Supporting Information

Benzimidazoles as potent and orally active mGlu5 receptor antagonists with a good PK/PD relationship

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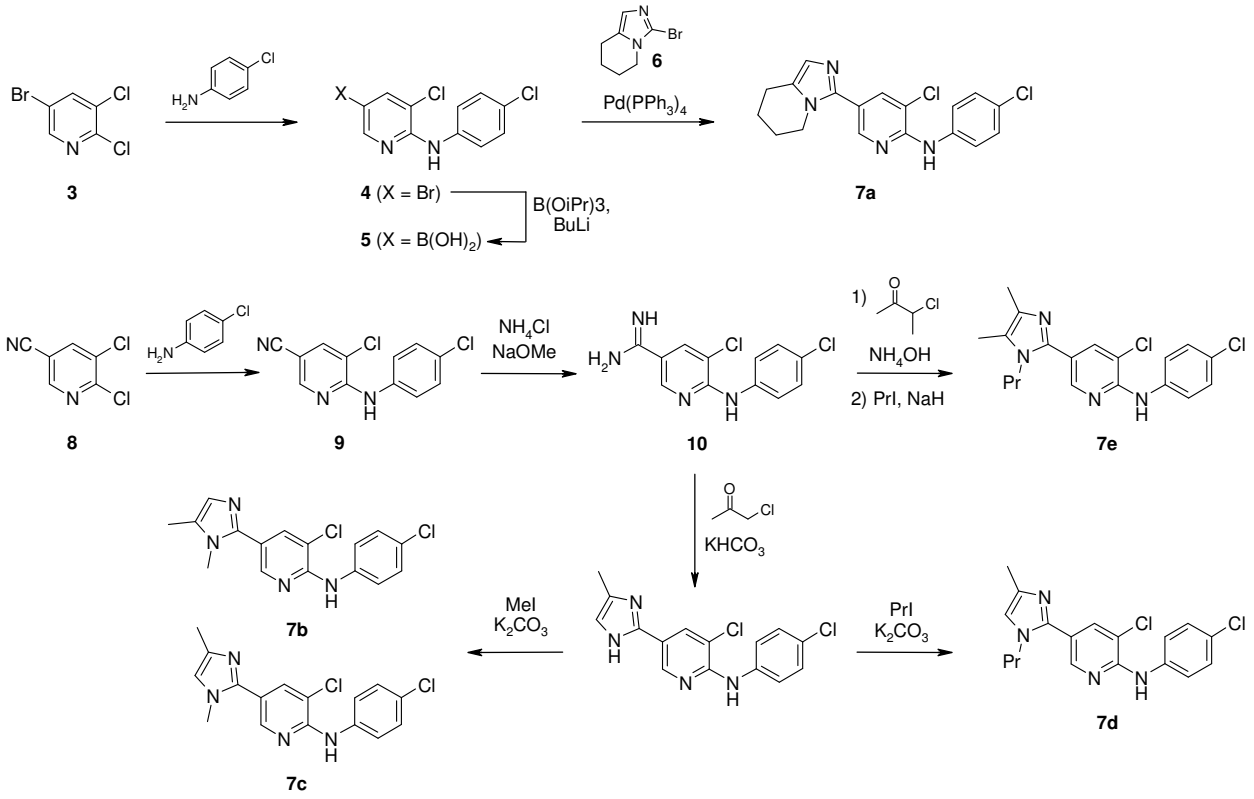
Supporting information includes experimental procedures and characterization of new compounds, as well as details about the biological experiments (*in vitro* and *in vivo*)

General: All commercially available reagents and solvents were used without further purification unless otherwise stated. Automated flash chromatography was performed on Flashmaster II (Jones chromatography) using SiO_2 (mesh 230-400) self-packed cartridges with UV peak detection at 254 nm. 1H-NMR spectra were recorded on a Varian Oxford-AS400 instrument operating at 400 MHz with residual protonated solvent used as a reference. Analytical LC was performed on a Waters Acquity UPLC system equipped with sample manager and a PDA detector operating at a wavelength range between 220 and 400 nm (column Acquity UPLC BEH C18 (1.7 µm, 50 x 2.1 mm), temperature: 35 °C, flow: 0.6 mL min-1. Eluents: Water + 0.1% TFA / Acetonitrile + 0.1% TFA from 95/5 to 0/100 over 2 min), an HPLC Agilent 1100 Series, LC-MSD system equipped with a MS detector (Column Agilent Zorbax SB-C18 (1.8µm, 3 x 30mm), temperature 35 °C, flow: 0.7 mL min-1. Eluents: Water + 0.05% TFA / Acetonitrile + 0.05% TFA from (a) 100/0 to 0/100 over 3.25' – 0/100 over 0.75' – 0/100 to 90/10 over 0.25'; (b) 90/10 to 0/100 over 3.25' – 0/100 over 0.75' – 0/100 to 70/30 over 0.25', or an HPLC Agilent 1100 Series, LC-MSD system equipped with a MS detector (Column Nucleosil C-18HD (1.8µm, 4 x 70mm), temperature 35 °C, flow: 1.0 mL min-1. Eluents: Water + 0.05% TFA / Acetonitrile + 0.05% TFA from (a) 80/20 to 0/100 over 6.00' – 0/100 over 1.50' – 0/100 to 80/20 over 0.50'; Low resolution mass spectra were recorded using the Agilent HPLC machines mentioned above coupled to a MSD using electrospray ionization. The high resolution mass spectra were recorded using positive electrospray ionization (ESI) on a LTQ Orbitrap XL (Thermo Scientific) mass spectrometer.

Scheme S1: preparation of **7a-e**:



(5-bromo-3-chloro-pyridin-2-yl)-(4-chloro-phenyl)-amine (**4**):

To a solution a 4-chloroaniline (11.1g, 86.1 mmol) in dry THF (200 mL) was added NaH (95%, 2.13 g, 84.0 mmol) portionwise. The mixture was stirred for 1 h at RT, and a solution of 5-bromo-2,3-dichloropyridine (10.0 g, 43.2 mmol) was then added. The mixture was heated to reflux for 14 h, and then allowed to cool to RT. The mixture was poured onto a saturated aqueous Na_2CO_3 solution and extracted twice with EtOAc. The combined organic phases were dried over Na_2SO_4, filtered and concentrated *in vacuo*. Purification by flash chromatography (SiO_2, Hex/EtOAc 100:0 to 80:20) gave 9.3g (68 % yield) of the title compound. UPLC (5-100% CH_3CN): t_R = 1.989 min; MS (ESI): *m/z* 318 [M^+H].

5-chloro-6-(4-chlorophenylamino)pyridin-3-ylboronic acid (**5**):

A solution of **4** (1.0 g, 3.14 mmol) in dry THF (10 mL) was cooled to -78 °C, and then treated with a solution of BuLi (1.6M, 4.9 mL, 7.8 mmol). The mixture was stirred at this temperature for 30 minutes, and a solution of $B(OiPr)_3$ (0.89 mL, 3.77 mmol) in dry THF (5 mL) was added. The mixture was allowed to warm to RT over 2 h, and then quenched by slowly adding H_2O. The THF was evaporated in vacuo, and the aqueous phase was neutralized with an aqueous solution of HCl (2M) and extracted three times with Et_2O. The combined organic phases were dried over Na_2SO_4, filtered and concentrated *in vacuo*. Purification by flash chromatography (SiO_2, CH_2Cl_2/MeOH 9:1 + 1% AcOH) provided the title

compound (312 mg, 35% yield). HPLC (System b, 10-100% CH_3CN): t_R = 2.923 min; MS (ESI): *m/z* 283 [M^+H].

3-bromo-5,6,7,8-tetrahydroimidazo[1,5-a]pyridine (**6**):

A solution of 5,6,7,8-tetrahydro-imidazo[1,5-a]pyridine (190 mg, 1.56 mmol) in CH_3CN (5 mL) was treated with BrCN (173 mg, 1.63 mmol). The mixture was stirred at RT overnight, and then concentrated *in vacuo*. The crude product was diluted with H_2O and extracted twice with EtOAc. The combined organic phases were dried over Na_2SO_4, filtered and concentrated *in vacuo*. Purification by flash chromatography (SiO_2, CH_2Cl_2/MeOH 100:0 to 90:10) afforded 63 mg (20 % yield) of the title compound. HPLC (System a, 0-100% CH_3CN): t_R = 2.240 min; MS (ESI): *m/z* 201 [M^+H].

3-chloro-N-(4-chlorophenyl)-5-(5,6,7,8-tetrahydroimidazo[1,5-a]pyridin-3-yl)pyridin-2-amine (**7a**):

A microwave reaction tube was charged with **5** (60.0 mg, 0.212 mmol), **6** (51 mg, 0.255 mmol), $Pd(PPh_3)_4$ (24 mg, 0.021 mmol), Na_2CO_3 (2M, 0.6 mL, 1.2 mmol), benzene (1.5 mL) and methanol (0.5 mL), degassed in the ultrasound bath with a flux of Ar, and then heated to 140 °C for 40 min in a microwave reactor. The aqueous phase was then separated and the organic phase was diluted with EtOAc, dried over Na_2SO_4, filtered and concentrated *in vacuo*. Purification by flash chromatography (SiO_2, CH_2Cl_2/MeOH 100:0 to 95:5) gave 20 mg (26% yield) of the title compound. HPLC (System c, 20-100% CH_3CN): t_R = 4.090 min; 1H NMR (CDCl3, 400 MHz) δ 8.39 (s, 1H), δ8.04 (s, 1H), δ7.63 (d, J = 9.0 Hz, 2H), δ 7.38 (d, J = 9.0 Hz, 2H), δ 7.15 (s, 1H), δ 6.96 (s, 1H), δ 4.11 (m, 2H), δ 2.92 (m, 2H), δ 2.02 (m, 2H), δ 1.91 (m, 2H); MS (ESI): *m/z* 359 [M^+H].

5-chloro-6-(4-chlorophenylamino)nicotinonitrile (**9**):

A solution of $Pd(OAc)_2$ (58.0 mg, 0.253 mmol) and *rac*-BINAP (162 mg, 0.255 mmol) in degassed toluene (10 mL) was stirred for 10 min at RT and 4-chloroaniline (1.53 g, 11.9 mmol) and 5,6-dichloro-nicotinonitrile (1.4 g, 7.93 mmol) were added. The mixture was stirred for another 10 min at RT, and K_2CO_3 (5.54 g, 39.7 mmol) was added. The mixture was heated to 100 °C for 16 h, and then concentrated *in vacuo*. Purification by flash chromatography (SiO_2, Hex/CH_2Cl_2 100:0 to 0:100) provided the title compound **9** (1.48 g, 71% yield). UPLC (5-100% CH_3CN): t_R = 1.635 min; MS (ESI): *m/z* 264 [M^+H].

5-chloro-6-(4-chlorophenylamino)nicotinamidine (**10**):

A solution of **9** (800 mg, 3.03 mmol) and NaOMe (253 mg, 4.54 mmol) in MeOH (20 mL) was stirred at RT for 16 h, and NH_4Cl (180 mg, 3.33 mmol) was then added. The mixture was heated to 65 °C for 2h, allowed to cool to RT and concentrated *in vacuo*. The crude product was then suspended in EtOH, stirred for 2 h at RT, and then filtered to give the title compound (520 mg, 61 %). UPLC (5-100% CH_3CN): t_R = 1.020 min; MS (ESI): *m/z* 281 [M^+H].

3-chloro-N-(4-chlorophenyl)-5-(1,5-dimethyl-1H-imidazol-2-yl)pyridin-2-amine (**7b**)

3-chloro-N-(4-chlorophenyl)-5-(1,4-dimethyl-1H-imidazol-2-yl)pyridin-2-amine (**7c**)

3-chloro-N-(4-chlorophenyl)-5-(4-methyl-1-propyl-1H-imidazol-2-yl)pyridin-2-amine (**7d**)

A biphasic solution of **10** (1.5 g, 1.97 mmol) and $KHCO_3$ (2.0 g, 19.8 mmol) in THF (20 mL) and H_2O (5 mL) was heated to 80 °C and chloroacetone (0.21 mL, 2.37 mmol) was added. The mixture was stirred at 80 °C for 5 h, allowed to cool to RT, diluted with H_2O, and extracted with EtOAc. The combined organic phases were dried over Na_2SO_4, filtered and concentrated *in vacuo* to give 650 mg of 3-chloro-N-(4-chlorophenyl)-5-(4-methyl-1H-imidazol-2-yl)pyridin-2-amine, which was used as it is in the next steps.

A solution of 3-chloro-N-(4-chlorophenyl)-5-(4-methyl-1H-imidazol-2-yl)pyridin-2-amine (150 mg, 0.470 mmol), MeI (22 μL, 0.343 mmol), K_2CO_3 (96 mg, 0.686 mmol) in dry DMF (5 mL) was stirred at RT for 16 h. The mixture was then diluted with H_2O, and extracted twice with EtOAc. The combined organic phases were dried over Na_2SO_4, filtered and concentrated *in vacuo*. Purification by flash chromatography (SiO_2, Hex/EtOAc 100:0 to 20:80) provided:

16 mg (10 % yield) of **7b**: UPLC (5-100% CH_3CN): t_R = 1.136 min; 1H NMR (d6-DMSO, 400 MHz) δ 8.73 (s, 1H), δ 8.34 (s, 1H), δ 8.00 (s, 1H), δ 7.76 (d, J = 9.0 Hz, 2H), δ 7.34 (d, J = 9.0 Hz, 2H); δ 6.75 (s, 1H), δ 3.57 (s, 3H), δ 2.20 (s, 3H); MS (ESI): *m/z* 333 [M^+H].

45 mg (29 % yield) of **7c**: UPLC (5-100% CH_3CN): t_R = 1.133 min; 1H NMR (d6-DMSO, 400 MHz) δ 8.73 (s, 1H), δ 8.37 (s, 1H), δ 8.04 (s, 1H), 7.75 (d, J = 9.0 Hz, 2H), δ 7.34 (d, J = 9.0 Hz, 2H), δ 6.93 (s, 1H), δ 3.68 (s, 3H), δ 2.09 (s, 3H); MS (ESI): *m/z* 333 [M^+H].

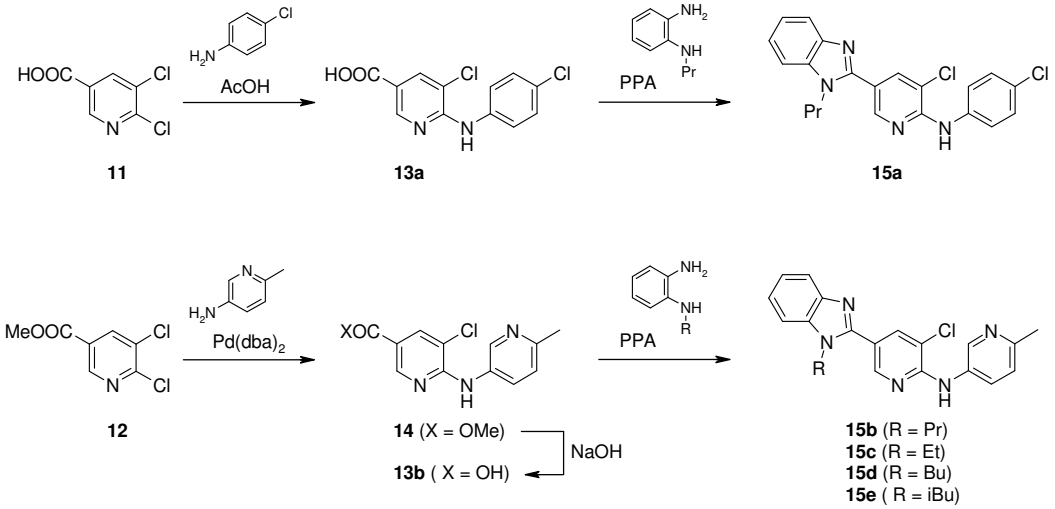
A solution of 3-chloro-N-(4-chlorophenyl)-5-(4-methyl-1H-imidazol-2-yl)pyridin-2-amine (120 mg, 0.376 mmol) in dry DMF (4 mL) was treated with NaH (95%, 10 mg, 0.410 mmol), stirred for 20 min at RT, and PrI (87 μL, 0.752 mmol) was then added. The mixture was stirred at RT for 30 min, then diluted with H_2O, and extracted twice with EtOAc. The combined organic phases were dried over Na_2SO_4, filtered and concentrated *in vacuo*. Purification by flash chromatography (SiO_2, Hex/EtOAc 100:0 to 40:60) gave 65 mg of **7d** (48% yield). UPLC (5-100% CH_3CN): t_R = 1.281 min; 1H NMR (d6-DMSO, 400 MHz) δ 8.73 (s, 1H), δ 8.27 (s, 1H), δ 7.96 (s, 1H), δ 7.75 (d, J = 8.6 Hz, 2H), δ 7.34 (d, J = 8.6 Hz, 2H), δ 7.00 (s, 1H), δ 3.91 (m, 2H), δ 2.10 (s, 3H), δ 1.65 (m, 2H), δ 0.76 (m, 3H); MS (ESI): *m/z* 361 [M^+H].

3-chloro-N-(4-chlorophenyl)-5-(4,5-dimethyl-1-propyl-1H-imidazol-2-yl)pyridin-2-amine (**7e**):

A solution of **10** (1.5 g, 1.97 mmol), 3-chloro-2-butanone (0.82 mL, 7.90 mmol) in aqueous NH_4OH (26%, 150 mL) was heated to reflux for 16 h, and then allowed to cool to RT. The mixture was filtered and the precipitate was washed with H_2O, and then dried *in vacuo* to give 320 mg of [3-chloro-5-(4,5-dimethyl-1H-imidazol-2-yl)-pyridin-2-yl]-(4-chloro-phenyl)-amine, which was used as it is in the next step.

A solution of [3-chloro-5-(4,5-dimethyl-1H-imidazol-2-yl)-pyridin-2-yl]-(4-chloro-phenyl)-amine (70 mg, 0.210 mmol) in dry DMF (4 mL) was treated with NaH (95%, 6 mg, 0.22 mmol), stirred for 30 min at RT, and PrI (49 µL, 0.42 mmol) was then added. The mixture was stirred at RT for 16 h, then diluted with H_2O, and extracted twice with EtOAc. The combined organic layers were then dried over Na_2SO_4, filtered and concentrated *in vacuo*. Purification by flash chromatography (SiO_2, Hex/EtOAc 100:0 to 40:60) afforded **7e** (6 mg, 8% yield). UPLC (5-100% CH_3CN): t_R = 1.320 min; 1H NMR (CD_3OD, 400 MHz) δ 8.30 (s, 1H), δ 8.02 (s, 1H), δ 7.69 (d, J = 9.0 Hz, 2H), δ 7.34 (d, J = 9.0 Hz, 2H), δ 4.09 (m, 2H), δ 2.35 (s, 3H), δ 2.33 (s, 3H), δ 1.76 (m, 2H), δ 0.89 (m, 3H); MS (ESI): *m/z* 375 [M^+H].

Scheme S2: preparation of **15a-e**:



5-chloro-6-(4-chlorophenylamino)nicotinic acid (**13a**):

A mixture of 5,6-dichloronicotinic acid (4.0 g, 20.8 mmol) and 4-chloroaniline (3.22 g, 25.0 mmol) in acetic acid (20 ml) was heated to 150 °C for 75 min in a microwave reactor. After cooling to RT, the precipitate was filtered off. The filtrate was then treated with EtOAc and another precipitate formed, which was filtered. Purification by re-crystallization from 2-PrOH gave the title compound (1.77 g, 30% yield). UPLC (5-100% CH_3CN): t_R = 1.483 min; MS (ESI): *m/z* 283 [M^+H].

3-chloro-N-(4-chlorophenyl)-5-(1-propyl-1H-benzo[d]imidazol-2-yl)pyridin-2-amine (**15a**):

A flask was charged with **13a** (1.0 g, 3.53 mmol), *N*-propylbenzene-1,2-diamine (637 mg, 4.24 mmol) and PPA (10 mL). The mixture was heated to 210 °C for 20 min in a microwave reactor. The mixture was then allowed to cool to RT, poured slowly onto H_2O, the pH was adjusted to 8 with an aqueous solution of NaOH (20%). The mixture was extracted twice with EtOAc, and the combined organic phases were dried over Na_2SO_4, filtered and concentrated *in vacuo* to give a brown oil. Purification by flash chromatography (SiO_2, Hex/EtOAc 100:0 to 75:25) and recrystallization from Hex provided the title compound (522 mg, 37% yield). HPLC (System b, 10-100% CH_3CN): t_R = 3.416 min; 1H NMR (d6-

DMSO, 400 MHz) δ 8.90 (s, 1H), δ 8.48 (s, 1H), δ 8.20 (s, 1H), 7.78 (d, J = 8.6 Hz, 2H), δ 7.65 (m, 2H), δ 7.37 (d, J = 8.6 Hz, 2H), δ 7.25 (m, 2H), δ 4.28 (m, 2H), δ 1.70 (m, 2H), δ 0.75 (m, 3H); MS (ESI): *m/z* 397 [M^+H].

methyl 5-chloro-6-(6-methylpyridin-3-ylamino)nicotinate (**14**):

A suspension of 5,6-dichloro-nicotinic acid methyl ester (50.0 g, 243 mmol), 3-amino-6-methylpyridine (40.2 g, 364 mmol), *rac*-BINAP (9.05 g, 14.5 mmol), $Pd_2(dba)_3$ (11.1 g, 12.1 mmol) and K_2CO_3 (101.0 g, 731 mmol) in toluene (800 mL) was heated to 120 °C for 16 h. The mixture was allowed to cool to RT and concentrated *in vacuo*. Purification by re-crystallization from toluene/EtOAc provided the title compound (37.8 g, 56%). UPLC (5-100% CH_3CN): t_R = 0.832 min; MS (ESI): *m/z* 278 [M^+H].

5-chloro-6-(6-methylpyridin-3-ylamino)nicotinic acid (**13b**):

A solution of **14** (42.0 g, 151 mmol) in MeOH (500 ml) was treated slowly with an aq. soln. of 1N NaOH (300 ml, 300 mmol). The solution was stirred for 1 h at RT and the mixture was then neutralized by adding 4N aq. HCl. The title compound precipitated and was collected by filtration (38.0 g, 95 %). UPLC (5-100% CH_3CN): t_R = 0.647 min; MS (ESI): *m/z* 264 [M^+H].

3-chloro-N-(6-methylpyridin-3-yl)-5-(1-propyl-1H-benzo[d]imidazol-2-yl)pyridin-2-amine (**15b**):

A flask was charged with 13b (5.0 g. 19.0 mmol), N-propylbenzene-1,2-diamine (3.42 g, 22.8 mmol) and PPA (50 mL) and the mixture was heated to 180 °C for 16 h. The mixture was then allowed to cool to RT, poured slowly onto H_2O, the pH was adjusted to 8 with an aqueous solution of NaOH (20%). The mixture was extracted twice with EtOAc, and the combined organic phases were dried over Na_2SO_4, filtered and concentrated in vacuo to give a brown oil. Purification by flash chromatography (SiO_2, CH_2Cl_2/MeOH 100:0 to 90:10) and recrystallization from Hex/EtOAc provided the title compound (2.90 g mg, 41 % yield). UPLC (5-100% CH_3CN): t_R = 0.810 min; 1H NMR (d6-DMSO, 400 MHz) δ 8.91 (s, 1H), δ 8.71 (m, 1H), δ 8.44 (m, 1H), δ 8.19 (m, 1H), δ 7.99 (m, 1H), δ 7.65 (m, 1H), δ 7.22 (m, 3H), δ 4.27 (m, 2H), δ 2.42 (s, 3H), δ 1.69 (m, 2H), δ 0.74 (m, 3H); MS (ESI): *m/z* 378 [M^+H].

3-chloro-5-(1-ethyl-1H-benzo[d]imidazol-2-yl)-N-(6-methylpyridin-3-yl)pyridin-2-amine (**15c**):

A mixture of **13b** (200 mg, 0.76 mmol) and N-ethyl-benzene-1,2-diamine (124 mg, 0.91 mmol) in PPA (3 ml) was heated to 210 °C in a microwave oven for 8 min. The mixture was then poured onto H_2O and stirred at RT for 18 h. The pH of the solution was adjusted to 8 with an aq. soln. of 2N NaOH, and the mixture was extracted with EtOAc. The combined org. layers were dried over Na_2SO_4, filtered and concentrated *in vacuo*. Purification by flash chromatography (SiO_2, Hex/EtOAc 100:0 to 0:100), followed by crystallization from Hex to provide the title product (107 mg, 39%). HPLC (System b, 10-100%

CH_3CN): t_R = 1.017 min; 1H NMR (d6-DMSO, 400 MHz) δ 8.92 (s, 1H), δ 8.71 (m, 1H), δ 8.44 (m, 1H), δ 8.18 (m, 1H), δ 7.99 (m, 1H), δ 7.64 (m, 2H), δ 7.24 (m, 3H), δ 4.33 (q, J = 7.2 Hz, 2H), δ 2.42 (s, 3H), δ 1.32 (t, J = 7.2 Hz, 3H); MS (ESI): *m/z* 364 [M^+H].

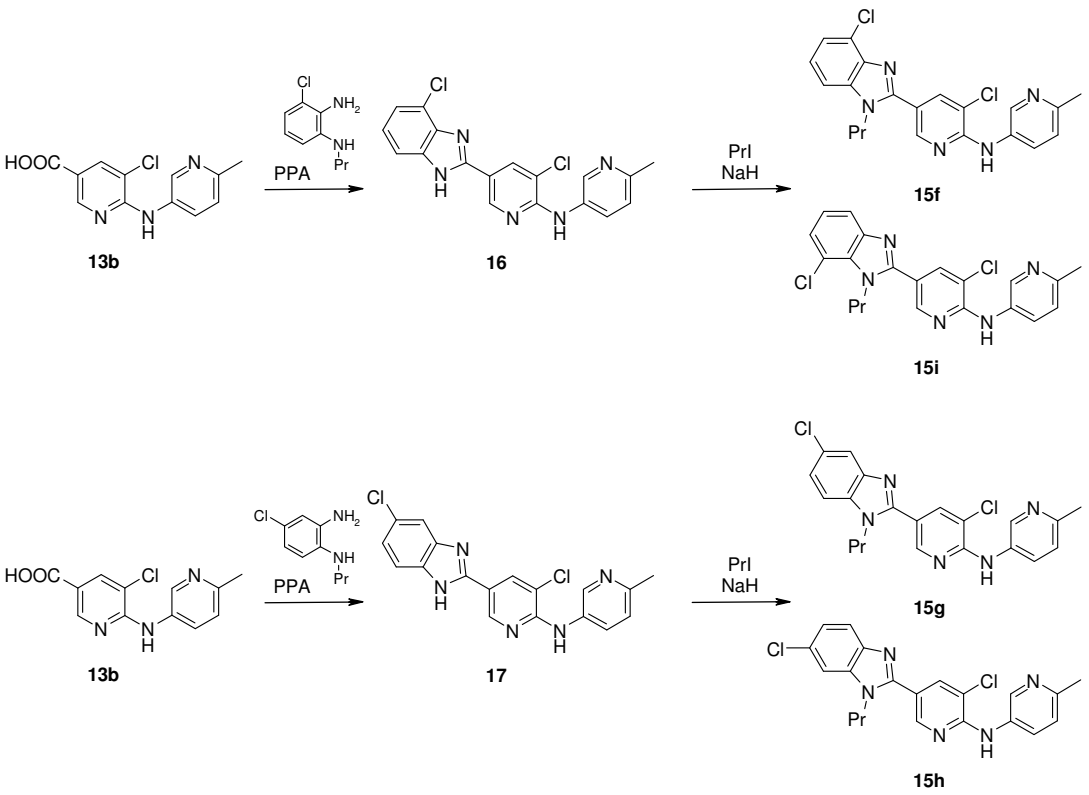
5-(1-butyl-1H-benzo[d]imidazol-2-yl)-3-chloro-N-(6-methylpyridin-3-yl)pyridin-2-amine (**15d**):

A mixture of **13b** (300 mg, 1.14 mmol) and N-ethyl-benzene-1,2-diamine (224 mg, 1.36 mmol) in PPA (3 ml) was heated to 210 °C in a microwave oven for 10 min. The mixture was then poured onto H_2O and stirred at RT for 18 h. The pH of the solution was adjusted to 8 with an aqueous solution of NaOH (20 %), and the mixture was extracted twice with EtOAc. The combined organic layers were dried over Na_2SO_4, filtered and concentrated *in vacuo*. Purification by flash chromatography (SiO_2, Hex/EtOAc 100:0 to 0:100), followed by crystallization from Hex/EtOAc gave the title product (151 mg, 34 %). HPLC (System b, 10-100% CH_3CN): t_R = 2.068 min; 1H NMR (d6-DMSO, 400 MHz) δ 8.91 (s, 1H), δ 8.71 (m, 1H), δ 8.45 (m, 1H), δ 8.19 (m, 1H), δ 7.98 (m, 1H), δ 7.64 (m, 2H), δ 7.24 (m, 3H), δ 4.30 (m, 2H), δ 2.42 (s, 3H), δ 1.65 (m, 2H), δ 1.16 (m, 2H), 0.78 (m, 3H); MS (ESI): *m/z* 392 [M^+H].

3-chloro-5-(1-isobutyl-1H-benzo[d]imidazol-2-yl)-N-(6-methylpyridin-3-yl)pyridin-2-amine (**15e**):

A mixture of **13b** (300 mg, 1.14 mmol) and *N*-isobutyl-benzene-1,2-diamine (224 mg, 1.36 mmol) in PPA (3 ml) was heated to 200 °C for 18 h. The mixture was then poured onto H_2O and stirred at RT for 18 h. The pH of the solution was adjusted to 8 with an aqueous solution of NaOH (20 %), and the mixture was extracted twice with EtOAc. The combined organic layers were dried over Na_2SO_4, filtered and concentrated *in vacuo*. Purification by flash chromatography (SiO_2, Hex/EtOAc 100:0 to 0:100), followed by recrystallization from Hex/EtOAc afforded the title product (76 mg, 17 % yield). HPLC (System b, 10-100% CH_3CN): t_R = 2.544 min; 1H NMR (d6-DMSO, 400 MHz) δ 8.89 (s, 1H), δ 8.71 (m, 1H), δ 8.47 (m, 1H), δ 8.21 (m, 1H), δ 7.99 (m, 1H), δ 7.65 (m 2H), δ 7.24 (m, 3H), δ 4.19 (m, 2H), δ 2.42 (s, 3H), δ 1.96 (m, 1H), δ 0.68 (d, J = 6.6 Hz, 6H); MS (ESI): *m/z* 392 [M^+H].

Scheme S3: preparation of **15f-i**:



3-chloro-5-(4-chloro-1H-benzo[d]imidazol-2-yl)-N-(6-methylpyridin-3-yl)pyridin-2-amine (**16**):

A mixture of **13b** (500 mg, 1.90 mmol) and 3-chloro-benzene-1,2-diamine (324 mg, 2.27 mmol) in PPA (10 ml) was heated to 200 °C for 16 h. The mixture was then poured onto H_2O and stirred at RT for 16 h. The pH of the solution was adjusted to 8 with an aqueous solution of NaOH (20 %), and the mixture was extracted twice with EtOAc. The combined organic layers were dried over Na_2SO_4, filtered and concentrated *in vacuo*. Purification by flash chromatography (SiO_2, Hex/EtOAc 100:0 to 0:100) gave the title product (550 mg, 74 %). UPLC (5-100% CH_3CN): t_R = 0.897 min; MS (ESI): *m/z* 370 [M^+H].

3-chloro-5-(4-chloro-1-propyl-1H-benzo[d]imidazol-2-yl)-N-(6-methylpyridin-3-yl)pyridin-2-amine (**15f**)

3-chloro-5-(7-chloro-1-propyl-1H-benzo[d]imidazol-2-yl)-N-(6-methylpyridin-3-yl)pyridin-2-amine (**15i**)

A solution of **16** (1.1 g, 2.82 mmol) in dry DMF (5 mL) was treated with NaH (95%, 84.0 mg, 3.39 mmol) and the mixture was stirred at RT for 1 h. PrI (0.40 mL, 3.96 mmol) was then added and the mixture was stirred at RT for 16 h. The reaction mixture was concentrated *in vacuo* and the crude product was purified by flash chromatography (SiO_2, Hex/EtOAc 100:0 to 50:50), followed by recrystallization from Hex/EtOAc to afford:

383 mg (33 % yield) of **15f**. UPLC (5-100% CH3CN): t_R = 1.043 min; 1H NMR (CD_3OD, 400 MHz) δ 8.77 (m, 1H), δ 8.44 (m, 1H), δ 8.15 (m, 2H), δ 7.57 (m, 1H), δ 7.32 (m, 3H), δ 4.31 (m, 2H), δ 2.52 (s, 3H), δ 1.83 (m, 2H), δ 0.85 (m, 3H); MS (ESI): *m/z* 412 [M+1].

115 mg (10 % yield) of **15i**. UPLC (5-100% CH_3CN): t_R = 1.082 min; 1H NMR (d6-DMSO, 400 MHz) δ 8.94 (s, 1H), δ 8.71 (m, 1H), δ 8.40 (m, 1H), δ 8.17 (m, 1H), δ 7.99 (m, 1H), δ 7.66 (m, 1H), δ 7.32 (m, 1H), δ 7.23 (2H), δ 4.43 (m, 2H), δ 2.43 (s, 3H), δ 1.70 (m, 2H), δ 0.69 (m, 3H); Hi Res Mass Spectrum (ESI, FTMS): m/z 412.1091 [M^+H, calculated for $C_{21}H_{20}Cl_2N_5$ 412.1090].

3-chloro-5-(5-chloro-1H-benzo[d]imidazol-2-yl)-N-(6-methylpyridin-3-yl)pyridin-2-amine (**17**):

A mixture of **13b** (100 mg, 0.38 mmol) and 4-chloro-benzene-1,2-diamine (68 mg, 0.46 mmol) in PPA (10 ml) was heated to 220 °C for 40 min in the microwave reactor. The mixture was then poured onto H_2O and stirred at RT for 16 h. The pH of the solution was adjusted to 8 with an aqueous solution of NaOH (20 %), and the mixture was extracted twice with EtOAc. The combined organic layers were dried over Na_2SO_4, filtered and concentrated *in vacuo*. Purification by flash chromatography (SiO_2, Hex/EtOAc Hex/EtOAc 100:0 to 0:100) to give the title product (40 mg, 27 %). UPLC (5-100% CH_3CN): t_R = 0.894 min; MS (ESI): *m/z* 370 [M^+H].

3-chloro-5-(5-chloro-1-propyl-1H-benzo[d]imidazol-2-yl)-N-(6-methylpyridin-3-yl)pyridin-2-amine (**15g**)

3-chloro-5-(6-chloro-1-propyl-1H-benzo[d]imidazol-2-yl)-N-(6-methylpyridin-3-yl)pyridin-2-amine (**15h**)

A solution of **17** (300 mg, 0.729 mmol) in dry DMF (5 mL) was treated with NaH (95%, 22 mg, 0.88 mmol) and the mixture was stirred at RT for 30 min. PrI (0.102 mL, 1.02 mmol) was then added and the mixture was stirred at RT for 4 h. The reaction mixture was concentrated *in vacuo* and the crude product was purified by flash chromatography (SiO_2, Hex/EtOAc 100:0 to 0:100) to afford:

35 mg (12 % yield) of **15g**. UPLC (5-100% CH_3CN): t_R = 1.006 min; 1H NMR (CD_3OD, 400 MHz) δ 9.46 (m, 1H), δ 8.79 (m, 1H), δ 8.64 (m, 1H), δ 8.32 (m, 1H), δ 7.97 (m 1H), δ 7.88 (m, 1H), δ 7.77 (m, 1H), δ 7.53 (m, 1H), δ 4.42 (t, J = 7.4 Hz, 2H), δ 2.76 (s, 3H), δ 1,89 (m, 2H), δ 0.91 (t, J = 7.4 Hz, 3H); MS (ESI): *m/z* 412 [M^+H].

25 mg (8 % yield) of **15h**. UPLC (5-100% CH_3CN): t_R = 1.005 min; 1H NMR (CD_3OD, 400 MHz) δ 8.76 (m, 1H), δ 8.43 (m, 1H), δ 8.13 (m, 2H), δ 7.67 (m, 1H), δ 7.60 (m, 1H), δ 7.33 (m, 2H), δ 4.31 (t, J = 7.4 Hz, 2H), δ 2.52 (s, 3H), δ 1.83 (m, 2H), δ 0.86 (t, J = 7.4 Hz, 3H); MS (ESI): *m/z* 412 [M^+H].

In vitro incubation with liver microsomes

Stock solution of **1** (2 mmol/L) was prepared in DMSO. Alamethicin solution was prepared (0.5 mmol/L) in ethanol. UDPGA solution (24 mmol/L) was prepared in phosphate buffer (100 mmol/L pH 7.4). In a further experiment, 15 mmol/L or 100 mmol/L glutathione in phosphate buffer (100 mmol/L pH 7.4) was

added as co-factor. For the generation of in vitro metabolites, **1** was incubated at 37°C for up to 60 minutes with liver microsomes from rat and human. 25 µL liver microsomes, containing 2 mg protein/mL, were mixed with 322 µL phosphate buffer (372 µL if no glutathione is added), 1.25 µL alamethicine solution and 50 µL UDPGA solution. Glutathione trapping was performed by adding 50 µL of a glutathione stock solution (final glutathione concentration: 1.5 mmol/L or 10 mmol/L) in phosphate buffer (100 mmol/L pH 7.4). The test compound **1** was added from the stock solutions (2 mM in DMSO, 1.25 µL) and preincubated for 3 min at 37°C. After pre-incubation, the incubation was started by addition of 50 µL of the NADPH-regenerating system, containing isocitrate-dehydrogenase (1 U/mL), NADP (1 mmol/L) and isocitrate (5 mmol/L). After 1 h the incubation were stopped with 500 µL of ice-cold acetonitrile and an internal standard (2 mM in DMSO; 10 µl solution = 5 µM final concentration) was added. The resulting mixture was stored at -80°C. Prior to injection the individual samples were diluted 1:5 by volume with starting buffer to be directly injected into HPLC.

In vivo PK studies in rats

The male Sprague Dawley rats were housed under standard housing condition and always had free access to standard food and water, ad libitum. The experimental procedure received prior approval from the City of Basel Cantonal Animal Protection Committee based on adherence to federal and local regulations on animal maintenance and testing.

For intravenous administration, **1** was dissolved in N-methyl pyrrolidone/PEG200 (30:70 v/v) and **15i** in PEG200/glucose 5%, 40/60, v/v. An intravenous dose of 10 µmol/kg of **1** and of 2.4 µmol/kg of **15i** were administered to rats by injection into the surgically exposed saphenous vein. For oral administration, both **1** and **15i** were suspended in Neoral Placebo (containing Cremophor RH40, corn oil glycerides, propylene glycol and ethanol)/distilled water, 10/90, v/v. An oral dose of 30 µmol/kg of **1** and of 3.6 µmol/kg of **15i** were administered to rats by gastric intubation.

For both routes, animals (n=3 per group) were sacrificed at various time points after dosing (up to 24 h post-dose). Whole blood was collected in anticoagulant-coated tubes and, following centrifugation, the plasma was separated. The brains were removed, cleaned and immediately frozen on dry ice. Plasma samples and brains were stored at -80°C until analysis.

Bioanalytical assays were conducted using specific liquid chromatography-tandem mass spectrometry methods, with quantification limits of 2 and 1 pmol/mL for **1** and **15i** in plasma, respectively, and 10 and 4 pmol/g for **1** and **15i** in brain homogenates, respectively.

The following pharmacokinetic parameters were estimated for plasma using pooled samples from each time point by noncompartmental analysis of composite concentration data: plasma clearance (CL, after i.v. dosage), mean resisdence time (MRT after i.v. dosage), apparent terminal elimination half-life (t1/2, z, after i.v. dosage), time to maximum concentration after oral dosage, dose-normalized maximum concentration (Cmax) observed after oral dosage and dose-normalized area under the curve (AUC) from 0 to infinity calculated after both routes of administration. The absolute oral bioavailability was estimated by the ratio of the dose-normalized oral/intravenous AUC values. For brain, the area under the curve

(AUC) from zero to infinite was also estimated for both routes of administration and the plasma/brain AUC ratios were determined for each compound after oral administration.

Metabolism investigations

The plasma obtained from the 3 rats treated either intravenously or orally were pooled to get one plasma sample for each route.

200 µL of ice-cold acetonitrile was added to 200 µL of plasma for protein precipitation. This procedure was repeated twice during 1 hour and, after centrifugation (10000 g, 10 min), 400 µL of the supernatant were reduced in volume. The remaining residue was recovered with water/acetonitrile, 95:5 (v/v) containing 0.1% formic acid and 0.02% trifluoroacetic acid, filtered and directly used for injection (10 µL).

Capillary HPLC/MS(n) analyses

In order to protect the analytical column and to enrich the target compounds, the samples were injected via a pre-column in back-flush mode. The system consisted of an automated injection device (HTS PAL, CTC Analytics, Zwingen, Switzerland) and a trapping column (Daiso CN, 15 mm x 0.06 mm, particle size 3 µm). The trapping column was preconditioned with 50 µL water/acetonitrile (95/5), 10 µL sample were injected and washed with 20 µL water/acetonitrile (95/5). Desorption time of the analytes was achieved with the mobile phase of the HPLC pump during 15 min. After desorption, the trapping column was cleaned by washing with 50 µL of acetonitrile /THF/ water (50:45:5, v/v/v).

The liquid chromatographic separation was performed using an Chorus-220 HPLC pump (CS Analytics, Beckenried, Switzerland) and a capillary column, 150 mm x 0.3 mm, filled with Reprosil-Pur ODS-3, particle size 3 µm (Morvay Analytik GmbH, Basel, Switzerland). Gradient mobile phase programming was used with a flow rate of 4.5 µL/min. Eluent A was acetonitrile/water (5/95) + 0.5 % formic acid + 0.02 % trifluoroacetic acid. Eluent B was acetonitrile/water (95/5) + 0.5 % formic acid + 0.02 % trifluoroacetic acid. The mobile phase was kept isocratic for 2 min at 5 % B, followed by a linear gradient from 5 % B to 95 % B over 28 min and a 5 min isocratic phase at 95 % B. The column temperature was set to 50°C.

The column effluent was introduced directly into the ion source of an ion trap mass spectrometer (ThermoFinnigan LCQ Deca or LTQ, ThermoFinnigan, San Jose, CA, USA). The ionization technique employed was positive electrospray (ES). Full scan mass spectra were recorded with a mass range of 150-700 Da with a scan time of 1sec. Product ion mass spectra were recorded with a normalized collision energy of 30 %, MSn experiments were done as appropriate at a normalized collision energy of 40 %.

In vivo pharmacology

The fear-potentiated startle paradigm was used to test in vivo efficiency. First, rats were fear-conditioned by 20 pairings of a light stimulus (5s) and an electric foot shock (0.5mA, 0.5s) on two successive days (mean intertrial interval: 120s, range: 90-150s). On day three, animals were orally treated (n=10/group) and, 1h later, the startle magnitude to a 102 dB SPL noise burst (50 ms) was measured in the presence of the light stimulus as well as without (12 presentations each after 12 noise bursts for acclimatization, 30s

intertrial interval). The startle magnitude was measured via a piezoelectric device (San Diego Instruments), and fed into a computer for further analysis,

Immediately after the behavioral test, i.e. 90 mins after drug dosage, the animals were sacrificed and brains and plasma were collected for determination of compound concentrations by LC/MS/MS analysis (see above).