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Discovery and SAR of novel series of imidazopyrimidinones and dihydroimidazopyrimidinones as positive allosteric modulators of the metabotropic glutamate receptor 5 ($mGlu_5$)

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ABSTRACT

We report the discovery and SAR of two novel series of imidazopyrimidinones and dihydroimidazopyrimidinones as metabotropic glutamate receptor 5 ($mGlu_5$) positive allosteric modulators (PAMs). Exploration of several structural features in the western and eastern part of the imidazopyrimidinone core and combinations thereof, revealed compound **4a** as a $mGlu_5$ PAM with good in vitro potency and efficacy, acceptable drug metabolism and pharmacokinetic (DMPK) properties and in vivo efficacy in an amphetamine-based model of psychosis. However, the presence of CNS-mediated adverse effects in preclinical species precluded any further in vivo evaluation.

Schizophrenia is a devastating psychiatric illness that afflicts approximately 1% of the world population. Current therapies, based on the direct modulation of the monoaminergic system (dopamine and serotonin), are associated with severe adverse effects and do not provide sufficient relief across all symptom domains of the disease.[1,2] Therefore, new treatments, possibly acting via different neurotransmitter systems are under investigation. Among the different existing alternatives, the modulation of the glutamatergic system has emerged as an obvious candidate.[3,4] Glutamate (L-glutamic acid) is the major excitatory neurotransmitter in the mammalian central nervous system (CNS), where it mediates its effects through three ionotropic receptors (D,L-α-amino-3-hydroxy-5-methyl-4-isoxazole propionic acid (AMPA), *N*-methyl-D-aspartate (NMDA) and kainate) and eight metabotropic glutamate receptors ($mGlu_1$ to $mGlu_8$).[5] Among the different mGlus, the $mGlu_5$ receptor is structurally and pharmacologically associated with the NMDA receptor playing prominent roles in synaptic plasticity, cognition, learning and memory process[1,6] and it has been reported that the activation of $mGlu_5$ potentiates NMDA receptor function.[7] Provided the advantages associated to PAMs over orthosteric agonists, that is, increased subtype selectivity, improved chemical tractability, better modulatory control of receptor function and low propensity for receptor desensitization; the potentiation of $mGlu_5$ via activation at allosteric sites could be a preferred pharmacological mechanism to indirectly correct the hypothesized NMDA receptor hypofunction in schizophrenia.[2,8] Consequently, the development of PAMs of $mGlu_5$ has emerged as a potentially viable way to treat positive symptoms[9,10] and also improve cognitive deficits[11,12] in schizophrenia.

After nearly a decade of $mGlu_5$ PAMs research, a variety of structurally different chemotypes have been reported.[13] Among

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them, we have recently described the discovery of phenoxy-containing $mGlu_5$ PAMs derived from dihydrothiazolopyridone[14] and naphthyridinone[15] bicyclic cores. As a progression of this work, and in order to further optimize the in vitro and in vivo potencies of dihydrothiazolopyridone **1**, we envisioned the replacement of the thiazolyl ring system by other aromatic five-membered rings (Fig. 1).

Our initial approach involved the direct replacement by an oxazolyl ring affording dihydrooxazolopyridones **2**. Encouraged by a comparable in vitro $mGlu_5$ PAM activity to dihydrothiazolopyridone **1** series, the most potent analogues were tested in the reversal of amphetamine-induced hyperlocomotion (AIH) in rats, a predictive model of antipsychotic activity.[10a,16] Unfortunately, none of the analogues tested showed robust in vivo activity after oral dosing. This lack of relevant in vivo activity could be explained by the limited absorption observed for these derivatives and thus, our subsequent exploration focused on the identification of other chemotypes with different properties (i.e., electronics, basicity, lipophilicity, etc.) that could eventually lead to an improvement in the in vivo efficacy. In this Letter we report our efforts towards the synthesis and optimization of imidazopyrimidinones **3** and dihydroimidazopyrimidinones **4** as novel series of $mGlu_5$ PAMs.

Synthesis of these 5,6-bicyclic systems was achieved following two parallel approaches that allowed the introduction of diverse substituents in the eastern and western part of the targeted molecules, as shown in Schemes 1 and 2. Our first strategy was focused on the preparation of the key compound **3a**, with the aim to survey the eastern diversity in the last step (Scheme 1). Condensation of chloropyrimidine **5** with 1,3-dichloroacetone under acidic conditions, following a procedure already reported in the literature afforded intermediate **6**.[17] Introduction of phenoxy group in the western part of the molecule using potassium carbonate as base was followed by the selective hydrolysis of the thiomethyl group yielding intermediate **7**. De-chlorination was performed under hydrogenation conditions affording analogue **3a**. This key compound was then derivatized to the desired *N*-aryl imidazopyrimidinones **3e** and **3h**–**3n**, by reaction either with *p*-fluoroiodobenzene (**3e**) or various heteroaryl halides (**3h**–**3n**) using a copper iodide–diamine ligand protocol to perform a modified Ullman-type lactam cross-coupling.

Unfortunately, functionalization in the eastern amide moiety with alkyl substituents or other aryl halides following the same Ullman-type conditions was unsuccessful and for this purpose, the approach outlined in Scheme 2 was used. This second strategy allowed the introduction of several alkyl and aryl moieties and also to survey the substitution in the western part of the molecule. Furthermore, this approach could be additionally applied to the introduction of small groups in the 7 and 8-positions of the imidazopyrimidinone core (Scheme 2). Thus, for the functionalization with alkyl and aryl groups in the eastern part of the molecule we started from commercially available cytosine derivatives **9**. The reaction of **9** with a variety of alkyl halides using tetrabutylammonium hydroxide as base allowed the selective alkylation on the lactam. On the other hand, for the functionalization with aryl moieties a Chan-Lam-type copper-catalyzed coupling reaction between cytosine derivatives **9** and the corresponding aryl boronic acids as depicted in Scheme 2 was followed.[18] Then, condensation with 1,3-dichloroacetone followed by introduction of the corresponding phenoxy or pyridyloxy group in the western part of the molecule, via reaction with the corresponding phenols or hydroxypyridines using potassium carbonate as base, allowed the preparation of analogues **3b**–**3g**, **3o**–**3s** and **3u**. The 8-methyl analogue **3v** was prepared by Suzuki-type coupling reaction between methylboronic acid and intermediate **13**, synthesized following a similar synthetic route as described before but starting from the corresponding substituted iodocytosine **12** as shown in Scheme 2.

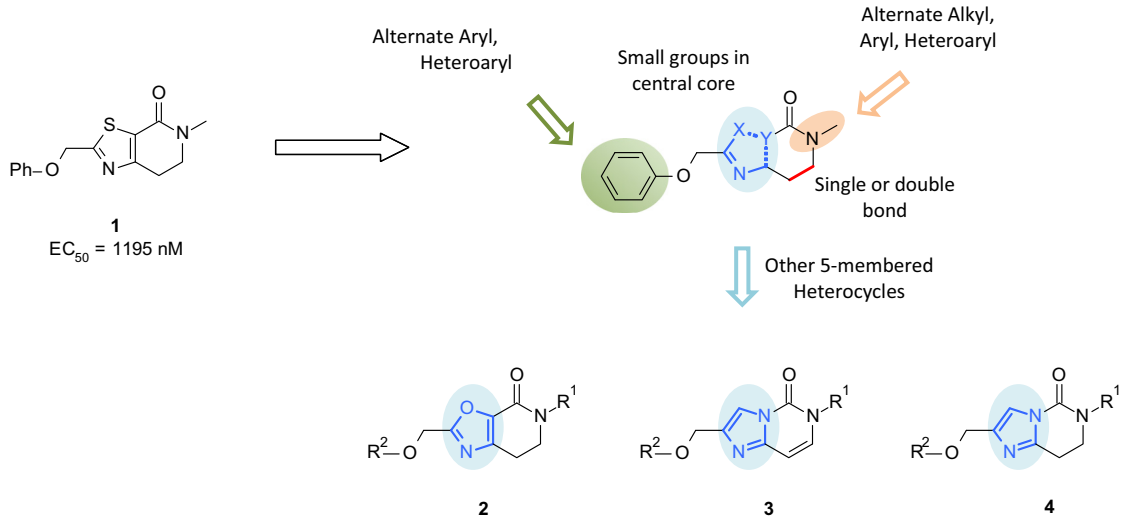
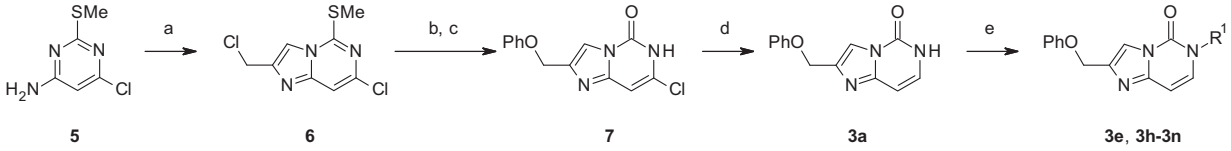
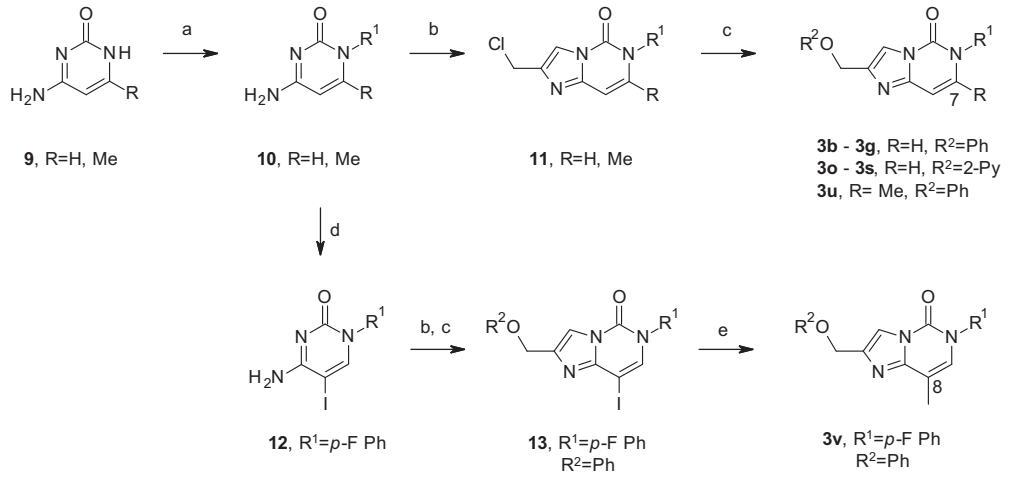


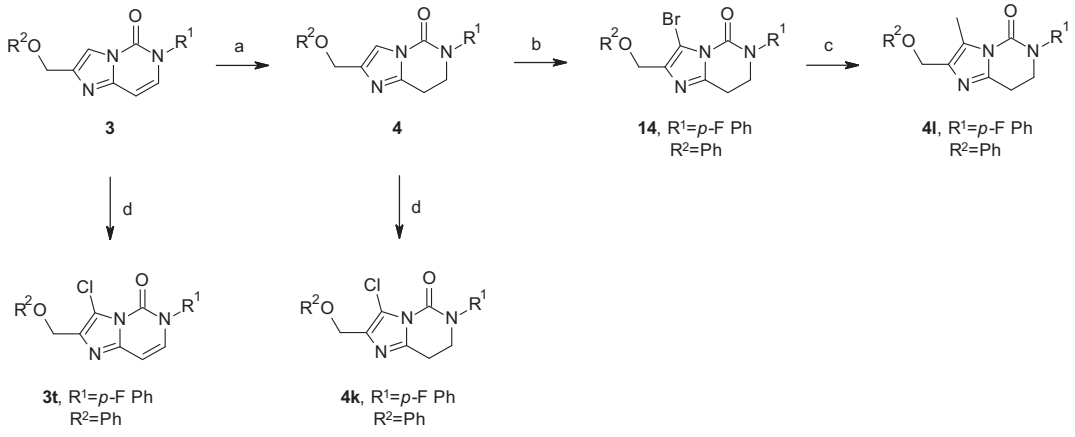
Figure 1. Evolution strategy of dihydrothiazolopyridone **1** to other 5,6-bicyclic systems.



Scheme 1. Reagents and conditions: (a) 1,3-dichloroacetone, AcOH, 110 °C, 16 h, 44%; (b) PhOH, K_2CO_3, ACN, 50 °C, 18 h, 53%; (c) LiOH, H_2O/THF, 50 °C, 4 h, 98%; (d) $Pd(OH)_2$/C, H_2 atm, Et_3N, AcOEt/MeOH, 50 °C, 45 psi, 16 h, in a Parr reactor, 92%; (e) R^1X, CuI, *N*,*N*′-dimethylethylenediamine, K_2CO_3, toluene, 120 °C, 16 h, 22–73%.



Scheme 2. Reagents and conditions: (a) for R^1 = Alkyl: R^1X, BuN_4OH, DMF, rt, 2 h, 57–77% and for R^1 = Aryl or heteroaryl: $R^1B(OH)_2$, $Cu(OAc)_2$, TMEDA, MeOH/H_2O, rt, 16 h, 48–65%; (b) 1,3-dichloroacetone, DMF, 150 °C, 30 min, microwave irradiation, 15–75%; (c) R^2OH, K_2CO_3, ACN, 90 °C, 18 h, 58–95%; (d) I_2, $NaIO_4$, H_2SO_4, AcOH/water, 80 °C, 4 h, 84%; (e) $MeB(OH)_2$, $Pd(PPh_3)_4$, K_2CO_3, 1,4-dioxane/DMF, 150 °C, 45 min, microwave irradiation, 72%.



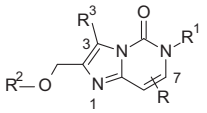
Scheme 3. Reagents and conditions: (a) $Pd(OH)_2/C$, H_2 atm, MeOH, 50 °C, 50 psi, 16 h, in a Parr reactor or Raney-Ni, 80 °C, full hydrogen mode, DMF/MeOH in an H-Cube reactor, 5–78%; (b) NBS, benzoyl peroxide, DCE, rt, 16 h, 35%; (c) $MeB(OH)_2$, $Pd(PPh_3)_4$, K_2CO_3, 1,4-dioxane/DMF, 150 °C, 45 min, microwave irradiation, 56%; (d) NCS, DMF, 150 °C, 15–20 min, microwave irradiation, 27–55%.

Hydrogenation of the double bond in analogues **3** was performed using palladium hydroxide on carbon or Raney nickel as catalysts at high temperatures (Scheme 3). All attempts to reduce the double bond in compounds with substitution in the 7 or 8-positions different than hydrogen were unsuccessful. Finally, functionalization in the imidazole ring was achieved by halogenation of the corresponding derivatives **3** and **4** with *N*-chlorosuccinimide (NCS) or *N*-bromosuccinimide (NBS). Subsequent Suzuki-type coupling reaction of **14** with methylboronic acid yielded analogue **4l** as depicted in Scheme 3.

The compounds synthesized were profiled in a human $mGlu_5$ low receptor expression cell line using a 'triple add' calcium mobilization assay, allowing the detection of agonism as well as positive and negative allosteric modulation simultaneously.[19] The most relevant SAR trends for the imidazopyrimidinones **3** are shown in Table 1. Initial exploration was focused on the introduction of substituents in the amide moiety with the aim to further improve the marginal activity observed for the unsubstituted analogue **3a** (EC_{50} >30 μM, 19% Glu Max). Following a similar strategy to the dihydrothiazolopyridone series (**1**),[14] we first targeted the introduction of alkyl substituents. Thus, N-methylation in compound **3b**, despite having a positive effect in efficacy (67% Glu Max) did not improve the in vitro potency (EC_{50} >10 μM), which was still significantly decreased compared with analogue **1**. According to previous work,[14] the functionalization with an alkyl chain of increased size such as cyclopropylmethyl (**3c**) interestingly induced a significant improvement in the in vitro $mGlu_5$ PAM activity, resulting in a potency in the same range as that of the dihydrothiazolopyridone prototype **1**, although with reduced efficacy. Comparable potency again but significantly improved efficacy (93% Glu Max) was achieved by the introduction of a bulkier aromatic benzyl group in **3d**.

We next evaluated the effects of the introduction of different substituted aryl and heteroaryl rings on the lactam. Based on our previous results,[14] SAR investigation started with the introduction of fluorine-containing phenyl rings (**3e**–**3g**) resulting in a remarkable improvement in the $mGlu_5$ PAM activity. Among this small set, the *para*- and the 3,4-substitutions (**3e** and **3g**) proved to be more beneficial than the *meta*- (**3f**) in terms of potency and efficacy. Additionally, the *meta*-substitution was also found detrimental for the metabolic stability in rat liver microsomes (RLM). In a second step, we studied the effects of the replacement of the phenyl ring by more polar and weakly basic pyridines prioritizing the 2-substituted pyridine analogues over the 3 or 4 regioisomers

Table 1
Structures and activities of substituted imidazopyrimidinones **3**



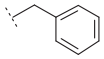
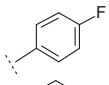
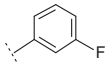
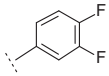
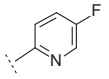
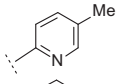
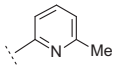
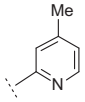
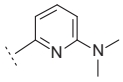
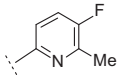
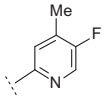
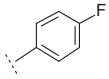
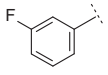
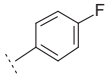
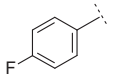
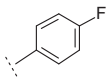
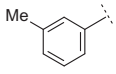
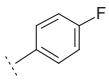
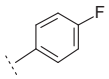
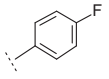
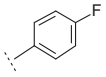
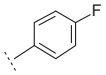
Compd	R^1	R^2	R^3	R	$mGlu_5$ pEC_{50}[a] (±SEM)	$mGlu_5$ EC_{50} (nM)	% Glu max[b] (±SEM)	HLM[c] (%)	RLM[c] (%)
3a	H		H	H	<4.52[d]	>30,000	19	n.t.[e]	n.t.[e]
3b			H	H	<5[d]	<10,000	67 ± 4.2	n.t.[e]	n.t.[e]
3c			H	H	5.95 ± 0.03	1120	47 ± 3.7	n.t.[e]	n.t.[e]
3d			H	H	5.80 ± 0.06	1590	93 ± 5.0	n.t.[e]	n.t.[e]
3e			H	H	6.51 ± 0.15	310	83 ± 9.1	14	27
3f			H	H	6.29 ± 0.02	514	69 ± 0.9	47	71
3g			H	H	6.64 ± 0.05	230	93 ± 11.7	n.t.[e]	n.t.[e]
3h			H	H	6.23 ± 0.05	587	87 ± 10.2	n.t.[e]	n.t.[e]
3i			H	H	5.69 ± 0.14	2000	84 ± 15.4	n.t.[e]	n.t.[e]
3j			H	H	5.45 ± 0.11	3580	64 ± 10.3	n.t.[e]	n.t.[e]
3k			H	H	5.29 ± 0.07	5100	64 ± 9.0	n.t.[e]	n.t.[e]
3l			H	H	6.55 ± 0.08	284	87 ± 13.6	66	90
3m			H	H	6.43 ± 0.05	375	77 ± 8.0	8	50
3n			H	H	6.36 ± 0.07	435	77 ± 11.9	17	49
3o			H	H	6.64 ± 0.05	230	91 ± 11.1	13	28
3p			H	H	6.43 ± 0.06	375	85 ± 11.6	11	29
3q			H	H	6.46 ± 0.11	344	30 ± 2.8	n.t.[e]	n.t.[e]
3r			H	H	5.50 ± 0.09	3170	88 ± 12.4	n.t.[e]	n.t.[e]
3s			H	H	<5[d]	<10,000	65 ± 5.5	n.t.[e]	n.t.[e]
3t			Cl	H	6.66 ± 0.08[f]	218[f]	5 ± 0.0.5[f]	6	73

Table 1 (*continued*)

Compd	R^1	R^2	R^3	R	$mGlu_5$ pEC_{50}[a] (±SEM)	$mGlu_5$ EC_{50} (nM)	% Glu max[b] (±SEM)	HLM[c] (%)	RLM[c] (%)
3u			H	7-Me	5.68 ± 0.08	2070	60 ± 4.5	n.t.[e]	n.t.[e]
3v			H	8-Me	6.00 ± 0.06[f]	1000[f]	16 ± 3.8[f]	36	71

[a] Calcium mobilization assay using HEK293 cells stably expressing rat $mGlu_5$ receptors; values are the average of three or more independent determinations.
[b] Expressed as amplitude of response using 30 μM test compound (percentage of maximal response vs 100 μM glutamate).
[c] HLM and RLM data refer to % of compound metabolized after incubation of tested compound with human and rat microsomes, respectively, for 15 min at 1 μM concentration.
[d] Data obtained from a single experiment not replicated.
[e] Not tested.
[f] Antagonists/NAMs, data represents pIC_{50}/IC_{50} from EC_{80} window and % Glu represents E_{min} not E_{max}.

(**3h**–**3n**).[14] Thus, while only a slight decreased PAM activity was shown with compound **3h**, the corresponding 2-pyridyl analogue of **3e**; the introduction of a methyl substituent in 3-, 4- and 5-position of the 2-substituted pyridine ring (analogues **3i**–**3k**), revealed a substantial loss of potency and efficacy. The recovery of both properties was achieved with a more basic analogue (**3l**) although unfortunately, this was accompanied by a high metabolic turnover in human liver microsomes (HLM) and RLM. Similarly, despite the combination of fluorine and methyl in the disubstituted pyridyl congeners **3m** and **3n** resulted in good in vitro PAM activity and efficacy, the improvement in metabolic stability in RLM was only moderate.

Encouraged by the promising profile of **3e**, we next decided to evaluate the influence of the western aromatic ring in our molecules keeping constant for that purpose the *para*-fluorophenyl substitution on the amide moiety. Thus, the introduction of a fluorine atom in *meta*- and *para*-positions of the phenoxy ring resulted in similar PAM potencies and efficacies (EC_{50} = 230 nM, 91% Glu Max for **3o** and EC_{50} = 375 nM, 85% Glu Max for **3p**) to the parent analogue **3e**. Similarly, the introduction of a methyl group also retained the in vitro activity although a remarkable decrease in efficacy was observed (**3q**, 30% Glu Max). In contrast, replacement of the western phenyl by a pyridine was found to be clearly detrimental for $mGlu_5$ activity (compounds **3r** and **3s**).

Additionally, we also investigated the influence of small groups in different positions of the imidazopyrimidinone core, keeping again the *para*-fluorophenyl substitution on the lactam (compounds **3t**–**3v**). This brief SAR survey led to an unexpected 'molecular switch' that changed the mode of the pharmacology. Thus, the introduction of a chlorine atom in the 3-position (**3t**) or a methyl substituent in the 8-position (**3v**) provided weak to moderate $mGlu_5$ antagonists or negative allosteric modulators (NAMs) with IC_{50}s of 218 and 1000 nM, respectively, and with additionally reduced metabolic stability in RLM. On the contrary, the 7-methyl analogue **3u** still behaved as $mGlu_5$ PAM although with a greatly reduced potency (EC_{50} = 2070 nM, 60% Glu Max) compared to parent congener **3e**.

In parallel to our exploration of the imidazopyrimidinone series **3**, we also synthesized a number of the more flexible dihydroimidazopyrimidinones **4** by hydrogenation of a set of analogues **3** presenting identified preferred modifications. SAR of this series is summarized in Table 2. Alkyl analogues in the lactam were found unstable under basic conditions[20] and thus only aryl and heteroaryl substitutions were explored. Interestingly, this small library proved more productive than the previous one, providing several analogues with $mGlu_5$ PAM activities below 250 nM. The specific comparison among pairs of analogues presenting fluorine decorations in the eastern aromatic aryl confirmed this trend; with a noticeable ~3.5 fold increase in potency for the *para*-fluorophenyl derivative **4a** versus **3e** and less prominent 1.8–2.4 increase for the *meta*- and the 3,4-difluoro substituted analogues (**4b** and **4c** vs **3f** and **3g**, respectively). The same beneficial effect was also observed for the pyridyl-containing pairs (**4d**–**4f** vs **3h**,**i**,**m**) with $mGlu_5$ potency increases ranging from 1.6 to 4.1 fold, however counterparts **4g** and **3n** showed comparable activity. On the other hand, the increased flexibility in the central core did not result in any outstanding effect on microsomal stability.

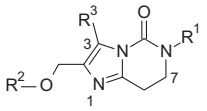
Analogously, the introduction of a fluorine atom in the *meta*- (**4h**) and *para*-position (**4i**) of the western aryl, greatly increased $mGlu_5$ potency up to ~3.3 fold versus series **3** corresponding counterparts, maintaining also a high efficacy. Gratifyingly, this exploration yielded analogue **4h**, the most potent $mGlu_5$ PAM across the two series described herein (EC_{50} = 69 nM, 72% Glu Max). However, the introduction of a methyl group in the *meta*-position (**4j**) contributed to a significant loss in efficacy in comparison with the other dihydroimidazopyrimidinones **4**, confirming the tendency previously observed for series **3** (47% Glu Max for **4j** vs 30% Glu Max for **3q**).

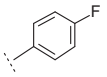
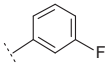
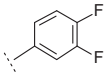
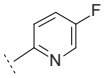
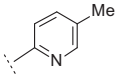
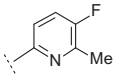
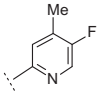
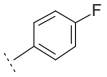
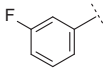
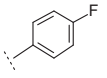
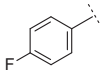
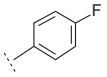
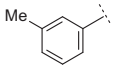
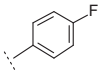
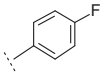
Similarly to chemotype **3**, the introduction of substituents in the 3-position of the dihydroimidazopyrimidinone core was responsible again for a change in the mode of pharmacology and resulted in compounds that behaved as antagonists/NAMs. Thus, the introduction of a chlorine atom in **4k** or a methyl group in **4l**, afforded $mGlu_5$ antagonists/NAMs of moderate potency (133 and 529 nM, respectively). These data demonstrated again that with very subtle structural changes, a reasonably potent PAM can be transformed into an antagonist/NAM of comparable potency, confirming the findings previously reported for other chemotypes.[15,21]

After this initial exploration, analogues **3e** and **4a** were selected as representative examples from both chemotypes for additional characterization (Table 3). Thus, besides increased flexibility, **4a** shows also a slightly reduced *c*Log*P* (0.5 log units). Both analogues were found adequately stable in HLM and RLM with no significant differences in the extent of metabolism. Additionally, in a cocktail assay using HLM and known substrates, **4a** did not show any relevant inhibition of the major human cytochrome P450 (CYP) enzymes (2C9, 2D6, 3A4, 1A2) (IC_{50}s >20 μM), while **3e** was found to display moderate CYP inhibitory activity on 1A2 (IC_{50} = 9.3 μM, others IC_{50} >20 μM). Finally, **4a** showed a slightly higher unbound fraction in both human and rat plasma compared with **3e**.

Subsequent selectivity profiling against the other mGlu receptors ($mGlu_{1-4,6-8}$) revealed that both compounds were also active as full $mGlu_3$ NAMs, (IC_{50} = 630 nM for **3e** and 290 nM for **4a**), resulting in moderate functional selectivities for $mGlu_5$ of ~2 fold for **3e** and ~3 fold for **4a** respectively (other mGlu EC_{50} >10 μM). An analogous dual profile had already been observed for similarly

Table 2
Structures and activities of substituted dihydroimidazopyrimidinones **4**



Compd	R^1	R^2	R^3	$mGlu_5$ pEC_{50}[a] (±SEM)	$mGlu_5$ EC_{50} (nM)	% Glu max[b] (±SEM)	HLM[c] (%)	RLM[c] (%)
4a			H	7.07 ± 0.02	86	70 ± 0.7	18	19
4b			H	6.66 ± 0.03	218	82 ± 9.2	20	78
4c			H	6.88 ± 0.06	130	91 ± 12.6	10	37
4d			H	6.44 ± 0.08	359	66 ± 2.1	1	14
4e			H	6.31 ± 0.02	485	74 ± 9.6	35	98
4f			H	6.74 ± 0.02	183	91 ± 13.0	31	93
4g			H	6.43 ± 0.08	371	87 ± 16.1	n.t.[d]	n.t.[d]
4h			H	7.16 ± 0.01	69	72 ± 0.7	0	29
4i			H	6.70 ± 0.04	197	88 ± 12.2	3	11
4j			H	6.55 ± 0.07	285	47 ± 3.3	n.t.[d]	n.t.[d]
4k			Cl	6.88 ± 0.05[e]	133[e]	14 ± 4.8[e]	n.t.[d]	n.t.[d]
4l			Me	6.28 ± 0.09[e]	529[e]	6 ± 1.4[e]	21	46

[a] Calcium mobilization assay using HEK293 cells stably expressing rat $mGlu_5$ receptors; values are the average of three or more independent determinations.
[b] Expressed as amplitude of response using 30 μM test compound (percentage of maximal response vs 100 μM glutamate).
[c] HLM and RLM data refer to % of compound metabolized after incubation of tested compound with human and rat microsomes respectively, for 15 min at 1 μM concentration.
[d] Not tested.
[e] Antagonists/NAMs, data represents pIC_{50}/IC_{50} from EC_{80} window and % Glu represents E_{min} not E_{max}.

Table 3
Comparative profile of compounds **3e** and **4a**

Compd	$mGlu_5$ EC_{50}[a] (nM)	% Glu max[b] (±SEM)	*c*log*P*[c]	$mGlu_3$ EC_{50} (nM)	HLM[d] (%)	RLM[d] (%)	hPPB[e] (%)	rPPB[e] (%)
3e	310	83 ± 9.1	3.95	630	14	27	96.4	93.1
4a	86	70 ± 0.7	3.42	290	18	19	90.3	88.1

[a] For the $mGlu_5$ PAM assay see Tables 1 and 2.
[b] Expressed as amplitude of response using 30 μM test compound (percentage of maximal response vs 100 μM glutamate).
[c] Calculated with Biobyte software.
[d] HLM and RLM data refer to % of compound metabolized after incubation of tested compound with human and rat microsomes respectively, for 15 min at 1 μM concentration.
[e] Plasma protein binding, human and rat.

substituted *N*-aryl analogues within our naphthyridinone series.[15] Although the biological relevance of the $mGlu_3$ receptor is still not well understood, inhibition of $mGlu_3$ has been hypothesized to have potential therapeutic utility in the treatment of neurological and psychiatric disorders.[22] Based on its $mGlu_5$ PAM potency and efficacy, higher selectivity versus $mGlu_3$, reduced *c*Log*P*, superior

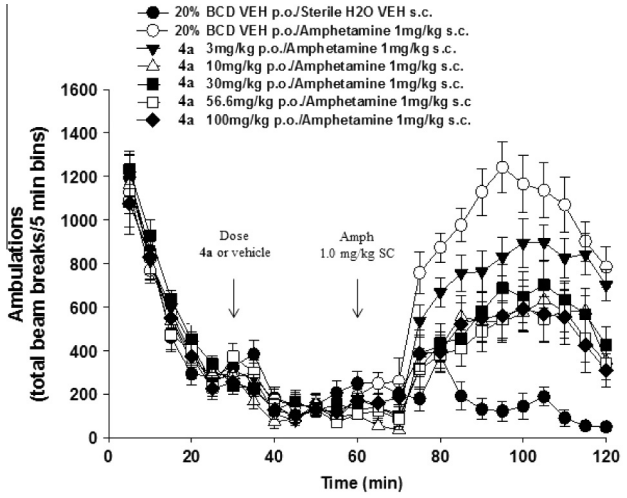


Figure 2. Dose-dependent effect and calculated terminal unbound brain of **4a** on the reversal of amphetamine-induced hyperlocomotion in rats.

CYP profile and higher unbound fraction in plasma, **4a** was selected for further in vivo pharmacokinetic (PK) and pharmacological evaluation.

Compound **4a** was formulated at 1 mg/mL in 20% hydroxypropyl-β-cyclodextrin (HP-β-CD) and evaluated in in vivo PK studies in rats (1 mg/kg IV, 10 mg/kg PO).[23] Compound **4a** showed a moderate clearance (CL_p = 23.3 mL/min/kg) and a terminal half-life ($t_{1/2}$) of 0.93 h. Evaluation of drug concentrations in portal vein, plasma and brain (0.5, 1.0, 2.0, 4.0 and 7.0 h) revealed a moderate first-pass effect (E_{hep} = 0.43) with relatively low volume of distribution (V_{ss} = 1.4 L/kg) and a good systemic exposure ($plasma_{AUC}$ = 7.2 μM-h). In addition, **4a** showed also a high brain exposure ($brain_{AUC}$ = 12.7 μM-h) with a very good distribution to the brain ($brain_{AUC}/plasma_{AUC}$ = 1.8), which together with a moderate unbound fraction in brain (rat f_u brain = 6.2%) contributed to reach high absolute brain levels (C_{max} = 6.05 μM). Encouraged by its overall profile, **4a** was evaluated for its ability to revert AIH in rats, an established model of antipsychotic activity.[10a,16] As seen in Figure 2, **4a** showed a robust dose-dependent reversal of amphetamine effects with a lowest active oral dose of 3 mg/kg, and a maximal effect of ~50% at the highest doses tested of 56.6 and 100 mg/kg. At these doses the average terminal unbound brain concentrations (382 and 363 nM, respectively) are well above the in vitro $mGlu_5$ PAM EC_{50}.[24]

In parallel to these explorations, potential $mGlu_5$ related CNS adverse-effect liabilities driven by excessive glutamate fold potentiation[25] or allosteric agonism[26,27] were reported, suggesting that PAMs with lower functional cooperativity with glutamate and devoid of allosteric agonism may be preferred for an adequate therapeutic index.[28] Concurrent with these findings and despite the fact that no significant adverse effects had previously been observed in PK and pharmacological experiments in rats up to 100 mg/kg p.o., investigation of **4a** in the modified Irwin neurological test battery in rats at a high dose (120 mg/kg) and in in vivo PK studies in dogs (0.5 mg/mL i.v.) revealed clear signs of CNS-mediated side effects (pro-convulsive behavior and dizziness, respectively) which excluded any further in vivo evaluation. Although further in vitro characterization of **4a** confirmed the lack of $mGlu_5$ agonistic activity up to the highest concentration tested of 30 μM, fold shift experiments highlighted a high degree of cooperativity with glutamate (12.5-fold leftward shift in the glutamate concentration response curve at 10 μM) that could eventually explain the observed adverse effects, although a potential contribution of $mGlu_5/mGlu_3$ synergistic effects cannot be completely ruled out.

In summary, further optimization of our initial dihydrothiazolopyridone lead **1** resulted in two novel series of imidazopyrimidinones and dihydroimidazopyrimidinones as $mGlu_5$ PAMs. Parallel SAR investigation of both series and further DMPK profiling of representative prototypes revealed dihydroimidazopyrimidinone **4a** as a highly potent and efficacious $mGlu_5$ PAM (EC_{50} = 86 nM, 70% Glu Max) and moderately potent $mGlu_3$ NAM (IC_{50} = 290 nM, 3% Glu Max). **4a** possessed an adequate PK profile in rats and showed robust dose-dependent effects in a preclinical model predictive of antipsychotic efficacy. However, the presence of CNS-mediated adverse effects in preclinical species precluded any further in vivo evaluation. Keeping in mind that more studies are necessary to address ongoing questions concerning the therapeutic index of $mGlu_5$, further efforts within these and related series are in progress and will be reported in due course.

Acknowledgments

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