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Atropisomeric 4-Phenyl-4*H*-1,2,4-triazoles as Selective Glycine Transporter 1 Inhibitors

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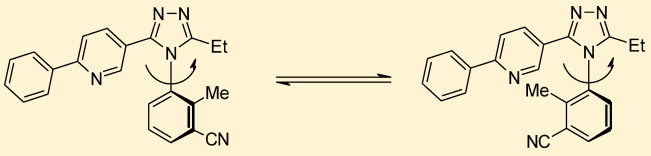
Supporting Information

ABSTRACT: We report on the optimization of 4*H*-1,2,4-triazole derivatives to increase their activity and selectivity as glycine transporter 1 (GlyT1) inhibitors. Structure−activity relationship exploration resulted in the identification of a 3-[3-ethyl-5-(6-phenylpyridin-3-yl)-4*H*-1,2,4-triazol-4-yl]-2-methylbenzonitrile (**14u**) compound with markedly higher selectivity for GlyT1. Physiochemical studies revealed that **14u** exists as a stable pair of atropisomers under physiological conditions. We successfully separated the atropisomers to obtain active enantiomer (***R***)-**14u**, which displayed favorable pharmacokinetic properties, as well as positive results in the mice Y-maze test.



INTRODUCTION

In the central nervous system, specific sodium/chloride-dependent transporters regulate glycine levels in synapses. Two such high-affinity glycine transporters, glycine transporters 1 (GlyT1, Slc6a9) and 2 (GlyT2, Slc6a5), terminate glycine activity by mediating its uptake.[1,2] GlyT1 is expressed in forebrain areas, such as the hippocampus and cerebral cortex, where the transporter is believed to modulate *N*-methyl-D-aspartate (NMDA) receptor activity by regulating glycine levels, which acts as a coagonist with glutamate.

Hypofunction of NMDA receptors is believed to be a contributing factor for the development of a number of human diseases, particularly schizophrenia.[3] A few clinical studies have demonstrated that positive, negative, and cognitive symptoms in schizophrenic patients can be ameliorated by the administration of glycine, D-serine (glycine site agonist of NMDA receptors), and sarcosine (*N*-methyl glycine, weak GlyT1 inhibitor) in conjunction with conventional therapy.[4−6] These clinical findings suggest that potentiating NMDA receptor function may be a useful approach for treating schizophrenia. In addition to their role in disease induction, NMDA receptors are involved in memory formation and learning.[7] Activation of NMDA receptors mediates long-term potentiation of synaptic transmission, which underlies memory formation and learning at the neuronal level.[8] Thus, agents that inhibit the activity of GlyT1 are expected to modulate NMDA receptor activity and may be useful for treating schizophrenia, dementia, and related disorders.

N-[(3*R*)-3-(4′-Fluorophenyl)-3-(4′-phenylphenoxy)propyl]-sarcosine (**1**),[9] an analogue of sarcosine, was reported as a selective inhibitor of GlyT1 (Figure 1), with preclinical in vivo studies in rodents demonstrating that **1** has a similar efficacy as clinically approved antipsychotics.[10,11] Due to these reported properties, a number of sarcosine analogues have been synthesized and characterized.[2,12−15] More recently, however, nonsubstrate-based GlyT1 inhibitors have attracted attention, and a wide variety of compounds without a carboxylate group have been disclosed.[2,12−15] Among these compounds, [4-(3-fluoro-5-trifluoromethylpyridin-2-yl)piperazin-1-yl][5-methanesulfonyl-2-((*S*)-2,2,2-trifluoro-1-methylethoxy)phenyl]-methanone (**2**)[16] was reported to have beneficial effects in schizophrenic patients in a recent phase II clinical trial.

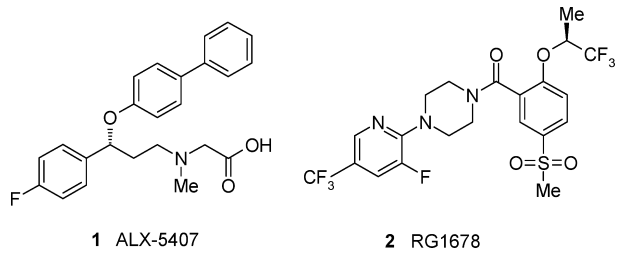


Figure 1. Structure of two GlyT1 inhibitors.

Our group is also actively studying a novel series of GlyT1 inhibitors derived from 3-biphenyl-4-yl-4-phenyl-4*H*-1,2,4-triazole (**3**, Figure 2), which was identified using a high-throughput screening (HTS) approach.[17] Modification of the 4-position of the triazole increased the GlyT1 inhibitory activity of the obtained compounds (**4** and **5**). Further modification of **5** by the introduction of a heterocycle at the 4-position led to the formation of isoquinoline analogue **6**, which exhibited 38-fold selectivity between GlyT1 and GlyT2 and effectively antagonized (+)-3-amino-1-hydroxypyrrolid-2-one [(+)-HA966]-induced hyperlocomotion in mice upon oral administration at 3 mg/kg.[18]

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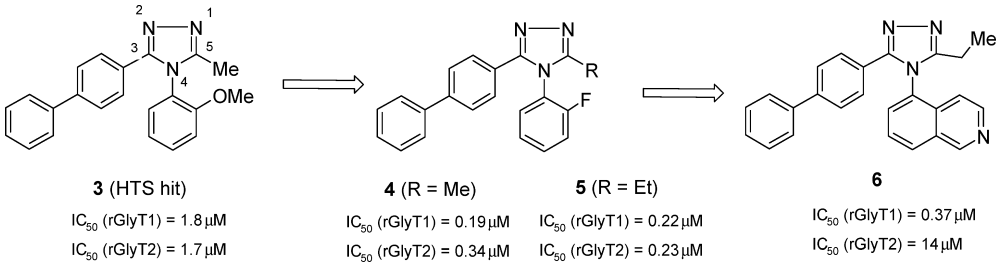
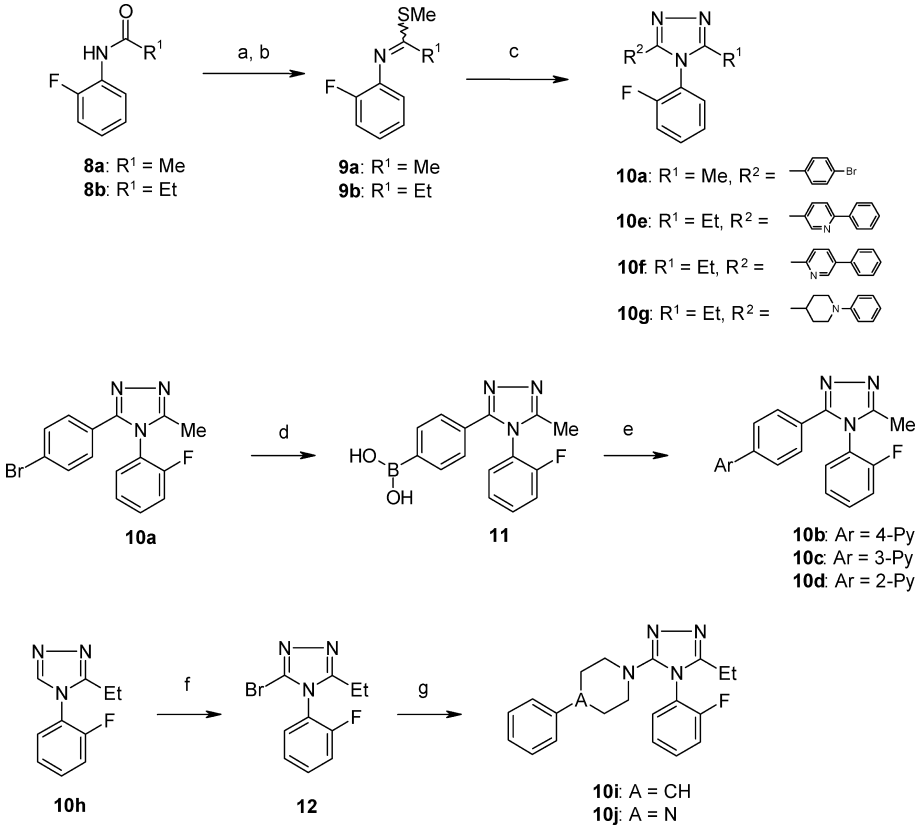


Figure 2. Structure of 1,2,4-triazole-based GlyT1 inhibitors.

Scheme 1. Synthesis of 1,2,4-Triazole Derivatives[a]



[a]Reagents and conditions: (a) Lawesson's reagent, toluene, 110 °C; (b) MeI, K_2CO_3, CH_3CN, 50 °C; (c) $R^2CONHNH_2$, $TsOH·H_2O$, DMF, 120 °C; (d) (i) *n*-BuLi, THF, −78 °C, (ii) $B(OMe)_3$; (e) ArBr, $Pd(PPh_3)_4$, Na_2CO_3, DME, reflux; (f) NBS, CCl_4, AcOH, reflux; (g) 4-phenylpiperidine or 1-phenylpiperadine.

In this paper, we describe the further optimization of this series of 1,2,4-triazole derivatives to improve selectivity for GlyT1 by modification of the 3- and 4-positions of triazole. In addition, we characterized the physicochemical properties of the optimized compound **14u**, which exist as separable atropisomers at physiological conditions. We also report the pharmacokinetic properties and in vivo behavioral effects of the optically active atropisomer of **14u**.

CHEMISTRY

The 1,2,4-triazole derivatives examined in this study were prepared as illustrated in Schemes 1 and 2. Scheme 1 shows the initial general synthesis method in which the thioimidates **9a,b** were utilized as intermediates for the synthesis of the target 1,2,4-triazole derivatives **10a,e−g**. Amides **8a,b** were first reacted with Lawesson's reagent to afford thioamides, which were then treated with MeI and K_2CO_3 in acetonitrile to obtain thioimidates **9a,b**. These compounds were coupled with acyl hydrazides and heated under acidic conditions to produce 1,2,4-triazole derivatives **10a,e−g**. Pyridine derivatives **10b−d** were prepared by the reaction of arylboronic acid **11** with the corresponding aryl bromides under Suzuki coupling conditions.[19] Piperidine **10i** and piperadine **10j** were prepared by bromination of **10h**, followed by substitution with the corresponding cyclic amines.

The second general synthesis method for 1,2,4-triazole derivatives utilized the reaction of 1,3,4-oxadiazoles **13a,b** with the corresponding anilines, as shown in Scheme 2.[20] **13a** and **13b** were heated with a variety of anilines under acidic conditions to yield **14a,b,e,f,k−p,r,t**. Aniline **14c** was prepared by the hydrogenation of the nitro derivative **14r**, which was subsequently methylated to give **14d**. Benzyl ether **14f** was converted to methanol **14g** using BCl_3, followed by chlorination to give intermediate **14h**. Methyl ether **14i**,

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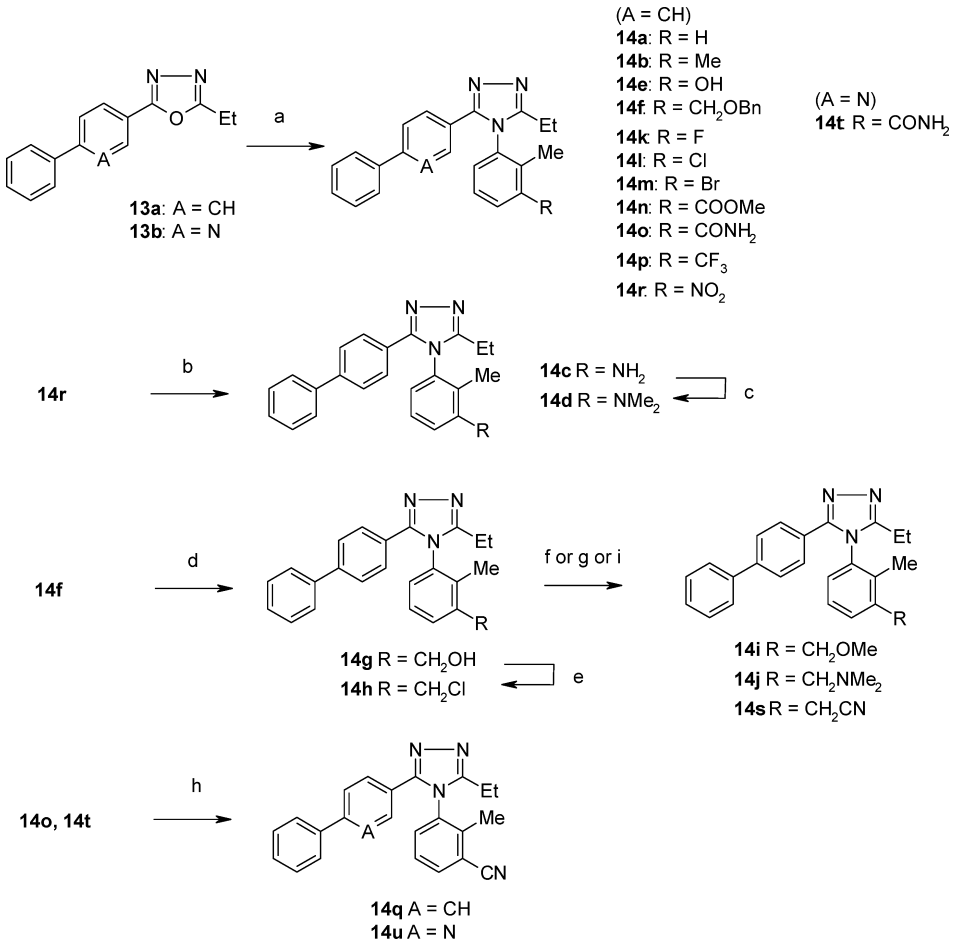
Scheme 2. Synthesis of 1,2,4-Triazole Derivatives[a]



[a]Reagents and conditions: (a) anilines, TsOH·H_2O, 160 °C; (b) Fe, 1 M HCl, EtOH, H_2O, 70 °C; (c) HCHO, $NaBH_3CN$, AcOH; (d) BCl_3, $CHCl_3$; (e) $SOCl_2$, $CHCl_3$; (f) NaOMe, MeOH, reflux; (g) Me_2NH, CH_3CN; (h) $POCl_3$, 100 °C; (i) KCN, 18-crown-6, CH_3CN, reflux.

benzylamine **14j**, and phenyl acetonitrile **14s** were readily prepared by the reaction of 14**h** with the corresponding nucleophiles. Nitriles **14q,u** were obtained from carboxamides **14o,t** using $POCl_3$ as a dehydrating agent.

RESULTS AND DISCUSSION

The GlyT1 inhibitory activities of the synthesized 4-phenyl-4*H*-1,2,4-triazoles were assayed by measuring [^{3}H]glycine uptake into rat C6 glioma cells according to reported protocols.[21] This assay method was validated using previously characterized GlyT1 inhibitors, including compound **1**, which showed potent inhibitory activity (IC_{50} = 0.0089 μM). GlyT2 inhibitory activities were evaluated in a similar manner to the GlyT1 assay using rat brainstem cells. The well-characterized GlyT2 inhibitor, 4-benzyloxy-3,5-dimethoxy-*N*-[1-(dimethylaminocyclopentyl)methyl]benzamide (Org-25543),[22] displayed potent activity (IC_{50} = 0.022 μM) in this assay, confirming the reliability of the assay.

We first attempted to modify the biphenyl group at the 3-position of triazole in compounds **4** and **5** to increase the GlyT1 inhibitory activity and selectivity over GlyT2. For exploration of the structure−activity relationship (SAR) of the biphenyl moiety, we postulated that a basic amine structure may improve the potency and selectivity for GlyT1, as a wide variety of cyclic amines were incorporated into the non-sarcosine-based GlyT1 inhibitors with high selectivity over GlyT2.[16,23,24] Therefore, we first modified the biphenyl moiety by incorporating aromatic and aliphatic amines and then assayed for GlyT1 inhibitory activity, as presented in Table 1.

Replacement of the terminal benzene ring of **4** with pyridine-2-yl groups resulted in a slight loss of GlyT1 inhibitory activity (**10d**, IC_{50} = 0.86 μM). In addition, a marked reduction of activity was observed upon replacement of the benzene ring with pyridine-3-yl and pyridine-4-yl groups (**10b**, IC_{50} = 5.9 μM; **10c**, IC_{50} = 7.0 μM). These results suggested that the terminal pyridines adopted an unfavorable structure for potent GlyT1 inhibitory activity. In contrast, the in vitro potency of was increased with translocation of a pyridine ring adjacent to the 1,2,4-triazole ring (**10e**,**10f**). In particular, **10e** showed equipotent GlyT1 inhibitory activity (IC_{50} = 0.12 μM) to the corresponding biphenyl analogue **5**. Interestingly, a slight reduction of GlyT2 inhibitory activity was observed for **10e** but resulted in a 6.6-fold increase in the selectivity compared to **5**. Aliphatic amines (**10g,i,j**), however, decreased GlyT1 inhibitory activity (IC_{50} = 2.6−6.6 μM), suggesting that the aromaticity of the biphenyl moiety is essential for potency.

In addition to a slight improvement of selectivity for **10e**, we observed a marked increase in solubility of this compound (**10e**, solubility: 31 μM in pH 6.8 aqueous buffer) compared to the corresponding biphenyl derivative (**5**, solubility: <1 μM in pH 6.8 aqueous buffer).

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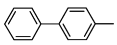
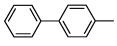
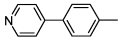
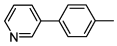
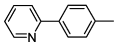
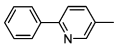
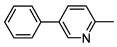
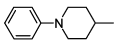
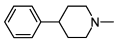
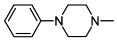
Table 1. In Vitro GlyT1 Inhibitory Activities of 4,5, and 10b–i

Compound	R^1	R^2	rGlyT1 IC_{50} (μM)[a]	rGlyT2 IC_{50} (μM)[a]	Selectivity[b]
4		Me	0.19 ± 0.073	0.34 ± 0.11	1.8
5		Et	0.22 ± 0.05	0.23 ± 0.092	1.0
10b		Me	5.9[c]	ND[d]	ND[d]
10c		Me	7.0[c]	ND[d]	ND[d]
10d		Me	0.86[c]	1.6[c]	1.9
10e		Et	0.12 ± 0.012	0.79 ± 0.15	6.6
10f		Et	0.70 ± 0.015	0.89[c]	1.3
10g		Et	6.6 ± 1.2	ND[d]	ND[d]
10i		Et	3.7 ± 0.35	ND[d]	ND[d]
10j		Et	2.6 ± 0.15	ND[d]	ND[d]

[a]Values are the mean ± standard error for three experiments. [b]Ratio of the IC_{50}(μM) values rGlyT2/rGlyT1. [c]Values are the means for two experiments. [d]ND: not determined.

In addition to modification of the biphenyl moiety at the 3-position of triazole, we also attempted to optimize 4-position to obtain more potent and selective GlyT1 inhibitors. This position was targeted based on the results of our previous SAR study, in which a marked improvement of selectivity was observed following the introduction of isoquinoline at the 4-position of triazole. The nitrogen atom in isoquinoline plays an important role in GlyT1 inhibitory activity and selectivity, as demonstrated by the potent GlyT1 inhibitory activity and highest selectivity (38-fold) exhibited by isoquinoline **6** (Figure 3) among all examined *N*-substitution patterns of quinolines and isoquinolines.[18] On the basis of the above findings for **6**, we hypothesized that a 2-methyl-3-substituted phenyl group would mimic the structure of isoquinoline and, further, that

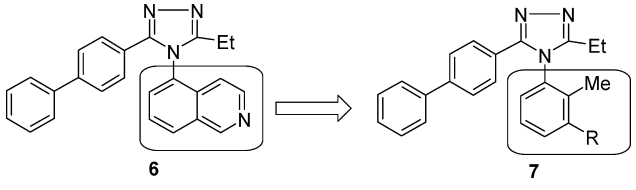
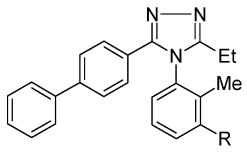


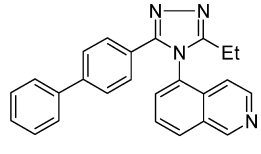
Figure 3. Synthetic plan for 2-methylbenzene analogues of isoquinoline.

incorporating a variety of R-functional groups could increase both the GlyT1 inhibitory activity and selectivity (Figure 3). We therefore synthesized a variety of 2-methylbenzene analogues of **6** and determined their GlyT1 inhibitory activities (Table 2).

Table 2. In Vitro GlyT1 Inhibitory Activities of 6,14a–e,g,i–s



14a-e, g, i-s



6

compd	R	rGlyT1 IC_{50} (μM)[a]	rGlyT2 IC_{50} (μM)[a]	selectivity[b]
6		0.37 ± 0.012	14 ± 5.3	38
14a	H	0.30 ± 0.030	0.70 ± 0.24	2.3
14b	Me	0.94 ± 0.030	1.2 ± 0.24	1.3
14c	NH_2	0.32 ± 0.040	0.34 ± 0.026	1.1
14d	NMe_2	3.1 ± 0.26	ND[c]	ND[c]
14e	OH	0.34 ± 0.046	1.3[d]	3.8
14g	CH_2OH	0.25 ± 0.080	3.1[d]	12
14i	CH_2OMe	2.4 ± 0.29	ND[c]	ND[c]
14j	CH_2NMe_2	29 ± 3.8	ND[c]	ND[c]
14k	F	0.63 ± 0.05	0.66 ± 0.028	1.0
14l	Cl	2.2 ± 0.18	ND[c]	ND[c]
14m	Br	2.2 ± 0.33	ND[c]	ND[c]
14n	CO_2Me	5.1 ± 0.79	ND[c]	ND[c]
14o	$CONH_2$	3.9 ± 0.74	ND[c]	ND[c]
14p	CF_3	>10 (38%)[e]	ND[c]	ND[c]
14q	CN	0.49 ± 0.040	>30 (43%)[f]	>61
14r	NO_2	0.80 ± 0.023	13 ± 2.3	16
14s	CH_2CN	2.8 ± 0.15	ND[c]	ND[c]

[a]Values are the mean ± standard error for three experiments. [b]Ratio of the IC_{50}(μM) values rGlyT2/rGlyT1. [c]ND: not determined. [d]Values are the mean for two experiments. [e]% inhibition at 10 μM (solubility limit). [f]% inhibition at 30 μM (solubility limit).

A wide range of substituents resulted in potent GlyT1 inhibitory activity. Both acidic (**14e**) and basic (**14c**) substituents led to potent GlyT1 inhibitors, which were equipotent to isoquinoline **6** (**14c**, IC_{50} = 0.32 μM; **14e**, IC_{50} = 0.34 μM). An unsubstituted analogue (**14a**) also retained potent inhibitory activity (**14a**, IC_{50} = 0.30 μM). A slight decrease in activity was observed when methyl, fluoro, cyano, and nitro groups were substituted as functional groups; however, the corresponding compounds retained moderate and acceptable GlyT1 inhibitory activities (**14b**, IC_{50} = 0.94 μM; **14k**, IC_{50} = 0.63 μM; **14q**, IC_{50} = 0.49 μM; and **14r**, IC_{50} = 0.80 μM). In contrast, introduction of halogens (**14l,m**), methyl ester (**14n**), carboxamide (**14o**), trifluoromethyl (**14p**), and cyanomethyl (**14s**) groups led to weakly active analogues (IC_{50} > 2 μM). Considering that potent GlyT1 inhibitory activity was obtained from both electron-withdrawing and -donating groups, the electrostatic properties of substituents appear to be unrelated to the inhibitory activity.

Although the aniline **14c** exhibited potent GlyT1 inhibitory activity, a 10-fold reduction in activity was observed for the dimethylamino derivative **14d** (IC_{50} = 3.1 μM). Moreover, introduction of a dimethylaminomethyl group resulted in a marked reduction of potency (**14j**, IC_{50} = 29 μM), which we

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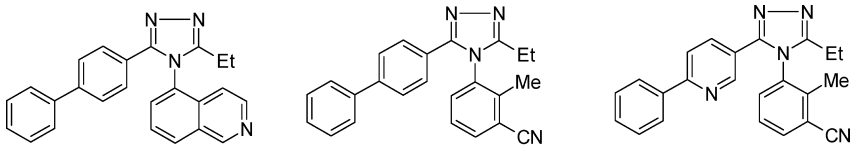
Table 3. In Vitro and In Vivo Profiles of 6, 14q, and 14u



6 **14q** **14u**

compd	rGlyT1 IC_{50} (μM)[a]	rGlyT2 IC_{50} (μM)[a]	selectivity[b]	(+)-HA966-induced hyperlocomotion in mice[c] % inhibition, dose
6	0.37 ± 0.040	14 ± 5.3	38	82%, 3 mg/kg
				8%, 1 mg/kg
14q	0.49 ± 0.040	>30	>61	44%, 3 mg/kg
14u	0.16 ± 0.030	>30	>187	91%, 3 mg/kg
				56%, 1 mg/kg

[a]Values are the mean ± standard error for three experiments. [b]Ratio of the IC_{50}(μM) values rGlyT2/rGlyT1. [c]Compound given orally, n = 16.

speculated was due to steric hindrance in the binding region of GlyT1. Interestingly, homologation of the hydroxyl group of **14e** retained the potent activity (**14g**, IC_{50} = 0.25 μM), whereas substitution of **14g** with a methyl group resulted in 10-fold reduction of activity (**14i**, IC_{50} = 2.4 μM). Together, these results suggested that the introduction of hydrophilic functional groups had little effect on GlyT1 inhibitory activity.

Compounds **14a−c,e,g,k,q,r**, which displayed equipotent GlyT1 inhibitory activity to that of isoquinoline **6**, were next assessed for GlyT2 inhibitory activity. Unsubstituted derivative **14a** and compounds with amino (**14c**), hydroxyl (**14e**), fluoro (**14k**), and methyl (**14b**) groups, exhibited potent GlyT2 inhibitory activity and poor selectivity (GlyT2/GlyT1 < 5). However, introduction of polar substituents, including hydroxymethyl (**14g**), cyano (**14q**), and nitro (**14r**) groups, led to a favorable reduction in GlyT2 inhibitory activity and resulted in higher GlyT1 selectivity. Notably, the nitrile **14q** exhibited a significant loss in GlyT2 inhibitory activity (**14q**, IC_{50} > 30 μM) and displayed excellent GlyT1 selectivity (>61-fold).

Having obtained the 3-cyano-2-methylphenyl derivative **14q** with potent and selective GlyT1 inhibitory activity, we next evaluated the aqueous solubility and in vivo behavioral effects of **14q** using a (+)-HA966-induced hyperlocomotion assay in mice (Tables 3 and 4).[25,26] Unfortunately, compound **14q** showed poor solubility in a pH 6.8 aqueous solution (<1 μM) and only inhibited hyperlocomotion by 44% at an oral dose of 3 mg/kg (po).

Table 4. Properties of 6, 14q, and 14u

compd	solubility (μM) pH 6.8	$LogD_{7.4}$	CLint, in vitro[a] human (mL/min/kg)	CLint, in vitro[a] mouse (mL/min/kg)
6	5	4.1	374	>1000
14q	<1	4.1	126	>1000
14u	98	3.0	48	467

[a]In vitro clearance using liver microsomes.

As these properties were inferior compared with compound **6**, we further modified **14q**. As shown in the SAR of the compounds in Table 1, we observed an improvement of solubility with a slight increase of GlyT1 selectivity. As we anticipated that these beneficial effects could be applied to **14q**, the biphenyl moiety of **14q** was replaced with a 2-phenyl-pyridine-5-yl group at the 3-positon of 1,2,4-triazole to generate **14u**. Compared to the biphenyl derivative **14q**, **14u** exhibited potent and selective GlyT1 inhibitory activity. In addition, a significant improvement in solubility (**14u**; 98 μM) was observed as expected, and **14u** showed better liver microsome clearances compared to those of **6** and **14q**. Lower lipophilicity of **14u** may have contributed to these beneficial drug properties of **14u** because measured $LogD_{7.4}$ of **14u** was lower than those of **6** and **14q**. Furthermore, the in vivo potency of **14u** was also increased to 56% inhibition at a dose of 1 mg/kg, a value that was much potent than that of **6** and **14q**.

Despite the favorable drug properties of **14u**, its development as a potential candidate of GlyT1 inhibitor was complicated by the structure of **14u**. The adjacent spaces to the bond between triazole and the 3-cyano-2-methylphenyl moiety were occupied with pyridine, ethyl, and methyl groups, which resulted in two enantiomeric rotational isomers of **14u** (Figure 4). Atropisomer chirality can have a significant impact

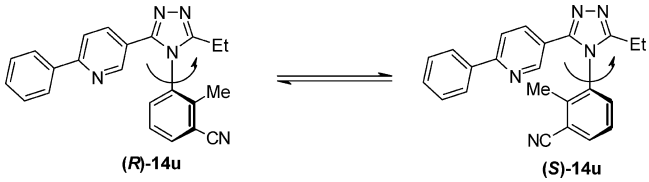


Figure 4. Compound **14u** with hindered rotation around the NC bond that creates a chiral axis.

on drug discovery and must be managed appropriately when assessing the efficacy, safety, and pharmacokinetic profiles of drug candidates.[27−29] Furthermore, racemization is often a concern in the manufacturing process, particularly when high temperature is necessary for the preparative and purification processes. Although only a few rotamers of 4-phenyl-1,2,4-triazoles have been reported to date,[30] numerous unsymmetrically substituted biphenyl derivative have been well-documented as separable atropisomers at a wide range temperatures.[31] It was suggested that atropisomers that equilibrate slowly (rotational barrier >30 kcal/mol) can be treated similarly to compounds with stereocenter-based chirarity.[28,29] Here, we therefore attempted to separate and determine the rotational barrier of the enantiomers of **14u** to decide whether **14u** should be treated as a single enantiomer or racemate in further preclinical studies.

The optical resolution of each atropisomer of **14u** was performed using chiral high-pressure liquid chromatography

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(HPLC). An iterative screening of available chiral HPLC columns, and conditions resulted in the separation of individual atropisomers of **14u** at 40 °C, as shown in Figure 5. The

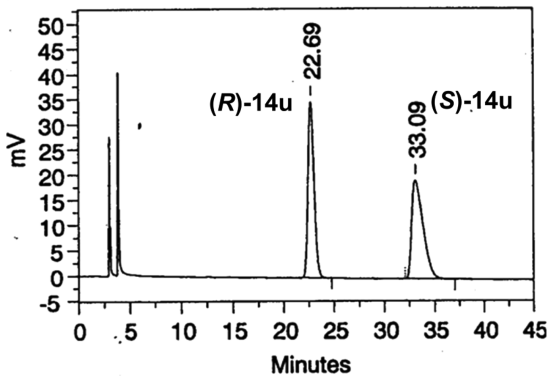

Figure 5. HPLC of atropisomers of **14u**. Column, CHIRALPAK AD 4.6 mm × 250 mm (Daicel Corporation); mobile phase, *n*-hexane/EtOH = 85/15; flow rate, 1.0 mL/min; and temperature, 40 °C. UV detection at 254 nm.

collected atropisomers, (***R***)-**14u** and (***S***)-**14u**,[32] appeared to be stable against racemization at room temperature over a period of months in the solution used in the HPLC analysis.

Having separated both atropisomers and confirming their thermal stability against racemization at ambient temperature, we next assessed the rate of interconversion at elevated temperatures. To evaluate the thermal stability of interconversion, a solution of (***S***)-**14u** in nitrobenzene was maintained at 79, 100, 112, 120, and 134 °C, and racemization was monitored by HPLC. Although racemization was observed at all examined temperatures, the process was quite slow.[33] The Gibbs energy of activation for racemization was estimated to be 31.4 kcal/mol at 25 °C using the Eyring equation.[33] On the basis of the estimated activation energy, the half-life of racemization at 37 °C was calculated to be greater than 21 years. Solvent dependency was not observed when 1,2-dichlorobenzene was used as a solvent (Gibbs energy: 31.2 kcal/mol at 25 °C). These results enabled (***R***)-**14u** and (***S***)-**14u** to be treated as single enantiomers, similar to the case of molecules with chiral carbon atoms.

We next evaluated the in vitro and in vivo profiles of each isomer of **14u**, as shown in Table 5. The (*R*)-isomer was found to be an active enantiomer with an IC_{50} value of 0.064 μM for rGlyT1, whereas the corresponding (*S*)-isomer displayed markedly less activity (previously reported data).[32] The GlyT1 selectivity of (***R***)-**14u** was greater than 469-fold, which was the highest selectivity among the compounds prepared in this series. In addition, we observed superior in vivo efficacy to the corresponding racemic compound, displaying 82% inhibition of hyperlocomotion induced by (+)-HA966.

On the basis of the high selectivity and activity of (***R***)-**14u**, PK study of the promising GlyT1 inhibitor were conducted in mice and monkeys (Table 6). (***R***)-**14u** showed moderate and acceptable oral bioavailability in both species (mouse, 32%; monkey, 23%). The in vitro metabolic stability of (***R***)-**14u** in liver microsomes and unbound drug ratio in plasma were also evaluated (Table 7). As the CLint value in humans was markedly lower than those from the other two species, we speculate that (***R***)-**14u** would exhibit good absorption on oral administration to humans. The unbound (***R***)-**14u** ratio in human and mouse plasma was similar (12% vs 11%, respectively), whereas slightly lower binding was observed in monkey (25% unbound).

The ability of **6**, **14q**, **14u**, and (***R***)-**14u** to act as substrates of P-glycoprotein (P-gp)-mediated transport (encoded by multidrug registrant gene [MDR1]) were evaluated in MDR1-expressing cells (LLC-GA5-COL150 cells) and LLC-PK1 control cells (Table 8). It was found that (***R***)-**14u** had excellent permeability profile (LLC-PK1 P_{app} AB = 35.4 × 10^{-6} cm/s) coupled with low potential for P-gp efflux as evidenced by low efflux ratio of 1.5 in MDR1-transfected LLC-PK1 cells. Compounds **6**, **14q**, and **14u** also showed excellent permeability with low P-gp liability, indicating that this series of GlyT1 inhibitors have favorable profiles for CNS agents. Actually, **14u** showed good total brain/plasma ratio of 0.7−1.4 in mouse.

Additional in vitro profiling revealed that (***R***)-**14u** inhibited hGlyT1 with IC_{50} value of 0.59 μM using human neuroblastoma SK-N-MC cells. Further, (***R***)-**14u** showed low activity toward non-GlyT1 targets, including receptors (NMDA, AMPA, GABA, strychnine-sensitive Gly, dopamine D_2, 5-$HT_{1A/1B/2A}$, muscarine, and vasopressin V_{1A} receptors) and transporters (glutamate, GABA, dopamine, norepinephrine, and 5-HT transporters), with less than half of the affinity or inhibitory activity at a concentration of 10 μM in all assays. With regard to drug−drug interactions, compound (***R***)-**14u** showed no significant inhibition of the major human CYP450 enzymes, such as hCYP1A2, 2D6, 2C9, 2C19, and 3A4 (IC_{50} >35 for all enzymes).

The favorable profiles of (***R***)-**14u** prompted us to evaluate the effect of this compound on spatial working memory using the Y-maze test.[36,37] Spontaneous alternation behavior has been proposed as a rudimentary animal model of working memory.[38] Hypofunction of NMDA receptors has been proposed as a causative factor underlying many schizophrenia symptoms, including working memory, and animals treated with (+)-5-methyl-10,11-dihydro-5*H*-dibenzo[*a*,*d*] cyclohepten-5,10-imine maleate (MK-801, a noncompetitive NMDA receptor antagonist) exhibit impaired spontaneous alternation in the Y-maze test.[39] Thus, MK-801-induced working memory deficit in the Y-maze test can be regarded as an animal model of cognitive impairment in schizophrenia. In addition, the total number of

Table 5. In Vitro and In Vivo Profiles of Atropisomers of 14u

compd	rGlyT1 IC_{50} (μM)[a]	rGlyT2 IC_{50} (μM)[a]	selectivity[b]	solubility (μM) pH 6.8	$LogD_{7.4}$	(+)-HA966-induced hyperlocomotion in mice[c] % inhibition, dose
(***RS***)-**14u**	0.16 ± 0.030	>30	>187	98	3.0	56%, 1 mg/kg
(***R***)-**14u**	0.064 ± 0.023	>30	>469	95	3.0	82%, 1 mg/kg
(***S***)-**14u**	20 ± 4.8	>30	>1.5	94	ND[d]	ND[d]

[a]Values are the mean ± standard error for three experiments. [b]Ratio of the IC_{50}(μM) values rGlyT2/rGlyT1. [c]Compound given orally, n = 16. [d]ND: not determined.

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Table 6. Pharmacokinetic Parameters for Compound (*R*)-14u[a]

species	route	dose (mg/kg)	$t_{1/2}$ (h)	AUC (ng·h/mL)	Vd[b] (L/kg)	CL[c] (mL/min·kg)	C_{max} (ng/mL)	*F* (%)
mouse	iv	1	0.22	248	1.0	67		
	po	3		235			319	32
monkey	iv	1	2.4	875	3.4	21		
	po	3		522			141	23

[a]Each value is the mean for $n = 3$ measurements. [b]Volume of distribution. [c]Total plasma clearance.

Table 7. CLint Values and Plasma Protein Binding Ratio of (*R*)-14u[a]

species	CLint in liver microsomes (mL/min/kg)	unbound ratio in plasma drug concentration (% unbound)
human	55	12
mouse	787	11
monkey	162	25

[a]Assays were performed in duplicate.

Table 8. Permeability Studies Using LLC-PK1 Cells

parameters	6	14q	14u	(*R*)-14u
LLC-PK1 P_{app} AB[a] (10^{-6} cm/s)	25.6	27.5	29.6	35.4
LLC-PK1 ER (BA/AB)[b]	0.9	1.0	1.0	0.8
LLC-GA5-COL150[c] ER (BA/AB)[b]	1.5	1.2	1.6	1.5

[a]Apparent membrane permeability (P_{app}) were calculated as the transported amount divided by initial donor concentration. [b]The efflux ratio (ER) was the ratio of P_{app} from apical to basal to that from basal to apical. [c]LLC-GA5-COL150 cells were established by transfection of human MDR1 cDNA into LLC-PK1 cells.[34,35]

arm entries in the Y-maze is correlated with locomotor activity. Because MK-801-induced hyperlocomotion is often used as an indicator of positive symptoms, the increase in total arm entries in the Y-maze induced by MK-801 could be used as an animal model of positive symptoms.

As shown in Figure 6, (*R*)-**14u** significantly improved MK-801-induced memory deficit at a dose range of 0.03−0.3 mg/kg po. In addition, (*R*)-**14u** reduced the total number of arm entries at a dose of 0.3 mg/kg po. These results suggest that (*R*)-**14u** may have beneficial effects on both cognitive impairment and positive symptoms in schizophrenia. We noted that the dose−response curve of (*R*)-**14u** is an inverted U-shape in this model, a characteristic shared by many other compounds with different mode of action when investigated in cognitive test.[40−42] For instance, previous studies have suggested that proper cognitive function requires dopamine levels be optimum, otherwise cognition is impaired.[43] Similarly, GlyT1 inhibition by agent as (*R*)-**14u** may also have an optimum level. Glycine levels in the brain after administration of (*R*)-**14u** were not measured, preventing us from drawing definitive conclusions on the effect of (*R*)-**14u** in Y-maze test. Detailed analysis using microdialysis is required to address the mode of action of (*R*)-**14u** in this model.

CONCLUSIONS

Through iterative chemical modifications of our previously identified series of GlyT1 inhibitors, we successfully improved the GlyT1 inhibitory activity and selectivity of several of these optimized compounds, leading to the identification of compound **14u**. Our analyses also revealed that **14u** exists as a mixture of stable atropisomers at ambient temperature, enabling them to be treated as single enantiomers, similar to chiral molecules with an asymmetric quaternary carbon. The active atropisomer (*R*)-**14u** displayed good PK profiles and also had the most potent in vivo effects in the (+)-HA966-induced hyperlocomotion assay in mice of all tested compounds in this series of GlyT1 inhibitors. Additionally, (*R*)-**14u** effectively attenuated working memory deficit and reduced total arm entry in the Y-maze test, suggesting that (*R*)-**14u** may have beneficial effects for the treatment of cognitive impairment and positive symptoms of schizophrenia based on the hypoglutamatergic theory.

EXPERIMENTAL SECTION

Chemistry: General. Melting points were determined using Büchi B-545 or Yanaco MP-500D micromelting apparatus and were uncorrected. ^{1}H NMR spectra were recorded on a JEOL JNM-LA-

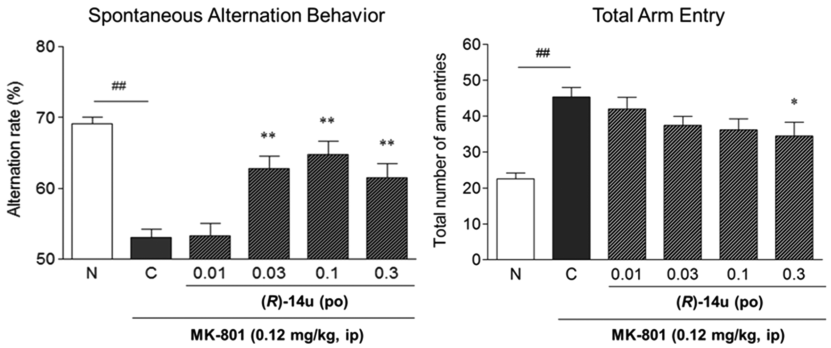

Figure 6. Effects of (*R*)-**14u** on MK-801-induced working memory deficit in the mouse Y-maze test. Left panel shows the alternation rate. Right panel shows the total number of arm entries. (*R*)-**14u** was administered orally, followed 10 min later by intraperitoneal injection of MK-801 (0.12 mg/kg). Then 20 min after MK-801 administration, each mouse was placed at the end of one arm and allowed to freely explore the apparatus for 8 min. Each value is the mean ± SEM ($n = 12$). N, normal group treated with saline; C, control group treated with MK-801. $^{\#\#}p < 0.01$; statistically significant compared with the normal group, as assessed by the Student's *t* test. $^{*}p < 0.05$, $^{**}p < 0.01$; statistically significant compared with the control group, as assessed by Dunnett's multiple comparison test.

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300 or JEOL JNM-EX-400 spectrometer, and the chemical shifts were expressed in δ (ppm) values using trimethylsilane as an internal reference (s = singlet, d = doublet, t = triplet, q = quartet, m = multiplet, and br = broad peak). Mass spectra (MS) were recorded on a Hitachi M-80 (EI) or JEOL JMS-LX2000 spectrometer (FAB). The purities of tested compounds were determined by elemental or HPLC analyses and were found to be above 95%, except for compound **10b**. HPLC analyses were conducted on a Hitachi D-7500 system using a TSKgel ODS-80TM column with UV-254 nm detection. Acetonitrile/[10 mmol/L KH_2PO_4 aq] (70:30) was used as eluent (flow rate, 1 mL/min; column temperature, 40 °C). Elemental analyses were performed with a Yanaco MT-5 CHN analyzer (C, H, N), Elementar Vario EL III elemental analyzer [or consider "instrument"] (C, H, N), and a Dionex DX-500 system (S, halogene) and were within ±0.4% of theoretical values.

Methyl *N*-(2-Fluorophenyl)ethanimidothioate (9a). To a suspension of 2-fluoroacetanilide (20.3 g, 133 mmol) in toluene (500 mL) was added Lawesson's reagent (26.8 g, 66.3 mmol), and the resultant mixture was stirred at 110 °C for 1.5 h. After cooling the mixture to room temperature, the solvent was evaporated in vacuo and the remaining residue was purified by column chromatography on silica gel ($CHCl_3$/MeOH = 20/1) to give crude 2-fluorothioacetanilide (13.0g, 56%) as a brown solid. The obtained solid (13.0 g, 75.0 mmol) was mixed with K_2CO_3 (10.5 g, 75.0 mmol) in CH_3CN (200 mL), to which was then added MeI (7.00 mL, 112 mmol), and the resultant mixture was stirred at 50 °C for 0.5 h. After cooling the mixture to room temperature, the mixture was evaporated in vacuo and the obtained residue was partitioned between EtOAc and water. The organic layer was washed with water, dried over $MgSO_4$, and concentrated in vacuo. The residue was purified by column chromatography on silica gel (hexane/EtOAc = 20/1) to give the title compound **9a** (10.4 g, 76%) as a pale-yellow oil. ^{1}H NMR (DMSO-d_6) δ 1.99 (3H, s), 2.36 (3H, s), 6.81−7.29 (4H, m). MS (EI) m/z 183 $[M]^+$.

Methyl *N*-(2-Fluorophenyl)propanimidothioate (9b). Compound **9b** was prepared from 2-fluoropropionanilide according to the procedure described for **9a**. Compound **9b** (25.7g, 90% in two steps) was obtained as a pale-yellow oil. ^{1}H NMR (DMSO-d_6) δ 0.96−1.25 (3H, m), 2.25−2.80 (5H, m), 6.76−7.26 (4H, m). MS (EI) m/z 197 $[M]^+$.

3-(4-Bromophenyl)-4-(2-fluorophenyl)-5-methyl-4*H*-1,2,4-triazole (10a). A mixture of **9a** (500 mg, 2.73 mmol), 4-bromobenzoic hydrazide (705 mg, 3.28 mmol), *p*-toluenesulfonic acid monohydrate (52.0 mg, 0.270 mmol), and *N*,*N*-dimethylformamide (DMF; 10 mL) was stirred at 120 °C for 1 h. The mixture was cooled to room temperature and then concentrated in vacuo. The obtained residue was purified by column chromatography on silica gel ($CHCl_3$/MeOH = 97/3) to give a crude solid, which was then triturated with EtOAc/hexane to give the title compound **10a** (550 mg, 61%) as a colorless solid. ^{1}H NMR (DMSO-d_6) δ 2.25 (3H, s), 7.28−7.75 (8H, m). MS (FAB) m/z 332 $[(M + H)^+]$.

4-{4-[4-(2-Fluorophenyl)-5-methyl-4*H*-1,2,4-triazol-3-yl]-phenyl}pyridine (10b). Compound **10b** was prepared from **10a** and 4-bromopyridine according to the procedure described for **10c**. The title compound **10b** (460 mg, 62%) was obtained as a white solid; mp 169−172 °C. ^{1}H NMR (DMSO-d_6) δ 2.26 (3H, s), 7.44−7.86 (10H, m), 8.61−8.67 (2H, m). MS (FAB) m/z 351 $[(M + H)^+]$; 92% pure by HPLC.

3-{4-[4-(2-Fluorophenyl)-5-methyl-4*H*-1,2,4-triazol-3-yl]-phenyl}pyridine (10c). To a solution of **10a** (500 mg, 1.51 mmol) in THF (15 mL) was added *n*-BuLi (1.57 M in hexane, 1.2 mL, 1.8 mmol) dropwise at −78 °C. After stirring for 20 min, trimethyl borate (0.50 mL, 4.5 mmol) was added to the reaction mixture at −78 °C, and the mixture was stirred for 3 h at room temperature, followed by the addition of 2 M HCl to a final pH of 2. The mixture was extracted with $CHCl_3$, and the combined organic layer was washed with brine, dried over $MgSO_4$, and concentrated in vacuo to give crude **11** as a pale-yellow oil. The obtained crude **11**, 3-bromopyridine (0.15 mL, 1.5 mmol), $Pd(PPh_3)_4$ (87 mg, 57 μmol), and 2 M Na_2CO_3 aqueous solution (1.5 mL, 3.0 mmol) were mixed in 1,2-dimethoxyethane (5 mL) and then refluxed for 2.5 h under an argon atmosphere. After cooling the mixture to room temperature, it was filtered through a Celite pad, and the filtrate was concentrated in vacuo. The residue was purified by column chromatography on silica gel ($CHCl_3$/MeOH = 98/2) to give the title compound **10c** (140 mg, 28%) as a colorless powder; mp 156−158 °C. ^{1}H NMR (DMSO-d_6) δ 2.26 (3H, s), 7.44−7.56 (5H, m), 7.63−7.70 (1H, m), 7.74−7.79 (3H, m), 8.07−8.11 (1H, m), 8.56−8.60 (1H, m), 8.88−8.92 (1H, m). MS (FAB) m/z 331 $[(M + H)^+]$. Anal. ($C_{20}H_{15}N_4F\cdot0.3H_2O\cdot0.1C_4H_8O_2$) C, H, N, F.

2-{4-[4-(2-Fluorophenyl)-5-methyl-4*H*-1,2,4-triazol-3-yl]-phenyl}pyridine (10d). Compound **10d** was prepared from **10a** and 2-bromopyridine according to the procedure described for **10c**. The title compound **10d** (200 mg, 27%) was obtained as a white solid; mp 156−158 °C. ^{1}H NMR (DMSO-d_6) δ 2.27 (3H, s), 7.34−7.38 (1H, m), 7.43−7.54 (4H, m), 7.63−7.69 (1H, m), 7.73−7.78 (1H, m), 7.86−7.91 (1H, m), 7.95−7.99 (1H, m), 8.06−8.11 (2H, m), 8.64−8.68 (1H, m). MS (FAB) m/z 351 $[(M + H)^+]$. Anal. ($C_{20}H_{15}N_4F\cdot0.1H_2O$) C, H, N, F.

5-[5-Ethyl-4-(2-fluorophenyl)-4*H*-1,2,4-triazol-3-yl]-2-phenylpyridine (10e). Compound **10e** was prepared from **9b** and 6-phenylnicotinic acid hydrazide according to the procedure described for **10a**. The title compound **10e** (198 mg, 43%) was obtained as a colorless crystal; mp 108−109 °C. ^{1}H NMR (DMSO-d_6) δ 1.19 (3H, t, J = 7.6 Hz), 2.50−2.72 (2H, m), 7.43−7.58 (5H, m), 7.65−7.73 (1H, m), 7.77−7.82 (2H, m), 8.01 (1H, d, J = 8.4 Hz), 8.05−8.12 (2H, m), 8.64 (1H, d, J = 2.0 Hz). MS (FAB) m/z 345 $[(M + H)^+]$. Anal. ($C_{21}H_{17}N_4F$) C, H, N, F.

2-[5-Ethyl-4-(2-fluorophenyl)-4*H*-1,2,4-triazol-3-yl]-5-phenylpyridine (10f). Compound **10f** was prepared from **9b** and 6-phenylnicotinic acid hydrazide according to the procedure described for **10a**. The title compound **10f** (16 mg, 3%) was obtained as a white solid; mp 130−131 °C. ^{1}H NMR (DMSO-d_6) δ 1.18 (3H, t, J = 7.6 Hz), 2.48−2.66 (2H, m), 7.36−7.51 (5H, m), 7.57−7.65 (2H, m), 7.70−7.76 (2H, m), 8.17−8.26 (2H, m), 8.57−8.60 (1H, m). MS (FAB) m/z 345 $[(M + H)^+]$; 98% pure by HPLC.

4-[5-Ethyl-4-(2-fluorophenyl)-4*H*-1,2,4-triazol-3-yl]-1-phenylpiperidine (10g). Compound **10g** was prepared from **9b** and 1-phenylpyperidine-4-carboxylic acid hydrazide according to the procedure described for **10a**. The title compound **10g** (154 mg, 33%) was obtained as a white solid; mp 141−142 °C. ^{1}H NMR (DMSO-d_6) δ 1.09 (3H, t, J = 7.6 Hz), 1.63−1.92 (4H, m), 2.35−2.70 (5H, m), 3.60−3.74 (2H, m), 6.73 (1H, t, J = 7.2 Hz), 6.90 (2H, d, J = 7.6 Hz), 7.18 (2H, t, J = 7.6 Hz), 7.47 (1H, t, J = 8.0 Hz), 7.59 (1H, t, J = 9.2 Hz), 7.65−7.77 (2H, m). MS (FAB) m/z 351 $[(M + H)^+]$. Anal. ($C_{21}H_{23}N_4F$) C, H, N, F.

3-Ethyl-4-(2-fluorophenyl)-4*H*-1,2,4-triazole (10h). Compound **10h** was prepared from **9b** and formic hydrazide according to the procedure described for **10a**. The title compound **10h** (10.4 g, 81%) was obtained as a white solid. ^{1}H NMR (DMSO-d_6) δ 1.15 (3H, t, J = 7.5 Hz), 2.60 (2H, q, J = 7.5 Hz), 7.40−7.70 (4H, m), 8.69 (1H, d, J = 0.8 Hz). MS (FAB) m/z 192 $[(M + H)^+]$.

1-[5-Ethyl-4-(2-fluorophenyl)-4*H*-[1,2,4]triazol-3-yl]-4-phenylpiperidine (10i). A mixture of **12** (1.1 g, 4.1 mmol) and 4-phenylpiperidine (5.80 g, 36 mmol) was stirred at 170 °C for 3 h, cooled to room temperature, and then partitioned between $CHCl_3$ and saturated $NaHCO_3$ aqueous solution. The organic phase was washed with saturated $NaHCO_3$ aqueous solution, dried over $MgSO_4$, and concentrated in vacuo. The obtained residue was purified by column chromatography on silica gel ($CHCl_3$/MeOH = 30/1) to give crude **10i** as a solid, which was then recrystallized with iPr_2O to give the title compound **10i** (856 mg, 60%) as a colorless crystal; mp 108−109 °C. ^{1}H NMR (DMSO-d_6) δ 1.07 (3H, t, J = 7.6 Hz), 1.40−1.58 (2H, m), 1.60−1.74 (2H, m), 2.40 (2H, q, J = 7.6 Hz), 2.56−2.70 (1H, m), 2.78−2.94 (2H, m), 3.17−3.26 (1H, m), 3.27−3.34 (1H, m), 7.14−7.22 (3H, m), 7.25−7.31 (2H, m), 7.44 (1H, t, J = 8.0 Hz), 7.55 (1H, t, J = 8.8 Hz), 7.60−7.70 (2H, m). MS (FAB) m/z 351 $[(M + H)^+]$. Anal. ($C_{21}H_{23}N_4F$) C, H, N, F.

1-[5-Ethyl-4-(2-fluorophenyl)-4*H*-[1,2,4]triazol-3-yl]-4-phenylpiperazine (10j). Compound **10j** was prepared from **12** and 1-phenylpiperadine according to the procedure described for **10i**. The

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title compound **10j** (50 mg, 12%) was obtained as a pale-brown crystal; mp 108−112 °C. ^{1}H NMR (DMSO-d_6) δ 1.07 (3H, t, J = 7.5 Hz), 2.40 (2H, q, J = 7.5 Hz), 2.99−3.14 (8H, m), 6.77 (1H, t, J = 7.5 Hz), 6.89 (2H, d, J = 7.5 Hz), 7.18 (2H, t, J = 7.5 Hz), 7.44 (1H, t, J = 7.5 Hz), 7.55 (1H, t, J = 7.5 Hz), 7.60−7.71 (2H, m). MS (FAB) m/z 352 [(M + H)$^+$]. Anal. ($C_{20}H_{22}N_5F$) C, H, N, F.

3-Bromo-5-ethyl-4-(2-fluorophenyl)-4*H*-[1,2,4]triazole (12). To a solution of **10h** (1.29 g, 6.75 mmol) in CCl_4 (30 mL)−AcOH (30 mL) was added NBS (1.8 g, 10.0 mmol), and the resulting mixture was refluxed for 1.5 h. After cooling to room temperature, the mixture was diluted with CH_2Cl_2, washed with 10% K_2CO_3 aq, dried over $MgSO_4$, and concentrated in vacuo. The residue was recrystallized with EtOAc−hexane to give the title compound **12** (955 mg, 52%) as a yellow crystal. ^{1}H NMR (DMSO-d_6) δ 1.12 (3H, t, J = 7.5 Hz), 2.54 (2H, q, J = 7.5 Hz), 7.48 (1H, t, J = 7.1 Hz), 7.56−7.65 (1H, m), 7.67−7.77 (2H, m). MS (FAB) m/z 270 [(M + H)$^+$].

3-Biphenyl-4-yl-5-ethyl-4-(2-methylphenyl)-4*H*-1,2,4-triazole (14a). To a mixture of 2-(biphenyl-4-yl)-5-ethyl-1,3,4-oxadiazole **13a** (230 mg, 0.92 mmol) and 2-methylaniline (250 mg, 2.33 mmol) was added *p*-toluenesulfonic acid monohydrate (30 mg, 0.16 mmol), and the resultant mixture was stirred at 160 °C for 3 h. After cooling the mixture to room temperature, it was purified by column chromatography on silica gel (AcOEt) to give a crude solid, which was then triturated with EtOAc/hexane to give the title compound **14a** (198 mg, 63%) as a white solid; mp 195−197 °C. ^{1}H NMR ($CDCl_3$) δ 1.27 (3H, t, J = 7.6 Hz), 1.93 (3H, s), 2.58 (2H, q, J = 7.6 Hz), 7.26−7.56 (13H, m). MS (FAB) m/z 340 [(M + H)$^+$]. Anal. ($C_{23}H_{21}N_3$·0.1H_2O) C, H, N.

3-Biphenyl-4-yl-4-(2,3-dimethylphenyl)-5-ethyl-4*H*-[1,2,4]-triazole (14b). Compound **14b** was prepared from **13a** and 2,3-dimethylaniline according to the procedure described for **14a**. The title compound **14b** (71 mg, 14%) was obtained as a white solid; mp 180−181 °C. ^{1}H NMR (DMSO-d_6) δ 1.15 (3H, t, J = 7.6 Hz), 1.75 (3H, s), 2.30 (3H, s), 2.40−2.47 (2H, m), 7.31−7.47 (8H, m), 7.62−7.68 (4H, m). MS (FAB) m/z 354 [(M + H)$^+$]. Anal. ($C_{24}H_{23}N_3$) C, H, N.

3-[3-(Biphenyl-4-yl)-5-ethyl-4*H*-1,2,4-triazol-4-yl]-2-methylaniline (14c). To a mixture of **14r** (1.6 g, 4.1 mmol), 1 M aqueous HCl (0.6 mL), EtOH (20 mL), and water (20 mL) was added Fe (powder, 2.9 g, 53 mmol), and the reaction mixture was stirred at 70 °C for 2 h. After the mixture was cooled to room temperature, it was diluted with $CHCl_3$ and filtered through a Celite pad. The obtained filtrate was mixed with 1 M aqueous NaOH (3.0 mL), and the organic layer was then separated, washed with brine, dried over $MgSO_4$, and concentrated in vacuo. The residue was purified by column chromatography on silica gel ($CHCl_3$/MeOH = 99/1) to give a crude solid, which was then recrystallized with EtOAc to give the title compound **14c** (330 mg, 23%) as a colorless powder; mp 200−202 °C. ^{1}H NMR (DMSO-d_6) δ 1.15 (3H, t, J = 7.6 Hz), 1.55 (3H, s), 2.43−2.49 (2H, m), 5.28−5.33 (2H, m), 6.62 (1H, d, J = 7.3 Hz), 6.81 (1H, d, J = 8.3 Hz), 7.11 (1H, t, J = 8.1 Hz), 7.34−7.39 (1H, m), 7.42−7.52 (4H, m), 7.63−7.69 (4H, m). MS (FAB) m/z 355 [(M + H)$^+$]. Anal. ($C_{23}H_{22}N_4$) C, H, N.

3-[3-(Biphenyl-4-yl)-5-ethyl-4*H*-1,2,4-triazol-4-yl]-*N*, *N*, 2-trimethylaniline (14d). To a solution of **14c** (248 mg, 0.70 mmol) and folmaldehyde (37% w/w in water, 0.13 mL) in AcOH (5 mL) was added $NaBH_3CN$ (53 mg, 0.84 mmol), and the reaction mixture was stirred for 3 h before diluting with $CHCl_3$. The organic layer then was separated, washed with saturated $NaHCO_3$ aqueous solution, dried over $MgSO_4$, and concentrated in vacuo. The residue was purified by column chromatography on silica gel ($CHCl_3$/MeOH = 99/1) to give a crude solid, which was recrystallized with iPr$_2$O to give the title compound **14d** (71 mg, 27%) as a colorless powder; mp 134−135 °C. ^{1}H NMR (DMSO-d_6) δ 1.16 (3H, t, J = 7.6 Hz), 1.73 (3H, s), 2.45−2.52 (2H, m), 2.61 (6H, s), 7.17−7.20 (1H, m), 7.26 (1H, d, J = 7.9 Hz), 7.34−7.48 (6H, m), 7.62−7.68 (4H, m). MS (FAB) m/z 383 [(M + H)$^+$]. Anal. ($C_{25}H_{26}N_4$) C, H, N.

3-[3-(Biphenyl-4-yl)-5-ethyl-4*H*-1,2,4-triazol-4-yl]-2-methylphenol (14e). Compound **14e** was prepared from **13a** and 3-amino-2-methylphenol according to the procedure described for **14a**. The title compound **14e** (36 mg, 7%) was obtained as a white solid; mp 298−300 °C. ^{1}H NMR (DMSO-d_6) δ 1.16 (3H, t, J = 7.6 Hz), 1.63 (3H, s), 2.44−2.50 (2H, m), 6.96 (1H, d, J = 7.8 Hz), 7.02 (1H, d, J = 7.8 Hz), 7.25 (1H, t, J = 7.8 Hz), 7.34−7.40 (1H, m), 7.42−7.49 (4H, m), 7.64−7.69 (4H, m), 10.03 (1H, s). MS (FAB) m/z 356 [(M + H)$^+$]. Anal. ($C_{23}H_{21}N_3O$·0.3H_2O) C, H, N.

4-{3-[(Benzyloxy)methyl]-2-methylphenyl}-3-(biphenyl-4-yl)-5-ethyl-4*H*-1,2,4-triazole (14f). Compound **14f** was prepared from **13a** and 3-[(benzyloxy)methyl]-2-methylaniline according to the procedure described for **14a**. The title compound **14f** (799 mg, 90%) was obtained as a solid. ^{1}H NMR (DMSO-d_6) δ 1.15 (3H, t, J l 7.5 Hz), 1.77 (3H, s), 2.40−2.52 (2H, m), 4.40−4.65 (4H, m), 7.26−7.65 (17H, m). MS (FAB) m/z 460 [(M + H)$^+$].

{3-[3-(Biphenyl-4-yl)-5-ethyl-4*H*-1,2,4-triazol-4-yl]-2-methylphenyl}methanol (14g). To a solution of **14f** (4.4 g, 9.6 mmol) in $CHCl_3$ (130 mL) was added 1 M BCl_3 solution in heptane (14 mL) dropwise at 0 °C. After it was stirred at room temperatrure for 3 h, an additional batch of BCl_3 (1 M solution in heptane, 24 mL) was added dropwise at 0 °C, and the resultant mixture was stirred overnight at room temperature. The reaction mixture was then poured into a 1 M aqueous NaOH solution (150 mL) at 0 °C and stirred for 1 h. The organic layer was separated, washed with brine, dried over $MgSO_4$, and concentrated in vacuo. The residue was purified by column chromatography on silica gel ($CHCl_3$/MeOH = 96/4) to give the title compound **14g** (2.32g, 66%) as a solid; mp 199−201 °C. ^{1}H NMR (DMSO-d_6) δ 1.15 (3H, t, J = 7.3 Hz), 1.76 (3H, s), 2.45 (2H, q, J = 7.3 Hz), 4.48−4.58 (2H, m), 5.30 (1H, t, J = 5.4 Hz), 7.34−7.47 (7H, m), 7.60−7.68 (5H, m). MS (FAB) m/z 370 [(M + H)$^+$]. Anal. ($C_{24}H_{23}N_3O$·0.3H_2O) C, H, N.

3-(Biphenyl-4-yl)-4-[3-(chloromethyl)-2-methylphenyl]-5-ethyl-4*H*-1,2,4-triazole (14h). To a solution of **14g** (1.0 g, 2.71 mmol) in $CHCl_3$ (20 mL) was added $SOCl_2$ (0.9 mL), and the resultant mixture was stirred for 30 min. The mixture was then evaporated in vacuo, and the obtained residue was diluted with $CHCl_3$, washed with saturated aqueous $NaHCO_3$ solution, dried over $MgSO_4$, and concentrated in vacuo to give the title compound **14h** (1.04 g, 99%) as a solid. ^{1}H NMR (DMSO-d_6) δ 1.15 (3H, t, J = 7.5 Hz), 1.89 (3H, s), 2.40−2.50 (2H, m), 4.86 (2H, s), 7.35−7.70 (12H, m); MS (FAB) m/z 388 [(M + H)$^+$].

3-(Biphenyl-4-yl)-5-ethyl-4-[3-(methoxymethyl)-2-methylphenyl]-4*H*-1,2,4-triazole (14i). To a mixture of **14h** (280 mg, 0.72 mmol), MeOH (15 mL), and THF (5 mL) was added NaOMe (390 mg, 7.2 mmol), and the reaction mixture was refluxed for 2 h. After it was cooled to room temparature, a 1 M aqueous solution of HCl (6.5 mL) was added to mixture, which was then concentrated in vacuo. The obtained residue was diluted with $CHCl_3$, washed with brine, dried over $MgSO_4$, and concentrated in vacuo. The residue was recrystallized with hexane/EtOAc/MeCN to give the title compound **14i** (120 mg, 43%) as a colorless powder; mp 152−154 °C. ^{1}H NMR (DMSO-d_6) δ 1.17 (3H, t, J = 7.6 Hz), 1.79 (3H, s), 2.45−2.51 (2H, m), 3.28 (3H, s), 4.43−4.51 (2H, m), 7.35−7.40 (1H, m), 7.42−7.48 (5H, m), 7.51−7.59 (2H, m), 7.64−7.68 (4H, m). MS (FAB) m/z 384 [(M + H)$^+$]. Anal. ($C_{25}H_{25}N_3O$·0.1H_2O) C, H, N.

1-{3-[3-(Biphenyl-4-yl)-5-ethyl-4*H*-1,2,4-triazol-4-yl]-2-methylphenyl}-*N*,*N*-dimethylmethanamine dihydrochloride (14j). To a solution of **14h** (287 mg, 0.74 mmol) in MeCN (12 mL) was added dimethylamine aqueous solution [50%(w/w), 330 mg, 3.7 mmol], and the resultant mixture was stirred for 1 h. After it was concentrated in vacuo, the residue was diluted with $CHCl_3$, washed with saturated $NaHCO_3$ aqueous solution, dried over $MgSO_4$, and concentrated in vacuo. The residue was purified by column chromatography on silica gel ($CHCl_3$/MeOH = 90/10) to give a solid, which was then dissolved in EtOAc (2 mL), followed by addition of 4 M HCl solution in EtOAc (0.45 mL). The resulting precipitate was filtered off, and it was recrystallized from EtOH/hexane to give the title compound **14j** (209 mg, 56%) as a colorless powder; mp 134−137 °C. ^{1}H NMR (DMSO-d_6) δ 1.22 (3H, t, J = 7.3 Hz), 2.06 (3H, s), 2.54−2.69 (5H, m), 2.73 (3H, d, J = 4.4 Hz), 4.32−4.48 (2H, m), 7.36−7.50 (5H, m), 7.57 (1H, t, J = 7.8 Hz), 7.63−7.74 (5H, m), 7.92 (1H, d, J = 7.8 Hz). MS (FAB) m/z 397 [(M + H)$^+$]. Anal. ($C_{26}H_{28}N_4$·2HCl·2H_2O) C, H, N, Cl.

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3-Biphenyl-4-yl-5-ethyl-4-(3-fluoro-2-methylphenyl)-4*H*-[1,2,4]triazole (14k). Compound **14k** was prepared from **13a** and 3-fluoro-2-methylaniline according to the procedure described for **14a**. The title compound **14k** (195 mg, 37%) was obtained as a solid; mp 161−162 °C. ^{1}H NMR (DMSO-d_6) δ 1.17 (3H, t, J = 7.6 Hz), 1.77 (3H, d, J = 2.0 Hz), 2.46−2.53 (2H, m), 7.34−7.40 (1H, m), 7.42−7.55 (7H, m), 7.64−7.70 (4H, m). MS (FAB) m/z 358 [(M + H)$^+$]. Anal. ($C_{23}H_{20}N_3F\cdot 0.1H_2O$) C, H, N, F.

3-Biphenyl-4-yl-5-ethyl-4-(3-chloro-2-methylphenyl)-4*H*-[1,2,4]triazole (14l). Compound **14l** was prepared from **13a** and 3-chloro-2-methylaniline according to the procedure described for **14a**. The title compound **14l** (182 mg, 31%) was obtained as a solid; mp 168−169 °C. ^{1}H NMR (DMSO-d_6) δ 1.17 (3H, t, J = 7.4 Hz), 1.88 (3H, s), 2.44−2.51 (2H, m), 7.35−7.40 (1H, m), 7.41−7.52 (5H, m), 7.60−7.64 (1H, m), 7.65−7.73 (5H, m). MS (FAB) m/z 374 [(M + H)$^+$]. Anal. ($C_{23}H_{20}N_3Cl$) C, H, N, Cl.

3-Biphenyl-4-yl-4-(3-bromo-2-methylphenyl)-5-ethyl-4*H*-1,2,4-triazole (14m). Compound **14m** was prepared from **13a** and 3-bromo-2-methylaniline according to the procedure described for **14a**. The title compound **14m** (1.40 g, 28%) was obtained as a solid; mp 173−174 °C. ^{1}H NMR (DMSO-d_6) δ 1.17 (3H, t, J = 7.8 Hz), 1.90 (3H, s), 2.47 (2H, q, J = 7.4 Hz), 7.35−7.47 (6H, m), 7.63−7.70 (5H, m), 8.87 (1H, d, J = 8.3 Hz). MS (FAB) m/z 418 [(M + H)$^+$]. Anal. ($C_{23}H_{20}N_3Br$) C, H, N, Br.

Methyl 3-[3-(Biphenyl-4-yl)-5-ethyl-4*H*-1,2,4-triazol-4-yl]-2-methylbenzoate (14n). Compound **14n** was prepared from **13a** and methyl 3-amino-2-methylbenzoate according to the procedure described for **14a**. The title compound **14n** (46 mg, 9%) was obtained as a solid; mp 194−196 °C. ^{1}H NMR (DMSO-d_6) δ 1.21 (3H, t, J = 7.5 Hz), 2.03 (3H, s), 2.50 (2H, q, J = 7.5 Hz), 3.88 (3H, s), 7.39−7.52 (5H, m), 7.63 (1H, t, J = 7.9 Hz), 7.69−7.73 (4H, m), 7.89 (1H, d, J = 7.8 Hz), 8.05 (1H, d, J = 7.8 Hz). MS (FAB) m/z 398 [(M + H)$^+$]. Anal. ($C_{25}H_{23}N_3O_2$) C, H, N.

3-[3-(Biphenyl-4-yl)-5-ethyl-4*H*-1,2,4-triazol-4-yl]-2-methylbenzamide (14o). Compound **14o** was prepared from **13a** and 3-amino-2-methylbenzamide according to the procedure described for **14a**. The title compound **14o** (597 mg, 39%) was obtained as a solid; mp 240−242 °C. ^{1}H NMR (DMSO-d_6) δ 1.18 (3H, t, J = 7.3 Hz), 1.88 (3H, s), 2.40−2.50 (2H, m), 7.34−7.52 (6H, m), 7.56−7.68 (7H, m), 7.96 (1H, br). MS (FAB) m/z 383 [(M + H)$^+$]. Anal. ($C_{24}H_{22}N_4O$) C, H, N.

3-Biphenyl-4-yl-5-ethyl-4-[2-methyl-3-(trifluoromethyl)-phenyl]-4*H*-1,2,4-triazole (14p). Compound **14p** was prepared from **13a** and 3-amino-2-methylbenzotrifluoride according to the procedure described for **14a**. The title compound **14p** (177 mg, 30%) was obtained as a solid; mp 174−175 °C. ^{1}H NMR (DMSO-d_6) δ 1.78 (3H, t, J = 7.6 Hz), 1.92 (3H, s), 2.44−2.51 (2H, m), 7.35−7.48 (5H, m), 7.65−7.73 (5H, m), 7.98 (2H, dd, J = 8.3, 3.0 Hz). MS (FAB) m/z 408 [(M + H)$^+$]. Anal. ($C_{24}H_{20}N_3F$) C, H, N, F.

3-[3-(Biphenyl-4-yl)-5-ethyl-4*H*-1,2,4-triazol-4-yl]-2-methylbenzonitrile (14q). A mixture of **14o** and $POCl_3$ was stirred at 100 °C for 1 h. The mixture was evaporated in vacuo, and the residue was diluted with $CHCl_3$. The solution was washed with a saturated $NaHCO_3$ aqueous solution, dried over $MgSO_4$, and concentrated in vacuo. The residue was recrystallized with EtOAc to give the title compound **14q** (532 mg, 44%) as a colorless powder; mp 199−200 °C. ^{1}H NMR (DMSO-d_6) δ 1.18 (3H, t, J = 7.8 Hz), 2.05 (3H, s), 2.45−2.52 (2H, m), 7.35−7.48 (5H, m), 7.64−7.70 (5H, m), 7.98 (1H, d, J = 7.8 Hz), 8.07 (1H, d, J = 7.9 Hz). MS (FAB) m/z 365 [(M + H)$^+$]. Anal. ($C_{24}H_{20}N_4$) C, H, N.

3-Biphenyl-4-yl-5-ethyl-4-(2-methyl-3-nitrophenyl)-4*H*-1,2,4-triazole (14r). Compound **14r** was prepared from **13a** and 2-methyl-3-nitroaniline according to the procedure described for **14a**. The title compound **14r** (799 mg, 33%) was obtained as a solid; mp 174−176 °C. ^{1}H NMR (DMSO-d_6) δ 1.19 (3H, t, J = 7.6 Hz), 1.97 (3H, s), 2.44−2.54 (2H, m), 7.35−7.40 (1H, m), 7.42−7.48 (4H, m), 7.65−7.75 (5H, m), 8.02 (1H, d, J = 6.9 Hz), 8.18−8.22 (1H, m). MS (FAB) m/z 385 [(M + H)$^+$]. Anal. ($C_{23}H_{20}N_4O_2$) C, H, N.

{3-[3-(Biphenyl-4-yl)-5-ethyl-4*H*-1,2,4-triazol-4-yl]-2-methylphenyl}acetonitrile (14s). To a solution of **14h** (287 mg, 0.74 mmol) in CH_3CN (10 mL) were added KCN (243 mg, 3.7 mmol) and 18-crown-6 (401 mg, 1.52 mmol), and the resultant mixture was refluxed for 2 h. After cooling the mixture to room temperature, it was concentrated in vacuo and then diluted with $CHCl_3$. The resultant organic solution was washed with a saturated $NaHCO_3$ aqueous solution, dried over $MgSO_4$, and concentrated in vacuo. The obtained residue was purified by column chromatography on silica gel ($CHCl_3$/MeOH = 98/2) to give a crude solid, which was recrystallized from iPr$_2$O/AcOEt to give the title compound **14s** (39 mg, 14%) as a colorless solid; mp 150−152 °C. ^{1}H NMR (DMSO-d_6) δ 1.15 (3H, t, J = 7.6 Hz), 1.81 (3H, s), 2.41−2.48 (2H, m), 4.13 (2H, s), 7.34−7.53 (6H, m), 7.56−7.67 (6H, m). MS (FAB) m/z 379 [(M + H)$^+$]. Anal. ($C_{25}H_{22}N_4$) C, H, N.

3-[3-Ethyl-5-(6-phenylpyridin-3-yl)-4*H*-1,2,4-triazol-4-yl]-2-methylbenzamide (14t). Compound **14t** was prepared from **13b** and 3-amino-2-methylbenzamide according to the procedure described for **14a**. The title compound **14t** (550 mg, 89%) was obtained as a solid. ^{1}H NMR (DMSO-d_6) δ 1.19 (3H, t, J = 7.2 Hz), 1.90 (3H, s), 2.40−2.55 (2H, m), 7.42−7.53 (4H, m), 7.59−6.61 (2H, m), 7.67 (1H, d, J = 7.6 Hz), 7.82 (1H, dd, J = 2.0, 8.0 Hz), 7.96 (1H, brs), 7.99 (1H, d, J = 8.8 Hz), 8.05−8.09 (2H, m), 8.60 (1H, d, J = 2.4 Hz). MS (FAB) m/z 384 [(M + H)$^+$].

3-[3-Ethyl-5-(6-phenylpyridin-3-yl)-4*H*-1,2,4-triazol-4-yl]-2-methylbenzonitrile (14u). Compound **14u** was prepared from **14t** according to the procedure described for **14q**. The title compound **14u** (242 mg, 46%) was obtained as a solid; mp 181−182 °C. ^{1}H NMR (DMSO-d_6) δ 1.19 (3H, t, J = 7.5 Hz), 2.08 (3H, s), 2.41−2.59 (2H, m), 7.43−7.52 (3H, m), 7.63 (1H, dd, J = 7.8, 8.3 Hz), 7.76 (1H, dd, J = 2.0, 8.3 Hz), 7.98−8.02 (1H, m), 7.99 (1H, d, J = 8.3 Hz), 8.05−8.12 (3H, m), 8.64 (1H, d, J = 2.0 Hz). MS (FAB) m/z 366 [(M + H)$^+$]. Anal. ($C_{23}H_{19}N_5$) C, H, N.

(*R*)-3-[3-Ethyl-5-(6-phenylpyridin-3-yl)-4*H*-1,2,4-triazol-4-yl]-2-methylbenzonitrile [(*R*)-14u] and (*S*)-3-[3-Ethyl-5-(6-phenylpyridin-3-yl)-4*H*-1,2,4-triazol-4-yl]-2-methylbenzonitrile [(*S*)-14u]. **14u** was separated on a preparative CHIRALPAK AD column (20 mm × 250 mm, flow rate: 5 mL/min wavelength: 254 nm) using n-hexane/EtOH (1:1) as an eluent to give (***R***)-**14u** as the first eluting product (retention time, 30 min) and (***S***)-**14u** as the second eluting product (retention time, 37 min).

(***R***)-**14u**: white solid, 100% ee, $[\alpha]_D^{25}$ −211° (c 1.0, $CHCl_3$), mp 162−163 °C. ^{1}H NMR (DMSO-d_6) δ 1.19 (3H, t, J = 7.4 Hz), 2.08 (3H, s), 2.41−2.59 (2H, m), 7.43−7.52 (3H, m), 7.66 (1H, t, J = 7.8 Hz), 7.76 (1H, dd, J = 1.5, 8.3 Hz), 7.97−8.02 (2H, m), 8.05−8.12 (3H, m), 8.64 (1H, d, J = 1.9 Hz). MS (FAB) m/z 366 [(M + H)$^+$]. Anal. ($C_{23}H_{19}N_5$) C, H, N.

(***S***)-**14u**: white solid, 99.7% ee, $[\alpha]_D^{25}$ +213° (c 1.0, $CHCl_3$), 100% ee, mp 159−160 °C. ^{1}H NMR (DMSO-d_6) δ 1.19 (3H, t, J = 7.4 Hz), 2.08 (3H, s), 2.41−2.59 (2H, m), 7.43−7.52 (3H, m), 7.66 (1H, J = 7.8 Hz), 7.76 (1H, dd, J = 2.4, 8.3 Hz), 7.98−8.02 (2H, m), 8.05−8.12 (3H, m), 8.64 (1H, d, J = 1.9 Hz). MS (FAB) m/z 366 [(M + H)$^+$]. Anal. ($C_{23}H_{19}N_5$) C, H, N.

Kinetic Measurements. The kinetics of rotamer interconversion of (***S***)-**14u** to its racemate in nitrobenzene were followed at 78.5, 100, 112, 120, and 134 °C by HPLC using the chromatographic conditions described in Figure 5. Fitting first-order kinetics gave rate constants for racemization at each temperature. The activation energy of racemizaton was calcurated using Eyling plots of the rate constant at each temperature.[33]

[^{3}H]Glycine Uptake Assay (GlyT1). The inhibitory effect of the compounds on rat GlyT1 was examined as previously described[21] with some modifications. Rat glioma C6 cells, which endogenously express GlyT1, were plated at a density of 2 × 10^4 cells per well in a white 96-well CulturPlate (PerkinElmer Inc.) and incubated for two days in a CO_2 incubator (37 °C, 5% CO_2). After washing with 4-(2-hydroxyethyl)-1-piperazineethanesulfonic acid (HEPES) buffer [NaCl (150 mmol/L), KCl (5 mmol/L), $MgCl_2$ (1 mmol/L), $CaCl_2$ (1 mmol/L), HEPES (10 mmol/L), D-glucose (10 mmol/L), and L-alanine (5 mmol/L), pH 7.4], the buffer was replaced with HEPES buffer containing the test compounds (for concentration ranges, see Supporting Information), and plates were incubated at 30 min at 37 °C. After incubation, [^{3}H]glycine (final concentration of [^{3}H]glycine:

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0.14 μM) was added, and the plate was further incubated for 10 min at 37 °C. After incubation, the wells were aspirated and washed three times with ice-cold phosphate-buffered saline (PBS). After solubilizing the cells with 17 μL of 0.1 mol/L NaOH, 100 μL of scintillant (MicroScint PS; PerkinElmer Inc.) was added to the wells, and the plate was counted in a Top Count microplate scintillation counter (Hewlett-Packard Company; Palo Alto, CA, USA). Nonspecific uptake was detected using 3 mmol/L sarcosine. We evaluated the effect of sarcosine on [^{3}H]glycine uptake in parallel as a reference GlyT1 inhibitor. The drug concentrations required to inhibit 50% of the specific [^{3}H]glycine uptake (IC_{50}) were obtained using nonlinear regression analysis and SAS software (SAS Institute Inc.; Cary, NC, USA), with the mean ± SEM obtained from three independent experiments.

[^{3}H]Glycine Uptake Assay (GlyT2). The inhibitory effect of the compounds on rat GlyT2 was examined using [^{3}H]glycine uptake into primary cultured cells, which endogenously express GlyT2. Pregnant rats (Wistar, 17 days gestation, Japan SLC) were euthanized under ether anesthesia by exsanguination by severing the carotid artery, and the infants were then obtained from the pregnant rats and their brainstems isolated. After digestion with papain, brainstem cells were dispersed into culture medium and plated at a density of 5×10^4 cells per well in white 96-well plates coated with poly-L-lysine and then incubated for 14−21 days in a CO_2 incubator (37 °C, 5% CO_2).

On the day of the assay, the cells were washed once with assay buffer (150 mmol/L NaCl, 5 mmol/L KCl, 1 mmol/L $MgCl_2$, 1 mmol/L $CaCl_2$, 10 mmol/L glucose, 5 mmol/L L-alanine, 3 mmol/L sarcosine, and 10 mmol/L HEPES, pH 7.4), and each well was then filled with 100 μL of assay buffer. After preincubation at 37 °C for 10 min, the assay buffer was then replaced with the reaction mixture containing the test compounds (for concentration ranges, see Supporting Information) and [^{3}H]glycine (final concentration of [^{3}H]glycine: 0.14 μM), and the plate was further incubated for 10 min at 37 °C, The reaction was then stopped by washing the cells four times with ice-cold buffer. After solubilizing the cells with 17 μL of 0.1 M NaOH, 100 μL of scintillant was added and the plate was counted using a TopCount microplate scintillation counter (Hewlett-Packard Company). Nonspecific [^{3}H]glycine uptake was determined with 3 mmol/L unlabeled glycine. The drug concentrations required to inhibit 50% of the specific [^{3}H]glycine uptake (IC_{50}) were determined using nonlinear regression analysis and SAS software (SAS Institute Inc.). We evaluated glycine in parallel as the reference, and the mean ± SEM from three independent experiments are reported as the final inhibition values.

(+)-HA966-Induced Enhancement of Locomotor Activity in Mice. Locomotor activity was assessed using a previously reported method with minor modifications.[44] Animal, male ICR mice (Japan SLC; age 5−7 weeks); drug, reserpine (Apoplon injection, 1 mg/mL; Daiichi Pharmaceuticals Co., Ltd., Tokyo, Japan), (+)-HA966, α-methyl-*p*-tyrosine methyl ester (Sigma, Inc.); equipment, Supermex (Muromachi Machine). Method. (1) Pharmaceutical drug-treated groups (n = 16) were defined as follows: [artificial cerebrospinal fluid (ACSF) + vehicle] group, [(+)-HA966 80 μg/mouse (icv) + vehicle] group, [(+)-HA966 80 μg/mouse (icv) + test compound] group. (2) Nineteen hours before (+)-HA966 administration, reserpine (10 mg/kg) was administered intraperitoneally. (3) Thirty minutes before (+)-HA966 administration, α-methyl-*p*-tyrosine methyl ester (250 mg/kg) was administered intraperitoneally. (4) Twenty minutes before (+)-HA966 administration, a test compound was administered orally. (5) (+)-HA966 was acutely administered bilaterally into the lateral ventricule using a two-step needle. Immediately after administration, each animal was placed in the measuring cage of an activity measurement apparatus. Activity per 90 min was measured immediately upon placing the animal in the cage. The integral value of locomotor activity per 90 min was selected as the data.

MK-801-Induced Working Memory Deficit in the Mouse Y-Maze Test. Male ddY mice (5−6 weeks old, Japan SLC, Inc.) were used for the experiments. The maze was constructed of gray polyvinyl chloride, with arms converging at equal angles. Arm dimensions were: 40 cm in length, 13 cm in height, and 3 and 10 cm in width at the bottom and top, respectively. Mice were acclimated in the experimental room for at least 1 h prior to testing. Test compounds were orally administered, and animals were returned to their home cages. Ten minutes after administration of (***R***)-**14u**, 0.12 mg/kg of MK-801 was administered intraperitoneally and animals were again returned to their home cages. Twenty minutes after administration of MK-801, each mouse was placed at the end of one arm and allowed to freely explore the apparatus for 8 min. The sequence and number of all arm entries were recorded for each animal throughout the test period. The sequence triads, in which all three arms were represented (including ABC, ACB, BAC, BCA, CAB, and CBA), were calculated as successful alternations to evaluate normal perception and working memory of the last arm entered. Alternation percentage was defined as entries into all three arms on consecutive occasions using the following formula:

$$\text{alternation}(\%) = 100 \times (\text{number of alternations} / (\text{number of total arm entries} - 2))$$

Data were eliminated in cases where the number of total arm entries was less than 10 or where the mouse escaped from the Y-maze field.

■ ASSOCIATED CONTENT

Ⓢ Supporting Information

Supplemental data for in vitro rGlyT1/2 assay, synthesis of the intermediates, plasma time curve, experimental method for PK studies, combustion analyses or HPLC data for the tested compound, and kinetic data for racemization. This material is available free of charge via the Internet at http://pubs.acs.org.

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Notes

The authors declare no competing financial interest.

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■ ABBREVIATIONS USED

ACSF, artificial cerebrospinal fluid; GlyT, glycine transporter; NMDA, *N*-methyl-D-aspartate; SAR, structure−activity relationship

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