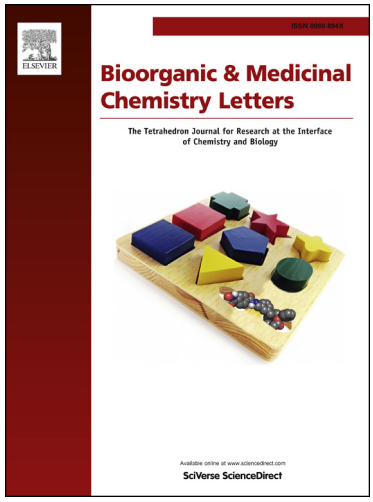

Preliminary investigation of 6,7-dihydropyrazolo[1,5-*a*]pyrazin-4-one derivatives as a novel series of $mGlu_5$ receptor positive allosteric modulators with efficacy in preclinical models of schizophrenia

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ABSTRACT

As part of our efforts to identify a suitable back-up compound to our recently disclosed $mGlu_5$ positive allosteric modulator (PAM) clinical candidate VU0490551/JNJ-46778212, this letter details the investigation and challenges of a novel series of 6,7-dihydropyrazolo[1,5-*a*]pyrazin-4-one derivatives. From these efforts, compound **4k** emerged as a potent and selective $mGlu_5$ PAM displaying overall attractive in vitro (pharmacological and ADMET) and PK profiles combined with in vivo efficacy in preclinical models of schizophrenia. However, further advancement of the compound was precluded due to severely limiting CNS-related side-effects confirming the previously reported association between excessive $mGlu_5$ activation and target-related toxicities.

In the search for improved schizophrenia therapeutics, restoration of dysfunctional *N*-methyl-D-aspartate (NMDA) receptor-mediated glutamatergic neurotransmission has gained increased attention as a potentially advantageous alternative to the modulation of the dopaminergic system exerted by current antipsychotic medications.[1-3] In this context, positive allosteric modulators (PAMs) of the metabotropic glutamate receptor subtype 5 ($mGlu_5$) hold high promise by virtue of the close physical and functional relationship between $mGlu_5$ and NMDA receptors in relevant brain areas.[4-8] Despite the recent association of excessive $mGlu_5$ receptor activation with target-related liabilities (neuronal necrosis[9] and seizure induction[10]), the observation that *in vivo* efficacy in several schizophrenia models has been confirmed for multiple $mGlu_5$ PAMs,[11-16] combined with the identification of potential alternatives (reduced cooperativity with glutamate,[17, 18] signal bias[16] or reduced residence-time and duration of target engagement[19]) to mitigate these burdens, suggests that further investigations on this novel mechanism are warranted. Resulting from our pioneering industrial-academic collaboration (Janssen Research and Development and the Vanderbilt Center for Neuroscience Drug Discovery (VCNDD))[20] we have recently reported on the identification[21] and preclinical characterization[16] of the $mGlu_5$ PAM advanced lead **1** (VU0409551/JNJ-46778212). Unfortunately, the progression of **1** was stopped as a consequence of toxicology findings in IND-enabling studies[16, 21] and we initiated a research campaign aimed to identify novel $mGlu_5$ PAMs either with a different pharmacological profile (vide supra) or from a different chemotype that could circumvent these issues. Preliminary efforts towards the identification of a suitable back-up compound within the same chemotype,[22] as well as within other exocyclic amide subseries,[19] failed to provide the combination of all of the desired attributes in a single molecule. As an alternative strategy, we turned our attention to other $mGlu_5$ PAM chemotypes reported by our labs.[23, 24] These chemical classes, represented by general structures **2** and **3**, share a structurally-related carbonyl-

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containing central scaffold, in contrast to the exocyclic amide moiety in **1**. SAR investigation of these series had already revealed aryls as preferred eastern and western substituents for potent $mGlu_5$ PAM activity and a prominent role of the central core for the modulation of physicochemical properties. As a continuation of this approach, we considered the 6,7-dihydropyrazolo[1,5-*a*]pyrazin-4-one scaffold **4** as a potential alternative core; in analogy with series **2** and **3**, this scaffold presents a similar geometry, a lactam-type carbonyl and an aromatic southern nitrogen. Our strategy involved a multidimensional optimization campaign focused on a broad range of modifications including the eastern and western aryl moieties, the ether linker, as well as the central core (**Figure 1**).

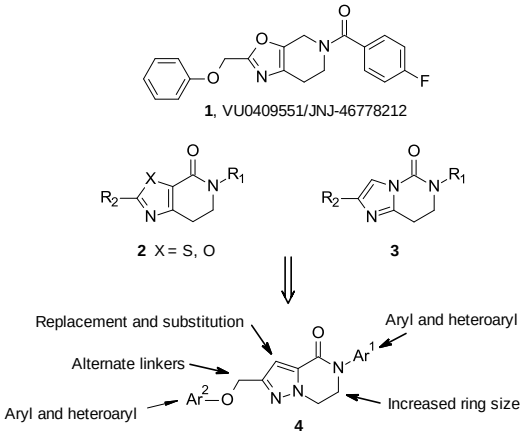
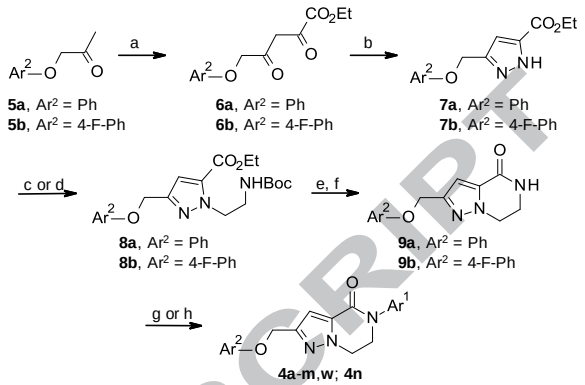


Figure 1. Structure of the clinical candidate **1** (VU0409551/JNJ-46778212). General structures of endocyclic amide-containing $mGlu_5$ PAM chemotypes and SAR exploration strategy for 6,7-dihydropyrazolo[1,5-*a*]pyrazin-4-ones **4**.

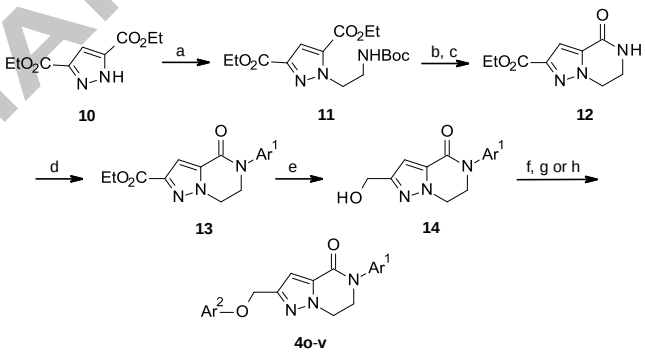
For this SAR exploration, different synthetic routes were developed. Compounds modified at the eastern and/or western aryl substituent were prepared following two different approaches. In the first one (**Scheme 1**), the western aryl was introduced at the first step of the synthesis from commercially available ketones **5**. Thus, in situ generated sodium enolates of **5** were reacted with diethyl carbonate to afford 2,4-diketone intermediates **6**. Further treatment of **6** with hydrazine afforded pyrazoles **7**. *N*-alkylation of **7**, via either a Mitsunobu or a nucleophilic substitution reaction, afforded a mixture of regioisomeric pyrazoles which after chromatographic separation provided the major regioisomers **8** pure. Boc protecting group removal in acidic media, followed by exposure to aqueous sodium carbonate, yielded bicyclic lactams **9**. Finally, *N*-arylation of **9** via copper (**4a-m**, **4w**) or palladium (**4n**) mediated couplings with the corresponding aryl halides furnished desired derivatives.

In the second approach (**Scheme 2**), symmetric diethyl 1*H*-pyrazole-3,5-dicarboxylate **10** was alkylated in basic media to afford intermediate **11** which after a deprotection-cyclization sequence analogous to the one described above, provided intermediate **12**. Eastern aryls were installed at this point via Goldberg-type coupling, resulting in intermediates **13**. Western aryls were introduced after reduction of the ester moiety in **13**, via either Mitsunobu reactions with the corresponding phenols or palladium- or copper-catalyzed coupling reactions with the corresponding aryl halides to give derivatives **4o-v**.

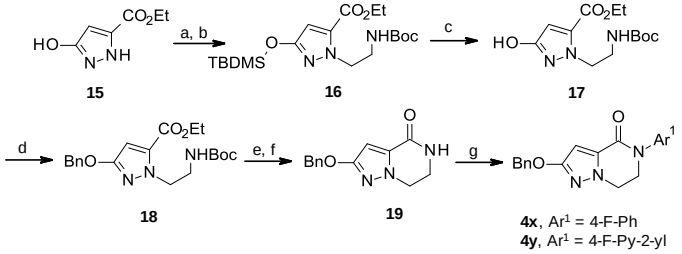
Compounds containing a -O-CH_2- linker were prepared following a similar procedure. 1*H*-pyrazole-derivative **15** was protected on the hydroxy substituent and *N*-alkylated to provide regiospecifically (likely due to the steric hindrance exerted by the TBDMS protecting group on the adjacent nitrogen) intermediate **16**. OH deprotection and alkylation with benzyl bromide furnished pyrazole **18**, which, after Boc-deprotection and base-promoted intramolecular amidation, yielded 6,7-dihydropyrazolo[1,5-*a*]pyrazin-4-one **19**. Eastern aryls were again introduced using copper-catalyzed couplings to afford derivatives **4x**,**y**.



Scheme 1. Reagents and conditions: (a) $(CO_2Et)_2$, Na, EtOH, 0 ºC to rt, 16 h, 38% (**5a**) and 30% (**5b**); (b) $N_2H_4{\cdot}H_2O$, EtOH, 80 ºC, 16 h, 98% (**7a**) and 44% (**7b**); (c) $BocNH(CH_2)_2OH$, DBAD, PPh_3, THF, 80 ºC, 16 h; (d) $BocHN(CH_2)_2Br$, K_2CO_3, DMF, rt, 16 h; (e) HCl, 1,4-dioxane, rt, 3 h; (f) Na_2CO_3, 1,4-dioxane/H_2O, rt, 16 h, 84% (**9a**) and 43% (**9b**) 3 steps; (g) ArX, CuI, $(MeHNCH_2)_2$, K_2CO_3, toluene, 120 ºC, 16 h, 60-97%; (h) 2-Cl-5-F-pyrimidine, $Pd(OAc)_2$, XPhos, Cs_2CO_3, 1,4-dioxane, 120 ºC, 16 h, 28%.



Scheme 2. Reagents and conditions: (a) $Br(CH_2)_2NHBoc$, Cs_2CO_3, DMF, rt, 16 h, 100%; (b) HCl, 1,4-dioxane, rt, 1.5 h; (c) Na_2CO_3, 1,4-dioxane/H_2O, rt, 3h, 99% 2 steps; (d) ArBr, K_2CO_3, CuI, $(MeHNCH_2)_2$, toluene, 130 ºC, 16 h, 92%; (e) $NaBH_4$, THF/MeOH, 0 ºC to rt, 2 h, 48%; (f) **4o-4r**, ArOH, DBAD, PPh_3, THF, 0 ºC to rt, 16 h, 4-34%; (g) **4s**,**v**, ArX, Cs_2CO_3, $Pd(OAc)_2$, $BiPhP{t}Bu_2$, toluene, 120 ºC, 16 h, 22 and 65%; (h) **4t-4u**, ArX, CuI, $Me_2NCH_2CO_2H$, Cs_2CO_3, 1,4-dioxane, 120 ºC, 16 h, 11-22%.



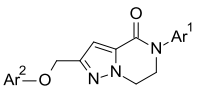
Scheme 3. Reagents and conditions: (a) TBDMSCl, imidazole, DCM, 0 ºC, 1 h, 59%; (b) $BocNH(CH_2)_2OH$, DBAD, PPh_3, THF, rt, 16 h, 76%; (c) TBAF, THF, rt, 16 h, 83%; (d) BnBr, Cs_2CO_3, DMF, rt, 16 h; (e) HCl, 1,4-dioxane, rt, 3 h; (f) Na_2CO_3, 1,4-dioxane/H_2O, rt, 16 h, 95% for 2 steps; (f) ArX, CuI, $(MeHNCH_2)_2$, K_2CO_3, toluene, 120 ºC, 16 h, 83-99%.

Analogs in which a methyl group was introduced on the -CH_2-O- spacer or carried modifications on the 6,7-dihydropyrazolo[1,5-*a*]pyrazin-4-one scaffold were prepared following similar procedures to the ones described herein (see **Supplementary Material** for further details).

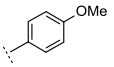
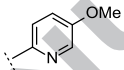
Gratifyingly, this approach proved successful, affording multiple potent $mGlu_5$ PAMs within this novel chemotype.

Representative eastern and western SAR for this series is summarized in **Table 1**. Keeping the western unsubstituted phenyl group constant, a fluorine walk around the eastern aryl resulted in potent (<100 nM) and highly efficacious $mGlu_5$ PAMs with some preference for para substitution (**4c**) over the ortho and meta congeners (**4a**,**b**). The introduction of an additional fluorine atom had no noticeable impact either in potency or in efficacy, as assessed by % maximal glutamate response (Glu Max%) (**4d**,**e**). Replacement of fluorine by methyl or methoxy groups resulted in comparable potencies (**4f**,**g** vs **4c**), yet both analogs displayed slightly reduced efficacy. The replacement of the phenyl by pyridyl led to $mGlu_5$ PAMs with moderate potency and good efficacy, with the pyridyl-2-yl (**4h**) the preferred regioisomer with a ~3-fold increased potency over the 3- and 4-isomers (**4i**,**j**). The activity was further improved by the introduction of a fluorine atom (**4k**) or a methyl group (**4l**) at the 5-position of the pyridine, while the introduction of a methoxy substituent at the same position had negligible effect (**4m** vs **4h**). Finally, the replacement of pyridine by pyrimidine resulted in a ~6-fold decrease in the $mGlu_5$ activity (**4n** vs **4k**). Selectivity screen of selected examples versus the other mGlu family members ($mGlu_{1\text{-}4,6\text{-}8}$)[25] revealed, similarly to imidazopyrimidinones **3**[24] and a closely related tetranaphthridinone series,[26] potent $mGlu_3$ antagonist activity for the phenyl-containing congeners (e.g. **4c**,**f** $mGlu_3$ EC_{50} = 74 and 150 nM respectively, full antagonism). Gratifyingly, the replacement by pyridine decreased the $mGlu_3$ antagonism (**4k**,**l** $mGlu_3$ EC_{50} = 6760 and >10000 nM respectively) resulting in >50 fold functional selectivity for $mGlu_5$ for **4k** and **4l**. Furthermore, none of the compounds tested showed relevant activities at any of the other mGlus (EC_{50} >10 μM vs $mGlu_{1,2,4,6,7,8}$). A focused surveillance of the western aryl group SAR was performed, fixing a 4-fluorophenyl group as the eastern amide substituent (**Table 1**). Akin to the eastern aryl exploration, the introduction of a fluorine atom yielded potent $mGlu_5$ PAMs with modest influence of the substitution pattern (**4o-q**). Replacement of fluorine by methyl (**4q** vs **4r**) resulted in a loss in both potency and efficacy. Finally, the introduction of pyridine had a clear detrimental effect on potency (**4s-u**) which, in this case, was not compensated by the introduction of a 3-fluoro substituent (**4v**). In contrast to the amide substituent, the nature of the eastern aryl had no noticeable impact on the $mGlu_3$ antagonist activity with representative examples (e.g. **4q**,**s** $mGlu_3$ ant. EC_{50} = 34 and 290 nM respectively, full antagonism) showing even more potent $mGlu_3$ than $mGlu_5$ activity. Additionally, despite the combination of one of the most potent western substituent (3-fluorophenyl) with the 5-fluoro-2-pyridyl amide in **4w**, resulting in a highly potent $mGlu_5$ PAM, the introduction of an eastern pyridyl moiety did not lead to improved selectivity versus $mGlu_3$ (EC_{50} = 140 nM).

Table 1. Structures and activities of analogs **4**.



4

Entry	Ar^1	Ar^2	pEC_{50} (±SEM)[a]	EC_{50} (nM)[a]	Glu Max % (±SEM)[a]
4a			6.80±0.11	160	86±5
4b			6.90±0.08	130	81±6
4c			7.19±0.02	65	79±3
4d			7.00±0.03	100	101±6
4e			7.06±0.02	87	81±4
4f			7.15±0.06	71	65±5
4g			6.94±0.03	110	58±8
4h			6.12±0.03	760	69±8
4i			5.68±0.11	2090	64±10
4j			5.68±0.15	2090	72±3
4k			6.90±0.04	130	72±5
4l			6.73±0.03	190	76±4
4m			6.21±0.06	620	74±6
4n			6.12±0.05	760	72±6
4o			6.83±0.02	150	66±8
4p			7.00±0.02	100	70±3
4q			6.98±0.04	100	72±5
4r			6.52±0.07	300	58±11
4s			6.31±0.05	490	68±3
4t			5.40±0.12	3980	69±2
4u			6.16±0.08	690	69±4
4v			5.1±0.17	7940	52±12
4w			7.04±0.03	91	62±9

[a]Calcium mobilization assay using HEK293 cells expressing human $mGlu_5$; values are the average of three or more independent determinations.

Other relevant SAR for this chemotype concerning modifications in the spacer and the central core is summarized in **Figure 2**. Replacement of the $-CH_2-O-$ linkage by the regioisomeric $-O-CH_2-$ resulted in a minor decrease in $mGlu_5$ potency (~2.5 fold) and efficacy for the match-pair **4x**-**4c** but in an improved selectivity over $mGlu_3$ ($mGlu_3$ ant. EC_{50} = 3390 nM). By contrast, the combination of this linker with the 4-F-2-pyridyl amide substituent resulted in an unexpected complete loss of activity (**4y** vs **4k**). Likewise, the introduction of a methyl group on the $-CH_2-O-$ linker (**4z**) significantly diminished the $mGlu_5$ potency by >100 fold. Finally, a small set of central core modifications were also surveyed. While increasing the ring size from piperazinone to homopiperazinone (**20**) resulted in a slight loss of activity at $mGlu_5$ (~3 fold) and a modestly improved selectivity ($mGlu_3$ EC_{50} = 540 nM), modifications in the pyrazole unit proved to be more deleterious. Thus, either replacement of

Table 2. Relevant comparative profiling data for analogs **4k** and **4x**.

Entry	cLogP	$mGlu_3$ ant EC_{50} (nM)[a]	$mGlu_3$ / $mGlu_5$ EC_{50} ratio	$mGlu_{1,2,4,6,7,8}$ EC_{50} (μM)[b]	Rat CL_{hep}[c] (mL/min/kg)	Rat $f_{u\ brain}$ (%)	AHL[d] (% reversal)	$[brain]_{u,90min}$[e] (nM)
4k	1.37	6760	52	> 10	34.5	5.5	50	313
4x	2.90	3390	21	> 10	34.6	6.5	12	376

[a]Calcium mobilization assay using HEK293 cells expressing human $mGlu_3$; values are the average of three or more independent determinations.
[b]Calcium mobilization assay using HEK293 cells expressing human expressing human $mGlu_1$, $mGlu_2$, $mGlu_7$, or $mGlu_8$ receptors and [^{35}S]-GTPγS functional assay using CHO cells expressing rat $mGlu_6$ receptors; values are the average of three or more independent determinations
[c]Predicted hepatic clearance based on microsomal CL_{int} data.
[d]Amphetamine-induced locomotion % reversal after a 10 mg/kg p.o. dose, vehicle 20% HP-β-CD.
[e]Based upon rat brain homogenate binding (equilibrium dialysis) fraction unbound and total terminal (90 min) brain levels.

the pyrazole by a triazole (**21a**), or the introduction of methyl or chlorine substituents at the 3 position of the 6,7-dihydropyrazolo-[1,5-*a*]pyrazin-4-one core (**21b**,**c**) resulted in a remarkable loss of $mGlu_5$ potency.

Since dual $mGlu_5$/$mGlu_3$ activities may mask or confound $mGlu_5$ associated pharmacology and/or toxicology, our selection criteria focused on compounds with potent $mGlu_5$ PAM activity combined with significant selectivity over $mGlu_3$ antagonist activity. Therefore, based on these selection principles, **4k** and **4x** stood out as candidates worthy of further characterization in support of eventual assessment in our primary rodent (rat) in vivo pharmacodynamic paradigm, the amphetamine-induced hyperlocomotion (AHL) model.[17] Comparative data for both compounds, included in **Table 2**, show that both compounds presented a favorable cLogP for a CNS drug,[27] >20 fold selectivity versus $mGlu_3$ antagonist activity and no relevant activities at any of the other mGlu receptors (EC_{50}s >10 μM). Furthermore, a moderate in vitro predicted rat hepatic clearance (rCL_{hep} <35 mL min^{-1} kg^{-1}) and a good fraction unbound in rat brain warranted the in vivo evaluation of both compounds. Thus **4k** and **4x** were evaluated in the AHL challenge model using an oral screening dose of 10 mg/kg (20% β-cyclodextrin (HP-β-CD), homogeneous suspension).[17] Interestingly, while both compounds had terminal unbound brain concentrations (n = 6 animals, T_{90}) above their corresponding in vitro $mGlu_5$ EC_{50}s, a robust reversal of AHL could only be demonstrated for **4k** (50%). This striking difference could be attributable to the lower PAM efficacy at $mGlu_5$ receptors for **4x** (54% vs 72% for **4k**) which may render this compound unable to effectively reverse the effects of amphetamine at the tested dose. Similar results have also been reported for other low efficacy $mGlu_5$ PAMs.[13, 14]

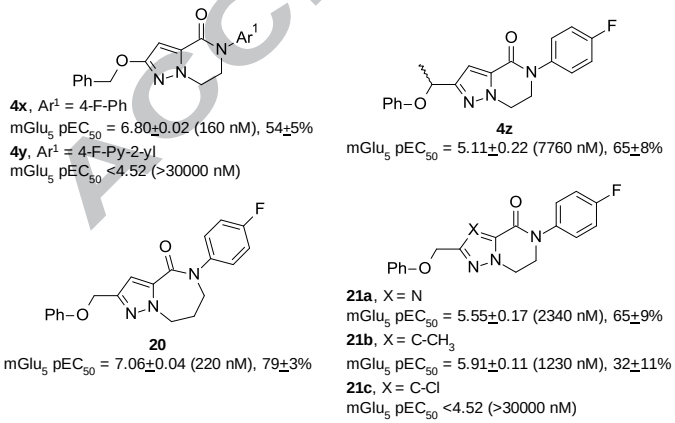


Figure 2. Structures and $mGlu_5$ activity of 6,7-dihydropyrazolo[1,5-*a*]pyrazin-4-one derivatives modified at the spacer and central core.

Thus, based on its promising preliminary profile as well as its good activity in our pharmacodynamic screen, **4k** was advanced to additional pharmacological and ADMET profiling, discrete PK (rat and dog), full dose-response studies in AHL to determine its minimal effective dose (MED), and evaluation in a preclinical cognition model, the contextual fear conditioning (CFC) paradigm.[16] Further pharmacological profiling of **4k** in glutamate concentration response curve (CRC) fold-shift (FS) experiments in human $mGlu_5$ expressing cells confirmed cooperativity at $mGlu_5$ receptors (7.7) (FS = 11 for **1**).[16] Additionally, **4k** was assessed in a broad ancillary screening panel of distinct GPCRs, ion channels and transporters[23] in which only weak interaction (57% inhibition of binding at 10 μM) with the human platelet activating factor (PAF) was found. Moreover, other relevant in vitro ADMET properties were also favorable, including moderate predicted human hepatic clearance (hCL_{hep} = 8.4 mL min^{-1} kg^{-1}), clean P450 inhibition profile (1A2 2C9, 2D6 and 3A4 IC_{50}s ≥ 30 μM), and low plasma protein binding (f_u (h,r): 8.2, 6.8). Additionally, and despite low solubility (FaSSIF = 13 μg/mL), **4k** was found to be well absorbed either from a 20% HP-β-CD solution or suspension (vide infra). In vitro preliminary toxicity assessment also confirmed a benign profile for **4k** in AMES, high content cytotoxicity assay (>100 μM), and in GSH/CN trapping studies, with no significant interactions on cardiovascular relevant ion channels (hERG, Ca, Na channels, IC_{50}s >10 μM). The in vivo PK profile for **4k** was assessed in rat and dog (**Table 3**), and moderate clearance and volume of distribution for both species were noted, with resulting moderate half-lives. A longer time to reach peak levels (T_{max}) in the rat likely reflects solubility-limiting absorption for this species. Oral bioavailability was good for both species (>45%) with an excellent 88% in the rat.

Table 3. Pharmacokinetic parameters for **4k**.

Parameters	rat[a]	dog[a]
Dose (mg kg-1) i.v./p.o.	2.5[b]/10[c]	0.5[b]/5[c]
CL_p (mL/min/kg) i.v.	19	25
Vd_{ss} (L/kg) i.v.	1.6	2.6
$t_{1/2}$ (h) i.v.	1.6	2.4
C_{max} (ng/mL) p.o.	730	519
T_{max} (h) p.o.	4	0.7
$AUC_{0\text{-}inf}$ (ng·h/mL) p.o.	6668	1536
F (%) p.o.	88	48

[a]Data reported as average of three animals.
[b]Dosed as 20% HP-β-CD solution.
[c]Dosed as 20% HP-β-CD suspension.

In full dose-response studies, **4k** produced a dose- and concentration-dependent reversal of amphetamine-induced hyperlocomotion upon oral administration with a MED of 3 mg/kg (**Figure 3**) and full reversal at 100 mg/kg. Terminal (1.5 h) plasma and brain concentrations in this study were linear for doses from 3 to 10 mg/kg and sub-linear from 30 to 100 mg/kg). Furthermore, in addition to its antipsychotic-like activity, **4k** also induced a dose-dependent enhancement of CFC acquisition in rats (MED = 0.3 mg/kg p.o.) demonstrating efficacy in an established measure of hippocampal-dependent cognitive function (**Supplementary Material**) which may be suggestive of efficacy across multiple domains of schizophrenia. In virtue of its attractive in vitro and in vivo properties, **4k** was considered

for advancement as a potential back-up candidate for **1**. Unfortunately, during a toxicology rat MTD study (25, 75, 150, 300 and 600 mg/kg, p.o.) evidence for signs for CNS-related side-effects (compulsive behavior (chewing and head shaking), pedaling movements of the forepaws and tremors) starting at the lowest tested dose, combined with the death of 2/6 animals at the highest dose, precluded further development and confirmed again the subtle relationship between mGu_5 activation and target related toxicities. As a consequence, and in order to identify suitable back-up candidates, our efforts have now shifted toward identifying compounds with an even further reduced efficacy at $mGlu_5$.

In conclusion, as part of our efforts towards a back-up compound for the $mGlu_5$ PAM clinical candidate **1** (VU0409551/JNJ-46778212) from a different chemotype, a scaffold hopping strategy resulted in the identification of a series of 6,7-dihydropyrazolo[1,5-*a*]pyrazin-4-ones as potent $mGlu_5$ PAMs. SAR investigations within this chemotype resulted in the identification of compound **4k** as a potential back-up candidate. Despite **4k** possessing attractive in vitro and in vivo pharmacological, ADMET and PK properties, rat toxicology MTD studies deemed the compound inadequate for further progression as a consequence of side-effects likely due to excessive $mGlu_5$ activation. Efforts aimed to identify less efficacious $mGlu_5$ PAMs, within this and other chemotypes will be reported in due course.

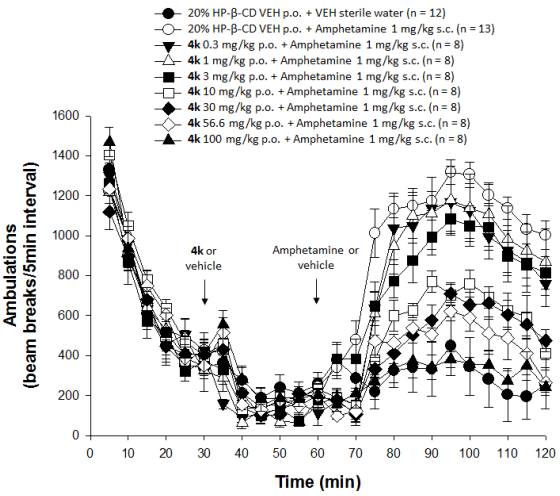


Figure 3. **4k** has antipsychotic-like activity in male, Sprague Dawley rats. **4k** dose-dependently (3-100 mg/kg, p.o.) reverses AHL. Vehicle is 20% HP-β-CD (3-10 mg/kg solution, 30-100 mg/kg suspension).

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Supplementary Material

Synthesis and experimental details for **4k**, synthesis routes and general conditions used to prepare analogs **4z**, **20** and **21a-c** and contextual fear conditioning data for **4k** are available as supplementary material.

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Preliminary investigation of 6,7-dihydropyrazolo-[1,5-*a*]pyrazine-4-one derivatives as a novel series of $mGlu_5$ receptor positive allosteric modulators with efficacy in preclinical models of schizophrenia

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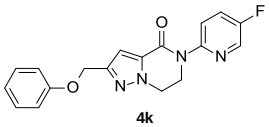

4k

$hmGlu_5$ PAM EC_{50} = 130 nM
$hmGlu_3$ ant. EC_{50} = 6760 nM
$hmGlu_5$ PAM FS = 7.7
AHL MED = 3 mg/kg p.o.
FC MED = 0.3 mg/kg p.o.