Supporting Information

Selective Small Molecule Probes for the Hypoxia Inducible Factor (HIF) Prolyl Hydroxylases

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Supplementary Schemes

Spectroscopic data for Tested Compounds

Supplementary Figures

Supplementary Tables

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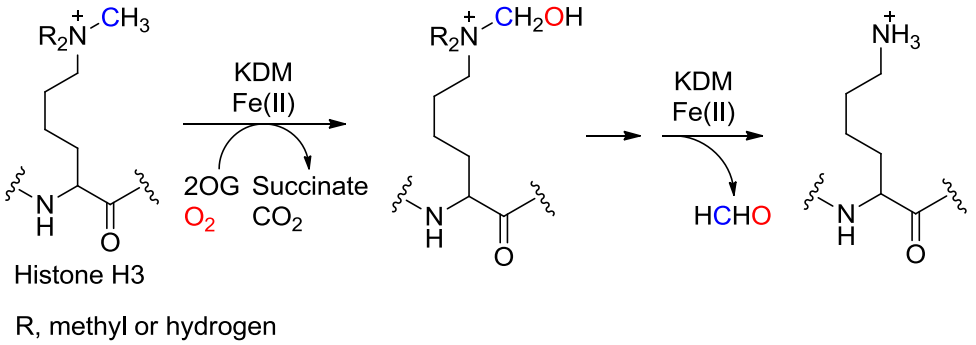
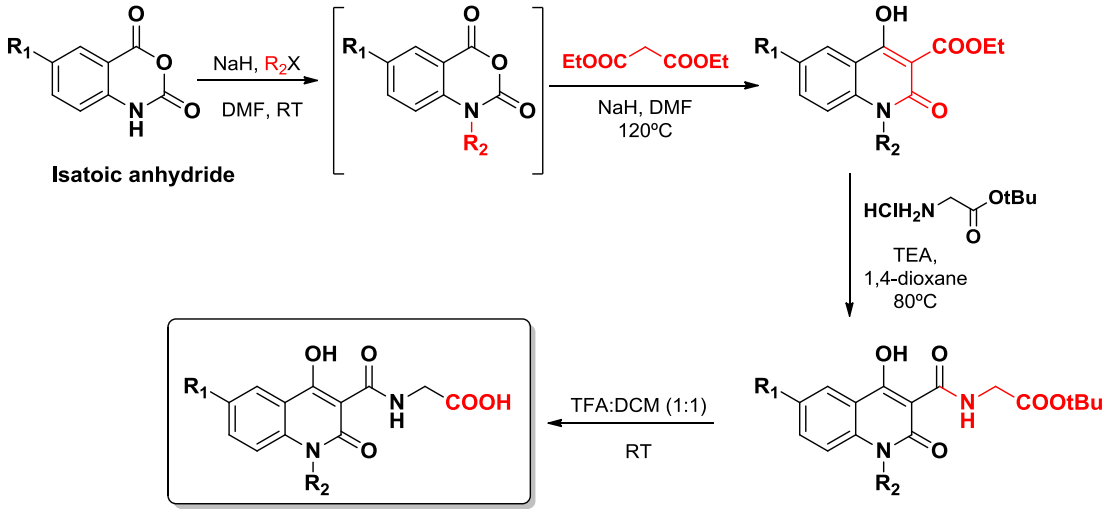
Supplementary Schemes

Supplementary Scheme 1. Catalysis of histone demethylation by 2OG dependent histone demethylases (KDMs).



Supplementary Scheme 2. Syntheses of 4HQ derivatives. Compounds **4**, **6**, **8**, **7** and **9** were synthesized as described in three steps starting from the commercially available isatoic anhydride or its 5-bromo derivative.[1] One pot reaction with benzyl/alkyl bromides, followed by treatment with diethyl malonate, under basic conditions, led to the dihydroquinoline derivatives. Coupling with *tert*-butyl glycinate hydrochloride at reflux and subsequent *tert*-butyl ester deprotection with CF_3COOH gave the desired compounds.



4: R_1= H, R_2= Me
6: R_1= H, R_2= Bn
8: R_1= H, R_2= octyl
7: R_1= H, R_2= allyl
9: R_1= Br, R_2= Bn

5: R_1= H, R_2= Me

Spectroscopic Data for Tested Compounds

*2-(4-Hydroxy-1-methyl-2-oxo-1,2-dihydroquinoline-3-carboxamido)acetic acid (**4**)*

^{1}H NMR (500 MHz, DMSO-d_6): δ = 16.98 (s, 1H, OH), 12.94 (bs, 1H, COOH), 10.56 (t, 1H, *J* = 5.5 Hz, NH), 8.08 (dd, 1H, *J* = 1.2, 8.0 Hz, ArCH), 7.81 (ddd, 1H, *J* = 1.4, 7.3, 8.6 Hz, ArCH), 7.62 (d, 1H, *J* = 8.5 Hz, ArCH), 7.38 (t, 1H, *J* = 7.4 Hz, ArCH), 4.14 (d, 2H, *J* = 5.6 Hz, CH_2), 3.63 (s, 3H, CH_3) ppm; ^{13}C NMR (125 MHz, DMSO-d_6): δ = 171.0, 170.7, 170.4, 161.4, 139.8, 134.4, 124.5, 122.5, 115.4, 114.9, 96.0, 40.9, 29.0, ppm; HRMS (ESI$^-$) calcd for $C_{13}H_{12}N_2NaO_5$ $(M-H)^-$ 299.0644; found, 299.0638.

tert-*Butyl 2-(4-hydroxy-1-methyl-2-oxo-1,2-dihydroquinoline-3-carboxamido)acetate (**5**)*

^{1}H NMR (500 MHz, $CDCl_3$): δ = 16.61 (s, 1H, OH), 10.77 (bs, 1H, NH), 8.21 (dd, 1H, *J* = 1.6, 8.0 Hz, ArCH), 7.69 (ddd, 1H, *J* = 1.6, 7.2, 8.6 Hz, ArCH), 7.36 (d, 1H, *J* = 8.6 Hz, ArCH), 7.30 (t, 1H, *J* = 7.6 Hz, ArCH), 4.14 (d, 2H, *J* = 5.4 Hz, CH_2), 3.70 (s, 3H, CH_3), 1.51 (s, 9H, $C(CH_3)_3$ ppm; ^{13}C NMR (125 MHz, $CDCl_3$): δ = 171.6, 171.1, 168.3, 162.6, 140.0, 133.8, 125.5, 122.3, 116.0, 114.2, 96.9, 82.3, 41.8, 29.2, 28.0 (3C) ppm; HRMS (ESI$^-$) calcd for $C_{17}H_{20}N_2O_5$ $(M-H)^-$ 331.1294; found, 331.1299.

*2-(4-Hydroxy-1-benzyl-2-oxo-1,2-dihydroquinoline-3-carboxamido)acetic acid (**6**)*

^{1}H NMR (500 MHz, DMSO-d_6): δ = 12.97 (bs, 1H, COOH), 10.54 (t, 1H, *J* = 5.3 Hz, NH), 8.13 (d, 1H, *J* = 8.1 Hz, ArCH), 7.71 (t, 1H, *J* = 8.0 Hz, ArCH), 7.48 (d, 1H, *J* = 8.7 Hz, ArCH), 7.39-7.20 (m, 6H, ArCH), 5.56 (s, 2H, $PhCH_2$), 4.16 (d, 2H, *J* = 5.5 Hz, CH_2) ppm; ^{13}C NMR (125 MHz, DMSO-d_6): δ = 171.5, 170.7, 170.4, 161.7, 139.2, 136.6, 134.4, 128.7 (2C), 127.1, 126.4 (2C), 124.8, 122.7, 115.7, 115.4, 95.9, 44.6, 41.0 ppm; HRMS (ESI$^-$) calcd for $C_{19}H_{16}N_2O_5$ $(M-H)^-$ 351.0981; found, 351.0986.

*2-(4-Hydroxy-1-allyl-2-oxo-1,2-dihydroquinoline-3-carboxamido)acetic acid (**7**)*

^{1}H NMR (500 MHz, DMSO-d_6): δ = 17.13 (bs, 1H, OH), 10.53 (s, 1H, NH), 8.12 (dd, 1H, *J* = 1.2, 8.2 Hz, ArCH), 7.79 (t, 1H, *J* = 7.8 Hz, ArCH), 7.54 (d, 1H, *J* = 8.5 Hz, ArCH), 7.38 (t, 1H, *J* = 7.4 Hz, ArCH), 5.97 (dddd, 1H, *J* = 4.5, 5.0, 10.5, 17.2 Hz,=CH), 5.16 (dd, 1H, *J* = 1.3, 10.5 Hz,=CH_2), 5.01 (dd, 1H, *J* = 1.3, 17.2 Hz,=CH_2), 4.94 (bs, 2H, CH_2) 4.12 (d, 2H, *J* = 5.5 Hz, CH_2) ppm; ^{13}C NMR (125 MHz, DMSO-d_6): δ = 171.4, 170.6, 170.3, 161.2, 139.1, 134.3, 132.4, 124.6, 122.6, 116.4, 115.8, 115.2, 95.8, 43.6, 41.2 ppm; HRMS (ESI$^-$) calcd for $C_{15}H_{14}N_2O_5$ $(M-H)^-$ 301.0825; found, 301.0830.

*2-(4-Hydroxy-1-octyl-2-oxo-1,2-dihydroquinoline-3-carboxamido)acetic acid (**8**)*

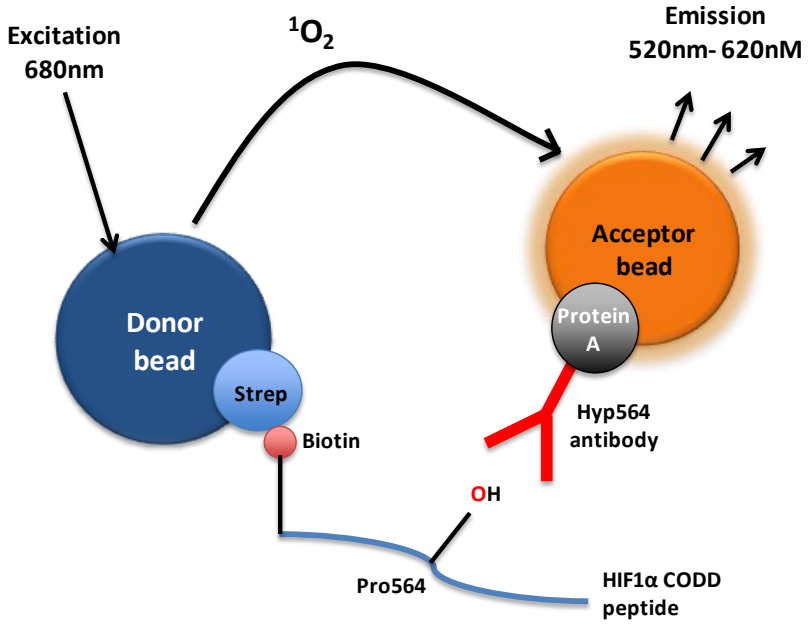
^{1}H NMR (500 MHz, DMSO-d_6): δ = 16.98 (s, 1H, OH), 12.94 (bs, 1H, COOH), 10.58 (t, 1H, *J* = 5.6 Hz, NH), 8.09 (dd, 1H, *J* = 1.4, 8.1 Hz, ArCH), 7.81 (ddd, 1H, *J* = 1.5, 7.5, 8.7 Hz, ArCH), 7.63 (d, 1H, *J* = 8.7 Hz, ArCH), 7.36 (t, 1H, *J* = 7.5 Hz, ArCH), 4.24 (t, 2H, *J* = 7.9 Hz, CH_2), 4.13 (d, 2H, *J* = 5.6 Hz, CH_2), 1.61 (quintet, 2H, *J* = 7.6 Hz, CH_2), 1.45-1.20 (m, 10H, CH_2), 0.85 (t, 3H, *J* = 7.1 Hz, CH_3) ppm; ^{13}C NMR (125 MHz, DMSO-d_6): δ = 171.0, 170.7, 170.4, 161.2, 139.0, 134.4, 124.7, 122.4, 115.2, 115.1, 95.9, 41.5, 40.9, 31.2, 28.7, 28.6, 27.2, 26.3, 22.0, 13.9 ppm; HRMS (ESI$^-$) calcd for $C_{20}H_{26}N_2O_5$ $(M-H)^-$ 397.1739; found, 397.1734.

*2-(6-bromo-4-Hydroxy-1-benzyl-2-oxo-1,2-dihydroquinoline-3-carboxamido)acetic acid (**9**)*

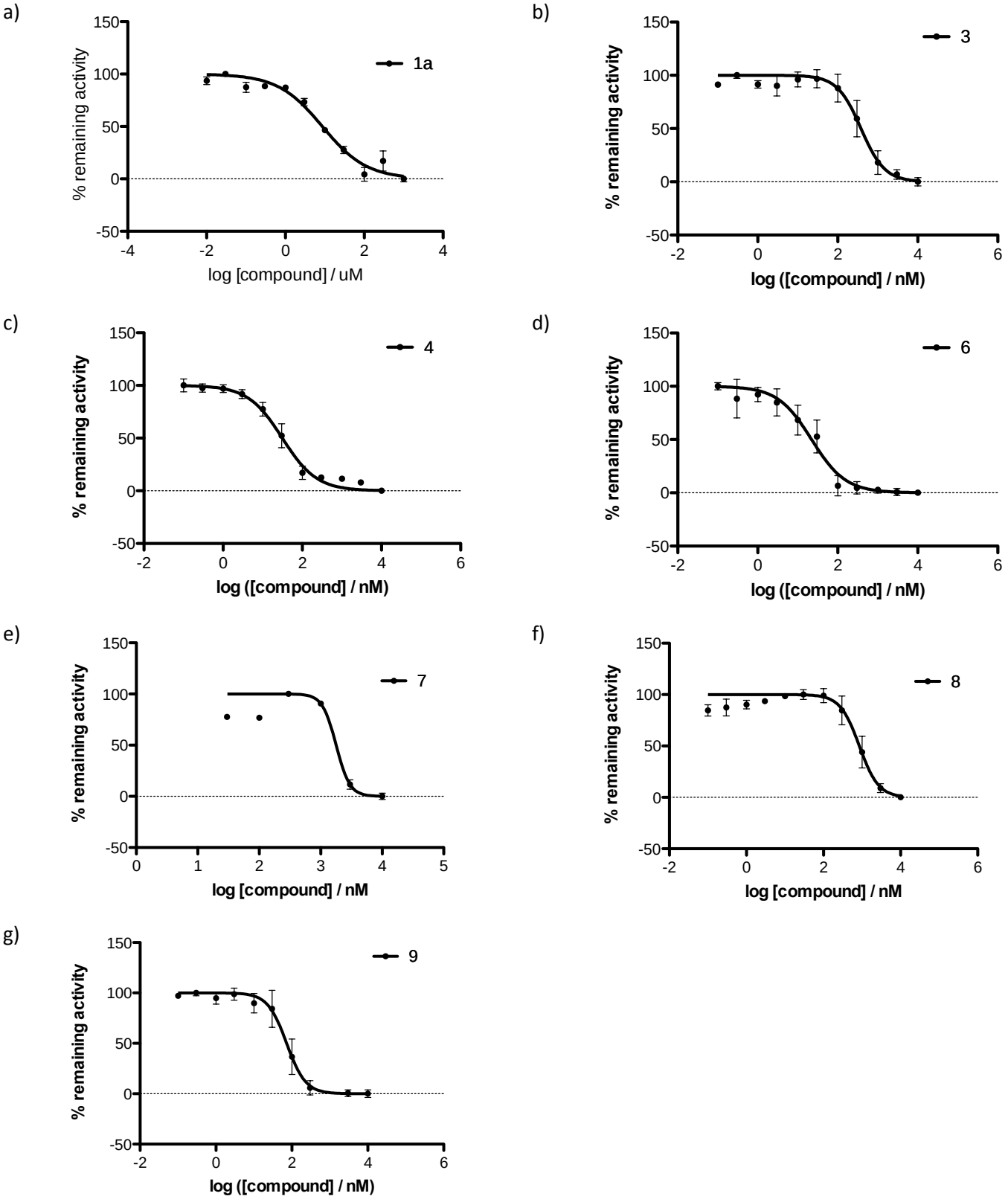
^{1}H NMR (500 MHz, DMSO-d_6): δ = 17.30 (bs, 1H, OH), 10.47 (t, 1H, *J* = 5.3 Hz, NH), 8.18 (d, 1H, *J* = 2.3 Hz, ArCH), 7.86 (dd, 1H, *J* = 2.3, 9.2 Hz, ArCH), 7.43 (d, 1H, *J* = 9.1 Hz, ArCH), 7.32 (t, 2H, *J* = 7.6 Hz, ArCH), 7.25 (t, 1H, *J* = 7.2 Hz, ArCH), 7.20 (d, 2H, *J* = 7.6 Hz, ArCH), 5.54 (bs, 2H, $PhCH_2$), 4.15 (d, 2H, *J* = 5.5 Hz, CH_2) ppm; ^{13}C NMR (125 MHz, DMSO-d_6): δ = 170.5, 170.4, 170.2, 161.5, 138.3, 136.7, 136.2, 128.7 (2C), 127.2, 126.6, 126.3 (2C), 118.2, 117.1, 114.8, 96.5, 44.8, 41.1 ppm; HRMS (ESI$^-$) calcd for $C_{19}H_{15}BrN_2O_5$ $(M-H)^-$ 429.0086; found, 429.0092.

Supplementary Figures

Supplementary Figure 1. Principles of the Amplified Luminescent Proximity Homogeneous Assay (AlphaScreen) for PHD2.

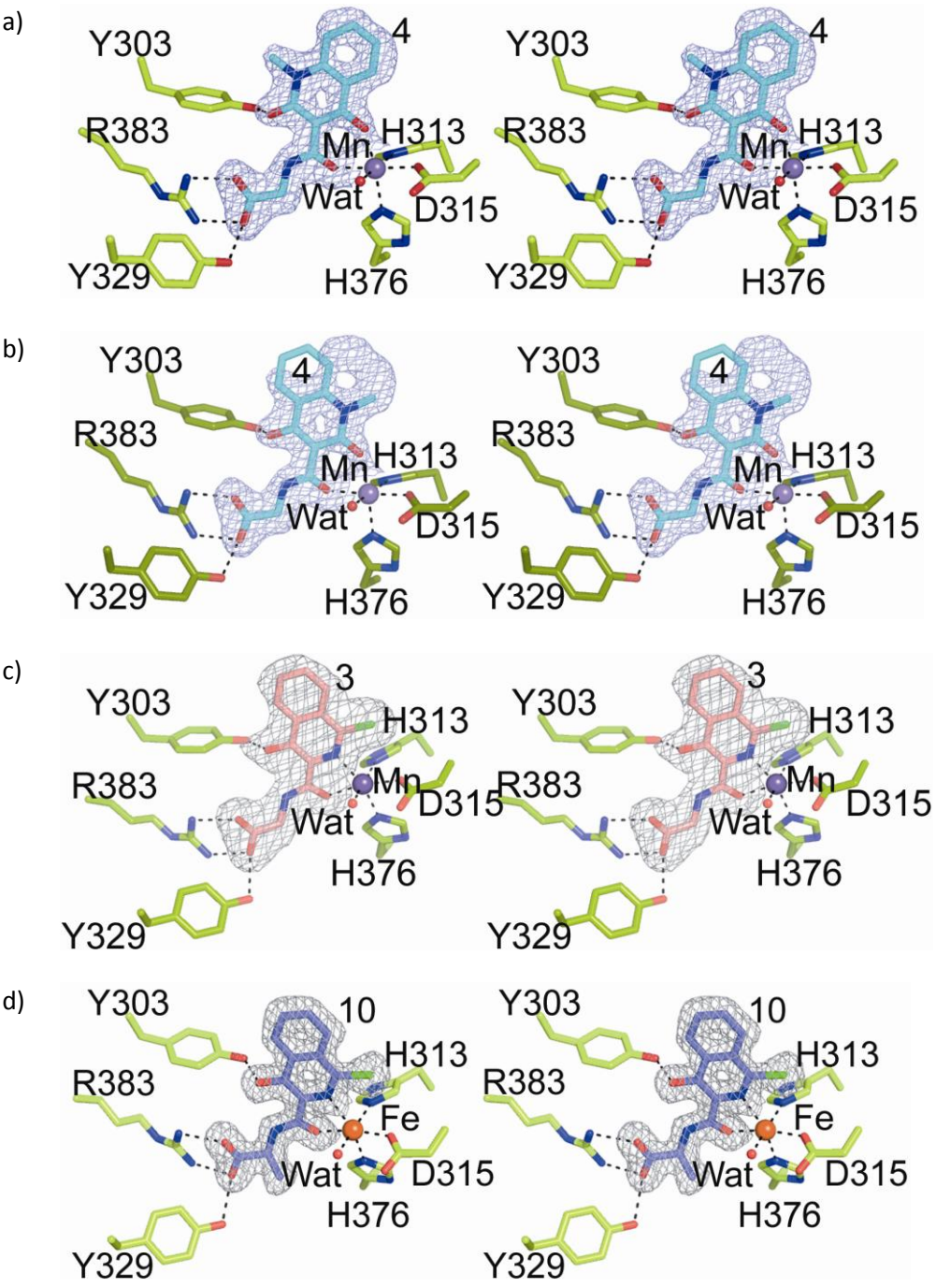


Supplementary Figure 2. Representative inhibition plots for compounds **1a**, **3**, **4**, **6**, **7**, **8** and **9** obtained using the PHD2 AlphaScreen assay. Values for each data point are averages ± standard deviation (n≥3).



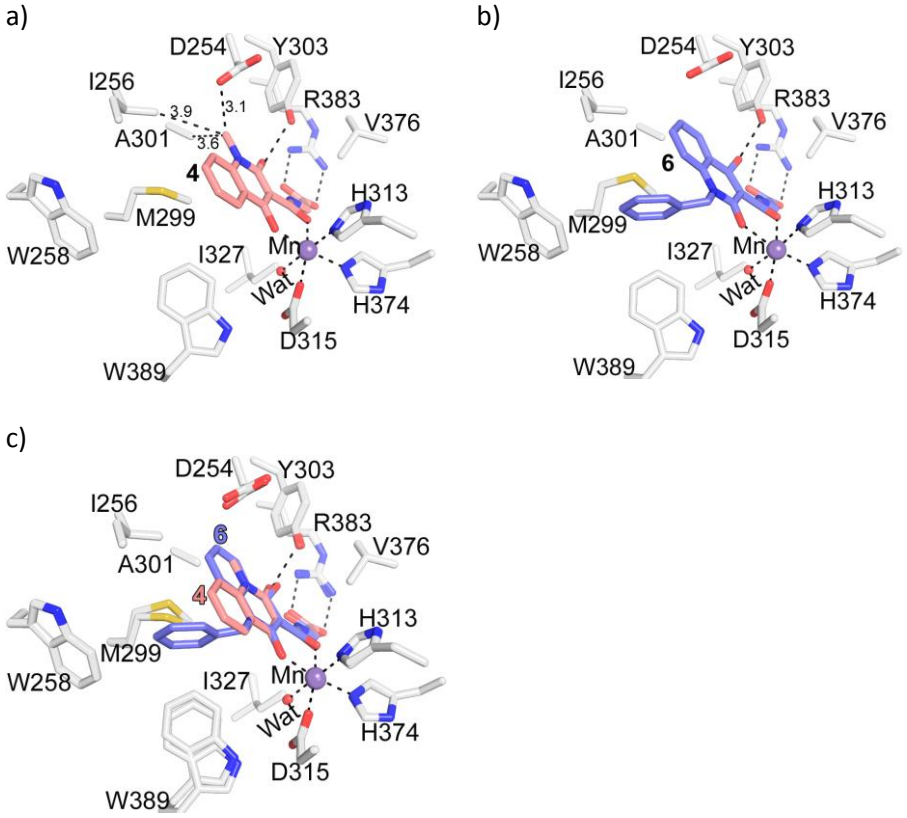
Supplementary Figure 3. Stereo-views from crystal structures of PHD2 in complex with **4** (a and b), **3** (c) and **15** (d)

showing the *Fo*-*Fc* OMIT map (contoured to 3σ) for the ligands. Because NMR evidence reveals that compound **4** can bind to the metal in the PHD2 active site in two modes (named 'X' and 'F' orientations) in solution,[2] attempts were made to model **4** in both these possible orientations ('X' as in S3A and 'F' as in S3B). Simulated annealed OMIT map analyses show that **4** only fits the map when in the 'X' orientation (as in S1a, map contoured to 3σ). Dotted lines indicate apparent hydrogen-bonds/ polar interactions.



Supplementary Figure 4. Comparison of binding modes for PHD inhibitors. View from a crystal structure of PHD2 in complex with **4** (a). A model predicting the binding mode for PHD2 in complex with Mn(II) and **6** (shown in b) was

generated using PHD2.Mn(II).**4** structure as the template. c) View from the superimposed PHD2.Mn(II).**4** crystal structure and a PHD2.Mn(II).**6** model. Parameter and topology files for **6** were generated using PRODRG.[3] The PHD2.Mn(II).**6** model was conjugate energy minimized using CNS (version 1.3)[4] without applying external energy terms. Note that the benzyl group of **6** is predicted to locate in a hydrophobic region.



Supplementary Table 1. Crystallographic data processing, refinement statistics of tPHD2 complexes with 3, 4 and 10.

Measurement	tPHD2.Mn(II).3	tPHD2.Mn(II).4	tPHD2.Fe(II).10
PDB acquisition codes	4BQX	4BQW	4BQY
Data collection			
Space Group	$P6_3$	$P6_3$	$P6_3$
Cell dimensions a,b,c (Å)	109.600	109.914	110.885
	109.600	109.914	110.885
	39.330	39.409	40.432
Resolution (Å)	54.8 – 1.79	23.8 – 1.79	36.3 – 1.55
	(1.89 – 1.79)*	(1.85 – 1.79)*	(1.62 – 1.55)*
No. of unique reflections	25470 (3690)*	25705 (2568)*	43534 (4284)*
Completeness (%)	99.0 (99.9)*	98.9 (99.9)*	95.5 (94.7)*
Redundancy	5.9 (5.9)*	5.3 (3.5)*	2.5 (2.3)*
R_{sym}**	0.067 (0.859)*	0.055 (0.681)*	0.051 (0.439)*
Mean I/σ(I)	11.3 (2.1)*	22.4 (2.1)*	16.1 (1.8)*
Wilson B value (Å^2)	27.1	26.2	22.5
Refinement			
R_{factor}	0.211	0.195	0.212
R_{free}	0.229	0.201	0.227
R.m.s. deviation			
Bond length, Å	0.006	0.006	0.01
Bond angle, °	1.2	1.5	1.5

*Highest resolution shell shown in parenthesis.

**$R_{sym} = \sum|I-\langle I\rangle|/\sum I$, where I is the intensity of an individual measurement and $\langle I\rangle$ is the average intensity from multiple observations.

$R_{factor} = \sum_{hkl}||F_{obs}(hkl)| - k|F_{calc}(hkl)||/\sum_{hkl}|F_{obs}(hkl)|$ for the working set of reflections; R_{free} is the R_{factor} for ~5% of the reflections excluded from refinement.

Additional supplementary references

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