Electronic Supplementary Information

Evidence for inhibition of HIF-1α Prolyl hydroxylase 3 activity by four biological active tetraazamacrocycles

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1. Experimental Details

(1) Details of activity reaction procedure

The reaction mixture contained 1 mM DTT, 0.6 mg/mL catalase, 2 mM ascorbate, 2 mg/mL BSA, 50 μM $FeCl_2$ (prepared as 500 mM stock in 20 mM HCl and diluted with water), HIF 19 peptide, enzyme and 20 mM PBS, pH 7.0, to a final volume of 96 μL, keeping on ice bath. The reaction was initiated by addition of 4 μL 600 μM 2OG to the reaction mixture. After warming to 37 °C for 20 min, the reaction was quenched by addition of 200 μL 0.5 M HCl. Derivatization was then achieved by the addition of 1 mL 1 mg/mL OPD in 0.5 M HCl and heating for 15 min at 75 °C. After centrifugation for 5 min (12000 rpm), the supernatant was stored at 4 °C for no more than 1 hour, then made basic by the addition of 1 mL 1.175 M NaOH for fluorescence detection.

(2) The preparation of compounds **1**-**4**

For use in enzyme activity assay and spectrometry studies, compounds **1**, **2** and **4** were prepared as 5 mM stock in Milli-Q deionized water and stored at 4 °C. **3** is prepared as 10 mM stock in acetonitrile and diluted with Milli-Q deionized water at room temperature.

2. Supplemented Figures

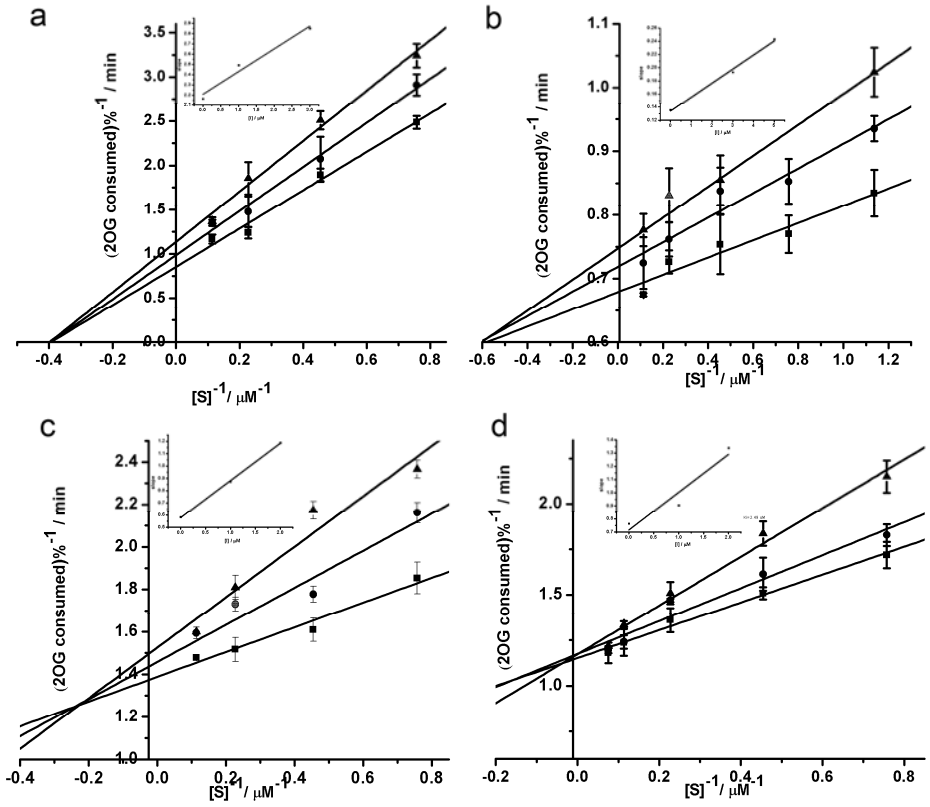

Fig. S1. Double reciprocal plots for the inhibition of hydroxylation activity by macrocyclic polyamines **1**-**4**. a Plots for **1** concentration of 0 μM (■), 1 μM (●), 3 μM (▲). b Plots for **2** concentration of 0 μM (■), 3 μM (●) and 5 μM (▲). c Plots for **3** concentration of 0 μM (■), 1 μM (●), 2 μM (▲). d Plots for **4** concentration of 0 μM (■), 1 μM (●) and 2 μM (▲). (inset) Replot of slopes versus concentration of inhibitors. Data were analyzed as mean ± S.D. of three independent experiments.

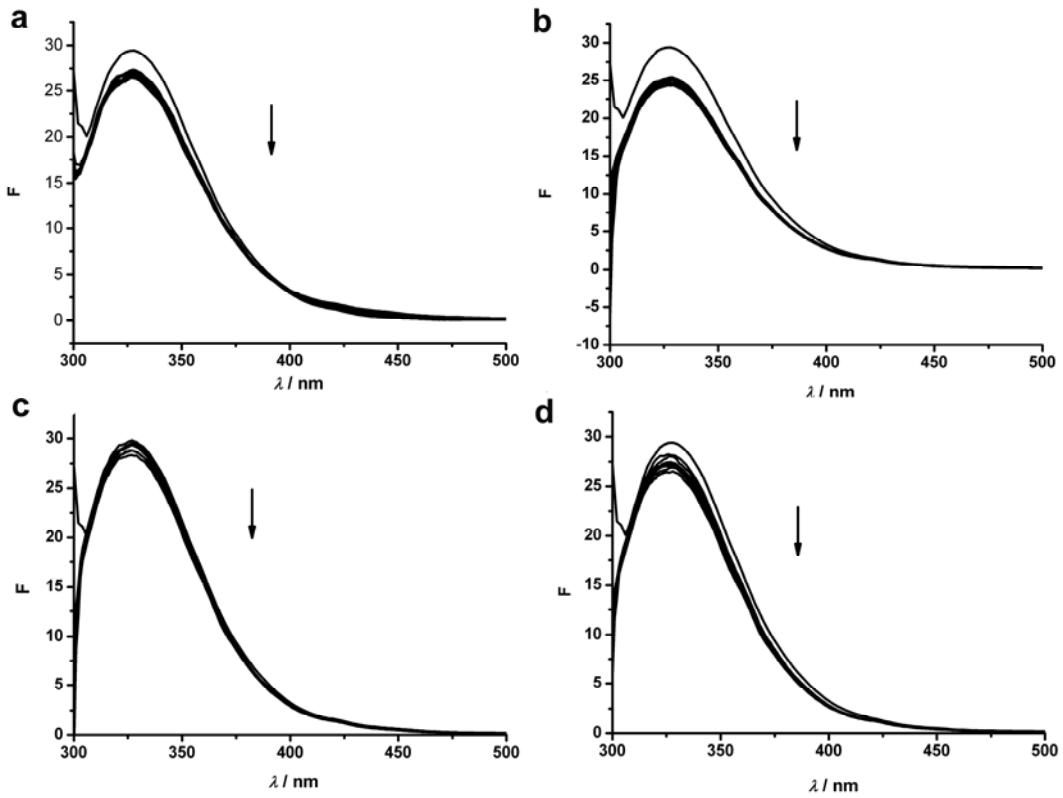

Fig. S2. Fluorescence emission spectra of PHD3 with increasing amounts of a **1**, b **2**, c **3** and d **4** in 50 mM PBS (pH 7.0) at 37 °C. λ (excitation) was 280 nm and maximum λ (emission) was 330 nm, respectively. The data indicates that the inhibitors have little effects on the fluorescence of the enzyme with absence of Fe^{2+}.

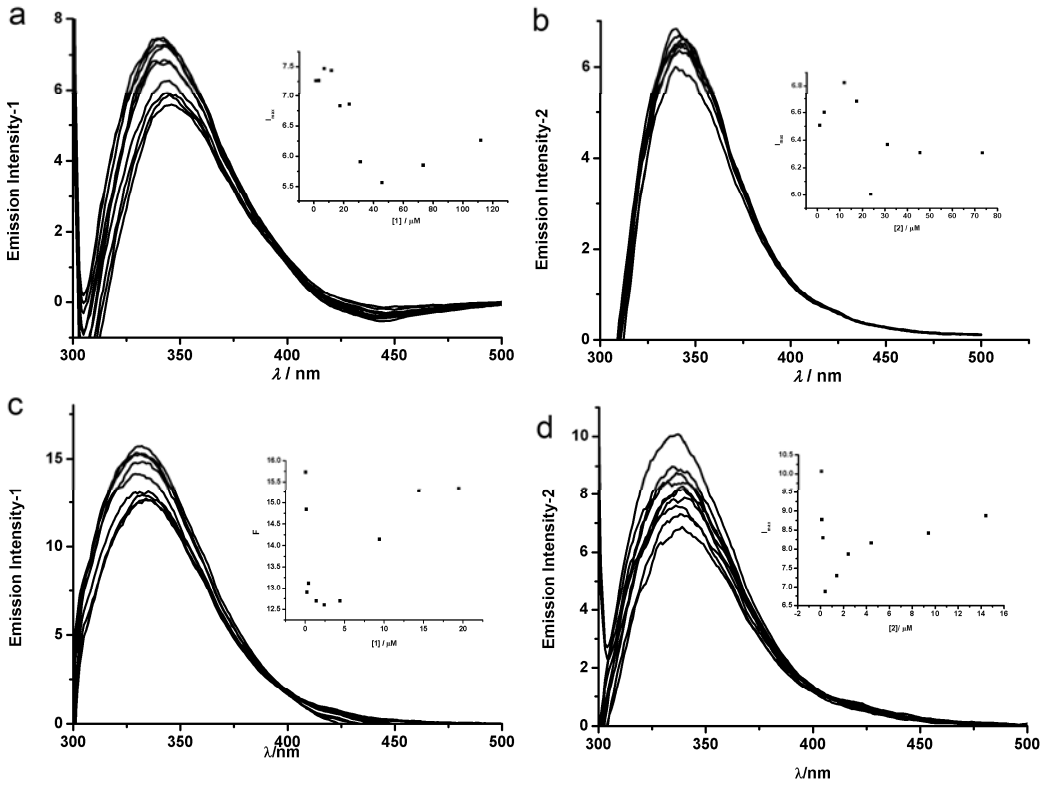

Fig. S3. Fluorescence emission spectra of PHD3-Fe^{2+} (a, b) and PHD3-Fe^{2+}-2OG (c, d) mixtures with increasing amounts of 1(a, c) and 2 (b, d) in 50 mM PBS (pH 7.0) at 37 °C. λ (excitation) was 280 nm. Inset: Effects of 1 (a,c) and 2 (b,d) on the maximum fluorescence emission intensity of PHD3-Fe^{2+} and PHD3-Fe^{2+}-2OG mixtures.

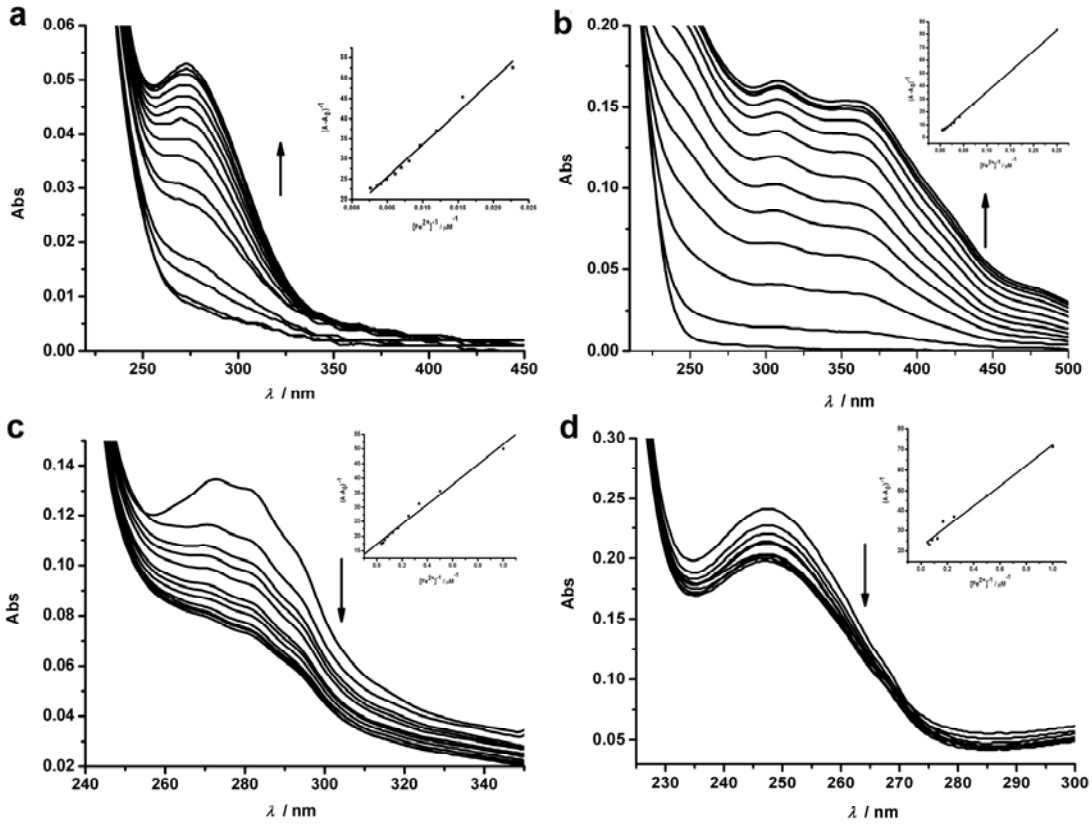

Fig. S4. Change in absorption spectra of a **1**, b **2**, c **3** and d **4** measured upon addition of Fe^{2+}. The concentrations of **1-4** were 130, 140, 25, 50 μM, respectively. The amounts of Fe^{2+} used for titration were from 10^{-4}-10^{-2} M. (inset) Determination of association constant (K_a) via Benesis-Hildebrand plot of λ_{max}. The four compounds coordinates with Fe^{2+} in a 1:1 stoichiometry, according to linear fit of Benesi-Hildebrand equation[1] (Eq S1).

$$1/(A-A_0)=1/[K_a*[L]*(A-A_0)]+1/(A-A_0) \quad (S1)$$

1. H. A. Benesi and J. H. Hildebrand, *J. Am. Chem. Soc.*, 1949, **71**, 2703-2707.

3. Computationally Optimized Energy and Coordinates

(1) Energy of optimized model for the complex of **4**-iron and residues of first coordination shell of PHD3 active site (a. u.)

-1990.7722236 a. u.

(2) Coordinates of optimized model for the complex of **4**-iron and residues of first coordination shell of PHD3 active site:

Standard orientation:

Center Number	Atomic Number	Atomic Type	Coordinates (Angstroms) X	Y	Z
1	6	0	4.724335	-1.738123	-0.215215
2	6	0	3.461462	-1.387963	0.256063
3	7	0	2.795785	-0.356304	-0.324343
4	6	0	3.163631	0.145828	-1.532142
5	6	0	4.414428	-0.170830	-2.054437
6	6	0	5.227809	-1.065068	-1.339107
7	6	0	2.519121	-2.186001	1.155491
8	6	0	1.964848	0.772405	-2.252684
9	7	0	1.168892	-2.020499	0.408147
10	6	0	-0.026698	-2.806814	0.861088
11	6	0	-1.046977	-2.877671	-0.292924
12	7	0	-1.631664	-1.543709	-0.730101
13	6	0	-1.460734	-1.032453	-2.130671
14	6	0	-0.589993	0.245399	-2.246012
15	7	0	0.780326	-0.027416	-1.690225
16	16	0	-3.238998	-1.329976	-0.040263

17	8	0	-3.197709	-1.622905	1.499303
18	8	0	-4.417261	-1.883465	-0.938736
19	1	0	5.283541	-2.543723	0.243236
20	1	0	4.734296	0.222276	-3.011233
21	1	0	6.212870	-1.311838	-1.715204
22	1	0	2.820391	-3.236329	1.218777
23	1	0	2.386464	-1.741656	2.141840
24	1	0	2.058352	0.650067	-3.337653
25	1	0	1.803369	1.821454	-2.008384
26	1	0	1.374638	-2.358630	-0.546046
27	1	0	0.251247	-3.839554	1.112540
28	1	0	-0.477665	-2.340110	1.731998
29	1	0	-0.577146	-3.301876	-1.188171
30	1	0	-1.843461	-3.570977	-0.000530
31	1	0	-2.438592	-0.850157	-2.590292
32	1	0	-1.000757	-1.840701	-2.712585
33	1	0	-0.538538	0.504393	-3.312771
34	1	0	-1.025371	1.075135	-1.692730
35	1	0	0.996652	-1.004140	-1.941676
36	26	0	1.052618	0.039396	0.348608
37	8	0	-0.763286	0.280697	1.380839
38	7	0	1.371265	2.060445	0.496133
39	6	0	-0.145724	-0.089893	2.486475
40	8	0	1.142343	-0.126923	2.406004
41	6	0	-0.887123	-0.539892	3.730786
42	7	0	1.116222	4.156126	1.187194
43	1	0	-1.853209	-0.957203	3.440982
44	1	0	-0.279219	-1.266074	4.273065
45	1	0	-1.062279	0.314934	4.396694
46	1	0	0.688888	4.984653	1.587331

47	7	0	-3.540894	0.537190	-0.418659
48	6	0	-2.878717	1.711355	-0.037290
49	1	0	-1.998840	1.634975	0.580802
50	6	0	-3.596088	2.747751	-0.575310
51	1	0	-3.443925	3.816306	-0.569910
52	6	0	-4.645772	0.835628	-1.134354
53	1	0	-5.343641	0.114715	-1.523857
54	6	0	2.485620	2.829057	0.087075
55	1	0	3.323552	2.395861	-0.423253
56	6	0	2.325536	4.125803	0.497026
57	1	0	2.937029	5.001849	0.337654
58	6	0	0.574273	2.901905	1.172441
59	1	0	-0.335543	2.627853	1.667803
60	7	0	-4.688914	2.173874	-1.246211
61	1	0	-5.406444	2.694101	-1.748648

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4. Table S1

Selected bond lengths (Å) and angles (º) of the computational model

Bond length		Bond angles	
N1-Fe	1.910	O3-Fe-O4	63.761
N2-Fe	2.064	N5-Fe-O3	89.187
N4-Fe	2.058	N4-Fe-N5	97.110
N5-Fe	2.051	N4-Fe-N1	76.409
O3-Fe	2.103	N1-Fe-N2	75.639
O4-Fe	2.066	N2-Fe-O4	83.599

5. Table S2

Average log P of compounds **1**-**4** calculated by **ALOGPS 2.1 program** from **Virtual Computational Chemistry Laboratory**[2]**.**

Inhibitors	Average logP
1	-4.58±1.93
2	-0.92±0.91
3	7.68±1.15
4	1.68±0.57

2. I. V. Tetko, J. Gasteiger, R. Todeschini, A. Mauri, D. Livingstone, P. Ertl, V. A. Palyulin, E. V. Radchenko, N. S. Zefirov, A. S. Makarenko, V. Y. Tanchuk and V. V. Prokopenko, *J. Comput. Aided. Mol. Des.*, 2005, 19, 453-463.