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Letter

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3-Amido-3-aryl-piperidines: A Novel Class of Potent, Selective, and Orally Active GlyT1 Inhibitors

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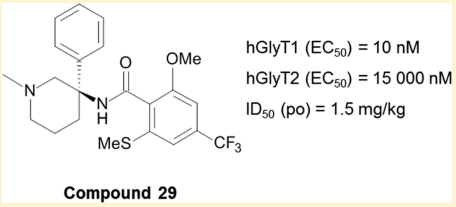
Supporting Information

ABSTRACT: 3-Amido-3-aryl-piperidines were discovered as a novel structural class of GlyT1 inhibitors. The structure−activity relationship, which was developed, led to the identification of highly potent compounds exhibiting excellent selectivity against the GlyT2 isoform, drug-like properties, and in vivo activity after oral administration.



KEYWORDS: *GlyT1 inhibitor, SAR, 3-amido-3-aryl-piperidines, Schizophrenia*

Schizophrenia is a devastating and chronic mental illness that affects close to 1% of the population. Symptoms of schizophrenia, which typically arise at young age (adolescence or early adulthood), are categorized as positive, negative, or cognitive. Currently approved drugs are efficacious in the treatment of positive symptoms but do not address the negative and cognitive symptoms. Numerous lines of evidence suggest that hypofunction of glutamatergic transmission via *N*-methyl-D-aspartate (NMDA) receptors may represent the final common pathway leading to symptoms in schizophrenic patients.[1] One approach to normalize the reduced NMDA receptor activity is to elevate the concentration of the coagonist glycine at its modulatory site on the receptor through blockade of the glycine transporter type 1 (GlyT1), which is colocalized with the NMDA receptor.[2] In the past decade, ample efforts have been focused on the discovery and development of selective GlyT1 inhibitors.[3] The first examples reported were sarcosine derivatives including **1**[4] and **2**[5] (Figure 1).

More recently, nonsarcosine-based compounds[6] have been described such as DCCCyB (**3**),[7] GSK1018921 (**4**),[8] and bitopertin (**5**),[9,10] which all progressed to clinical studies (Figure 1). In a phase II proof of concept study, Bitopertin demonstrated a beneficial effect in patients with schizophrenia characterized with predominant negative symtoms.[11] However, recently, Hoffmann-La Roche announced that two phase III studies of bitopertin did not meet their clinical end points.[12] Four additional phase III studies with bitopertin are ongoing.

As part of our continued effort to discover and develop novel and selective GlyT1 inhibitors, we considered the pyrrolidino-ethyl-amide **4** as a potential starting point for further structural modification. In the low energy conformation obtained for compound **4** (Figure 2), the carbon (C1) adjacent to the pyrrolidine nitrogen and the benzylic carbon (C2) bearing the phenyl and amide substituents appeared as suitable attachment points for forming, via a linker, a novel cyclic system (Figure 2).

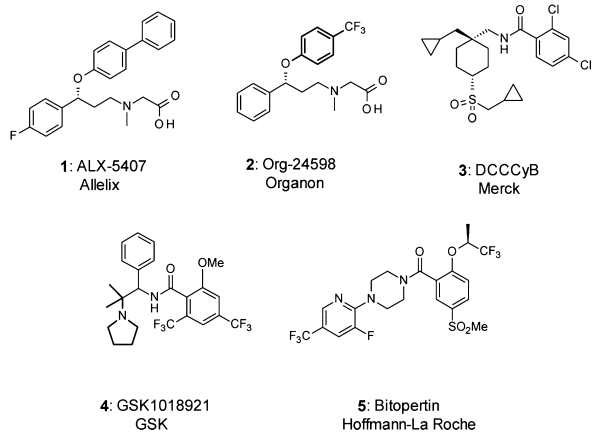


Figure 1. Selection of published GlyT1 inhibitors.

We thus decided to investigate this cyclization approach further. As connecting elements between C1 and C2, a bond, a methylene, and an ethylene unit were evaluated (in red, Figure 2). To make our designed molecules chemically tractable, the gem dimethyl group and the pyrrolidine ring present in **4** were deleted. As a result, our approach led us to the generation of the 3,3′-substituted-*N*-methyl-azetidine **6**, pyrrolidine **7**, and piperidine **8** (Figure 2). In a glycine uptake inhibition assay performed in CHO cells transfected with hGlyT1, azetine **6** and pyrrolidine **7** were found inactive or poorly active. In contrast, and to our delight, piperidine **8** displayed a promising GlyT1 potency with an IC_{50} of 140 nM. The X-ray crystal structure of **8**[13] (Figure 2) showed that the amide and phenyl substituents

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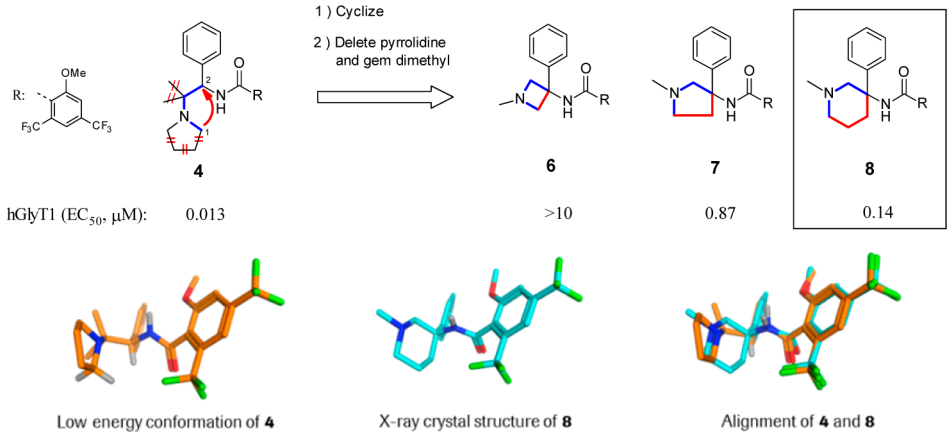
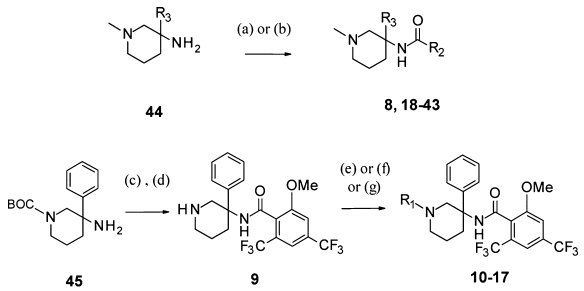


Figure 2. Design principles leading to the identification of active 3-amido-3-aryl-piperidine **8** using pyrrolidino-ethyl-amide **4** as a seed structure.

at the position 3 on the piperidine ring adopted, respectively, an axial and equatorial orientation. In addition, because of the presence of the two ortho groups on the benzamide side, the carbonyl function adopted a highly twisted orientation with a torsion angle of 78°. Overall, as depicted in Figure 2, the crystal structure of **8** aligned very well with the low energy conformation of **4**, a result perfectly in line with the good GlyT1 potency measured for **8**. A literature and patent search revealed that, although quite structurally simple, 3-amido-3-aryl-piperidines such as **8** had not been previously described. Thus, the discovery of **8** offered us the unique opportunity to develop a novel series of potent GlyT1 inhibitors having an exquisite intellectual proprietary status. In this letter, we report on our structure−activiy relationship (SAR) exploration effort in this series[14] that culminated in the identification of potent, selective, and orally active GlyT1 inhibitors such as compound **29**.

N-Methyl-3-amido-3-aryl-piperidine derivatives **8** and **18**−**43** were synthesized under standard amide coupling conditions from *N*-methyl-3-amino-3-aryl-piperidines **44** (Scheme 1).

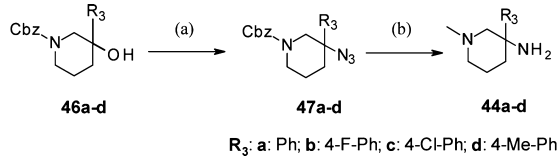
Scheme 1. Synthesis of 3-Amido-3-aryl-piperidines 8−43[a]



[a]Reagents and conditions: (a) R_2COCl, DIPEA, CH_2Cl_2, RT, 4−90%; (b) R_2CO_2H, HATU, DIPEA, DMF, RT, 18−68%; (c) 2- OMe,2′,4-$(CF_3)_2$-PhCOCl, DIPEA, CH_2Cl_2, RT, 86%; (d) HCl, dioxane, RT, 86%; (e) R_1I, DIPEA, CH_2Cl_2, RT, 58%; (f) ketone or aldehyde, $NaCNBH_3$, AcOH, MeOH, RT, 63−89%; (g) acyl- or sulfonyl-chloride, DIPEA, CH_2Cl_2, RT, 65−99%.

Derivatives **10**−**17** having different R_1 groups on the piperidine nitrogen were obtained upon alkylation, reductive amination, acylation, or sulfonylation of the unsubstituted piperidine **9**, itself prepared from *N*-Boc-protected-3-amino-3-phenyl-piperidine building block **45**. *N*-Methyl-3-amino-3-aryl-piperidine intermediates **44a**−**d** carrying the aromatic R_3 substituents indicated in Scheme 2 were prepared from the 3-aryl-3-piperidin-ol derivatives **46a**−**d** under modified Ritter reaction

Scheme 2. Synthesis of *N*-Methyl-3-amino-3-aryl-piperidines 44a−d[a]



[a]Reagents and conditions: (a) NaN_3, TFA, H_2O, RT, 87−100%; (b) $LiAlH_4$, THF, RT, 32−63%.

conditions[15] in the presence of sodium azide and trifluoroacetic acid, followed by reduction of the obtained azido intermediates **47a**−**d** with lithium aluminum hydride.

However, this synthetic approach turned out to be not versatile enough. In particular, the azidation reaction performed on 3-aryl-3-piperidinols carrying electron rich aryl R_3 groups such as 4-OMe-Ph failed. During the search of a more general access route, we discovered that *N*-methyl-3-amino-3-aryl-piperidine intermediates **44** could be efficiently prepared from easily accessible 4-aryl-4-nitro-butyric acid methyl esters **51** via a novel route involving as a key step a Mannich in situ lactamization reaction as depicted in Scheme 3. When performed in the presence of methylamine and formaldehyde, this sequence provided with good to excellent yields *N*-methyl-5-aryl-5-nitro-piperidin-2-one intermediates **52**, which led to the target building blocks **44** after the reduction of the nitro and carbonyl functions. In addition, the synthetic access route we have established for **44** was successfully applied to the preparation of *N*-Boc-3-amino-3-phenyl-piperidine building block **45** using ammonium acetate and formaldehyde as reagents in the key Mannich in situ lactamization reaction (Scheme 3).

Our first effort aimed at establishing the SAR at the piperidine nitrogen (Table 1). Starting from **8**, deletion of the methyl group (compound **9**) led to a 5-fold reduction in GlyT1 activity. A drop of activity was equally observed upon replacing the methyl group with larger alkyl (**10**, **11**) or cycloalkyl groups (**12**, **13**). Moreover, *N*-benzylation (**14**) as well as *N*-acylation and sulfonylation (**15**−**17**) practically abolished the GlyT1 potency suggesting that a certain level of basicity of the piperidine nitrogen is required for GlyT1 activity. Overall, our

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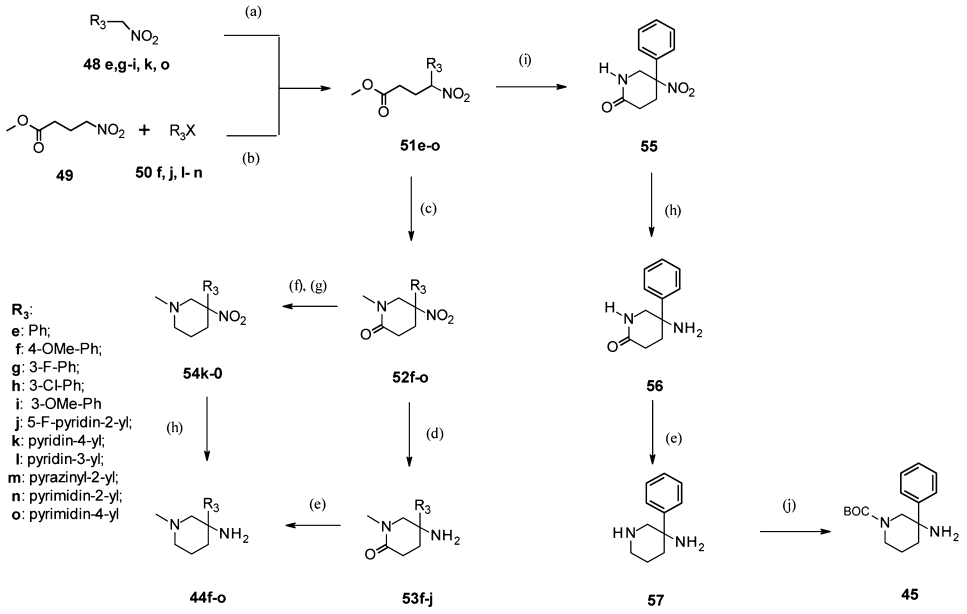
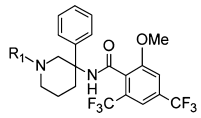
Scheme 3. Synthesis of *N*-Methyl-3-amino-piperidines 44f−o and *N*-Boc-3-amino-3-phenyl-piperidine 45[a]



[a]Reagents and conditions: (a) methyl acrylate, amberlyst A-21, dioxane, RT, 41−85%; (b) cat. Pd_2dba_3, cat. di-*tert*-butyl-(2′-methyl-biphenyl-2-yl)-phosphane, Cs_2CO_3, DME, reflux, 10−90%; (c) $MeNH_2$ (41% in water), CH_2O (36% in water), dioxane, RT then 65 °C, 49−94%; (d) zinc, HCl, dioxane, RT, 13−86%; (e) $LiAlH_4$, THF, RT, 71−86%; (f) Lawesson's reagent, toluene, 80 °C, 77−100%; (g) $NaBH_4$, MeOH, RT, 49−76%; (h) Ra−Ni, H_2 (1 atm.), THF, 0 °C, 87−100%; (i) NH_4OAc, CH_2O (36% in water), EtOH, reflux, 84%; (j) Boc_2O, Et_3N, CH_2Cl_2, RT, 70%.

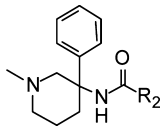
Table 1. In Vitro Inhibitory Activity of 9−17 at GlyT1[a]



compd	R_1	GlyT1 EC_{50} (μM)[b]
9	H	0.66
10	Et	0.23
11	*i*-Pr	0.23
12	*c*-C_5H_9	0.27
13	CH_2-*c*-Pr	0.38
14	CH_2−Ph	6.7
15	C(O)−Me	>10
16	C(O)−Ph	>10
17	SO_2Me	>10

[a]EC_{50} values are the average of at least two independent experiments. [b][^{3}H]-glycine uptake inhibition assay in cells transfected with hGlyT1.[10]

Table 2. In Vitro Inhibitory Activity of 18−30 at GlyT1[a]



compd	R_2	GlyT1 EC_{50} (μM)[b]
18	2-OMe, 6-CF_3−Ph	1.5
19	2-OMe, 4-CF_3−Ph	4.8
20	2-CF_3, 4-CF_3−Ph	0.54
21	4-CF_3−Ph	>10
22	2-CN, 4-CF_3−Ph	>10
23	2-F, 4-CF_3−Ph	9.9
24	2-Me, 4-CF_3−Ph	0.32
25	2-Br, 4-CF_3−Ph	0.30
26	2-SMe, 4-CF_3−Ph	0.067
27	2-SMe, 2′-OMe, 4-CF_3−Ph	0.024
28	*S*-enantiomer of **27**	0.18
29	***R*-enantiomer of 27**	**0.010**
30	2,4-Cl_2−Ph	0.67

[a]EC_{50} values are the average of at least two independent experiments. [b][^{3}H]-glycine uptake inhibition assay in cells transfected with hGlyT1.[10]

exploration at this position revealed a highly restricted SAR with the methyl group being the only well tolerated substituent.

Next, keeping the preferred *N*-methyl group in place, the SAR evaluation at the benzamide region was conducted (Table 2). Starting from **8**, selective deletion of the ortho, ortho′, and para substituents (compounds **18** to **21**) resulted in a significant drop of GlyT1 potency suggesting that substitution at all three positions on the benzamide moiety is important for reaching a good level of potency. In particular, the lack of activity observed for the *p*-CF_3 monosubstituted compound **21**, devoid of any ortho groups suggested that for GlyT1 activity the amide carbonyl group should adopt a twisted orientation as seen in the X-ray structure of our initial compound **8**. Next, starting from inactive compound **21** and keeping the *p*-CF_3 group in place, we explored various substituents at the ortho position (compounds **22−26**). No improvement of activity was observed with polar groups in place such as nitrile (**22**) as well as with small substituents like fluorine (**23**). With larger lipophilic groups such as methyl (**24**) or bromine (**25**), the activity improved by more than 30-fold. Further exploration in that direction resulted finally in the identification of the thiomethyl group as one of the best substituents at the ortho position providing a compound displaying a low nanomolar activity (**26**, 67 nM). Our initial SAR studies (vide supra) suggested that a further gain in activity could be predicted upon introducing a second ortho group on **26**. Pleasingly, by adding a

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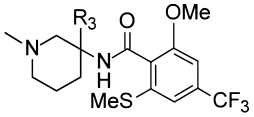
methoxy group (**27**), a very potent compound was generated demonstrating an EC_{50} of 24 nM. This compound existing as a racemic mixture was subsequently separated by chiral HPLC to provide the two enantiomers **28** ((*S*)-enantiomer) and **29** ((*R*)-enantiomer). The R-configurated isomer **29**[16] showed the best GlyT1 potency, reaching an EC_{50} as low as 10 nM at the hGlyT1 (5 nM at the mouse GlyT1).

Finally, the SAR around the aryl group at position 3 on the piperidine ring was explored (Table 3).

Table 3. In Vitro Inhibitory Activity of 31−43 at GlyT1[a]



compd	R_3	GlyT1 EC_{50} (μM)[b]
31	4-F−Ph	0.026
32	4-Cl−Ph	0.028
33	4-Me−Ph	0.23
34	4-OMe−Ph	0.17
35	3-F−Ph	0.048
36	3-Cl−Ph	0.025
37	3-OMe−Ph	0.110
38	pyridin-3-yl	0.054
39	pyridin-4-yl	0.060
40	5-F-pyridin-2-yl	0.180
41	pyrazinyl-2-yl	1.670
42	pyrimidin-2-yl	>10
43	pyrimidin-4-yl	1.7

[a]EC_{50} values are the average of at least two independent experiments. [b][^{3}H]-glycine uptake inhibition assay in cells transfected with hGlyT1.[10]

This study was performed keeping the optimized 2-thiomethyl-2-methoxy-4-trifluoromethyl-phenyl group in place at the benzamide moiety. A very similar SAR was seen at the para and meta position on the aryl moiety. Indeed, at both positions, introduction of EWGs like fluorine or chlorine (compounds **31−32** and **35−36**) was well tolerated (EC_{50} = 23−48 nM), whereas with EDGs such as methoxy group (**34**, **37**) the potency dropped significantly (EC_{50} = 110−170 nM). Moreover, a 6-membered-ring-heteroaryl scan revealed that the meta and para positions were the best centers to insert a nitrogen atom as exemplified with compounds **38** (54 nM) and **39** (60 nM). All the synthetized heteroaryl derivatives having a nitrogen atom in ortho (**40−43**) displayed much reduced potencies.

From our SAR and optimization studies, compound **29** emerged as the most potent congener from our newly discovered piperidine series. Further profiling revealed that **29** had excellent selectivity against GlyT2 (15 μM) as well as a fairly good selectivity profile in a CEREP screen performed against a panel of 80 targets including transmembrane and soluble receptors, ion channels, and monoamine transporters.[17] Moreover, as indicated in Table 4, **29** displayed good physicochemical properties with a moderate lipophilicity, a good aqueous solubility and membrane permeability. The metabolic stability of **29** in mouse and human liver microsomes was found to be intermediate with a measured intrinsic clearance of 54 and 19 μL/min/mg protein, respectively. Not surprisingly, in vitro metabolism studies on **29** revealed that the corresponding *N*-des-methyl piperidine derivative was a major

Table 4. In Vitro Properties of 29

parameter	value
log D[a]	3.12
solubility[b] (μg/mL)	147
pampa pe[c] (10^{-6} cm/s)	7.4
pK_a[d]	7.72
human, mouse CLint (μL/min/mg protein)	19, 54
hERG IC_{20}[e] (μM)	5.4
CYP450 $3A_4$, $2D_6$, $2C_9$, $2C_{19}$ IC_{50} (μM)	>50, 16, >50, 46
human, mouse P-gp ER[f]	0.9, 1.5

[a]Determined in 1-octanol/phosphate buffer, pH = 7.4. [b]Measured in a lyophilization solubility assay (LYSA) at pH = 6.5.[18] [c]Pe: PAMPA permeation constant through artificial membranes.[19] [d]Determined by capillary electrophoresis. [e]Patch clamp assay. [f]ER = efflux ratio in LLC-PK1 cells stably expressing human MDR1 and mouse MDR1A.

metabolite after incubation in human hepatocytes. In agreement with our SAR results (vide supra), this compound showed only weak GlyT1 activity (EC_{50} = 0.45 μM). Gratifyingly, **29** showed low inhibitory activity at the hERG potassium channel (IC_{20} = 5.4 μM), a result that may be attributed to the relatively low basicity of its piperidine nitrogen (pK_a = 7.72). For comparison, pyrrolidino-ethyl-amide seed structure **4**, which displayed a higher basicity (pK_a = 9.12), showed a significantly higher hERG activity (IC_{20} = 0.7 μM). Moreover, **29** demonstrated a low level of inhibition at the major drug metabolizing CYP450 enzymes (IC_{50} > 16 μM) and was inactive in the genotoxicity assays (Ames and MNT tests). Finally, **29** was not a substrate for the human and mouse P-gp efflux systems.

In vivo pharmacokinetic studies in mouse (Table 5) revealed, in addition, that **29** had an attractive profile, with, in particular,

Table 5. Pharmacokinetics Properties of 29 in Mouse

parameter	value[a]
iv dose (1.7 mg/kg)	
CL (mL/min/kg)	54
V_{ss} (L/kg)	8
po dose (7.7 mg/kg)	
bioavailability F (%)	100
$t_{1/2}$ (h)	4.2
brain/plasma (at 1.5 h)	4.3
PPB (% unbound)	1.7

[a]Values are the average of two independent experiments.

a complete oral bioavailability despite a medium systemic clearance and an excellent brain penetration (brain/plasma = 4.3), a result in agreement with its low P-gp efflux ratio (1.5) and large volume of distribution. Interestingly, **29** demonstrated a much superior brain penetration profile over seed compound **4** for which a P-gp efflux ratio of 3.9 and a brain/plasma ratio of only 0.4 was measured in the mouse.

These favorable data led us to evaluate the oral effect of **29** in the mouse L-687,414-induced hyperlocomotion assay, a novel and straightforward functional screening model,[20] that allows the detection of the in vivo activity of GlyT1 inhibitors (Figure 3). Pleasingly, **29** displayed a robust pharmacodynamic activity after oral administration and reached an ID_{50} (dose producing 50% inhibition of L-687,414-induced hyperlocomotion) of 1.5 mg/kg po. Noteworthy, the in vivo efficacy of **29** was reached at a very low plasma concentration (8 ng/mL), a result that can

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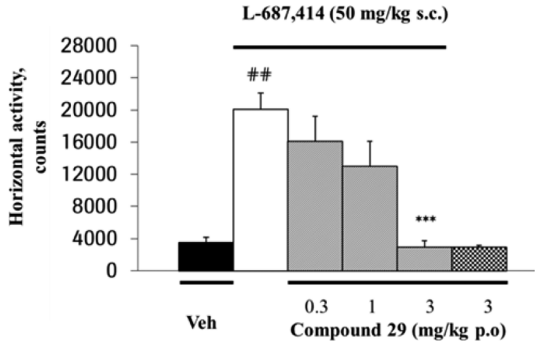

Figure 3. Dose-dependent inhibition of L-687,414-induced hyperlocomotion by **29** in mice. The data represent mean horizontal activity counts per group recorded over a 60 min time period; error bars indicate SEM (n = 8 per group). ##, $p < 0.01$ versus vehicle (Veh) alone; ***, $p < 0.001$ versus L-687,414 alone.

be explained by its high GlyT1 potency and its excellent brain penetration in the mouse.

In addition, the effect of **29** on the extracellular level of glycine in rat striatum in a microdialysis study was evaluated (Table 6). We were pleased to observe that, at an oral dose of 10 mg/kg, **29** produced a 1.7-fold glycine increase over basal levels.

Table 6. Effect of 29 on the Extracellular Glycine Levels in Rat Striatum at 10 mg/kg po[a] and Its Plasma and Brain Exposures

max. fold incr. of glycine	fold incr. of glycine at 3 h	plasma[b] (ng/mL)	brain[b] (ng/mL)	brain[b] GlyT1 EC_{50}
1.7	1.5	713	60	13

[a] n = 6. [b] Measured at 3 h postdosing.

After 3 h, at which time the extracellular fluid sampling was stopped, the PD effect was slightly diminished (1.5-fold). Determination of the plasma and brain exposures at 3 h postdosing revealed a much lower brain penetration (B/P: 0.08) for **29** in this rat experiment compared to its penetration measured in the mouse (B/P: 4.3) (vide supra). In spite of this, **27** achieved a total brain exposure above its GlyT1 EC_{50} (13-fold), sufficient to produce the effect on glycine levels observed at the 3 h time point.[21]

Noteworthy, in a dedicated mechanistic study performed in the rat, we observed that the brain/plasma ratio increased by 4-fold after coadministration of **29** with the P-gp inhibitor tariquidar[22] indicating that **29** is a rat P-gp substrate. This result is in sharp contrast with the low P-gp activity of **29** measured in the mouse (vide supra). The marked difference in brain penetration observed in the mouse and in the rat may thus be attributed to the differentiated P-gp activity profile of **29** in these two rodent species. Compound **29**, which is not a substrate of the human P-gp, is thus predicted to display in human an excellent brain penetration, quite similar to the one measured in the mouse.

In summary, we report here on the discovery of 3-amido-3-aryl-piperidines, a novel structural class of GlyT1 inhibitors designed using the previously reported pyrrolidino-ethyl-amide **4** as a seed structure. From the initial compound **8**, the exploration of the SAR at the three exit vectors has led to the identification of potent and selective compounds such as **29**, which demonstrated drug-like properties, a promising in vitro safety profile and in vivo efficacy in rodents after oral administration.

■ ASSOCIATED CONTENT

Supporting Information

Experimental details for the synthesis of compounds **8**–**43** and intermediates. This material is available free of charge via the Internet at http://pubs.acs.org.

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Author Contributions

The manuscript was written through contributions of all authors.

Notes

The authors declare no competing financial interest.

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■ ABBREVIATIONS

CYP, cytochrome P; dba, dibenzylideneacetone; DIPEA, *N*,*N*-diisopropylethylamine; EWG, electron withdrawing group; EDG, electron donating group; HATU, 1-[bis-(dimethylamino)methylene]-1*H*-1,2,3-triazolo[4,5-b]pyri-dinium 3-oxid hexafluorophosphate; hERG, human ether-à-go-go-related gene; MDR, multidrug resistance protein; TFA, trifluoro acetic acid

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