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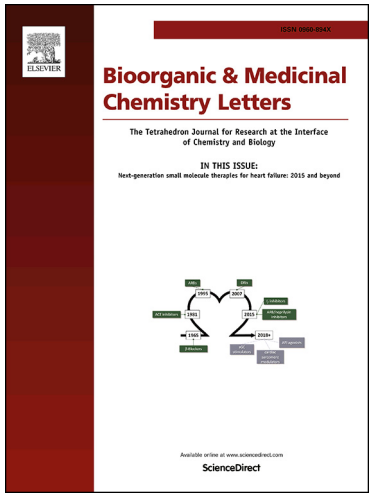

Surveying heterocycles as amide bioisosteres within a series of $mGlu_7$ NAMs: discovery of VU6019278

Carson W. Reed, Jordan P. Washecheck, Marc C. Quitlag, Matthew T. Jenkins, Alice L. Rodriguez, Darren W. Engers, Anna L. Blobaum, P. Jeffrey Conn, Colleen M. Niswender, Craig W. Lindsley

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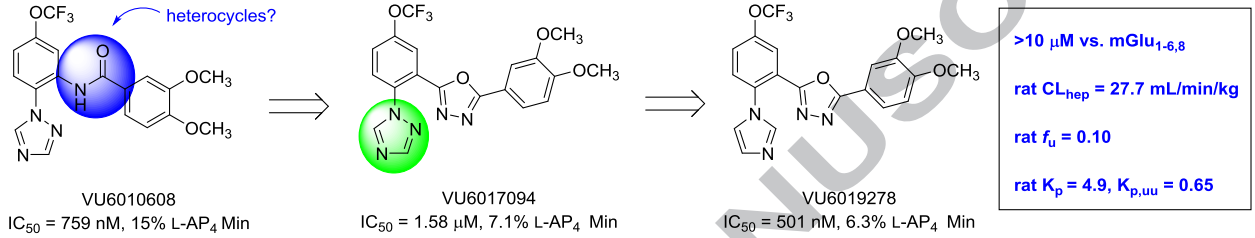

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Surveying heterocycles as amide bioisosteres within a series of $mGlu_7$ NAMs: discovery of VU6019278

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ABSTRACT

This letter describes a diversity-oriented library approach to rapidly assess diverse heterocycles as bioisosteric replacements for a metabolically labile amide moiety within a series of $mGlu_7$ negative allosteric modulators (NAMs). SAR rapidly honed in on either a 1,2,4- or 1,3,4-oxadizaole ring system as an effective bioisostere for the amide. Further optimization of the southern region of the $mGlu_7$ NAM chemotype led to the discovery of VU6019278, a potent $mGlu_7$ NAM (IC_{50} = 501 nM, 6.3% L-AP_4 Min) with favorable plasma protein binding (rat f_u= 0.10), low predicted hepatic clearance (rat CL_{hep} = 27.7 mL/min/kg) and high CNS penetration (rat K_p = 4.9, $K_{p,uu}$ = 0.65).

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Metabotropic glutamate receptor subtype 7 ($mGlu_7$) is an attractive therapeutic target for a number of CNS disorders, including ADHD, schizophrenia, depression, Rett syndrome, epilepsy, anxiety and autism.[1-13] However, of the eight mGlu receptors ($mGlu_{1-8}$), a lack of highly selective *in vivo* tool compounds has severely hindered the field from assessing the true therapeutic potential of activating or inhibiting $mGlu_7$ in the CNS.[14,15] In order to provide subtype selectivity amongst the mGlu family, efforts have largely focused on target modulation through allosteric mechanisms. To date, there are no selective $mGlu_7$ positive allosteric modulators (PAMs), but both *pan*-Group III PAMs and $mGlu_7$-preferring PAMs have been reported.[16] The literature with $mGlu_7$ negative allosteric modulators (NAMs) is more mature (e.g, **1**-**5**),[17-22] but only recently has a robust *in vivo* tool compound, **6** (VU6012962) been reported (**Fig. 1**).[23] Prior to the discovery of **6**, poor physiochemical and DMPK properties precluded key *in vivo* proof of concept studies. Along the path to **6**, NAM **5** was an important step,[22] demonstrating robust efficacy in native tissues (i.e., electrophysiology), but metabolic instability of the amide linker limited *in vivo* studies. Further optimization of **5** followed two parallel tracks: 1) functionalization of the eastern aryl ring to improve DMPK properties and 2) replacement of the metabolically labile amide linker with bioisosteres. Recently, we reported on the first approach, which led to the discovery of **6**.[23] Here, we will describe efforts examining a diverse array of small heterocycles to serve as replacement bioisosteres for the amide linker and the identification of an additional new tool compound and potential lead.

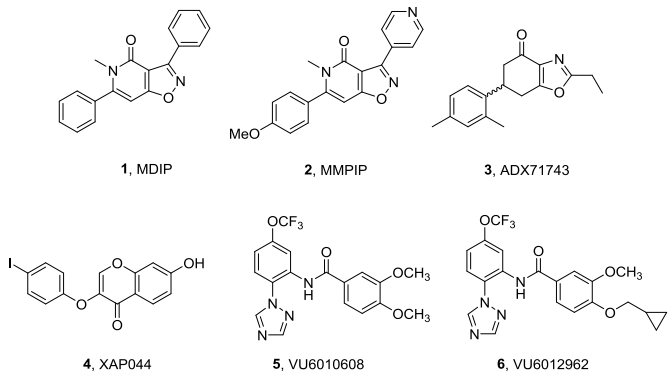

Figure 1. Structures of reported $mGlu_7$ NAMs **1**-**5**, and the key *in vivo* tool compound **6**.

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The optimization plan is depicted in **Figure 2**, where we envisioned surveying a diverse array of heterocycles as amide bioisosteres to afford analogs **7**. If a viable heterocyclic bioisostere could be identified, we would then optimize the substituents on the eastern aryl ring, and ensure that the southern 1,2,4-triazole remained the optimal moiety in the context of analogs **5**.[22]

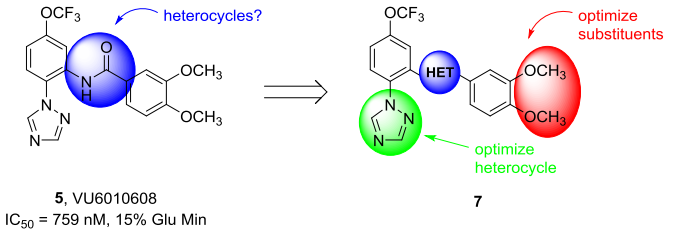


Figure 2. Optimization plan for the labile amide containing **5**. First, heterocyclic bioisoteres for the amide in **5** would be evaluated, followed by optimization of the southern 1,2,4-triazole and the eastern 3,4-dimethoxy phenyl ring.

To survey a wide array of potential heterocyclic bioisosteres for the amide linker in **5**, we held the western (R_1) and eastern (R_2) moieties of **5** constant, and surveyed 13 heterocycles with varying hydrogen-bond donating and accepting capabilities in analogs **7** (**Fig. 3**). SAR was steep, with many inactive analogs ($mGlu_7$ IC_{50} > 30 μM, e..g, **7a**, **7j-m**), a number of weak NAMs (e.g., **7b-d**) and a few with modest activity (e.g., **7e-g**). Interestingly, a 1,3,4-oxadiazole analog **7h** proved to be an acceptable amide replacement ($mGlu_7$ IC_{50} = 1.58 μM, 7.1% L-AP_4 Min), as did a 1,2,4-oxadiazole **7i** ($mGlu_7$ IC_{50} = 1.32 μM, 14.5% L-AP_4 Min); moreover, both analogs were within two-fold of the parent **5**.[22] Prior to optimizing the R_1 and R_2 positions of these oxadiazole biosiosteres, we evaluated their *in vitro* DMPK properties to ensure further consideration was warranted.

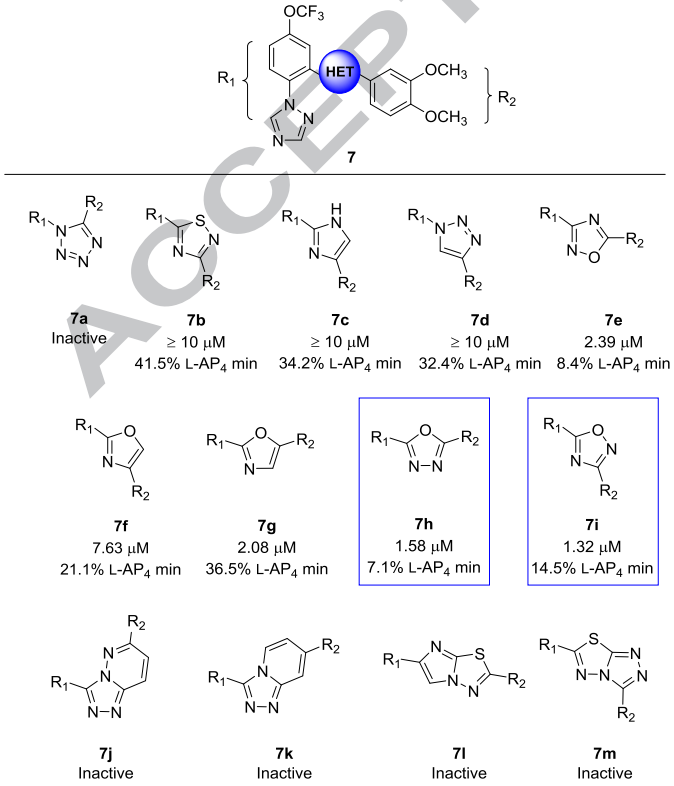
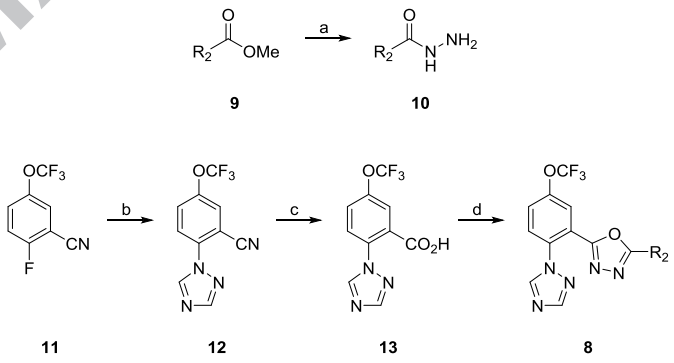


Figure 3. Diversity library assessing a wide array of heterocycles **7** as replacements for the amide linker in **5**. A 1,3,4-oxadiazole **7h** and a 1,2,4-oxadiazole **7i** stood out as viable replacements.

Both NAMs displayed favorable profiles, with predicted hepatic clearances (CL_{hep}) in rat of 27.2 and 26.8 mL/min/kg for **7h** and **7i**, respectively, moderate plasma protein binding (rat f_us of 0.14 and 0.31 for **7h** and **7i**, respectively) and, in the case of **7h**, favorable rat brain homogenate binding (f_u = 0.022).[24] Based on these data, further optimization was pursued around both **7h** and **7i**.

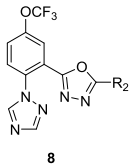
SAR around the 1,2,4-oxadiazole core was steep, with very few analogs displaying even modest $mGlu_7$ NAM potency, and no analogs were more potent than **7i**. Therefore, all attention shifted to a focus on optimization of the 1,3,4-oxadiazole **7h**. A rapid, four-step, microwave-assisted route to analogs **8** was developed (**Scheme 1**) to quickly assess diverse aryl (R_2) moieties. Commercial esters **9** were treated with hydrazine under microwave conditions to provide the analogous acyl hydrazides **10** in 81-88% yields. In parallel, commercial 2-fluoro-5-(trifluoromethoxy)benzonitrile **11** undergoes an S_NAr reaction with 1*H*-1,2,4-triazole to deliver **12** in 67% yield, followed by hydrolysis of the nitrile to the acid **13**. Finally, condensation of acyl hydrazides **10** with acid **13** with T3P® under microwave conditions provided analogs **8**.

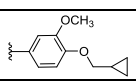
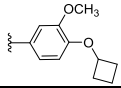
Scheme 1. Synthesis of putative $mGlu_7$ NAM analogs **8**.[a]



[a]Reagents and conditions: (a) $H_2NNH_2{\cdot}H_2O$, EtOH, 160 °C, mw, 1h, 81-88%; (b) 1*H*-1,2,4-triazole, K_2CO_3, DMF, 150 °C, mw, 30 min, 67%; (c) KOH, EtOH:H_2O (1:1), 160 °C, mw, 30 min, 51%; (d) **10**, propylphosphonic anhydride (T3P®), Et_3N, 150 °C, mw, 1h, 27-42%.

Table 1. Structure and $mGlu_7$ NAM activities of analogs **8**.



Cmpd	Het	$mGlu_7$ IC_{50} (μM)[a]	$mGlu_7$ % L-AP_4 Min
8a		1.31	13.2
8b		1.55	10.4
8c		>30	-

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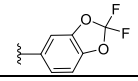
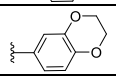
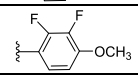
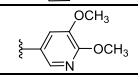
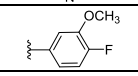
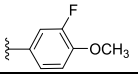
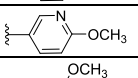
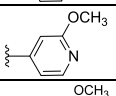
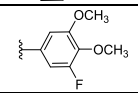
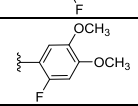
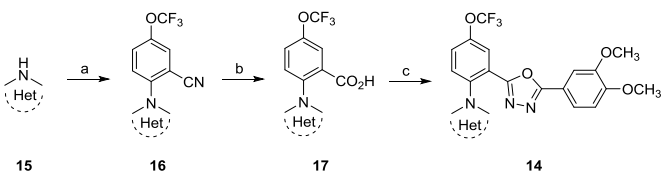
8d		>30	-
8e		>30	-
8f		>30	-
8g		4.38	13.5
8h		>30	-
8i		>30	-
8j		>30	-
8k		≥10	68.9
8l		>30	-
8m		≥10	72.8

[a]For SAR determination, calcium mobilization assays with rat $mGlu_7/G_{\alpha 15}$/HEK cells performed in the presence of an EC_{80} fixed concentration of glutamate; values represent (n = 1) independent experiments performed in triplicate.

As shown in **Table 1**, SAR amongst analogs **8** was very steep, affording few active $mGlu_7$ NAMs. Of the very few active compounds, **8a** ($mGlu_7$ IC_{50} = 1.31 μM, 13.2% L-AP_4 Min) was a 1,3,4-oxadiazole analog of **6**, and a cyclobutyl ether congener **8b** of **5** was also of comparable activity ($mGlu_7$ IC_{50} = 1.55 μM, 10.4% L-AP_4 Min). Alternate functionality and/or incorporation of a heteroatom (**8g**, **8j** and **8k**) all led to inactive or a significant diminution in NAM activity. To compare with **7h**, we evaluated the DMPK profile of **8a**. NAM **8a** displayed very low predicted hepatic clearance in rat (CL_{hep} = 2.9 mL/min/kg) in combination with favorable plasma protein binding (f_u = 0.038). Moreover, a rat plasma:brain level (PBL) study demonstrated that **8a** was CNS penetrant (K_p = 2.0, $K_{p,uu}$ = 0.43). This positive disposition data supported further optimization efforts to enhance $mGlu_7$ NAM potency, while hopefully, maintaining an attractive DMPK profile.

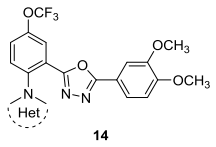
Based on these data, we elected to hold the 3,4-dimethoxyphenyl moiety of **7h** constant and surveyed saturated and unsaturated nitrogen-containing heterocycles as replacements for the 1*H*-1,2,4-triazole in *N*-linked analogs **14**. Once again, an S_NAr with **11**, and various saturated and unsaturated nitrogen-containing heterocycles **15**, provided *N*-linked derivatives **16**. Hydrolysis of the nitrile delivers acid **17**, which undergoes a T3P®-mediated microwave–assisted condensation with 3,4-dimethoxybenzohydrazide to provide putative $mGlu_7$ NAMs **14**.

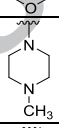
Scheme 2. Synthesis of putative $mGlu_7$ NAM analogs **14**.[a]



[a]Reagents and conditions: (a) **11**, K_2CO_3, DMF, 150 °C, mw, 30 min, 54-72%; (b) KOH, EtOH:H_2O (1:1), 160 °C, mw, 30 min, 38-51%; (c) 3,4-dimethoxybenzohydrazide, propylphosphonic anhydride (T3P®), Et_3N, 150 °C, mw, 1h, 22-62%.

Table 2. Structure and $mGlu_7$ NAM activities of analogs **14**.



Cmpd	Het	$mGlu_7$ IC_{50} (μM)[a]	$mGlu_7$ % L-AP_4 Min
14a		>30	-
14b		5.86	65.4
14c		1.18	23.1
14d		>30	-
14e		0.57	6.3
14f		2.04	50.6

[a]For SAR determination, calcium mobilization assays with rat $mGlu_7/G_{\alpha 15}$/HEK cells performed in the presence of an EC_{80} fixed concentration of glutamate; values represent (n = 1) independent experiments performed in triplicate.

As shown in **Table 2**, analogs **14** proved more tractable towards producing $mGlu_7$ NAMs. Saturated azacines, such as pyrrolidine (**14a**) lacked $mGlu_7$ NAM activity, but ring expansion to the piperidine derivative (**14b**) afforded a weak partial NAM, while a morpholine congener (**14c**) proved as potent ($mGlu_7$ IC_{50} = 1.18 μM, 23.1% L-AP_4 Min) as **7h**. In contrast, a basic piperazine analog was inactive. Replacement of the 1,2,4-triazole with an imidazole ring (i.e., **14e**, VU6019278) was highly successful, affording the most potent and efficacious $mGlu_7$ NAM ($mGlu_7$ IC_{50} = 571 nM, 6.3% L-AP_4 Min) discovered during this campaign, and more potent than **5**. When assayed in triplicate, the potency of **14e** further improved (IC_{50} = 501 nM, pIC_{50} = 6.30±0.11, 6.3±1.0 L-AP_4 min). Moreover, NAM **14e** displayed low predicted hepatic clearance in rat (CL_{hep} = 27.7 mL/min/kg) in combination with favorable plasma protein binding (f_u = 0.10) and rat brain homogenate binding (f_u = 0.013). Finally, a rat plasma:brain level (PBL) study demonstrated that **14e** was highly CNS penetrant (K_p = 4.9, $K_{p,uu}$ = 0.65), offering improvements over NAM **5**.[22] Finally, NAM **14e** was also selective versus the other seven mGlu receptors (>10 μM vs. $mGlu_{1\text{-}6,8}$).

Since the *N*-linked southern azacines **14** proved attractive, we elected to explore the viability of C-linked heterocyclic congeners **18** as $mGlu_7$ NAMs. Analogs **18** were readily prepared in two steps from commercial benzoic acid **19** (**Scheme 3**). A T3P®-mediated microwave–assisted condensation with 3,4-dimethoxybenzohydrazide and **19** delivers **20** in acceptable yield (43%). Next, a Suzuki coupling with various heteroaryl boronic acids provides putative $mGlu_7$ NAMs **18** in yields ranging from 36-60%.

Representative structures and activities for tricyclic analogs **18** are highlighted in **Table 3**. While not as productive as the *N*-

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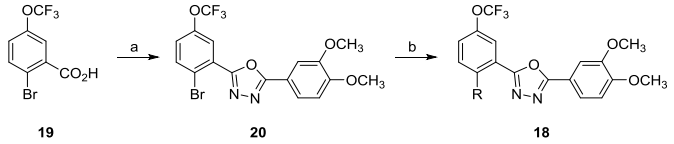
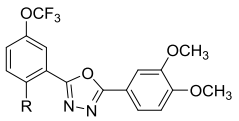
liked congeners **14**, interesting SAR resulted. C-linked 6-membered heterocycles, both pyridines (**18a**,**b**) and a pyridazine (**18c**) were inactive. A 4-pyrazole derivative (**18d**) was a modest $mGlu_7$ NAM ($mGlu_7$ IC_{50} = 2.24 μM, 10.2% L-AP_4 Min), whereas the *N*-Me congener (**18e**) lost all $mGlu_7$ NAM activity. However, an *N*-Me, 5-pyrazole analog (**18f**), a regioisomer of inactive **18e**, proved to be the most potent $mGlu_7$ NAM in this subseries ($mGlu_7$ IC_{50} = 1.32 μM, 20.6% L-AP_4 Min).

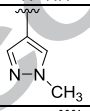
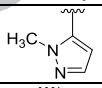
Scheme 3. Synthesis of putative $mGlu_7$ NAM analogs **18**.[a]



[a]Reagents and conditions: (a) 3,4-dimethoxybenzohydrazide, propylphosphonic anhydride (T3P®), Et_3N, 150 °C, mw, 1h, 43%; (b) R-$B(OH)_2$, Cs_2CO_3, $Pd(dppf)Cl_2$, 1,4-dioxane:H_2O (9:1), 90 °C, 16h, 36-60%.

Table 3. Structure and $mGlu_7$ NAM activities of analogs **18**.



Cmpd	R	$mGlu_7$ IC_{50} (μM)[a]	$mGlu_7$ % L-AP_4 Min
18a		>30	-
18b		≥10	27.9
18c		≥10	27.6
18d		2.24	10.3
18e		>30	-
18f		1.32	20.6
18g		3.53	49.2

[a]For SAR determination, calcium mobilization assays with rat $mGlu_7/G_{\alpha 15}$/HEK cells performed in the presence of an EC_{80} fixed concentration of glutamate; values represent (n = 1) independent experiments performed in triplicate.

In summary, we detailed a diversity-oriented library approach to rapidly assess diverse heterocycles as bioisosteric replacements for a metabolically labile amide moiety within a series of $mGlu_7$ negative allosteric modulators (NAMs), exemplified by **5**. SAR rapidly identified a 1,3,4-oxadiazole ring system as an effective bioisostere for the amide linker. Further optimization of the southern heterocycle of this new chemotype led to the discovery of VU6019278 (**14e**), a potent $mGlu_7$ NAM (IC_{50} = 501 nM, 6.3% L-AP_4 Min) with favorable plasma protein binding (rat f_u= 0.10), low predicted hepatic clearance in microsomal incubations (rat CL_{hep} = 27.7 mL/min/kg) and high CNS penetration (rat K_p = 4.9, $K_{p,uu}$ = 0.65). Not only was **14e** more potent than **5**, at the 0.2 mg//kg IV PBL dose, **14** achieve total brain levels of 456 nM, comparable to the *in vitro* IC_{50} (501 nM), whereas **5** was relegated as an *in vitro* tool due to low total brain concentrations. However, free brain levels for **14e** from the PBL cassette study, based on rat brain homogenate binding, suggest ~6 nM free brain concentration at 0.2 mg/kg (if dose linear, doses of 10-30 mg/kg would afford free brain levels of **14e** above the *in vitro* IC_{50}). NAM **14e** represented an improvement over the prototypical NAMs **1**-**5**, and argued for additional exploration of this subseries. Efforts in this vein are underway, and results will be reported in due course.

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HIGHLIGHTS

- Novel series of selective and CNS penetrant $mGlu_7$ NAMs
- Identified a 1,3,4-oxadiazole as biosiostere for a labile amide linker
- Iterative libraries quickly optimized this novel tricyclic series.

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