Supporting Information

Synthesis and Biological Evaluation of 4-(Aminomethyl)-1-hydroxypyrazole Analogues of Muscimol as γ-Aminobutyric Acid$_A$ Receptor Agonists

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Elemental analysis data

Compound		Elemental analysis calculated (found)		
		C	H	N
2a	$C_4H_7N_3O \cdot HBr \cdot 0.1H_2O$	24.53 (24.62)	4.22 (3.95)	21.46 (21.16)
2b	$C_4H_6N_3OBr \cdot 1.1HBr$	17.10 (17.60)	2.55 (2.56)	14.95 (14.62)
2c	$C_4H_6N_3OCl \cdot HBr$	21.03 (21.13)	3.09 (2.92)	18.39 (18.07)
2d	$C_5H_9N_3O \cdot 1HBr \cdot 0.2H_2O$	28.37 (28.52)	4.95 (4.54)	19.85 (19.43)
2e	$C_6H_{11}N_3O \cdot 1.45HBr$	27.88 (28.17)	4.85 (4.46)	16.26 (15.88)
2f	$C_4H_6IN_3O \cdot HBr$	15.02 (15.11)	2.21 (2.08)	13.13 (13.04)
2g	$C_5H_9N_3O \cdot 1.1HBr$	27.78 (27.77)	4.71 (4.45)	19.44 (19.00)
2h	$C_6H_{11}N_3O \cdot 1.45HBr$	27.88 (28.24)	4.85 (4.59)	16.26 (15.74)
2j	$C_{10}H_{11}N_3O \cdot HBr$	44.46 (44.40)	4.48 (4.38)	15.56 (15.33)
2k	$C_{11}H_{13}N_3O \cdot 1.3HBr$	42.84 (43.21)	4.67 (4.76)	13.62 (13.60)
2l	$C_{12}H_{15}N_3O \cdot 1.1HBr$	47.06 (47.17)	5.30 (5.02)	13.72 (13.34)
2m	$C_{11}H_{14}N_4O \cdot 2HBr \cdot 1H_2O$	33.19 (33.08)	4.56 (3.95)	14.07 (13.56)
2n	$C_{16}H_{15}N_3O \cdot HBr \cdot 1.5H_2O$	51.49 (51.53)	5.13 (5.09)	11.26 (10.44)
2o	$C_{16}H_{15}N_3O \cdot 1.8HBr$	46.76 (46.39)	4.12 (4.16)	10.22 (9.98)
16	$C_6H_9N_3O \cdot HBr$	32.75 (32.76)	4.58 (4.66)	19.09 (18.46)
17	$C_6H_9N_3O \cdot HBr$	32.75 (32.07)	4.58 (4.51)	19.09 (18.23)
24	$C_5H_8N_2O_2 \cdot HBr$	28.73 (29.00)	4.34 (4.22)	13.40 (13.40)

Experimental details

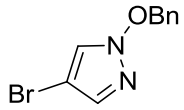
Preperative supercritical fluid chromatopgraphy, preperative and analytical HPLC

Preparative supercritical fluid chromatography (prep. SFC) was performed on a PrepStar SD-1 system (Varian Analytical Instruments) connected to a PrepStar Methanol pump and CO_2 pump, a peak detector (Spectrophotometer K-2501, Knauer, 210 nm), a Berger heater, nozzle, syringe pump and separator. The system was equipped with a Chiralcel AD-H column (250 × 20 mm, 5 μm). The CO_2 mobile phase was modified with 20 % ethanol (additive 0.1 % diethylamine and a flow of 50 mL/min). Preparative HPLC (prep. HPLC) was performed using a Jasco M880 pump, a Rheodyne 7125 injector, a 5 mL loop, a Shimadzu SPD-6A UV spectrophotometer (210 or 225 nm) and a Hitachi D-2000 Chromato-Integrator using an X-terra Prep MS C_{18} column (300 × 10 mm, 10 μm) (Waters) in a serial connection with a Gemini C_{18} column (10 × 250 mm, 5 μm) (Phenomenex). The flow rate was 5.0 mL/min with aqueous TFA (0.1%) as mobile phase. Analytical HPLC (anal. HPLC) was performed on a Merck-Hitachi HPLC system consisting of an L-7100 pump, an L-7200 autosampler, and an L-7400 UV detector (254 nm), using an X-Terra column (4.6 × 150 mm) eluted at a flow rate of 0.8 mL/min. A linear gradient elution was performed with eluent A (H_2O/TFA 100:0.1) containing 0 % of solvent B (MeCN/H_2O/TFA, 90:10:0.01) rising to 100 % of B during 5 min. Data were acquired and processed using the EZChrom Elite Software version 3.1.7 by Hitachi.

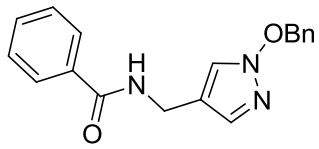
pKa measurement of compound 2a

The pKa value were measured potentiometrically using a Sirius GlpKa Auto-titrator and data were analyzed with Sirius pKaLOGP software (version 5.2). Multiple runs were performed using MeOH/H_2O mixtures. The derived pKa values were extrapolated to zero organic solvent using Yasuda-Shedlovsky plots.[1, 2] The samples were run from low pH to high pH using first HCl (aq) to obtain a pH of 2 or 3.5 and then titrated with KOH (aq).

Preparation of N-((1-benzyloxy)-1H-pyrazol-4-yl)methylbenzamide



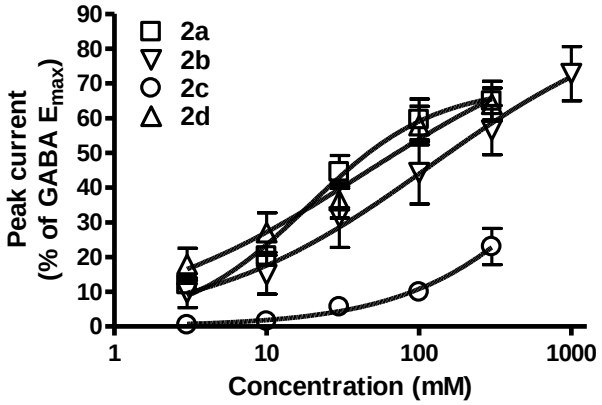
1-(benzyloxy)-4-bromo-1H-pyrazole. Prepared by a procedure described in reference.[3] mp 33.9-37.1 °C. R_f (Heptane/EtOAc 2:1) 0.54.^{1}H-NMR ($CDCl_3$) δ 7.37-7.23 (m, 6H), 7.00 (s, 1H), 5.25 (s, 2H).^{13}C-NMR ($CDCl_3$) δ 133.97, 133.28, 129.60, 129.43, 128.71, 122.91, 90.42, 80.76.



N-((1-(benzyloxy)-1H-pyrazol-4-yl)methyl)benzamide. Prepared by a procedure described in reference.[4] R_f (Heptane/EtOAc 1:1) 0.27. ^{1}H-NMR ($CDCl_3$) δ 7.71 (d, *J* = 8.5 Hz, 2H), 7.51-7.24 (m, 9H), 7.04 (s, 1H), 6.18 (b s, 1H).^{13}C-NMR ($CDCl_3$) δ 167.06, 134.18, 133.62, 132.70, 131.56, 129.61, 129.25, 128.61, 128.55, 126.82, 122.18, 115.78, 80.59, 76.58, 34.57.

Concentration-response curves for 2a-d at $\alpha_1\beta_2\gamma_2$ $GABA_A$ receptors

Concentration-response curves for **2a-d** at $\alpha_1\beta_2\gamma_2$ $GABA_A$ receptors transiently expressed in HEK-293 cells. Responses were obtained using whole-cell patch-clamp electrophysiology and quantified using the peak currents. The responses are given as % of the maximum response of GABA. Each point represents the mean +/- SEM of five to seven different cells.



X-ray crystallographic analysis of compound 17

Crystal data: single crystals suitable for X-ray diffraction studies were grown from a solution in acetic acid, $C_6H_{10}N_3OBr$, M_r 220.08, orthorhombic, space group *Pbca* (No 61), a = 9.7548(8), b = 12.3201(13), c = 14.1744(6) Å, V = 1703.5(2) Å^3, Z = 8, D_c = 1.716 Mg/m^3, F(000) = 880, μ(MoKα) = 4.774 mm^{-1}, crystal size: 0.37 x 0.33 x 0.3 mm.

Data collection and reduction: a single crystal was mounted and immersed in a stream of nitrogen gas [T = 122.0(5)K]. Data were collected, using graphite monochromated MoKα radiation source (λ = 0.71073 Å) on a KappaCCD diffractometer. Data collection and cell refinement were performed using COLLECT[5] and DIRAX[6]. Data reduction was performed using EvalCCD.[7] Correction for absorption was performed using Gaussian integration.[8, 9]

Structure solution and refinement: positions of all non-hydrogen atoms were found by direct methods (SHELXS97).[10] Full-matrix least squares refinements (SHELXL97)[10] were performed on F^2, minimizing $\Sigma w(F_o^2\text{-}kF_c^2)^2$, with anisotropic displacement parameters of the non-hydrogen atoms. The position of the hydrogen atoms were located in subsequent difference electron density maps and refined with fixed isotropic displacement parameters (U_{iso} = 1.2U_{eq} for CH, CH_2, and NH_2, U_{iso} = 1.5U_{eq} for OH). Refinement (130 parameters, 3751 unique reflections) converged at R_F = 0.023, wR_F^2 = 0.046 [3135 reflections with $F_o > 4\sigma(F_o)$; $w^{-1} = (\sigma^2(F_o^2)+(0.0159P)^2 + 1.0517P)$, where $P = (F_o^2 + 2F_c^2)/3$; S = 1.188]. The residual electron density varied between -0.58 and 0.60 e Å^{-3} (close to bromid). Complex scattering factors for neutral atoms were taken from International Tables for Crystallography as incorporated in SHELXL97. [10, 11]

Fractional atomic coordinates, list of anisotropic displacement parameters and a complete list of geometrical data for compound 18 have been deposited in Cambridge Crystallographic Data Centre (No. CCDC 901038).

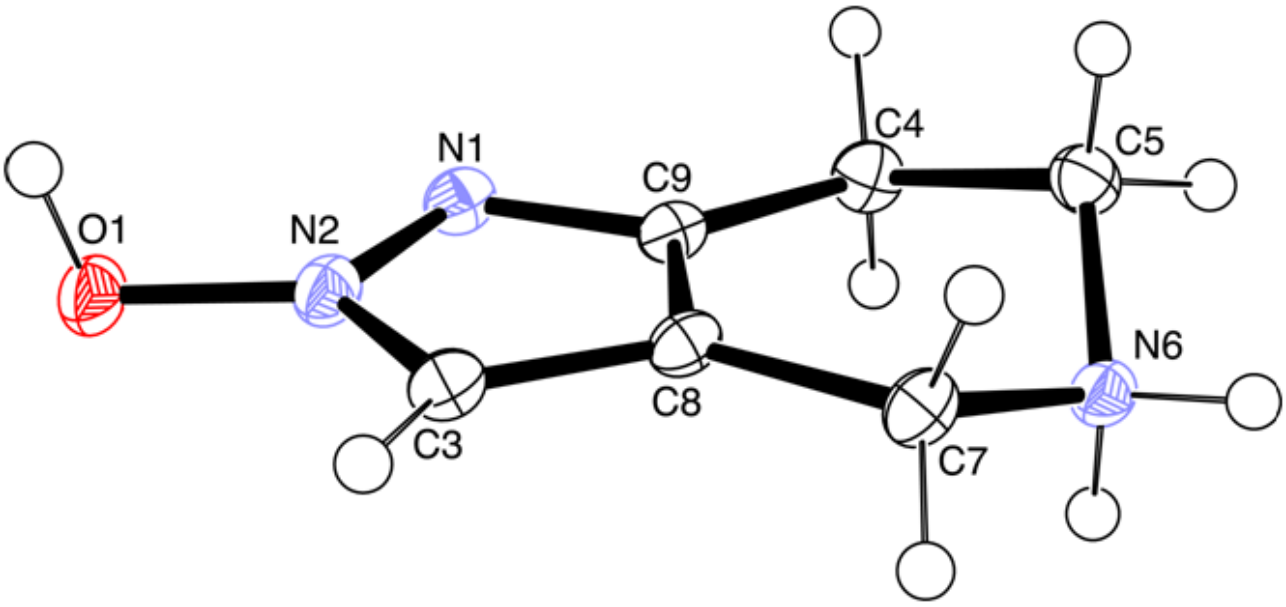


Figure 1. Perspective drawing[12] of compound 18. Displacement ellipsoids of the non-hydrogen atoms are shown at the 50% probability level. Hydrogen atoms are represented by spheres of arbitrary size.

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