Electronic Supplementary Material for Chemical Communications

Supplementary Information

The Inhibition of Factor Inhibiting Hypoxia-Inducible Factor (FIH) by β-Oxocarboxylic acids

Synthesis

Materials and methods:

Reagents and solvents used were obtained from commercial sources unless otherwise stated. Flash chromatography was performed using silica gel (0.125-0.25 mm, 60-120 mesh) as the stationary phase. Thin layer chromatography (TLC) was performed on aluminium plates pre-coated with silica gel (Merck silica gel 60 F_{254} 1.05554), which were visualized by the quenching of UV fluorescence (using an irradiation wavelength λ_{max} = 254nm), and/or by staining with iodine or $KMnO_4$ in solution, followed by heating. Proton magnetic resonance spectra (^{1}H NMR) were recorded on Brüker DQX 400 (400MHz), Brüker DRX500 (500MHz), and Brüker AMX500 (500MHz) spectrometers at ambient temperature. Carbon magnetic resonance spectra (^{13}C NMR) were recorded on Brüker DPX 400 (100.6MHz), Brüker DQX 400 (100.6MHz), Brüker DRX500 (125.8MHz), and Brüker AMX500 (125.8MHz) spectrometers at ambient temperature. Coupling constant (J) are ± 0.5 Hz. Chemical shifts (δ) are quoted in parts per million (ppm) and are referenced to the residual solvent peak. High-resolution mass spectra were recorded on a VG Autospec spectrometer by chemical ionization or on a Micromass LCT electrospray ionization mass spectrometer operating at a resolution of 5000 full width half height. Synthetic procedures follow those in the literature[1] except where stated.

General procedure for the condensation of amines with diethylethoxymethylene malonate:[1]

A mixture of amine (1 eq) and diethylethoxymethylene malonate (1 eq) were heated at 80^{o}C for an hour under nitrogen. On cooling to room temperature, in some cases a solid appeared, which was recrystallised from ethanol, filtered, dried and directly used for the next step. In cases where the reaction mixture was a liquid, it was purified by column chromatography (silica gel; EtOAc: petroleum ether) to give the desired compounds (**2** or **6**) in 80-85% yield.

General procedure for the cyclisation to keto-esters:[1]

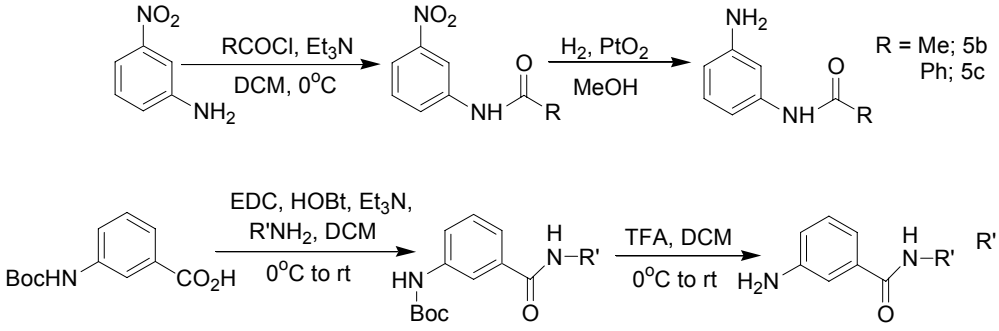
To the secondary amine (**2** or **6**) was added diphenylether (12-15 ml/gm); the solution was slowly heated to refluxing temperature and heated for 1-1.5 hr. On cooling to room temperature, a solid appeared which was then washed thoroughly with hexane, filtered and dried. The product thus obtained was directly used for the saponification step.

General procedure for the saponification of the keto-esters:[1]

To the keto-ester (1 eq) was added 10% potassium hydroxide solution (2ml/mmol) and it was refluxed for 0.5-1 h, cooled to room temperature, washed with ethyl acetate. The aqueous layer was acidified with 1N

HCl to pH~2 when a solid appeared which was filtered and washed thoroughly with water and dried under vacuum.

Preparation of the primary amines for the coupling with diethylethoxymethylene malonate:[2]



General procedure for the amide coupling reactions:

To a solution of the acid (1 mmol) in dichloromethane (10 mL) at 0°C was added 1-(3-dimethylaminopropyl)-3-ethylcarbodiimide hydrochloride (1.2 mmol), followed by 1-hydroxy benzotriazole (1.2 mmol) and Et_3N (1.5 mmol) and it was stirred under nitrogen for 5 minutes. Then to this the amine (1.5 mmol) was added, followed by another portion of Et_3N (1.5 mmol). The reaction mixture was stirred for 7-8 hs under nitrogen at room temperature. After this it was washed with saturated sodium bicarbonate solution (3 x 10 mL), followed by saturated citric acid solution (3 x 10 mL) and finally by water. Drying over sodium sulfate, filtration and evaporation of the solvent under reduced pressure yielded the crude amide, which was column chromatographed (silica gel; EtOAC : petroleum ether) to give the purified product in 90% yield.

Analytical data: Diethyl-[(quinolin-8-ylamino)methylene]malonate **2a:** δ_H (400MHz; $CDCl_3$) 12.5 (1 H, d, *J* 14.5), 9.0 (1 H, brs), 8.81 (1 H, d, *J* 14.5), 7.57-7.48 (4 H, m), 7.27 (1H, s), 4.42 (2 H, q, *J* 7), 4.30 (2 H, q, *J* 7), 1.47 (3 H, t, *J* 7), 1.38 (3 H, t, *J* 7); δ_C (100MHz; $CDCl_3$) 175, 168, 166.2, 149.5, 148.9, 136, 128.5, 126.5, 122.5, 122.2, 110.5, 95.3, 60.4, 60.2, 14.4, 14.3; m/z (EI) 313.1188 (M^+ - H^+ for $C_{17}H_{17}N_2O_4^-$ requires 313.1188). Ethyl-4-oxo-1,4-dihydro-1,10-phenanthroline-3-carboxylate **3a:** δ_H (400MHz; $CDCl_3$) 8.99 (1 H, d, *J* 3), 8.48 (1 H, d, *J* 9), 8.31 (1 H, d, *J* 7), 7.76 (1 H, d, *J* 9), 7.36-7.01 (3 H, m), 4.46 (2 H, q, *J* 7), 1.45 (3 H, t, *J* 7). δ_C (100MHz; $CDCl_3$) 175.5, 175.43, 149.6, 136.4, 136.9, 129.7, 124, 123.2, 122.2, 121.8, 118.8, 116.9, 114.9, 61.2, 14.4; m/z (EI) 269.0926 (M^+ + H^+ for $C_{15}H_{13}N_2O_3^+$ requires 269.0926). 4-Oxo-1,4-dihydro-1,10-phenanthroline-3-carboxylic acid **4a:** δ_H (400MHz; DMSO-d_6) 15.37 (1 H, brs), 9.06 (1 H, d, *J* 3.5), 8.62 (1 H, s), 8.52 (1 H, d, *J* 8), 8.10 (1 H, d, *J* 9), 7.92 (1 H, d, *J* 9), 7.84 (1 H, m); δ_C (100MHz; DMSO-d_6; Me_4Si) 178.5, 166.9, 151.3, 144.4, 139, 137.77, 137.69, 130.36, 126, 125.9, 124.2, 121.9, 111; m/z (EI) 241.0613 (M^+ + H^+ for $C_{13}H_9N_2O_3^+$ requires 241.0613). Diethyl-[(1-naphthylamino)methylene]malonate **2b:** δ_H (400MHz; $CDCl_3$) 11.80 (1 H, d, *J* 13), 8.68 (1 H, d, *J* 13), 8.05

(1 H, d, *J* 8.5), 7.90 (1 H, d, *J* 7.5), 7.71 (1 H, d, *J* 8.5), 7.65-7.55 (3 H, m), 7.5 (1 H, t, *J* 8), 4.39 (2 H, q, *J* 7), 4.28 (2 H, q, *J* 7), 1.43 (3 H, t, *J* 7), 1.35 (3 H, t, *J* 7); δ_C (100MHz; $CDCl_3$) 175.2, 153.5, 149.5, 148.9, 136, 128.6, 126.9, 126.7, 125.7, 125.5, 122.5, 120.5, 110.4, 110, 60.5, 60.2, 14.5, 14.4; m/z (EI) 314.1392 ($M^+ + H^+$ for $C_{18}H_{20}NO_4^+$ requires 314.1392). Ethyl-4-oxo-1,4-dihydrobenzo[*h*]quinoline-3-carboxylate **3b:** δ_H (400MHz; DMSO-d_6) 12.63 (1 H, bs), 8.67 (1 H, bs), 8.18 (1 H, dd, *J* 3.5, 5), 7.81-7.78 (2 H, m), 7.39 (1 H, dd, *J* 2.5, 5), 7.13 (1 H, dd, *J* 7.5, 6.5), 7.01-7.0 (1 H, m), 4.24 (2 H, q, *J* 7), 1.31 (3 H, t, *J* 7); δ_C (100MHz; DMSO-d_6) 156, 144.2, 138.1, 135.6, 130.9, 129.7, 129.6, 128.1, 127.5, 124.2, 123.2, 122.6, 118.4, 110.6, 60.4, 15.2; m/z (EI) 266.0817 ($M^+ - H^+$ for $C_{16}H_{12}NO_3^-$ requires 266.0817). 4-Oxo-1,4-dihydrobenzo[*h*]quinoline-3-carboxylic acid **4b:** δ_H (400MHz; DMSO-d_6) 8.75 (2 H, bs), 8.21 (1 H, d, *J* 9), 8.15 (1 H, d, *J* 9), 8.0 (1 H, d, *J* 9), 7.87 (1 H, d, *J* 3.5), 7.86 (1 H, d, *J* 9); δ_C (100MHz; DMSO-d_6) 178.6, 167.1, 144.3, 138.1, 135.7, 130.7, 129.9, 128.8, 127.6, 124, 123.1, 122.7, 121.2, 110.6; m/z (EI) 238.0504 ($M^+ - H^+$ for $C_{14}H_8NO_3^-$ requires 238.0504). Diethyl-(anilinomethylene)malonate **6a:** δ_H (400MHz; $CDCl_3$) 11.01 (1 H, d, *J* 13), 8.53 (1 H, d, *J* 13), 7.37 (1 H, d, *J* 8), 7.35 (1 H, d, *J* 7.5), 7.16-7.12 (3 H, m), 4.31 (2 H, q, *J* 7), 4.24 (2 H, q, *J* 7), 1.38 (3 H, t, *J* 7), 1.32 (3 H, t, *J* 7); δ_C (100MHz; $CDCl_3$) 169, 165.7, 151.9, 139.2, 129.8, 124.9, 117.1, 93.58, 60.4, 60, 14.5, 14.3; m/z (EI) 264.1236 ($M^+ + H^+$ for $C_{14}H_{18}NO_4^+$ requires 264.1236). Diethyl-({[3-(acetylamino)phenyl]amino} methylene)malonate **6b:** δ_H (400MHz; $CDCl_3$) 10.96 (1 H, d, *J* 13.5), 8.48 (1 H, d, *J* 13.5), 7.68 (1 H, brs), 7.53 (1 H, brs), 7.30-7.26 (1 H, m), 7.15 (1 H, d, *J* 8), 6.86 (1 H, d, *J* 8), 4.28 (2 H, q, *J* 7), 4.24 (2 H, q, *J* 7), 2.19 (3 H, s), 1.36 (3 H, t, *J* 7), 1.32 (3 H, t, *J* 7); δ_C (100MHz; $CDCl_3$) 168.8, 168.6, 165.7, 151.7, 139.9, 139.5, 130.2, 115.8, 112.6, 108.6, 93.7, 60.5, 60.2, 24.6, 14.5, 14.3; m/z (EI) 321.1450 ($M^+ + H^+$ for $C_{16}H_{21}N_2O_5^+$ requires 321.1450). Diethyl-({[3-(benzoylamino)phenyl]amino}methylene)malonate **6c:** δ_H (400MHz; $CDCl_3$) 11.01 (1 H, d, *J* 13.5), 8.51 (1 H, d, *J* 13.5), 8.06 (1 H, brs), 7.9 (1 H, brs), 7.88 (1 H, brs), 7.67 (1 H, s), 7.54-7.21 (5H, m), 6.93-6.90 (1 H, m), 4.30 (2 H, q, *J* 7), 4.25 (2 H, q, *J* 7), 1.37 (3 H, t, *J* 7), 1.33 (3 H, t, *J* 7); δ_C (100MHz; $CDCl_3$) 168.8, 165.8, 165.7, 151.7, 140, 139.5, 134.5, 132, 130.3, 128.8, 127, 116.3, 112.9, 109, 93.8, 60.4, 60.2, 14.5, 14.3; m/z (EI) 383.1607 ($M^+ - H^+$ for $C_{21}H_{23}N_2O_5^-$ requires 383.1607). Diethyl-({[3-(anilinocarbonyl)phenyl]amino}methylene)malonate **6d:** δ_H (400MHz; $CDCl_3$) 11.11 (1 H, d, *J* 13.5), 8.53 (1 H, d, *J* 13.5), 8.07 (1 H, brs), 7.68-7.66 (3 H, m), 7.59 (1 H, d, *J* 7.5), 7.48 (1 H, t, *J* 7.5), 7.40 (1 H, d, *J* 7.5), 7.38 (1 H, d, *J* 7), 7.28 (1 H, d, *J* 9), 7.17 (1 H, t, *J* 7), 4.29 (2 H, q, *J* 7), 4.25 (2 H, q, *J* 7), 1.36 (3 H, t, *J* 7), 1.28 (3 H, t, *J* 7); δ_C (100MHz; $CDCl_3$) 168.9, 165.5, 164.8, 151.2, 139.8, 137.7, 136.9, 130.2, 129.1, 124.8, 122.7, 120.3, 120, 115.9, 94.6, 60.6, 60.3, 14.5, 14.3; m/z (EI) 383.1607 ($M^+ - H^+$ for $C_{21}H_{23}N_2O_5^-$ requires 383.1607). Diethyl-({[3-(benzylaminocarbonyl)phenyl]amino}-methylene)-malonate **6e:** δ_H (400MHz; $CDCl_3$) 11.09 (1 H, d, *J* 13.5), 8.53 (1 H, d, *J* 13.5), 7.64-7.23 (9H, m), 6.56 (1 H, brs), 4.65 (2 H, d, *J* 6), 4.33 (2 H, q, *J* 7), 4.26 (2 H, q, *J* 7), 1.37 (3 H, t, *J* 7), 1.32 (3 H, t, *J* 7); δ_C (100MHz; $CDCl_3$) 168.9, 166.4, 165.4, 151.3, 139.7, 137.8, 136.2, 130, 128.8, 127.9, 127.7, 122.5, 119.9, 115.9, 94.4, 60.5, 60.2, 44.3, 14.5, 14.3; m/z (EI) 397.1763 ($M^+ + H^+$ for $C_{22}H_{25}N_2O_5^+$ requires 397.1770). Diethyl-{[(3-cyanophenyl)amino]methylene}malonate **6f:** δ_H (400MHz; $CDCl_3$) 11.08 (1 H, d, *J* 13), 8.45 (1 H, d, *J* 13),

7.51-7.26 (4H, m), 4.32 (2 H, q, *J* 7), 4.26 (2 H, q, *J* 7), 1.38 (3 H, t, *J* 7), 1.34 (3 H, t, *J* 7); δ_C (100MHz; $CDCl_3$) 168.7, 165.2, 150.6, 140.2, 130.8, 127.9, 121.3, 119.8, 117.9, 114, 95.7, 60.8, 60.5, 14.4, 14.2; m/z (EI) 311.1008 ($M^+ + Na^+$ for $C_{15}H_{16}N_2O_4$ requires 311.1008). Ethyl-4-oxo-1,4-dihydroquinoline-3-carboxylate **7a:** δ_H (400MHz; DMSO-d_6) 12.31 (1 H, brs), 8.54 (1 H, d, *J* 4.5), 8.15 (1 H, d, *J* 8.5), 7.70-7.67 (1 H, m), 7.60 (1 H, d, *J* 8.5), 7.41-7.38 (1 H, m), 4.21 (2 H, q, *J* 7), 1.28 (3 H, t, *J* 7); δ_C (100MHz; DMSO-d_6) 174.3, 165.4, 145.7, 140.6, 133.1, 128.1, 126.5, 125.5, 119.6, 111, 60.4, 15.2; m/z (EI) 216.0661 ($M^+ - H^+$ for $C_{12}H_{10}NO_3^-$ requires 216.0661). Ethyl- 7-(acetylamino)-4-oxo-1,4-dihydroquinoline-3-carboxylate **7b:** δ_H (400MHz; DMSO-d_6) 13.57 (1 H, brs), 8.53 (1 H, brs), 8.42 (1 H, d, *J* 6), 7.63 (1 H, t, *J* 7), 7.25 (1 H, d, *J* 6), 4.23 (2 H, q, *J* 6), 2.16 (3 H, s), 1.28 (3 H, t, *J* 6); δ_C (100MHz; DMSO-d_6) 178.6, 169.2, 164.7, 144.9, 141.6, 140.7, 133.9, 114.3, 113.4, 112.7, 111, 60.3, 25.8, 15; m/z (EI) 275.1032 ($M^+ + H^+$ for $C_{14}H_{15}N_2O_4^+$ requires 275.1034). Ethyl-7-(benzoylamino)-4-oxo-1,4-dihydroquinoline-3-carboxylate **7c:** δ_H (400MHz; $CDCl_3$) 14.48 (1 H, brs), 11.99 (1 H, brs), 8.86 (1 H, d, *J* 8.5), 8.43 (1 H, d, *J* 7), 8.17-8.10 (2 H, m), 7.59-7.27 (5 H, m), 4.33 (2 H, q, *J* 7), 1.35 (3 H, t, *J* 7); δ_C (100MHz; $CDCl_3$) 178.5, 169.8, 164.5, 144.8, 141.6, 140.5, 134.5, 132, 130.4, 128.8, 127, 116.3, 114.2, 112.9, 111, 60.5, 14.5; m/z (EI) 337.1188 ($M^+ + H^+$ for $C_{19}H_{17}N_2O_4^+$ requires 337.1189). Ethyl-7-(anilinocarbonyl)-4-oxo-1,4-dihydroquinoline-3-carboxylate **7d:** δ_H (500MHz; DMSO-d_6) 13.19 (1 H, brs), 10.63 (1 H, brs), 8.97 (1 H, brs), 8.64 (1 H, brs), 8.47-7.17 (7 H, m), 3.05-2.51 (2 H, m), 1.29-1.28 (3 H, m); δ_C (125MHz; DMSO-d_6) 173.5, 165, 163, 146, 140.7, 139.2, 138, 129.2, 127.7, 126.5, 124.4, 123.5, 120.8, 118.9, 110.8, 60.2, 14.8; m/z (EI) 337.1188 ($M^+ + H^+$ for $C_{19}H_{17}N_2O_4^+$ requires 337.1191). Ethyl-7-[(benzylamino)carbonyl]-4-oxo-1,4-dihydroquinoline-3-carboxylate **7e:** δ_H (400MHz; DMSO-d_6) 10.43 (1 H, brs), 9.4 (1 H, brs), 8.21 (1 H, d, *J* 8.5), 8.42-7.87 (3 H, m), 7.41-6.99 (5 H, m), 4.51 (2H, d, *J* 6), 4.22 (2H, q, *J* 7), 1.28 (3 H, t, *J* 7); δ_C (100MHz; DMSO-d_6) 177, 166, 164, 157, 146, 145, 140.6, 139.7, 139.2, 138.3, 130.5, 128.9, 127.8, 123.9, 110, 60.1, 40.2, 14.8; m/z (EI) 351.1345 ($M^+ + H^+$ for $C_{20}H_{19}N_2O_4^+$ requires 351.1338). Ethyl 7-cyano-4-oxo-1,4-dihydroquinoline-3-carboxylate **7f:** δ_H (400MHz; $CDCl_3$) 11.09 (1 H, d, *J* 13), 8.46 (1 H, d, *J* 13), 7.52-7.35 (3H, m), 4.32 (2 H, q, *J* 7), 1.39 (3 H, t, *J* 7); δ_C (100MHz; $CDCl_3$) 175.7, 175.6, 150.6, 130.8, 127.9, 121.2, 119.8, 114, 110, 109.8, 106.7, 60.8, 14.3; m/z (EI) 241.0613 ($M^+ - H^+$ for $C_{13}H_9N_2O_3^-$ requires 241.0613). 4-Oxo-1,4-dihydroquinoline-3-carboxylic acid **8a:** δ_H (400MHz; DMSO-d_6) 13.43 (1 H, brs), 8.91 (1 H, d, *J* 6.5), 8.29 (1 H, d, *J* 7), 7.91-7.81 (2 H, m), 7.63-7.59 (1 H, m); δ_C (100MHz; DMSO-d_6) 179.2, 167.2, 146, 140.3, 134.8, 127, 125.9, 125.2, 120.5, 108.4; m/z (EI) 188.0348 ($M^+ - H^+$ for $C_{10}H_6NO_3^-$ requires 188.0348). 7-Amino-4-oxo-1,4-dihydroquinoline-3-carboxylic acid **8b:** δ_H (400MHz; DMSO-d_6) 8.61 (2 H, brs), 7.90 (1 H, d, *J* 9), 7.47 (2 H, brs), 7.41 (1 H, d, *J* 8), 6.70 (1 H, d, *J* 8), 6.57 (1 H, d, *J* 8); δ_C (100MHz; DMSO-d_6) 167.3, 154.9, 152, 145.5, 135.5, 127.3, 110.7, 110.2, 107.2, 104.3; m/z (EI) 203.0457 ($M^+ - H^+$ for $C_{10}H_7N_2O_3^-$ requires 203.0457). 7-(Benzoylamino)-4-oxo-1,4-dihydroquinoline-3-carboxylic acid **8c:** δ_H (400MHz; DMSO-d_6) 15.20 (1 H, brs), 13.63 (1 H, s), 8.62 (1 H, s), 8.05-8.03 (1 H, m), 7.89 (1 H, m), 7.68-7.65 (1 H, m), 7.53-7.39 (4 H, m), 6.70 (1 H, d, *J* 6.5), 6.57 (1 H, d, *J* 6.5); δ_C (100MHz; DMSO-d_6) 181.9, 166.8, 165.4, 151.7, 145, 141.5, 135, 132.8, 129.6, 127.5, 115, 110.2, 108.6,

106, 103.8; m/z (EI) 307.0719 (M^+ - H^+ for $C_{17}H_{11}N_2O_4^-$ requires 307.0711). 7-(Anilinocarbonyl)-4-oxo-1,4-dihydroquinoline-3-carboxylic acid **8d:** δ_H (500MHz; DMSO-d_6) 10.66 (1 H, brs), 9.01 (1 H, d, *J* 9), 8.36 (1 H, m), 8.32 (1 H, brs), 8.10-7.99 (1 H, m); 7.80 (1 H, d, *J* 8), 7.74 (1 H, d, *J* 7.5), 7.4 (1 H, d, *J* 8.5), 7.37 (1 H, d, *J* 8.5), 7.16 (1 H, m), δ_C (125MHz; DMSO-d_6) 178.4, 166.8, 164.8, 146.9, 140, 139.2, 135.7, 129.2, 127.4, 126.6, 126.5, 126, 124.9, 122, 108.8; m/z (EI) 307.0719 (M^+ - H^+ for $C_{17}H_{11}N_2O_4^-$ requires 307.0719). 7-[(Benzylamino)carbonyl]-4-oxo-1,4-dihydroquinoline-3-carboxylic acid **8e**: δ_H (400MHz; DMSO-d_6) 15.22 (1 H, s), 9.43 (1 H, t, *J* 6), 8.95 (1 H, s), 8.38-8.36 (m, 1H), 8.29-8.27 (1 H, m), 8.04-8.01 (1 H, m), 7.37-7.23 (6 H, m), 4.53 (2 H, d, *J* 6); δ_C (100MHz; DMSO-d_6) 178.8, 167, 166, 140.2, 129.2, 128, 127.7, 126.8, 126.3, 125.9, 125.6, 124.8, 123.4, 122.5, 109, 43.7; m/z (EI) 321.0875 (M^+ - H^+ for $C_{18}H_{13}N_2O_4^-$ requires 321.0874). 7-Cyano-4-oxo-1,4-dihydroquinoline-3-carboxylic acid **8f:** δ_H (400MHz; DMSO-d_6) 13.67 (1 H, brs), 8.99 (1 H, d, *J* 6), 8.39 (1 H, d, *J* 11), 8.10 (1 H, d, *J* 8), 8.06-7.98 (1 H, m); δ_C (100MHz; DMSO-d_6) 178.4, 166.5, 146.6, 140.7, 135.05, 127.3, 126.2, 121.6, 118.3, 108.7; m/z (EI) 213.0300 (M^+ - H^+ for $C_{11}H_5N_2O_3^-$ requires 213.0302).

Molecular modelling

AutoDock 3.0[3] was employed to perform the docking studies. The FIH structure,[4,5] accession PDB-IB 1H2L FIH.Fe(II).2OG.HIF were used for all docking experiments. The substrate, cosubstrate, water molecules and sulphate ions were removed from the protein structure and the active site iron was retained. Kollman united atom charges and desolvation parameters were assigned using AutodockTools (ADT). The grid maps (box size 60 x 60 x 60 points and spacing 0.375 Å) include the entire active site of the enzyme and were generated using AutoGrid. Quantum mechanical calculations were performed to obtain the charge for Fe(II) (0.935) in the protein environment. Atomic solvation parameters and fragmental volumes for the protein were assigned using the AddSol utility of ADT. The 3D structures of the ligands were generated using ChemDraw Pro 8.0 and Chem3D Ultra 8.0 (CambridgeSoft) and minimized using the MOPAC feature within Chem 3D Ultra 8.0. Nonpolar hydrogens were removed and partial atomic charges were added using ADT. All rotatable bonds in the ligands were allowed and defined using AutoTors. Docking was conducted using the Lamarckian genetic algorithm with a population size 100, 10,000,000 generations, 10,000,000 energy evaluations and 10 docking runs.

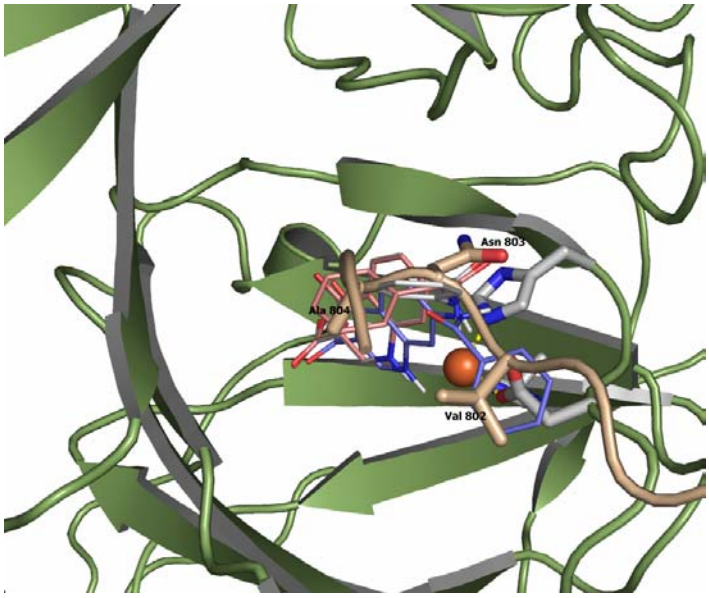


Figure S1. Superposition of the preferred binding mode of **8c** (purple, 8 out of 10 runs), **8d** (white, 8 out of 10 runs) and **8e** (salmon, 9 out of 10 runs) and the HIF substrate (beige). The orange sphere represents the iron.

Enzyme preparation and assay

The HIF-1α CAD substrate fragment (GST-HIF$_{786-826}$) was expressed in *E. coli* as a fusion protein with glutathione S transferase.[6] Synthetic CAD peptide, corresponding to HIF-1α residues 788-822 was purchased from Peptide Protein Research Ltd. (Fareham, UK). FIH and *N*-terminally truncated PHD2 were expressed in *E. coli* and purified as the His$_6$ tagged enzyme.[6,7] In all assays mentioned below, PHD2 was assayed in 50mM HEPES pH7.0 and FIH in 50mM Tris/HCl pH7.5. Screens of the test compounds were carried out using an assay dependent on a post-reaction derivatisation of 2OG with *o*-phenylenediamine.[7] Each analogue was present in the reaction mixture at 1mM, with 1mM DTT, 0.6mg/ml catalase, then 4μM FIH + 50μM iron(II) + 500μM 2OG + 500μM CAD OR 4μM PHD2 + 50μM iron(II) + 300μM 2OG + 100μM HIF1α peptide. Incubation was at 37°C for 12 or 20 minutes for FIH and PHD2 respectively. Inhibition is reported as the difference in 2OG consumption in the presence and absence of test compound. Values are (at least) the mean of three independent measurements. IC$_{50}$ values were determined by varying the concentration of the test compound from 0 – 1mM using the assay conditions above. Decay curves were drawn by hand onto the graphs and the IC$_{50}$ estimated as the concentration at which the activity is half that in the absence of inhibitor.

ESI-Mass Spectrometry

Soft electrospray ionisation mass spectrometry (ESI-MS) was conducted using a Micromass (now Waters) Q-TOFmicro quadrupole-time of flight mass spectrometer. The standard Micromass source was replaced

with an Advion BioSciences NanoMate^{TM} chip-based nano-ESI source. Protein samples (10 μM) were sprayed from 10 mM NH_4OAc (pH 7.0) using a chip nozzle voltage of 1.70 kV, and a cone voltage of 80 V. Collisional cooling of ions was achieved by partially closing a valve on the rotary vacuum pump, leading to an increased pressure in the intermediate vacuum region of the mass spectrometer. CsI was used for calibration.

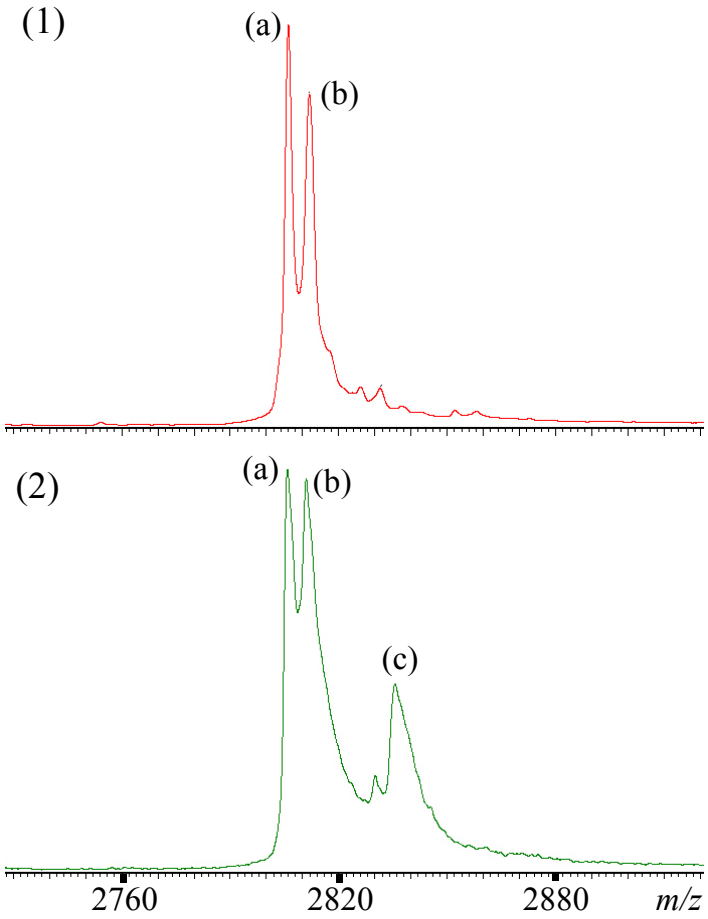


Figure S2. Soft ionisation MS analyses of (1) PHD2 and (2) PHD2 with addition of 10 μM **4a** focussed on charge state 10: (a) Apo-PHD2 m/z = 2805.6, (b) PHD2.Fe(II) m/z = 2811.0; (c) PHD2.Fe(II).**4a** m/z = 2835.6.

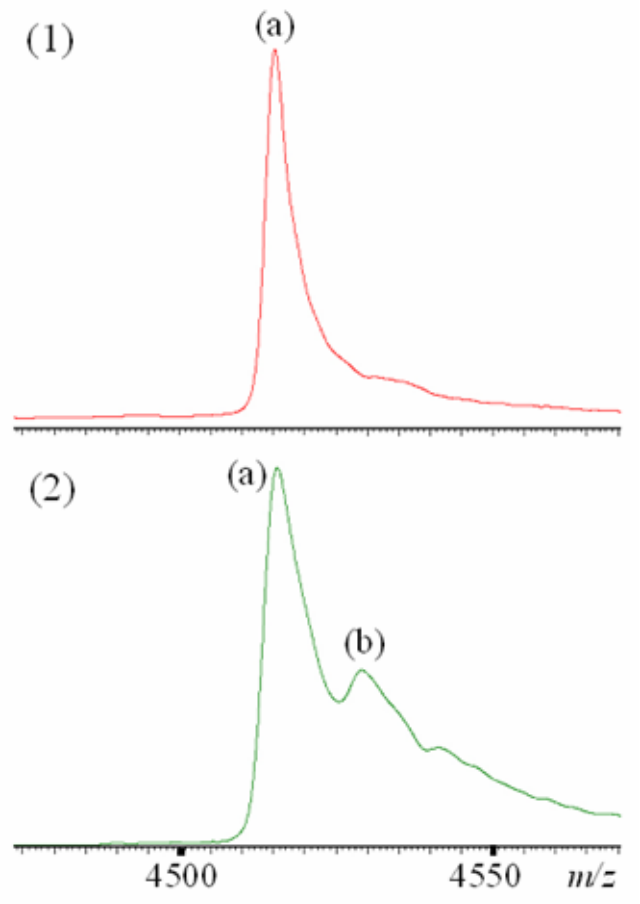


Figure S3. Soft ionisation MS analyses of (1) FIH and (2) FIH with addition of 5 μM **4a** (part dissolved in DMSO + NH_4OH) focussed on charge state 18: (a) Metallo-FIH dimer *m/z* = 4515.3, (b) Metallo-FIH dimer.**4a** *m/z* = 4528.8;

References

1 (a) H. R. Snyder and F. H. Freier, *J. Am. Chem. Soc.*, 1946, **68**, 1320; J. K. Shah and A. E. Coats, *J. Med. Chem.*, 1977, **20**, 1001; F. C. K. Chiu, R. T. C. Brownlee and D. R. Phillips, *Tetrahedron*, 1994, **50**, 889; C. C. Price, H. R. Snyder, O. H. Bullitt and P. Kovacic, *J. Am. Chem. Soc.* 1947, **69**, 374; M. I. Crespo, J. Gracia, C. Puig, A. Vega, J. Bou, J. Beleta, T. Domenech, H. Ryder, V. Segarra and J. M. Palacios, *Bioorg. Med. Chem. Lett.,* 2000, **10**, 2661; B. R. Baker and R. R. Bramhall, *J. Med. Chem.*, 1972, **15**, 235.

2 M. Ogata, H. Matsumoto, S. Shimizu, S. Kida, T. Wada, M. Shiro and K. Sato, *J. Med. Chem.*, 1986, **29**, 417.

3 G. M. Morris, D. S. Goodsell, R. S. Halliday, R. Huey, W. E. Hart, R. K. Belew and A. J. Olson, *J. Comput. Chem.,* 1998, **19**, 1639.

4 J. M. Elkins, K. S. Hewitson, L. A. McNeill, J. F. Seibel, I. Schlemminger, C. W. Christopher, P. J. Ratcliffe and C. J. Schofield, *J. Biol. Chem.*, 2003, **278**, 1802.

5 H. M. Berman, J. Westbrook, Z. Feng, G. Gilliland, T.N. Bhat, H. Weissig, I. N. Shindyalov and P.E. Bourne, *Nucleic Acids Research*, 2000**, 28** , 235.

6 K. S. Hewitson, L. A. McNeill, M. V. Riordan, Y. M. Tian, A. N. Bullock, R. W. D. Welford, J. M. Elkins, N. J. Oldham, S. Battacharya, J. Gleadle, P. J. Ratcliffe, C. W. Pugh and C. J. Schofield, *J. Biol. Chem.,* 2002, **277**, 26351.

7 L. A. McNeill, L. Bethge, K. S. Hewitson and C. J. Schofield, *Anal. Biochem.*, 2005, **336**, 125.