# Discovery of 8-Trifluoromethyl-3-cyclopropylmethyl-7-[(4-(2,4-difluorophenyl)-1-piperazinyl)methyl]-1,2,4-triazolo[4,3-a]pyridine (JNJ-46356479), a Selective and Orally Bioavailable mGlu2 receptor Positive Allosteric Modulator (PAM)

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## Supporting Information

**Contents**



## Melting points

Values are peak values, and are obtained with experimental uncertainties that are commonly associated with this analytical method.

Mettler FP62 apparatus (A):For a number of compounds, melting points were determined in open capillary tubes on a Mettler FP62 apparatus. Melting points were measured with a temperature gradient of 1, 3, 5 or 10 °C/minute. Maximum temperature was 300 °C. The melting point was read from a digital display.

Mettler FP 81HT / FP90 apparatus (B): For a number of compounds, melting points were determined in open capillary tubes on a Mettler FP 81HT / FP90 apparatus. Melting points were measured with a temperature gradient of 1, 3, 5 or 10 °C/minute. Maximum temperature was 300 °C. The melting point was read from a digital display

## LCMS

*General procedure*

The High Performance Liquid Chromatography (HPLC) measurement was performed using a LC pump, a diode-array (DAD) or a UV detector and a column as specified in the respective methods. If necessary, additional detectors were included (see table of methods below).

Flow from the column was brought to the Mass Spectrometer (MS) which was configured with an atmospheric pressure ion source. It is within the knowledge of the skilled person to set the tune parameters (e.g. scanning range, dwell time…) in order to obtain ions allowing the identification of the compound's nominal monoisotopic molecular weight (MW) and/or exact mass monoisotopic molecular weight. Data acquisition was performed with appropriate software.

Compounds are described by their experimental retention times ($R_t$) and ions. If not specified differently in the table of data, the reported molecular ion corresponds to the $[M+H]^+$ (protonated molecule) and/or $[M-H]^-$ (deprotonated molecule). In case the compound was not directly ionizable the type of adduct is specified (i.e. $[M+NH_4]^+$, $[M+HCOO]^-$, $[M+CH_3COO]^-$ etc…). For molecules with multiple isotopic patterns (Br, Cl), the reported value is the one obtained for the lowest isotope mass. All results were obtained with experimental uncertainties that are commonly associated with the method used.

Hereinafter, "UPLC" means Ultra Performance Liquid Chromatography, "DAD" Diode Array Detector, "MSD" Mass Selective Detector, "SQD" Single Quadrupole Detector, "RT" room temperature, "BEH" bridged ethylsiloxane/silica hybrid.

**Table 1.** LC-MS Methods (Flow expressed in mL/min; column temperature (T) in °C; Run time in minutes).

| Compound | Method | Instrument | Column | Mobile phase | Gradient | Flow<br>-------<br>Col T | Run time |
|---|---|---|---|---|---|---|---|
| **12, 13, 15, 16, 18, 19, 20, 21, 22, 23, 24, 25, 26, 27,** | **1** | Waters: Acquity® UPLC® -DAD and SQD | Waters: BEH C18 (1.7µm, 2.1x50mm) | A: 95% $CH_3CO_2NH_4$ 6.5mM + 5% $CH_3CN$, B: $CH_3CN$ | From 95% A to 40% A in 3.8min, to 5% A in 0.8min, held for 0.4min | 1<br>-------<br>50 | 5 |

| Compound | Method | Instrument | Column | Mobile phase | Gradient | Flow<br>-------<br>Col T | Run time |
|---|---|---|---|---|---|---|---|
| **28, 29** | | | | | | | |
| **14** | **2** | Waters: Acquity® UPLC® -DAD and SQD | Waters: BEH C18 (1.7µm, 2.1x50mm) | A: 95% $CH_3CO_2NH_4$ 6.5mM + 5% $CH_3CN$, B: $CH_3CN$ | From 95% A to 40% A in 2.8min, to 5% A in 0.8min, held for 0.2min, back to 95% A in 0.2min, held for 1.0min | 1<br>-------<br>50 | 5 |
| **17** | **3** | Agilent: HP1100-DAD, Micromass: LCT TOF | Agilent: Eclipse Plus C18 (3.5µm, 2.1x30mm) | A: 95% $CH_3CO_2NH_4$ 6.5mM + 5% $CH_3CN$, B: $CH_3CN$ + $CH_3OH$, 1/1 | 95% A for 0.2 min, to 0% A in 2.8min, held for 0.15min, back to 95% A in 0.15min, held for 1.7min | 1<br>-------<br>60 | 5 |
| **--** | **4** | Agilent: HP1100-DAD, Waters: SQD | Agilent: Eclipse Plus C18 (3.5µm, 2.1x30mm) | A: 95% $CH_3CO_2NH_4$ 6.5mM + 5% $CH_3CN$, B: $CH_3CN$ + $CH_3OH$, 1/1 | 95% A kept for 0.2 min, to 0% A in 2.8min, held for 0.15min, back to 95% A in 0.15min, held for 1.7min | 1<br>------<br>60 | 5 |
| **--** | **5** | Waters: Acquity® UPLC® -DAD and SQD | Waters: BEH C18 (1.7µm, 2.1x50mm) | A: 95% $CH_3CO_2NH_4$ 6.5mM + 5% $CH_3CN$, B: $CH_3CN$ | From 95% A to 40% A in 1.2min, to 5% A in 0.6min, held for 0.2min | 1<br>-------<br>50 | 2 |
| **--** | **6** | Waters: Acquity® IClass UPLC® -DAD and | Waters: BEH C18 (1.7µm, 2.1x50mm) | A: 95% $CH_3CO_2NH_4$ 6.5mM + 5% $CH_3CN$, B: | From 95% A to 40 % A in 1.2min, to 5% A in 0.6min, held | 1<br>-------<br>50 | 2 |

| Compound | Method | Instrument | Column | Mobile phase | Gradient | Flow<br>-------<br>Col T | Run time |
|---|---|---|---|---|---|---|---|
| | | SQD | | $CH_3CN$ | for 0.2min | | |

## Molecular Modeling

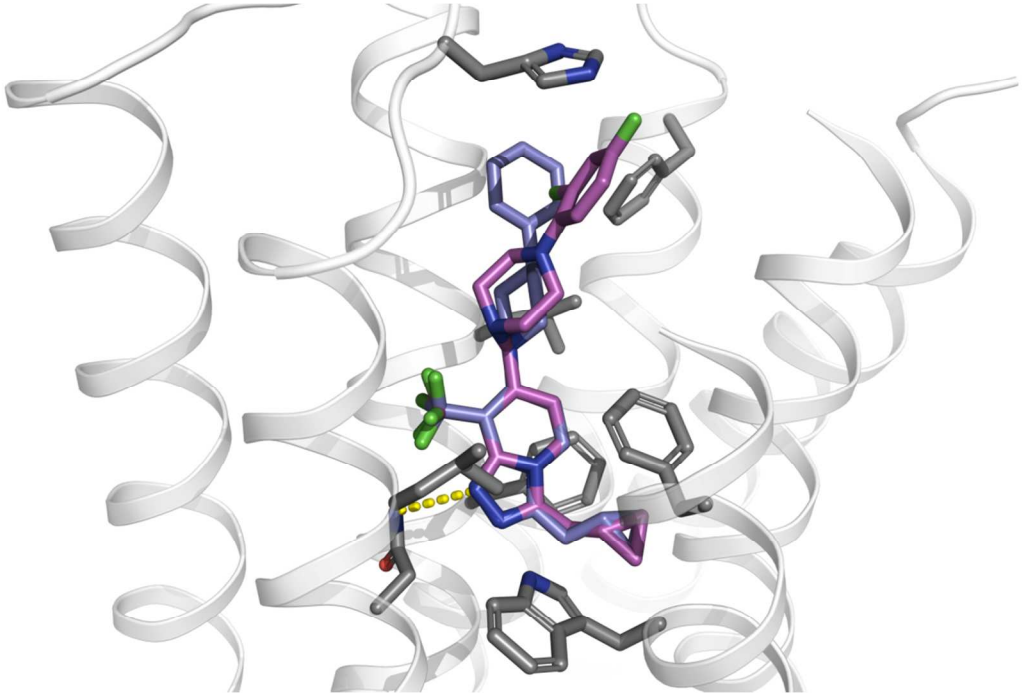

**Figure S1.** Comparison of the docking binding modes of **6** (purple) and **27** (magenta) in the mGlu2 receptor model. The figure shows the high structural overlap with most deviation occurring at the distal phenyl end of each molecule, towards the extracellular side of the receptor.