SUPPLEMENTARY INFO

Substituted 4-hydroxy-1,2,3-triazoles: synthesis, characterization and first drug design applications through bioisosteric modulation and scaffold hopping approaches.

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Table of Contents

Compound Characterization

Melting points (m.p.) were measured on a capillary apparatus (Büchi 540). The final m.p. determination was achieved by placing the sample in a bath at a temperature 10 °C below the m.p., and applying a heating rate of 2° C min^{-1}. All compounds were routinely checked by ^{1}H NMR (Bruker Avance 300) and mass spectrometry (Finnigan-Mat TSQ-700, 70 eV, direct inlet). For coupling patterns, the following abbreviations are used: *br* = broad, *s* = singlet, *d* = doublet, *dd* = doublet of doublets, *t* = triplet, *q* = quartet, *m* = multiplet. Chemical shifts (δ) are given in parts per million (ppm). The entire sample batch described showed a purity > 95%, checked using two analytical methods. For compounds **1a**-**13** and **23**-**27**, HPLC analyses were performed on an UHPLC chromatographic system (Perkin Elmer, Flexar). The analytical column was an UHPLC Acquity CSH Fluoro-Phenyl (2.1x100 mm, 1.7 um particle size) (Waters). Compounds were dissolved in CH_3CN and injected through a 20uL loop. The mobile phase consisted of CH_3CN/water with 0.1% trifluoroacetic acid (ratio between 60/40 and 40/60, depending on the compound's retention factor). HPLC retention times were obtained at flow rates of 0.5 mL/min, and the column effluent was monitored at 215 and 254 nm, referenced against a 360 nm wavelength. For compounds **17** and **18,** purity rate was determined by potentiometric titration.

Ethyl 5-hydroxy-1-methyl-1H-1,2,3-triazole-4-carboxylate (1a). White solid (m.p. 102.0 - 104.2°C). Quantitative yield. 1H-NMR (300 MHz, DMSO): δ 1.28 (3H, t, J = 7.1 Hz, -CH2CH3), 3.73 (3H, s, -NCH3), 4.26 (2H, q, J = 7.1 Hz, -CH2CH3); MS (CI) 172 (M+1).

Ethyl 1-benzyl-5-hydroxy-1*H*-1,2,3-triazole-4-carboxylate (1b). White solid (m.p. 101.8 - 102.9°C). Yield 70%. ^{1}H-NMR (300 MHz, $CDCl_3$): δ 1.33 (3H, *t*, J = 7.2 Hz, -CH_2CH_3), 4.35 (2H, *q,* J = 7.2 Hz, -CH_2CH_3), 5.36 (2H, *s*, -NCH_2-), 7.27 - 7.38 (5H, *m*, *aromatic protons*), 8.91 (1H, *br s*, -O*H*); MS (CI) 248 (M+1).

Ethyl 5-hydroxy-2-methyl-2*H*-1,2,3-triazole-4-carboxylate (2a). White solid (m.p. 114.3 - 115.3°C, from diisopropyl ether). Yield 90%. ^{1}H-NMR (300 MHz, DMSO): δ 1.27 (3H, *t*, J = 7.1 Hz, -CH_2CH_3), 4.02 (3H, *s*, -NCH_3), 4.25 (2H, *q,* J = 7.1 Hz, -CH_2CH_3), 11.30 (1H, s, -O*H*); MS (CI) 172 (M+1).

Ethyl 2-benzyl-5-hydroxy-2*H*-1,2,3-triazole-4-carboxylate (**2b**). White solid (m.p. 77.1 - 77.8°C, from diisopropyl ether). Yield 92%. ^{1}H-NMR (300 MHz, DMSO): δ 1.25 (3H, *t*, J = 7.2 Hz, -CH_2CH_3), 4.25 (2H, *q*, J = 7.2 Hz, -CH_2CH_3), 5.49 (2H, *s*, -NCH_2-), 7.28 - 7.45 (5H, s, *aromatic protons*), 11.41 (s br, -O*H*); MS (CI) 248 (M+1).

Ethyl 4-hydroxy-1-methyl-1*H*-1,2,3-triazole-5-carboxylate (3a). White solid. (m.p. 155.5 - 158.5°C, from diisopropyl ether). Yield 91%. ^{1}H-NMR (300 MHz, DMSO): δ 1.29 (3H, *t*, J = 7.1 Hz, -CH_2CH_3), 4.11 (3H, *s*, -NCH_3), 4.28 (2H, *q*, J = 7.1 Hz, -CH_2CH_3), 11.38 (1H, *s*, -O*H*). MS (CI) 172 (M+1).

Ethyl 1-benzyl-4-hydroxy-1*H*-1,2,3-triazole-5-carboxylate (**3b**). White solid (m.p. 153.6 - 155.2°C, from diisopropyl ether). Yield 89 %. ^{1}H-NMR (300 MHz, DMSO): δ 1.21 (3H, *t*, J = 7.1 Hz, -CH_2CH_3), 4.23 (2H, *q*, J = 7.1 Hz, -CH_2CH_3), 5.77 (2H, *s*, -NCH_2-), 7.17 - 7.38 (5H, s, *aromatic protons*), 11.55 (*s br*, -O*H*); MS (CI) 248 (M+1).

Ethyl 5-(benzyloxy)-1-methyl-1H-1,2,3-triazole-4-carboxylate (4a). White solid (m.p. 63.2 - 63.9°C, from isopropyl alcohol). Yield 93%. ^{1}H-NMR (300 MHz, $CDCl_3$): δ 1.45 (3H, *t* J = 7.0 Hz, -CH_2CH_3), 3.62 (3H, *s*, -CH_3), 4.47 (2H, *q* J = 6.7 Hz, -CH_2CH_3), 5.55 (2H, *s*, -OCH_2Ph), 7.36 (5H, *s*, *aromatic protons*); MS (CI) 262 (M+1).

Ethyl 1-benzyl-5-(benzyloxy)-1*H*-1,2,3-triazole-4-carboxylate (4b). Colorless oil. Yield 83 %. ^{1}H-NMR (300 MHz, $CDCl_3$): δ 1.35 (3H, *t,* J = 7.1 Hz, -CH_2CH_3), 4.37 (2H, *q,* J = 7.1 Hz, -CH_2CH_3), 5.12 (2H, *s*, -NCH_2), 5.32 (2H, *s*, -OCH_2Ph), 7.07 - 7.26 (10H, *m*, *aromatic protons*); MS (CI) 338 (M+1).

Ethyl 5-(benzyloxy)-2-methyl-2*H*-1,2,3-triazole-4-carboxylate (5a). White solid (m. p. 51.9 - 56.2°C, triturated with diisopropyl ether). Yield 47%. ^{1}H-NMR (300 MHz, $(CD_3)_2CO$): δ 1.32 (3H, *t*, J = 7.2 Hz, -CH_2CH_3), 4.09 (3H, *s,* -NCH_3), 4.30 (2H, *q,* J = 7.2 Hz, -CH_2CH_3), 5.33 (*2H*, *s*, -OCH_2), 7.34 -7.52 (5H, *m*, *aromatic protons*); MS (CI) 262 (M+1).

Ethyl 2-benzyl-5-(benzyloxy)-2*H*-1,2,3-triazole-4-carboxylate (5b). Colorless oil. Yield 45%. ^{1}H-NMR (300 MHz, $CDCl_3$): δ 1.38 (3H, *t,* J = 7.2 Hz, -CH_2CH_3), 4.40 (2H, *q,* J = 7.2 Hz, -CH_2CH_3), 5.33 (2H, *s*, -OCH_2-), 5.45 (2H, *s*, -NCH_2-), 7.25 - 7.45 (10H, *m*, *aromatic protons*); MS (CI) 338 (M+1).

Ethyl 4-(benzyloxy)-1-methyl-1*H*-1,2,3-triazole-5-carboxylate (6a) White solid (m. p. 75.9 - 79.7°C, triturated with diisopropyl ether). Yield 30%. ^{1}H-NMR (300 MHz, $(CD_3)_2CO$): δ 1.35 (3H, *t,* J = 7.2 Hz, -CH_2CH_3), 4.21 (3H, *s,* -NCH_3), 4.34 (2H, *q*, J = 7.2 Hz, -CH_2CH_3), 5.48 (2H, *s*, -OCH_2), 7.32 -7.54 (5H, *m*, *aromatic protons*); MS (CI) 262 (M+1).

Ethyl 1-benzyl-4-(benzyloxy)-1*H*-1,2,3-triazole-5-carboxylate (6b). Colorless oil, Yield 30%. ^{1}H-NMR (300 MHz, $CDCl_3$): δ 1.31 (3H, *t,* J = 7.2 Hz, -CH_2CH_3), 4.30 (2H, *q,* J = 7.2 Hz, -CH_2CH_3), 5.53 (2H, *s*, -OCH_2-), 5.82 (2H, *s*, -NCH_2-), 7.26 - 7.49 (10H, *m*, *aromatic protons*); MS (CI) 338 (M+1).

Ethyl 5-hydroxy-1-(4-methoxybenzyl)-1*H*-1,2,3-triazole-4-carboxylate (8).[1] White solid (m. p 112.0 - 112.8°C from chloroform-petroleum ether). Yield 71%. ^{1}H-NMR (300 MHz, DMSO): δ 1.27 (3H, *t*, J = 7.1 Hz, -CH_2CH_3), 3.73 (3H, *s*, -OCH_3), 4.26 (2H, *q*, J = 7.1 Hz, -CH_2CH_3), 5.26 (2H, *s*, -NCH_2-), 6.92 - 7.21 (4H, *m*, *aromatic protons*); MS (CI) 278 (M+1).

Ethyl 5-(benzyloxy)-1-(4-methoxybenzyl)-1*H*-1,2,3-triazole-4-carboxylate (9). Colorless oil. Yield 55%. ^{1}H-NMR (300 MHz, $CDCl_3$): δ 1.43 (3H, *t,* J = 7.2 Hz, -CH_2CH_3), 3.78 (3H, *s,* -OCH_3), 4.45 (2H, *q,* J = 7.2 Hz, -CH_2CH_3), 5.14 (2H, *s*, -NCH_2-), 5.41 (2H, *s*, -OCH_2Ph), 6.81 – 7.13 (4H, *m*, *aromatic protons*), 7.28 - 7.36 (5H, *m*, *aromatic protons*); MS (CI) 368 (M+1).

5-(Benzyloxy)-1*H*-1,2,3-triazole-4-carboxylate (10). White solid (m.p. 92.8 - 93.5°C). Yield 35%. ^{1}H-NMR (300 MHz, DMSO): δ 1.28 (3H, *t,* J = 6.0 Hz, -CH_2CH_3), 4.28 (2H, *q*, J = 6.0 Hz, -CH_2CH_3), 5.35 (2H, *s*, -OCH_2-), 7.35 - 7.49 (5H, *m*, *aromatic protons*); MS (CI) 248 (M+1).

Ethyl 5-hydroxy-1-methyl-1*H*-1,2,3-triazole-4-carboxylate methylammonium salt (13). White solid (m.p. 191.9 - 192.7°C). Yield 79%. ^{1}H-NMR (300 MHz, DMSO): δ 1.21 (3H, *t*, J = 7.1 Hz, -CH_2CH_3), 2.37 (3H, *s*, $CH_3NH_3^+$), 3.34 (3H, *s*, -NCH_3), 4.11 (2H, *q*, J = 7.1 Hz, -CH_2CH_3), 8.06 (3H, $CH_3NH_3^+$).

Ethyl 3-hydroxy-1-methyl-1H-pyrazole-4-carboxylate (14).[2] **W**hite solid (m.p. 144.2 - 146.0°C). Yield 63%. ^{1}H-NMR (300 MHz, ($CDCl_3$): δ 1.35 (3H, *t*, J = 7.1 Hz, -CH_2CH_3), 3.77 (3H, *s*, -NCH_3), 4.33 (2H, *q*, J = 7.1 Hz, -CH_2CH_3), 7.56 (1H, s, *aromatic proton*). MS (CI) 171 (M+1).

Ethyl 4-hydroxy-1,2,5-thiadiazole-3-carboxylate (15). White solid (m. p. 61.0 - 62.9°C). ^{1}H-NMR (300 MHz, DMSO): δ 1.31 (3H, *t*, J = 7.1 Hz, -CH_2CH_3), 4.33 (2H, *q*, J = 7.1 Hz, -CH_2CH_3), 13.09 (1H, *s br*, -O*H*); MS (CI) 175 (M+1).

Ethyl 4-hydroxy-1,2,5-oxadiazole-3-carboxylate (16). Sticky solid. ^{1}H-NMR (300 MHz, (DMSO): δ 1.32 (3H, *t*, J= 7.0 Hz, -CH_2CH_3), 4.37 (2H, *q*, J= 7.0 Hz, -CH_2CH_3), 4.62 (1H, *s br*, -O*H*).

(*RS*)-2-Amino-3-(5-hydroxy-2-methyl-triazol-4-yl)propanoic acid chlorhydrate (17). Brown solid (m.p. 216.7-217.4°C). Yield 100%. ^{1}H-NMR (300 MHz, D_2O): δ 3.22 (2H, *dd*, ^{3}J = 6.0 Hz, -CH_2CH-), 3.90 (3H, *s*, NCH_3), 4.29 (1H, *t*, J = 6.0 Hz, -CH_2CH).

(*RS*)-2-Amino-3-(5-hydroxy-3-methyl-triazol-4-yl)propanoic acid hydrochloride (18). Brown solid (m.p. 130.3-131.7°C). Yield 100%. ^{1}H-NMR (300 MHz, D_2O): δ 3.31 (2H, *dd*, ^{3}J = 6.2Hz, ^{4}J = 0.82, Hz, -CH_2CH-), 3.90 (3H, *s*, NCH_3), 4.36 (1H, *t*, J = 6.2 Hz, -CH_2CH).

4-hydroxy-1-methyl-*N*-(4-phenoxyphenyl)-1*H*-1,2,3-triazole-5-carboxamide (23).
White solid (m.p. 243.9-244.6°C). Yield 99%. ^{1}H-NMR (300 MHz, DMSO): δ 4.18 (3H, *s*, -CH_3), 6.98 - 7.68 (9H, *m, aromatic protons*), 9.61 (1H, s, O*H*).

1-(cyclohexylmethyl)-4-hydroxy-*N*-(4-phenoxyphenyl)-1*H*-1,2,3-triazole-5-carboxamide (24). White solid (m.p. 224.2-224.8°C). Yield 98%. ^{1}H-NMR (300 MHz, DMSO): δ 0.97 - 1.78 (10H, *m*, -C_6H_{11}), 1.82 - 1.90 (1H, *m*, -$CH_2C_6H_{11}$), 4.46 (2H, *d,* J = 7.2 Hz, -$CH_2C_6H_{11}$), 6.98 - 7.68 (9H, m, *aromatic protons*), 9.70 (1H, *s*, N*H*), 12.96 (1H, *br s*, O*H*).

4-(benzyloxy)-1-(cyclohexylmethyl)-*N*-(4-phenoxyphenyl)-1*H*-1,2,3-triazole-5-carboxamide (25). Colorless sticky oil. Yield 42%. ^{1}H-NMR (300 MHz, $CDCl_3$): δ 1.00 - 1.25 (10H, *m*, -$CH_2C_6H_{11}$), 1.97 - 2.05 (1H, *m*, -$CH_2C_6H_{11}$), 4.61 (2H, *d,* J = 7.4 Hz, -$CH_2C_6H_{11}$, 5.61 (2H, *s*, -CH_2Ph), 6.96 - 7.54 (14H, *m*, *aromatic protons*), 8.65 (1H, *s*, N*H*).

2-benzyl-5-(benzyloxy)-*N*-(4-phenoxyphenyl)-2*H*-1,2,3-triazole-4-carboxamide (26). Colorless sticky oil. Yield 57%. ^{1}H-NMR (300 MHz, $CDCl_3$): δ 5.39 (2H, *s*, NCH_2Ph), 5.47 (2H, *s*, -OCH_2Ph), 6.96 - 7.55 (19H, m, *aromatic protons*) 8.41 (1H, *s*, N*H*).

1-benzyl-4-(benzyloxy)- *N*-(4-phenoxyphenyl)-1*H*-1,2,3-triazole-5-carboxamide (27). Pale yellow solid (m.p. 101.3-101.9°C). Yield 65%. ^{1}H-NMR (300 MHz, $CDCl_3$): δ 5.60 (2H, *s*, NC*H*$_2$Ph), 5.97 (2H, *s*, -OC*H*$_2$Ph), 6.95 - 7.44 (19H, m, *aromatic protons*) 8.57 (1H, *s*, N*H*).

Determination of ionization constants

The ionization constants of compounds **1a**-**3a**, **1b-3b**, **14-16** (Table 1 in the article) were determined by potentiometric titration using a GLpK_a apparatus (Sirius Analytical Instruments Ltd., Forest Row,East Sussex, UK). Because of the low aqueous solubility, the compounds required titrations in the presence of MeOH as co-solvent. At least five different hydro-organic solutions (ionic strength adjusted to 0.15 M with KCl) of the compounds (20 mL, ~1 mM in 20–60 wt% MeOH) were initially acidified to pH 1.8 with 0.5 M HCl and then titrated with standardized 0.5 M KOH to pH 12.2 at 25°C under N_2. The apparent ionization constants in the H_2O–MeOH mixtures (psK_a) were obtained, and aqueous pK_a values were calculated by extrapolation to zero content of the co-solvent, following the Yasuda–Shedlovsky procedure.[3] For **1a** and **1b**, titration failed probably due to the instability of these hydroxylic tautomers. [4,5] The pK_a value of these two compounds was obtained by acqueous titration of their methyl and benzyl ammonium salts, respectively.

The ionization constants of compounds **17** and **18** (Table 2 in the article) were measured by potentiometric titration as previously described.[6] The products show three dissociation constants in the ranges pK_{a1} = 2.04 - 2.12, pK_{a2} = 6.11- 6.42, and pK_{a3} = 9.63 - 9.64. The first two ranges are related to the dissociation of the two acid functions, while the third is related to pK_a values of the basic -NH_2 function. A comparison of these data with the pK_a values of Glu, Asp, and Gly [7] indicates that it is reasonable to assign the first and the second sets to the prevalent dissociation of the COOH function and of the hydroxyl-triazole moiety, respectively. The third set is then assigned to the NH_3^+ group.

Radioligand Binding at iGluRs

Binding Affinity at Native iGluRs of the racemates **17** and **18**: Affinities for AMPA, KA, and NMDA receptors in rat cortical synaptosomes were determined using 5 nM [^{3}H]AMPA,[8] 5 nM [^{3}H]KA,[9] and 2 nM [^{3}H](*RS*)-(*E*)-2-amino-4-phosphonomethyl-3-heptenoic acid ([^{3}H]CGP 39653),[10] respectively, with minor modifications as previously described.[11] Rat brain membrane preparations used in these receptor binding experiments were prepared according to a method previously described.[12]

Chitinase Inhibition Assay

The procedure for the determination of IC_{50} values of OvCHT1 inhibition was the same as described previously.[13]

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