Article

New α-Hydroxy-1,2,3-triazoles and 9*H*-Fluorenes-1,2,3-triazoles: Synthesis and Evaluation as Glycine Transporter 1 Inhibitors

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Two series of new compounds containing 1,2,3-triazole moiety were designed as putative GlyT1 inhibitors aiming the discovery of new hits with activity in cognitive disorders. 1,4-Disubstituted α-hydroxy-1,2,3-triazoles were obtained as racemates in moderate to good yields by the copper-catalyzed azide-alkyne cycloaddition (CuAAC) reaction (click chemistry) as the key step between propargyl alcohols and aryl azides, previously prepared from anilines or boronic acids. Benzo[*c*]chromene-triazoles were planned to be obtained by palladium-catalyzed C−H activation using [bis(trifluoroacetoxy)iodobenzene] (PhI(TFA)$_2$) of some α-hydroxy-1,2,3-triazoles, since benzo[*c*]chromenes are also privileged groups with several biological activities, including to the central nervous system. Unexpectedly, 9*H*-fluorenes-1,2,3-triazoles, instead of benzo[*c*]chromene-triazoles, were obtained by Friedel-Crafts alkylation reaction. The two series of compounds were tested for inhibition of the glycine transporter (rat GlyT1 isoform) but only the α-hydroxy-1,2,3-triazole **9b** was active (half maximal inhibitory concentration (IC$_{50}$) = 8.0 μM).

Keywords: 1,4-disubstituted-1,2,3-triazoles, 9*H*-fluorenes-1,2,3-triazoles, click chemistry, Friedel-Crafts alkylation, schizophrenia, glycine transport

Introduction

Several compounds containing the 1,2,3-triazole moiety have pharmacological properties potentially useful for antifungal and antibacterial,[1] anticancer[2] and antiviral[3] therapy and, in some cases, for the treatment of mental disorders including depression, anxiety, schizophrenia and epilepsy.[4,5]

Different reports indicate that triazoles have affinity not only for dopaminergic, adrenergic and serotonergic receptors[4,6] but also inhibit the glycine transporter GlyT1, considered as an important target for the treatment of schizophrenia, cognitive disorders, alcohol dependence and pain.[7-9] Figure 1 shows some examples of GlyT1 inhibitors such as ALX 5407 (**1**), a potent, selective and irreversible compound[10] without clinical use but largely employed as a pharmacological tool.

Bitopertin (**2**) (Hoffmann-La Roche) was the first of the non-sarcosine derivative GlyT1 inhibitors to be tested in schizophrenia preclinical models and phase II and III clinical trials.[11] This compound is a potent and selective non-competitive inhibitor of the GlyT1 subtype and does not act on other molecular targets.[12] Among the compounds containing a triazole moiety, methyl-1,2,3-triazole **3** stands out since it is a non-competitive inhibitor with very high potency and good metabolic stability *in vitro*.[9] It is important to notice that among the compounds evaluated by these authors,[9] the 1,2,3-triazoles were more potent than

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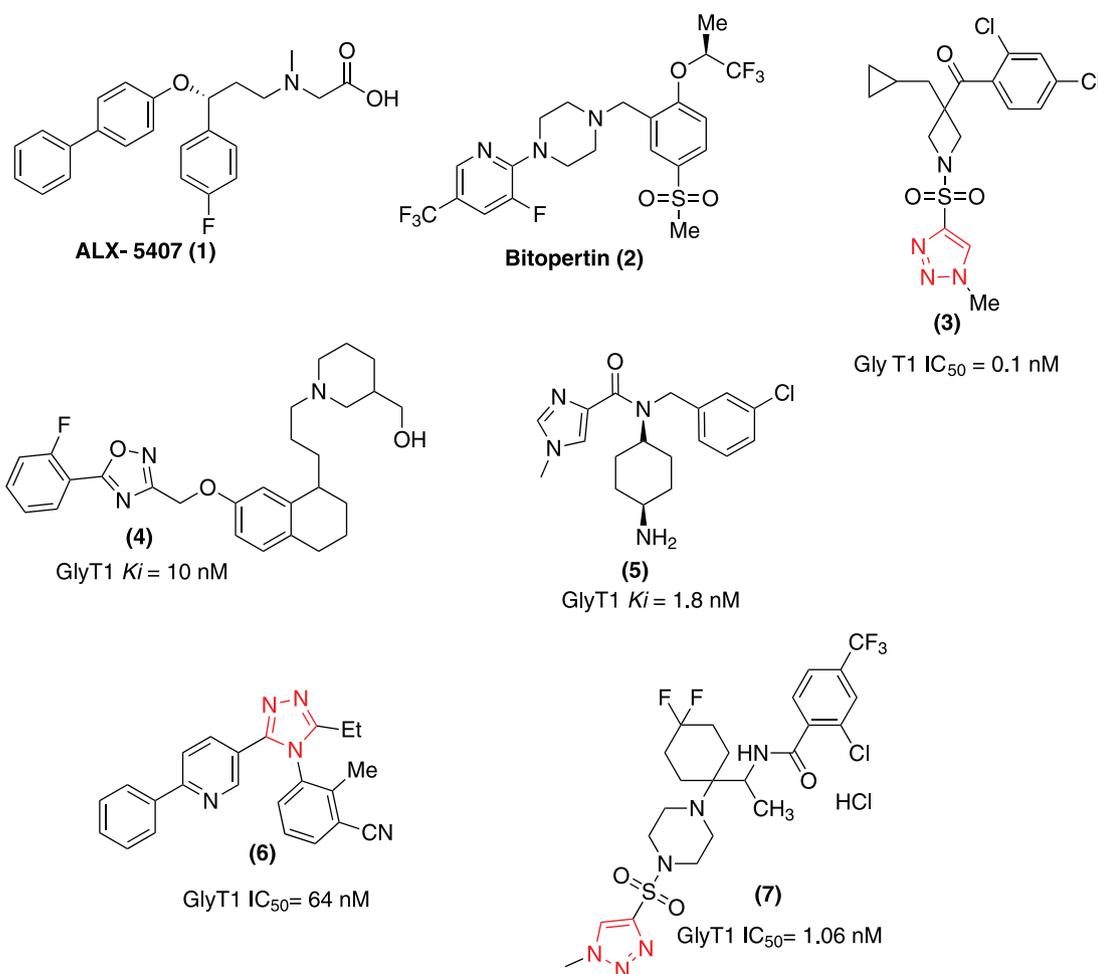
Figure 1. GlyT1 inhibitors.

the 1,2,4-triazoles. Compounds **4** and **5** were described by the Pfizer company as very potent and selective GlyT1 inhibitors. Compound **4** exhibited potent *in vivo* activity in the cerebral spinal fluid (CSF) glycine model, with excellent drug-like physical properties due to its lipophilicity, whereas compound **5** showed good selectivity and high affinity for the GlyT1 (K_i (inhibitor constant) = 1.8 nM).[13,14] New classes of the 4*H*-1,2,4-triazole derivatives were reported selective GlyT1 inhibitors.[8] Compound **6** was moderately potent (half maximal inhibitory concentration (IC_{50}) = 64 nM), had a good pharmacokinetics (PK) profile and was the most potent in the induced hyperlocomotion assay.[8] More recently, it was reported[15] another kind of structure based on 1,2,3-triazoles exemplified by compound **7**, with excellent inhibitory activity (IC_{50} = 1.06 nM).

The most common method for the synthesis of 1,2,3-triazole is the copper(I) catalyzed azide-alkyne [3 + 2] cycloaddition reaction (CuAAC), a kind of "click" reaction.[16,17] In a previous work,[18] we described the use of 1,2,3-triazoles as linker to combine different groups in a bifunctional molecules active for cancer

human glioblastoma cells (GBM), including highly drug-resistant human cell lines GBM-02 and GBM-95. In this work, we have planned new α-hydroxy-1,2,3-triazoles and benzo[*c*]chromene-triazoles, two other kinds of bifunctional molecules linked by the 1,2,3-triazole moiety. Unexpectedly, we obtained 9*H*-fluorene linked to the 1,2,3-triazole moiety instead of benzo[*c*]chromene-triazoles. As 9*H*-fluorenes are interesting class of polycyclic aromatic hydrocarbons that can be found in many naturally compounds and pharmaceutically active substances as well as organic materials used in the development of photoelectric devices,[19,20] we decided to evaluate them together with the α-hydroxy-1,2,3-triazoles as non-sarcosine based GlyT1 inhibitors (Figure 2).

Results and Discussion

Synthesis of α-hidroxy-1,2,3 triazoles

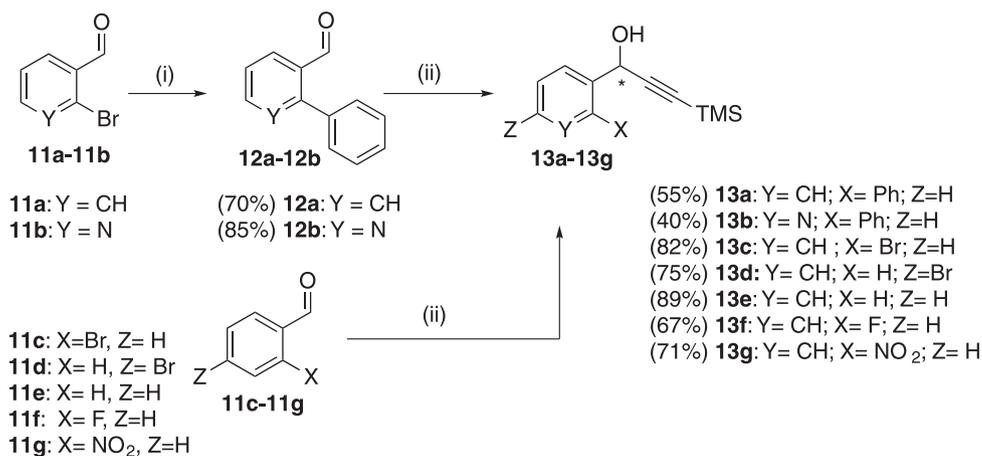The propargyl alcohols **13a**-**13b** were obtained in two steps with good yields by cross-coupling reaction between
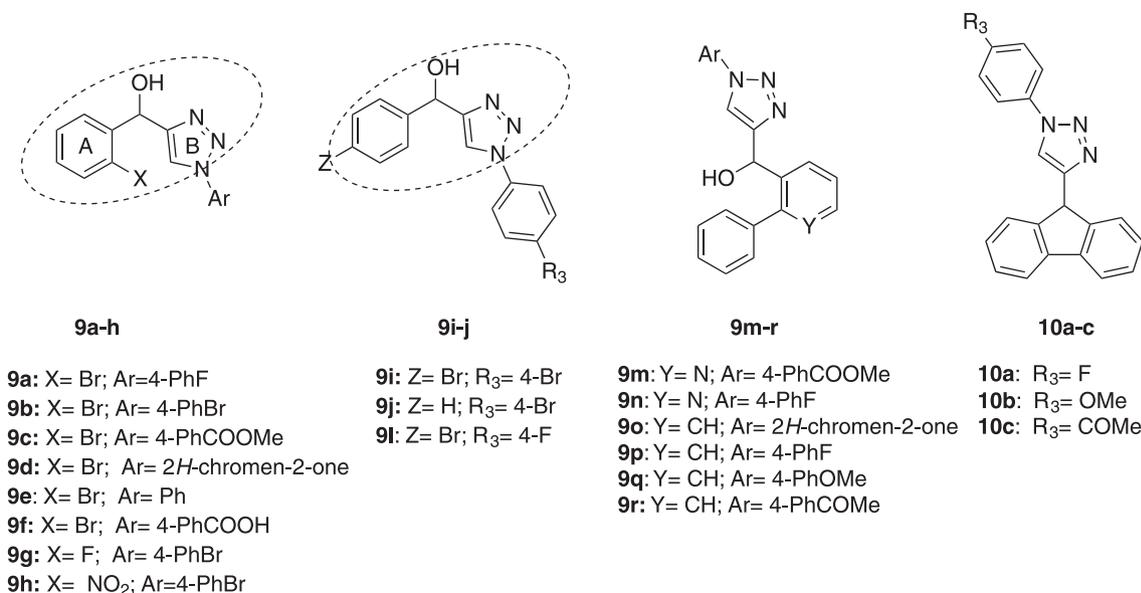
Figure 2. Structures of α-hydroxy-1,2,3-triazoles and 9H-fluorenes-1,2,3-triazoles prepared in this work.

9a-h

9a: X= Br; Ar=4-PhF
9b: X= Br; Ar= 4-PhBr
9c: X= Br; Ar= 4-PhCOOMe
9d: X= Br; Ar= 2H-chromen-2-one
9e: X= Br; Ar= Ph
9f: X= Br; Ar= 4-PhCOOH
9g: X= F; Ar= 4-PhBr
9h: X= NO$_2$; Ar=4-PhBr

9i-j

9i: Z= Br; R$_3$= 4-Br
9j: Z= H; R$_3$= 4-Br
9l: Z= Br; R$_3$= 4-F

9m-r

9m: Y= N; Ar= 4-PhCOOMe
9n: Y= N; Ar= 4-PhF
9o: Y= CH; Ar= 2H-chromen-2-one
9p: Y= CH; Ar= 4-PhF
9q: Y= CH; Ar= 4-PhOMe
9r: Y= CH; Ar= 4-PhCOMe

10a-c

10a: R$_3$= F
10b: R$_3$= OMe
10c: R$_3$= COMe

11a-**11b** and phenylboronic acid followed by addition of ethynyl trimethylsilane to the aldehydes **12a**-**12b** (Scheme 1). On the other hand, the propargyl alcohols **13c**-**13g** were obtained in one step by addition of ethynyl trimethylsilane to the aldehydes **12c**-**12g** in yields ranging from 67 to 89% (Scheme 1). The lowest yields observed for compounds **13a** and **13b** were possibly due to the steric hindrance by the phenyl group. The characterization of the compounds was performed by ^{1}H nuclear magnetic resonance (NMR) and Fourier transform infrared spectroscopy (FTIR). The ^{1}H NMR spectrum exhibited a singlet signal at δ 5.41-5.97 ppm relative to the benzyl alcohol (CH) and another sharp singlet around δ 0.2 ppm to the ethynyl trimethylsilane.

The new 1,4-disubstituted α-hydroxy-1,2,3-triazoles **9a**-**9r** were obtained in good yields by the copper-catalyzed azide-alkyne cycloaddition (CuAAC) reaction (Scheme 2). Although the aryl azides can be prepared by different synthetic approaches, the diazotization reaction of aromatic amines is one of the most commonly used methods. Thus, the aryl-azides **15a**-**15e** were prepared from commercial anilines **14a**-**14d** by diazotization reaction with NaNO$_2$ followed by substitution with sodium azide (NaN$_3$) in yields ranging from 60 to 94%. In order to avoid a reactional step, aryl azide substituted by OCH$_3$ or F in the position 4 or unsubstituted aryl azide were generated *in situ* by copper(II)-catalyzed conversion of aryl boron acids, also known as Cham-Lam coupling.[21] In these cases, aryl boronic acids **16a**-**16c** were used in presence of NaN$_3$ in methanol at 55 °C for 1-2 h. The main advantage of this method is to avoid the isolation of aryl azides, which are sometimes unstable and photosensitive.[22] Thus α-hydroxy-

11a: Y = CH
11b: Y = N

(70%) **12a:** Y = CH
(85%) **12b:** Y = N

11c: X=Br, Z= H
11d: X= H, Z= Br
11e: X= H, Z=H
11f: X= F, Z=H
11g: X= NO$_2$, Z=H

(55%) **13a:** Y= CH; X= Ph; Z=H
(40%) **13b:** Y= N; X= Ph; Z=H
(82%) **13c:** Y= CH ; X= Br; Z=H
(75%) **13d:** Y= CH; X= H; Z=Br
(89%) **13e:** Y= CH; X= H; Z= H
(67%) **13f:** Y= CH; X= F; Z= H
(71%) **13g:** Y= CH; X= NO$_2$; Z=H

Scheme 1. Synthesis of propargyl alcohols **13a**-**13g**. Reagents and conditions: (*i*) 10 mol% Pd(OAc)$_2$, 20 mol% PPh$_3$, phenylboronic acid, 2 M K$_2$CO$_3$, toluene, 5 h, 100 °C; (*ii*) ethynyl-trimethylsilane, *n*-BuLi, THF dry, −5 °C, 3 h.

1,2,3-triazole derivatives **9a**-**9r** were prepared in one step by reaction of propargyl alcohols **13a**-**13g** in the presence of sodium methoxide (NaOMe) as deprotection agent of the trimethylsilyl group (TMS) and sodium ascorbate as reduction agent.[23] The presence of sodium ascorbate favors the *in situ* formation of Cu[I], avoiding the formation of agglomerates, and also avoids atmospheric oxygen interference, that may hinder the reaction process when directly using sources of copper(I).[24] Compounds **9a**-**9r** were characterized by ^{1}H and ^{13}C NMR. The ^{1}H NMR spectrum exhibited a sharp singlet signal in the region δ 7.7 ppm confirming the presence of a triazole proton. Although compounds **9f**, **9h** and **9q** were obtained in low yields, there is not any relationship of these yields with the pattern of substitution of aryl azides or alkynes. As described in the literature,[24] the reaction CuAAC is not significantly affected by the steric and electronic properties of the groups attached to the azide and alkyne centers.
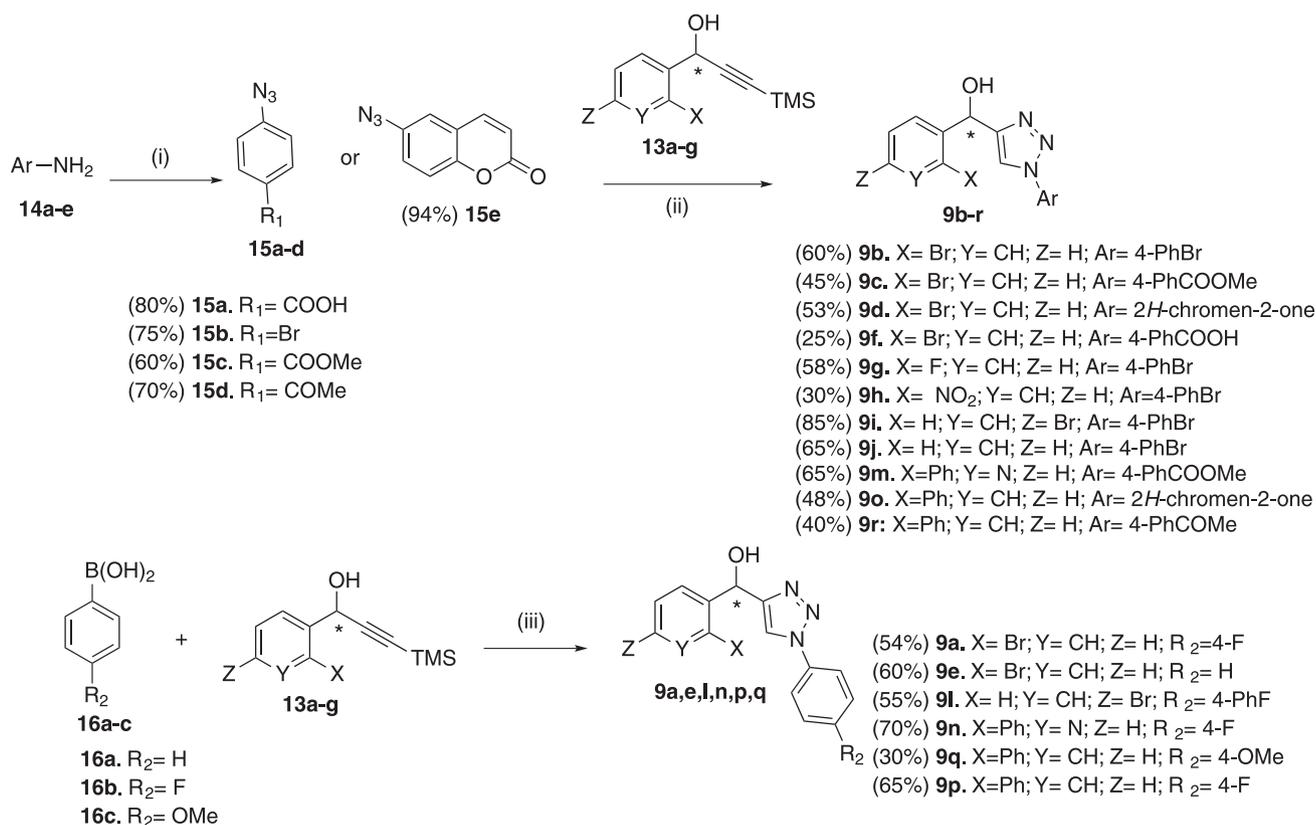
Aiming the synthesis of benzo[*c*]chromene-triazoles, the α-hydroxy-1,2,3-triazoles (**9p**-**9r**) were submitted to cyclization conditions using the Pd-catalyzed β-carbon cleavage by the coordination of hydroxy group for obtaining C−O bond.[25] However, instead of benzochromenes-triazoles

14a-**14c**, new 9*H*-fluorenes-1,2,3-triazoles **10a**-**10c** were obtained with yields ranging from 25 to 40%, by Friedel-Crafts alkylation (Scheme 3). Similar results have been reported in the literature[26,27] using Lewis acid. The characterization of the 9*H*-fluorenes-1,2,3-triazoles was performed by IR, ^{1}H and ^{13}C NMR. In the IR spectrum, the absence of the broadband in the 3300 cm^{−1} region referring to the stretching of the O−H bond of alcohols occurs. The ^{13}C NMR spectrum exhibited signals in the region δ 45 and 118 ppm, relative to the presence of benzyl and triazole (CH).

Biological evaluation

The capacity of each compound to inhibit the glycine transporter GlyT1 was evaluated and a maximal concentration of ALX 5407 (0.5 μM) was used as a positive control. At the high concentration tested (10 μM), only the α-hydroxy-1,2,3-triazole **9b** presented a statistically significant effect (Table 1).

In order to determine the potency of **9b**, we performed a full concentration-response curve (Figure 3) and measured the mean inhibitory concentration (IC$_{50}$), i.e., the concentration inhibiting by 50% the specific transport


(80%) **15a**. R$_1$= COOH
(75%) **15b**. R$_1$=Br
(60%) **15c**. R$_1$= COOMe
(70%) **15d**. R$_1$= COMe

(60%) **9b**. X= Br; Y= CH; Z= H; Ar= 4-PhBr
(45%) **9c**. X= Br; Y= CH; Z= H; Ar= 4-PhCOOMe
(53%) **9d**. X= Br; Y= CH; Z= H; Ar= 2*H*-chromen-2-one
(25%) **9f**. X= Br; Y= CH; Z= H; Ar= 4-PhCOOH
(58%) **9g**. X= F; Y= CH; Z= H; Ar= 4-PhBr
(30%) **9h**. X= NO$_2$; Y= CH; Z= H; Ar=4-PhBr
(85%) **9i**. X= H; Y= CH; Z= Br; Ar= 4-PhBr
(65%) **9j**. X= H; Y= CH; Z= H; Ar= 4-PhBr
(65%) **9m**. X=Ph; Y= N; Z= H; Ar= 4-PhCOOMe
(48%) **9o**. X=Ph; Y= CH; Z= H; Ar= 2*H*-chromen-2-one
(40%) **9r**: X=Ph; Y= CH; Z= H; Ar= 4-PhCOMe

16a-**c**
16a. R$_2$= H
16b. R$_2$= F
16c. R$_2$= OMe

(54%) **9a**. X= Br; Y= CH; Z= H; R$_2$=4-F
(60%) **9e**. X= Br; Y= CH; Z= H; R$_2$= H
(55%) **9l**. X= H; Y= CH; Z= Br; R$_2$= 4-PhF
(70%) **9n**. X=Ph; Y= N; Z= H; R$_2$= 4-F
(30%) **9q**. X=Ph; Y= CH; Z= H; R$_2$= 4-OMe
(65%) **9p**. X=Ph; Y= CH; Z= H; R$_2$= 4-F

Scheme 2. Synthesis of α-hydroxy-1,2,3-triazole derivatives (**9a**-**9r**). Reagents and conditions: (*i*) H$_2$SO$_4$, H$_2$O, NaNO$_2$, NaN$_3$, 0 °C, 0.5-1 h; (*ii*) 10 mol% sodium ascorbate, 5 mol% Cu(OAc)$_2$, MeOH, 1 M NaOMe, rt, 20 h; (*iii*) 10 mol% Cu(OAc)$_2$, NaN$_3$, MeOH, 55 °C, 1-2 h; 20 mol% sodium ascorbate, 1 M NaOMe, rt, 20 h.

(40%) **10a**: R_3= F
(30%) **10b**: R_3= OMe
(25%) **10c**: R_3= COMe
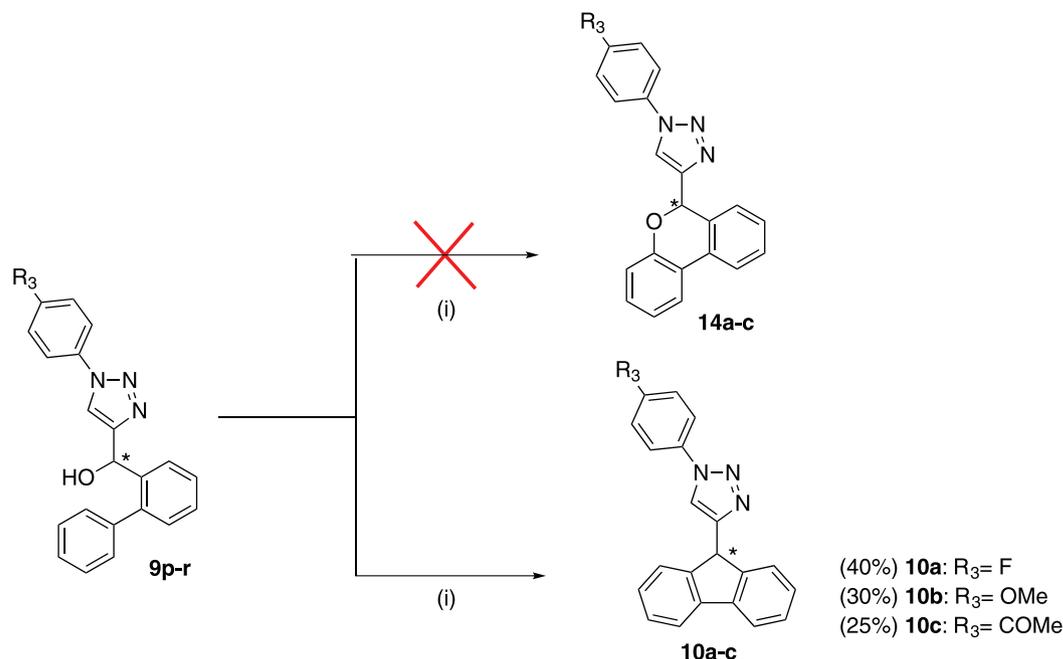
Scheme 3. Synthesis of 9H-fluorenes-1,2,3-triazoles. Reagents and conditions: (*i*) 5 mol% Pd(OAc)$_2$, Na$_2$CO$_3$, PhI(TFA)$_2$, DCE, 100 °C, 36 h.

Table 1. Effects of compounds on [³H]-glycine uptake by HEK293 cells expressing GlyT1. Transport rates were measured as described in Experimental section. Endogenous glycine uptake by parental HEK cells was subtracted from every value. The uptake assays were performed in the presence of compounds (10 μM) or ALX5407 (0.5 μM) and compared to control. Data from this screening assay are means ± S.E. from two independent experiments, each performed in duplicate

Compound	[³H]-glycine uptake / (% control)
ALX-5407	1.4 ± 0.1
9a	83.4 ± 2.5
9b	41.8 ± 2.7
9c	98.4 ± 9.2
9d	112.9 ± 6.5
9e	114.2 ± 11.6
9f	100.8 ± 16.2
9g	101.6 ± 6.6
9h	83.6 ± 3.8
9i	85.5 ± 2.4
9j	77.3 ± 1.6
9l	76.5 ± 8.4
9m	93.8 ± 5.7
9n	114.0 ± 3.8
9o	96.8 ± 7.5
9p	89.8 ± 0.5
9q	98.3 ± 3.6
9r	97.1 ± 5.5
10a	93.5 ± 6.5
10b	94.8 ± 4.6
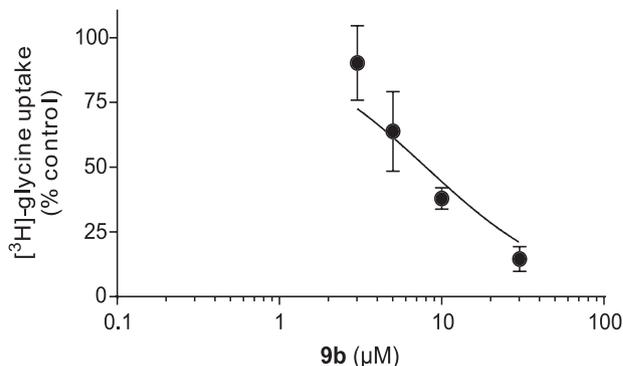
Figure 3. Concentration-response curve for inhibition of [³H]-glycine uptake by HEK293 cells expressing GlyT1. Transport rates were measured as described in Experimental section. Data are means ± S.E. from three independent experiments, each performed in duplicate. The curve was plotted using the parameters fitted by non-linear regression, fixing top and bottom to 100 and 0%, respectively.

of glycine through GlyT1. The IC$_{50}$ of **9b** was 8.0 μM, indicating a relatively low potency as compared to different compounds reported in the literature[9,15] that have IC$_{50}$ values in the nanomolar range (see Introduction section and Table 1). As only compound **9b** was active, we were not able to establish some structure-activity relationship. However, as **9b** had some activity contrarily to **9a**, we can infer that the bromine plays an important role for the pharmacological activity.

Conclusions

In summary, we have synthesized novel α-hydroxy-1,2,3-triazoles under mild conditions in yields ranging

from 25 to 85%. The conditions of Pd-catalyzed C−O bond did not lead to the expected benzo[*c*]chromene-triazoles but to new 9*H*-fluorenes-1,2,3-triazoles by Friedel-Crafts alkylation reaction. All the synthesized compounds were evaluated as glycine transporter (GlyT1) inhibitors. As **9b** was the only active compound with a relatively low potency ($IC_{50} = 8.0 \mu M$), we have to conclude that the α-hydroxy-1,2,3-triazole scaffold is not a good starting point for the discovery of new inhibitors of the GlyT1, a putative molecular target for the treatment of cognitive disorders.

Experimental

General information

For the structural elucidation of the synthesized compounds, ^{1}H and ^{13}C NMR spectra were recorded at ambient temperature on a Bruker Advance III spectrometer (operating at 400 MHz for ^{1}H NMR and 101 MHz for ^{13}C NMR, with $CDCl_3$ or dimethyl sulfoxide (DMSO-d_6) as solvent). The chemical shifts (δ) were given in parts *per* million from internal tetramethylsilane on the δ scale, multiplicity (s = singlet, d = doublet, t = triplet, q = quartet, m = multiplet). All coupling constants (*J* values) were given in Hz. Melting points (mp) were determined with an electrothermal, analog model. Infrared spectra were performed using a Varian-3100 spectrometer with ATR (attenuated total reflection). High resolution mass spectra (HRMS) were obtained using a Bruker, MicrOTOF II instrument. Reactions were monitored by thin layer chromatography (TLC) using TLC silica gel 60 F254 (Merck, São Paulo, Brazil). Silica gel column chromatography was performed over Merck silica gel 60 Å (particle size: 0.040-0.063 mm, 230-400 mesh ASTM). All reagents used were commercially obtained (Merck, São Paulo, Brazil and Oakwood Chemical, San Diego, CA, USA) and, where necessary, purified prior to use, such as tetrahydrofuran (THF), that was dried by metallic sodium.

Synthesis of compounds 12a-12b

In a 10 mL round-bottomed flask, it was added 2-bromobenzaldehyde (2.16 mmol), phenylboronic acid (0.38 g, 3.11 mmol), $Pd(OAc)_2$ (0.048 g, 0.216 mmol), triphenylphosphine (0.11 g, 0.432 mmol), toluene (6.5 mL) and K_2CO_3 (0.28 g, 2.16 mmol). The reaction mixture was stirred for 5 h at 100 °C under N_2 atmosphere. At the end of the reaction toluene was evaporated under reduced pressure and the mixture was extracted with EtOAc (2 × 20 mL), washed with brine (20 mL), dried (Na_2SO_4) and filtered.

The solvent was evaporated under reduced pressure and the residue was purified by flash chromatography using ethyl acetate/hexane (5:95) as eluent.

[1,1'-Biphenyl]-2-carbaldehyde (12a)

Yield 70%; colorless oil; ^{1}H NMR (400 MHz, $CDCl_3$) δ 9.99 (s, 1H), 8.04 (dd, *J* 7.8, 1.3 Hz, 1H), 7.65 (td, *J* 7.5, 1.4 Hz, 1H), 7.54-7.44 (m, 5H), 7.41-7.37 (m, 2H).

2-Phenylnicotinaldehyde (12b)

Yield 85%; brown oil; ^{1}H NMR (400 MHz, $CDCl_3$) δ 10.06 (d, *J* 0.8 Hz, 1H), 8.89 (dd, *J* 4.7, 1.8 Hz, 1H), 8.31 (dd, *J* 7.9, 1.8 Hz, 1H), 7.62-7.57 (m, 2H), 7.55-7.51 (m, 3H), 7.45 (ddd, *J* 7.9, 4.7, 0.8 Hz, 1H).

Synthesis of propargyl alcohols 13a-13g

The products **13a**-**13g** were prepared following the procedure of Wienhold *et al.*[28] *n*-BuLi (2.5 M in hexane, 2.8 mL, 7.0 mmol) was added to a stirred solution of (trimethylsilyl)acetylene (0.99 mL, 7.02 mmol) in anhydrous THF (7 mL) at −5 °C under an N_2 atmosphere. After 15 min, benzaldehyde (5.4 mmol) was added and the reaction mixture was stirred for 3 h at 0 °C. The reaction was quenched with saturated aqueous $NaHCO_3$ (10 mL) and the layers were separated. The aqueous phase was extracted with Et_2O (2 × 10 mL) and washed with brine (5 mL). The combined organic layers were dried over Na_2CO_3, filtered, and evaporated to dryness under reduced pressure. When necessary the products were purified by flash chromatography using ethyl acetate/hexane (10:90) as eluent.

1-([1,1'-Biphenyl]-2-yl)-3-(trimethylsilyl)prop-2-yn-1-ol (13a)

Yield 55%; colorless oil; IR (KBr) ν_{max} / cm^{-1} 3349 (OH), 2173 (C≡C); ^{1}H NMR (400 MHz, $CDCl_3$) δ 7.89 (dd, *J* 7.7, 1.3 Hz, 1H), 7.48-7.39 (m, 7H), 7.31 (dd, *J* 7.5, 1.3 Hz, 1H), 5.47 (s, 1H), 0.20 (s, 9H).

1-(2-Phenylpyridin-3-yl)-3-(trimethylsilyl)prop-2-yn-1-ol (13b)

Yield 40%; pale yellow solid; mp 126-127 °C; IR (KBr) ν_{max} / cm^{-1} 3201 (OH), 2174 (C≡C); ^{1}H NMR (400 MHz, $CDCl_3$) δ 8.69 (dd, *J* 4.7, 1.7 Hz, 1H), 8.21 (dd, *J* 7.9, 1.7 Hz, 1H), 7.67-7.62 (m, 2H), 7.52-7.45 (m, 3H), 7.39 (dd, *J* 7.9, 4.7 Hz, 1H), 5.54 (d, *J* 4.4 Hz, 1H), 0.20 (s, *J* 3.5 Hz, 9H).

1-(2-Bromophenyl)-3-(trimethylsilyl)prop-2-yn-1-ol (13c)

Yield 80%; yellow oil; IR (KBr) ν_{max} / cm^{-1} 3340 (OH), 2172 (C≡C); ^{1}H NMR (400 MHz, $CDCl_3$) δ 7.80 (dd, *J* 7.7,

1.7 Hz, 1H), 7.59 (dd, *J* 8.0, 1.2 Hz, 1H), 7.39 (td, *J* 7.6, 1.1 Hz, 1H), 7.22 (td, *J* 7.7, 1.7 Hz, 1H), 5.81 (s, 1H), 2.53 (s, 1H), 0.23 (s, *J* 3.5 Hz, 9H).

1-(4-Bromophenyl)-3-(trimethylsilyl)prop-2-yn-1-ol (13d)

Yield 75%; colorless oil; IR (KBr) v_{max} / cm^{-1} 3342 (OH), 2174 (C≡C); ^{1}H NMR (400 MHz, CDCl$_3$) δ 7.51 (d, *J* 8.5 Hz, 2H), 7.41 (d, *J* 8.5 Hz, 2H), 5.41 (s, 1H), 0.20 (s, *J* 3.5 Hz, 9H).

1-Phenyl-3-(trimethylsilyl)prop-2-yn-1-ol (13e)

Yield 90%; yellow light oil; IR (KBr) v_{max} / cm^{-1} 3343 (OH), 2173 (C≡C); ^{1}H NMR (400 MHz, CDCl$_3$) δ 7.57-7.53 (m, 1H), 7.41-7.33 (m, 3H), 5.46 (d, *J* 5.8 Hz, 1H), 0.21 (s, 9H).

1-(2-Fluorophenyl)-3-(trimethylsilyl)prop-2-yn-1-ol (13f)

Yield 67%; colorless oil; IR (KBr) v_{max} / cm^{-1} 3343 (OH), 2173 (C≡C); ^{1}H NMR (400 MHz, CDCl$_3$) δ 7.65 (td, *J* 7.6, 1.8 Hz, 1H), 7.34-7.26 (m, 1H), 7.16 (td, *J* 7.6, 1.0 Hz, 1H), 7.08-7.01 (m, 1H), 5.72 (s, 1H), 0.19 (s, 9H).

1-(2-Nitrophenyl)-3-(trimethylsilyl)prop-2-yn-1-ol (13g)

Yield 71%; brown oil; IR (KBr) v_{max} / cm^{-1} 3389 (OH), 2175 (C≡C); ^{1}H NMR (400 MHz, CDCl$_3$) δ 7.94 (td, *J* 7.9, 1.3 Hz, 2H), 7.67 (td, *J* 7.6, 1.3 Hz, 1H), 7.51 (td, *J* 7.9, 1.4 Hz, 1H), 5.97 (s, 1H), 0.19 (s, 9H).

Preparation of aryl azides 15a-15e

The aryl azides **15a**-**15e** were prepared following the procedure of Wilkening *et al.*[29] The aniline derivative (7.5 mmol) was dissolved in 5 mL of water and concentrated sulfuric acid (98%, 1.5 mL) and additional water (1.5 mL) was added. The suspension was cooled to 0 °C and a solution of NaNO$_2$ (0.5 g, 7.6 mmol) in water (1.5 mL) was slowly added under constant stirring. After 15 min, NaN$_3$ (0.6 g, 9.3 mmol) was added and the mixture was stirred for additional 0.5-1 h. The reaction mixture was extracted with ethyl acetate (3 × 20 mL) and the combined organic fractions were washed with water (50 mL). The organic layer was dried over Na$_2$SO$_4$ and concentrated under reduced pressure. The desired azides **15a**-**15e** were obtained without further purification.

4-Azidobenzoic acid (15a)

Yield 80%; pale yellow solid; mp 185 °C; IR (KBr) v_{max} / cm^{-1} 2105 (N≡N), 1284 (C−O); ^{1}H NMR (400 MHz, DMSO-d_6) δ 12.98 (s, 1H, OH), 8.54-7.70 (m, 2H, CH), 7.58-6.88 (m, 2H, CH).

1-Azido-4-bromobenzene (15b)

Yield 85%; brown liquid; IR (KBr) v_{max} / cm^{-1} 2114 (N≡N); ^{1}H NMR (400 MHz, CDCl$_3$) δ 7.48 (d, *J* 8.8 Hz, 2H, Ar-H), 6.93 (d, *J* 8.8 Hz, 2H, Ar-H).

Methyl 4-azidobenzoate (15c)

Yield 70%; white solid; mp 118-119 °C; IR (KBr) v_{max} / cm^{-1} 2113 (N≡N), 1716 (C=O); ^{1}H NMR (400 MHz, CDCl$_3$) δ 8.03 (d, *J* 8.8 Hz, 1H), 7.07 (d, *J* 8.8 Hz, 1H), 3.91 (s, 1H).

1-(4-Azidophenyl)ethanone (15d)

Yield 70%; orange solid; mp 118-119 °C; IR (KBr) v_{max} / cm^{-1} 2091 (N≡N), 1668 (C=O); ^{1}H NMR (400 MHz, CDCl$_3$) δ 7.96 (d, *J* 8.7 Hz, 2H), 7.09 (d, *J* 8.7 Hz, 1H), 2.58 (s, 1H).

6-Azido-2*H*-chromen-2-one (15e)

Yield 94%; orange solid; mp 159-161 °C; IR (KBr) v_{max} / cm^{-1} 2105 (N≡N), 1711 (C=O); ^{1}H NMR (400 MHz, CDCl$_3$) δ 7.66 (d, *J* 9.6 Hz, 1H, =CH), 7.34 (d, *J* 8.8 Hz, 1H, Ar-H), 7.20 (dd, *J* 8.8, 2.6 Hz, 1H, Ar-H), 7.12 (d, *J* 2.6 Hz, 1H, Ar-H), 6.48 (d, *J* 9.6 Hz, 1H, =CH).

Synthesis of α-hydroxy-1,2,3-triazoles 9a-9r

The triazoles were prepared following the procedure of Oikawa *et al.*[23]

Procedure A

In a 25 mL round-bottomed flask, aryl azide was prepared using arylboronic acid (0.5 mmol), NaN$_3$ (0.75 mmol), Cu(OAc)$_2$ (0.05 mmol) and MeOH (3 mL). Then, the mixture was stirred at 55 °C for 1.5 h under aerobic condition. After cooling to room temperature, sodium ascorbate (0.1 mmol), NaOMe (1 M, 0.5 mmol) and alkyne (0.5 mmol) were added to the resultant mixture, which was stirred at room temperature for 24 h under N$_2$ atmosphere. At the end of the reaction, the solution was bubbled with air at ambient temperature for 1 h to oxidize the residual organoboron compound. The mixture was extracted with EtOAc (3 × 10 mL), washed with brine (20 mL), dried (Na$_2$SO$_4$), and filtered. The solvent was evaporated under reduced pressure and the residue was purified by flash chromatography using ethyl acetate/hexane (30:70) as eluent. The solvents used to perform TLC plate analysis were ethyl acetate/hexane (40:60).

Procedure B

In a 10 mL round-bottomed flask, it was added aryl azide (0.5 mmol), Cu(OAc)$_2$ (0.025 mmol), sodium ascorbate

(0.05 mmol), alkyne (0.5 mmol), MeOH (3 mL) and NaOMe (1 M, 0.5 mmol). The reaction mixture was stirred at room temperature for 24 h. At the end of the reaction, the mixture was extracted with EtOAc (3 × 10 mL), washed with brine (20 mL), dried (Na_2SO_4), and filtered. The solvent was evaporated under reduced pressure and the residue was purified by flash chromatography using ethyl acetate/hexane (30:70) as eluent.

(2-Bromophenyl)(1-(4-fluorophenyl)-1*H*-1,2,3-triazol-4-yl)methanol (**9a**)

Procedure A: the product (**9a**) was purified by flash chromatography using ethyl acetate/hexane (30:70) as eluent to give a white solid in 54% yield. mp 102-104 °C; ^{1}H NMR (400 MHz, $CDCl_3$) δ 7.79-7.73 (m, 1H), 7.68-7.60 (m, 3H), 7.59-7.54 (m, 1H), 7.40 (t, *J* 7.5 Hz, 1H), 7.23-7.14 (m, 3H), 6.44 (s, 1H); ^{13}C NMR (101 MHz, $CDCl_3$) δ 163.77, 161.23, 150.52, 140.69, 133.18, 132.84, 129.56, 128.25, 127.98, 122.68, 122.59, 122.21, 120.11, 116.79, 116.56, 67.76; HRMS(electrospray ionization (ESI)) *m/z*, calcd. for $C_{15}H_{11}BrFN_3O$ + Na [M + Na]$^+$: 369.9967, found: 369.9964.

(2-Bromophenyl)(1-(4-bromophenyl)-1*H*-1,2,3-triazol-4-yl)methanol (**9b**)

Procedure B: the product (**9b**) was purified by flash chromatography using ethyl acetate/hexane (30:70) as eluent to give a white solid in 65%. mp 160-161 °C; ^{1}H NMR (400 MHz, DMSO-d_6) δ 8.67 (s, 1H), 7.89 (d, *J* 8.9 Hz, 2H), 7.81-7.73 (m, 3H), 7.59 (d, *J* 8.7 Hz, 1H), 7.46 (t, *J* 7.5 Hz, 1H), 7.26 (t, *J* 7.6 Hz, 1H), 6.31 (d, *J* 4.8 Hz, 1H), 6.15 (d, *J* 4.7 Hz, 1H); ^{13}C NMR (101 MHz, DMSO-d_6) δ 151.49, 142.63, 136.29, 133.18, 132.71, 129.82, 129.32, 128.33, 122.34, 122.19, 121.61, 121.42, 67.22; HRMS(ESI) *m/z*, calcd. for $C_{15}H_{11}Br_2N_3O$ + Na [M + Na]$^+$: 429.9269, found: 429.9161.

Methyl 4-(4-((2-bromophenyl)(hydroxy)methyl)-1*H*-1,2,3-triazol-1-yl)benzoate (**9c**)

Procedure B: the product (**9c**) was purified by flash chromatography using ethyl acetate/hexane (20:80 to 40:60) as eluent to give a white solid in 45% yield. mp 135-136 °C; ^{1}H NMR (400 MHz, $CDCl_3$) δ 8.18 (d, *J* 8.8 Hz, 2H), 7.80 (d, *J* 8.8 Hz, 2H), 7.76 (s, 1H), 7.72 (dd, *J* 7.8, 1.6 Hz, 1H), 7.59 (dd, *J* 8.0, 1.1 Hz, 1H), 7.41 (td, *J* 7.7, 1.1 Hz, 1H), 7.25-7.20 (m, 1H), 6.45 (d, *J* 3.5 Hz, 1H), 3.95 (s, 3H), 3.21 (d, *J* 3.9 Hz, 1H); ^{13}C NMR (101 MHz, $CDCl_3$) δ 165.89, 150.82, 140.67, 139.93, 132.87, 131.28, 130.28, 129.63, 128.26, 128.00, 122.25, 119.96, 119.75, 67.74, 52.48.

6-(4-((2-Bromophenyl)(hydroxy)methyl)-1*H*-1,2,3-triazol-1-yl)-2*H*-chromen-2-one (**9d**)

Procedure B: the product (**9d**) was purified by flash chromatography using ethyl acetate/hexane (50:50) as eluent to give a yellow pale solid in 53% yield. mp 75-76 °C; ^{1}H NMR (400 MHz, $CDCl_3$) δ 7.91 (d, *J* 2.5 Hz, 1H), 7.84 (dd, *J* 8.9, 2.5 Hz, 1H), 7.80-7.73 (m, 3H), 7.61 (d, *J* 8.5 Hz, 1H), 7.49 (d, *J* 8.9 Hz, 1H), 7.44 (t, *J* 7.2 Hz, 1H), 7.28-7.23 (m, 1H), 6.57 (d, *J* 9.6 Hz, 1H), 6.48 (s, 1H); ^{13}C NMR (101 MHz, $CDCl_3$) δ 159.72, 153.60, 142.39, 140.64, 133.24, 132.90, 129.68, 128.23, 128.03, 123.72, 122.22, 119.59, 119.54, 118.48, 118.35, 67.86.

(2-Bromophenyl)(1-phenyl-1*H*-1,2,3-triazol-4-yl)methanol (**9e**)

Procedure A: the product (**9e**) was purified by flash chromatography using ethyl acetate/hexane (20:80) as eluent to give a yellow solid in 60% yield. mp 113-115 °C; ^{1}H NMR (400 MHz, $CDCl_3$) δ 7.76 (d, *J* 7.3 Hz, 1H), 7.68 (d, *J* 7.8 Hz, 3H), 7.57 (d, *J* 8.8 Hz, 1H), 7.49 (t, *J* 7.6 Hz, 2H), 7.41 (dd, *J* 13.2, 7.0 Hz, 2H), 7.21 (t, *J* 7.6 Hz, 1H), 6.46 (s, 1H), 3.69 (s, 1H); ^{13}C NMR (101 MHz, $CDCl_3$) δ 139.80, 135.95, 131.83, 128.69, 128.53, 127.83, 127.25, 126.94, 121.22, 119.62, 66.94.

4-(4-((2-Bromophenyl)(hydroxy)methyl)-1*H*-1,2,3-triazol-1-yl)benzoic acid (**9f**)

Procedure B: the product (**9f**) was purified by flash chromatography using ethyl acetate/hexane (30:70) as eluent to give a white solid in 25% yield. ^{1}H NMR (400 MHz, DMSO-d_6) δ 8.74 (s, 1H), 8.10 (d, *J* 8.7 Hz, 2H), 8.05 (d, *J* 8.3 Hz, 2H), 7.76 (d, *J* 7.7 Hz, 1H), 7.59 (d, *J* 7.9 Hz, 1H), 7.46 (t, *J* 7.5 Hz, 1H), 7.26 (t, *J* 7.6 Hz, 1H), 6.17 (s, 1H); ^{13}C NMR (101 MHz, DMSO-d_6) δ 151.61, 142.60, 139.82, 132.72, 131.48, 129.83, 129.33, 128.34, 122.20, 121.52, 120.09, 67.22.

(1-(4-Bromophenyl)-1*H*-1,2,3-triazol-4-yl)(2-fluorophenyl)methanol (**9g**)

Procedure B: the product (**9g**) was purified by flash chromatography using ethyl acetate/hexane (30:70) as eluent to give a white solid in 58% yield. mp 148-150 °C; ^{1}H NMR (400 MHz, $CDCl_3$) δ 7.76 (s, 1H), 7.65-7.61 (m, 3H), 7.59 (d, *J* 9.0 Hz, 2H), 7.37-7.29 (m, 1H), 7.21 (td, *J* 7.6, 1.0 Hz, 1H), 7.11-7.05 (m, 1H), 6.37 (s, 1H); ^{13}C NMR (101 MHz, $CDCl_3$) δ 161.11, 158.66, 135.91, 132.89, 129.85, 129.77, 127.99, 127.96, 124.61, 124.57, 122.54, 121.99, 115.66, 115.45, 63.41; HRMS(ESI) *m/z*, calcd. for $C_{15}H_{11}BrFN_3O$ + Na [M + Na]$^+$: 369.9967, found: 369.9964.

(1-(4-Bromophenyl)-1*H*-1,2,3-triazol-4-yl)(2-nitrophenyl)methanol (**9h**)

Procedure B: the product (**9h**) was isolated by filtration and purified on a celite column to give a white solid in 30% yield. mp 190-191 °C; ^{1}H NMR (400 MHz, DMSO-d_6) δ 8.70 (s, 1H), 7.98 (ddd, *J* 7.7, 6.6, 1.2 Hz, 2H), 7.87 (d, *J* 9.0 Hz, 2H), 7.81 (dd, *J* 7.5, 1.2 Hz, 1H), 7.77 (d, *J* 9.0 Hz, 2H), 7.58 (ddd, *J* 8.2, 7.5, 1.4 Hz, 1H), 6.49 (s, 1H); ^{13}C NMR (101 MHz, DMSO-d_6) δ 151.22, 148.09, 138.15, 136.25, 134.01, 133.13, 129.25, 129.14, 124.65, 122.35, 121.68, 121.42, 63.69.

(4-Bromophenyl)(1-(4-bromophenyl)-1*H*-1,2,3-triazol-4-yl)methanol (**9i**)

Procedure B: the product (**9i**) was purified by flash chromatography using ethyl acetate/hexane (30:70) as eluent to give a white solid in 85% yield. mp 153-155 °C; ^{1}H NMR (400 MHz, DMSO-d_6) δ 8.68 (s, 1H), 7.88 (d, *J* 8.9 Hz, 2H), 7.76 (d, *J* 8.9 Hz, 2H), 7.54 (d, *J* 8.5 Hz, 2H), 7.42 (d, *J* 8.4 Hz, 2H), 6.25 (d, *J* 4.6 Hz, 1H), 5.90 (d, *J* 4.5 Hz, 1H); ^{13}C NMR (101 MHz, DMSO-d_6) δ 152.85, 143.47, 136.30, 133.19, 131.50, 129.20, 122.36, 121.63, 120.87, 120.74, 67.66; HRMS(ESI) *m/z*, calcd. for $C_{15}H_{11}Br_2N_3O$ + Na [M + Na]$^+$: 429.9269, found: 429.9161.

(1-(4-Bromophenyl)-1*H*-1,2,3-triazol-4-yl)(phenyl)methanol (**9j**)

Procedure B: the product (**9j**) was purified by flash chromatography using ethyl acetate/hexane (30:70) as eluent to give a white solid in 60% yield. mp 155-156 °C; ^{1}H NMR (400 MHz, CDCl$_3$) δ 7.69 (s, 1H), 7.63 (d, *J* 9.0 Hz, 2H), 7.58 (d, *J* 9.0 Hz, 2H), 7.51 (d, *J* 7.1 Hz, 1H), 7.40 (t, *J* 7.3 Hz, 1H), 7.34 (t, *J* 7.2 Hz, 1H), 6.12 (s, 1H); ^{13}C NMR (101 MHz, CDCl$_3$) δ 141.64, 135.94, 132.88, 128.74, 128.23, 126.43, 122.40, 121.92, 69.17; HRMS(ESI) *m/z*, calcd. for $C_{15}H_{12}BrN_3O$ + H [M + H]$^+$: 330.0242, found: 330.0236.

(4-Bromophenyl)(1-(4-fluorophenyl)-1*H*-1,2,3-triazol-4-yl)methanol (**9l**)

Procedure A: the product (**9l**) was purified by flash chromatography using ethyl acetate/hexane (30:70) as eluent to give a white solid in 55% yield. mp 105-107 °C; ^{1}H NMR (400 MHz, CDCl$_3$) δ 7.68-7.63 (m, 3H), 7.52 (d, *J* 8.4 Hz, 2H), 7.39 (d, *J* 8.4 Hz, 2H), 7.20 (t, *J* 9.0 Hz, 2H), 6.08 (s, 1H); ^{13}C NMR (101 MHz, CDCl$_3$) δ 163.75, 161.27, 140.72, 133.10, 131.77, 128.72, 128.15, 126.42, 122.61, 122.52, 122.05, 119.77, 116.86, 116.63, 68.35.

Methyl 4-(4-(hydroxy(2-phenylpyridin-3-yl)methyl)-1*H*-1,2,3-triazol-1-yl)benzoate (**9m**)

Procedure B: the product (**9m**) was purified by flash chromatography using ethyl acetate/hexane (20:80 to 40:60) as eluent to give a white solid in 64% yield. mp 78-80 °C; ^{1}H NMR (400 MHz, CDCl$_3$) δ 8.60 (d, *J* 4.4 Hz, 1H), 8.17 (d, *J* 8.7 Hz, 2H), 8.03 (d, *J* 7.9 Hz, 1H), 7.76 (d, *J* 8.6 Hz, 2H), 7.70 (s, 1H), 7.55-7.49 (m, 2H), 7.41-7.37 (m, 3H), 7.32 (dd, *J* 7.9, 4.8 Hz, 1H), 6.20 (s, 1H), 3.95 (s, 3H); ^{13}C NMR (101 MHz, CDCl$_3$) δ 165.84, 157.85, 151.57, 149.07, 139.87, 139.24, 136.20, 135.07, 131.36, 130.39, 129.23, 128.53, 128.39, 122.85, 119.87, 119.48, 65.40, 52.49; HRMS(ESI) *m/z* calcd. for $C_{22}H_{18}N_4O_3$ + Na [M + Na]$^+$: 409.1379, found: 409.1270.

(1-(4-Fluorophenyl)-1*H*-1,2,3-triazol-4-yl)(2-phenylpyridin-3-yl)methanol (**9n**)

Procedure A: the product (**9n**) was purified by flash chromatography using ethyl acetate/hexane (20:80 to 40:60) as eluent to give a white solid in 70% yield. mp 74-76 °C; ^{1}H NMR (400 MHz, CDCl$_3$) δ 8.59 (d, *J* 3.5 Hz, 1H), 8.04 (d, *J* 7.9 Hz, 1H), 7.62 (dd, *J* 9.0, 4.6 Hz, 2H), 7.56 (s, 1H), 7.52-7.47 (m, 2H), 7.41-7.35 (m, 3H), 7.31 (dd, *J* 7.9, 4.8 Hz, 1H), 7.19 (t, *J* 8.5 Hz, 2H), 6.18 (s, 1H); ^{13}C NMR (101 MHz, CDCl$_3$) δ 157.75, 151.37, 148.90, 139.24, 136.27, 135.32, 129.23, 128.47, 128.34, 122.83, 122.53, 122.45, 119.86, 116.87, 116.64, 65.20; HRMS(ESI) *m/z*, calcd. for $C_{20}H_{15}FN_4O$ + H [M + H]$^+$: 347.1308, found: 347.1304.

6-(4-([1,1'-Biphenyl]-2-yl(hydroxy)methyl)-1*H*-1,2,3-triazol-1-yl)-2*H*-chromen-2-one (**9o**)

Procedure B: the product (**9o**) was purified by flash chromatography using ethyl acetate/hexane (50:50) as eluent to give an orange solid in 48% yield. mp 80-82 °C; ^{1}H NMR (400 MHz, CDCl$_3$) δ 7.88 (d, *J* 2.4 Hz, 1H), 7.80 (dd, *J* 8.9, 2.4 Hz, 1H), 7.75 (d, *J* 9.6 Hz, 1H), 7.69 (d, *J* 7.3 Hz, 2H), 7.48-7.39 (m, 3H), 7.40-7.35 (m, 5H), 7.31 (d, *J* 7.3 Hz, 1H), 6.53 (d, *J* 9.6 Hz, 1H), 6.18 (s, 1H); ^{13}C NMR (101 MHz, CDCl$_3$) δ 159.77, 153.53, 142.44, 141.26, 140.29, 138.98, 133.33, 130.30, 129.46, 128.32, 128.19, 128.15, 127.45, 127.34, 123.59, 119.55, 119.44, 118.48, 118.37, 65.93; HRMS(ESI) *m/z*, calcd. for $C_{24}H_{17}N_3O_3$ + Na [M + Na]$^+$: 418.1168, found: 418.1163.

[1,1'-Biphenyl]-2-yl(1-(4-fluorophenyl)-1*H*-1,2,3-triazol-4-yl)methanol (**9p**)

Procedure A: the product (**9p**) was purified by flash chromatography using ethyl acetate/hexane (30:70) as eluent to give a white solid in 70% yield. mp 114-116 °C; ^{1}H NMR (400 MHz, CDCl$_3$) δ 7.69 (d, *J* 7.2 Hz, 1H),

7.65-7.60 (m, 2H), 7.58 (s, 1H), 7.45-7.36 (m, 7H), 7.29 (dd, *J* 7.4, 1.2 Hz, 1H), 7.17 (t, *J* 8.5 Hz, 2H), 6.15 (s, 1H); ^{13}C NMR (101 MHz, CDCl$_3$) δ 163.63, 161.15, 141.18, 140.37, 139.12, 133.22, 130.22, 129.45, 128.27, 128.09, 128.05, 127.38, 127.36, 122.52, 122.43, 119.90, 116.80, 116.57, 65.81; HRMS(ESI) *m/z*, calcd. for C$_{21}$H$_{16}$FN$_3$O + H [M + H]$^+$: 346.1356, found: 346.1350.

[1,1'-Biphenyl]-2-yl(1-(4-methoxyphenyl)-1*H*-1,2,3-triazol-4-yl)methanol (9q)

Procedure A: the product (**9q**) was purified by flash chromatography using ethyl acetate/hexane (30:70) as eluent to give a white solid in 30% yield. mp 108-110 °C; ^{1}H NMR (400 MHz, CDCl$_3$) δ 7.72 (d, *J* 7.5 Hz, 1H), 7.56 (d, *J* 8.9 Hz, 3H), 7.52 (s, 1H), 7.45-7.33 (m, 9H), 7.30 (d, *J* 7.3 Hz, 1H), 6.99 (d, *J* 8.9 Hz, 2H), 6.15 (s, 1H), 3.85 (s, 4H); ^{13}C NMR (101 MHz, CDCl$_3$) δ 159.81, 141.21, 140.40, 139.23, 130.45, 130.18, 129.46, 128.27, 128.07, 127.99, 127.38, 127.36, 122.16, 119.78, 114.73, 65.92, 55.63; HRMS(ESI) *m/z*, calcd. for C$_{22}$H$_{19}$N$_3$O$_2$ + H [M + H]$^+$: 358.1556, found: 358.1549.

1-(4-(4-([1,1'-Biphenyl]-2-yl(hydroxy)methyl)-1*H*-1,2,3-triazol-1-yl)phenyl)ethanone (9r)

Procedure B: the product (**9r**) was purified by flash chromatography using ethyl acetate/hexane (30:70) as eluent to give a white solid in 45% yield. mp 118-120 °C; ^{1}H NMR (400 MHz, CDCl$_3$) δ 8.10 (d, *J* 8.7 Hz, 2H), 7.82 (d, *J* 8.7 Hz, 2H), 7.73-7.65 (m, 2H), 7.47-7.42 (m, 1H), 7.42-7.35 (m, 6H), 7.31 (dd, *J* 7.5, 1.5 Hz, 1H), 6.18 (s, 1H), 2.65 (s, 3H); ^{13}C NMR (101 MHz, CDCl$_3$) δ 196.64, 141.29, 140.31, 140.02, 138.97, 136.82, 130.28, 130.07, 129.46, 128.30, 128.17, 128.14, 127.43, 127.33, 119.99, 119.41, 65.98, 26.68; HRMS(ESI) *m/z*, calcd. for C$_{23}$H$_{19}$N$_3$O$_2$ + H [M + H]$^+$: 370.1556, found: 370.1551.

Synthesis of 9*H*-fluorenes-1,2,3-triazoles 10a-10c

Compounds **10a**-**10c** were prepared according to the procedure described by Wang *et al.*[25] In a 20 mL Schlenk tube, 2.0 mL dichloroethane were added to a mixture of α-hydroxy-1,2,3-triazoles (0.2 mmol, 1.0 equiv.), Pd(OAc)$_2$ (0.01 mmol, 0.05 equiv., 5 mol%), Na$_2$CO$_3$ (0.3 mmol, 1.5 equiv.), and PhI(TFA)$_2$ (0.3 mmol, 1.5 equiv.) under air. The reaction mixture was stirred at 100 °C for 36 h. After cooling to room temperature, the reaction mixture was diluted with diethyl ether (15 mL), filtered through celite, washed with diethyl ether (2 × 10 mL), carefully concentrated under vacuum and the residue was purified by column chromatography using ethyl acetate/hexane (20:80) as eluent.

4-(9*H*-Fluoren-9-yl)-1-(4-fluorophenyl)-1*H*-1,2,3-triazole (10a)

The product (**10a**) was obtained as a white solid in 40% yield. mp 131-132 °C; ^{1}H NMR (400 MHz, CDCl$_3$) δ 7.81 (d, *J* 7.6 Hz, 2H), 7.65 (d, *J* 7.4 Hz, 2H), 7.63-7.58 (m, 2H), 7.43 (t, *J* 7.5 Hz, 2H), 7.36-7.30 (m, 3H), 7.17-7.11 (m, 2H), 5.51 (s, 1H); ^{13}C NMR (101 MHz, CDCl$_3$) δ 163.57, 161.07, 149.05, 145.17, 140.85, 127.89, 127.57, 125.40, 122.38, 122.30, 120.12, 118.56, 116.70, 116.47, 45.40; HRMS(ESI) *m/z*, calcd. for C$_{21}$H$_{14}$FN$_3$ + Na [M + Na]$^+$: 350.1072, found: 350.1064.

4-(9*H*-Fluoren-9-yl)-1-(4-methoxyphenyl)-1*H*-1,2,3-triazole (10b)

The product (**10b**) was obtained as a white solid in 30% yield. mp 124-125 °C; ^{1}H NMR (400 MHz, CDCl$_3$) δ 7.84 (d, *J* 7.5 Hz, 2H), 7.68 (d, *J* 7.5 Hz, 2H), 7.55 (d, *J* 9.1 Hz, 2H), 7.45 (t, *J* 7.2 Hz, 2H), 7.38-7.33 (m, 3H), 6.96 (d, *J* 9.1 Hz, 2H), 5.54 (s, 1H), 3.85 (s, 3H); ^{13}C NMR (101 MHz, CDCl$_3$) δ 159.68, 145.35, 140.84, 130.56, 127.76, 127.53, 125.44, 122.01, 120.07, 118.57, 114.64, 55.59, 45.47; HRMS(ESI) *m/z*, calcd. for C$_{22}$H$_{17}$N$_3$O + Na [M + Na]$^+$: 362.1372, found: 362.1364.

1-(4-(4-(9*H*-Fluoren-9-yl)-1*H*-1,2,3-triazol-1-yl)phenyl)ethanone (10c)

The product (**10c**) was obtained as a white solid in 25% yield. ^{1}H NMR (400 MHz, CDCl$_3$) δ 8.07 (d, *J* 8.7 Hz, 2H), 7.85 (d, *J* 7.4 Hz, 2H), 7.79 (d, *J* 8.7 Hz, 2H), 7.67 (d, *J* 7.6 Hz, 2H), 7.50 (s, 1H), 7.47 (t, *J* 7.5 Hz, 2H), 7.37 (t, *J* 7.1 Hz, 2H), 5.55 (s, 1H), 2.65 (s, 3H); ^{13}C NMR (101 MHz, CDCl$_3$) δ 196.58, 149.44, 144.99, 140.86, 136.68, 129.98, 127.96, 127.60, 125.37, 120.17, 119.86, 118.16, 45.34, 29.70.

Biological evaluation

Plasmid corresponding to rat GlyT1b in the pcDNA3 vector was transiently transfected into HEK293 cells using lipofectamine (Invitrogen), as described before.[30] Twenty-four hours after transfection, cells were replated in polylysine-covered 24-well plates. Subconfluent transfected HEK293 cells growing overnight in Dulbecco's Modified Eagle Medium (DMEM) supplemented with 10% fetal bovine serum and non-essential amino acids at 37 °C and 5% CO$_2$ were washed with (4-(2-hydroxyethyl)-1 piperazineethanesulfonic acid (HEPES)-buffered saline (HBS; 10 mM HEPES-Tris buffer containing 150 mM NaCl, 1 mM CaCl$_2$, 2.5 mM KCl, 2.5 mM MgSO$_4$, and 10 mM (+) D-glucose at pH 7.4). Experiments were performed as previously described,[30] with some modifications. Cells were incubated for 5 min at 22 °C

with the test compounds before addition of [³H]-glycine. After 5 min incubation with 10 μM [³H]-glycine (0.01 μM stock solution, 49.9 Ci mmol⁻¹, PerkinElmer, Boston, USA, diluted with 10 μM unlabeled glycine), the cells were washed twice with HBS and dissolved in 0.2 mL of 0.2 M NaOH. Aliquots of each well were taken for scintillation counting (Packard TriCarb 1600 TR liquid scintillation analyzer, PerkinElmer) and protein concentration determination (BCA™ Protein Assay Kit, Pierce). Results are means ± standard error (S.E.) of two independent experiments performed in duplicate and are expressed as percentage of control uptake. Note that the glycine accumulation in non-transfected HEK293 cells was subtracted from that of transfected cells in order to measure specifically the glycine uptake due to GlyT1. Statistically significant differences were determined by one-way analysis of variance (ANOVA) followed by Dunett's multiple comparisons test (*vs.* control). To estimate the concentration causing 50% inhibition of glycine uptake (IC_{50}), compound **9b** was tested at different concentrations and the concentration-response curve was analyzed by non-linear regression (One site - Fit logIC50) using GraphPad Prism 5.0.[31] This experiment was performed in duplicate and replicated twice (n = 3).

Supplementary Information

Supplementary data (¹H NMR and ¹³C NMR spectra) are available free of charge at http://jbcs.sbq.org.br as PDF file.

Acknowledgments

Author Contributions

Veronica D. da Silva, Rafaela R. Silva, João Gonçalves Neto were responsible for the data curation and investigation; François Noël and Camilla D. Buarque for the formal analysis, funding acquisition, conceptualization and writing review and editing; Camilla D. Buarque was also responsible for the project administration, resources, validation and visualization; Veronica D. da Silva, Rafaela R. Silva for the writing of original draft; Beatriz López-Corcuera, Marilia Z. Guimarães for the writing review and editing.

References

1. Mady, M. F.; Awad, G. E. A.; Jørgensen, K. B.; *Eur. J. Med. Chem.* **2014**, *84*, 433.

2. Yadav, P.; Lal, K.; Kumar, A.; Guru, S. K.; Jaglan, S.; Bhushan, S.; *Eur. J. Med. Chem.* **2017**, *126*, 944.

3. Mohammed, I.; Kummetha, I. R.; Singh, G.; Sharova, N.; Lichinchi, G.; Dang, J.; Stevenson, M.; Rana, T. M.; *J. Med. Chem.* **2016**, *59*, 7677.

4. Keck, T. M.; Banala, A. K.; Slack, R. D.; Burzynski, C.; Bonifazi, A.; Okunola-Bakare, O. M.; Moore, M.; Deschamps, J. R.; Rais, R.; Slusher, B. S.; Newman, A. H.; *Bioorg. Med. Chem.* **2015**, *23*, 4000.

5. Ayati, A.; Emami, S.; Foroumadi, A.; *Eur. J. Med. Chem.* **2016**, *109*, 380.

6. Caliendo, G.; Fiorino, F.; Perissutti, E.; Severino, B.; Scolaro, D.; Gessi, S.; Cattabriga, E.; Borea, P. A.; Santagada, V.; *Eur. J. Pharm. Sci.* **2002**, *16*, 15.

7. Harvey, R. J.; Yee, B. K.; *Nat. Rev. Drug Discovery* **2013**, *12*, 866.

8. Sugane, T.; Tobe, T.; Hamaguchi, W.; Shimada, I.; Maeno, K.; Miyata, J.; Suzuki, T.; Kimizuka, T.; Sakamoto, S.; Tsukamoto, S.; *J. Med. Chem.* **2013**, *56*, 5744.

9. Thomson, J. L.; Blackaby, W. P.; Jennings, A. S. R.; Goodacre, S. C.; Pike, A.; Thomas, S.; Brown, T. A.; Smith, A.; Pillai, G.; Street, L. J.; Lewis, R. T.; *Bioorg. Med. Chem. Lett.* **2009**, *19*, 2235.

10. Atkinson, B. N.; Bell, S. C.; De Vivo, M.; Kowalski, L. R.; Lechner, S. M.; Ognyanov, V. I.; Tham, C. S.; Tsai, C.; Jia, J.; Ashton, D.; Klitenick, M. A.; *Mol. Pharmacol.* **2001**, *60*, 1414.

11. Singer, P.; Dubroqua, S.; Yee, B.; *Curr. Pharm. Des.* **2015**, *21*, 3771.

12. Alberati, D.; Moreau, J.-L.; Lengyel, J.; Hauser, N.; Mory, R.; Borroni, E.; Pinard, E.; Knoflach, F.; Schlotterbeck, G.; Hainzl, D.; Wettstein, J. G.; *Neuropharmacology* **2012**, *62*, 1152.

13. Lowe, J. A.; Hou, X.; Schmidt, C.; Tingley, F. D.; McHardy, S.; Kalman, M.; DeNinno, S.; Sanner, M.; Ward, K.; Lebel, L.; Tunucci, D.; Valentine, J.; *Bioorg. Med. Chem. Lett.* **2009**, *19*, 2974.

14. Lowe, J.; Drozda, S.; Qian, W.; Peakman, M.-C.; Liu, J.; Gibbs, J.; Harms, J.; Schmidt, C.; Fisher, K.; Strick, C.; Schmidt, A.; Vanase, M.; Lebel, L.; *Bioorg. Med. Chem. Lett.* **2007**, *17*, 1675.

15. Cioffi, C. L.; Liu, S.; Wolf, M. A.; Guzzo, P. R.; Sadalapure, K.; Parthasarathy, V.; Loong, D. T. J.; Maeng, J.-H.; Carulli, E.; Fang, X.; Karunakaran, K.; Matta, L.; Choo, S. H.; Panduga, S.; Buckle, R. N.; Davis, R. N.; Sakwa, S. A.; Gupta, P.; Sargent, B. J.; Moore, N. A.; Luche, M. M.; Carr, G. J.; Khmelnitsky, Y. L.; Ismail, J.; Chung, M.; Bai, M.; Leong, W. Y.; Sachdev, N.; Swaminathan, S.; Mhyre, A. J.; *J. Med. Chem.* **2016**, *59*, 8473.

16. Rostovtsev, V. V.; Green, L. G.; Fokin, V. V.; Sharpless, K. B.; *Angew. Chem., Int. Ed.* **2002**, *41*, 2596.

17. Kolb, H. C.; Sharpless, B.; *Drug Discovery Today* **2003**, *8*, 1128.

18. da Silva, V. D.; de Faria, B. M.; Colombo, E.; Ascari, L.; Freitas, G. P. A.; Flores, L. S.; Cordeiro, Y.; Romão, L.; Buarque, C. D.; *Bioorg. Chem.* **2019**, *83*, 87.

19. Shi, Y.; Gao, S.; *Tetrahedron* **2016**, *72*, 1717.

20. Kaiser, R. P.; Caivano, I.; Kotora, M.; *Tetrahedron* **2019**, *75*, 2981.

21. Grimes, K.; Gupte, A.; Aldrich, C.; *Synthesis* **2010**, *2010*, 1441.

22. Tao, C.-Z.; Cui, X.; Li, J.; Liu, A.-X.; Liu, L.; Guo, Q.-X.; *Tetrahedron Lett.* **2007**, *48*, 3525.

23. Oikawa, A.; Kindaichi, G.; Shimotori, Y.; Hoshi, M.; *Tetrahedron* **2016**, *72*, 4205.

24. Hein, J. E.; Fokin, V. V.; *Chem. Soc. Rev.* **2010**, *39*, 1302.

25. Wang, X.; Lu, Y.; Dai, H.; Yu, J.; *J. Am. Chem. Soc.* **2010**, *132*, 12203.

26. Li, G.; Wang, E.; Chen, H.; Li, H.; Liu, Y.; Wang, P. G.; *Tetrahedron* **2008**, *64*, 9033.

27. Killander, D.; Sterner, O.; *Eur. J. Org. Chem.* **2014**, *2014*, 6507.

28. Wienhold, F.; Claes, D.; Graczyk, K.; Maison, W.; *Synthesis* **2011**, 4059.

29. Wilkening, I.; del Signore, G.; Hackenberger, C. P. R.; *Chem. Commun.* **2011**, *47*, 349.

30. Herdon, H. J.; Godfrey, F. M.; Brown, A. M.; Coulton, S.; Evans, J. R.; Cairns, W. J.; *Neuropharmacology* **2001**, *41*, 88.

31. *GraphPad Prism 5.0*; GraphPad Software, Inc., CA, USA, 2014.