Supporting Information

Synthesis, Evaluation and Radiolabeling of New Potent Positive Allosteric Modulators of the Metabotropic Glutamate Receptor 2 as Potential Tracers for Positron Emission Tomography Imaging

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General procedure A for HP 1100-MS instruments (MSD, SQD or TOF, Agilent-Waters)

The HPLC measurement was performed using an HP 1100 (Agilent Technologies) system comprising a pump (quaternary or binary) with degasser, an autosampler, a column oven, a diode-array detector (DAD) and a column as specified in the respective methods with flow split to the MS detector. The MS detector (MSD, SQD or TOF) was configured with an electrospray ionization source. Nitrogen was used as the nebulizer gas. The source temperature was maintained either at 100°C (MSD) or 140ºC (SQD or TOF). Data acquisition was performed either with Chemstation-Agilent Data Browser software or MassLynx-Openlynx software.

A1: Mass spectra were acquired on a single quadrupole MSD detector by scanning from 100 to 1000 in 0.99 seconds, step size of 0.30 and peak width of 0.10 minutes. The capillary needle voltage was 1.0 Kv, the fragmentor voltage was 70V for positive and negative ionization modes.

A2: Mass spectra were acquired on a single quadrupole SQD detector by scanning from 100 to 1000 in 0.1 second using an inter-channel delay of 0.08 second. The capillary needle voltage was 3.0 kV. The cone voltage was 20 V for positive ionization mode and 30 V for negative ionization mode.

A3: Mass spectra were acquired on a Time of Flight (TOF) detector by scanning from 100 to 750 in 0.5 seconds using a dwell time of 0.3 seconds. The capillary needle voltage was 2.5 kV for positive ionization mode and 2.9 kV for negative ionization mode. The cone voltage was 20 V for both positive and negative ionization modes.

Method 1: In addition to the general procedure A1: Reversed phase HPLC was carried out on a XDB-C18 cartridge (1.8 μm, 2.1 x 30 mm) from Agilent, with a flow rate of 0.8 ml/min, at 60ºC. The gradient conditions used are: 90% A (6.5 mM NH_4AcO/H_2O), 10%

B (CH_3CN/ CH_3OH 1/1), kept 0.2 minutes, to 100% B in 3.0 minutes, kept till 3.15 minutes and equilibrated to initial conditions at 3.3 minutes until 5.0 minutes. The injection volume was 2.0 µl.
This method was used for compound **12b.**
Method 2: In addition to the general procedure A1: Reversed phase HPLC was carried out on an XBridge-C18 column (2.5 µm, 2.1 x 30 mm) from Waters, with a flow rate of 1.0 ml/min, at 60ºC. The gradient conditions used are: 90% A (6.5 mM NH_4AcO/H_2O), 10% B (CH_3CN/CH_3OH 1/1), kept 0.2 minutes, to 100% B in 3.5 minutes, kept till 3.65 minutes and equilibrated to initial conditions at 3.8 minutes until 5.0 minutes. The injection volume was 2.0 µl.
This method was used for compound **7b**.
Method 3: In addition to the general procedure A1: Reversed phase HPLC was carried out on an Eclipse Plus-C18 column (3.5 µm, 2.1 x 30 mm) from Agilent, with a flow rate of 1.0 ml/min, at 60ºC. The gradient conditions used are: 95% A (6.5 mM NH_4AcO in H_2O/CH_3CN 95/5), 5% B (CH_3CN/CH_3OH 1/1), kept 0.2 minutes, to 100% B in 3.0 minutes, kept till 3.15 minutes and equilibrated to initial conditions at 3.3 minutes until 5.0 minutes. The injection volume was 2.0 µl.
This method was used for compounds **6c**, **6e**, **6g**, **15c**.
Method 4: In addition to the general procedure A3: Reversed phase HPLC was carried out on an XDB-C18 cartridge (1.8 µm, 2.1 x 30 mm) from Agilent, with a flow rate of 1 ml/min, at 60ºC. The gradient conditions used are: 90% A (6.5 mM NH_4AcO/H_2O), 5% B (CH_3CN), 5% C (CH_3OH), kept 0.2 minutes, to 50% B, 50% C in 3.5 minutes, kept till 3.65 minutes and equilibrated to initial conditions at 3.8 minutes until 5.0 minutes. The injection volume was 2.0 µl.
This method was used for compound **10d**.
Method 5: In addition to the general procedure A2: Reversed phase HPLC was carried out on a Sunfire-C18 column (2.5 µm, 2.1 x 30 mm) from Waters, with a flow rate of 1.0 ml/min, at 60ºC. The gradient conditions used are: 95% A (6.5 mM NH_4AcO in H_2O/CH_3CN 95/5), 5% B (CH_3CN/CH_3OH 1/1), kept 0.2 minutes, to 100% B in 3.0 minutes, kept till 3.15 minutes and equilibrated to initial conditions at 3.30 minutes until 5.0 minutes. The injection volume was 2 µl. The cone voltage was 20 V and 50 V for positive ionization mode and 30 V for negative ionization mode.
This method was used for compounds **6f**, **13c**.
Method 6: In addition to the general procedure A2: Reversed phase HPLC was carried out on an Eclipse Plus-C18 column (3.5 µm, 2.1 x 30 mm) from Agilent, with a flow rate of 1.0 ml/min, at 60ºC. The gradient conditions used are: 95% A (6.5 mM NH_4AcO in H_2O/CH_3CN 95/5), 5% B (CH_3CN/CH_3OH 1/1), kept 0.2 minutes, to 100% B in 3.0 minutes, kept till 3.15 minutes and equilibrated to initial conditions at 3.30 minutes until 5.0 minutes. The injection volume was 2 µl. The cone voltage was 20 V and 50 V for positive ionization mode and 30 V for negative ionization mode.
This method was used for compounds **11b**, **11c**, **13b**, and precursor of **7f**.
Method 7: In addition to the general procedure A2: Reversed phase HPLC was carried out on an Eclipse Plus-C18 column (3.5 µm, 2.1 x 30 mm) from Agilent, with a flow rate of 1.0 ml/min, at 60ºC. The gradient conditions used are: 95% A (6.5 mM NH_4AcO in H_2O/CH_3CN 95/5), 5% B (CH_3CN/CH_3OH 1/1), to 100% B in 5.0 minutes, kept to 5.15

minutes and equilibrated to initial conditions at 5.30 minutes until 7.0 minutes. The injection volume was 2 µl.
This method was used for compound **17b**.
Method 8: In addition to the general procedure A3: Reversed phase HPLC was carried out on a Sunfire-C18 column (2.5 µm, 2.1 x 30 mm) from Waters, with a flow rate of 1.0 ml/min, at 60ºC. The gradient conditions used are: 95% A (6.5 mM NH_4AcO in H_2O/CH_3CN 95/5), 2.5% B (CH_3CN), 2.5% C (CH_3OH) to 50% B, 50%C in 6.5 minutes, kept till 7.0 minutes and equilibrated to initial conditions at 7.3 minutes until 9.0 minutes. The injection volume was 2 µl.
This method was used for compounds **20c**, **16d**.

General procedure B for Acquity-SQD instrument
The UPLC (Ultra Performance Liquid Chromatography) measurement was performed using an Acquity UPLC (Waters) system comprising a sampler organizer, a binary pump with degasser, a four column's oven, a diode-array detector (DAD) and a column as specified in the respective methods without flow split to the MS detector. The MS detector was configured with an electrospray ionization source. Mass spectra were acquired on a single quadrupole SQD detector scanning from 100 to 1000 in 0.1 second using an inter-channel delay of 0.08 second. The capillary needle voltage was 3.0 kV. The source temperature was maintained at 140 °C. Nitrogen was used as the nebulizer gas. Data acquisition was performed with MassLynx-Openlynx software.
B1: The cone voltage was 20 V for positive ionization mode and 30 V for negative ionization mode.
B2: The cone voltage was 25 V for positive ionization mode and 30 V for negative ionization mode.
Method 9: In addition to the general procedure B1: Reversed phase UPLC was carried out on a BEH-C18 column (1.7 µm, 2.1 x 50 mm) from Waters, with a flow rate of 0.8 ml/min, at 60ºC. The gradient conditions used are: 95% A (6.5 mM NH_4AcO in H_2O/CH_3CN 95/5), 5% B (CH_3CN/CH_3OH 1/1), to 20% A, 80 %B in 4.9 minutes, to 100% B in 5.3 minutes, kept till 5.8 minutes and equilibrated to initial conditions at 6.0 minutes until 7.0 minutes. The injection volume was 0.5 µl.
This method was used for compound **22b**.
Method 10: In addition to the general procedure B1: Reversed phase UPLC was carried out on a BEH-C18 column (1.7 µm, 2.1 x 50 mm) from Waters, with a flow rate of 0.8 ml/min, at 60ºC. The gradient conditions used are: 95% A (6.5 mM NH_4AcO in H_2O/CH_3CN 95/5), 5% B (CH_3CN/CH_3OH 1/1), kept 0.2 minutes, to 20% A, 80% B in 3.5 minutes, to 100% B in 3.8 minutes, kept till 4.15 minutes and equilibrated to initial conditions at 4.3 minutes until 5.0 minutes. The injection volume was 2.0 µl.
This method was used for compounds **6b**, **7f**, **21f**, **15b**.
Method 11: In addition to the general procedure B2: Reversed phase UPLC was carried out on a BEH-C18 column (1.7 µm, 2.1 x 50 mm) from Waters, with a flow rate of 1.0 ml/min, at 50ºC. The gradient conditions used are: 95% A (6.5 mM NH_4AcO in H_2O/CH_3CN 95/5), 5% B (CH_3CN), to 40% A, 60% B in 4.4 minutes, to 5% A, 95% B in 5.6 minutes, kept till 5.8 minutes and equilibrated to initial conditions at 6.0 minutes until 7.0 minutes. The injection volume was 0.5 µl.

This method was used for compounds **22c**, **22g, 22h**, **23f**, **20b**, **17c**, **20h**, **20e**, **20d**, **20g**, **20a**, **21d**.

Method 12: In addition to the general procedure B2: Reversed phase UPLC was carried out on a BEH-C18 column (1.7 µm, 2.1 x 50 mm) from Waters, with a flow rate of 1.0 ml/min, at 50ºC. The gradient conditions used are: 95% A (6.5 mM NH_4AcO in H_2O/CH_3CN 95/5), 5% B (CH_3CN), to 40% A, 60% B in 2.8 minutes, to 5% A, 95% B in 3.6 minutes, kept till 3.8 minutes and equilibrated to initial conditions at 4.0 minutes until 5.0 minutes. The injection volume was 0.5 µl.

This method was used for compounds **22f**, **20f**, **7g**.

Method 13: In addition to the general procedure B2: Reversed phase UPLC was carried out on a RRHD Eclipse Plus-C18 (1.8 µm, 2.1 x 50 mm) from Agilent, with a flow rate of 1.0 ml/min, at 50ºC. The gradient conditions used are: 95% A (6.5 mM NH_4AcO in H_2O/CH_3CN 95/5), 5% B (CH_3CN), to 40% A, 60% B in 3.8 minutes, to 5% A, 95% B in 4.6 minutes, kept to 5.0 minutes. The injection volume was 2.0 µl.

This method was used for compounds **6d**, **6h**, **7d**, **7e**.

Method 14: In addition to the general procedure B2: Reversed phase UPLC was carried out on a RRHD Eclipse Plus-C18 (1.8 µm, 2.1 x 50 mm) from Agilent, with a flow rate of 1.0 ml/min, at 50ºC. The gradient conditions used are: 95% A (6.5 mM NH_4AcO in H_2O/CH_3CN 95/5), 5% B (CH_3CN), to 40% A, 60% B in 1.2 minutes, to 5% A, 95% B in 1.8 minutes, kept to 2.0 minutes. The injection volume was 2.0 µl.

This method was used for compounds **7h**, **7c**.

GCMS- methods:

General procedure for Agilent GC/MSD instrument

The GC measurement was performed using a 6890 Series Gas Chromatograph (Agilent Technologies) system comprising a 7683 Series injector and autosampler, a column oven and a column as specified in the respective methods below, coupled to a 5973N MSD Mass Selective Detector (single quadrupole, Agilent Technologies). The MS detector was configured with an electronic impact ionization source / chemical ionization source (EI/CI). EI low-resolution mass spectra were acquired by scanning from 50 to 550 at a rate of 14.29 scans/s. The source temperature was maintained at 230°C. Helium was used as the nebulizer gas. Data acquisition was performed with Chemstation-Open Action software.

Method 1 In addition to the general procedure: GC was carried out on a J&W HP-5MS column (30 m x 0.25 mm, 0.25 µm) from Agilent Technologies, with a flow rate of 1.2 ml/min. The temperature gradient applied was: initial temperature 50ºC, hold for 3 min, then a 20ºC/min ramp applied for 10 min until 250ºC and hold for 2 min in a 15 min run. Front inlet temperature was 250ºC. Split injection mode was used, 1 µl injection volume, with a 50/1 ratio into the GC/MS system.

This method was used for compounds: 2-bromo-1,4-difluoro-3-methoxybenzene (precursor of **6f**), 6-bromo-2,3-difluorophenol and 1-bromo-3,4-difluoro-2-methoxybenzene (both precursors of **6e**).

Method 2 In addition to the general procedure: GC was carried out on a J&W HP-5MS column (20 m x 0.18 mm, 0.18 µm) from Agilent Technologies, with a flow rate of 0.7 ml/min. The temperature gradient applied was: initial temperature 50ºC, hold for 0.8 min, then a 60ºC/min ramp applied for 4.17 min until 300ºC and hold for 3.0 min in a 8 min run.

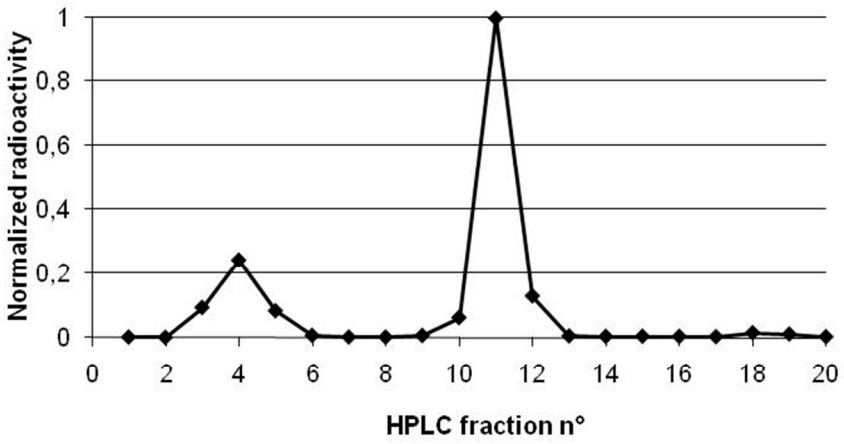
Front inlet temperature was 250ºC. Split injection mode was used, 0.2 µl injection volume, with a 50/1 ratio into the GC/MS system.
This method was used for 2-bromo-1,5-difluoro-3-methoxybenzebe (precursor of **6h**).

Biodistribution of [^{11}C]**20f** in normal rats at 2, 30 and 60 min post tracer injection.

	%ID [a]		
	2 min	30 min	60 min
urine	0.1 ± 0.0	0.3 ± 0.1	0.3 ± 0.1
kidneys	6.6 ± 0.7	4.3 ± 1.0	2.1 ± 0.2
liver	33.5 ± 1.4	22.7 ± 3.0	20.1 ± 7.0
spleen + pancreas	1.4 ± 0.1	1.4 ± 0.2	0.7 ± 0.0
lungs	1.5 ± 0.1	1.1 ± 0.5	0.6 ± 0.1
heart	4.6 ± 0.6	2.5 ± 0.8	1.2 ± 0.2
stomach	1.4 ± 0.2	3.7 ± 0.3	1.7 ± 0.4
intestines	8.5 ± 0.3	10.4 ± 1.2	15.6 ± 2.7
striatum	0.032 ± 0.008	0.047 ± 0.008	0.033 ± 0.008
hippocampus	0.028 ± 0.008	0.045 ± 0.005	0.024 ± 0.006
cortex	0.097 ± 0.019	0.118 ± 0.041	0.080 ± 0.022
rest of cerebrum	0.535 ± 0.121	0.704 ± 0.112	0.421 ± 0.010
cerebrum total	0.691 ± 0.146	0.914 ± 0.140	0.558 ± 0.042
cerebellum	0.174 ± 0.039	0.291 ± 0.088	0.142 ± 0.029
blood	4.3 ± 0.6	2.7 ± 0.9	2.0 ± 0.0
carcass	38.4 ± 2.6	50.9 ± 3.4	55.8 ± 9.4

[a] Percentage of injected dose calculated as cpm in organ/ total cpm recovered. Data are expressed as mean ± SD; n = 3 per time point.

Reconstructed radiochromatogram corresponding to a rat plasma analysis at 30 min post injection of [^{11}C]**20f**.



Reconstructed radiochromatograms corresponding to a rat perfused cerebrum and cerebellum analysis at 30 min post injection of [^{11}C]**20f**.