Substituted 1-Phenyl-3-(pyridin-2-yl)urea Negative Allosteric Modulators of mGlu₅: Discovery of a New Tool Compound VU0463841 with Activity in Rat Models of Cocaine Addiction

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ABSTRACT: Cocaine is a powerful and highly addictive stimulant that disrupts the normal reward circuitry in the central nervous system (CNS), producing euphoric effects. Cocaine use can lead to acute and life threatening emergencies, and abuse is associated with increased risk for contracting infectious diseases. Though certain types of behavioral therapy have proven effective for treatment of cocaine addiction, relapse remains high, and there are currently no approved medications for the treatment of cocaine abuse. Evidence has

29 (VU0463841) Cocaine Self-Administration

29 (VU0463841)
mGlu₅ IC₅₀ = 13 nM
other mGlus > 30 μM

continued to accumulate that indicates a critical role for the metabotropic glutamate receptor subtype 5 (mGlu₅) in the modulation of neural circuitry associated with the addictive properties of cocaine. While the small molecule mGlu₅ negative allosteric modulator (NAM) field is relatively advanced, investigation into the potential of small molecule mGlu₅ NAMs for the treatment of cocaine addiction remains an area of high interest. Herein we describe the discovery and characterization of a potent and selective compound **29** (VU0463841) with good CNS exposure in rats. The utility of **29** (VU0463841) was demonstrated by its ability to attenuate drug seeking behaviors in relevant rat models of cocaine addiction.

KEYWORDS: mGlu₅, negative allosteric modulator, noncompetitive antagonist, CNS, cocaine, addiction
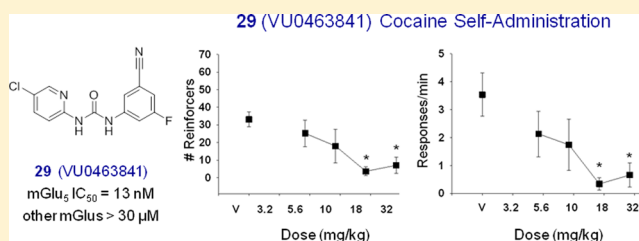
Cocaine is a naturally occurring chemical that can be isolated from coca leaves and is one of the oldest known psychoactive compounds. It is a powerful and highly addictive stimulant that produces its effects in the central nervous system (CNS) by binding to the dopamine transporter (DAT) thereby blocking the normal recycling process and resulting in an accumulation of dopamine in the synapse. Such effects disrupt the normal reward circuitry and produce the euphoric effects associated with the drug. Repeated cocaine use leads to profound changes in the brain's reward pathway and increased tolerance to the drug's effects, leading many abusers to increase their dose and/or frequency of use. Cocaine use can lead to acute and life threatening emergencies such as cardiac arrest, stroke, and seizure. Furthermore, cocaine abuse is associated with increased risk for contracting infectious diseases such as human immunodeficiency virus (HIV) and hepatitis C virus (HCV). Certain types of behavioral therapy have proven effective for treatment of cocaine addiction and the prevention of relapse. Unfortunately, due to the intensely addictive nature of cocaine, relapse remains high, even following long periods of abstinence. Moreover, there are currently no medications that have been approved by the Food and Drug Administration (FDA) for the treatment of cocaine abuse. Hence, interest in

the discovery and evaluation of new pharmacological approaches to treatment of the disease remains high in the research community.[1,2]

Accumulating evidence has indicated a critical role for the metabotropic glutamate receptor subtype 5 (mGlu₅) in the modulation of cortical and limbic neural circuitry that underlie the addictive properties of many common drugs of abuse, including cocaine.[3] The metabotropic glutamate receptors (mGlus) are a family of eight related G-protein-coupled receptors (GPCRs) that have been classified into three groups according to their structure, preferred signal transduction mechanisms, and pharmacology. The Group I receptors include mGlu₁ and mGlu₅, which are both coupled to Gαq, an event that leads to an increase in intracellular calcium.[4,5] While the development of selective orthosteric mGlu ligands has often been complicated by the highly conserved binding site across the family, substantial success has been realized of late with the development of allosteric modulators of the mGlus.[6−9]
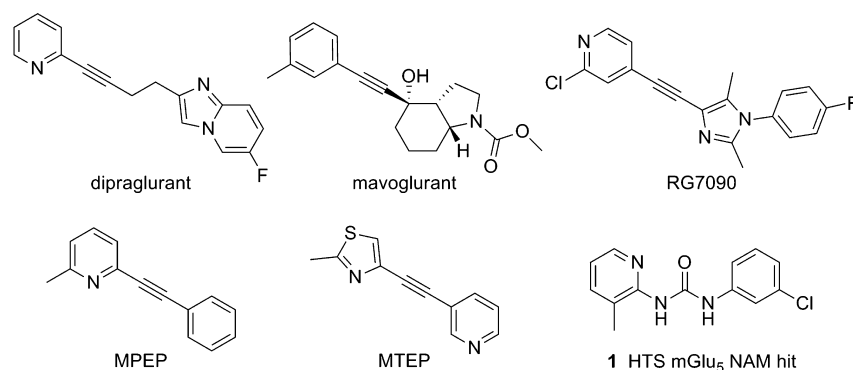
Figure 1. Top row: mGlu$_5$ NAMs in active clinical trials. Bottom row: mGlu$_5$ NAM tools and urea HTS hit.

Negative allosteric modulators (NAMs), also termed noncompetitive antagonists, of mGlu$_5$ are one of the more well developed areas within this field. A number of small molecule mGlu$_5$ NAMs have been developed and evaluated in behavioral models of a variety of CNS disorders including addiction, anxiety, autism, depression, fragile X syndrome (FXS), pain, and Parkinson's disease levodopa induced dyskinesia (PD-LID).[10−14] Furthermore, multiple mGlu$_5$ NAMs have advanced to clinical trials, and at least three compounds, dipraglurant, mavoglurant, and RG7090 (Figure 1), remain active in the clinic.[10] Nevertheless, none of the current trials either ongoing or recruiting for these mGlu$_5$ NAMs include studies in patients suffering from cocaine addiction.[15]

Mutant mGlu$_5$-null mice do not self-administer cocaine[16,17] and are insensitive to its locomotor-stimulating effects. Consistent with this phenotype, the well-known mGlu$_5$ NAM tool compounds MPEP[18] and MTEP[19] (Figure 1) have demonstrated efficacy in several animal models relevant to cocaine abuse. Included among these accounts are decreased cocaine self-administration,[16,17,20−23] cocaine-induced place preference,[24] and cue-induced reinstatement of cocaine self-administration.[20,25−27] In contrast, neither mGlu$_5$ gene deletions nor mGlu$_5$ NAMs alter responding for food in rodents.[16,28] These data suggest that mGlu$_5$ has an important function in modulating the positive reinforcing properties of cocaine and potentially other drugs of abuse without affecting general reward mechanisms that are critical for feeding. However, certain aspects of addiction seem to be differentially affected by mGlu$_5$ NAMs. For example, researchers have shown that MTEP selectively decreases cocaine self-administration when compared with sweetened condensed milk (SCM).[26] However, the effects of MTEP on cue-induced reinstatement postextinction appeared to be nonselective for cocaine when compared with SCM. This would suggest that treatment with an mGlu$_5$ NAM may have greater efficacy in blocking the reinforcing effects of cocaine, rather than nonselectively blocking reinstatement for rewarding stimuli. Taken together, the development of selective mGlu$_5$ NAMs for the reduction of cocaine reward and compulsive drug seeking behaviors in humans represents an exciting novel approach for the treatment of addiction.
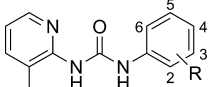
As can be seen above (Figure 1), the mGlu$_5$ NAMs in ongoing clinical trials as well as the tools MPEP and MTEP possess a common disubstituted alkyne structural motif. One strategy that can help guard against unforeseen future development issues when pursuing a target where advanced compounds are already known is the pursuit of structural diversity. We have been actively engaged in the design and development of novel mGlu$_5$ NAMs from within chemotypes devoid of an alkyne functional group. We have recently reported several successful examples of our work in this area that involved rapid optimization of hits identified in a functional cell-based high-throughput screen (HTS) of a collection of 160,000 compounds.[29−31] In addition to HTS hit optimization, we have also employed rational design approaches[32−34] and a virtual screening strategy[35] toward this end. As we previously disclosed, concentration response curves (CRCs) collected from the 624 putative mGlu$_5$ antagonists identified in the HTS produced 345 confirmed mGlu$_5$ antagonists with urea **1** representing a compound of interest (Figure 1).[29−31] Herein we disclose the optimization program initiated from urea **1** that culminated in the identification of a potent and selective compound **29** (VU0463841) with good CNS exposure following intraperitoneal (IP) dosing in rats. Finally, the utility of **29** (VU0463841) as a tool compound was demonstrated by its ability to attenuate drug seeking behaviors in relevant rat models of cocaine addiction.

■ RESULTS AND DISCUSSION

Among the features that made urea **1** an attractive starting point for a medicinal chemistry optimization program was its low molecular weight and the simple chemistry required to prepare a diverse set of analogues within the chemotype. In fact, most analogues of interest were prepared in a single step through the reaction of an appropriate amine with an isocyanate.[36] Our first library of analogues held the western 3-methylpyridin-2-yl group constant while varying substituents on the eastern aniline ring (Table 1). The functional activity of new analogues was measured in our primary assay for mGlu$_5$ NAM lead optimization. This assay measures the ability of the compound to block the mobilization of calcium induced by an EC$_{80}$ concentration of glutamate in HEK293A cells expressing rat mGlu$_5$. Compounds were tested in at least three separate experiments, and the results were averaged. This small library contained compounds with a variety of pharmacological profiles (Figure 2). Several compounds (**3**, **4**, **7**, **10**, and **13**) were inactive at 30 μM, which was the highest concentration tested. Other compounds (**1**, **2**, **5**, and **9**) were classified as weak antagonists. Weak antagonists clearly inhibit the glutamate response at higher concentrations; however, the CRC does not plateau. Compounds **6** and **8** were full antagonists and demonstrated a typical antagonist CRC. On the other hand, 2-fluoro analogue **11** was a partial antagonist, possessing a CRC that reached a plateau well above baseline. We have noted partial antagonists within the MPEP[37,38] and other scaffolds[32−34] previously, though instances of potent partial

Table 1. Initial Urea SAR

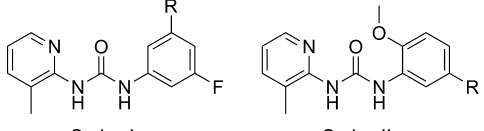| entry	R	mGlu$_5$ pIC$_{50}$ ($\pm$SEM)[a]	mGlu$_5$ IC$_{50}$ (nM)	% Glu Max[ab]
1	3-Cl	<5.0[c]	>10 000	26.0 ± 8.8
2	H	<5.0[c]	>10 000	20.4 ± 7.3
3	2-Cl	<4.5	>30 000	
4	4-Cl	<4.5	>30 000	
5	2-CH$_3$	<5.0[c]	>10 000	57.9 ± 10.5
6	3-CH$_3$	5.91 ± 0.30	1220	2.1 ± 0.5
7	4-CH$_3$	<4.5	>30 000	
8	2-OCH$_3$	6.47 ± 0.10	338	1.8 ± 0.3
9	3-OCH$_3$	<5.0[c]	>10 000	22.9 ± 10.0
10	4-OCH$_3$	<4.5	>30 000	
11	2-F	5.72 ± 0.11	1910	23.0 ± 4.9
12	3-F	5.83 ± 0.22	1460	10.5 ± 4.5
13	4-F	<4.5	>30 000	

[a]Calcium mobilization mGlu$_5$ assay; values are average of $n \geq 3$. [b]Amplitude of response in the presence of 30 μM test compound as a percentage of maximal response (100 μM glutamate); average of $n \geq 3$. [c]CRC does not plateau.

Table 2. Disubstituted Aniline Urea SAR

Series I   Series II

entry	series	R	mGlu$_5$ pIC$_{50}$ ($\pm$SEM)[a]	mGlu$_5$ IC$_{50}$ (nM)	% Glu Max[ab]
14	I	F	6.70 ± 0.09	201	19.1 ± 6.7
15	I	CH$_3$	6.81 ± 0.05	154	14.2 ± 7.9
16	I	CN	7.08 ± 0.21	83	38.2 ± 10.4
17	II	CH$_3$	6.65 ± 0.04	224	2.2 ± 0.8
18	II	Cl	6.88 ± 0.22	132	26.4 ± 14.4

[a]Calcium mobilization mGlu$_5$ assay; values are average of $n \geq 3$. [b]Amplitude of response in the presence of 30 μM test compound as a percentage of maximal response (100 μM glutamate); average of $n \geq 3$.

antagonism was relatively rare within these series. As will be made clear herein, the prevalence of potent partial antagonists within this urea series was higher than typically observed in other mGlu$_5$ NAM chemotypes that we have explored. Although a clear explanation for this observation was not forthcoming, care was taken to ensure that issues such as solubility did not provide misleading results. For example, we ensured that the compounds remained in solution at the highest concentrations for each of the examples reported herein. We typically classify compounds as partial antagonist when the CRC plateaus above 10% of the glutamate maximum, which consequently makes 3-fluoro analogue **12** a borderline case as far as categorization. Some important structure–activity relationships (SAR) were also gleaned from this initial library. We saw that substitution of the 4-position was detrimental to activity, while substitution at both the 2- and 3-positions could improve potency depending on the nature of the substituent. The full antagonist 2-methoxy analogue **8** represented a major step forward, demonstrating an improvement of at least 30-fold relative to both hit compound **1** and unsubstituted analogue **2**.

A follow up library was executed based on these initial results (Table 2). Given the improved activity observed with the 3-methyl (**6**), 2-methoxy (**8**), and 3-fluoro (**12**) analogues, we decided to prepare disubstituted aniline analogues that also

contained at least one of these functional groups. Gratifyingly, this strategy proved effective for further enhancing the potency of compounds within this chemotype. For example, the 3,5-difluoro analogue **14** was approximately 7-fold more potent than 3-fluoro analogue **12**, though **14** does appear to be a clear partial antagonist. A similar observation was made with 3-fluoro-5-methyl analogue **15** which was a borderline partial antagonist. Compound **15** was more than 9-fold potent than **12** and approximately 8-fold more potent than **6**. Further potency enhancement was observed with 3-cyano-5-flouro analogue **16**, which was a partial antagonist that demonstrated a more than 17-fold improvement in potency relative to **12**. The 3-cyano-5-fluorophenyl motif has been noted as a common advantageous structural feature across multiple chemotypes within the mGlu$_5$ NAM field.[33,34,39−43] Incorporation of a methyl (**17**) and a chloro (**18**) group in combination with the 2-methoxy substituent provided small potency enhancements relative to analogue **8**.

Our third library was designed to evaluate the SAR around the western pyridine ring while holding the eastern aniline constant as either the 3,5-difluoroaniline or the 3-cyano-5-fluoroaniline (Table 3). Deletion of the 3-methyl group found in analogue **14** resulted in complete loss of potency (**19**), thus illustrating the impact that subtle structural modifications can have on activity. Although neither the 4-methyl (**20**) nor the 6-methyl (**22**) could restore this lost potency, near restoration was observed with 5-methyl analogue **21**. Compound **21** was a potent partial antagonist with a clear plateau of the CRC well above baseline. Replacement of the 3-methyl group found in analogue **14** with chloro (**25**) and methoxy (**26**) resulted in only moderate loss of potency, while the 3-trifluoromethyl (**23**)
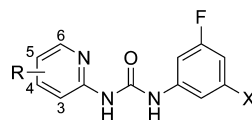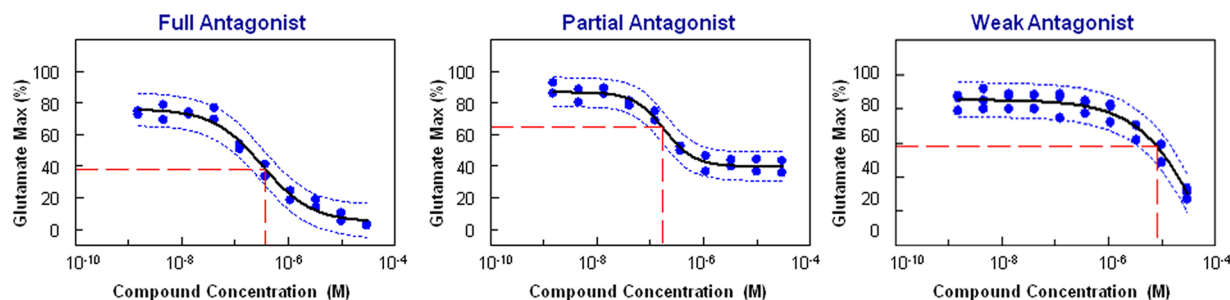

**Figure 2.** CRC curves exemplifying various pharmacological profiles.

Table 3. 2-Aminopyridine Urea SAR

entry	X	R	mGlu$_5$ pIC$_{50}$ ($\pm$SEM)^a	mGlu$_5$ IC$_{50}$ (nM)	% Glu Max^{ab}
19	F	H	<4.5	>30 000	
20	F	4-CH$_3$	<4.5	>30 000	
21	F	5-CH$_3$	6.55 $\pm$ 0.10	284	52.7 $\pm$ 9.4
22	F	6-CH$_3$	<4.5	>30 000	
23	F	3-CF$_3$	5.97 $\pm$ 0.14	1070	39.8 $\pm$ 1.1
24	F	3-F	<5.0^c	>10 000	58.4 $\pm$ 14.3
25	F	3-Cl	6.39 $\pm$ 0.07	411	14.9 $\pm$ 9.9
26	F	3-OCH$_3$	6.43 $\pm$ 0.11	374	12.4 $\pm$ 8.2
27	CN	3-OCH$_3$	6.42 $\pm$ 0.24	379	10.6 $\pm$ 7.6
28	CN	5-CH$_3$	7.96 $\pm$ 0.06	11	1.5 $\pm$ 0.2
29	CN	5-Cl	7.88 $\pm$ 0.07	13	2.5 $\pm$ 0.3

^{a}Calcium mobilization mGlu$_5$ assay; values are average of $n \geq 3$. ^{b}Amplitude of response in the presence of 30 μM test compound as a percentage of maximal response (100 μM glutamate); average of $n \geq 3$. ^{c}CRC does not plateau.

and 3-fluoro (**24**) analogues each suffered from more dramatic loss of activity. Several analogues not depicted here with the 3-cyano-5-fluoroaniline eastern ring suffered from poor solubility, which made determination of reliable CRCs difficult; however, three analogues (**27**-**29**) did not suffer from such an issue. Fortunately, the 5-methyl analogue **28** and the 5-chloro analogue **29** (VU0463841) were among these compounds, and both represented significant improvements in potency. A final piece of SAR was established with *N*-methylated analogues **30** and **31**, which were both inactive at 30 μM (Figure 3).

30 R^1 = CH$_3$ R^2 = H
31 R^1 = H   R^2 = CH$_3$
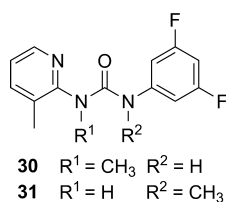
Figure 3. *N*-methylated analogues.

During the course of our optimization program, a number of analogues were evaluated in two in vitro drug metabolism and pharmacokinetics (DMPK) assays that helped identify an optimal compound for in vivo evaluation (Table 4). The intrinsic clearance of the compounds was measured in rat liver microsomes to predict hepatic clearance (CL$_{hep}$), and the fraction of unbound (F_u) compound in the presence of rat plasma proteins was also measured. Determination of the unbound fraction was deemed important since nonspecific binding of drug to protein can potentially reduce the fraction of compound available to interact with the receptor of interest.[44] Unfortunately, the majority of compounds examined in the intrinsic clearance assay were predicted to be highly cleared by rat P450s. Still, three compounds, **17**, **26**, and **29** (VU0463841), were predicted to exhibit more moderate clearance. Ideally, at least one of the partial antagonists and one of the full antagonists would have emerged with the balance of properties suitable for evaluation in vivo; however, analogue **29** (VU0463841) was clearly and uniquely superior

Table 4. Urea in Vitro DMPK Results

entry	mGlu$_5$ IC$_{50}$ (nM)	% Glu Max	rat CL$_{hep}$ (mL/min/kg)^a	rat PPB (F_u)^b
8	338	1.8	63.3	0.038
14	201	16	57.7	0.074
15	154	14	67.0	0.035
16	83	38	64.1	0.077
17	224	2.2	44.9	0.012
18	132	32	67.7	0.004
25	411	15	59.0	0.003
26	374	12	31.2	0.014
27	379	11	62.9	0.036
28	11	1.5	64.1	0.032
29	13	1.7	32.1	0.012

^{a}Predicted hepatic clearance based on intrinsic clearance measured in rat liver microsomes in the presence of NADPH. ^{b}Fraction unbound in rat plasma proteins as determined using equilibrium dialysis apparatus.

among this set of compounds. Other analogues were not as highly bound to plasma proteins as **29** (VU0463841); however, this factor is more than offset by the superior potency and reduced intrinsic clearance observed with this compound. The nonspecific binding of analogue **29** (VU0463841) was also measured in the presence of rat brain homogenates, and binding was quite high (F_u = 0.005). Still, whether such a feature was severe enough to prevent efficacy in vivo would hinge on the extent to which the compound could penetrate the CNS.

Given the predicted moderate clearance of **29** (VU0463841), IP dosing was chosen as a convenient route of administration that would also be amenable to future behavioral studies (Table 5). We were pleased to discover that the CNS penetration of

Table 5. Rat PK Results for 29 (VU0463841)^{a,b}
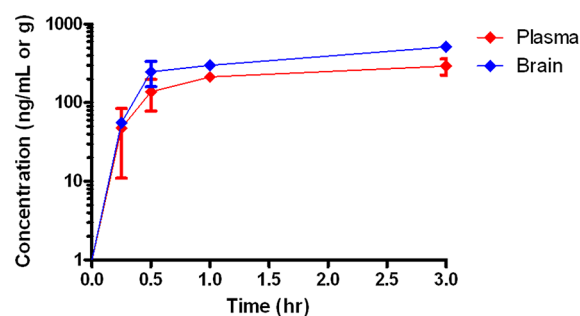
T_{max}	3 h
plasma C_{max}	292 ng/mL
brain C_{max}	516 ng/g
AUC$_{plasma}$	616 ng·h/mL
AUC$_{brain}$	990 ng·h/g
B/P ratio	1.6

^{a}10 mg/kg IP dose; 10% Tween 80 formulation. ^{b}Male Sprague–Dawley rats, n = 2 per time point.

the compound was excellent (B/P = 1.6) and the overall exposure in the brain was quite good (~1.8 μM) at the C_{max}. In fact, consideration of the brain levels in conjunction with the aforementioned brain homogenate binding data was predictive of an unbound fraction near the functional IC$_{50}$ value. Furthermore, the exposure was sustained at roughly the same level from the 30 min to 3 h time points. Such a profile was

deemed acceptable and warranted progression of the compound into multiple behavioral models related to cocaine use and addiction. Prior to investigation in such assays, an assessment of the selectivity profile of **29** (VU0463841) was required. We were pleased to find that the compound was inactive up to the top concentration of 30 μM when tested in full CRC mode against mGlu$_{1-4}$ and mGlu$_{7-8}$. In these experiments, full CRCs were performed in the presence of concentrations of agonist that elicited responses that were 80% of the relative maximal response for that receptor.[45] Finally, **29** (VU0463841) was submitted to a commercially available radioligand binding assay panel of 67 clinically relevant GPCRs, ion channels, kinases, and transporters,[46] and no significant responses were found at a 10 μM concentration of compound.[47]

To validate novel therapeutics such as the selective urea mGlu$_5$ NAM **29** (VU0463841) for their potential to treat addiction, several preclinical models can be used that model the underlying imbalances in neural circuitry that mediates reward (e.g., nucleus accumbens and ventral tegmental area), drug seeking/motivation (e.g., amygdala), and cue/context-induced reinstatement/relapse (e.g., prefrontal cortex).[48-52] Two of the most common paradigms used for detecting changes in drug-seeking behavior and the rewarding effects of drugs of abuse are drug self-administration and conditioned place preference (CPP). In drug self-administration, animals are generally trained to operate manipulanda (e.g., lever, key, nose-poke) to obtain infusions of drug (i.e., drug-seeking behavior). Lights, tones, and other stimuli can be paired with drug self-administration to signal availability of the reinforcer and can be used to investigate cue-induced reinstatement of drug seeking (i.e., relapse). Drug self-administration data collected in rodents has been shown to be highly predictive of abuse liability in humans, as animals will readily self-administer most drugs of abuse.[53] The CPP paradigm utilizes a paired-conditioning protocol to investigate the rewarding properties of a drug. Generally, a rewarding stimulus (e.g., cocaine) is administered in close temporal proximity to a neutral stimulus (e.g., a distinct chamber in the CPP apparatus), resulting in the neutral stimulus developing secondary rewarding properties and exerting control over behavior. In the CPP assay, increased time spent in the drug-paired chamber indicates that the administered compound may have rewarding properties.[54]

To assess the effects of **29** (VU0463841) on cocaine intake, rats were first trained to press a lever on an FR10 schedule of food reinforcement. After a brief extinction trial, cocaine replaced the food reward as the reinforcer. Once responding under this schedule stabilized (i.e., number of reinforcers did not vary by more than 20% of the mean for three days), drug studies with **29** (VU0463841) were initiated. The effects of increasing doses of **29** (VU0463841) administered IP on the number of reinforcers and response rate (response/min) during cocaine self-administration are shown in Figure 4. As indicated by one-way ANOVA, **29** (VU0463841) dose-dependently attenuated both the number of reinforcers [$F_{(4,26)} = 8.295$, $P < 0.001$] and response rate [$F_{(4,26)} = 4.944$, $P = 0.004$] of rats in the self-administration paradigm. Holm–Sidak posthoc tests indicated that both 18 and 32 mg/kg **29** (VU0463841) were significantly different from vehicle injections. Subsequently, we evaluated whether **29** (VU0463841) would dose-dependently attenuate the reinforcing effects of cocaine in the CPP paradigm. First, a dose–response curve was generated to determine if **29** (VU0463841) produced a CPP when
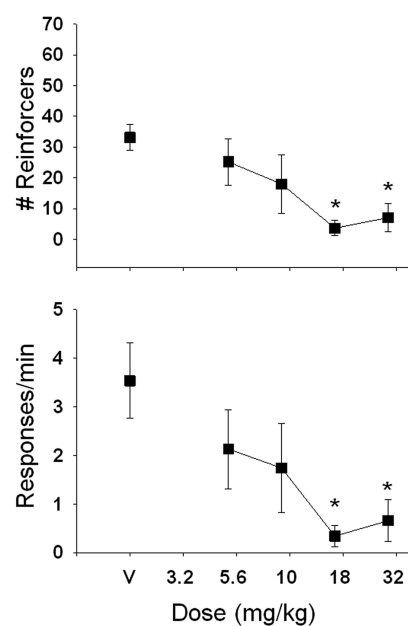

Figure 4. mGlu$_5$ NAM **29** (VU0463841) dose-dependently reduces cocaine self-administration in rats. Effects of **29** (VU0463841) ($n = 14$) on cocaine self-administration were evaluated in male Sprague–Dawley rats trained to lever press under a fixed ratio 10 (FR10) for a 45 mg food pellet. Animals were then catheterized and 0.5 mg/kg/injection of cocaine IV served as a reinforcer. Total number of reinforcers and response rate served as the dependent variables. Values represent the mean ± SEM. Asterisks indicate significant differences from vehicle administration ($p < 0.05$).

administered alone (Figure 5A). As depicted here, **29** (VU0463841) produced no effect on CPP when administered alone. A two-way ANOVA revealed no significant differences in time spent in the drug-paired compartment at any dose [$F_{(4,45)} = 2.456$, $P = 0.059$], no differences between pre- and post-conditioning test [$F_{(1,45)} = 2.076$, $P = 0.157$], or interaction between test day and drug dose [$F_{(1,45)} = 2.076$, $P = 0.123$]. Next, we evaluated the ability of increasing doses of **29** (VU0463841) to block the CPP observed with a 10 mg/kg dose of cocaine (Figure 5B). As indicated by a two-way ANOVA, there was a significant effect of dose [$F_{(4,44)} = 3.910$, $P = 0.008$], test day [$F_{(1,44)} = 21.406$, $P < 0.001$], and an interaction between dose and test day [$F_{(4,44)} = 6.577$, $P < 0.001$]. Holm–Sidak posthoc tests found that, within test day, the time spent in the cocaine-paired chamber was significantly different from time spent in the vehicle-paired chamber (58.7% increase from pretest), indicating a cocaine-induced place preference at 10 mg/kg. Holm–Sidak posthoc tests also found differences between vehicle and the 18 (40.6% increase from pretest) and 32 (69.9% increase from pretest) mg/kg doses of **29** (VU0463841) in combination with 10 mg/kg cocaine, but not between vehicle and 56 mg/kg (0.9% decrease from pretest) of **29** (VU0463841), indicating a blockade of the cocaine-induced place preference at the highest dose tested.

While **29** (VU0463841) produced dose related reversal of cocaine self-administration at doses lower than those that blocked CPP, two possible explanations for these effects remained. The data may reflect a true attenuation of the reinforcing effects of cocaine, or alternatively this mGlu$_5$ NAM could produce a potentiation of the reinforcing effects of cocaine[55] and/or impairment of general locomotor function.
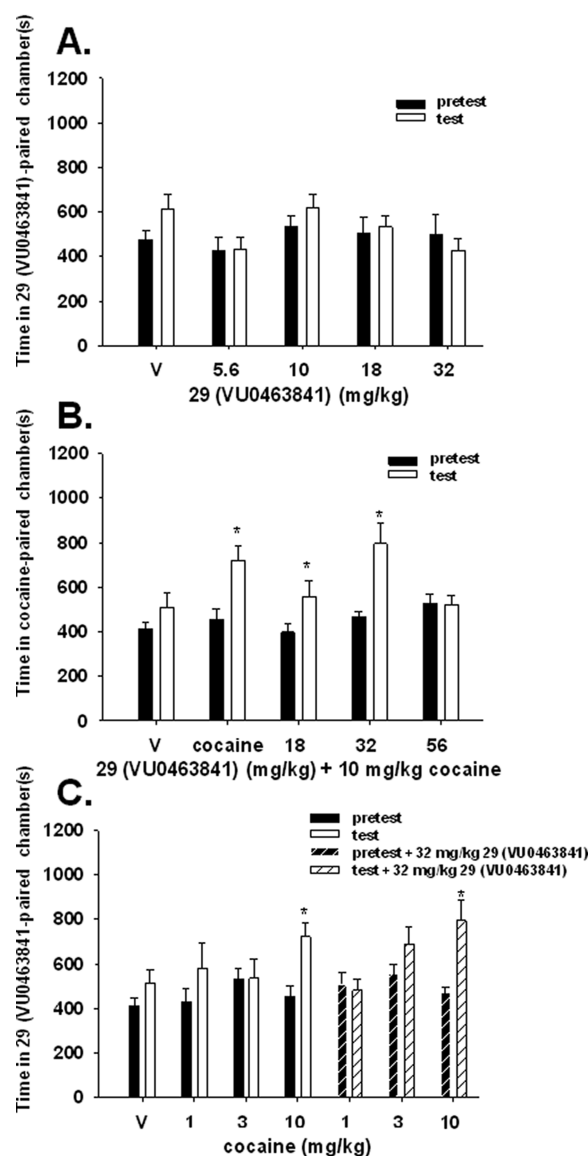
29 (VU0463841) CPP Results

Figure 5. mGlu$_5$ NAM **29** (VU0463841) dose-dependently reduces cocaine-induced place preference in rats. Using a three-compartment CPP paradigm, drug was paired with the nonpreferred chamber for all compounds and vehicle was paired with the preferred compartment. Mean time spent in the nonpreferred compartment (seconds) for each group (n = 10 rats/dose) was plotted ± SEM. (A) **29** (VU0463841) did not increase the amount of time spent in the nonpreferred chamber. (B) Effects of cocaine alone and in combination with three doses of **29** (VU0463841) (18−56 mg/kg). (C) **29** (VU0463841) in combination with cocaine did not potentiate cocaine's effects in the CPP paradigm. Asterisks indicate significant differences from vehicle administration (p < 0.05).

For example, recent research found that the diaryl alkyne tool mGlu$_5$ NAM MPEP potentiated the conditioned place preference of both addictive and nonaddictive substances and concluded that MPEP may produce a "substitution-like" effect, rather than a true potentiation. Another study by the same group found that MPEP is readily self-administered by drug-naïve rats, in addition to producing a conditioned place preference when administered alone.[56] Interestingly, **29** (VU0463841) did not produce a place preference within the

dose range tested. In order to further understand the nature of the observed effects of **29** (VU0463841), we assessed whether a dose of 32 mg/kg **29** (VU0463841) could potentiate the effects of increasing doses of cocaine (e.g., 1, 3, and 10 mg/kg) to induce a CPP (Figure 5C). A two-way ANOVA revealed a significant effect of dose [$F_{(6,61)}$ = 3.076, P = 0.011], test day [$F_{(6,61)}$ = 14.825, P < 0.001], and an interaction between dose and test day [$F_{(6,61)}$ = 3.767, P = 0.003]. Holm−Sidak posthoc tests found significant differences between 10 mg/kg cocaine (58.7% increase from pretest) and the combination of 10 mg/kg cocaine and 32 mg/kg **29** (VU0463841) (69.6% increase from pretest) when compared to vehicle (24% increase from pretest). These findings further support the interpretation that **29** (VU0463841) blocks the reinforcing effect of cocaine since our mGlu$_5$ NAM did not produce a significant potentiation of the effects of cocaine across the doses tested when given in combination. In addition, we assessed the effects of **29** (VU0463841) on general locomotor activity during the CPP task, to control for potential sedation of general motor output (Figure 6), using an analysis of the mean locomotor

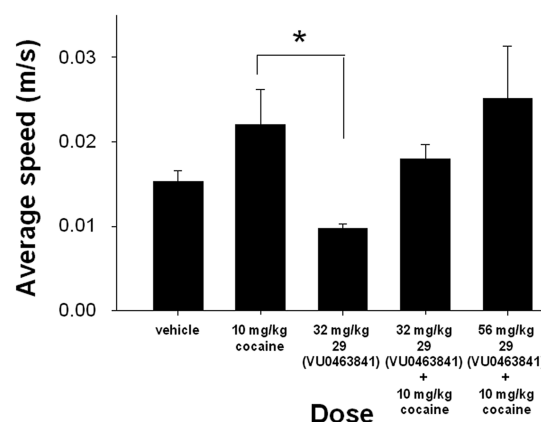## 29 (VU0463841) Effects on Locomotor Activity

Figure 6. **29** (VU0463841) has no significant effect on general locomotor activity. Effects of vehicle, 10 mg/kg cocaine, 32 mg/kg **29** (VU0463841), and the combination of cocaine and two doses of **29** (VU0463841) on mean speed (m/s) during drug conditioning sessions (n = 10/dose). Holm−Sidak posthoc test detected no significant difference between vehicle and the three treatment groups. However, a pairwise comparison detected a significant difference between 10 mg/kg cocaine and 32 mg/kg **29** (VU0463841).

speed (m/s) under vehicle and drug conditions. Vehicle, **29** (VU0463841) (32 mg/kg), cocaine (10 mg/kg), and two combinations (32 and 56 mg/kg in combination with cocaine) were analyzed using a one-way ANOVA, which revealed a significant difference between the five groups [$F_{(4,155)}$ = 3.589, P = 0.008]. Interestingly, Holm−Sidak posthoc tests found no differences between vehicle and each drug condition, but rather a significant difference was detected between 10 mg/kg cocaine and 32 mg/kg **29** (VU0463841). This would indicate no significant effect of 32 mg/kg **29** (VU0463841) (i.e., the highest dose tested in self-administration) on locomotor activity when compared with vehicle controls and no significant effect of 56 mg/kg in combination with 10 mg/kg cocaine (i.e., the highest doses tested in CPP).

In summary, we have developed a novel mGlu$_5$ NAM **29** (VU0463841) from a non-MPEP urea scaffold identified through a HTS. This new tool is potent and selective for the

mGlu$_5$ receptor, both versus other mGlu receptors and with regard to ancillary pharmacology. IP dosing of **29** (VU0463841) in rats leads to exposures in the brain that are therapeutically relevant. Finally, **29** (VU0463841) dose-dependently reduces cocaine self-administration and cocaine place preference, and these reductions are likely not due to changes in the reinforcing properties of cocaine or changes in locomotor function. While conclusive identification of the underlying mechanisms behind the observed effects with **29** (VU0463841) remains to be determined, one potential interpretation is that our selective mGlu$_5$ NAM inhibits central glutamatergic signaling critical for maintaining the associative memory between drug seeking behavior and environmental cues. Others have suggested that after extensive exposure to a drug that produces dependence, such as cocaine, many drug seeking behaviors become predominately mediated by different neurocircuitry than in naïve or nondrug dependent individuals (e.g., the prefrontal cortex may provide a larger neuronal input in drug seeking behavior than the amygdala, ventral tegmental area, or nucleus accumbens).[3,25,49] Future studies will investigate the effects of **29** (VU0463841) and other mGlu$_5$ NAMs on the formation of such associative memories.

■ METHODS

Synthesis and Characterization of 29 (VU0463841). 3-Amino-5-fluorobenzonitrile (1.00 g, 7.35 mmol, 1.0 equiv) was dissolved in ethyl acetate (17 mL) and added dropwise in portions to a solution of triphosgene (1.09 g, 3.67 mmol, 0.50 equiv) in ethyl acetate (43 mL). The reaction was refluxed for 4 h, cooled, and concentrated. The product was used crude without further purification. The crude isocyante (1.43 g, 8.82 mmol, 1.0 equiv) and 2-amino-5-chloropyridine (1.13 g, 8.82 mmol, 1.0 equiv) were dissolved in methylene chloride (44 mL) and stirred until the reaction was deemed complete by LCMS (∼ 30 min.). The reaction was filtered and washed with diethyl ether to afford 1.19 g (47%) of the title compound as a white solid. ^{1}H NMR (400 MHz, DMSO-d_6) δ 10.43 (s, 1H), 9.75 (s, 1H), 8.33 (d, J = 2.3 Hz, 1H), 7.88 (dd, J = 2.6, 8.9 Hz, 1H), 7.80 (d, J = 11.3 Hz, 1H), 7.76 (s, 1H), 7.64 (d, J = 9.0 Hz, 1H), 7.46 (d, J = 8.0 Hz, 1H). HPLC (Method 2) R_T = 0.778 min. ES−MS [M+H]$^+$: 291.1. HRMS (TOF, ES$^+$) $C_{13}H_8ClFN_4O$ [M+H]$^+$ calculated, 291.0449; found, 291.0448.

Calcium Mobilization Assay. HEK 293A cells stably expressing rat mGlu$_5$ were plated in black-walled, clear-bottomed, poly-D-lysine coated 384-well plates (BD Biosciences, San Jose, CA) in 20 μL of assay medium (DMEM containing 10% dialyzed FBS, 20 mM HEPES, and 1 mM sodium pyruvate) at a density of 20K cells/well. The cells were grown overnight at 37 °C in the presence of 5% CO$_2$. The next day, medium was removed and the cells incubated with 20 μL of 2 μM Fluo-4, AM (Invitrogen, Carlsbad, CA) prepared as a 2.3 mM stock in DMSO and mixed in a 1:1 ratio with 10% (w/v) pluronic acid F-127 and diluted in assay buffer (Hank's balanced salt solution, 20 mM HEPES and 2.5 mM Probenecid (Sigma-Aldrich, St. Louis, MO)) for 45 min at 37 °C. Dye was removed, 20 μL of assay buffer was added, and the plate was incubated for 10 min at room temperature. Ca^{2+} flux was measured using the Functional Drug Screening System (FDSS6000, Hamamatsu, Japan). Compounds were serially diluted 1:3 into 10 point CRC (30 μM to 1 nM final) and transferred to daughter plates using the Echo acoustic plate reformatter (Labcyte, Sunnyvale, CA). Compounds were diluted into assay buffer to a 2× stock using a Thermo Fisher Combi (Thermo Fisher, Waltham, MA) which was applied to cells at t = 3 s. Cells were incubated with the test compounds for 140 s and then stimulated with an EC$_{20}$ concentration of glutamate; 74 s later, an EC$_{80}$ concentration of glutamate was added and readings taken for an additional 40 s. Data were collected at 1 Hz. Concentration response curves were generated using a four point logistical equation with XLfit curve fitting software for Excel (IDBS, Guildford, U.K.).

Stability in Rat Liver Microsomes. Hepatic microsomes (0.5 mg/mL) and 1 μM test compound were incubated in 100 mM potassium phosphate pH 7.4 buffer with 3 mM MgCl$_2$ at 37 °C with constant shaking. After a 5 min preincubation, the reaction was initiated by addition of NADPH (1 mM). At selected time intervals (0, 3, 7, 15, 25, and 45 min), 50 μL aliquots were taken and subsequently placed into a 96-well plate containing 150 μL of cold acetonitrile with internal standard (50 ng/mL carbamazepine). Plates were then centrifuged at 3000 rcf (4 °C) for 10 min, and the supernatant was transferred to a separate 96-well plate and diluted 1:1 with water for LC/MS/MS analysis. The in vitro half-life ($T_{1/2}$, min, eq 1), intrinsic clearance (CL$_{int}$, mL/min/kg, eq 2), and subsequent predicted hepatic clearance (CL$_{hep}$, mL/min/kg, eq 3) were determined employing the following equations:

$$T_{1/2} = \frac{\ln(2)}{k} \tag{1}$$

where k represents the slope from linear regression analysis of the natural log percent remaining of test compound as a function of incubation time.

$$CL_{int} = \frac{0.693}{\text{in vitro } T_{1/2}} \times \frac{\text{mL incubation}}{\text{mg microsomes}} \times \frac{45 \text{ mg microsomes}}{\text{gram liver}}$$
$$\times \frac{20^a \text{ gram liver}}{\text{kg body wt}} \tag{2}$$

where the superscript "a" indicates scale-up factors of 20 (human) or 45 (rat).

$$CL_{hep} = \frac{Q_h \times CL_{int}}{Q_h + CL_{int}} \tag{3}$$

where Q_h (hepatic blood flow, mL/min/kg) is 21 (human) or 70 (rat).

Binding to Rat Plasma Protein and Brain Homogenates. The protein binding of each compound was determined in plasma via equilibrium dialysis employing RED Plates (ThermoFisher Scientific, Rochester, NY). Plasma (220 μL) was added to the 96-well plate containing test compound (5 μL) and mixed thoroughly for a final concentration of 5 μM. Subsequently, 200 μL of the plasma–compound mixture was transferred to the *cis* chamber (red) of the RED plate, with an accompanying 350 μL of phosphate buffer (25 mM, pH 7.4) in the *trans* chamber. The RED plate was sealed and incubated for 4 h at 37 °C with shaking. At completion, 50 μL aliquots from each chamber were diluted 1:1 (50 μL) with either plasma (*cis*) or buffer (*trans*) and transferred to a new 96-well plate, at which time ice-cold acetonitrile containing internal standard (50 ng/mL carbamazepine) (2 volumes) was added to extract the matrices. The plate was centrifuged (3000 rcf, 10 min), and supernatants transferred and diluted 1:1 (supernatant: water) into a new 96-well plate, which was then sealed in preparation for LC/MS/MS analysis. Each compound was assayed in triplicate within the same 96-well plate. Fraction unbound was determined using the following equation:

$$F_u = \frac{\text{conc}_{buffer}}{\text{conc}_{plasma}}$$

The brain homogenate binding of each compound was determined in brain homogenate via equilibrium dialysis employing RED Plates (ThermoFisher Scientific, Rochester, NY). Brain tissue homogenate was prepared by diluting one volume of whole brain tissue with three volumes of phosphate buffer (25 mM, pH 7.4). The mixture was then subjected to mechanical homogenation employing a Mini-Beadbeater and 1.0 mm zirconia/silica beads (BioSpec Products). Brain homogenate (220 μL) was added to the 96-well plate containing test compound (5 μL) and mixed thoroughly for a final concentration of 5 μM. Subsequently, 200 μL of the brain homogenate–compound mixture was transferred to the *cis* chamber (red) of the RED plate, with an accompanying 350 μL of phosphate buffer (25 mM, pH 7.4) transferred to the *trans* chamber. The RED plate was sealed and incubated for 4 h at 37 °C with shaking. At completion, 50 μL aliquots

from each chamber were diluted 1:1 (50 μL) with either brain homogenate (*cis*) or buffer (*trans*) and transferred to a new 96-well plate, at which time ice-cold acetonitrile containing internal standard (50 ng/mL carbamazepine) (2 volumes) was added to extract the matrices. The plate was centrifuged (3000 rcf, 10 min), and supernatants transferred and diluted 1:1 (supernatant: water) into a new 96-well plate, which was then sealed in preparation for LC/MS/MS analysis. Each compound was assayed in triplicate within the same 96-well plate. Fraction unbound was determined using the following equation:

$$F_{\text{u,tissue}} = \frac{1/D_f}{(1/F_{\text{u,hom}} - 1) + 1/D_f}$$

where $F_{\text{u,hom}}$ represents the measured fraction unbound in the diluted homogenate and D_f represents dilution factor.

LC/MS/MS Bioanalysis of Samples from in Vitro Assays. In vitro samples were analyzed on a Thermo Electron TSQ Quantum Ultra triple quad mass spectrometer (San Jose, CA) via electrospray ionization (ESI) with two Themo Electron Accella pumps (San Jose, CA), and a Leap Technologies CTC PAL autosampler (Carrboro, NC). Analytes were separated by gradient elution on a dual column system with two Thermo Hypersil Gold (2.1 × 30 mm, 1.9 μm) columns (San Jose, CA) thermostatted at 40 °C. HPLC mobile phase A was 0.1% formic acid in water, and mobile phase B was 0.1% formic acid in acetonitrile. The gradient started at 10% B after a 0.2 min hold and was linearly increased to 95% B over 0.8 min; hold at 95% B for 0.2 min; returned to 10% B in 0.1 min. The total run time was 1.3 min, and the HPLC flow rate was 0.8 mL/min. While pump 1 ran the gradient method, pump 2 equilibrated the alternate column isocratically at 10% B. Compound optimization, data collection, and processing were performed using Thermo Electron's QuickQuan software (v2.3) and Xcalibur (v2.0.7 SP1).

Rat Pharmacokinetic Study. This study was carried out in accordance with the Institutional Animal Care and Use Committee of Vanderbilt University and the guidelines of the Committee on Care and Use of Laboratory Animal Resources, as adopted and promulgated by the U.S. National Institutes of Health. Compound **29** (VU0463841) was formulated in 10% Tween 80 in sterile water and administered IP to male Sprague–Dawley rats weighing 275–300 g (Harlan, Indianapolis, IN) at the dose of 10 mg/kg. The blood (cardiac puncture) and brain were collected at 0.25, 0.5, 1, and 3 h. Animals were euthanized and decapitated, and the brains were removed, thoroughly washed in cold phosphate-buffered saline, and immediately frozen on dry ice. Plasma was separated by centrifugation (4000 rcf, 4 °C) and stored at −80 °C until analysis. On the day of analysis, frozen whole-rat brains were weighed and diluted with 1:3 (w/w) parts of 70:30 isopropanol/water. The mixture was then subjected to mechanical homogenation employing a Mini-Beadbeater and 1.0 mm zirconia/silica beads (BioSpec Products) followed by centrifugation. The sample extraction of plasma (20 μL) or brain homogenate (20 μL) was performed by a method based on protein precipitation using three volumes of ice-cold acetonitrile containing an internal standard (50 ng/mL carbamazepine). The samples were centrifuged (4000 rcf, 5 min), and supernatants transferred and diluted 1:1 (supernatant:water) into a new 96-well plate, which was then sealed in preparation for LC/MS/MS analysis.

LC/MS/MS Bioanalysis of Samples from in Vivo Studies. In vivo samples were analyzed via electrospray ionization (ESI) on an AB Sciex API-5500 QTrap (Foster City, CA) instrument that was coupled with Shimadzu LC-20AD pumps (Columbia, MD) and a Leap Technologies CTC PAL autosampler (Carrboro, NC). Analytes were separated by gradient elution using a Fortis C18 3.0 × 50 mm, 3 μm column (Fortis Technologies Ltd., Cheshire, U.K.) thermostatted at 40 °C. HPLC mobile phase A was 0.1% formic acid in water (pH unadjusted), and mobile phase B was 0.1% formic acid in acetonitrile (pH unadjusted). The gradient started at 10% B after a 0.2 min hold and was linearly increased to 90% B over 0.8 min, held at 90% B for 0.5 min, and returned to 10% B in 0.1 min, followed by a re-equilibration (0.9 min). The total run time was 2.5 min, and the

HPLC flow rate was 0.5 mL/min. The source temperature was set at 500 °C, and mass spectral analyses were performed using multiple reaction monitoring (MRM), with transitions specific for each compound utilizing a Turbo-Ionspray source in positive ionization mode (5.0 kV spray voltage). The calibration curves were constructed, and linear response was obtained in the range of 0.5−5000 ng/mL, by spiking known amounts of **29** (VU0463841) in blank brain homogenate or plasma. All data were analyzed using AB Sciex Analyst software v1.5.1.

Behavioral Assays. *Subjects.* Male Sprague−Dawley rats (Harlan Laboratories, Indianapolis, IN), weighing approximately 280−300 g for CPP studies and 340−380 g for self-administration studies. Rats were group housed (three animals per cage), with the exception of the animals in the self-administration paradigm, which were individually housed in a colony room on a 12 h light/12 h dark cycle (lights on at 0600 h CST). Standard rodent chow (Purina Mills, Richmond, Indiana) was provided ad libitum for all animals used in the conditioned place preference. For the self-administration studies, rats were food restricted and maintained at 85−90% of their free-feeding weight. Water was available ad libitum for all rats in their home cage. This study was carried out in accordance with the Institutional Animal Care and Use Committee of Vanderbilt University and the guidelines of the Committee on Care and Use of Laboratory Animal Resources, as adopted and promulgated by the U.S. National Institutes of Health.

Apparatus. Twelve identical modular test chambers (MED Associates, Inc., St. Albans, VT) configured specifically for rodents were used. Located on the front wall of each chamber were a houselight, speaker, pellet trough (2 cm above the floor), two response levers (6.5 cm above the floor, 5 cm left and right of the pellet trough), and one stimulus light above each lever. The response lever required a minimum force of 0.25 N for activation, and a feeder, located behind the operant chamber, dispensed pellets to the pellet trough. Each chamber was enclosed within a sound-attenuating cubicle equipped with a fan for ventilation. A syringe pump (PHM-100, MED Associates, Inc., VT) was located outside the sound attenuating cubicle and was attached to a single channel stainless steel swivel (Instech Laboratories, Inc. Plymouth Meeting, PA), which was mounted on a drug delivery arm (PHM-110-SAI) centered above the chamber. All test chambers were connected to a computer programmed in MED-PC for Windows, Version IV (MED Associates, Inc., VT). The three-chamber conditioned place preference boxes were constructed from clear Plexiglas (Williams Machine Co., Greenbrier, TN). Outer chambers were 15″ × 15″ and were separated by a clear middle chamber (7 3/4″ × 9 1/4″). Each chamber had either a striped black and white pattern on the walls and a smooth floor, or a checkered black and white pattern on the walls and a coarse floor.

Surgical Procedure. Each rat was implanted with a chronic silastic jugular cannula which passed subcutaneously to a silicone assembly mounted on the animal's back. The technique used to construct and implant the cannula was an adaption of that of Thomsen and Caine.[57]

Self-Administration Behavioral Procedure. Rats were initially trained to respond on two levers, in order to receive a sugar pellet. Rats were trained 5 days/week, and each session was 2 h or 120 reinforcers, whichever occurred first. Animals were placed into the operant chamber, and illumination of the houselight served as a cue for reinforcer availability. Initially, responding on either lever produced a food pellet until animals earned >100 reinforcers. Reinforced responding was then restricted to one of the two levers (i.e., the active lever) each session and alternated each session until animals earned >100 reinforcers on both levers. Responding on the inactive lever was recorded but had no programmed consequence. Finally, the active lever was assigned to either the left or right lever (counter-balanced across animals) for the remainder of the study, and a 20 s timeout was introduced after each complete ratio. During the timeout, the light above the active lever was illuminated and reinforcement was no longer available (i.e., responding had no consequence). After responding had stabilized under this continuous reinforcement schedule, the number of active lever responses necessary for reinforcement was incrementally increased until animals were responding under a fixed-ratio 10 (FR-10) schedule of reinforcement

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(i.e., 10 responses on the active lever resulted in the delivery of a reinforcer). After responding stabilized under this schedule of reinforcement, extinction sessions would occur in which the pellet dispenser was disconnected and all other stimuli remained the same. Extinction sessions would continue until the total number of reinforcers earned per session was less than 30% of the mean of the 3 days prior to extinction training. Animals would then undergo jugular vein catheterization followed by a regimen of 5 mg/kg/day enrofloxacin for 5 days. Afterward, the animal would be placed back into the operant chamber and each completed fixed-ratio would result in a cocaine infusion (0.5 mg/kg). Once responding stabilized, the rats were tested periodically with a compound, up to 10 sessions per month for the remainder of the study.

CPP and Locomotor Behavioral Procedures. Rats were initially placed into a three-chamber shuttle box and allowed to move freely between compartments. Time spent in each compartment was recorded. On day 2−5, each rat was pretreated either 0 min (cocaine/vehicle) or 20 min (**29 (VU0463841)**/vehicle) prior to conditioning with an alternating dose of vehicle or drug and confined to a single compartment for 30 min. Each drug was dosed IP, and the animal was confined to the nonpreferred chamber, while the vehicle was paired with the preferred chamber in a biased design. The rat was returned to its home cage after each 30 min session. On day 6, rats were allowed to roam freely between the three chambers for 30 min to assess preference. Total time spent in the drug-paired compartment and locomotor activity during drug conditioning sessions were recorded and analyzed. Average speeds in meters per second were pooled from the drug conditioning sessions across all animals in order to detect disruptions in locomotor activity when compared to vehicle conditioning days. A biased design was chosen, as there are several studies reporting that a biased design is capable of detecting preference with drugs that have long been known to produce dependence, while nonbiased designs tend to be less consistent.[58,59]

Drugs and Drug Administration. The acute effects of cocaine (1−10 mg/kg) and **29 (VU0463841)** (5.6−56 mg/kg) were determined in CPP experiments and after responding under cocaine self-administration had stabilized. Cocaine was dissolved in sterile saline. Compound **29 (VU0463841)** was dissolved in 10% Tween 80 and sterile water. The injection volume was always 0.1 mL/100 g, except for the highest dose of **29 (VU0463841)**, which was less soluble at the highest concentration (56 mg/kg) and was injected 0.2 mL/100 g. For the self-administration experiments, all the dosages for a given drug were administered in a mixed order and responding was always allowed to return to baseline levels before administration of the next dose. However, to avoid the development of acute tolerance or any "carry over" effects, the highest doses of each drug were administered only once per week. Enrofloxacin was administered 5 days postsurgery for animals in the self-administration studies at 5 mg/kg and was dissolved in sterile saline to prevent a bacterial infection. For CPP experiments, each animal received two drug injections prior to conditioning sessions with an identical dose of compound or combination of these compounds.

Statistical Analysis. A one-way repeated measures analysis of variance (ANOVA) using SigmaStat (SYSTAT Software Inc., Point Richmond, CA) determined the effects of **29 (VU0463841)** on the number of reinforcers and response rate for cocaine self-administration. Two-way ANOVA tests were used to determine the effects of **29 (VU0463841)** and cocaine alone and in combination for time spent in the drug-paired compartment (drug × pretest/test) in the conditioned place preference paradigm. One-way ANOVAs were used to analyze locomotor activity in the conditioned place preference paradigm. Holm−Sidak posthoc tests were used to compare drug sessions with control (saline or vehicle) sessions. The same posthoc tests were used to carry out all pairwise comparisons when differences from the control were not significant but there was a main effect. Significance was accepted at α of 0.05 for all statistical tests.

■ ASSOCIATED CONTENT

⑤ Supporting Information

Synthesis and characterization information for analogues as well as complete results from the ancillary pharmacology screen of **29 (VU0463841)**. This material is available free of charge via the Internet at http://pubs.acs.org.

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Author Contributions

K.A.E. and C.W.L. oversaw and designed the chemistry. A.S.F. performed synthetic chemistry work. P.J.C. and C.M.N. oversaw and designed the molecular pharmacology experiments. A.L.R. oversaw, designed, performed, and interpreted the molecular pharmacology experiments. D.F.V. performed the molecular pharmacology experiments. J.S.D. oversaw and designed the in vitro and in vivo DMPK experiments. R.D.M. designed, performed, and interpreted the in vitro DMPK experiments. F.W.B. performed the in vivo PK studies. C.K.J. oversaw, designed, and interpreted the data from the behavioral studies. R.J.A. designed, performed, and interpreted the data from the behavioral studies.

Notes

The authors declare no competing financial interest.

■ ABBREVIATIONS

CNS, central nervous system; DAT, dopamine transporter; HIV, human immunodeficiency virus; HCV, hepatitis C virus; FDA, Food and Drug Administration; mGlu, metabotropic glutamate receptor; GPCR, G-protein-coupled receptor; NAM, negative allosteric modulators; FXS, fragile X syndrome; PD-LID, Parkinson's disease levodopa induced dyskinesia; MPEP, 2-methyl-6-(phenylethynyl) pyridine; MTEP, 3-[(2-methyl-1,3-thiazol-4-yl)ethynyl]pyridine; SCM, sweetened condensed milk; HTS, high-throughput screen; CRC, concentration response curve; IP, intraperitoneal; EC, effective concentration; HEK, human embryonic kidney; SAR, structure activity relationships; DMPK, drug metabolism and pharmacokinetics; IV, intravenous; F_u, fraction unbound; B/P, brain to plasma; IC$_{50}$, half maximal inhibitory concentration; Glu Max, amplitude of response in the presence of 30 μM test compound

as a percentage of maximal response (100 μM glutamate); CL_{hep}, predicted hepatic clearance; T_{max}, time at maximum concentration; C_{max}, maximum concentration; AUC, area under the curve; CPP, conditioned place preference; ANOVA, analysis of variance; g, grams; mmol, millimoles; equiv, equivalents; mL, milliliters; LCMS, liquid chromatography–mass spectrometry; ^{1}H NMR, proton nuclear magnetic resonance; MHz, megahertz; DMSO, dimethyl sulfoxide; s, singlet; d, doublet; dd, doublet of doublets; ES–MS, electrospray ionization mass spectrometry; HRMS, high–resolution mass spectrometry; TOF, time-of-flight; μL, microliter; DMEM, Dulbecco's modified Eagle's medium; FBS, fetal bovine serum; HEPES, N'-2-hydroxyethylpiperazine N'-2-ethanesulfonic acid; mM, millimolar; NADPH, nicotinamide adenine dinucleotide phosphate; rcf, relative centrifugal force; LC/MS/MS, liquid chromatography tandem mass spectrometry; $T_{1/2}$, half-life; CL_{int}, intrinsic clearance; HPLC, high performance liquid chromatography; RED, rapid equilibrium dialysis; MRM, multiple reaction monitoring; Tween 80, polyoxyethylene (20) sorbitan monooleate; cm, centimeter; N, normal; mg/kg, milligrams per kilogram

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