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Syntheses of 2-Amino and 2-Halothiazole Derivatives as High-Affinity Metabotropic Glutamate Receptor Subtype 5 Ligands and Potential Radioligands for In Vivo Imaging

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Abstract

The structure of the potent selective mGlu₅ ligand, SP203 (**1**, 3-fluoro-5-[[2-(fluoromethyl)thiazol-4-yl]ethynyl]benzonitrile), was modified by replacing the 2-fluoromethyl substituent with an amino or halo substituent and by variation of substituents in the distal aromatic ring to provide a series of new high-affinity mGlu₅ ligands. In this series, among the most potent ligands obtained, the 2-chloro-thiazoles **7a** and **7b** and the 2-fluorothiazole **10b** showed sub-nanomolar mGlu₅ affinity. **10b** also displayed >10,000-fold selectivity over all other metabotropic receptor subtypes plus a wide range of other receptors and binding sites. The 2-fluorothiazoles **10a** and **10b** were labeled using [¹⁸F]fluoride ion (*t*_{1/2} = 109.7 min) in moderately high radiochemical yield to provide potential radioligands that may resist troublesome radiodefluorination during the imaging of brain mGlu₅ with position emission tomography. The iodo compound **9b** has nanomolar affinity for mGlu₅ and may also serve as a lead to a potential ¹²³I-labeled ligand for imaging brain mGlu₅ with single photon emission computed tomography.

Introduction

Glutamate, the predominant excitatory neurotransmitter in the central nervous system, promotes fast-synaptic transmission and synaptic plasticity by activating any one of three ion channel ionotropic glutamate receptors, namely NMDA, AMPA or kainate receptors.¹ Glutamate also has a modulatory influence on neuronal excitability by activating metabotropic glutamate receptors (mGluRs), which are members of the G protein-coupled seven-membrane-domain class of receptors.² Eight mGluR subtypes have been identified and classified according to their sequence homology, signal transduction, and pharmacology.³ Among these subtypes, the metabotropic glutamate receptor subtype 5 (mGlu₅) plays key roles in a variety of normal brain functions, and is also putatively involved in several neuropsychiatric disorders. Activation of mGlu₅ stimulates phospholipase C, and this results in phosphoinositide hydrolysis and an increase in intracellular Ca²⁺ levels.^{3,4}

Modulation of mGlu₅ receptors has potential for the treatment of schizophrenia⁵, Fragile X syndrome⁶ and Alzheimer's disease⁷. Further evidence supports the use of antagonists or modulators of mGlu₅ in the treatment of anxiety⁸, depression⁹ and pain¹⁰. mGlu₅ may also play an important role in drug-related behaviors, particularly drug abuse,¹¹ drug addiction¹² and alcohol withdrawal.¹³ Hence, mGlu₅ antagonists may turn out to be useful therapeutics for a variety of central nervous system disorders.

We previously reported the discovery of a very potent and highly selective ligand for mGlu₅ which we dubbed SP203 (**1**, 3-fluoro-5-[[2-(fluoromethyl)thiazol-4-yl]ethynyl] benzonitrile; Chart 1).¹⁴ [¹⁸F]**1**, which has a fluorine-18 ($t_{1/2}$ = 109.7 min) label in the 2-fluoromethyl position, is an effective radioligand for imaging brain mGlu₅ receptors in human subjects in vivo without major complications arising from radiometabolites.¹⁵ Only a low amount of radioactivity appears in skeleton and that radioactivity is associated with marrow rather than bone, thereby showing this radioactivity is not present as [¹⁸F]fluoride ion and that metabolic radiodefluorination is virtually absent.¹⁶ [¹⁸F]**1** is significantly easier to prepare than previous ¹⁸F-labeled mGlu₅ radioligands, such as the structurally related [¹⁸F]F 3-methyl-5-[(2-methyl-1,3-thiazol-4-yl)ethynyl]benzonitrile ([¹⁸F]F-MTEB17, [¹⁸F]**2**; Chart 1), and has broad potential utility, ranging from receptor occupancy studies preceding drug clinical trials to studies of mGlu₅ receptor expression in neuropsychiatric patients.

In rat and in monkey, [¹⁸F]**1** also shows acceptable brain entry and a sizeable receptor-specific signal.¹⁴ However, unlike in human subjects, radiodefluorination occurs,¹⁸ leading to steady accumulation of fluorine-18 in bones and skull. This radioactivity confounds measurement of rat or monkey brain mGlu₅ receptors with this radioligand and positron emission tomography (PET).

Although metabolic defluorination often occurs from an aliphatic carbon,¹⁹ this is relatively rare from an aryl carbon. The thiazole moiety is strongly aromatic. We therefore considered that a 2-fluoro-thiazole moiety might resist defluorination in vivo, unlike the 2-fluoromethylthiazole moiety of **1** when in monkey or rat. One report suggests that fluorination on a thiazole ring might increase stability towards ring metabolism.²⁰

Here we report the synthesis of a series of compounds related to **1** in which the 2-fluoromethylthiazole moiety in **1** was replaced with a halogen, including fluorine, or with an amino group. The substitution pattern in the distal phenyl ring was also varied. We report the affinities of these new ligands towards mGlu₅ receptors. Finally, we describe the labeling of some of the novel 2-fluorothiazoles to produce candidate ¹⁸F-labeled PET mGlu₅ radioligands.

Results and Discussion

Our primary goal was to produce high-affinity mGlu₅ ligands that would be suitable for development as ¹⁸F-labeled ligands for imaging brain mGlu₅ in animals without being susceptible to troublesome radiodefluorination. We considered that this aim might be achieved by modifying [¹⁸F]**1** so that the fluorine-18 atom was directly bound to the thiazole ring at the 2-position. Computation indicated that this modification, the replacement of a fluoromethyl group with a single fluorine atom, would not greatly alter lipophilicity. For example, the *cLogD* value of the 2-fluoro compound **10b** is 3.58 and similar to that of the 2-fluoromethyl analogue **1** (*cLogD* = 3.31). These values are within the range usually considered desirable for a prospective brain imaging agent.²¹ In accord with this guideline, [¹⁸F]**1**, in rodents and primates, shows good penetration of the blood-brain barrier, has high peak uptake in brain and shows relatively low non-specific binding.¹⁴

Chemistry

The 2-amino compounds (**6a–d**, Scheme 1) were prepared by Sonogashira cross-coupling of the aminothiazole **4** with the appropriate haloarenes. Yields were low from bromoarenes (< 30%), but moderate to high from iodoarenes (60 to ~ 90%). Therefore, iodoarenes were used in all coupling reactions. The amines **6a–d** were then halogenated regioselectively²² with CuX (X = Cl, Br, I) in the presence of *n*-butyl nitrite to give the 2-chloro (**7a–d**), 2-bromo (**8a–d**) and 2-iodo derivatives (**9a–d**) (Table 1). Yields increased with halogen size. Thus,

yields for chlorination were low and typically in the range 15–25%. Yields for bromination and iodination were usually over 50% (Table 1).

Because the 2-chloro derivatives **7a–d** could only be obtained in small amounts and their purifications were tedious, we decided to explore an alternative synthesis to improve their yields. We considered that the use of the 2-chlorothiazole synthon **5** (Scheme 1) in a Sonogashira cross-coupling reaction might lead to higher yields. Synthon **5** was prepared by reaction of the easily accessible compound **4** with CuCl and *n*-butyl nitrite and submitted to Sonogashira cross-coupling. Yields of the 2-chloro compounds **7a–d** from cross-coupling reactions with **5** were greatly improved (48–67%). The purifications of the 2-chloro products were also easier than for the amino compounds **6a–d**. Noticeably, during the cross-coupling reactions, no coupling was observed at position 2 of the 2-chloro-thiazole acetylene **5**.

With the 2-chloro, 2-bromo, and 2-iodo compounds available, we aimed to prepare the corresponding 2-fluorothiazoles. The use of electrophilic reagents, either Accufluor23 or Selectfluor24, gave almost negligible yields (1–5%) of the desired fluorinated products. Therefore, we next investigated direct nucleophilic substitution in the halo precursors with potassium fluoride. We recently reported a procedure for the rapid fluorination of 2-chloro and 2-bromothiazole derivatives under microwave irradiation²⁵ and we looked to apply this method to obtain the target 2-fluoro ligands. By treating bromides **8a–d** with a potassium fluoride-Kryptofix 2.2.2 (K 2.2.2) complex in DMSO under microwave irradiation (30 W, 130 °C, 8–15 min), the respective 2-fluoro compounds **10a–d** were obtained in useful 24–43% yields (Table 1).

When the 2-chloro-precursors **7a–d** were treated similarly (KF-K 2.2.2, DMSO, MW, 130 °C, 10–15 min), the fluorination yields were dramatically improved, reaching 78% in the case of **10c**. By contrast, only negligible product was detected when the iodo precursor **9a** was submitted to nucleophilic fluorination (yield, < 5%). These results accord with our previous observations on leaving group ability in the nucleophilic fluorination of simpler 2-halo-thiazoles.²⁵

For the synthesis of **10a**, we also experimented with the use of a KF-AgF with the bromo precursor **8a** under microwave irradiation (Scheme 2). After eight minutes irradiation, the yield of **10a** reached 62%, as measured with HPLC. However, this fluorination method was less reproducible and we also observed the reduction of DMSO into the irritant and volatile dimethyl sulfide.

The 2-chloro and 2-bromo-(4-phenylacetylene)thiazoles **7e** and **8e** were also subjected to fluorination with KF in DMSO, as reported previously, to give the fluoro derivative **10e** in 78 and 46% yields, respectively.²⁵

Pharmacology

The affinity of each new ligand for rat brain tissue mGlu₅ was determined in a binding assay with [³H]MPEP ([³H]6-methyl-2-phenylethenylpyridine) as reference radioligand. The majority of the prepared 2-halo-thiazoles exhibited high affinity with *K_i* values in the nanomolar range. In general, the 2-iodo compounds had lower affinity than their bromo, chloro or fluoro analogs. Unexpectedly, the amino compound intermediates **6a–d** also exhibited high to very high affinity for mGlu₅. The substituent pattern in the distal aryl group strongly influenced affinity for mGlu₅. Thus, the combination of fluoro and nitrile substituents in meta position to the alkynyl group, as also exists in **1**, generally gave the highest affinity among each subset of 2-substituted thiazoles. Removal of the meta-fluoro substituent generally had little effect. A single meta cyanomethyl or methoxy group reduced affinity appreciably, especially a methoxy group.

The high-affinity 2-fluoro ligand **10b** was screened for affinity at other mGluR subtypes (mGlu₁, mGlu₂, mGlu₄, mGlu₆, mGlu₇, and mGlu₈) and a panel of other brain receptors, binding sites and ion channels as listed under Experimental. In all cases, the affinity of **10b** was found to >10,000 nM. Therefore, replacement of the 2-fluoromethyl group in **1** with a fluoro substituent retained excellent mGlu₅ selectivity. Such excellent selectivity is highly desirable in a prospective PET radioligand.

Currently, no effective radioligand exists for imaging brain mGlu₅ with SPECT (single photon emission computed tomography). Interestingly, the iodo compounds **9a** and **9b** show mGlu₅ affinity in the nanomolar range and are possibly suitable for labeling with iodine-123 to provide candidate radioligands for imaging brain mGlu₅ with single photon emission computed tomography (SPECT). The highest affinity iodinated ligand **9b** has affinity that almost matches that of PET radioligands, such as [¹¹C]M-MTEB ([¹¹C]3-methyl-5-[(2-methyl-1,3-thiazol-4-yl)ethynyl] benzonitrile) and [¹⁸F]F-PEB ([¹⁸F]3-fluoro-5-[(2-methyl-1,3-thiazol-4-yl)ethynyl] benzonitrile), that have been shown to give strong receptor-specific signals in monkeys.¹⁷ The computed lipophilicity of **9b** appears quite high for a prospective SPECT radioligand. However, this computed value, and also those for the other ligands may be considerably over-estimated, since the true value for ligand **1** is much lower than for its computed value (Table 1).

Radiochemistry

We previously reported reaction conditions for the preparation of [¹⁸F]2-fluorothiazole derivatives with cyclotron-produced no-carrier-added (NCA) [¹⁸F]fluoride ion under thermal and microwave heating conditions.²⁵ We established that simple 2-chlorothiazole structures label efficiently with [¹⁸F]fluoride ion in the presence of K⁺-K 2.2.2 in DMSO or MeCN. The radiochemical yield was slightly higher for 2-chlorothiazoles than for 2-bromothiazoles. These conditions were applied successfully for the radiosynthesis of [¹⁸F]**10a** from the chloro compound **7a**. Thus, at 130 °C in DMSO under inert atmosphere and with brief microwave heating (90 W, 150 °C, 10 min), [¹⁸F]**10a** was obtained in 36% decay-corrected radiochemical yields (RCY) and with a measured specific activity of 2.1 Ci/μmol at the end of synthesis.

Because of its high affinity, we then selected **10b** as the major target for labeling with fluorine-18. The chloro compound **7b** was selected as precursor for radiofluorination. The conversion of **7b** into [¹⁸F]**10b** was initially attempted using the reaction conditions mentioned above. Reaction for 10 min at 130 °C gave decay-corrected RCYs of 26% (Scheme 3). Unexpectedly, under these conditions some exchange of the aryl fluoro group with [¹⁸F]fluoride ion was also observed and [¹⁸F]**7b** was detected as a by-product of radiofluorination. Typically, with the use of anhydrous DMSO at 130 °C, the ratio of [¹⁸F]**10b** to [¹⁸F]**7b** was 45 to 55. The use of anhydrous acetonitrile under similar conditions (K 2.2.2 - K₂CO₃; MW, 130 °C), switched the major labeled product to [¹⁸F]**10b** ([¹⁸F]**10b**: [¹⁸F]**7b**; 82: 18). In acetonitrile, when the temperature of reaction was lowered to 110 °C, the incorporation of [¹⁸F]fluoride ion was up to 48%, with the ratio of [¹⁸F]**10b** to [¹⁸F]**7b** being 92: 8.

The radioligands were easily purified by HPLC in high chemical purity (> 95%) and high radiochemical purity (> 99%). The specific activity of the target compound [¹⁸F]**10b**, 0.5 Ci/μmol at the end of synthesis (about 150 min from the end of radionuclide production) was somewhat lower than for [¹⁸F]**10a**, probably because of the ¹⁸F for F exchange occurring in precursor **7b**. Nonetheless this new radioligand now merits further evaluation with PET in animals to assess its resistance to radiodefluorination and efficacy for imaging brain mGlu₅.

Conclusions

Several new 2-amino and 2-halo-thiazole-4(alkynyl)benzenes were synthesized and they showed moderate to high affinity as ligands at mGlu₅ in rat brain tissue. The labeling of some of the 2-fluorothiazole derivatives with [¹⁸F]fluoride ion was shown to be feasible to provide new candidate radioligands, [¹⁸F]**10a** and [¹⁸F]**10b**, for mGlu₅ imaging with PET. A candidate, **9b** for development as a SPECT radioligand for imaging brain mGlu₅, was also identified.

Experimental

Materials and general methods

5-Amino-3-fluorobenzonitrile was obtained from Oakwood Products, Inc. All other reagents and solvents were obtained from Sigma-Aldrich in the highest purity available (> 98%) and were used as purchased. All reactions were performed under argon atmosphere unless otherwise indicated. 4-((Trimethylsilyl)ethynyl)thiazol-2-amine (**4**)²⁷, 3-((2-fluorothiazol-4-yl)ethynyl) benzonitrile (**8a**)²², and 2-fluoro-4-(phenylethynyl)thiazole (**10e**)²⁵ were prepared as described previously. Thin layer chromatography (TLC) was performed with silica gel layers (type 60 F254; 400–630 mesh) with compounds visualized under UV light ($\lambda = 254$ nm). Column chromatography was performed on silica gel with hexane-EtOAc mixtures as eluents. Constituent proportions in chromatographic mobile phases are expressed by volume. Separation of compounds with HPLC was performed on a Luna column (10 μ m, 250 $\times$ 10 mm, Phenomenex, Torrance, CA) eluted with H₂O-MeCN. The purities of all new compounds were determined by HPLC on a Luna C18 column (5 μ m, 250 $\times$ 4.6 mm, Phenomenex) eluted isocratically with H₂O-MeCN and were greater than 95%. All HPLC eluates were monitored for absorbance at 254 nm. Yields were recorded for chromatographically pure materials (> 98%).

The ¹H-NMR (400 MHz) and ¹³C-NMR (100 MHz) spectra of all compounds were acquired in CDCl₃ using the chemical shift of residual deuterated solvent as internal standard; chemical shifts (δ) for the proton and carbon resonance are reported in parts per million (ppm) downfield from TMS ($\delta = 0$). The following abbreviations were used to describe peak splitting patterns when appropriate: s = singlet, d = doublet, t = triplet, q = quartet, m = multiplet, dd = double of doublet. ¹⁹F-NMR (376.5 MHz) spectra were acquired in CDCl₃ in the presence of CFC₃ as internal standard ($\delta = 0$). Mass spectra were acquired with either a GC-MS instrument equipped with a capillary RTX-5ms column (30 m $\times$ 0.25 mm; flow rate, 1 mL/min; carrier gas, He) or by LC-MS. LC-MS analyses were performed on a LCQ Deca MS instrument interfaced with a Surveyor HPLC pump and autosampler (Thermo Fisher Scientific, Waltham, MA). High-resolution mass spectra were acquired with ESI.

3-Fluoro-5-iodobenzonitrile (3)—3-Amino-5-fluorobenzonitrile (2.72 g, 20 mmol) was treated with *n*-butyl nitrite (3.09 g, 35.6 mmol) and CuI (5.71 g, 30 mmol) in MeCN (30 mL) at 80 °C. The mixture was refluxed for 20 min, and allowed to cool to r.t. Then the solvent was evaporated off. The residue was suspended in water (20 mL) and extracted twice with DCM (2 $\times$ 25 mL). The organic layers were combined, washed with brine (15 mL), dried over MgSO₄ and evaporated to dryness under vacuum. Chromatography of the residue on silica gel (hexane-EtOAc; 90: 10, v/v) gave **3** as yellow crystals (2.32 g; yield, 47%). m.p.: 29–30 °C; ¹H-NMR: δ 7.80 (s, 1H), 7.71 (m, 1H), 7.36 (m, 1H); ¹³C-NMR: δ 161.6 (d, $J = 256.03$ Hz), 136.7 (d, $J = 3.8$ Hz), 129.1 (d, $J = 23.20$ Hz), 118.7 (d, $J = 24.34$ Hz), 115.9 (d, $J = 3.23$ Hz), 115.3 (d, $J = 9.59$ Hz), 93.9 (d, $J = 8.23$ Hz); ¹⁹F-NMR: δ –107.54 (s, 1F); GC-MS *m/z*: 247.94 [M + H]⁺.

2-Chloro-4-((trimethylsilyl)ethynyl)thiazole (5)—4-(Trimethylsilyl)ethynylthiazol-2-amine (**4**; 2.25 g, 11.48 mmol) and CuCl (2.85 g, 28.78 mmol) were dissolved in MeCN (25 mL) at r.t. *n*-Butyl nitrite (1.6 mL, 13.7 mmol) was added with stirring, and the solution heated to 75 °C. TLC showed the reaction to be complete after 45 min. The reaction mixture was then evaporated to dryness under vacuum. The residue was dissolved in ethyl acetate (20 mL) and washed with ammonia solution (0.1M; 2 × 50 mL). The organic layer was dried over MgSO₄ and evaporated to dryness under vacuum. Chromatography of the residue on silica gel (hexane-EtOAc; 95: 5, v/v) gave **5** as a white powder (750 mg; yield, 30%). m.p.: 67–68 °C; ¹H-NMR: δ 7.34 (s, 1H), 0.25 (s, 9H); ¹³C-NMR: δ 151.1, 136.7, 125.2, 97.4, 96.3, 0.0; HRMS (ESI+): calc'd for C₈H₁₁NCISSi 207.0070, found 207.0067. HPLC purity: 98.45%.

3-[(2-Aminothiazol)-4-ethynyl]benzonitrile (6a)—Compound **4** (375 mg, 1.91 mmol), 3-iodobenzonitrile (650 mg, 2.85 mmol), CuI (25 mg, 0.12 mmol), Pd(PPh₃)₄ (74 mg, 0.7 mmol), and Et₃N (1.2 mL) were added to dimethylethylene glycol (10 mL) under argon. Argon was bubbled into the resulting dark solution while it was heated to 80 °C. A solution of TBAF (1.0 M) in THF (2.5 mL) was then added via syringe over 25 min. The reaction mixture was heated at 80 °C until TLC showed no starting material present (15 min), and then cooled to r.t. and evaporated to dryness. The residue was dissolved in EtOAc (25 mL), and then washed with water (2 × 40 mL) and brine (1 × 40 mL). The combined organic fractions were dried over MgSO₄, and evaporated to dryness in vacuo. Chromatography of the residue on silica gel (hexane-EtOAc, 70: 30, v/v) gave **6a** (230 mg; yield, 54%) as a brown solid; m.p.: 154–155 °C; ¹H-NMR: δ 7.52 (m, 2H), 7.33 (m, 2H), 6.78 (s, 1H); ¹³C-NMR: δ 166.6, 133.1, 131.7, 128.5, 128.3, 122.6, 113.15, 88.1, 83.9; HRMS (ESI+): calc'd for C₁₁H₉N₂S 201.0486, found 201.0485. HPLC purity: 99.75%.

3-[(2-Aminothiazol)-4-ethynyl]-5-fluorobenzonitrile (6b)—This compound was prepared from **4** and 3-fluoro-5-iodobenzonitrile with the method and chromatography described for **6a** and gave **6b** as a brown powder (288 mg; yield, 62%); m.p.: 209–210 °C; ¹H-NMR: δ 7.64 (m, 1H), 7.59 (s, 1H), 7.55 (m, 1H), 7.44 (m, 1H); ¹³C-NMR: δ 166.5 (d, *J* = 256.10 Hz), 160.7, 152.3, 135.1, 131.2 (d, *J* = 3.56 Hz), 125.9, 123.1 (d, *J* = 22.86 Hz), 118.9 (d, *J* = 24.76 Hz), 116.7, 115.3 (d, *J* = 10.10 Hz), 85.9, 84.6; ¹⁹F-NMR: δ –109.18 (s, 1F); HRMS (ESI+): calc'd for C₁₁H₉N₂S 244.0345, found 244.0334. HPLC purity: 97.32%.

2-(3-((2-Aminothiazol-4-yl)ethynyl)acetonitrile (6c)—This compound was prepared from **4** and 2-(3-iodophenyl)acetonitrile with the method and chromatography described for **6a** and gave **6c** as a brown powder (395 mg; yield, 86%); m.p.: 154–156 °C; ¹H-NMR: δ 7.47 (m, 2H), 7.30 (t, *J* = 7.80 Hz, 1H), 7.31 (t, *J* = 7.8 Hz, 1H), 6.82 (s, 1H), 3.75 (s, 2H); ¹³C-NMR: δ 166.7, 159.5, 133.3 (2 C), 129.6, 124.4, 123.8, 116.5, 115.5, 113.5, 88.3, 83.9, 22.4; HRMS (ESI+): calc'd for C₁₂H₇FN₃S 240.0591, found 240.0595. HPLC purity: 96.40%.

4-(3-Methoxyphenyl)ethynyl(thiazol)-2-amine (6d)—This compound was prepared from **4** and 1-iodo-3-methoxybenzene with the method and chromatography described for **6a** and gave **6d** as a yellow powder (392 mg; yield, 89%); m.p.: 94–95 °C; ¹H-NMR: δ 7.24 (t, *J* = 7.64 Hz, 1H), 7.12 (d, *J* = 7.60 Hz, 1H), 7.05 (s, 1H), 6.89 (m, 1H), 6.78 (s, 1H), 3.80 (s, 3H); ¹³C-NMR: δ 166.8, 159.5, 133.3, 129.6, 124.4, 123.8, 116.5, 115.5, 113.5, 88.3, 83.9, 55.5; HRMS (ESI+): calc'd for C₁₂H₁₁N₂OS 231.0585, found 231.0592. HPLC purity: 97.22%.

3-((2-Chlorothiazol)-4-yl)ethynyl)benzonitrile (7a)—Compound **5** (431 mg, 2.0 mmol), 3-iodobenzonitrile (650 mg, 2.85 mmol), CuI (25 mg, 0.12 mmol), Pd(PPh₃)₄ (74 mg, 0.7 mmol), and Et₃N (1.2 mL) were added to dimethylethylene glycol (10 mL) under argon. Argon was bubbled into the resulting dark solution while it was heated to 80 °C. A solution of TBAF (1.0 M) in THF (2.5 mL) was added via syringe over 25 min. The reaction mixture was stirred at 80 °C. After 15 min TLC showed no starting material present. The reaction mixture was then cooled to r.t. and evaporated to dryness. The residue was dissolved in EtOAc (25 mL), and then washed with water (2 × 40 mL) and brine (1 × 40 mL). The combined organic fractions were dried over MgSO₄, and evaporated to dryness in vacuo. Chromatography of the residue on silica gel (hexane-EtOAc, 85: 15, v/v) gave **7a** as a brown solid (274 mg; 56%); m.p.: 118–120 °C; ¹H-NMR: δ 7.81 (t, *J* = 1.4 Hz, 1H), 7.75 (dt, *J*₁ = 7.88 Hz, *J*₂ = 1.34 Hz, 1H), 7.65 (dt, *J*₁ = 7.84 Hz, *J*₂ = 1.33 Hz, 1H), 7.58 (s, 1H), 7.49 (t, *J* = 7.86 Hz, 1H); ¹³C-NMR: δ 138.1, 136.6, 135.2, 133.2, 132.7, 129.9, 126.1, 123.7, 117.1, 114.4, 87.5, 84.8; HRMS (ESI+): calc'd for C₁₂H₆⁷⁹BrN₂S 244.9936, found 244.9940. HPLC purity: 99.11%.

3-((2-Chlorothiazol)-4-yl)ethynyl)-5-fluorobenzonitrile (7b)—This compound was prepared from **5** and 3-fluoro-5-iodobenzonitrile with the method and chromatography described for **7a** and gave **7b** as a yellow powder (335 mg; yield, 64%); m.p.: 120–121 °C; ¹H-NMR: δ 7.62 (s, 1H), 7.49 (s, 1H), 7.46 (m, 1H), 7.36 (m, 1H); ¹³C-NMR: δ 161.9 (d, *J* = 251.85 Hz), 152.3, 135.1, 131.2 (d, *J* = 3.56 Hz), 125.9, 123.2 (d, *J* = 23.07 Hz), 119.5 (d, *J* = 24.80 Hz), 116.7, 114.4 (d, *J* = 10.22 Hz), 85.9, 84.6; ¹⁹F-NMR: δ –109.12 (s, 1F); HRMS (ESI+): calc'd for C₁₂H₅ClFN₂S 262.9841, found 262.9844. HPLC purity: 98.75%.

2-(3-((2-Chlorothiazol)-4-yl)ethynyl)-phenyl)acetonitrile (7c)—This compound was prepared from **5** and 2-(3-iodophenyl)acetonitrile with the method and chromatography described for **7a** and gave **7c** as a beige powder (346 mg; yield, 67%); m.p.: 108–110 °C; ¹H-NMR: δ 7.52 (m, 1H), 7.50 (m, 1H), 7.43 (s, 1H), 7.36 (m, 2H), 3.75 (s, 2H); ¹³C-NMR: δ 150.7, 135.0, 130.4, 130.1, 129.3, 128.3, 127.4, 123.5, 122.0, 116.3, 87.6, 82.2, 22.4; HRMS (ESI+): calc'd for C₁₃H₈ClN₂S 259.0097, found 259.0098. HPLC purity: 98.62%.

2-Chloro-4-((3-methoxyphenyl)ethynyl)thiazole (7d)—This compound was prepared from **5** and 1-iodo-3-methoxybenzene with the method and chromatography described for **7a** and gave **7d** as a light yellow powder (238 mg; yield, 48%); m.p.: 88 °C; ¹H-NMR: δ 7.39 (s, 1H), 7.26 (t, *J* = 7.96 Hz, 1H), 7.15 (m, 1H), 7.07 (m, 1H), 6.93 (m, 1H), 3.81 (s, 3H); ¹³C-NMR: δ 159.3, 151.6, 136.4, 129.5, 124.4, 124.1, 122.9, 116.5, 115.9, 89.7, 82.1, 55.3; HRMS (ESI+): calc'd for C¹²H⁹CINOS 250.0093, found 250.0097. HPLC purity: 99.08%.

3-((2-Bromothiazol)-4-ethynyl)-5-fluorobenzonitrile (8b)—The 2-aminothiazole derivative **6b** (0.245 g, 1 mmol) and CuBr (0.2 g, 1.39 mmol) were dissolved in MeCN (8 mL) at r.t. *n*-Butyl nitrite (162 μL, 1.39 mmol) was added with stirring, and the solution heated to 60 °C. The reaction was complete after 15 min, as monitored by TLC. The reaction mixture was then evaporated to dryness in vacuo. The residue was dissolved in EtOAc (20 mL) and washed with ammonia solution (0.1 M; 2 × 50 mL). The organic layer was dried over MgSO₄ and evaporated to dryness in vacuo. Chromatography of the residue on silica gel (hexane-EtOAc; 97: 3, v/v) gave **8b** (150 mg; yield, 48%); m.p.: 139–140 °C; ¹H-NMR: δ 7.62 (t, *J*₁ = 1.28 Hz, 1H), 7.54 (s, 1H), 7.47 (ddd, *J*₁ = 8.72 Hz, *J*₂ = 1.36 Hz, *J*₃ = 1.12 Hz, 1H), 7.36 (ddd, *J*₁ = 7.82 Hz, *J*₂ = 1.40 Hz, *J*₃ = 1.12 Hz, 1H); ¹³C-NMR: δ 161.9 (d, *J* = 251.71 Hz), 136.6, 136.3 (d, *J* = 32.20 Hz), 131.2 (d, *J* = 3.56 Hz), 127.6, 125.7 (d, *J* = 10.08

Hz), 123.2 (d, $J = 23.50$ Hz), 119.5 (d, $J = 24.57$ Hz), 116.7, 114.4 (d, $J = 10.20$ Hz), 86.2 ($J = 3.45$ Hz), 85.3; ^{19}F -NMR: $\delta -108.7$ (s, 1F); HRMS (ESI+) calc'd for $\text{C}_{12}\text{H}_5\text{BrFN}_2\text{S}$ 306.9335, found 306.9338. HPLC purity: 98.45%.

2-(3-((2-Bromothiazol-4-yl)ethynyl)phenyl)acetonitrile (8c)—This compound was prepared from the 2-aminothiazole derivative **6c** with the method described for **8b**. Chromatography (hexane-EtOAc, 95: 5, v/v) gave **8c** as a white powder (185 mg; yield, 61%); m.p.: 110–111 °C; ^1H -NMR δ 7.51 (m, 2H), 7.47 (s, 1H), 7.37 (m, 2H), 3.76 (s, 2H); ^{13}C -NMR δ 137.9, 136.1, 131.7, 131.4, 130.5, 129.5, 128.7, 126.4, 123.3, 117.5, 89.2, 83.2, 23.6; HRMS (ESI+): calc'd for $\text{C}_{13}\text{H}_8\text{BrN}_2\text{S}$ 302.9592, found 302.9587. HPLC purity: 98.08%.

2-Bromo-4-((3-methoxyphenyl)ethynyl)thiazole (8d)—This compound was prepared from the amino-thiazole derivative **6d** with the method and chromatography described for **8c** and gave **8d** as a white powder (175 mg; yield, 59%); m.p.: 98 °C; ^1H -NMR: δ 7.44 (s, 1H), 7.25 (t, $J = 8.01$ Hz, 1H), 7.14 (m, 1H), 7.08 (m, 1H), 6.92 (m, 1H), 3.81 (s, 3H); ^{13}C -NMR: δ 159.5, 138.2, 135.9, 129.7, 126.0, 124.5, 123.1, 116.7, 116.1, 90.2, 82.0, 55.5; HRMS (ESI+): calc'd for $\text{C}_{12}\text{H}_9\text{BrNOS}$ 293.9588, found 293.9594. HPLC purity: 97.91 %.

3-[(2-Iodothiazol)-4-ethynyl]-benzonitrile (9a)—This compound was prepared from **6a** with the method described for **8b** but with CuI in place of CuBr. Chromatography (hexane-EtOAc, 95: 5, v/v) gave **9a** as a beige powder (280 mg; yield 83%); m.p.: 118–120 °C; ^1H -NMR: δ 7.81 (t, $J = 1.4$ Hz, 1H), 7.76 (dt, $J_1 = 7.60$ Hz, $J_2 = 1.32$ Hz, 1H), 7.65 (dt, $J_1 = 7.64$ Hz, $J_2 = 1.32$ Hz, 1H), 7.53 (s, 1H), 7.49 (t, $J = 8.00$ Hz, 1H); ^{13}C -NMR: δ 138.1, 136.6, 135.2, 133.2, 132.7, 129.9, 126.1, 123.7, 117.1, 114.4, 87.5, 84.8; HRMS (ESI+): calc'd for $\text{C}_{12}\text{H}_6\text{IN}_2\text{S}$ 336.9296, found 336.9307. HPLC purity: 98.25%.

3-((2-Iodothiazol)-4-ethynyl)-5-fluorobenzonitrile (9b)—This compound was prepared from **6b** with the method described for **9a**. Chromatography (hexane-EtOAc, 90: 10, v/v) gave **9b** as a light yellow powder (250 mg; yield 71%). m.p.: 158–160 °C; ^1H -NMR: δ 7.62 (s, 1H), 7.56 (s, 1H), 7.47 (m, 1H), 7.36 (m, 1H); ^{13}C -NMR: δ 161.9 (d, $J = 251.76$ Hz), 138.5, 131.2 (d, $J = 3.51$ Hz), 130.1, 125.8 (d, $J = 10.01$ Hz), 123.2 (d, $J = 22.95$ Hz), 119.4 (d, $J = 24.76$ Hz), 116.7 (d, $J = 3.05$ Hz), 114.4 (d, $J = 10.18$ Hz), 100.7, 86.5 (d, $J = 3.31$ Hz), 85.0; ^{19}F -NMR: $\delta -108.7$ (s, 1F); HRMS (ESI+): calc'd for $\text{C}_{12}\text{H}_5\text{FIN}_2\text{S}$ 354.9202, found 354.9192. HPLC purity: 99.05%.

2-(3-((2-Iodothiazol-4-yl)ethynyl)phenyl)acetonitrile (9c)—This compound was prepared from **6c** with the method described for **9b**. Chromatography (hexane-EtOAc, 90: 10, v/v) gave **9c** as a beige powder (215 mg; yield 62%). m.p.: 120–122 °C; ^1H -NMR: δ 7.52 (m, 2H), 7.50 (s, 1H), 7.37 (m, 2H), 3.76 (s, 2H); ^{13}C -NMR: δ 139.7, 131.7, 131.4, 130.5, 129.5, 128.9, 128.6, 123.3, 117.5, 100.3, 89.5, 82.8, 23.6; HRMS (ESI+): calc'd for $\text{C}_{13}\text{H}_8\text{IN}_2\text{S}$ 350.9453, found 350.9443. HPLC purity: 97.77%.

2-Iodo-4-((3-methoxyphenyl)ethynyl)thiazole (9d)—This compound was prepared from **6d** with that the method described for **9a**. Chromatography (hexane-EtOAc, 90: 10, v/v) gave **9d** as a pale yellow (170 mg; yield 50%). m.p. 106–108 °C; ^1H -NMR: δ 7.46 (s, 1H), 7.26 (t, $J = 7.95$ Hz, 1H), 7.15 (dt, $J_1 = 7.6$ Hz, $J_2 = 1.16$ Hz, 1H), 7.08 (m, 1H), 6.92 (m, 1H), 3.81 (s, 3H); ^{13}C -NMR: δ 159.3, 139.9, 129.5, 128.2, 124.4, 123.0, 116.5, 115.8, 99.9, 90.3, 81.4, 55.3; HRMS (ESI+): calc'd for $\text{C}_{12}\text{H}_9\text{INOS}$ 341.9450, found 341.9449. HPLC purity: 98.65%.

3-((2-Fluorothiazol-4-yl)ethynyl)benzonitrile (10a)—8a (105 mg, 0.36 mmol) was placed in a microwave vial with Kryptofix@2.2.2 (135 mg, 0.36 mmol), anhydrous KF (42 mg, 0.72 mmol) and DMSO (1 mL). The solution was irradiated with microwaves (30 W, 130 °C 10 min). Reaction was monitored with ¹H-NMR until disappearance of the signal of the proton in C5 in the thiazole ring. The reaction mixture was then poured into water (10 mL) and extracted with EtOAc (3 × 25 mL). The combined organic phases were washed with water (3 × 10 mL) and then brine (10 mL). The organic fraction was dried over MgSO₄, evaporated to dryness under vacuum and chromatographed on silica gel (DCM-hexane, 50: 50, v/v) to give **10a** as an off-white powder (30 mg; yield, 24%); m.p. 113–114 °C; ¹H-NMR: δ 7.81 (t, *J* = 1.4 Hz, 1H), 7.75 (dt, *J*₁ = 7.88 Hz, *J*₂ = 1.34 Hz, 1H), 7.65 (dt, *J*₁ = 7.84 Hz, *J*₂ = 1.33 Hz, 1H), 7.48 (t, *J* = 8.0 Hz, 1H) 7.18 (d, *J*₂ = 1.6 Hz, 1H); ¹³C-NMR: δ 169.1 (d, *J* = 285.7 Hz), 135.8, 135.0, 132.2, 130.0 (d, *J* = 19.2 Hz), 129.4, 123.6, 119.7 (d, *J* = 5.03 Hz), 117.8, 113.1, 86.6, 84.9; ¹⁹F-NMR: δ –77.7 (s); HRMS (ESI+): calc'd for C₁₂H₆FN₂S 229.0231, found 229.0236. HPLC purity: 99.85%.

3-((2-Fluorothiazol-4-yl)ethynyl)-5-fluorobenzonitrile (10b)—The method described for **10a** was applied to **7b** and gave **10b** as a white powder (65 mg; yield, 72%); m.p.: 128–130 °C; ¹H-NMR: δ 7.81 (t, *J* = 1.4 Hz, 1H), 7.75 (dt, *J*₁ = 7.88 Hz, *J*₂ = 1.34 Hz, 1H), 7.65 (dt, *J*₁ = 7.84 Hz, *J*₂ = 1.33 Hz, 1H), 7.48 (t, *J* = 8.0 Hz, 1H) 7.18 (d, *J*₂ = 1.6 Hz, 1H); ¹³C-NMR: δ 169.2 (d, *J* = 286.0 Hz), 161.9 (d, *J* = 251.7 Hz), 131.2 (d, *J* = 3.5 Hz), 129.6 (d, *J* = 19.1 Hz), 125.7 (d, *J* = 9.7 Hz), 123.2 (d, *J* = 22.8 Hz), 120.5 (d, *J* = 4.9 Hz), 119.5 (d, *J* = 24.8 Hz), 115.4, 114.4 (d, *J* = 10.2 Hz), 86.6, 84.9 (d, *J* = 3.6 Hz); ¹⁹F-NMR: δ –109.02 (s, 1F), –77.2 (1F, s); HRMS (ESI+): calc'd for C₁₂H₅F₂N₂S 247.0142, found 247.0133. HPLC purity: 100.00%.

2-(3-((2-Fluorothiazol-4-yl)ethynyl)phenyl)acetonitrile (10c)—The method described for **10a** was applied to **7c** and gave **10c** as a beige powder (68 mg; yield, 78%); m.p.: 106–108 °C; ¹H-NMR: δ 7.52 (m, 2H), 7.47 (s, 1H), 7.35 (m, 2H), 3.76 (s, 2H); ¹³C-NMR: δ 170.1 (d, *J* = 285.1 Hz), 137.9, 136.1, 131.5 (d, *J* = 30.1 Hz), 131.4, 130.5, 129.5, 128.7, 126.4, 123.3, 117.5, 89.2, 83.2, 23.6; ¹⁹F-NMR: δ –78.05 (s, 1F); HRMS (ESI+): calc'd for C₁₃H₈FN₂S 243.0389, found 243.0383. HPLC purity: 98.85%.

2-Fluoro-4-((3-methoxyphenyl)ethynyl)thiazole (10d)—The method described for **10a** was applied to **7d** and gave **10d** as a white crystals (70 mg; yield 65%); m.p.: 64–66 °C. ¹H-NMR: δ 7.26 (t, 1H, *J* = 7.94 Hz), 7.12 (dt, 1H, *J* = 7.96 Hz, *J* = 1.20 Hz), 7.10 (s, 1H), 7.06 (dd, 1H, *J* = 2.6 Hz, *J* = 1.40 Hz), 6.92 (ddd, 1H, *J* = 8.32 Hz, *J* = 2.60 Hz), 3.81 (s, 3H); ¹³C-NMR: δ 169.2 (d, *J* = 284.80 Hz), 159.5, 130.9 (d, *J* = 18.87 Hz), 129.7, 124.6, 123.1 (1C), 118.6 (d, *J* = 4.70 Hz), 116.7, 116.0, 89.4, 82.7, 55.5; ¹⁹F-NMR: δ –78.23 (s); HRMS (ESI+): calc'd for C₁₂H₉FNOS 234.0389, found 234.0383. HPLC purity: 98.95%.

mGlu₅ Binding assay and pharmacological screen

The prepared ligands were assayed for binding to rat brain mGlu₅ receptors by the National Institute of Mental Health (NIMH) Psychoactive Drug Screening Program (PDSP) in an assay using [³H]MPEP (1.0 nM) as radioligand.

The high-affinity ligand **10b** was also screened by the PDSP for binding to a wide variety of receptor and binding sites (5-HT_{1A}, 1B, 1D, 1E, 2A, 2B, 2C, 3, 5A, 6, 7, α_{1A}, 1B, 1D, 2A, 2B, 2C, β_{1–3}, BZP rat brain site, D_{1–5}, DAT, DOR, GABA_A, KOR, H_{1–4}, M_{1–5}, mGlu₁, mGlu₂, mGlu₄, mGlu₆, mGlu₈, MOR, NET, NMDA PCP site, SERT, σ 1–2). Full details of the assays used by the PDSP may be found at their website (<http://pdsp.med.unc.edu/indexR.html>).

Radiochemistry

NCA [^{18}F]fluoride ion was obtained through the $^{18}\text{O}(\text{p},\text{n})\ ^{18}\text{F}$ nuclear reaction by irradiating [^{18}O]water (95 atom %) for 90 to 120 min with a proton beam (17 MeV; 20 μA) produced with a PET trace cyclotron (GE Medical Systems, Milwaukee, MI). Radioactivity was measured with a calibrated ionization calibrator (Atomlab 300; Biodex Medical Systems, Shirley, NY), and corrected for background and physical decay. Radiochemistry was performed in a lead-shielded hot-cell for personnel safety with a Synthia MKII Lab System apparatus equipped with a microwave-heated reactor cavity.²⁶ The reactor cavity was linked via an RF coaxial cable to a controller of both irradiation time and power placed on the outside of the hot-cell. Reactions were performed in a V-vial (1-mL) equipped with a screw-on cap and septum (Tuf-Bond Teflon/silicone). The septum was pierced with a vent needle that was connected to a glass vial (20 mL) to collect any emitted solvents, and also to a charcoal trap to retain any break-through of volatile radioactive species. Liquid handling was achieved with an Aspec auto-injector/dispenser which forms part of the Synthia apparatus. Other operations in the radiosyntheses and purification procedures were controlled by a Visual Chemistry-based recipe.

Cyclotron-produced [^{18}F]fluoride ion (50–100 mCi) in ^{18}O -enriched water (350–500 μL) was dried in the V-vial in the presence of Kryptofix 2.2.2 (13 μmol ; 5 mg), K_2CO_3 (3.6 μmol , 0.5 mg) in $\text{MeCN-H}_2\text{O}$ (9: 1 v/v, 100 μL). Microwave heating (90 W in 3 pulses of 2 min) was applied under nitrogen gas flow (200 mL/min) to speed up the removal of $\text{MeCN-H}_2\text{O}$ azeotrope. The acetonitrile (500 μL) addition and evaporation were repeated twice. Precursor for labeling (~ 1.0–1.5 mg) in MeCN (100 μL) was then introduced into the sealed V-vial and heated at 130 $^\circ\text{C}$ (50 to 90 W in five pulses of 2 min). The reaction mixture was then diluted with water (0.75 mL) and injected onto a Luna C18 column (5 μm , 250×10 mm) eluted at 4 mL/min with $\text{MeCN-H}_2\text{O}$ with MeCN at 45% (v/v) for 2 min, then 75% for 20 min and finally 25% for 30 min. The radioactive product was collected and measured for the calculation of decay-corrected radiochemical yield.

Measurement of specific radioactivity

The specific radioactivity of [^{18}F]**10b** was calculated by injecting a sample of known radioactivity content (0.1–0.4 mCi) into a mass-calibrated analytical HPLC system, comprising a Luna C18 column (5 μm , 4.6×250 mm; Phenomenex) eluted with $\text{MeCN-10 mM HCOONH}_4$ (60: 40 v/v) at 2 mL/min with eluate monitored for absorbance at 254 nm. Specific radioactivity was calculated as the measured mass of **10b** divided by the activity of [^{18}F]**10b**, corrected for physical decay to the time of product isolation. The specific radioactivity of [^{18}F]**10a** was calculated similarly.

Abbreviations

AMPA	α -amino-3-hydroxyl-5-methyl-4-isoxazole-propionate
DCM	dichloromethane
F-PEB	3-fluoro-5-[2-methyl-1,3-thiazol-4-yl]ethynyl]benzonitrile
mGlu	metabotropic glutamate
mGluR	metabotropic glutamate receptor
M-MTEB	3-methyl-5-[(2-methyl-1,3-thiazol-4-yl)ethynyl]benzonitrile
MPEP	2-methyl-6(phenylethynyl)pyridine

NCA	no-carrier-added
NMDA	<i>N</i> -methyl-D-aspartate
PET	positron emission tomography
RCY	decay-corrected radiochemical yields
SPECT	single photon emission computed tomography

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References

1. a) Nakanishi S. Molecular diversity of glutamate receptors and implications for brain function. *Science*. 1992; 258:597–603. [PubMed: 1329206] b) Watkins JC. L-Glutamate as a central neurotransmitter: looking back. *Biochem. Soc. Trans.* 2000; 28:297–310. [PubMed: 10961913]
2. Brauner-Osborne H, Egebjerg J, Nielsen EO, Madsen U, Krosgaard-Larsen P. Ligands for glutamate receptors: design and therapeutic prospects. *J. Med. Chem.* 2000; 43:2609–2645. [PubMed: 10893301]
3. Conn PJ, Pin J-P. Pharmacology and functions of metabotropic glutamate receptors. *Ann. Rev. Pharmacol. Toxicol.* 1997; 37:205–237. [PubMed: 9131252]
4. Pin J-P, Acher F. The metabotropic glutamate receptors: structure, activation mechanism and pharmacology. *Curr. Drug Targets: CNS Neurol. Disord.* 2002; 1:297–317. [PubMed: 12769621]
5. Pietraszek M, Nagel J, Gravius A, Schäfer D, Danysz W. The role of group I metabotropic glutamate receptors in schizophrenia. *Amino Acids*. 2006; 32:173–178. [PubMed: 16699816]
6. Maccarrone M, Rossi S, Bari M, De Chiara V, Rapino C, Musella A, Bernardi G, Bagni C, Centonze D. Abnormal mGlu5 receptor/endocannabinoid coupling in mice lacking FMRP and BC1 RNA. *Neuropsychopharmacol.* 2010; 35:1500–1509.
7. (a) Tsai VWW, Scott HL, Lewis RJ, Dodd PR. The role of group I metabotropic glutamate receptors in neuronal excitotoxicity in Alzheimer's disease. *Neurotoxicity Res.* 2005; 7:125–141. b) Westmark CJ, Westmark PR, Malter JS. Alzheimer's disease and Down syndrome rodent models exhibit audiogenic seizures. *J. Alzheimer's Disease*. 2010; 20:1009–1013.
8. Brodtkin J, Busse C, Sukoff SJ, Varney MA. Anxiolytic-like activity of the mGlu5 antagonist MPEP: a comparison with diazepam and buspirone. *Pharmacol. Biochem. Behav.* 2002; 73:359–366. [PubMed: 12117590]
9. Cosford NDP, Tehrani L, Roppe J, Schweiger E, Smith ND, Anderson JJ, Bristow L, Brodtkin J, Jiang XH, McDonald I, Rao S, Washburn M, Varney MA. 3-[(2-Methyl-1,3-thiazol-4-yl)ethynyl]-pyridine: a potent and highly selective metabotropic glutamate subtype 5 receptor antagonist with anxiolytic activity. *J. Med. Chem.* 2003; 46:204–206. [PubMed: 12519057]
10. Walker K, Bowes M, Panesar M, Davis A, Gentry C, Kesingland A, Gasparini F, Spooren WPJM, Stoeckl N, Pagano A, Flor PI, Varanasic I, Lingenhoehl K, Johnson EC, Varney M, Urban L, Kuhn R. Metabotropic glutamate receptor subtype 5 (mGlu5) and nociceptive function I. Selective blockade of mGlu5 receptors in models of acute, persistent and chronic pain. *Neuropharmacology*. 2001; 40:1–9. [PubMed: 11077065]
11. a) Tessari M, Pilla M, Andreoli M, Hutcheson DM, Heidbreder CA. Antagonism at metabotropic glutamate 5 receptors inhibits nicotine- and cocaine-taking behaviours and prevents nicotine-triggered relapse to nicotine-seeking. *Eur. J. Pharmacol.* 2004; 499:121–133. [PubMed: 15363959] b) Chiamulera C, Epping-Jordan MP, Zocchi A, Marcon C, Cottiny C, Tacconi S, Corsi M, Orzi F,

- Conquet F. Reinforcing and locomotor stimulant effects of cocaine are absent in mGlu5 null mutant mice. *Nature Neurosci.* 2001; 4:873–874. [PubMed: 11528416]
12. Kenny PJ, Markou A. The ups and downs of addiction: role of metabotropic glutamate receptors. *Trends Pharmacol. Sci.* 2004; 25:265–272. [PubMed: 15120493]
13. a) Olive OM, Mcgeehan AJ, Kinder JR, McMahon T, Hodge CW, Janak PH, Messing RO. The mGlu5 antagonist 6-methyl-2-(phenylethynyl)pyridine decreases ethanol consumption via a protein kinase C-epsilon-dependent mechanism. *Mol. Pharmacol.* 2005; 67:349–355. [PubMed: 15548766] b) Backstrom P, Bachteler D, Koch S, Hyytia P, Spanagel R. mGlu5 antagonist MPEP reduces ethanol-seeking and relapse behavior. *Neuropsychopharmacol.* 2004; 29:921–928.
14. Siméon FG, Brown AK, Zoghbi SS, Patterson VM, Innis RB, Pike VW. Synthesis and simple ^{18}F -labeling of a high affinity 2-(fluoromethyl)thiazole derivative (^{18}F]SP203) as a radioligand for imaging brain metabotropic glutamate subtype-5 receptors with PET. *J. Med. Chem.* 2007; 50:3256–3266. [PubMed: 17571866]
15. Brown AK, Kimura Y, Zoghbi SS, Siméon FG, Liow J-S, Kreisl WC, Taku A, Fujita M, Pike VW, Innis RB. Metabotropic glutamate subtype-5 (mGlu5) receptors quantified in human brain with a novel radioligand for positron emission tomography. *J. Nucl. Med.* 2008; 49:2042–2048. [PubMed: 19038998]
16. Kimura Y, Brown AK, Siméon FG, Liow J-S, Gladding RL, Mozley PD, Pike VW, Innis RB, Fujita M. Biodistribution and radiation dosimetry of a positron emission tomography ligand, ^{18}F -SP203, to image metabotropic glutamate subtype 5 (mGlu5) receptor in humans. *Eur J. Nucl. Med. & Mol. Imaging.* 2010; 37:1943–1949. [PubMed: 20585776]
17. Hamill TG, Krause S, Ryan C, Bonnefous C, Govek S, Seiders TJ, Cosford NDP, Roppe J, Kamenecka T, Patel S, Gibson RE, Sanabria S, Riffel K, Eng W, King C, Yang X, Green MD, O'Malley SS, Hargreaves R, Burns HD. Synthesis, characterization, and first successful monkey imaging studies of metabotropic glutamate receptor subtype 5 (mGlu5) PET radiotracers. *Synapse.* 2005; 56:205–216. [PubMed: 15803497]
18. Shetty HU, Zoghbi SS, Siméon FG, Liow J-S, Brown AK, Kannan P, Innis RB, Pike VW. Radiodefluorination of 3-fluoro-5-(2-(2- ^{18}F](fluoromethyl)-thiazol-4-yl)ethynyl) benzonitrile (^{18}F]SP203), a radioligand for imaging brain metabotropic glutamate subtype-5 receptors with positron emission tomography, occurs by glutathionylation in rat brain. *J. Pharmacol. Exper. Ther.* 2008; 327:727–735. [PubMed: 18806125]
19. a) Banks, RE. Fluorine the Enabler. In: Banks, RE.; Lowe, KC., editors. *Fluorine in Medicine in the 21st Century*. New York: Chemserve (UMIST); 1994. b) Campbell TF, Stephens CE. Nuclear fluorination of 2,4-diarylthiazoles with Accufluor. *J. Fluorine Chem.* 2006; 127:1591–1594.
20. Briner PH, Fyfe MCT, Martin P, Murray PJ, Naud F, Procter M. Practical synthesis of 2-amino-5-fluorothiazole hydrochloride. *J. Org. Process. Res. Develop.* 2006; 10:346–348.
21. Pike VW. Positron-emitting radiotracers for studies *in vivo* – probes for human psychopharmacology. *J. Psychopharmacol.* 1993; 7:139–158. [PubMed: 22290661]
22. Siméon FG, Wendahl MT, Pike VW. Efficient and regioselective halogenations of 1,3-thiazoles with copper salts. *J. Org. Chem.* 2009; 74:2578–2581. [PubMed: 19231816]
23. Campbell TF, Stephens CE. Nuclear fluorination of 2,4-diarylthiazoles with Accufluor. *J. Fluor. Chem.* 2006; 127:1591–1594.
24. Nyffeler PT, Gonzalez Durn S, Burkart MD, Vincent SP, Wong C-H. Selectfluor: mechanistic insight and applications. *Angew. Chem. Int. Ed.* 2005; 44:192–212.
25. Siméon FG, Wendahl MT, Pike VW. The ^{18}F 2-fluoro-1,3-thiazolyl moiety - an easily-accessible structural motif for prospective molecular imaging radiotracers. *Tet. Lett.* 2010; 51:6034–6036.
26. Lazarova N, Siméon FG, Musachio JL, Lu SY, Pike VW. Integration of a microwave reactor with Synthia to provide a fully automated radiofluorination module. *J. Label. Compd. Radiopharm.* 2007; 50:463–465.
27. Iso Y, Grajkowska E, Wroblewski JT, Davis J, Goeders NE, Johnson KM, Sanker S, Roth BL, Tueckmantel W, Kozikowski AP. Synthesis and structure-activity relationships of 3-[(2-methyl-1,3-thiazol-4-yl)ethynyl]pyridine analogues as potent, noncompetitive metabotropic glutamate receptor subtype 5 antagonists; search for cocaine medications. *J. Med. Chem.* 2006; 49:1080–1100. [PubMed: 16451073]

Scheme 1^a

^aReagents and conditions: i) CuCl , $n\text{-BuNO}_2$; 30–67% ii) PdPPh_4 , ArI , Et_3N , TBA, DME, 62–89%; iii) CuX , $n\text{-BuNO}_2$ (X = Br or I), 32–83%, See Table 1 for identify of R^1 and R^2 .

Scheme 3^a

^aReactions and conditions. i) $[^{18}\text{F}]\text{FK}$, K 2.2.2., DMSO or MeCN, MW, 130 °C, 10 min, 26 % RCY; ii) $[^{18}\text{F}]\text{FK}$, K 2.2.2., MeCN, MW, 110 °C, 10 min, 26–48 % RCY.

1, SP203 **[¹⁸F]1** **2, F-MTEB** **[¹⁸F]2**

Chart 1.
Structures of some mGlu₅ ligands and derived PET radioligands.

Table 1

Ligand yields, lipophilicities and mGlu₅ affinities.

Entry	X	R ¹	R ²	Yield (%)	cLogD ^a	K _i (nM) ^b
1	FCH ₂	F	CN	NA	3.66 (2.18) ¹⁴	0.03614
2	Me	F	CN	NA	3.42	0.08 ± 0.0217
6a	NH ₂	H	CN	71	2.52	0.90 ± 0.19
6b	NH ₂	F	CN	62	3.06	0.25 ± 0.04
6c	NH ₂	H	CH ₂ CN	86	2.49	5.90 ± 0.95
6d	NH ₂	H	OMe	89	2.72	18.6 ± 0.38
7a	Cl	H	CN	56 ^c	2.88	0.27 ± 0.04
7b	Cl	F	CN	64 ^c	3.41	0.40 ± 0.09
7c	Cl	H	CH ₂ CN	67 ^c	2.99	3.10 ± 0.46
7d	Cl	H	OMe	48 ^c	3.44	8.10 ± 1.36
8a	Br	H	CN	32 ^d	3.38	3.70 ± 0.25
8b	Br	F	CN	48	3.95	0.95 ± 0.10
8c	Br	H	CH ₂ CN	61	3.48	3.30 ± 0.53
8d	Br	H	OMe	59	3.86	22.3 ± 0.50
9a	I	H	CN	83	3.56	4.20 ± 0.50
9b	I	F	CN	71	4.21	1.30 ± 0.23
9c	I	H	CH ₂ CN	62	3.63	23.3 ± 3.53
9d	I	H	OMe	50	3.96	132.9 ± 2.54
10a	F	H	CN	24, 75 ^e	3.12	1.60 ± 0.11 [*]
10b	F	F	CN	35, 72 ^e	3.58	0.28 ± 0.05 [*]
10c	F	H	CH ₂ CN	43, 78 ^e	3.18	3.90 ± 0.56

Entry	X	R ¹	R ²	Yield (%)	cLogD ^a	K _i (nM) ^b
10d	F	H	OMe	33, 65 ^e	3.56	22.7 ± 0.28
10e	F	H	H	46, 78 ^f	3.38	122 ± 3.3

^aThe cLogD was calculated with Pallas 3.4.0 software (Compudrug), except value in parenthesis which is a measured value.

^bValues were determined in triplicate from rat brain homogenates, except those marked

* (n = 6).

^cObtained by coupling from compound **5**.

^dValue reported in reference 22.

^eFrom the 2-bromo and 2-chloro-thiazoles, respectively (each treated with KF in DMSO at 130 °C).

^fFrom the 2-bromo- and 2-chloro-thiazoles, respectively.²⁴

NA = Not applicable.