Supporting Information

Synthesis, *in vitro* evaluation and co-crystal structure of 4-oxo-[1]benzopyrano[4,3-c]pyrazole *Cryptosporidium parvum* inosine 5'-monophosphate dehydrogenase (*Cp*IMPDH) inhibitors

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Chemistry Materials and Methods. All starting materials, reagents and solvents were purchased as high-grade commercial products and used without further purification. Reactions sensitive to moisture and/or oxygen were carried out under an inert atmosphere of anhydrous argon. Melting points were determined using a Thomas Hoover capillary melting point apparatus and were uncorrected. Analytical thin-layer chromatography (TLC) was performed on Baker-flex® 4449-02 silica gel sheets, and spots were visualized with UV light (λ = 254 nm) or stained with aqueous potassium permanganate or 10% phosphomolybdic acid solution in ethanol. Flash column chromatography was performed using silica gel 60 (230-400 mesh, Merck) or a Combiflash®RF column chromatography workstation. ^{1}H and ^{13}C NMR were collected in $CDCl_3$ or DMSO-d_6. The chemical shifts are reported in ppm from TMS. Coupling constants (*J*) are expressed in hertz (Hz). High-resolution mass spectrum was obtained from an AccuTOF mass spectrometer (JEOL, USA) with a DART source (IonSense, USA) using helium as the ionization gas (University of Connecticut). HPLC purity assessment was performed using a Thermo Finnigan LCQ Deca XP system and a Phenomenex Kinetex 5u XB-C18, 100A, 50 x 2.1 mm column. Gradient solvent consisted 0.1% formic acid and 5- 90% acetonitrile in water. Collecting time was 10 min and the flow rate was 200 μL/min. All tested compounds were found to have > 95% purity.

Preparation of 4-chloro-3-methoxy-*N*-methylaniline (2l). 4-Chloro-3-methoxyaniline was refluxed with di-*t*-butyl dicarbonate (1.5 equiv) in toluene for 4 h.[1] After evaporation, the crude product was treated with sodium hydride (4 equiv) and methyliodide (2 equiv) in DMF at 0 ºC and then stirred at room temperature for 8 h. The reaction mixture was partitioned between aqueous NH_4Cl and ethyl acetate. The organic layer was dried over anhydrous $MgSO_4$, filtered and concentrated. *N*-Boc-4-chloro-3-methoxyaniline was purified by silica gel column (0-10% ethyl acetate in hexane). This material was treated with trifluoroacetic acid in CH_2Cl_2 (1:4) and then concentrated *in vacuo* to give **2l** as an oil (65% yield). ^{1}H NMR (500 MHz, $CDCl_3$) δ 7.12-7.10 (d, *J* = 8.6 Hz, 1H), 6.15-6.14 (m, 1H), 6.12-6.10 (dd, *J* = 8.6, 2.3 Hz, 1H), 3.82 (s, 3H), 2.78 (s, 3H). ^{13}C NMR (100 MHz, $CDCl_3$) δ 155.4, 149.3, 130.1, 109.8, 104.7, 96.9, 55.7, 30.7.

2-Bromo-*N*-(3-methoxyphenyl)acetamide (**4a**). Prepared from 3-anisidine and bromoacetyl chloride to give a solid (96% yield). ^{1}H NMR (400 MHz, $CDCl_3$) δ 8.17 (bs, 1H), 7.18-7.14 (m, 2H), 6.95-6.93 (d, *J* = 7.8 Hz, 1H), 6.65-6.63 (d, *J* = 7.3 Hz, 1H), ^{13}C NMR (100 MHz, $CDCl_3$) δ 163.6, 160.1, 138.0, 129.8, 112.1, 110.9, 105.7, 55.3, 29.5.

2-Bromo-*N*-(3-chlorophenyl)acetamide (**4b**). Prepared from 3-chloroaniline and bromoacetyl chloride to give a solid (92% yield). ^{1}H NMR (500 MHz, $CDCl_3$) δ 8.30 (bs, 1H), 7.64 (s, 1H), 7.39-7.36 (d, *J* = 8.6 Hz, 1H), 7.28-7.25 (dd, *J* = 8.6, 8.0 Hz, 1H), 7.15-7.13 (d, *J* = 8.0 Hz, 1H), 4.01 (s, 2H). ^{13}C NMR (125 MHz, $CDCl_3$) δ 163.9, 137.9, 134.7, 130.0, 125.4, 120.3, 118.1, 29.2.

2-Bromo-*N*-(2-chlorophenyl)acetamide (**4c**). Prepared from 2-chloroaniline and bromoacetyl chloride to give a solid (96% yield). ^{1}H NMR (400 MHz, $CDCl_3$) δ 8.81 (bs, 1H), 8.34-8.32 (d, *J* = 8.2 Hz, 1H), 7.41-7.39 (d, *J* = 7.8 Hz, 1H), 7.32-7.28 (dd, *J* = 8.2, 7.3 Hz, 1H), 7.12-7.07 (ddd, *J* = 8.2, 7.3, 1 Hz, 1H), 4.08 (s, 2H). ^{13}C NMR (100 MHz, $CDCl_3$) δ 163.4, 133.8, 129.1, 127.7, 125.4, 123.4, 121.1, 29.6.

2-Bromo-*N*-(4-fluorophenyl)acetamide (**4d**). Prepared from 4-fluoroaniline and bromoacetyl chloride to give a solid (87% yield). ^{1}H NMR (400 MHz, $CDCl_3$) δ 7.26 (bs, 1H), 7.51-7.48 (m, 2H), 7.08-7.03 (dd, *J* = 8.7, 8.2 Hz, 2H), 4.02 (s, 2H). ^{13}C NMR (100 MHz, $CDCl_3$) δ 163.5, 161.0, 158.6, 122.0, 121.9, 115.9, 115.7, 29.3.

2-Bromo-*N*-(4-(trifluoromethyl)phenyl)acetamide (**4e**). Prepared from 4-trifluomethylaniline and bromoacetyl chloride to give a solid (92% yield). ^{1}H NMR (400 MHz, $CDCl_3$) δ 10.77 (bs, 1H), 7.82-7.79 (d, *J* = 8.7 Hz, 2H), 7.72-7.70 (d, *J* = 8.7 Hz, 2H), 4.09 (s, 2H). ^{13}C NMR (100 MHz, $CDCl_3$) δ 165.5, 142.2, 126.2, 119.2, 30.3.

2-Bromo-*N*-(3,4-dichlorophenyl)acetamide (**4f**). Prepared from 3,4-dichloroaniline and bromoacetyl chloride to give a solid (92% yield). ^{1}H NMR (400 MHz, $CDCl_3$) δ 8.23 (bs, 1H), 7.51-7.50 (m, 2H), 7.16-7.15 (dd, *J* = 1.8, 1.4 Hz, 1H), 4.02 (s, 2H). ^{13}C NMR (100 MHz, $CDCl_3$) δ 163.7, 138.6, 135.4, 125.2, 118.2, 29.1.

2-Bromo-*N*-(2,4-dichlorophenyl)acetamide (**4g**). Prepared from 2,4-dichloroaniline and bromoacetyl chloride to give a solid (90% yield). ^{1}H NMR (400 MHz, $CDCl_3$) δ 8.77 (bs, 1H), 8.32-8.30 (d, *J* = 8.7 Hz, 1H), 7.42-7.41 (d, *J* = 2.3 Hz, 1H), 7.29-7.26 (dd, *J* = 8.7, 2.3 Hz, 1H), 4.07 (s, 2H). ^{13}C NMR (100 MHz, $CDCl_3$) δ 163.4, 132.6, 130.0, 128.9, 127.9, 123.9, 121.8, 29.5.

2-Bromo-*N*-(2,3-dihydrobenzo[b][1,4]dioxin-6-yl)acetamide (**4h**)**.** Prepared from 1,4-benzodioxan-6-amine and bromoacetyl chloride to give a solid (97% yield). ^{1}H NMR (500 MHz, DMSO-d_6) δ 10.23 (s, 1H), 7.22-7.21 (d, *J* = 2.3 Hz, 1H), 6.97-6.95 (dd, *J* = 8.6, 2.3 Hz, 1H), 6.81-6.79 (d, *J* = 8.6 Hz, 1H), 4.23-4.19 (m, 4H), 3.99 (s, 2H). ^{13}C NMR (125 MHz, DMSO-d_6) 164.3, 143.0, 139.7, 132.2, 116.9, 112.4, 108.3, 64.2, 63.9, 39.2.

2-Bromo-*N*-(3,4-dimethoxyphenyl)acetamide (**4i**). Prepared from 4-aminoveratrole and bromoacetyl chloride to give a solid (92% yield). ^{1}H NMR (400 MHz, $CDCl_3$) δ 8.07 (bs, 1H), 7.28 (d, *J* = 2.8 Hz, 1H), 6.96-6.92 (dd, *J* = 8.7, 2.8 Hz, 1H), 6.84-6.82 (d, *J* = 8,7 Hz, 1H), 4.03 (s, 2H), 3.89 (s, 3H), 3.87 (s, 3H). ^{13}C NMR (100 MHz, $CDCl_3$) δ 163.3, 149.0, 146.4, 130.3, 112.3, 111.1, 104.8, 56.0, 55.9, 29.5.

2-Bromo-*N*-(naphthalen-2-yl)acetamide (**4j**). Prepared from 2-naphthylamine and bromoacetyl chloride to give a solid (65% yield). ^{1}H NMR (400 MHz, $CDCl_3$) δ 8.32 (bs, 1H), 8.20 (d, *J* = 1.8 Hz, 1H), 7.83-7.79 (m, 3H), 7.50-7.44 (m, 3H), 4.08 (s, 2H). ^{13}C NMR (100 MHz, $CDCl_3$) δ 163.6, 134.3, 133.7, 131.0, 129.0, 127.8, 127.6, 126.7, 125.5, 119.6, 117.1, 29.6.

2-Bromo-*N*-(4-chloro-3-methoxyphenyl)acetamide (**4k**). Prepared from 4-chloro-3-methoxyaniline and bromoacetyl chloride to give a solid (75% yield). ^{1}H NMR (400 MHz, $CDCl_3$) δ 8.23 (bs, 1H), 7.45 (s, 1H), 7.31-7.29 (d, *J* = 8.2 Hz, 1H), 6.90-6.87 (dd, *J* = 8.2, 1Hz, 1H), 4.02 (s, 2H), 3.90 (s, 3H). ^{13}C NMR (100 MHz, $CDCl_3$) δ 163.6, 155.2, 136.6, 130.1, 118.4, 112.3, 104.3, 56.2, 29.4.

2-Chloro-*N*-(4-chloro-3-methoxyphenyl)-N-methylacetamide (**4l**)**.** Prepared from **2l** and chloroacetyl chloride to give a solid (92 % yield). ^{1}H NMR (500 MHz, $CDCl_3$) δ 7.43-7.42 (d, *J* = 8.6 Hz, 1H), 6.84-6.83 (m, 1H), 6.81-6.79 (dd, *J* = 8.6, 2.3Hz, 1H), 3.92 (s, 3H), 3.86 (s, 2H), 3.30 (s, 3H). ^{13}C NMR (100 MHz, $CDCl_3$) δ 166.2, 155.9, 142.2, 131.2, 122.7, 119.6, 110.9, 56.3, 41.2, 38.0.

2-Bromo-*N*-(4-chloro-3-methoxyphenyl)propanamide (**4m**). Prepared from 4-chloro-3-methoxyaniline and 2-bromopropionyl chloride to give a solid (89% yield). ^{1}H NMR (400 MHz, $CDCl_3$) δ 8.21 (bs, 1H), 7.51-7.50 (d, *J* = 1.4 Hz, 1H), 7.29-7.27 (d, *J* = 8.7 Hz, 1H), 6.88-6.86 (dd, *J* = 8.7, 1.4 Hz, 1H), 4.58-4.54 (q, *J* = 6.9 Hz, 1H), 3.90 (s, 3H), 1.98-1.94 (d, *J* = 6.9 Hz, 3H). ^{13}C NMR (100 MHz, $CDCl_3$) δ 167.5, 155.2, 136.9, 130.0, 118.1, 112.2, 104.3, 56.1, 44.9, 22.7.

***t*-Butyl 2-(2-((3-methoxyphenyl)amino)-2-oxoethyl)hydrazinecarboxylate** (**5a**). Prepared from **4a** to give a solid (84% yield). ^{1}H NMR (500 MHz, $CDCl_3$) δ 9.74 (bs, 1H), 7.41-7.39 (s, 1H), 7.22-7.20 (d, *J* = 9.2 Hz, 1H), 6.67-6.64

(dd, J = 9.2, 2.3 Hz, 1H), 6.52 (s, 1H), 4.35 (bs, 1H), 3.79 (s, 3H), 3.61 (s, 2H), 1.45 (s, 9H). ^{13}C NMR (125 Hz, $CDCl_3$) δ 168.4, 159.9, 156.9, 139.0, 129.5, 112.0, 110.1, 105.2, 81.2, 56.1, 55.2, 28.2.

***t*-Butyl 2-(2-((3-chlorophenyl)amino)-2-oxoethyl)hydrazinecarboxylate (5b)**. Prepared from **4b** to give a solid (83% yield). ^{1}H NMR (400 MHz, DMSO-d_6) δ 10.25 (bs, 1H), 8.54 (bs, 1H), 7.84 (s, 1H), 7.53-7.51 (d, J = 8.2 Hz, 1H), 7.36-7.31 (t, J = 8.3 Hz, 1H), 7.11-7.09 (dd, J = 7.8, 1 Hz, 1H), 5.38 (bs, 1H), 3.44 (s, 2H), 1.37 (s, 9H). ^{13}C NMR (100 MHz, DMSO-d_6) δ 169.4, 157.1, 139.9, 133.1, 130.5, 123.1, 118.6, 117.5, 79.1, 55.0, 28.2.

***t*-Butyl 2-(2-((2-chlorophenyl)amino)-2-oxoethyl)hydrazinecarboxylate (5c)**. Prepared from **4c** to give a solid (82% yield). ^{1}H NMR (400 MHz, DMSO-d_6) δ 9.83 (bs, 1H), 8.56 (bs, 1H), 8.08-8.06 (d, J = 7.3 Hz, 1H), 7.52-7.50 (d, J = 8.2 Hz, 1H, 7.36-7.32 (t, J = 7.3 Hz, 1H), 7.17-7.14 (dd, J = 7.3, 6.9 Hz, 1H), 5.47 (bs, 1H), 3.49-3.48 (d, J = 3.2 Hz, 2H), 1.37 (s, 9H). ^{13}C NMR (100 MHz, DMSO-d_6) δ 169.2, 156.3, 134.5, 129.3, 127.6, 125.4, 124.2, 122.9, 78.8, 55.0, 28.1.

***t*-Butyl 2-(2-((4-fluorophenyl)amino)-2-oxoethyl)hydrazinecarboxylate (5d)**. Prepared from **4d** to give a solid (86% yield). ^{1}H NMR (400 MHz, DMSO-d_6) δ 10.17 (bs, 1H), 8.55 (bs, 1H), 7.70-7.67 (dd, J = 9.2, 5.0 Hz, 2H), 7.20-7.15 (t, J = 8.7 Hz, 2H), 5.38 (bs, 1H), 3.44 (d, J = 3.2 Hz, 2H), 1.39 (s, 9H). ^{13}C NMR (100 MHz, DMSO-d_6) δ 168.9, 159.2, 157.0, 156.9, 134.9, 120.7, 115.5, 115.2, 79.0, 55.0, 28.2.

***t*-Butyl 2-(2-oxo-2-((4-(trifluoromethyl)phenyl)amino)ethyl)hydrazinecarboxylate (5e)**. Prepared from **4e** to give a solid (75% yield). ^{1}H NMR (400MHz, DMSO-d_6) δ 10.45 (bs, 1H), 8.57 (bs, 1H), 7.88-7.86 (d, J = 8.7 Hz, 2H), 7.72-7.70 (d, J = 8.7 Hz, 2H), 5.42 (bs, 1H), 3.49-3.48 (d, J = 2.8 Hz, 2H), 1.37 (s, 9H). ^{13}C NMR (100 MHz, DMSO-d_6) δ 169.8, 157.1, 142.0, 126.1, 123.6, 123.3, 118.9, 79.1, 55.1, 28.2.

***t*-Butyl 2-(2-((3,4-dichlorophenyl)amino)-2-oxoethyl)hydrazinecarboxylate (5f).** Prepared from **4f** to give a solid (84% yield). ^{1}H NMR (400 MHz, $CDCl_3$) δ 10.06 (bs, 1H), 7.97 (bs, 1H), 7.53-7.51 (dd, J = 8.7, 2.3 Hz, 1H), 7.37-7.35 (d, J = 8.7 Hz, 1H), 6.39 (bs, 1H), 3.63 (s, 2H), 1.46 (s, 9H). ^{13}C NMR (100 MHz, $CDCl_3$) δ 168.4, 157.1, 137.4, 132.5, 130.3, 126.7, 121.3, 119.0, 81.7, 56.2, 28.3.

***t*-Butyl 2-(2-((2,4-dichlorophenyl)amino)-2-oxoethyl)hydrazinecarboxylate (5g)**. Prepared from **4g** to give a solid (77% yield). ^{1}H NMR (400 MHz, DMSO-d_6) δ 9.89 (bs, 1H), 8.56 (bs, 1H), 8.12-8.10 (d, J = 8.7 Hz, 1H), 7.70-7.69 (d, J = 2.3 Hz, 1H), 7.45-7.42 (dd, J = 8.7, 2.3 Hz, 1H), 5.49 (bs, 1H), 3.49 (d, J = 3.7 Hz, 2H), 1.36 (s, 9H). ^{13}C NMR (125 MHz, DMSO-d_6) δ 170.5, 157.3, 134.0, 129.5, 128.3, 126.8, 125.4, 120.1, 80.0, 55.4, 28.6.

***t*-Butyl 2-(2-((2,3-dihydrobenzo[b][1,4]dioxin-6-yl)amino)-2-oxoethyl)hydrazinecarboxylate (5h).** Prepared from **4h** to give a solid (82% yield). ^{1}H NMR (400 MHz, DMSO-d_6) δ 9.94 (bs, 1H), 8.50 (bs, 1H), 7.28-7.27 (d, J = 2.3 Hz, 1H), 7.03-7.01 (dd, J = 8.7, 2.3 Hz, 1H), 6.80-6.78 (d, J = 8.7 Hz, 1H), 5.31 (bs, 1H), 4.21-4.19 (m, 4H), 3.38-3.37 (d, J = 3.2 Hz, 2H), 1.39 (s, 9H). ^{13}C NMR (100 MHz, DMSO-d_6) δ 168.4, 157.0, 142.9, 139.4, 132.2, 116.8, 112.2, 108.1, 79.0, 64.2, 63.9, 54.9, 28.1.

***t*-Butyl 2-(2-((3,4-dimethoxyphenyl)amino)-2-oxoethyl)hydrazinecarboxylate (5i)**. Prepared from **4i** to give a solid (77% yield). ^{1}H NMR (400 MHz, DMSO-d_6) δ 9.97 (bs, 1H), 8.52 (bs, 1H), 7.33-7.32 (d, J = 2.3 Hz, 1H), 7.19-7.16 (dd, J = 8.7, 2.3 Hz, 1H), 6.91-6.89 (d, J = 8.7 Hz, 1H), 5.34 (bs, 1H), 3.73 (s, 3H), 3.71 (s, 3H), 3.40-3.39 (d, J = 3.2 Hz, 2H), 1.38 (s, 9H). ^{13}C NMR (100 MHz, DMSO-d_6) δ 168.4, 156.5, 148.5, 144.9, 132.1, 112.0, 110.9, 104.1, 79.0, 55.7, 55.3, 54.9, 28.1.

***t*-Butyl 2-(2-(naphthalen-2-ylamino)-2-oxoethyl)hydrazinecarboxylate** (**5j**). Prepared from **4j** to give a solid (65% yield). ^{1}H NMR (400 MHz, DMSO-d_6) δ 10.27 (bs, 1H), 8.58 (bs, 1H), 8.34 (s, 1H), 7.88-7.79 (m, 3H), 7,64-7,62 (dd, *J* = 8.7, 2.3 Hz, 1H), 7.50-7.45 (ddd, *J* = 8.3, 6.9, 1.4 Hz, 1H), 7.43-7.39 (ddd, *J* = 8.3, 6.9, 1.4 Hz, 1H), 5.40 (bs, 1H), 3.48 (d, *J* = 2.8 Hz, 2H), 1.38 (s, 9H). ^{13}C NMR (100 MHz, DMSO-d_6) δ 169.4, 157.2, 136.1, 133.5, 130.0, 128.6, 127.6, 127.4, 126.7, 124.8, 120.0, 115.1, 79.3, 55.2, 28.3.

***t*-Butyl 2-(2-((4-chloro-3-methoxyphenyl)amino)-2-oxoethyl)hydrazinecarboxylate** (**5k**). Prepared from **4k** to give a solid (86% yield). ^{1}H NMR (400 MHz, DMSO-d_6) δ 10.23 (bs, 1H), 8.56 (bs, 1H), 7.54-7.53 (d, *J* = 2.3 Hz, 1H), 7.37-7.35 (d, *J* = 8.7 Hz, 1H), 7.27-7.24 (dd, *J* = 8.7, 2.3 Hz, 1H), 5.38 (bs, 1H), 3.82 (s, 3H), 3.44 (s, 2H), 1.39 (s, 9H). ^{13}C NMR (100 MHz, DMSO-d_6) δ 169.2, 157.0, 154.4, 138.7, 129.8, 115.0, 111.8, 104.6, 79.1, 55.7, 55.0, 28.2.

***t*-Butyl 2-(2-((4-chloro-3-methoxyphenyl)(methyl)amino)-2-oxoethyl)hydrazinecarboxylate** (**5l**). Prepared from **4l** to give a solid (66% yield). ^{1}H NMR (500 MHz, $CDCl_3$) δ 7.40-7.38 (d, *J* = 9.2 Hz, 1H), 7.27 (s, 1H), 6.74-6.72 (m, 1H), 6.47 (bs, 1H), 3.90 (s, 3H), 3.40 (bs, 2H), 3.26 (s, 3H), 1.43 (s, 9H). ^{13}C NMR (125 MHz, $CDCl_3$) δ 169.8, 156.0, 152.5, 141.8, 131.1, 122.2, 119.9, 110.9, 80.3, 56.3, 53.2, 37.2, 28.3.

***t*-Butyl 2-(1-((4-chloro-3-methoxyphenyl)amino)-1-oxopropan-2-yl)hydrazinecarboxylate** (**5m**). Prepared from **4m** to give a solid (83% yield). ^{1}H NMR (500 MHz, $CDCl_3$) δ 9.8 (bs, 1H), 7.60-7.59 (d, *J* = 2.3 Hz, 1H), 7.27-7.25 (d, *J* = 8.6 Hz, 1H), 7.15-7.13 (m, 1H), 6.48 (s, 1H), 3.97 (s, 1H), 3.90 (s, 3H), 3.61-3.57 (q, *J* = 6.9 Hz, 1H), 1.42 (s, 9H), 1.40-1.39 (d, *J* = 6.9 Hz, 1H). ^{13}C NMR (125 MHz, $CDCl_3$) δ 171.6, 156.8, 154.9, 137.9, 129.8, 116.8, 112.2, 104.0, 81.3, 61.8, 56.0, 28.2, 17.1.

General procedure of Vilsmeier-Haack reactions to prepare 7b and 10a-b. 4-Hydroxycoumarins, 4-chromanone or α-tetralone (5.1 mmol) and DMF (10.2 mmol) were dissolved in 10 mL 1,2-dichloroethane. To this solution was added $POCl_3$ (4.5 mmol). The reaction mixture was heated at 80 ºC for 4 h (for **7b**) or for 1 h followed by room temperature for another 3 h (for **10a-b**). The reaction was stopped by the addition of saturated aqueous sodium acetate. The reaction mixture was extracted with ethyl acetate, dried over anhydrous $MgSO_4$, filtered and concentrated. The obtained product was used in the next stage without further purification.

4-Chloro-3-formyl-7-methylcoumarin (**7b**). Prepared from 4-hydroxy-7-methylcoumarin to give a solid (91% yield) ^{1}H NMR (400 MHz, $CDCl_3$) δ 10.37 (s, 1H), 8.01-7.99 (d, *J* = 8.3 Hz, 1H), 7.25-7.22 (d, *J* = 8.3 Hz, 1H), 7.20 (s, 1H), 2.53 (s, 3H). ^{13}C NMR (100 MHz, $CDCl_3$) δ 186.9, 158.7, 153.8, 153.3, 148.1, 127.3, 126.9, 117.2, 117.0, 116.0, 22.0.

4-Chloro-2H-chromene-3-carbaldehyde (**10a**). Prepared from 4-chromanone to give a solid (87% yield) ^{1}H NMR (500 MHz, $CDCl_3$) δ 10.14 (s, 1H), 7.69 (dd, *J* = 8.0, 1.2 Hz, 1H), 7.38-7.35 (ddd, *J* = 8.6, 7.5, 1.2 Hz, 1H), 7.06-7.03 (ddd, *J* = 8.0, 7.5 Hz, 1H), 6.91-6.89 (d, *J* = 8.6 Hz, 1H), 4.99 (s, 2H). ^{13}C NMR (125 MHz, $CDCl_3$) δ 187.7, 156.5, 143.2, 134.0, 126.5, 124.7, 122.1, 120.4, 116.6, 64.2.

1-Chloro-3,4-dihydronaphthalene-2-carbaldehyde (**10b**). Prepared from α-tetralone to give a solid (98% yield). ^{1}H NMR (500 MHz, $CDCl_3$) δ 10.39 (s, 1H), 7.87-7.86 (d, *J* = 7.5 Hz, 1H), 7.39-7.36 (ddd, *J* = 7.5, 7.5, 1.7 Hz, 1H), 7.35-7.32 (ddd, *J* = 7.5, 6.3, 1.2 Hz, 1H), 7.23-7.22 (d, *J* = 7.5 Hz, 1H), 2.87-2.84 (dd, *J* = 8.6, 7.5 Hz, 2H), 2.66-2.63 (dd, *J* = 8.6, 7.5 Hz, 2H). ^{13}C NMR (125 MHz, $CDCl_3$) δ 190.7, 145.9, 138.9, 132.0, 131.9, 131.4, 127.7, 127.1,

126.3, 27.0, 21.5.

4-Oxo-*N*-(3-methoxyphenyl)-[1]benzopyrano[4,3-c]pyrazole-1(4H)acetamide (8a). Prepared from **5a** and **7a** to give a yellow solid (35% yield). Mp 220-222 °C. ^{1}H NMR (500 MHz, DMSO-d_6) δ 10.60 (s, 1H), 8.37 (s, 1H), 8.06-8.04 (d, *J* = 8.0 Hz, 1H), 7.67-7.65 (dd, *J* = 8.0, 7.5 Hz, 1H), 7.57-7.55 (d, *J* = 8.6 Hz, 1H), 7.44-7.41 (dd, *J* = 8.0, 7.5 Hz, 1H), 7.26-7.22 (m, 2H), 7.11-7.09 (d, *J* = 8.0 Hz, 1H), 6.69-6.67 (dd, *J* = 10.3, 2.3 Hz, 1H), 5.66 (s, 2H), 3.70 (s, 3H). ^{13}C NMR (125 MHz, DMSO-d_6) δ 164.4, 159.6, 156.4, 152.2, 141.5, 139.5, 138.5, 131.4, 129.8, 124.6, 123.4, 119.4, 117.7, 111.5, 109.4, 107.6, 104.9, 55.1, 55.0. HRMS $[M+H]^+$, $C_{19}H_{15}N_3O_4$, calc. 350.1141, obs. 350.1128. HPLC retention time: 6.65 min.

4-Oxo-*N*-(3-chlorophenyl)-[1]benzopyrano[4,3-c]pyrazole-1(4H)acetamide (**8b**). Prepared from **5b** and **7a** to give a yellow solid (33% yield). Mp 236-238 °C. ^{1}H NMR (500 MHz, DMSO-d_6) δ 10.82 (s, 1H), 8.37 (s, 1H), 8.05-8.03 (d, *J* = 8.6 Hz, 1H), 7.76-7.75 (dd, *J* = 2.3, 1.7 Hz, 1H), 7.67-7.63 (ddd, *J* = 8.6, 6.9, 1.2 Hz, 1H), 7.56-7.55 (d, *J* = 8.6 Hz, 1H), 7.46-7.42 (m, 2H), 7.40-7.36 (dd, *J* = 8.0, 8.0 Hz, 1H), 7.17-7.16 (d, *J* = 8.6 Hz, 1H), 5.68 (s, 2H). ^{13}C NMR (125 MHz, DMSO-d_6) δ 164.8, 152.2, 141.5, 139.7, 138.6, 135.7, 133.2, 131.4, 130.7, 124.7, 123.6, 123.3, 122.5, 118.8, 117.7, 114.3, 111.3, 55.0. HRMS, $[M+H]^+$, $C_{18}H_{12}ClN_3O_3$, calc. 354.0645, obs. 354.0644. HPLC retention time: 6.99 min.

4-Oxo-*N*-(2-chlorophenyl)-[1]benzopyrano[4,3-c]pyrazole-1(4H)acetamide (**8c**). Prepared from **5c** and **7a** to give a yellow solid (31% yield). Mp 245-247 °C. ^{1}H NMR (500MHz, DMSO-d_6) δ 10.24 (s, 1H), 8.37 (s, 1H), 8.07-8.05 (d, *J* = 8.0 Hz, 1H), 7.69-7.64 (m, 2H), 7.57-7.55 (dd, *J* = 8.6, 1.2 Hz, 1H), 7.54-7.52 (dd, *J* = 8.0, 1.2 Hz, 1H), 7.46-7.43 (ddd, *J* = 8.6, 6.9, 1.2 Hz, 1H), 7.39-7.31 (dd, *J* = 7.5, 6.9 Hz, 1H), 7.25-7.21 (dd, *J* = 8.0, 7.5 Hz, 1H), 5.79 (s, 2H). ^{13}C NMR (125 MHz, DMSO-d_6) δ 165.1, 156.3, 152.2, 141.4, 138.5, 134.3, 131.4, 129.7, 127.6, 127.0, 126.7, 126.2, 124.6, 123.3, 117.7, 111.2, 107.7, 54.8. HRMS, $[M+H]^+$, $C_{18}H_{12}ClN_3O_3$, calc. 354.0645, obs. 354.0653. HPLC retention time: 6.83 min.

4-Oxo-*N*-(4-fluorophenyl)-[1]benzopyrano[4,3-c]pyrazole-1(4H)acetamide (**8d**). Prepared from **5d** and **7a** to give a yellow solid (35% yield). Mp 250-252 °C. ^{1}H NMR (500 MHz, DMSO-d_6) δ 10.67 (s, 1H), 8.37 (s, 1H), 8.06-8.04 (dd, *J* = 8.6, 1.7 Hz, 1H), 7.67-7.63 (ddd, *J* = 8.6, 6.9, 1.2 Hz, 1H), 7.60-7.55 (m, 3H), 7.44-7.41 (ddd, *J* = 8.0, 7.5, 1.2 Hz, 1H), 7.19-7.16 (m, 2H), 5.67 (s, 2H). ^{13}C NMR (125 MHz, DMSO-d_6) δ 164.9, 157.0, 152.6, 142.0, 139.1, 135.2, 131.9, 125.1, 123.9, 121.6, 121.5, 118.1, 116.1, 116.0, 111.9, 108.2, 55.5. HRMS, [M+H]+, $C_{18}H_{12}FN_3O_3$, calc. 338.0941, obs. 338.0956. HPLC retention time: 6.41 min.

4-Oxo-*N*-(4-trifluoromethylphenyl)-[1]benzopyrano[4,3-c]pyrazole-1(4H)acetamide (**8e**). Prepared from **5e** and **7a** to give a yellow solid (36% yield). Mp 274-276 °C. ^{1}H NMR (500 MHz, DMSO-d_6) δ 11.00 (s, 1H), 8.38 (s, 1H), 8.07-8.05 (dd, *J* = 8.0, 1.2 Hz, 1H), 7.80-7.78 (d, *J* = 8.6 Hz, 1H), 7.72-7.70 (d, *J* = 8.6 Hz, 1H), 7.65-7.63 (ddd, *J* = 8.0, 7.5, 1.2 Hz, 1H), 7.57-7.56 (d, *J* = 8.0 Hz, 1H), 7.44-7.41 (ddd, *J* = 8.0, 7.5, 1.2 Hz, 1H), 5.72 (s, 2H). ^{13}C NMR (125 MHz, DMSO-d_6) δ 165.0, 156.4, 152.2, 141.9, 141.6, 138.6, 131.4, 126.3, 126.2, 124.6, 123.7, 123.3, 119.3, 117.7, 111.4, 107.7, 51.1. HRMS, $[M+H]^+$, $C_{19}H_{12}F_3N_3O_3$, calc. 388.0909, obs. 388.0926. HPLC retention time: 6.63 min.

4-Oxo-*N*-(3,4-dichlorophenyl)-[1]benzopyrano[4,3-c]pyrazole-1(4H)acetamide (**8f**). Prepared from **5f** and **7a** to give a yellow solid (32% yield). Mp 275-277 °C. ^{1}H NMR (500 MHz, DMSO-d_6) δ 10.96 (s, 1H), 8.37 (s, 1H),

8.05-8.03 (dd, *J* = 8.0, 1.2 Hz, 1H), 7.94-7.93 (d, *J* = 2.9 Hz, 1H), 7.67-7.64 (ddd, *J* = 8.6, 6.9, 1.2 Hz, 1H), 7.62-7.60 (d, *J* = 8.6 Hz, 1H), 7.57-7.55 (dd, *J* = 8.6, 1.2 Hz, 1H), 7.50-7.48 (dd, *J* = 9.2, 2.9 Hz, 1H), 7.44-7.41 (ddd, *J* = 8.0, 7.5, 1.2 Hz, 1H), 5.69 (s, 2H). ^{13}C NMR (125 MHz, DMSO-d_6) δ 165.5, 156.9, 152.7, 142.1, 139.2, 138.9, 132.0, 131.7, 131.5, 125.9, 125.2, 123.9, 121.1, 120.0, 118.2, 112.0, 108.2, 55.6. HRMS, $[M+H]^+$, $C_{18}H_{11}Cl_2N_3O_3$, calc. 388.0256, obs. 388.0262. HPLC retention time: 6.56 min.

4-Oxo-*N*-(2,4-dichlorophenyl)-[1]benzopyrano[4,3-c]pyrazole-1(4H)acetamide (**8g**). Prepared from **5g** and **7a** to give a yellow solid (36% yield). Mp 244-246 °C. ^{1}H NMR (500 MHz, DMSO-d_6) δ 10.32 (s, 1H), 8.37 (s, 1H), 7.92-7.91 (d, *J* = 7.5 Hz, 1H), 7.82-7.81 (d, *J* = 8.6 Hz, 1H), 7.71-7.69 (d, *J* = 2.9 Hz, 1H), 7.67-7.64 (ddd, *J* = 8.6, 7.5, 1.7 Hz, 1H), 7.47-7.44 (dd, *J* = 8.6, 2.9 Hz, 1H), 7.33-7.29 (m, 2H), 5.79 (s, 2H). ^{13}C NMR (125 MHz, DMSO-d_6) δ 169.2, 158.2, 154.1, 134.3, 133.5, 131.4, 129.6, 129.1, 129.0, 127.7, 127.6, 127.2, 127.1, 126.7, 124.6, 124.0, 116.9, 53.0. HRMS, $[M+H]^+$, $C_{18}H_{11}Cl_2N_3O_3$, calc. 388.0256, obs. 388.0223. HPLC retention time: 6.58 min.

4-Oxo-*N*-(2,3-dihydro-1,4-benzodioxin-6-yl-[1]benzopyrano[4,3-c]pyrazole-1(4H)acetamide (**8h**). Prepared from **5h** and **7a** to give a yellow solid (35% yield). Mp 265-267 °C. ^{1}H NMR (500 MHz, DMSO-d_6) δ 10.43 (s, 1H), 8.35 (s, 1H), 8.03-8.02 (d, *J* = 8.0 Hz, 1H), 7.66-7.63 (dd, *J* = 8.0, 7.5 Hz, 1H), 7.57-7.55 (d, *J* = 8.6 Hz, 1H), 7.44-7.41 (dd, *J* = 8.0, 7.5 Hz, 1H), 7.17-7.16 (d, *J* = 2.3 Hz, 1H), 6.97-6.94 (dd, *J* = 8.6, 2.3 Hz, 1H), 6.82-6.80 (d, *J* = 8.6 Hz, 1H), 5.61 (s, 2H), 4.21-4.19 (m, 4H).). ^{13}C NMR (100 MHz, DMSO-d_6) δ 163.8, 156.3, 152.2, 143.0, 141.5, 139.8, 138.5, 132.0, 131.4, 124.6, 123.4, 117.6, 117.0, 112.5, 111.5, 108.4, 107.6, 64.2, 63.9, 55.0. HRMS, $[M+H]^+$, $C_{20}H_{15}N_3O_5$, calc. 378.1090, obs. 378.1081. HPLC retention time: 6.39 min.

4-Oxo-*N*-(3,4-dimethoxyphenyl)-[1]benzopyrano[4,3-c]pyrazole-1(4H)acetamide (**8i**). Prepared from **5i** and **7a** to give a yellow solid (36% yield). Mp 231-233 °C. ^{1}H NMR (400 MHz, DMSO-d_6) δ 10.48 (s, 1H), 8.36 (s, 1H), 8.08-8.05 (dd, *J* = 8.2, 1.4 Hz, 1H), 7.67-7.63 (ddd, *J* = 8.7, 6.9, 1.4 Hz, 1H), 7.56-7.55 (dd, *J* = 8.2, 1Hz, 1H), 7.47-7.43 (m, 1H), 7.29-7.28 (d, *J* = 2.3 Hz, 1H), 7.06-7.04 (dd, *J* = 8.7, 2.3 Hz, 1H), 6.93-6.90 (m, 1H), 5.63 (s, 2H), 3.71 (s, 3H), 3.69, (s, 3H). ^{13}C NMR (100 MHz, DMSO-d_6) δ 163.8, 156.4, 152.2, 148.6, 145.2, 141.5, 138.5, 131.9, 131.4, 124.6, 123.4, 117.6, 112.0, 111.5, 111.1, 107.9, 104.2, 55.7, 55.3, 55.0. HRMS, $[M+H]^+$, $C_{20}H_{17}N_3O_5$, calc. 380.1246, obs. 380.1245. HPLC retention time: 6.32 min.

4-Oxo-*N*-(2-naphthyl)-[1]benzopyrano[4,3-c]pyrazole-1(4H)acetamide (**8j**)**.** Prepared from **5j** and **7a** to give a brown solid (37% yield). Mp 146-148 °C. ^{1}H NMR (400 MHz, DMSO-d_6) δ 10.83 (s, 1H), 8.39 (s, 1H), 8.29-8.27 (d, *J* = 7.8 Hz, 1H), 8.12-8.10 (d, *J* = 8.2 Hz, 1H), 7.92-7.90 (d, *J* = 9.2 Hz, 1H), 7.87-7.85 (d, *J* = 7.8 Hz, 1H), 7.80-7.78 (d, *J* = 8.2 Hz, 1H), 7.65-7.56 (m, 3H), 7.48-7.39 (m, 3H), 5.74 (s, 2H). ^{13}C NMR (100 MHz, DMSO-d_6) δ 164.6, 156.4, 152.2, 141.6, 138.6, 135.9, 133.3, 131.4, 130.0, 128.6, 127.5, 127.4, 126.6, 124.9, 124.8, 124.7, 123.5, 122.4, 119.8, 117.7, 115.6, 55.1. HRMS, $[M+H]^+$, $C_{22}H_{15}N_3O_3$, calc. 370.1192, obs. 370.1192. HPLC retention time: 7.18 min.

4-Oxo-*N*-(4-chloro-3-methoxyphenyl)-[1]benzopyrano[4,3-c]pyrazole-1(4H)acetamide (**8k**). Prepared from **5k** and **7a** as a yellow solid (39% yield). Mp 243-245 °C. ^{1}H NMR (500 MHz, DMSO-d_6) δ 10.78 (s, 1H), 8.37 (s, 1H), 8.07-8.05 (dd, *J* = 8.0, 1.2 Hz, 1H), 7.67-7.64 (ddd, *J* = 8.6, 6.9, 1.2 Hz, 1H), 7.57-7.55 (m, 1H), 7.51-7.50 (d, *J* = 2.3 Hz, 1H), 7.44-7.41 (ddd, *J* = 8.0, 7.5, 1.2 Hz, 1H), 7.38-7.36 (d, *J* = 8.6 Hz, 1H), 7.12-7.10 (dd, *J* = 8.6, 2.3 Hz, 1H), 5.67 (s, 2H), 3.78 (s, 3H). ^{13}C NMR (125 MHz, DMSO-d_6) δ 164.6, 156.4, 154.5, 152.2, 141.5, 138.6, 138.5,

131.4, 129.9, 124.7, 123.4, 117.7, 115.5, 111.9, 111.4, 107.7, 103.8, 55.8, 55.1. HRMS, $[M+H]^+$, $C_{19}H_{14}ClN_3O_4$, calc. 384.0751, obs. 384.0758. HPLC retention time: 6.93 min.

4-Oxo-*N*-(4-chloro-3-methoxyphenyl)-*N*-methyl-[1]benzopyrano[4,3-c]pyrazole-1(4H)acetamide (**8l**). Prepared from **5l** and **7a** to give a red solid (33% yield). Mp 151-153 °C. ^{1}H NMR (500 MHz, DMSO-d_6) δ 8.25 (s, 1H), 7.83-7.81 (d, *J* = 8.0 Hz, 1H), 7.64-7.61 (ddd, *J* = 7.5, 7.5, 1.2 Hz, 1H), 7.56-7.54 (d, *J* = 8.6 Hz, 1H), 7.50-7.45 (m, 3H), 7.23-7.21 (dd, *J* = 8.6, 1.7 Hz, 1H), 5.37 (s, 2H), 3.95 (s, 3H), 3.23 (s, 3H). ^{13}C NMR (125 MHz, DMSO-d_6) δ 165.0, 156.3, 155.4, 152.1, 141.7, 141.2, 138.2, 131.2, 130.8, 124.6, 123.2, 120.9, 119.9, 117.6, 112.2, 111.3, 107.5, 56.6, 54.0, 48.6. HRMS, $[M+H]^+$, $C_{20}H_{16}ClN_3O_4$, calc. 398.0908, obs. 398.0882. HPLC retention time: 7.39 min.

4-Oxo-*N*-(4-chloro-3-methoxyphenyl)-2-([1]benzopyrano[4,3-c]pyrazole-1(4H))propanamide (**8m**). Prepared from **5m** and **7a** to give a yellow solid (21% yield). Mp 65-67 °C. ^{1}H NMR (500 MHz, DMSO-d_6) δ 10.43 (s, 1H), 8.24-8.23 (dd, *J* = 8.0, 1.7 Hz, 1H), 8.20 (s, 1H), 7.88-7.85 (ddd, *J* = 8.6, 6.9, 1.7 Hz, 1H), 7.74-7.72 (d, *J* = 8.6, Hz, 1H), 7.57-7.54 (dd, *J* = 8.0, 6.9 Hz, 1H), 7.50-7.49 (d, *J* = 2.3 Hz, 1H), 7.36-7.34 (d, *J* = 8.6 Hz, 1H), 7.17-7.15 (dd, *J* = 8.6, 2.3 Hz, 1H), 5.52-5.48 (q, *J* = 7.5 Hz, 1H), 3.82 (s, 3H), 1.95-1.89 (d, *J* = 7.5 Hz, 3H). ^{13}C NMR (500 Hz, DMF) δ 171.7, 167.3, 154.4, 153.9, 138.7, 135.2, 134.6, 129.8, 129.7, 126.3, 125.4, 122.4, 118.1, 115.4, 112.2, 105.2, 104.1, 57.6, 55.8, 16.3. HRMS, $[M+H]^+$, $C_{20}H_{16}ClN_3O_4$, calc. 398.0908, obs. 398.0882. HPLC retention time: 7.25 min.

4-Oxo-*N*-(4-chloro-3-methoxyphenyl)-(7-methyl-[1]benzopyrano[4,3-c]pyrazole-1(4H))acetamide (**8n**). Prepared from **5k** and **7b** to give a yellow solid (33% yield). Mp 248-250 °C. ^{1}H NMR (400 MHz, DMSO-d_6) δ 10.76 (s, 1H), 8.33 (s, 1H), 7.94-7.92 (d, *J* = 8.2 Hz, 1H), 7.51-7.50 (d, *J* = 2.3 Hz, 1H), 7.39-7.36 (m, 2H), 7.27-7.24 (m, 1H), 7.12-7.09 (dd, *J* = 8.7, 2.3 Hz, 1H). 5.64 (s, 2H), 3.78 (s, 3H), 2.42 (s, 3H). ^{13}C NMR (125 MHz, DMSO-d_6) δ 164.6, 156.3, 154.5, 152.3, 142.1, 141.5, 138.6, 129.9, 125.6, 123.0, 117.7, 115.5, 111.9, 108.8, 107.8, 103.7, 55.8, 55.0, 21.0. HRMS, $[M+H]^+$, $C_{20}H_{16}ClN_3O_4$, calc. 398.0908, obs. 398.0881. HPLC retention time: 7.31 min.

3-[(Dimethylamino)methylene]chroman-2,4-dione (**9b**). ^{1}H NMR (500 MHz, $CDCl_3$) δ 9.96 (s, 1H), 7,86-7.82 (dd, *J* = 9.2, 1 Hz, 1H), 7.57-7.53 (m, 1H), 7.36-7.34 (d, *J* = 8.24 Hz, 1H), 7.28-7.26 (m, 1H), 3.5 (s, 6H). ^{13}C NMR (125 MHz, $CDCl_3$) δ 185.9, 164.1, 160.9, 153.3, 132.8, 126.8, 123.2, 118.4, 117.6, 100.2, 47.6.

***N*-(3-methoxyphenyl)-[1]benzopyrano[4,3-c]pyrazol-4(2H)-one** (**9c**)**.** Mp 208-210 °C. ^{1}H NMR (500 MHz, DMSO-d_6) δ 10.51 (s, 1H), 8.87 (s, 1H), 8.00-7.98 (d, *J* = 7.5 Hz, 1H), 7.58-7.55 (ddd, *J* =8.0, 6.9, 1.7 Hz, 1H), 7.46-7.44 (d, *J* = 8.6 Hz, 1H), 7.40-7.37 (ddd, *J* = 7.5, 7.5, 1.2 Hz, 1H), 7.32-7.30 (t, *J* = 2.3 Hz, 1H), 7.26-7.23 (t, *J* = 8.0 Hz, 1H), 7.13-7.11 (d , *J* = 8 Hz, 1H), 6.69-6.67 (dd, *J* = 8.6, 2.3 Hz, 1H), 5.32 (s, 2H), 3.72 (s, 3H). ^{13}C NMR (125 MHz, DMSO-d_6) δ 164.3, 159.6, 156.9, 152.4, 148.1, 139.6, 135.8, 130.6, 129.8, 124.8, 122.4, 117.4, 114.3, 111.5, 109.3, 106.8, 104.9, 55.6, 55.0. HRMS $[M+H]^+$, $C_{19}H_{16}N_3O_4$, calc. 350.1141, obs. 350.1126.

Ethyl 4-oxo-[1]benzopyrano[4,3-c]pyrazole-1(4H)acetate (**11a**)**.** Prepared from **7a** to give a yellow solid (63% yield). ^{1}H NMR (500 MHz, DMSO-d_6) δ 8.37 (s, 1H), 7.94-7.92 (d, *J* = 8.0 Hz, 1H), 7.67-7.65 (dd, *J* = 8.0, 7.5 Hz, 1H), 7.57-7.55 (d, *J* = 8.6 Hz, 1H), 7.46-7.45 (d, *J* = 8.0, 7.5 Hz, 1H), 5.75 (s, 2H), 4.21-4.18 (q, *J* = 6.9 Hz, 1H), 1.21-1.19 (t, *J* = 6.9 Hz, 1H). ^{13}C NMR (125 MHz, DMSO-d_6) δ 167.4, 156.3, 152.2, 141.2, 138.7, 131.6, 124.7, 123.1, 117.7, 111.1, 107.9, 61.8, 53.5, 14.0.

Ethyl [1]benzopyrano[4,3-c]pyrazole-1(4H)acetate (**11b**). Prepared from **10a** as a yellow solid (76% yield). ^{1}H NMR (400 MHz, DMSO-d_6) δ 7.41-7.40 (m, 2H), 7.26-7.23 (ddd, *J* = 8.2, 7.3, 1.4 Hz, 1H), 7.01-6.99 (m, 2H), 5.35 (s, 2H), 5.21 (s, 2H), 4.16-4.13 (q, *J* = 6.9 Hz, 2H), 1.19-1.14 (t, *J* = 6.9 Hz, 3H). ^{13}C NMR (100 MHz, $CDCl_3$) δ 168.0, 153.3, 133.8, 133.3, 129.6, 122.1, 121.9, 117.4, 115.9, 113.5, 63.6, 61.3, 52.7, 14.0.

Ethyl 4,5-dihydro-1H-benz[g]indazole-1-acetate (**11c**)**.** Prepared from **10b** to give a brown oil (72% yield). ^{1}H NMR (400 MHz, DMSO-d_6) δ 7.42 (s, 1H), 7.27-7.25 (m, 4H), 5.18 (s, 2H), 4.28-4.23 (q, *J* = 7.3 Hz, 2H), 2.90-2.88 (dd, *J* = 7.8, 6.9 Hz, 2H), 2.73-2.71 (dd, *J* = 8.3, 6.9 Hz, 2H), 1.26-1.23 (t, *J* = 7.3 Hz, 3H). ^{13}C NMR (100 MHz, DMSO-d_6) δ 168.1, 138.5, 137.8, 136.7, 128.9, 127.5, 126.8, 121.5, 119.0, 61.8, 53.0, 30.7, 19.6, 14.1.

[1]Benzopyrano[4,3-c]pyrazole-1(4H)acetic acid (**12a**). ^{1}H NMR (400 MHz, DMSO) δ 8.35 (s, 1H), 7.95-7.92 (d, *J* = 8.2 Hz, 1H), 7.69-7.65 (dd, *J* = 8.3, 7.3 Hz, 1H), 7.57-7.53 (d, *J* = 8.2 Hz, 1H), 7.48-7.44 (dd, *J* = 8.0, 7.5 Hz, 1H), 5.63 (s, 2H). ^{13}C NMR (100 MHz, DMSO-d_6) δ 168.8, 156.4, 152.2, 141.1, 138.5, 131.5, 124.7, 123.1, 117.7, 111.2, 107.8, 53.7.

[1]Benzopyrano[4,3-c]pyrazole-1(4H)acetic acid (**12b**). Prepared from **11b** to give a yellow solid (95% yield).^{1}H NMR (400 MHz, DMSO-d_6) δ 13.3 (bs, 1H), 7.44-7.41 (dd, *J* = 7.3, 1.4 Hz, 1H), 7.40 (s, 1H), 7.26-7.21 (ddd, *J* = 7.8, 7.8, 1.8 Hz, 1H), 7.05-6.99 (m, 2H), 5.25 (s, 2H), 5.22 (s, 2H). ^{13}C NMR (100 MHz, DMSO-d_6) δ 170.0, 153.3, 133.5, 133.1, 129.5, 122.1, 121.9, 117.4, 116.1, 113.3, 63.6, 52.8.

4,5-Dihydro-1H-benz[g]indazole-1-acetate (**12c**). Prepared from **11c** to give a yellow solid (93% yield). ^{1}H NMR (500 MHz, DMSO-d_6) δ 13.23 (bs, 1H), 7.40-7.38 (m, 2H), 7.36-7.35 (d, *J* = 7.5 Hz, 1H), 7.31-7.28 (ddd, *J* = 8.0, 7.5, 1.2 Hz, 1H), 7.25-7.22 (ddd, *J* = 7.5, 7.5, 1.2 Hz, 1H), 5.21 (s, 2H), 2.86-2.83 (dd, *J* = 8.0, 6.9 Hz, 2H), 2.65-2.62 (dd, *J* = 8.0, 6.9 Hz, 2H). ^{13}C NMR (125 MHz, DMSO-d_6) δ 169.5 153.3, 133.5, 133.1, 129.5, 122.1, 121.9, 117.4, 116.1, 113.3, 63.6, 52.8.

4-Oxo-*N*-(1H-benzo[d]imidazol-6-yl)-[1]benzopyrano[4,3-c]pyrazole-1(4H)acetamide (**13a**). Prepared from **12a** and 1H-benzo[d]imidazol-6-amine to give a brown solid (45% yield). Mp 268-270 °C. ^{1}H NMR (400 MHz, DMSO-d_6) δ 10.61 (s, 1H), 8.38 (s, 1H), 8.17 (s, 1H), 8.10-8.08 (dd, *J* = 8.2, 1.4 Hz, 1H), 7.97-7.96 (d, *J* = 7.8 Hz, 1H), 7.67-7.63 (ddd, *J* = 8.7, 6.9, 1.4 Hz, 1H), 7.58-7.55 (m, 2H), 7.45-7.4 (ddd, *J* = 7.8, 7.3, 1Hz, 1H), 7.28-7.24 (dd, *J* = 11.2, 8.7 Hz, 1H), 5.69 (s, 2H), 3.09-3.07 (m, 1H). ^{13}C NMR (100 MHz, DMSO-d_6) δ 164.0, 162.3, 156.5, 152.2, 142.4, 142.3, 141.5, 138.5, 133.0, 131.4, 124.6, 123.4, 117.7, 114.8, 114.7, 111.5, 111.4, 107.7, 55.1. HRMS, $[M+H]^+$, $C_{19}H_{13}N_5O_3$, calc. 360.1097, obs. 360.1112. The target peak is weak in high-resolution mass spectrum. HPLC retention time: 5.20 min.

4-Oxo-*N*-(1H-indol-6-yl)-[1]benzopyrano[4,3-c]pyrazole-1(4H)acetamide (**13b**). Prepared from **12a** and 6-aminoindole to give a yellow solid (75% yield). Mp 262-264 °C. ^{1}H NMR (500 MHz, DMSO-d_6) δ 11.03 (s, 1H), 10.50 (s, 1H), 8.37 (s, 1H), 8.10-8.09 (dd, *J* = 8.0, 1.2 Hz, 1H), 7.87 (s, 1H), 7.67-7.63 (ddd, *J* = 8.0, 7.5, 1.2 Hz, 1H), 8.58-7.57 (d, *J* = 7.5 Hz, 1H), 7.48-7.46 (d, *J* = 8.6 Hz, 1H), 7.44-7.41 (ddd, *J* = 8.0, 7.5, 1.2 Hz, 1H), 7.28-7.27 (t, *J* = 2.9 Hz, 1H), 7.06-7.04 (dd, *J* = 8.6, 1.7 Hz, 1H), 6.36 (s, 1H), 5.67 (s, 2H). ^{13}C NMR (125 MHz, DMSO-d_6) δ 163.7, 156.5, 152.2, 141.6, 138.5, 135.8, 132.4, 131.4, 125.3, 124.6, 124.3, 123.5, 120.0, 117.6, 112.2, 111.5, 107.6, 102.4, 101.0, 55.1. HRMS, $[M+H]^+$, $C_{20}H_{14}N_4O_3$, calc. 359.1144, obs. 359.1168. HPLC retention time: 7.83 min

4-Oxo-*N*-(1H-indol-5-yl)-[1]benzopyrano[4,3-c]pyrazole-1(4H)acetamide (**13c**). Prepared from **12a** and 5-aminoindole to give a brown solid (35% yield). Mp 244-246 °C. ^{1}H NMR (500 MHz, DMSO-d_6) δ 11.07 (s, 1H), 10.43 (s, 1H), 8.37 (s, 1H), 8.11-8.09 (d, *J* = 8.0 Hz, 1H), 7.83 (s, 1H), 7.66-7.63 (t, *J* = 7.5 Hz, 1H), 7.57-7.55 (d, *J* = 8.6 Hz, 1H), 7.45-7.42 (t, *J* = 7.45 Hz, 1H), 7.35-7.31 (m, 2H), 7.23-7.21 (dd, *J* = 8.6, 1.2 Hz, 1H), 6.36 (s, 1H), 5.66 (s, 2H). ^{13}C NMR (125 MHz, DMSO-d_6) δ 163.6, 156.5, 152.2, 141.5, 138.5, 133.0, 131.3, 130.3, 127.5, 126.1, 124.6, 123.5, 117.6, 114.7, 111.5, 111.4, 110.9, 107.6, 101.1, 55.1. HRMS, $[M+H]^+$, $C_{20}H_{14}N_4O_3$, calc. 359.1144, obs. 359.1169. HPLC retention time: 7.46 min.

4-Oxo-*N*-(quinolin-6-yl)-[1]benzopyrano[4,3-c]pyrazole-1(4H)acetamide (**13d**). Prepared from **12a** and 6-aminoquinoline to give a brown solid (15% yield). Mp 238-240 °C. ^{1}H NMR (500 MHz, DMSO-d_6) δ 10.98 (s, 1H), 8.79 (bs, 1H), 8.37(s, 1H), 8.34-8.33 (d, *J* = 1.7 Hz, 1H), 8.25-8.24 (d, *J* = 8.6 Hz, 1H), 8.10-8.09 (d, *J* = 7.5 Hz, 1H), 8.00-7.99 (d, *J* = 9.2 Hz, 1H), 7.81-7.79 (dd, *J* = 9.2, 2.3 Hz, 1H), 7.65-7.62 (t, *J* = 7.5 Hz, 1H), 7.56-7.54 (d, *J* = 8.0 Hz, 1H), 7.47-7.46 (dd, *J* = 8.0, 4.0 Hz, 1H), 7.43-7.30 (t, *J* = 7.5 Hz, 1H), 5.76 (s, 2m). ^{13}C NMR (125 MHz, DMSO-d_6) δ 164.8, 156.5, 152.2, 149.4, 144.9, 141.6, 138.6, 136.2, 135.6, 131.4, 129.8, 128.1, 124.7, 123.4, 123.2, 121.9, 117.7, 115.5, 111.5, 108.0. 54.9. HRMS, $[M+H]^+$, $C_{21}H_{14}N_4O_3$, calc. 371.1144, obs. 371.1137. HPLC retention time: 5.74 min

***N*-(4-Chloro-3-methoxyphenyl)-[1]benzopyrano[4,3-c]pyrazole-1(4H)acetamide** (**13e**). Prepared from **12b** and 4-chloro-3-methoxyaniline to give a yellow solid (41% yield). Mp 184-186 °C. ^{1}H NMR (400 MHz, DMSO-d_6) δ 10.64 (s, 1H), 7.57-7.56 (dd, *J* = 8.0, 1.7 Hz, 1H), 7.54-7.53 (d, *J* = 2.3 Hz, 1H), 7.43 (s, 1H), 7.37-7.36 (d, *J* = 8.6 Hz, 1H), 7.26-7.22 (ddd, *J* = 8.0, 7.5, 1.2 Hz, 1H), 7.13-7.11 (dd, *J* = 8.6, 2.3 Hz, 1H), 7.03-7.01 (m, 2H), 5.31 (s, 2H), 5.23 (s, 2H), 3.80 (s, 3H). ^{13}C NMR (100 MHz, DMSO-d_6) δ 165.6, 154.5, 153.3, 138.7, 133.7, 129.9, 129.5, 122.4, 121.8, 117.3, 116.2, 115.3, 113.2, 111.8, 103.7, 63.6, 55.8, 54.2. HRMS, $[M+H]^+$, $C_{19}H_{16}ClN_3O_3$, calc. 370.0958, obs. 370.0941. HPLC retention time: 7.54 min

***N*-(4-Chloro-3-methoxyphenyl)-4,5-dihydro-1H-benz[g]indazole-1-acetamide** (**13f**)**.** Prepared from **12c** and 4-chloro-3-methoxyaniline to to give a yellow solid (45% yield). Mp 194-196 °C. ^{1}H NMR (400 MHz, DMSO-d_6) δ 10.63 (s, 1H), 7.56-7.55 (m, 2H), 7.42-7.38 (m, 2H), 7.36 (s, 1H), 7.31-7.27 (ddd, *J* = 7.8, 7.3, 1.4 Hz, 1H), 7.25-7.21 (ddd, *J* = 7.8, 7.3, 1.4 Hz, 1H), 7.17-7.14 (dd, *J* = 8.2, 2.3 Hz, 1H), 5.30 (s, 2H), 3.81 (s, 3H), 2.87-2.83 (dd, *J* = 7.8, 6.9 Hz, 2H), 2.67-2.63 (dd, *J* = 7.8, 6.9 Hz, 2H). ^{13}C NMR (100 MHz, DMSO-d_6) δ 166.0, 154.5, 138.8, 137.9, 137.3, 135.9, 129.9, 128.8, 127.4, 126.9, 126.7, 122.1, 118.1, 115.3, 111.9, 103.7, 55.8, 54.3, 30.2, 19.3. HRMS, $[M+H]^+$, $C_{20}H_{18}ClN_3O_2$, calc. 368.1166, obs. 368.1159. HPLC retention time: 7.90 min.

Determination of *Cp*IMPDH IC_{50} values.[2, 3] Inhibition of recombinant *Cp*IMPDH, purified from *E. coli*,[4-6] was assessed by monitoring the production of NADH by fluorescence at varying inhibitor concentrations (25 pM - 5 μM). IMPDH was incubated with inhibitor for 5 min at room temperature prior to addition of substrates. The following conditions were used: 50 mM Tris-HCl, pH 8.0, 100 mM KCl, 3 mM EDTA, 1 mM dithiothreitol (assay buffer) at 25 °C, 10 nM *Cp*IMPDH, 300 μM NAD and 250 μM IMP. IC_{50} values were calculated for each inhibitor according to Equation 1 using the SigmaPlot program (SPSS, Inc.):

$$\upsilon_i = \upsilon_o/(1 + [I]/IC_{50}) \qquad \text{(Eq. 1)}$$

where υ_i is initial velocity in the presence of inhibitor (I) and υ_o is the initial velocity in the absence of inhibitor. Inhibition at each inhibitor concentration was measured in quadruplicate and averaged; this value was used as υ_i. The IC_{50} values were determined three times; the average and standard deviations are reported.

Assessment of *h*IMPDH2 inhibition. Inhibition of recombinant *h*IMPDH2 was assessed by monitoring the production of NADH (fluorescence) at a test compound concentration of 5 μM. Assays were performed in 50 mM Tris-HCl, pH 8.0, 100 mM KCl, 3 mM EDTA, 1 mM dithiothreitol (assay buffer), 70 nM *h*IMPDH2, 100 μM NAD and 250 μM IMP at 25 °C. None of the compounds significantly inhibited *h*IMPDH2 at 5 μM.

Gene cloning, protein expression, purification and crystallization. The 90-134-deletion variant of *Cp*IMPDH enzyme was cloned, expressed, and purified as previously described.[2] Crystallizations were set up with the help of a Mosquito liquid dispenser (TTP LabTech) using the sitting-drop vapor-diffusion method in 96-well CrystalQuick plates (Greiner Bio-One). The INDEX screen (Hampton Research) and MCSG1-4 screens were used (Microlytic) for the initial screening. For each condition, 0.4 μl protein solution and 0.4 μl crystallization formulation were mixed and equilibrated against a 135 μl reservoir of crystallization formulation. Diffraction quality crystals with IMP and **8k** were obtained after optimization of the condition consisting of 0.1 M Tris pH 7.0 and 15% ethanol in a hanging drop format. The crystals appeared after 2 days from a solution containing 10 mg/ml protein, 5 mM IMP and 1.5 mM **8k** at 16 °C. The crystals were mounted on LithoLoops (Molecular Dimension) and flash-cooled in liquid nitrogen. The cryoprotectant consisted of 20% ethylene glycol, 0.1 M Tris pH 7.0 and 15% ethanol.

Data collection and structure determination. Diffraction data were collected at 100 °K at the 19-ID beamline of the Structural Biology Center at the Advanced Photon Source, Argonne National Laboratory.[7] The single wavelength data at 0.9793 Å up to 2.40 Å were collected from a single crystal of *Cp*IMPDH in complex with IMP and **8k**. The crystal was exposed for 3 s per 1.0° rotation of ω with the crystal to detector distance of 330 mm. The data were recorded on a CCD detector Q315r from ADSC scanning 160º. The SBC-Collect program was used for all data collection and visualization. Data collection strategy, integration, and scaling were performed with the HKL3000 program package.[8] Summary of the crystallographic data can be found in Table S1.

The structure was determined by molecular replacement using chain A of the structure of *Cp*IMPDH (PDB ID 3FFS; 3.19 Å)[9] as a search model with HKL3000 (Molrep/refmac) using the data to 2.40 Å. Rigid-body refinement was done at 3.0 Å and the initial refinement was done at 2.40 Å as part of HKL300 molecular replacement procedure.[8] The initial model contained 4 copies of the search model and there was extra electron density for additional protein residues that were not part of the search model. The presence of IMP and **8k** in the active site was apparent from the initial electron density map (F_o). Extensive manual model building with coot[10] and the subsequent refinement using phenix.refine[11] was performed against the full data set up to 2.40 Å until the structure converged to the *R* factor (R_{work}) of 0.202, and R_{free} of 0.258 with the r.m.s. bond distances of 0.010 and the r.m.s. bond angles of 1.445°. The asymmetric unit contains four protein chains, A, B, C and D. Several C-terminal and some N- terminal residues that were introduced as a cloning artifact (SNA)[12] are missing in the final model due to disorder. Chain A is comprised of residues 2-92 and 135-399, chain B includes residues 0-92 and 135-399, chain C contains residues 0-92 and 135-399 and chain D is comprised of residues -1-92 and 135-399. In addition, several residues within the active site flap are disordered and are not modeled. These include residues 310-323 of chain A, 311-323 of chain B, 311-323 of chains C and D. The SGG linker that replaces residues 90-134 in the *Cp*IMPDH variant[9] is visible in all chains. The final model includes four IMP molecules, four **8k** molecules, 133 ordered water molecules and five other small molecules such as ethylene glycol, acetic acid, formic acid used in the purification and crystallization. The stereochemistry of the structure was checked with PROCHECK[13] and the Ramachandran plot. Atomic coordinates and experimental structure factors of the structure have been deposited in the PDB under the ID code 4QJ1.

Table S1: Statistics for data collection and refinement

DATA COLLECTION	
X-ray wavelength (Å)	0.9793
Temperature (°K)	100
Space group	$P2_1$
Unit cell (Å, °)	$a = 90.412$, $b = 92.337$, $c = 91.914$, $\beta = 104.24$
Resolution (Å)[a]	2.40 (2.40-2.44)
Total no. of reflections	55,500 (2649)
‹I/σ(I)›	16.9 (2.1)
Completeness of data (%)	99.1 (95.6)
[b]R_{merge}(%)	0.150 (0.664)
REFINEMENT	
Resolution range (Å)	39.6-2.4
Reflections used (working/free)	47418/2554
[c]R_{work} /R_{free}(%)	20.2/25.8
Total number of non-hydrogen atoms in asymmetric unit	20880
R.m.s. deviations from ideal geometry	
Bond length (Å)	0.010
Bond angles (deg)	1.445
Mean B value (Å^2)	53.2
PDB accession code	4QJ1

[a] Values in parenthesis correspond to the highest-resolution shell.
[b] $R_{merge} = \Sigma_{hkl}\Sigma_i|I_i(\text{hkl}) - \langle I(hkl)\rangle|/\Sigma_{hkl}\Sigma_i|\langle I_i(hkl)\rangle|$, expressed as %, where I_i(hkl) is the intensity for the ith measurement of an equivalent reflection with indices h, k, and l.
[c] $R = \Sigma_{hkl}\,||F_o| - |F_c||/\,\Sigma_{hkl}|F_o|$, expressed as %, where F_o and F_c are observed and calculated factors, respectively. R_{free} is calculated analogously for the test reflections, which were randomly selected and excluded from refinement.

Supporting Information References

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