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Acyl dihydropyrazolo[1,5-*a*]pyrimidinones as metabotropic glutamate receptor 5 positive allosteric modulators

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Abstract

We report the optimization of a series of metabotropic glutamate receptor 5 (mGlu₅) positive allosteric modulators (PAMs) from an acyl dihydropyrazolo[1,5-*a*]pyrimidinone class. Investigation of exocyclic amide transpositions with this unique 5,6-bicyclic core were conducted in attempt to modulate physicochemical properties and identify a suitable backup candidate with a reduced half-life. A potent and selective PAM, 1-(2-(phenoxymethyl)-6,7-dihydropyrazolo[1,5-*a*]pyrimidin-4(5*H*)-yl)ethanone (**9a**, VU0462807), was identified with superior solubility and efficacy in the acute amphetamine-induced hyperlocomotion (AHL) rat model with a minimum

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Supporting information

Synthesis and experimental details for **9a** and **20**, along with routes and conditions used to prepare heterocycles **15-19** are available as supporting information.

effective dose of 3 mg/kg. Attempts to mitigate oxidative metabolism of the western phenoxy of **9a** through extensive modification and profiling are described.

Graphical Abstract

Keywords

metabotropic glutamate receptor 5; mGlu₅; positive allosteric modulator (PAM)

The pursuit of positive allosteric modulators (PAMs) of mGlu₅ as a promising therapeutic approach to treat cognitive deficits in schizophrenia has been stimulated by evidence from several antipsychotic and cognitive animal models.¹⁻⁷ Recently, we reported identification of a mGlu₅ PAM clinical candidate VU0409551/JNJ-46778212 (**1**)⁸ and subsequent efforts⁹ towards a backup candidate within the (2(phenoxyethyl)-6,7-dihydrooxazolo[5,4-c]pyridine-5(4H)-yl(aryl)methanone series focused on enhancing in vivo efficacy through improvements in PK and/or in vitro potency within the series. Prior findings from Merck-Addex¹⁰ and our laboratories¹¹ unveiled a target-mediated CNS adverse-effect liability; which appeared to be driven by excessive PAM cooperativity or allosteric agonism, suggesting that PAMs with lower cooperativity and lacking allosteric agonism may be preferable for obtaining an improved therapeutic index.^{12,13} Interestingly, electrophysiological studies with **1** indicate that signal bias may allow a path for an enhanced therapeutic window, even for PAMs with high efficacy (cooperativity/fold-shift).¹⁴ Although no neurotoxicity was observed for **1** in 14 day oral dose-limiting studies in rat at 450 mg/kg/day (273-440 μ M-h), IND-enabling studies at 360 mg/kg/day for 30 days (253-416 μ M-h) induced Fluoro-Jade C staining in a small number of rats, raising concerns about potential neurotoxicity sensitivity of an mGlu₅ PAM in man.^{8,14}

As one differentiator from the lead series we undertook the development of mGlu₅ PAMs with a low cooperativity profile.¹³ However, in addition to this approach and based on our findings with **1** we wished to pursue back-up contenders specifically within other exocyclic amide subseries (**4**) that might have 1) cooperativity and signaling profiles comparable to **1**, and 2) support a shorter predicted elimination half-life in man (e.g. $T_{1/2}$ 4-6 h versus 12-24 h). Compounds with such a profile may provide a mechanism to test the hypothesis that a short-acting mGlu₅ PAM with a reduced in vivo residence-time and duration of action could avoid CNS toxicity while maintaining a favorable and sufficient efficacy profile. In this case, our screening paradigm consisted of traditional in vitro testing (e.g. calcium mobilization, glutamate fold-shift, mGlu selectivity assays), amphetamine-induced hyperlocomotion (AHL) challenge in rat at a single oral dose, and preclinical PK in rat and dog followed by signaling pathway profiling in vitro and in more native systems for selected compounds. Previous studies in a highly efficient ketone series exemplified by PAM **2**¹⁵ and

the Astra-Zeneca 2,3-dihydro-1*H*-pyrido[2,3-*b*][1,4]oxazine **3**¹⁶ provided the rationale to initiate an amide translocation as illustrated by **4**. Compounds within template **2** were found to have high metabolic turnover relative to **1** despite excellent ligand efficiency and physicochemical properties. In the case of **2** and related congeners this was shown to be primarily connected to the oxidative liability at the benzylic ether. Therefore, backup efforts within **4** appeared to be an attractive starting point to identify shorter acting compounds with the target pharmacological profile. Similar to studies focused within ketone series **2**, the dihydropyrazolo[1,5-*a*]pyrimidine core was utilized to define a preferred translocated exocyclic amide with the target profile.

To this end we focused initially on synthesizing target **9** positioning the amide in an orientation similar to that represented by Astra-Zeneca template **3** (Scheme 1). Synthesis was envisioned to proceed in a strategy similar to that utilized in the preparation of **2**, via incorporation of the western phenoxy and building the 5,6-ring system from left to right. Initial cross-Claisen condensation using ethyl 2-phenoxyacetate and acetonitrile afforded cyano ketone **5** in 24% yield. Treatment of **5** with hydrazine in MeOH generated aminopyrazole **6** in 82% yield. Using **6**, a tandem Michael-lactam formation using methylacrylate in pyridine and water at elevated temperature proceeded in good yield to give key intermediate **7**. Lactam **7** was reduced with borane dimethyl sulfide complex to give amine **8** and subsequently coupled with acids or acyl chlorides to afford targets **9a-r**. Alternatively, treatment of lactam **7** with Boc₂O, followed by Grignard addition (R₂MgBr) and TFA treatment, furnished the desired 5-substituted 6,7-dihydropyrazolo[1,5-*a*]pyrimidine via elimination of the acyl hemiaminal. Reduction of the 6,7-dihydropyrimidine ring using Pd/C with atmospheric hydrogen followed by acylation afforded alpha 5-substituted targets **10a-b**.

To access western phenoxy and various linker analogs in parallel, compound **9a** was deprotected using BBr₃ at low temperature to afford bromide **11** in moderate yield (55%, Scheme 2). Displacement of bromide **11** with alcohols, thiols, and amines proceeded smoothly to afford analogs (**12-14**). Sulfide **13a** could be subsequently oxidized using one or two equivalents of MCPBA to afford sulfone **13b** and sulfoxide **13c**, respectively.

Gratifyingly, the translocated exocyclic amide concept **4** proved successful within the dihydropyrazolo pyrimidine core as acetylated analog **9a** (Table 1), a close comparator to AZ-ether **3**, was a PAM with an EC₅₀ of 360 nM and good efficacy (65% Glu Max). Importantly, no allosteric agonism was detected in cell lines over expressing mGlu₅ or in rat astrocytes, which represents a native tissue setting in which mGlu₅ is endogenously expressed (vide infra **Fig. 4**). In addition, based on potency, MW, and physicochemical properties (cLogP = 2.1), **9a** displayed excellent ligand efficiency metrics (LE = 0.44, LELP = 4.7) comparable to **2** and **3**, thus making it an attractive starting point for further optimization. With exception of ethyl congener **9b** (EC₅₀ = 332 nM), higher alkyl homologs of **9a** proved deleterious; with diminished potency tracking with increasing steric bulk near the amide (**9c** versus **9d**). Saturated carbocycles revealed a similar trend (**9e-9g**); cyclopropyl was preferred (**9e**, EC₅₀ = 559 nM) with potency comparable to methyl (**9a**) and ethyl (**9b**) congeners. Interestingly, phenyl acetate analog **9h** produced a PAM with potency

similar to lead **9a** (EC_{50} = 378 nM) at the obvious cost of ligand efficiency. With respect to aryl moieties, SAR was flat (**9i-9m**) with no general improvements in activity. A low efficacy thiazoly amide **9r** (EC_{50} = 257 nM, Glu Max = 24%) was identified; however, heterocyclic amides more typically displayed weak (isoxazolyl **9q**, pyridyl **9n**, **9o**) or inactive (furan **9p**) profiles. In contrast to SAR within the ketone series **2**, introduction of alpha substituents within the 6,7-dihydropyrazolo[1,5-a]pyrimidine (**Fig. 2**) including 5-methyl (**10a**) and 5-phenyl (**10b**) were flat or lost activity versus **9a**, possibly indicating disruption of a preferred amide conformation.

Utilizing **11** and the method described in Scheme 2 a thorough examination of western phenoxy SAR was undertaken and is shown in Table 2. Fluorine walk within series **12** revealed a strict preference for 3-fluoro congener (**12b**, EC_{50} = 266 nM) as 2-fluoro (**12a**) and 4-fluoro (**12c**) derivatives were weak or inactive. In addition, di-substitution as either difluoro (**12d**) or 3-cyano-5-fluoro (**12e**) afforded weak PAMs and pyridyl derivatives **12f-g** were unproductive. Intermediate **11** was also utilized to revisit broader linker SAR and, in the case of thioether **13a**, weak PAM activity was observed, while the corresponding sulfone (**13b**) and sulfoxide (**13c**) were inactive.

A brief survey of amines proved more interesting as ether linkage replacements. Aniline **14a** demonstrated weak PAM activity; however, the *N*-methyl aniline **14b** displayed robust efficacy (Glu Max = 74%) and an EC_{50} of 700 nM. Attempts to replace the aniline moiety with a [1.1.1] propellane to afford **14c** proved unsuccessful. A series of constrained anilines were then explored to expand **14b**. Indoline **14d** was inactive; however, tetrahydroquinoline (**14e**) displayed weak PAM activity. Based on the weak activity of **14e**, benzoxazine **14f** was prepared to further explore alternative six-member constraints. Gratifyingly, **14f** demonstrated comparable potency and efficacy versus the

acyclic PAM **14b** with an EC_{50} 725 nM.

We next evaluated the in vitro DMPK profiles of selected analogs including **9a**, **12b**, **14b**, and **14f** (Table 3). In hepatic microsomal intrinsic clearance assays, both phenoxy PAMs **9a** and **12b** displayed moderate to high in vitro metabolism with predicted hepatic clearance (CL_{HEP}) > 35 mL min⁻¹ kg⁻¹ in rat and > 12 mL min⁻¹ kg⁻¹ in human. Both amino congeners **14b** and **14f** displayed higher turnover in rat, with similar turnover in human near 50% maximum liver blood flow. Plasma protein binding experiments revealed high fraction unbound in both rat and human plasma for all four compounds (24-43% unbound in rat plasma, 17-46% unbound in human plasma). A rat PK study (0.2 mg/kg, intravenous cassette paradigm, male Sprague-Dawley, n = 2) using **9a** and **12a** confirmed high clearance in vivo with super hepatic clearance (rat CL_p > 70 mL min⁻¹ kg⁻¹). Follow up rat plasma stability studies indicated both **9a** and **12a** were stable (4 h incubation; 37 °C). Further

stability studies in simulated gastric fluid for 1 h (SGF solubility 315 µg/mL, 37 °C) or in vehicle formulations (20% β-CD pH 6, 4 mg/mL) for up to five days also indicated the external amide remained intact with < 5% decomposition. In parallel, both **9a** and **12a** were evaluated in vivo in an amphetamine-induced hyperlocomotion (AHL) challenge model using an oral screening dose of 10 mg/kg.¹⁷ Interestingly as shown in Table 3, **9a** demonstrated a robust 20% reversal of AHL while **12a** was without effect relative to vehicle control (<10% reversal). Terminal mean brain and plasma levels ($n = 6$ animals, T_{90}) were assessed from these studies to inform interpretation of the screening results. PAM **9a** exhibited a terminal brain concentration of 2.25 µM and plasma concentration of 2.15 µM, corresponding to an estimated unbound brain concentration of 1.16 µM (based on a rat $f_{u, \text{brain}} = 0.51$ from brain homogenate binding assay), while the corresponding 3-fluoro derivative **12a** was not detected in brain samples. Thus, based on the performance of **9a** in our pharmacodynamic screen and its apparent enhanced CNS penetration (calculated $K_{p, \text{uu}}$ of 1.4),¹⁸ we opted to further profile **9a** in additional pharmacological endpoints, discrete PK (rat and dog), and in full dose-response studies in rat AHL to determine its minimal effective dose (MED). The dose response profile for **9a** in AHL with unbound terminal brain concentrations is shown in Figure 3 along with an extensive profile overview in Figure 4.

As seen from Figure 3, **9a** displayed a maximum reversal of 55% at a dose of 56.6 mg/kg and a 22% reversal at an MED of 3 mg/kg. At the 3 mg/kg dose, this corresponds to a terminal estimated unbound brain concentration of 199 nM, a three-fold multiple of the rat mGlu₅ potency of 60 nM (Figure 4). To the best of our knowledge, only **9a** and one other dihydroimidazopyrimidinone mGlu₅ PAM from a lactam class¹⁹ have demonstrated an MED of 3 mg/kg (PO) in the AHL antipsychotic efficacy model; however, in the latter case, dihydroimidazopyrimidinone mGlu₅ PAMs¹⁹ showed clear CNS-mediated side effects in dog and in rat modified Irwin test batteries thought to be attributed to high cooperativity and/or mixed mGlu₅/mGlu₃ activity. Evaluation of **9a** in a rat modified Irwin study using a 30 mg/kg PO dose demonstrated no observable alterations in behavior across 30 distinct autonomic and somatomotor endpoints. In this study, **9a** reached an impressive brain concentration of 71 µM 1.0 h post-dose (est. $C_{b, u} = 36$ µM). At a dose of 56.6 mg/kg, 113 µM of **9a** was measured in the brain (est. $C_{b, u} = 67$ µM) and a modified Racine score of 1 noted over a 6.0 h observational period, with animals generally demonstrating decreased motor activity and coordination. Based on the concentrations achieved in the modified Irwin studies and in the AHL dose-response study at the MED, **9a** appeared to provide a large safety window, in this case 100-fold or greater on the basis of these acute testing paradigms. Thus, PAM **9a** represented a considerable improvement relative to candidate **1** (MED of 10 mg/kg)⁸ or related back-up candidates (MEDs of 30 mg/kg)⁹ on the basis of AHL and behavioral CNS toxicity.

Further pharmacological profiling of **9a** in glutamate concentration response curve (CRC) fold-shift experiments with human or rat mGlu₅ receptor expressing cell lines revealed modest maximal leftward fold-shifts (FS) of 5.0 and 4.2 respectively, using 10 µM of PAM (**Fig. 4**). PAM **9a** was also highly selective versus other mGlu family members (mGlu_{1-4,6-8} FS < 1.2) and no noted activity was observed in a broad panel ancillary screen against 68 distinct GPCRs, ion channels and transporters (Eurofins Inc.). In addition, **9a** had an

excellent profile against the major CYP₄₅₀ enzymes (>30 μ M IC₅₀s). Evaluation of **9a** in discrete rat and dog pharmacokinetic studies confirmed the inherent moderate to high clearance of **9a** in both species (**Fig. 4**); however, in rat the elimination $T_{1/2}$ was 6.5 h, a profile consistent with this particular backup effort. After oral administration (10 mg/kg, 20% β -CD, 1 mg/mL) to male Sprague-Dawley rats, **9a** reached an average maximal concentration ($C_{\max}$) of 2.5 μ M with a corresponding time to reach $C_{\max}$ ($T_{\max}$) of 1.0 h and an AUC_{0-8h} of 3.31 μ M-h, thus affording an absolute %F of 29. Unfortunately, in dog both IV and oral PK proved disappointing, with **9a** exhibiting both poor exposure and low bioavailability and a short elimination $T_{1/2}$ of less than 30 minutes.

Despite promising CNS penetration and acute efficacy in vivo in rat AHL, ongoing backup efforts progressing PAM **9a** or related members of the series were considered challenging given the poor dog PK and steep SAR. Similar to prior studies in lead candidate series **1** and within the ketone series **2**, in vitro metabolic soft-spot analysis revealed oxidative metabolism of the electron rich western phenoxy moiety, in addition to the C(4) pyrazole carbon, as major contributors to the observed metabolic instability.

In light of the limited success in addressing the metabolic soft-spot through modification of the western phenoxy ring both herein (e.g. Table 2) and in previous subseries,^{9,15} the medicinal chemistry campaign focused on attempting to mitigate metabolic turnover through modifications of the pyrazole core itself and through modification of the pyrazolo C(4) carbon. Efforts in this vein are briefly summarized in Figure 5. Despite considerable attempts to modify the nature of the heterocyclic core structure (**Fig. 5, 15-19**) or substitute the C(4) position of **9a** with fluorine (**20**), these efforts generally resulted in compounds that suffered from diminished PAM activity or from metabolic and/or plasma instability.

In summary, we have discovered a set of potent mGlu₅ PAMs with high ligand efficiency and excellent CNS penetration inspired by the exocyclic amide **3** and conceptually by the hybrid 5,6-bicyclic template **4**. In an effort to identify PAMs with reduced half-lives and pharmacological target profiles most similar to candidate **1**, PAM **9a** (VU0462807) was identified as a selective and moderate fold-shift mGlu₅ PAM having an exquisite efficacy profile in the acute AHL rat model. Despite our success in identifying a centrally penetrant compound with both improved in vivo potency and a reduced half-life, the overall metabolic instability and correspondingly poor oral F in higher species was deemed unacceptable for development and thus backup efforts in this area were suspended. Ongoing studies utilizing **9a** for other indications and additional chronic studies to better understand PK/PD and duration of action are ongoing and will be communicated in due course.

Supplementary Material

Refer to Web version on PubMed Central for supplementary material.

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References and Notes

1. Awad H, Hubert GW, Smith Y, Levey AI, Conn PJ. *J Neurosci.* 2000; 20:7871.
2. Chavez-Noriega LE, Schaffhauser H, Campbell UC. *Curr Drug Targets CNS Neurol. Disord.* 2002; 1:261.
3. Perroy J, Raynaud F, Homburger V, Rousset MC, Telley L, Bockaert J, Fagni LJ. *Biol. Chem.* 2008; 283:6799.
4. Conn PJ, Lindsley CW, Jones CK. *Trends Pharmacol. Sci.* 2009; 30:25. [PubMed: 19058862]
5. Rosenbrock H, Kramer G, Hobson S, Koros E, Grundl M, Grauert M, Reymann KG, Schroder UH. *Eur J Pharmacol.* 2010; 639:40. [PubMed: 20371241]
6. Stauffer SR. *ACS Chem. Neurosci.* 2011; 2:450. [PubMed: 22860171]
7. Lindsley CW, Stauffer SR. *Pharmaceutical Patent Analyst.* 2013; 2:93. [PubMed: 24236973]
8. Conde-Ceide S, Martinez-Vituro CM, Alcazar J, Garcia-Barrantes PM, Lavreysen H, Mackie C, Vinson PN, Rook JM, Bridges TM, Daniels JS, Megens A, Langlois X, Drinkenburg WH, Ahnaou A, Niswender CM, Jones CK, Macdonald GJ, Steckler T, Conn PJ, Stauffer SR, Bartolome-Nebreda JM, Lindsley CW. *ACS Med. Chem. Letters.* 2015; 6:716.
9. Zhou Y, Malosh C, Conde-Ceide S, Martinez-Vituro CM, Alcazar J, Lavreysen H, Mackie C, Bridges TM, Daniels JS, Niswender CM, Jones CK, Macdonald GJ, Steckler T, Conn PJ, Stauffer SR, Bartolome-Nebreda JM, Lindsley CW. *Bioorg. Med. Chem. Lett.* 2015; 17:3515–3519. [PubMed: 26183084]
10. Parmentier-Batteur S, Hutson PH, Menzel K, Uslaner JM, Mattson BA, O'Brien JA, Magliaro BC, Forest T, Stump CA, Tynebor RM, Anthony NJ, Tucker TJ, Zhang XF, Gomez R, Huszar SL, Lambeng N, Faure H, Le Poul E, Poli S, Rosahl TW, Rocher JP, Hargreaves R, Williams TM. *Neuropharmacology.* 2014; 82:161–173. [PubMed: 23291536]
11. Rook JM, Noetzel MJ, Pouliot WA, Bridges TM, Vinson PN, Cho HP, Zhou Y, Gogliotti RD, Manka JT, Gregory KJ, Stauffer SR, Dudek FE, Xiang Z, Niswender CM, Daniels JS, Jones CK, Lindsley CW, Conn PJ. *Biol. Psychiatry.* 2013; 73:501. [PubMed: 23140665]
12. Turlington M, Noetzel MJ, Chun A, Zhou Y, Gogliotti RD, Nguyen ED, Gregory KJ, Vinson PN, Rook JM, Gogi KK, Xiang Z, Bridges TM, Daniels JS, Jones C, Niswender CM, Meiler J, Conn PJ, Lindsley CW, Stauffer SR. *J. Med. Chem.* 2013; 56:7976. [PubMed: 24050755]
13. Turlington M, Malosh C, Jacobs J, Manka JT, Noetzel MJ, Vinson PN, Jadhav S, Herman EJ, Lavreysen H, Mackie C, Bartolome-Nebreda JM, Conde-Ceide S, Martin-Martin ML, Tong HM, Lopez S, Macdonald GJ, Steckler T, Daniels JS, Weaver C, Niswender CM, Jones C, Conn JP, Lindsley CW, Stauffer SR. *J. Med. Chem.* 2014; 57:5620. [PubMed: 24914612]
14. Rook JM, Xiang Z, Lv X, Ghoshal A, Dickerson JW, Bridges TM, Johnson KA, Foster DJ, Gregory KJ, Vinson PN, Thompson AD, Byun N, Collier RL, Bubser M, Nedelcovych MT, Gould RW, Stauffer SR, Daniels JS, Niswender CM, Lavreysen H, Mackie C, Conde-Ceide S, Alcazar J, Bartolome-Nebreda JM, Macdonald GJ, Talpos JC, Steckler T, Jones CK, Lindsley CW, Conn PJ. *Neuron.* 2015; 86:1029. [PubMed: 25937172]
15. Turlington M, Noetzel MJ, Bridges TM, Vinson PN, Steckler T, Lavreysen H, Mackie C, Bartolome-Nebreda JM, Conde-Ceide S, Tong HM, Macdonald GJ, Daniels JS, Jones CK, Niswender CM, Conn PJ, Lindsley CW, Stauffer SR. *Bioorg. Med. Chem. Lett.* 2014; 24:3641. [PubMed: 24961642]
16. Varnes JG, Marcus AP, Mauger RC, Throner SR, Hoesch V, King MM, Wang X, Sygowski LA, Spear N, Gadiant R, Brown DG, Campbell JB. *Bioorg. Med. Chem. Lett.* 2011; 21:1402. [PubMed: 21295468]
17. Kinney GG, O'Brien JA, Lemaire W, Burno M, Bickel DJ, Clements MK, Chen TB, Wisnoski DD, Lindsley CW, Tiller PR, Smith S, Jacobson MA, Sur C, Duggan ME, Pettibone DJ, Conn PJ, Williams DL Jr. *J. Pharmacol. Exp. Ther.* 2005; 313:199. [PubMed: 15608073]
18. Di L, Rong H, Feng B. *J. Med. Chem.* 2013; 56:2. [PubMed: 23075026]
19. Martin-Martin ML, Bartolome-Nebreda JM, Conde-Ceide S, Alonso de Diego SA, Lopez S, Martinez-Vituro CM, Tong HM, Lavreysen H, Macdonald GJ, Steckler T, Mackie C, Bridges TM, Daniels JS, Niswender CM, Noetzel MJ, Jones CK, Conn PJ, Lindsley CW, Stauffer SR. *Bioorg. Med. Chem. Lett.* 2015; 25:1310. [PubMed: 25683622]

4a
mGlu₅ EC₅₀ = 86 nM
FS = 12.5 (10 μM)

20. Bridges TM, Rook JM, Noetzel MJ, Morrison RD, Zhou Y, Gogliotti RD, Vinson PN, Xiang Z, Jones CK, Niswender CM, Lindsley CW, Stauffer SR, Conn PJ, Daniels JS. Drug Metab. Dispos. 2013; 41:1703. [PubMed: 23821185]

Figure 1.
Structures, mGlu₅ PAM activities, and ligand efficiency metrics of clinical candidate **1** (VU0409551/JNJ-46778212), Vanderbilt ketone **2**, AZ exocyclic amide **3**, and proposed translocated amide system **4**.

Scheme 1.

Reagents and conditions: (a) NaH, CH₃CN, rt-55 °C, 4 h, 24%; (b) H₂NNH₂, EtOH, 85 °C, 16 h, 82%; (c) methylacrylate, pyridine, H₂O, 135 °C, 48 h, 75%; (d) BH₃-SMe₂, THF, 0 – 60 °C, 16 h, 95%; (e) R¹COCl, DCM, Et₃N or R¹CO₂H, Ghosez reagent, DMF, DIPEA, 0 °C – rt, 25-70%; (f) Boc₂O, THF, 76%; (g) R₂MgBr, THF, 0 °C, 33-61%; (h) TFA, DCM, 0 °C, 64-71%; (i) Pd/C, H₂, EtOAc, 82%; (j) R¹COCl, DCM, Et₃N, 0 °C – rt, 22-58%.

Scheme 2.

Reagents and conditions: (a) BBr₃, DCM, 0 °C, 3 h, 55% (b) R₁OH, DMF, K₂CO₃, 140 °C μ wave, 20 min., 33-84; (c) R¹SH, CH₃CN, 120 °C μ wave, 20 min., 81%; (d) MCPBA 1 or 2 equiv, DCM, -20 °C – rt, 2 hr, 24-68, (e) R₂R₃NH, DMF, K₂CO₃, rt, 4-16h, 45-76%.

Figure 2.
Structures and activities of 5-substituted analogs **10**.

Figure 3.

Dose-dependent reversal effect and calculated terminal unbound brain levels of **9a** in AHL in male, SD rats after oral administration ($n = 8$ animals/group).

MW = 271, cLogP = 2.1
LE = 0.44, LELP = 4.7

SGF sol. = 315 µg/mL
Fassif sol. (24h) = 215 µg/mL

hmGlu₅ EC₅₀ = 360 nM, 65% Glu Max
rmGlu₅ EC₅₀ = 60 nM, 65% Glu Max
rat astrocytes EC₅₀ = 360 nM, 57% Glu Max
Fold Shift (h, r) = 5.0, 4.2; mGlu_{1-4,6-8} > 10 µM

CL_{HEP} (h, r, d): 12, 36, 24 mL/min/kg
PPB *f_u* (h, r): 0.26, 0.37; Rat brain *f_u* = 0.52
CYPs (2C9, 3A4, 1A2, 2D6): IC₅₀ > 30 µM

Rat PK (IV/PO, 2.5/10 mg/kg):
CL_p = 54 mL/min/kg, V_{ss} = 3.9 L/kg, t_{1/2} = 6.5 h
Plasma_{AUC} = 3.3 µM-h, C_{max} = 2.5 µM, %F ~ 29

Dog PK (IV/PO, 1.0/5.0 mg/kg):
CL_p = 37 mL/min/kg, V_{ss} = 1.1 L/kg, t_{1/2} = 0.4 h
Plasma_{AUC} = 0.1 µM-h, C_{max} < 0.1 µM, %F < 5

Figure 4.
In vitro and in vivo profile summary of **9a**.

Figure 5.
Isosteric replacements **15-19** and fluoro derivative **20**.

Table 1Structures and activities of analogs **9**.

9				
Entry	R ¹	pEC ₅₀ ^a	EC ₅₀ (nM) ^a	Glu Max % ^a
9a		6.44±0.04	360	65±3
9b		6.48±0.03	332	48±5
9c		5.77±0.11	1,710	59±4
9d		6.05±0.09	882	64±2
9e		6.28±0.05	559	61±6
9f		5.91±0.12	1,220	41±8

9				
Entry	R ¹	pEC ₅₀ ^a	EC ₅₀ (nM) ^a	Glu Max % ^a
9g		5.76±0.11	1,750	41±11
9h		6.43±0.03	378	72±4
9i		6.21±0.06	610	63±5
9j		5.95±0.08	1,130	62±5
9k		6.23±0.07	591	61±6
9l		6.22±0.05	596	63±8
9m		6.41±0.04	390	68±3

9				
Entry	R ¹	pEC ₅₀ ^a	EC ₅₀ (nM) ^a	Glu Max % ^a
9n		<5.00	>10,000	44±11
9o		<5.00	>10,000	51±9
9p		<4.5	Inactive	<15
9q		5.56±0.11	2780	22±7
9r		6.59±0.02	257	24±7

^a Calcium mobilization assay using HEK293 cells expressing human mGlu5; values are the mean±SEM of three or more independent determinations performed in triplicate.

Table 2Structures and activities of linker analogs **12-14**.

Entry	R ¹ X-	pEC ₅₀ ^a	EC ₅₀ (nM) ^a	Glu Max % ^a
12a		5.63±0.13	2,370	60±6
12b		6.62±0.03	266	58±8
12c		<5.00	>10,000	56±4
12d		5.90±0.06	1,270	63±3
12e		<5.00	>10,000	39±9
12f		<4.5	Inactive	<15
12g		<4.5	Inactive	<15
13a		5.41±0.16	3,900	39±8
13b		<4.5	Inactive	<15

Entry	R ¹ X-	pEC ₅₀ ^a	EC ₅₀ (nM) ^a	Glu Max % ^a
13c		<4.5	Inactive	<15
14a		<5.00	>10,000	20±6
14b		6.15±0.06	700	74±3
14c		<4.5	Inactive	<15
14d		<4.5	Inactive	<15
14e		<5.00	>10,000	48±12
14f		6.14±0.07	725	67±4

^a Calcium mobilization assay using HEK293 cells expressing human mGlu5; values are the mean±SEM of three or more independent determinations performed in triplicate.

Table 3

In vitro DMPK, rat IV pharmacokinetics, and rat amphetamine-induced locomotion % reversal from 10 mg/kg PO: **9a**, **12b**, **14c**, and **14f**.^a

Entry	CL _{int} (h, r) ^b	CL _{hep} (h, r) ^b	PPB <i>f</i> _u (h, r)	Rat CL _p ^c	AHL T ₉₀ % reversal ^d
9a	21, 74	12, 36	0.26, 0.37	118	20
12a	23, 62	11, 42	0.17, 0.24	103	<10
14b	18, 269	9, 56	0.46, 0.35	nt	nt
14f	25, 225	12, 53	0.35, 0.43	nt	nt

^a human (h), rat (r), dog (d)

^b mL/min/kg, see Ref. 13 and 17 for methods

^c mL/min/kg, rat IV dose 0.2 mg/kg

^d amphetamine-induced locomotion % reversal see Ref 12, vehicle 20% HP-β-CD; nt= not tested.