2c** in DMSO-$d_6$

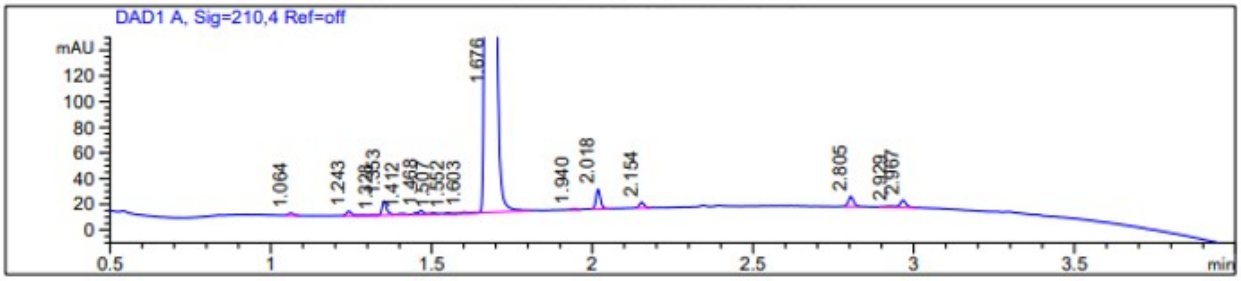


HRMS Spectra for **2c**



HPLC Chromatogram of **2c** (purity: 95.8%; $t_R$ = 1.676 min)

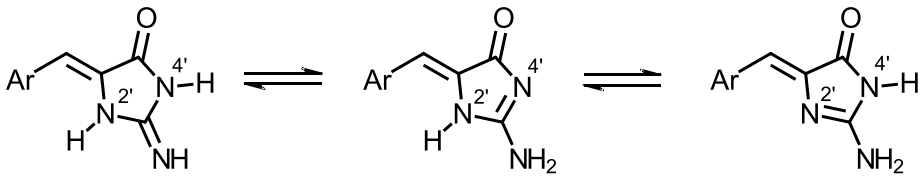



| # | RT | Symmetry | Width | Height | Height % | Area | Area % |
|---|---|---|---|---|---|---|---|
| 1 | 1.06 | 0.795 | 0.020 | 1.76 | 0.16 | 2.1393 | 0.1248 |
| 2 | 1.24 | 0.542 | 0.023 | 3.74 | 0.34 | 5.2060 | 0.3038 |
| 3 | 1.33 | 6226.791 | 0.025 | 0.48 | 0.04 | 0.7073 | 0.0413 |
| 4 | 1.35 | 0.778 | 0.017 | 11.29 | 1.04 | 11.7810 | 0.6874 |
| 5 | 1.41 | 1.356 | 0.027 | 1.21 | 0.11 | 1.9815 | 0.1156 |
| 6 | 1.47 | 1.532 | 0.023 | 3.25 | 0.30 | 4.4893 | 0.2620 |
| 7 | 1.51 | 0.717 | 0.018 | 1.26 | 0.12 | 1.3315 | 0.0777 |
| 8 | 1.55 | 0.497 | 0.027 | 1.21 | 0.11 | 1.9646 | 0.1146 |
| 9 | 1.60 | 0.436 | 0.035 | 1.27 | 0.12 | 2.7055 | 0.1579 |
| 10 | 1.68 | 0.461 | 0.027 | 1025.87 | 94.39 | 1642.1398 | 95.8208 |
| 11 | 1.94 | 0.434 | 0.022 | 0.96 | 0.09 | 1.2958 | 0.0756 |
| 12 | 2.02 | 0.795 | 0.016 | 15.61 | 1.44 | 15.0299 | 0.8770 |
| 13 | 2.15 | 0.905 | 0.018 | 4.55 | 0.42 | 4.8228 | 0.2814 |
| 14 | 2.81 | 0.894 | 0.019 | 7.87 | 0.72 | 8.8412 | 0.5159 |
| 15 | 2.93 | 1.136 | 0.033 | 0.82 | 0.08 | 1.6368 | 0.0955 |
| 16 | 2.97 | 0.820 | 0.022 | 5.71 | 0.53 | 7.6889 | 0.4487 |

## IV. Assignments of Tautomeric Form and Tentative Assignments of *E*/*Z* Geometries

There are three possible tautomeric forms of **2a**, **2c**, and **3a**:



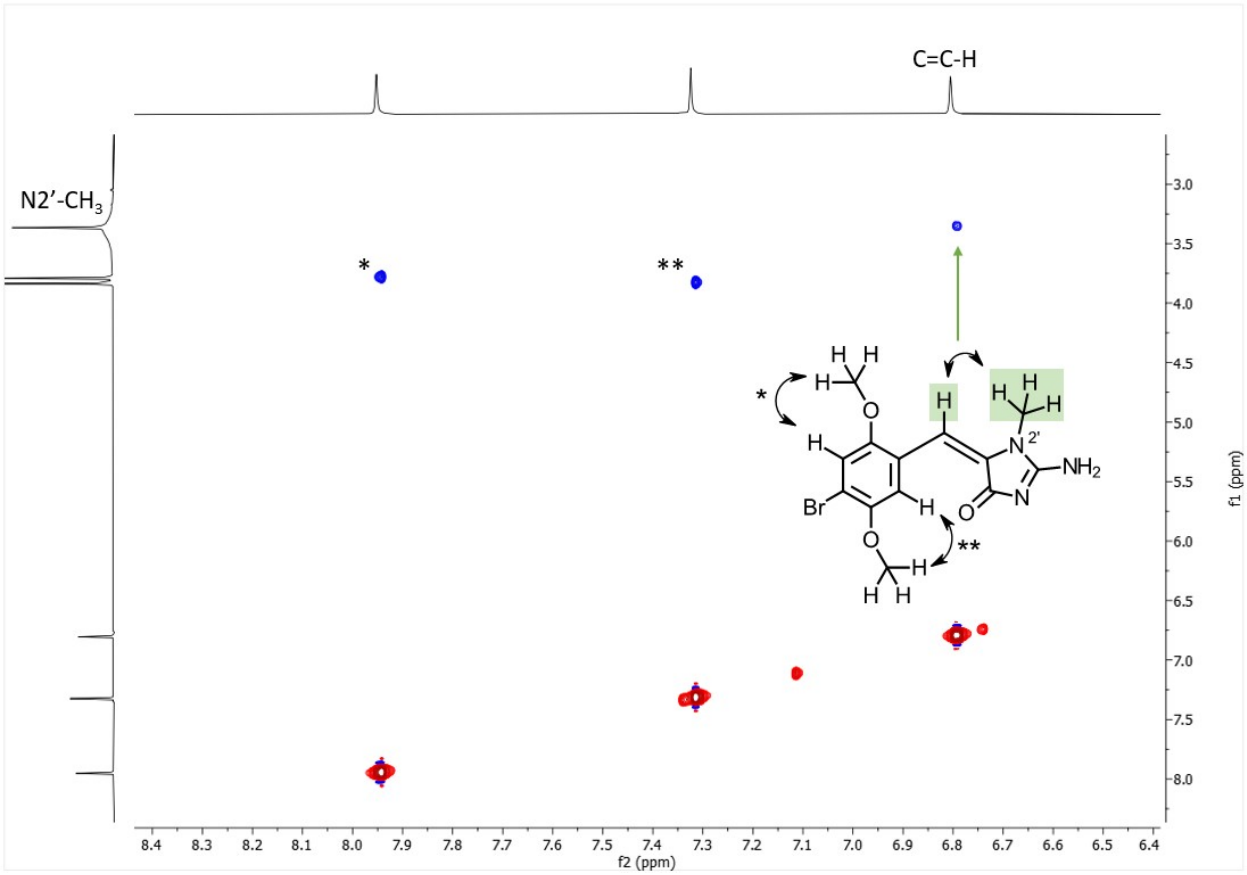

*exo* **(A)** *endo* **(B)** *endo* **(C)**

Early investigators of acylguanidine chemistry concluded that in glycocyamidine, *endo* tautomer **B** is preferred because of conjugation of the endocyclic C=N with the carbonyl.[2] However, in **2a**, **2c**, and **3a**, both *endo* tautomers are fully conjugated in an extended π-system. Another possible confounding effect is potential steric clash between the N2'–H and the aryl 6–H in tautomer **B**. For consistency, **2a**, **2c**, and **3a** are drawn herein as *endo* tautomer **C**, but this assignment is tentative.

In principle, APMIs can also exist in two geometrically isomeric forms (*E* and *Z*) based on the stereochemistry of the double bond linking the aryl and imidazolone moieties. In aplysinopsin-type compounds, the presence or absence of an N2' substituent is the dominant factor that determines the stereochemical outcomes of the reaction of the aryl aldehyde with the imidazolone.[5] For example, Guella and coworkers' crystallographic and NMR studies concluded that in N2'-methylated aplysinopsins, significant steric repulsion between the N2' methyl and the indole core in the rate-limiting transition state of condensation/elimination leads to the selective formation of the *E* isomer (*E*:*Z* ratio > 95:5).[6] Similarly, in aplysinopsins lacking a N2' methyl (i.e., in which N2' is unsubstituted), steric repulsions between the imidazolone C=O and the indole core lead to the selective formation of the *Z* isomer (*Z*:*E* ratio > 95:5).

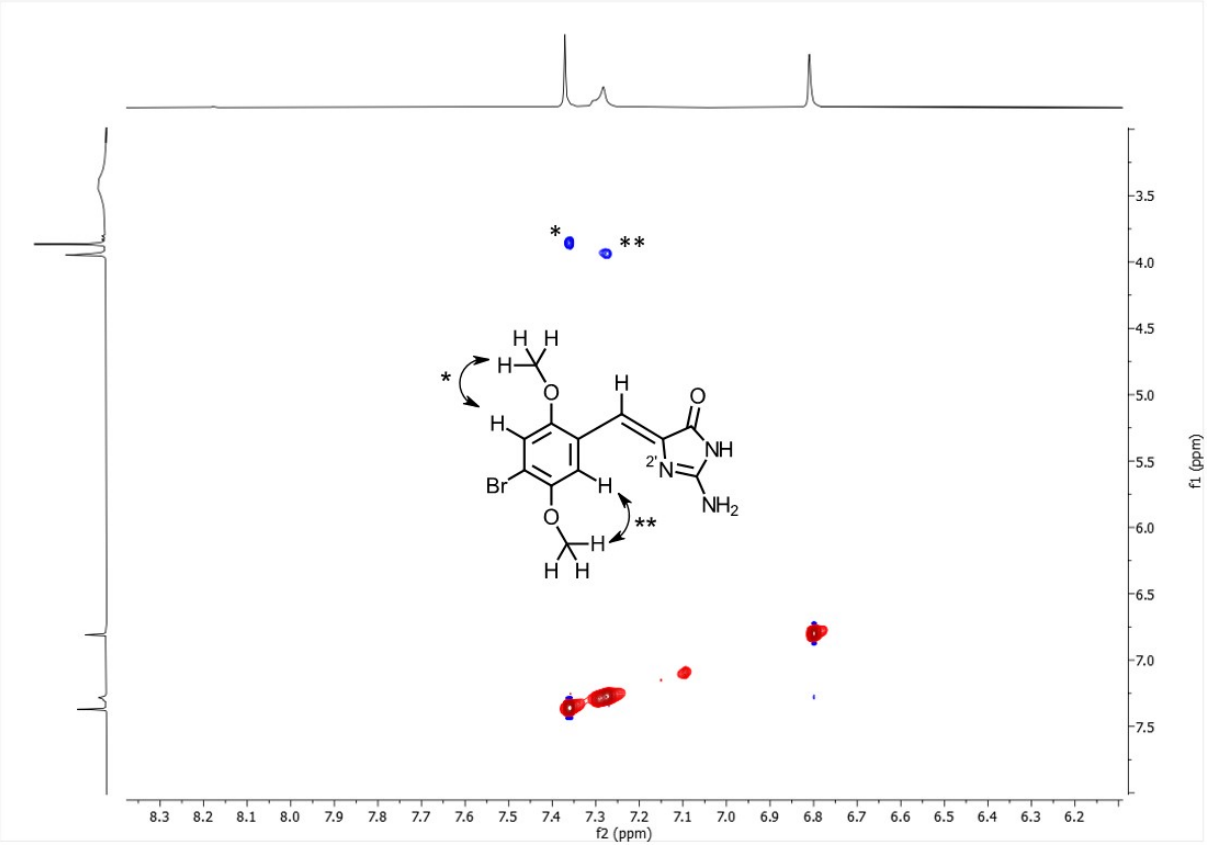
It was hypothesized that the compounds herein would follow a similar trend, given that for each compound, the available spectroscopic and chromatographic data only indicated the presence of a single isomer. Trends in *E*/*Z* geometry were investigated and tentatively assigned by 2D Nuclear Overhauser Effect Spectroscopy (NOESY). Below is a representative NOESY spectrum of **2d** in DMSO-$d_6$, which shows an NOE correlation between the methylidene proton and the N2' methyl, suggesting that this compound has *E* geometry.

*NOESY spectrum (400 MHz) of **2d** in DMSO-*$d_6$

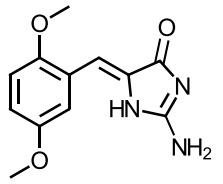



As expected, the NOESY spectra for compounds that are unsubstituted at N2' (i.e., **2a**, **2c**, and **3a**) do not show any NOE correlations between the methylidene proton and any other peaks. Below is a representative NOESY spectrum of **2c** in DMSO-$d_6$, which shows no NOE correlations between the methylidene proton and any other signals.

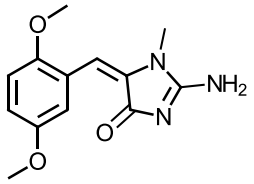
*NOESY spectrum (400 MHz) of **2c** in DMSO-*$d_6$



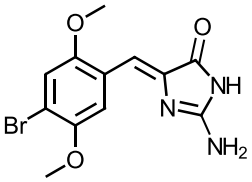

Therefore, NOESY could not be used for assigning the geometry of these compounds. Furthermore, it must be emphasized that any geometric assignment based on NOESY must be considered tentative until confirmed by crystallography (e.g., single-crystal XRD). Based on the results of the NOESY experiments for the N2'-methylated compounds, and the agreement of these results with the reported trends of *E*/*Z* geometry in aplysinopsin-type compounds, the geometries of the compounds were tentatively assigned as follows:



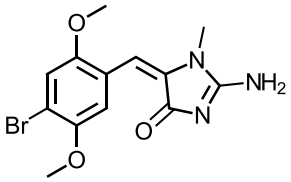
(*E*)-2a



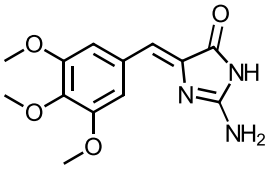
(*Z*)-2b



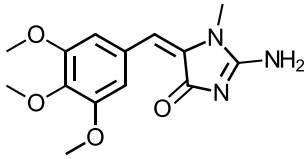
(*E*)-2c



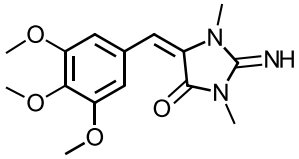
(*Z*)-2d



(*E*)-3a



(*Z*)-3b



(*Z*)-3c

## V. Kinetic Solubility Assays

The kinetic solubility assays were performed by Pharmaron, Inc. (Beijing, China) according to their standard procedures.

*Solution Preparation:*

Stock solutions of test compounds were prepared by dissolving each test compound in DMSO (10 mM).

Standard solutions were prepared by first diluting the 10 mM stock solutions prepared as described above to a final concentration of 300 μM in DMSO. 10 μL aliquots of each of the diluted stock solutions were transferred into vials, then mixed with 980 μL of methanol and 10 μL of pH 7.4 PBS buffer solution to obtain standard solutions with a final concentration of 3 μM.

*Assay Procedure:*

A stock solution of the positive control compound (progesterone) was prepared in DMSO (30 mM). Duplicate 30 μL aliquots of each stock solution were transferred into vials, then 970 μL of pH 7.4 PBS buffer solution was added to each vial. A stir stick was added to each vial and then the vials were sealed using a molded PTDE/SIL 96-Well Plate Cover. The plate was moved to a Thermomixer plate shaker and incubated at room temperature for 2 hours with shaking at 1100 rpm. After 2 hours incubation, the stir sticks were removed using a magnet and all samples were vacuum filtered to remove any insoluble compound. 10 μL aliquots of each of the filtered samples were diluted with 980 μL of methanol and 10 μL of DMSO.

*Data Analysis:*

The filtered solutions were analyzed and quantified against the standard solutions using liquid chromatography-tandem mass spectrometry (LC-MS/MS) operating in multiple reaction monitoring (MRM) mode using a Shizmadu LC-30AD system coupled to a AB Sciex Triple Quad™ 5500 instrument with a capillary voltage of 5.5 kV and an ESI mode positive ion trap detector. The column was an XSelect Hss T3 2.5μM (2.1 × 30 mm) XP column coupled with a preguard column. The chromatography conditions and MS parameters were:

- Mobile phase: 0.1% formic acid in water (A) and 0.1% formic acid in acetonitrile (B)

| Time (min) | 0 | 0.5 | 0.8 | 0.81 | 1.0 |
|---|---|---|---|---|---|
| %B | 5 | 100 | 100 | 5 | 5 |

- Injection volume: 20 μL
- Flow rate: 1.00 mL/min
- Column temperature: 40 °C
- MS parameters:

- Ion source: Turbo spray
- Ionization model: ESI
- Scan type: MRM
- Collision gas: 6 L/min
- Curtain gas: 30 L/min
- Nebulize gas: 50 L/min
- Auxiliary gas: 50 L/min
- Temperature: 500 °C
- Ionspray voltage: +5500 V (positive MRM)

Concentrations of the test compounds were calculated according to the following equation:

$$[test] = \frac{AREA_{test} \times INJVOL_{STD} \times DF_{test} \times [STD]}{AREA_{STD} \times INJVOL_{test}}$$

(DF = Dilution Factor)

*Results:*

**Table S1. Kinetic solubility values for control and test compounds**

| ***Compound*** | ***Solubility (μM) in pH 7.4 PBS*** |
|---|---|
| *Progesterone (control)* | *18.58* |
| **2a** | >300* |
| **2b** | >300* |
| **2c** | 20.17 |
| **2d** | 56.03 |
| **3a** | 290.89 |
| **3b** | >300* |
| **3c** | >300* |

*The upper limit of the assay was 300 μM.

## VI. *In Vitro* PAMPA-BBB Permeability Assays

The PAMPA-BBB assays were performed by Pharmaron, Inc. (Beijing, China) according to their standard procedures.

*Solution Preparation:*

Stock solutions of controls (testosterone and methotrexate) and test compounds were prepared by dissolving the compounds in DMSO at a concentration of 10 mM, then diluting with pH 7.4 PBS buffer solution to a final concentration of 10 μM.

*Assay Procedure:*

A 2% (w/v) solution of Porcine brain polar lipid extract was prepared in dodecane solvent, with sonication to ensure complete dissolution. 5 μL of this solution was pipetted into the top compartment of each acceptor plate well. Immediately after application of the artificial membrane (i.e., within 10 min), 300 μL of pH 7.4 PBS solution was added to each well of the acceptor plate. Then, 300 μL of the test compound solutions was added to the bottom compartment of each well of the donor plate. The assay was conducted in triplicate. The acceptor plate was placed into the donor plate, ensuring good contact between the underside of the membrane and the test compound solutions in all wells. The plate lid was installed and the plate was incubated at 25 °C for 16 h. After incubation, 50 μL aliquots from each well of the acceptor and donor plates were transferred into a 96-well plate. 200 μL of methanol (containing100 nM alprazolam, 200nM caffeine, and 200 nM diclofenac as internal standards) was added into each well. The plate lid was reinstalled and the plate was vortexed at 750 rpm for 100 s. Each sample was then centrifuged at 3,220 g for 20 min.

$$Log\ P_e = Log\left\{ C \times \left[ -ln\left(1 - \frac{[drug]_{acceptor}}{[drug]_{equilibrium}}\right)\right]\right\}$$

*Data Analysis:*

The concentrations of compounds were determined by LC-MS/MS as described above. The effective permeabilities ($P_e$; cm/s) were calculated according to the following equation:

where:

$C = V_D \times V_A / [(V_D + V_A) \times t \times A]$

$V_D$ = volume of donor compartment (0.30 mL)

$V_A$ = volume of acceptor compartment (0.30 mL)

A = filter area (0.24 $cm^2$ for Multi-Screen Permeability Filter plate)

*Results:*

**Table S2. Permeability results for controls and test compounds**

| Compound | $P_e$ (cm $s^{-1}$) | -Log Pe | Recovery (%) |
|---|---|---|---|
| *Methotrexate (control)* | *2.19 x 10$^{-9}$* | *>8.66* | *>98.26* |
| *Testosterone (control)* | *2.57 x 10$^{-5}$* | *4.59* | *75.18* |
| **2a** | 1.10 x 10$^{-6}$ | 5.96 | 96.47 |
| **2b** | 2.29 x 10$^{-6}$ | 5.64 | 104.82 |
| **2c** | 6.03 x 10$^{-6}$ | 5.22 | 70.24 |
| **2d** | 9.33 x 10$^{-6}$ | 5.03 | 97.60 |
| **3a** | 1.86 x 10$^{-7}$ | 6.73 | 100.28 |
| **3b** | 5.62 x 10$^{-7}$ | 6.25 | 100.81 |
| **3c** | 3.09 x 10$^{-5}$ | 4.51 | 89.37 |

## VII. *In Vitro* Radioligand Binding Affinity Assays

The radioligand binding affinity assays were performed by Wuxi Apptec (Shanghai, China) according to their standard procedures.

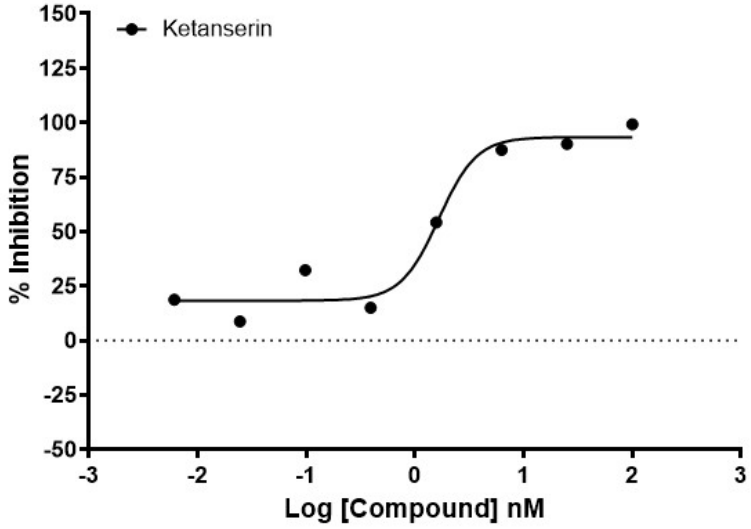
Using a multi-channel pipette, 1 μL of serial diluted reference compound (ketanserin) and test compounds was transferred to assay plates. Then 1 μL of the nonspecific binding compounds was transferred to the assay plate, according to the plate map, for nonspecific binding. Next, 100 μL of membrane stocks were distributed into the plate. To this, 100 μL of [$^3$H]ketanserin was added. The plates were sealed and shaken at 300 rpm. The Unifilter-96 GF/C filter plates were soaked with 50 μL of 0.3% PEI per well for at least 0.5 h at room temperature. When the binding assays completed, the reaction mixture was filtered through GF/B plates using Perkin Elmer Filtermate Harvester, and then each plate was washed 4 times with 250 μL cold wash buffer. The filter plates were dried for 1 h at 50 °C. After drying, the bottom of the filter plate wells was sealed using Perkin Elmer Unifilter-96 backing seal tape. 50 μL of Perkin Elmer Microscint 20 cocktail was added. The top of filter plates was sealed with Perkin Elmer TopSeal-A sealing film. $^3$H trapped on the filter was counted using a Perkin Elmer MicroBeta2 Reader. Data were analyzed with GraphPad Prism 5.0 (GraphPad software).

*Results:*

**Table S3. Radioligand binding assay results for control and test compounds**

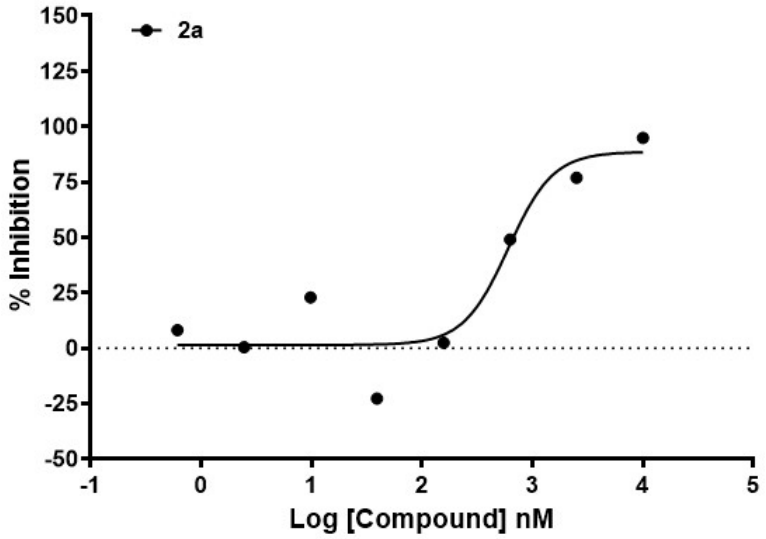
| Compound | $IC_{50}$ (nM) | $K_i$ (nM) | Max Dose (nM) | %Inh @ MaxDose |
|---|---|---|---|---|
| *Ketanserin (control)* | *1.65* | *0.64* | *100* | *99.23* |
| **2a** | 601.7 | 233.33 | 10000 | 94.94 |
| **2b** | ＞10000 | ＞3877.8 | 10000 | 31.66 |
| **2c** | 48.03 | 18.63 | 10000 | 97.12 |
| **2d** | ＞10000 | ＞3877.8 | 10000 | 19.13 |
| **3a** | ＞10000 | ＞3877.8 | 10000 | 6.61 |
| **3b** | ＞10000 | ＞3877.8 | 10000 | -6.54 |
| **3c** | ＞10000 | ＞3877.8 | 10000 | -0.35 |

Dose-response curves and non-linear fit parameters for ketaneserin (control):



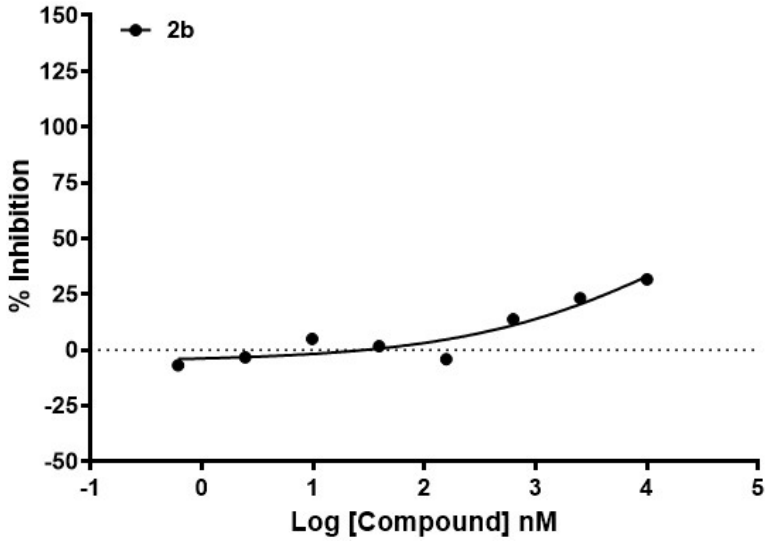

| log(inhibitor) vs. response -- Variable slope | |
|---|---|
| Best-fit values | |
| Bottom | 18.34 |
| Top | 93.35 |
| LogIC50 | 0.2175 |
| HillSlope | 2.499 |
| IC50 | 1.650 |

Dose-response curves and non-linear fit parameters for **2a**:



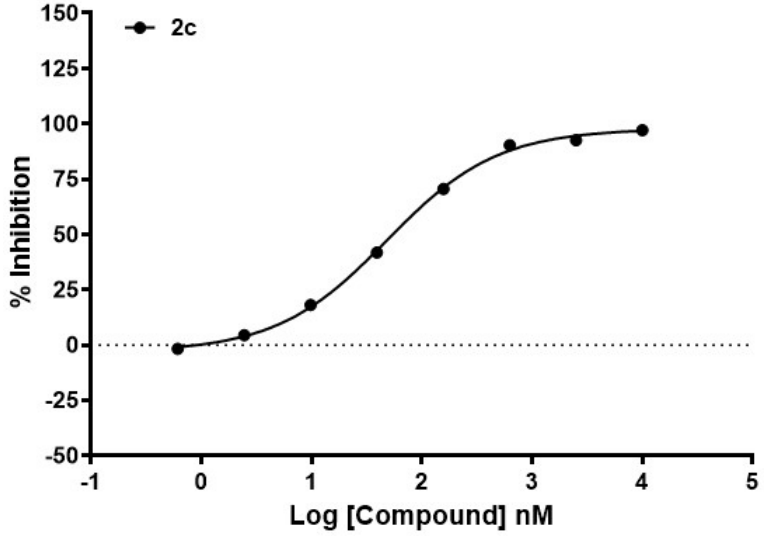

| log(inhibitor) vs. response -- Variable slope | |
|---|---|
| Best-fit values | |
| Bottom | 1.468 |
| Top | 88.59 |
| LogIC50 | 2.779 |
| HillSlope | 2.146 |
| IC50 | 601.7 |

Dose-response curves and non-linear fit parameters for **2b**:



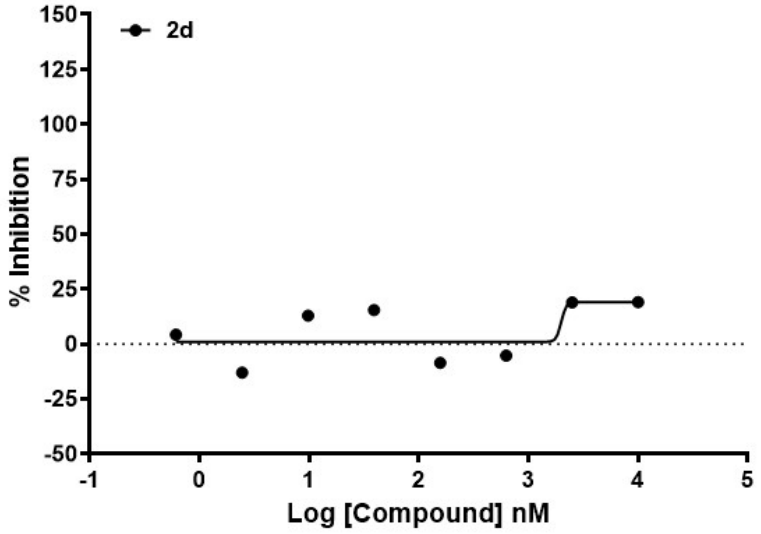

| log(inhibitor) vs. response -- Variable slope | |
|---|---|
| Best-fit values | |
| Bottom | -5.091 |
| Top | = 100.0 |
| LogIC50 | 4.590 |
| HillSlope | 0.4122 |
| IC50 | 38926 |

Dose-response curves and non-linear fit parameters for **2c**:



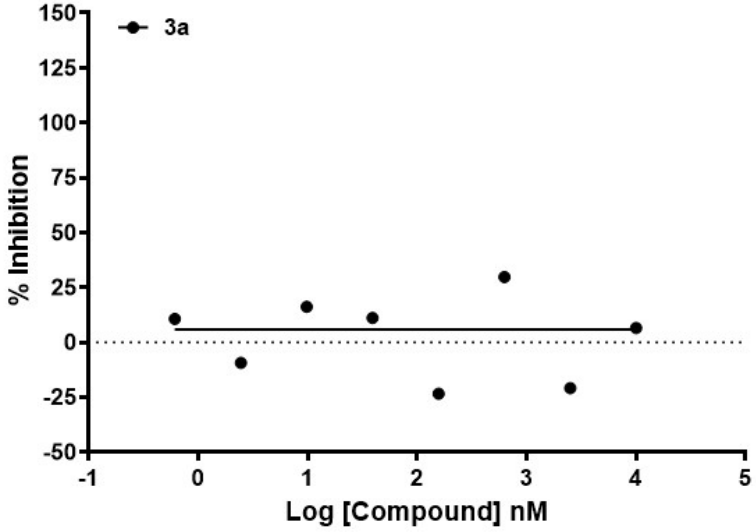

| log(inhibitor) vs. response -- Variable slope | |
|---|---|
| Best-fit values | |
| Bottom | -3.150 |
| Top | 97.78 |
| LogIC50 | 1.681 |
| HillSlope | 0.8656 |
| IC50 | 48.03 |

Dose-response curves and non-linear fit parameters for **2d**:



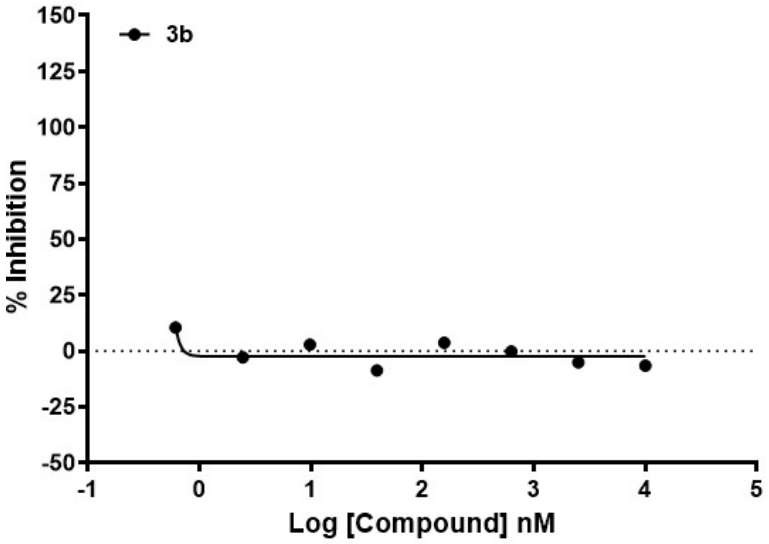

| log(inhibitor) vs. response -- Variable slope | Ambiguous |
|---|---|
| Best-fit values | |
| Bottom | 0.9592 |
| Top | 19.13 |
| LogIC50 | ~ 3.298 |
| HillSlope | ~ 19.53 |
| IC50 | ~ 1988 |

Dose-response curves and non-linear fit parameters for **3a**:



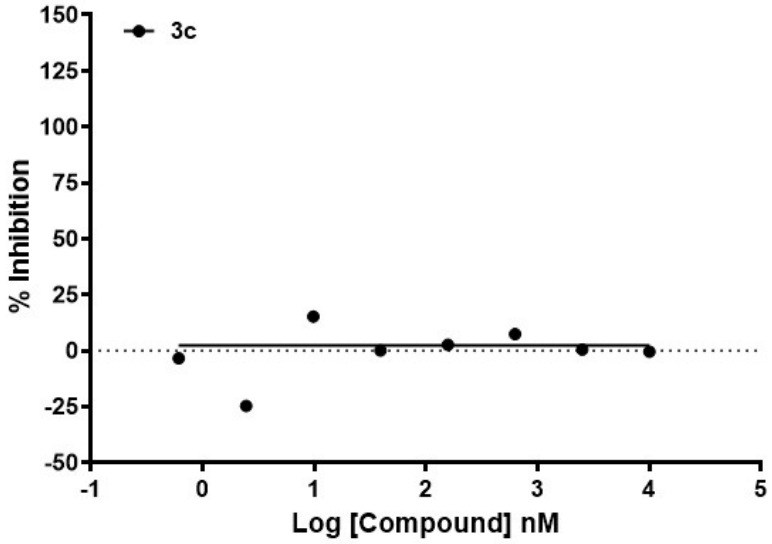

| log(inhibitor) vs. response -- Variable slope | Ambiguous |
|---|---|
| Best-fit values | |
| Bottom | 5.829 |
| Top | ~ 1.265 |
| LogIC50 | ~ 3.949e+023 |
| HillSlope | ~ 5.999e+026 |
| IC50 | ~ +infinity |

Dose-response curves and non-linear fit parameters for **3b**:




| log(inhibitor) vs. response -- Variable slope | Ambiguous |
|---|---|
| Best-fit values | |
| Bottom | -2.389 |
| Top | ~ 186604 |
| LogIC50 | ~ -0.6384 |
| HillSlope | ~ -9.813 |
| IC50 | ~ 0.2299 |

Dose-response curves and non-linear fit parameters for **3c**:




| log(inhibitor) vs. response -- Variable slope | Ambiguous |
|---|---|
| Best-fit values | |
| Bottom | ~ -7.357 |
| Top | 2.510 |
| LogIC50 | ~ -4.675e+052 |
| HillSlope | ~ 7.099e+055 |
| IC50 | ~ 0.000 |

## VIII. *In Vitro* IP-One Assays

Assessment of 5-$HT_{2A}$ $G_{\alpha q}$ signaling was conducted by WuXi Apptec (Shanghai, China) using the homogeneous time-resolved fluorescence-based IP-One immunoassay kit (Cisbio Bioassays, Codolet, France) in accordance with their standard procedures and the kit manufacturer's instructions.

Cells were plated onto 384-well plates (7,500 cells per well) and treated with varying doses of test compounds or reference compounds (serotonin for the agonist assays; methiothepin mesylate salt for the antagonist assays).

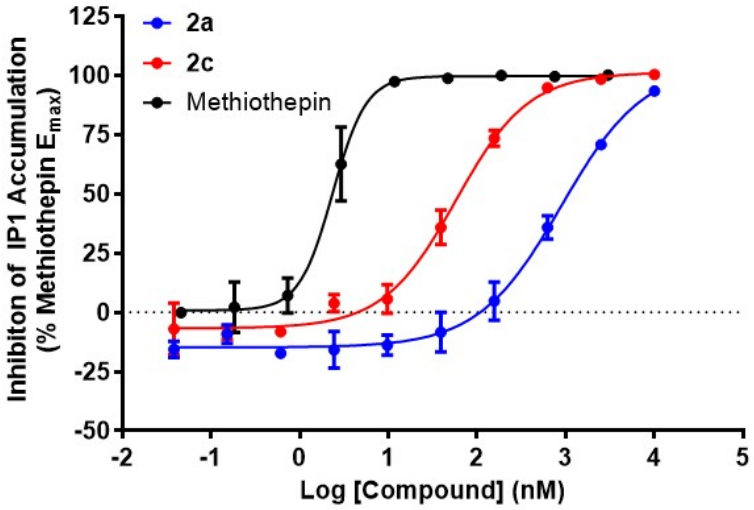
The standard curve was prepared according to the manufacturer's instructions, starting at 7,700 nM IP1 in stimulation buffer and diluted 4-fold to create 9 points in duplicate. Each point of the standard curve was added to an empty assay plate in a volume of 14µL. 14 µL of stimulation buffer was added as a negative control.

The plates were incubated for 1 h at 37 °C, lysed by addition of the supplied buffer containing d2-labeled IP1, followed by addition of terbium cryptate-labeled anti-IP1 antibody, according to the manufacturer's instructions. Plates were incubated for 1 h at room temperature and fluorescence signals were measured at 615 and 665 nm using an EnVision® Multimode Plate Reader (PerkinElmer). Data were analyzed with GraphPad Prism 5.0 (GraphPad software).

*Results: Agonist Mode*

**Table S4. IP-One (Agonist) assay results for control and test compounds**

| Compound | $EC_{50}$ (nM) | Max Dose (nM) | %Activity @ MaxDose |
|---|---|---|---|
| *Serotonin (control)* | *7.904* | *5000* | *100.73* |
| **2a** | >10,000 | 10000 | -1.28 |
| **2c** | >10,000 | 10000 | 3.06 |




Non-linear fit parameters for serotonin (5-HT):

| log(agonist) vs. response -- Variable slope | |
|---|---|
| Best-fit values | |
| Bottom | 0.04122 |
| Top | 94.94 |
| LogEC50 | 0.8979 |
| HillSlope | 1.174 |
| EC50 | 7.904 |

Non-linear fit parameters for **2a**:

| log(inhibitor) vs. response -- Variable slope | |
|---|---|
| Best-fit values | |
| Bottom | -14.87 |
| Top | 104.3 |
| LogIC50 | 2.948 |
| HillSlope | 0.9280 |
| IC50 | 887.4 |

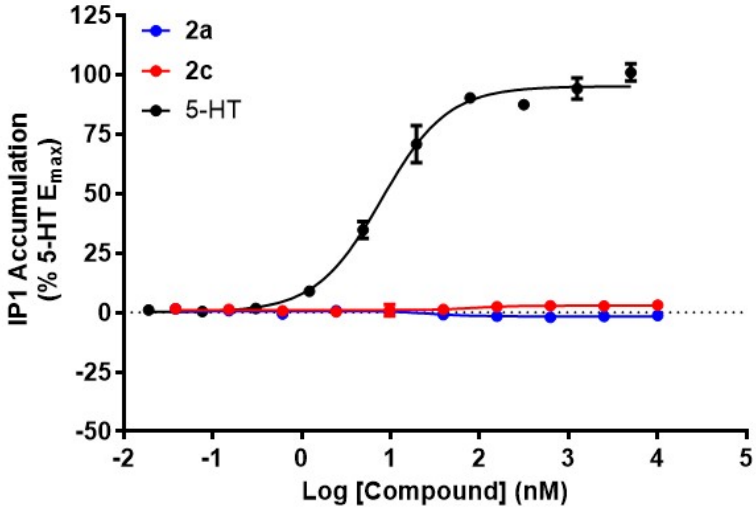
Non-linear fit parameters for **2c**:

| log(inhibitor) vs. response -- Variable slope | |
|---|---|
| Best-fit values | |
| Bottom | -6.804 |
| Top | 101.1 |
| LogIC50 | 1.761 |
| HillSlope | 1.061 |
| IC50 | 57.66 |

*Results: Antagonist Mode*

**Table S5. IP-One (Antagonist) assay results for control and test compounds**

| Compound | $IC_{50}$ (nM) | Max Dose (nM) | %Inh @ MaxDose |
|---|---|---|---|
| *Methiothepin (control)* | *2.439* | *3000* | *100.03* |
| **2a** | 887.4 | 10000 | 93.24 |
| **2c** | 57.66 | 10000 | 100.21 |




Non-linear fit parameters for methiothepin mesylate:

| log(inhibitor) vs. response -- Variable slope | |
|---|---|
| Best-fit values | |
| Bottom | 2.930 |
| Top | 99.38 |
| LogIC50 | 0.3872 |
| HillSlope | 2.573 |
| IC50 | 2.439 |

Non-linear fit parameters for **2a**:

| log(agonist) vs. response -- Variable slope | |
|---|---|
| Best-fit values | |
| Bottom | -1.675 |
| Top | 0.5710 |
| LogEC50 | 1.434 |
| HillSlope | -1.848 |
| EC50 | 27.15 |

Non-linear fit parameters for **2c**:

| log(agonist) vs. response -- Variable slope | |
|---|---|
| Best-fit values | |
| Bottom | 0.9085 |
| Top | 2.829 |
| LogEC50 | 1.925 |
| HillSlope | 1.864 |
| EC50 | 84.12 |

## IX. References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