Supporting Information

Synthesis and preliminary studies of $^{11}$C-labeled tetrahydro-1,7-naphthyridine-2-carboxamides for PET imaging of metabotropic glutamate receptor 2

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Figure S1. Concentration–response curves of mGlu NAMs compounds 14a-14g in mGlu GIRK or mGlu GIRK functional assays.
A. Baseline studies of $[^{11}\text{C}]14\text{a}$

B. Baseline studies with pretreatment of 14a

**Figure S2.** Time-activity curves of $[^{11}\text{C}]14\text{a}$ in rat brains. *Blocking conditions: 14a (1 mg/kg), 30 min i.v. before radioligand injection.*
Figure S3. Radiometabolite analysis of \([^{11}\text{C}]14\text{a}\) in rat brain (average two runs)

Table S1. Radiometabolite and parent (unchanged) fraction of \([^{11}\text{C}]14\text{a}\) in rat brain and plasma

<table>
<thead>
<tr>
<th></th>
<th>Metabolite (%)</th>
<th>Unchanged (%)</th>
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<tr>
<td>Plasma 5 min–1</td>
<td>11.96</td>
<td>88.04</td>
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<tr>
<td>Plasma 5 min–2</td>
<td>17.06</td>
<td>82.94</td>
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<tr>
<td>Plasma 20 min–1</td>
<td>44.51</td>
<td>55.49</td>
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<tr>
<td>Plasma 20 min–2</td>
<td>38.26</td>
<td>61.74</td>
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<tr>
<td>Brain 5 min–1</td>
<td>0.06</td>
<td>99.94</td>
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<tr>
<td>Brain 5 min–2</td>
<td>0.49</td>
<td>99.51</td>
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<tr>
<td>Brain 20 min–1</td>
<td>7.3</td>
<td>92.7</td>
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<tr>
<td>Brain 20 min–2</td>
<td>5.76</td>
<td>94.24</td>
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Figure S4. RadioHPLC chromatogram in the brain and plasma 5 min post injection of \([^{11}\text{C}]14\text{a}\)
Figure S5. RadioHPLC chromatogram in the brain and plasma 20 min post injection of $^{14}$C14a

Figure S6. Radiometabolite analysis of $^{14}$C14b in rat brain (average two runs)

Table S2. Radiometabolite and parent (unchanged) fraction of $^{14}$C14b in rat brain and plasma

<table>
<thead>
<tr>
<th></th>
<th>Metabolite (%)</th>
<th>Unchanged (%)</th>
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<tbody>
<tr>
<td>Plasma 5 min-1</td>
<td>21.32</td>
<td>76.68</td>
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<td>Plasma 5 min-2</td>
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<td>Plasma 20 min-1</td>
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<td>Plasma 20 min-2</td>
<td>67.60</td>
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<td>Brain 5 min-1</td>
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<td>99.72</td>
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<td>Brain 5 min-2</td>
<td>0.04</td>
<td>99.96</td>
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<tr>
<td>Brain 20 min-1</td>
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<tr>
<td>Brain 20 min-2</td>
<td>1.22</td>
<td>98.78</td>
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Figure S7. RadioHPLC chromatogram in the brain and plasma 5 min post injection of $[^{11}C]14b$
Figure S8. RadioHPLC chromatogram in the brain and plasma 20 min post injection of $^{11}$C\textsuperscript{14}b
Figure S9. Image of the ROIs used for quantification of in vitro autoradiography. (A) Brain sections were treated with $[^{11}\text{C}]14\text{a}$; (B) Brain sections were pre-treated with 14a (10 μM), followed by $[^{11}\text{C}]14\text{a}$; (C) Brain sections were pre-treated with QCA (10 μM), followed by $[^{11}\text{C}]14\text{a}$; (A) Brain sections were treated with $[^{11}\text{C}]14\text{b}$; (B) Brain sections were pre-treated with 14b (10 μM), followed by $[^{11}\text{C}]14\text{b}$; (C) Brain sections were pre-treated with QCA (10 μM), followed by $[^{11}\text{C}]14\text{b}$.

Figure S10. Functional (agonist and antagonist) assays of compound 14b towards mGlu receptors, including mGlu1, mGlu4, mGlu5, mGlu6 and mGlu8.
Figure S11. Off-target pharmacological evaluation of compound 14b at a concentration of 10 μM against major CNS targets, including common GPCRs, enzymes, ion channels and transporters: Initial screening at a concentration of 10 μM. All data are mean ± SD (n = 4). No significant off-target binding (>50%) was observed at 10 μM compound testing concentration.
Figure S12. Representative PET/MRI fused images (summed at 0-10 min, 10-30 min and 30-60 min) and time-activity curves of $[^{11}C]14b$ under baseline and blocking conditions in SD rat brain. Blocking conditions: 14b (1 mg/kg), 30 min i.v. before radioligand injection; Blocking conditions: 14a (3 mg/kg), 30 min i.v. before radioligand injection. Data are presented as mean ± SEM (n = 3).
NMR spectra of synthesized compounds

$^1$H spectrum of 11a

![11a](image_url)
$^{13}$C spectrum of 11a

![Chemical Structure](image)

11a
$^1$H spectrum of 11b
$^{13}$C spectrum of 11b
$^1$H spectrum of 11c
$^{13}$C spectrum of 11c

![Chemical Structure of 11c]
$^1$H spectrum of 11d
$^{13}$C spectrum of 11d

11d

94.55
69.52
$^1$H spectrum of 11g
$^{13}$C spectrum of 11g
$^1$H spectrum of 13a
$^{13}$C spectrum of 13a
$^1$H spectrum of 13b

13b
$^{13}$C spectrum of 13b
$^1$H spectrum of 13c
$^{13}C$ spectrum of 13c
$^1$H spectrum of 13d
$^{13}$C spectrum of 13d
$^1$H spectrum of $^{13}e$
$^{13}$C spectrum of 13e
$^1$H spectrum of 13f
$^{13}\text{C}$ spectrum of 13f
$^{1}$H spectrum of 13g
$^{13}$C spectrum of 13g
$^1$H spectrum of 14a
$^{13}$C spectrum of 14a
$^1$H spectrum of 14b
$^{13}$C spectrum of 14b
$^1$H spectrum of 14c
$^{13}$C spectrum of 14c
$^1$H spectrum of 14d
$^{13}$C spectrum of 14d
$^1$H spectrum of 14e
$^{13}$C spectrum of 14e
$^1$H spectrum of 14f
$^{13}$C spectrum of 14f
$^{1}H$ spectrum of 14g
$^{13}$C spectrum of 14g
$^{1}$H spectrum of 16
$^{13}$C spectrum of 16

![Chemical Structure Image]
$^{1}$H spectrum of 17
$^{13}$C spectrum of 17
$^1$H spectrum of 18
$^{13}\text{C}$ spectrum of 18