# Supporting Information for:

# Benzimidazole-2-pyrazole HIF Prolyl 4-Hydroxylase Inhibitors as Oral Erythropoietin Secretagogues

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Contents:

**General Experimental Details:** All reagents were used as supplied. Solvents were dried and purified by passing through a column of activated alumina.[1] Routine chromatographic purifications were performed via semi-automated MPLC using prepacked RediSep 35-60 μm silica gel columns on ISCO Sg100 systems with uv peak detection. Standard mass spectra were recorded on an Agilent single quadrupole electrospray MSD mass detector using multimode electrospray and chemical ionization (ESI/CI). High-resolution mass spectra (HRMS) were recorded on a Bruker ESI microTOF instrument using lithium formate cluster ions as reference. Proton NMR spectra were recorded at either 400 or 500 MHz using Bruker DPX-400 and DPX-500 spectrometers. Analytical HPLC data were collected on an Agilent/HP1100 LC system with diode array uv (220 and 254 nm) detection and a MeCN/water/0.05% TFA solvent gradient running on an Agilent Eclipse XDB-C8 5 μm column, and results are reported as retention time in minutes, % purity. Quantitative elemental analyses were perfomed by Numega Resonance Labs, San Diego, CA.

**Representative procedure for the synthesis of benzimidazole-2-glycine amides 3a-h. [(1H-Benzoimidazole-2-carbonyl)-amino]-acetic acid (3a)**:

**A. [(1H-Benzoimidazole-2-carbonyl)-amino]-acetic acid, methyl ester (2a)**: Triethylamine (0.77 mL, 5.5 mmol) was added dropwise to a mixture of 1H-benzimidazole-2-carboxylic acid (0.20 g, 1.2 mmol), glycine methyl ester hydrochloride (0.17 g, 1.4 mmol), HATU (0.57 g, 1.5 mmol), and DMF (10 mL). The reaction was allowed to proceed for 16 h at 23 °C. Water (25 mL) was added, and the resulting precipitate was collected and dried to provide **2a** (0.18 g, 61%). $^1$H NMR (500 MHz, DMSO-$d_6$): δ 13.29 (s, 1H), 9.23 (t, $J$ = 6.1 Hz,

1H), 7.75 (d, *J* = 7.2 Hz, 1H), 7.55 (d, *J* = 7.3 Hz, 1H), 7.31 (m, 2H), 4.08 (d, *J* = 6.1 Hz, 2H), 3.68 (s, 3H). MS (ESI/CI) *m/z* 234.1 $[M+H]^+$.

**B. [(1H-Benzoimidazole-2-carbonyl)-amino]-acetic acid (3a)**: A solution of $LiOH \cdot H_2O$ (0.090 g, 2.1 mmol) and $H_2O$ (2 mL) was added to a mixture of **2a** (0.10 g, 0.43 mmol) and THF (5 mL). The resulting mixture was stirred rapidly for 30 min, then the THF was removed *in vacuo*. A solution of 1M HCl (3 mL) was added, and the resulting precipitate was collected to provide the titled compound (0.085 g, 90%). $^1$H NMR (500 MHz, DMSO-$d_6$): δ 12.40-12.90 (broad s, 2H), 9.06 (t, *J* = 6.10, 6.1 Hz, 1H), 7.64 (s, 1H), 7.31 (m, 2H), 3.99 (d, *J* = 6.1 Hz, 2H). MS (ESI/CI) *m/z* 220.0 $[M+H]^+$. HRMS (ESI) $[M+H]^+$ *m/z* calcd. for $C_{10}H_{10}N_3O_3$, 220.0717; found, 220.0723. HPLC: 6.11 min, >99%.

**[(5,6-Dichloro-1H-benzoimidazole-2-carbonyl)-amino]-acetic acid (3b)**: The titled compound was prepared in a manner analogous to **3a**. $^1$H NMR (400 MHz, DMSO-$d_6$): δ 13.66 (s, 1H), 12.76 (s, 1H), 9.23 (t, *J* = 6.1 Hz, 1H), 8.05 (s, 1H), 7.75 (s, 1H), 3.97 (d, *J* = 6.1 Hz, 2H). MS (ESI/CI) *m/z* 288.0 $[M+H]^+$. HRMS (ESI) $[M+H]^+$ *m/z* calcd. for $C_{10}H_8Cl_2N_3O_3$, 287.9937; found, 287.9930. HPLC: 7.56 min, >99%.

**[(5-Bromo-1H-benzoimidazole-2-carbonyl)-amino]-acetic acid (3c)**: The titled compound was prepared in a manner analogous to **3a**. $^1$H NMR (400 MHz, DMSO, tautomeric mixture) δ 13.47 (d, *J* = 30.5 Hz, 1H), 12.70 (s, 1H), 9.12 (s, 1H), 8.02 – 7.34 (m, 3H), 3.98 (d, *J* = 6.1 Hz, 2H). MS (ESI/CI) *m/z* 298.0 $[M+H]^+$. HRMS (ESI) $[M+H]^+$ *m/z* calcd. for $C_{10}H_9BrN_3O_3$, 297.9822; found, 297.9820. HPLC: 6.90 min, 94%.

**[(5-Fluoro-1H-benzoimidazole-2-carbonyl)-amino]-acetic acid (3d)**: The titled compound was prepared in a manner analogous to **3a**. $^1$H NMR (400 MHz, DMSO, tautomeric mixture) δ 8.99 (t, *J* = 6.1 Hz, 1H), 7.52 (dd, *J* = 8.9, 4.9 Hz, 1H), 7.27 (dd, *J* = 9.2, 2.3 Hz, 1H), 7.04 (td, *J* = 9.6, 2.5 Hz, 1H), 3.83 (d, *J* = 6.1 Hz, 2H). MS (ESI/CI) *m/z* 238.0 $[M+H]^+$. HRMS (ESI) $[M+H]^+$ *m/z* calcd. for $C_{10}H_9FN_3O_3$, 238.0622; found, 238.0631. HPLC: 6.29 min, >99%.

**[(5-Methoxy-1H-benzoimidazole-2-carbonyl)-amino]-acetic acid (3e)**: The titled compound was prepared in a manner analogous to **3a**. $^1$H NMR (400 MHz, DMSO-$d_6$, mixture of tautomers): δ 13.22 (broad s, 1H), 12.75 (broad s, 1H), 9.02 (t, *J* = 6.0 Hz, 1H), 7.65-6.94 (m, 3H), 3.99 (d, *J* = 5.6 Hz, 2H), 3.84 (s, 3H). MS (ESI/CI) *m/z* 250.1 $[M+H]^+$. HRMS (ESI) $[M+H]^+$ *m/z* calcd. for $C_{11}H_{12}N_3O_4$, 250.0822; found, 250.0824. HPLC: 5.95 min, >99%.

**[(5,7-Bis-trifluoromethyl-1H-benzoimidazole-2-carbonyl)-amino]-acetic acid (3f)**: The titled compound was prepared in a manner analogous to **3a**. $^1$H NMR (400 MHz, DMSO-$d_6$ ): δ 1H NMR (600 MHz, DMSO-$d_6$): 14.39 (s, 1H), 12.83 (s,1H), 9.16 (s, 1H), 8.15 (s, 1H), 7.94-7.93 (m, 1H), 4.03 (d, *J* = 6.1 Hz, 2H). MS (ESI/CI) *m/z* 354.0 $[M-H]^-$. HRMS (ESI) $[M+H]^+$ *m/z* calcd. for $C_{12}H_8F_6N_3O_3$, 356.0464; found, 356.0467. HPLC: 8.28 min, >99%.

**(*S*)-2-[(5,7-Bis-trifluoromethyl-1H-benzoimidazole-2-carbonyl)-amino]-propionic acid (3g)**: The titled compound was prepared in a manner analogous to **3a**. $^1$H NMR (500 MHz, DMSO-$d_6$): δ 9.06 (d, *J* = 7.6 Hz, 1H), 8.18 (s, 1H), 7.91 (s, 1H), 4.54 ( q, *J* = 7.2 Hz, 1H), 1.48 (d, *J* =

7.3 Hz, 3H). MS (ESI/CI) *m/z* 368.0 $[M-H]^-$. HRMS (ESI) $[M+H]^+$ *m/z* calcd. for $C_{13}H_{10}F_6N_3O_3$, 370.0621; found, 370.0622. HPLC: 8.48 min, 98%.

**(*R*)-2-[(5,7-Bis-trifluoromethyl-1H-benzoimidazole-2-carbonyl)-amino]-propionic acid (3h)**: The titled compound was prepared in a manner analogous to **3a**. $^1$H NMR (500 MHz, DMSO) δ 9.08 (d, *J* = 7.6 Hz, 1H), 8.18 (s, 1H), 7.92 (s, 1H), 4.64 – 4.45 (m, *J* = 7.3 Hz, 1H), 1.48 (d, *J* = 7.3 Hz, 3H). MS (ESI/CI) *m/z* 368.0 $[M-H]^-$. HRMS (ESI) $[M+H]^+$ *m/z* calcd. for $C_{13}H_{10}F_6N_3O_3$, 370.0621; found, 370.0264. HPLC: 8.48 min, >99%.

**Representative procedure for the synthesis of benzimidazole-2-pyrazoles 6a–h. 1-(1H-Benzoimidazol-2-yl)-1H-pyrazole-4-carboxylic acid (6a)**:

**A. 1-(1H-Benzoimidazol-2-yl)-1H-pyrazole-4-carboxylic acid ethyl ester hydrochloride (5a)**: A mixture of NaH (60% dispersion in oil, 0.40 g, 9.8 mmol) and THF (10 mL) was cooled to 0 °C, then solid 2-chlorobenzoimidazole (1.0 g, 6.5 mmol) was added portion wise over 10 min. The resulting mixture was stirred at 0 °C for 1 h, then 2-trimethylsilylethoxymethyl chloride (1.5 mL, 8.5 mmol) was added. The reaction mixture was allowed to warm to 23 °C and was stirred 16 h. The mixture was carefully poured over ice (ca. 200 g) and then was extracted with $Et_2O$ (3 × 100 mL). The combined organic extracts were dried and concentrated, and the residue was chromatographed (1:99 to 15:85 EtOAc/hexanes) to provide 2-chloro-1-(2-trimethylsilanyl-ethoxymethyl)-1H-benzoimidazole, which has been previously described: PCT Int. Appl. (2005), 465 pp. CODEN: PIXXD2 WO 2005012297 A1 20050210 CAN 142:219286 AN 2005:120926.

A portion of the above 2-chloro-1-(2-trimethylsilanyl-ethoxymethyl)-1H-benzoimidazole (0.34 g, 1.2 mmol), ethyl pryazole-4-carboxylate (0.24 g, 1.7 mmol), cesium carbonate (0.78 g, 2.4 mmol), and anhydrous DMF (2.5 mL) was stirred at 100 °C for 5 h. The mixture was allowed to cool to 23 °C and was diluted with EtOAc, then filtered through a pad of silica gel. The resulting solution was concentrated and the residue was chromatographed (5:95 to 40:60 EtOAc/hexanes) to provide 1-[1-(2-trimethylsilanyl-ethoxymethyl)-1H-benzoimidazol-2-yl]-1H-pyrazole-4-carboxylic acid ethyl ester (0.36 g, 77%). $^1$H NMR (500 MHz, $CDCl_3$): δ 8.88 (s, 1H), 8.18 (s, 1H), 7.77-7.69 (m, 1H), 7.60-7.50 (m, 1H), 7.40-7.30 (m, 2H), 6.03 (s, 2H), 4.34 (q, *J* = 7.1 Hz, 2H), 3.57-3.50 (m, 2H), 1.37 (t, *J* = 7.1, Hz, 3H), 0.87-0.80 (m, 2H), -0.11 (s, 9H).

A solution of HCl and dioxane (4M, 2 mL, 8 mmol) was added to a portion of the above 1-[1-(2-trimethylsilanyl-ethoxymethyl)-1H-benzoimidazol-2-yl]-1H-pyrrole-3-carboxylic acid ethyl ester (0.30 g, 0.78 mmol) and EtOH (4 mL). The reaction mixture was heated to reflux for 30 min, then cooled to 23 °C. $Et_2O$ was added (20 mL), and the mixture was cooled to 0 °C for 10 min. The resulting precipitate was collected by filtration and washed well with $Et_2O$ to afford the titled compound (0.18 g, 91%). $^1$H NMR (400 MHz, DMSO-$d_6$): δ 8.96 (s, 1H), 8.33 (s, 1H), 7.56 (s, 2H), 7.28-7.21 (m, 2H), 4.30 (q, *J* = 7.1 Hz, 2H), 1.32 (t, *J* = 7.1 Hz, 3H). MS (ESI/CI) *m*/*z* 257.1 $[M+H]^+$.

**B. 1-(1H-Benzoimidazol-2-yl)-1H-pyrazole-4-carboxylic acid (6a)**: A solution of LiOH and $H_2O$ (1.0 M, 1.0 mL, 1.0 mmol) was added to a mixture of 1-(1H-benzoimidazol-2-yl)-1H-pyrrole-3-carboxylic acid ethyl ester hydrochloride (0.040 g, 0.16 mmol) and THF (2.0 mL), and the reaction mixture was stirred at 23 °C for 16 h. The THF was removed *in vacuo* and then aqueous HCl (1.0 M, 2 mL, 2 mmol) was added at 0 °C. The resulting precipitate was collected and washed with water to give the titled compound (0.033 g, 90%). $^1$H NMR (500

MHz, DMSO-$d_6$): δ 13.32 (s, 1H), 13.00-12.86 (br. s, 1H), 8.90 (d, $J$ = 0.6 Hz, 1H), 8.28 (d, $J$ = 0.6 Hz, 1H), 7.64 (d, $J$ = 4.6 Hz, 1H), 7.49 (d, $J$ = 5.5 Hz, 1H), 7.28-7.20 (m, 2H). MS (ESI/CI) $m/z$ 229.0 $[M+H]^+$. HRMS (ESI) $[M+H]^+$ $m/z$ calcd. for $C_{11}H_9N_4O_2$, 229.0720; found, 229.0724. HPLC: 6.84 min, >99%.

**1-(5-Bromo-1H-benzoimidazol-2-yl)-1H-pyrazole-4-carboxylic acid (6b)**: The titled compound was prepared in a manner analogous to **6a**. $^1$H NMR (400 MHz, DMSO-$d_6$, tautomeric mixture): δ 8.82 (d, $J$ = 0.5 Hz, 1H), 8.22 (s, 1H), 7.67 (d, $J$ = 1.2 Hz, 1H), 7.45 (d, $J$ = 8.5 Hz, 1H), 7.32 (dd, $J$ = 8.5, 1.9 Hz, 1H). MS (ESI/CI) $m/z$ 307.0 $[M+H]^+$. HRMS (ESI) $[M+H]^+$ $m/z$ calcd. for $C_{11}H_8BrN_4O_2$, 306.9825; found, 306.9825. HPLC: 7.69 min, >99%.

**1-(5-Methoxy-1H-benzoimidazol-2-yl)-1H-pyrazole-4-carboxylic acid (6c):** The titled compound was prepared in a manner analogous to **6a**. $^1$H NMR (500 MHz, DMSO-$d_6$, tautomeric mixture): δ 13.16 (s, 1H), 12.91 (s, 1H), 8.84 (s, 1H), 8.26 (s, 1H), 6.83-7.54 (m, 3H), 3.80 (s, 3H). MS (ESI/CI) $m/z$ 259.1 $[M+H]^+$. HRMS (ESI) $[M+H]^+$ $m/z$ calcd. for $C_{12}H_{11}N_4O_3$, 259.0826; found, 259.0822. HPLC: 6.62 min, 98%.

**1-(5-Chloro-6-fluoro-1H-benzoimidazol-2-yl)-1H-pyrazole-4-carboxylic acid (6d)**: The titled compound was prepared in a manner analogous to **6a**. $^1$H NMR (500 MHz, DMSO-$d_6$, tautomeric broadening): δ 14.21-12.25 (br. s, 2H), 8.88 (d, $J$ = 0.6 Hz, 1H), 8.30 (d, $J$ = 0.6 Hz, 1H), 7.81-7.67 (br. s, 1H), 7.65-7.52 (br. s, 1H). MS (ESI/CI) $m/z$ 279.0 $[M-H]^-$. HRMS (ESI) $[M+H]^+$ $m/z$ calcd. for $C_{11}H_7ClFN_4O_2$, 281.0236; found, 281.0232. HPLC: 7.65 min, >99%.

**1-(5-Trifluoromethoxy-1*H*-benzoimidazol-2-yl)-1*H*-pyrazole-4-carboxylic acid (6e)**: The titled compound was prepared in a manner analogous to **6a**. $^1$H NMR (400 MHz, DMSO-$d_6$, tautomeric broadening): δ 8.91 (s, 1H), 8.31 (s, 1H), 7.83-7.41 (m, 2H), 7.30-7.21 (m, 1H). MS (ESI/CI) *m*/*z* 313.1 $[M+H]^+$. HPLC: 7.08 min, >99%.

**1-(4-Bromo-6-trifluoromethoxy-1*H*-benzoimidazol-2-yl)-1*H*-pyrazole-4-carboxylic acid (6f)**: The titled compound was prepared in a manner analogous to **6a**.[2] $^1$H NMR (400 MHz, DMSO-$d_6$): δ 13.98 (s, 1H), 12.94 (s, 1H), 8.90 (s, 1H), 8.32 (d, *J* = 0.4 Hz, 1H), 7.57–7.52 (m, 1H), 7.48 (s, 1H). MS (ESI/CI) *m*/*z* 391.0 $[M+H]^+$. HRMS (ESI) $[M+H]^+$ *m*/*z* calcd. for $C_{12}H_7BrF_3N_4O_3$, 390.9648; found, 390.9649. HPLC: 8.31 min, >99%.

**1-(5-Chloro-6-trifluoromethyl-1*H*-benzoimidazol-2-yl)-1*H*-pyrazole-4-carboxylic acid (6g)**: The titled compound was prepared in a manner analogous to **6a**. $^1$H NMR (500 MHz, DMSO-$d_6$): δ 8.93 (s, 1H), 8.34 (s, 1H), 7.99 (s, 1H), 7.85 (s, 1H). $^{13}$C NMR (151 MHz, DMSO-$d_6$) δ 162.85, 147.79, 143.0, 131.71, 124.02, 123.44 (q, *J* = 272.3 Hz), 120.56 (q, *J* = 30.5), 118.63.[4] MS (ESI/CI) *m*/*z* 331.0 $[M+H]^+$. HRMS (ESI) $[M+H]^+$ *m*/*z* calcd. for $C_{12}H_7ClF_3N_4O_2$, 331.0204; found, 331.0202. HPLC: 8.15 min, >99%. Anal. Calcd for $C_{12}H_6ClF_3N_4O_2$: C, 43.59; H, 1.83; N, 16.94. Found: Found: C, 43.34; H, 2.04; N, 16.82.

**1-(5-Fluoro-6-trifluoromethyl-1*H*-benzoimidazol-2-yl)-1*H*-pyrazole-4-carboxylic acid (6h)**: The titled compound was prepared in a manner analogous to **6a**.[2] $^1$H NMR (400 MHz, $CD_3OD$,

tautomeric broadening): δ 8.93 (s, 1H), 8.20 (s, 1H), 7.87 (broad s, 1H), 7.49 (broad s, 1H). $^{13}$C NMR (151 MHz, DMSO-$d_6$) δ 162.85, 155.35 (d, $J$ = 244.5 Hz), 147.71, 143.88, 131.63, 123.25 (q, $J$ = 271.27 Hz), 118.53, 111.75 – 111.00 (m).[4] MS (ESI/CI) $m/z$ 315.1 $[M+H]^+$. HRMS (ESI) $[M+H]^+$ $m/z$ calcd. for $C_{12}H_7F_4N_4O_2$, 315.0500; found, 315.0500. HPLC: 7.92 min, >99%. Anal. Calcd for $C_{12}H_6F_4N_4O_2$: C, 45.87; H, 1.92; N, 17.83. Found: C, 45.78; H, 2.20; N, 17.62.

**Assay Details**

*PHD2 enzymatic assay*

The PHD2 enzymatic assay was performed as previously described.[2]

*In-vitro EPO assay by MSD*

Hep3B cells were plated at 20,000 cells per well in 96-well plate, in 100 µl of DMEM, 10% serum, supplemented with NEAA and antibiotics. 24 hours after plating, 1.1 µl of a solution of compound and 100% DMSO was added, followed by incubation for an additional 24 hours after compound addition. 50µl of the supernatant was tested by using MSD Hypoxia plate. EPO was measured using Meso Scale Discovery's MSD Hypoxia plates, an ELISA-like assay based on a combination of electrochemiluminescence detection and patterned array, which can simultaneously detect EPO, IGFBP-1 and VEGF. Concentration was determined according to manufacturer's instructions. Briefly, 50 µl of the supernatant is transferred to a BSA blocked MSD Hypoxia plate and incubated at room temperature in an orbital shaker for 2 h. 25 µl of

antibody solution containing 0.5 µg/ml of anti-EPO, anti-VEGF and anti-IGFBP-1 detection antibody is added and incubated for an additional 2 h. at room temperature in an orbital shaker. After 4 washes in PBS, 150 µl of 1X read buffer is added and the plate is then read on the SECTOR™ instrument. The reported percent stimulation at 100 uM is relative to reference compound 7-[(4-chlorophenyl)-(5-methylisoxazol-3-ylamino)-methyl]-quinolin-8-ol (CAS # 496013-45-7) at 10uM.

*In-vitro HIF assay by MSD*

Hep3B cells were plated at 20,000 cells per well in 96-well plate, in 100 µl of DMEM, 10% serum, supplemented with NEAA and antibiotics. 24 hours after plating, 1.1 µl of a solution of compound and 100% DMSO was added, followed by incubation for an additional 24 hours after compound addition. The supernatant was removed and the cells were lysed for HIF measurement by using MSD human HIF-1a plate. 55 µl of MSD lysis buffer containing protease inhibitors was added to plate. 45 µl of the cell lysate was then transferred to a blocked MSD HIF-1α detection plate and incubated at room temperature in an orbital shaker for 2 h. After 4 washes in MSD Tris wash buffer, 25 µl of 10 nM anti-HIF-1α detection antibody was added for 1 hour at room temperature in an orbital shaker. After 4 washes in MSD Tris wash buffer, 150 µl of 1X read buffer was added and the plate was then read on the SECTOR™ instrument. The reported percent stimulation at 100 uM is relative to reference compound 7-[(4-chlorophenyl)-(5-methylisoxazol-3-ylamino)-methyl]-quinolin-8-ol (CAS # 496013-45-7) at 10uM.

*In-vivo epo release*

*In-vivo* studies were performed using male Balb-C mice (BALB/c AnNCrl) obtained from Charles River laboratory (strain code 028). Animals were placed on 5008 Lab Diet brand standard chow upon arrival in a 12-hour/12-hour dark cycle (6am-6pm for light portion). The animals arrived at 8 weeks of age and the study was performed two weeks post-arrival. Body weights were recorded on the morning of the study prior to dosing. Compound was formulated using 5% NMP and 20% Hp-b-cd and pH balanced using 1M NaOH. Animals were housed four per cage and were assigned to either a vehicle group or treatment group. Animals were orally dosed at a dose volume of 10ml/kg and a concentration of 100umol/kg. One-hour and six-hours post-dose the appropriate groups of animals were euthanized using carbon dioxide and blood was taken via a cardiac puncture. Blood was collected in EDTA tubes and spun at 4 °C for four-minutes prior to plasma removal. Epo level determination was performed for the six hour samples vs. vehicle group. Epo levels were quantified using MSD MULTI-SPOT Assay: Mouse/Rat Hypoxia Panel. All data are presented as means ± standard error. Comparisons among groups were made using a one-way ANOVA followed by a Dunnett's test. A p value of less than 0.05 was considered to be statistically significant.

**X-ray Crystallography**

**PHD/2-Oxoglutarate:** PHD2-R127 at 12mg/ml was co-crystallized with 2-OG by sitting drop vapor diffusion against 100 mM MES, pH 6.4, 0.2M Ammonium Sulfate, 28% PEG 8000. The dataset for the 2-OG structure was collected at the Argonne Advanced Photon Source using beamline 23ID. The data were reduced with HKL2000 [Otwinowski 1997 Methods Enzymology 276, 307-326] and molecular replacement (Molrep) was done using the CCP4 suite [1994 Acta

Cryst D50, 760-763] and the 2G19 model [McDonough, M.A. 2006 PNAS 103, 9814-9819]. Model building and refinement were done using Coot [Emsley 2004 Acta Cryst D 60, 2126-2132] and Refmac5 [Murshudov 1997 Acta Cryst D 53, 240-255].

**PHD2/Compound 3b:** PHD2-R717 at 12mg/ml was co-crystallized with **3b** by sitting drop vapor diffusion against 100 mM Na Acetate, pH 5.5, 0.2M Ammonium Sulfate, 2% PEG 400. The dataset for the 40787422 structure was collected at the Argonne Advanced Photon Source using beamline 24ID. The data were reduced with HKL2000 [Otwinowski 1997 Methods Enzymology 276, 307-326] and molecular replacement (Molrep) was done using the CCP4 suite [1994 Acta Cryst D50, 760-763] and a model derived from the 2G19 model [McDonough, M.A. 2006 PNAS 103, 9814-9819]. Model building and refinement were done using Coot [Emsley 2004 Acta Cryst D 60, 2126-2132] and Refmac5 [Murshudov 1997 Acta Cryst D 53, 240-255].

**PHD2/Compound 6d:** PHD2-R127 at 15mg/ml was co-crystallized with **6d** by sitting drop vapor diffusion against 100mM Imidazole pH 6.50, 2M Ammonium Sulfate 100mM Magnesium Sulfate, 10% Glycerol. The dataset for the 41536014 structure was collected at the Stanford Synchrotron Radiation Lab using beamline 7-1 (ADSC Q315R detector). The data were reduced with HKL2000 [Otwinowski 1997 Methods Enzymology 276, 307-326] and molecular replacement (Molrep) was done using the CCP4 suite [1994 Acta Cryst D50, 760-763] and a model derived from the 2G19 model [McDonough, M.A. 2006 PNAS 103, 9814-9819]. Model building and refinement were done using Coot [Emsley 2004 Acta Cryst D 60, 2126-2132] and Refmac5 [Murshudov 1997 Acta Cryst D 53, 240-255].

**References and Notes**

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(2) For preparation of 2-chloro-4-bromo-6-trifluoromethylbenzimidazole and 2-chloro-5-fluoro-6-trifluoromethylbenzimidazole, see: Hocutt, F. M.; Leonard, B.E.; Peltier, H.M.; Phuong, V.K.; Rabinowitz, M.H.; Rosen, M.D.; Tarantino, K.T.; Venkatesan, H.; Zhao, L.X. Preparation of Benzoimidazoles as Prolyl Hydroxylase Inhibitors. PCT Int. Appl. (2009), 213pp. CODEN: PIXXD2 WO 2009134750 A1 20091105 CAN 151:528765 AN 2009:1363853 CAPLUS.

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(4) In most cases, tautomeric and rotomeric broadening was observed in the NMR spectra. In the case of poorly resolved examples, broadening prevented the observation of all carbon atoms, particularly the carbon atoms closest to the tautomeric nitrogen atoms (carbons 2, 3a and 7a, benzimidazole numbering). See: (a) Kihel, A. E.; Ahbala, M.; Essassi, E. M.; Danion-Bougot, R.; Bauchat, P. *Phys. Chem. News* **2005**, *25*, 130-134. (b) Sridharan, V.; Saravanan, S.; Muthusubramanian, S.; Sivasubramanian, S. *Magn. Reson. Chem.* **2005**, *43*, 551-556.