SUPPORTING INFORMATION

Novel, potent small molecule inhibitors of the molecular chaperone Hsp90 discovered through structure-based design

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1. Experimental Procedures for the Synthesis of Compounds 1 - 12

1.1 General Points

^1H (400 MHz) and ^{13}C (100 MHz) Nuclear magnetic resonance (NMR) analyses were performed using a Bruker DPX-400 MHz NMR spectrometer. The spectral reference was the known chemical shift of the sample solvent. ^1H nmr data is reported indicating the chemical shift (δ), the integration (e.g. 1H), the multiplicity (s, singlet; d, doublet; t, triplet; q, quartet; m, multiplet; br, broad; dd, doublet of doublets etc.), the coupling constant (J) in Hz (app implies apparent coupling on broadened signals), and the assignment. ^{13}C data is reported indicating the chemical shift (δ). Deuterated solvents were obtained from the Sigma-Aldrich Chemical Company or Fluorochem.

LCMS analyses were performed on a HP1100 instrument, with a Luna 3 μM , C18(2), 30 mm $\times$ 4.6 mm i.d. column from Phenomenex at a temperature of 22 $^\circ\text{C}$, at a flow rate of 2 mL min $^{-1}$ using the following solvent systems (Solvents purchased from Romil UK, Waterbeach, UK; <http://www.romil.com>):

Solvent A: HPLC grade Water + 10 mM ammonium acetate + 0.08% v/v formic acid.

Solvent B: 95% v/v HPLC grade acetonitrile + 5% v/v Solvent A + 0.08% v/v formic acid.

Gradient: 95:5 Solvent A : Solvent B, 0.00 to 0.25 mins; 95 : 5 to 5 : 95 Solvent A : Solvent B, 0.25 to 2.50 mins; 5 : 95 Solvent A : Solvent B, 2.50 to 3.75 mins.

UV detection was at 230 nm, 254 nm and 270 nm. Mass spectrometer was a HP1100MSD, Series A instrument, operating in positive or negative ion electrospray ionisation mode. Molecular weight scan range is 120 to 1000. Samples were supplied as a 1 mM solution in DMSO, with 5 μL partial loop fill injection.

Preparative HPLC purifications were performed on a Waters FractionLynx MS Autopurification system with a Gemini $^{\text{®}}$ 5 μM C18(2), 100 mm $\times$ 20 mm i.d. column from Phenomenex, running at a flow rate of 20 mL min $^{-1}$ with UV diode array detection (210 – 400 nm) and mass-directed collection. Gradients used for each compound are shown in Table 1. Solvents A and B are as for the analytical conditions above. The mass spectrometer was a Waters Micromass ZQ2000 spectrometer operating in positive or

negative ion electrospray ionisation modes, with a molecular weight scan range of 150 to 1000.

Table 1 Preparative HPLC gradients

Time /min	%B for Compound no.			
	8	9	11	12
0.0	5	5	5	5
0.5	20	25	30	35
7.0	40	45	50	55
7.5	95	95	95	95
9.5	95	95	95	95
10	5	5	5	5

Flash chromatography was performed using pre-packed silica gel cartridges (Strata Si-1, 61 Å from Phenomenex, Cheshire UK, or IST Flash II, 54 Å from Argonaut, Hengoed UK). Thin layer chromatography was conducted with 5 × 10 cm plates coated with Merck Type 60 F₂₅₄ silica gel to a thickness of 0.25 mm.

Microanalyses were performed by Medac Ltd, Egham UK (<http://www.medacLtd.com>).

All reagents obtained from commercial sources were used without further purification. Anhydrous solvents were obtained from the Sigma-Aldrich Chemical Company Ltd., and used without further drying.

The following abbreviations are used in the text: DCM, dichloromethane; DMF *N,N*-dimethylformamide; EDCI, *N*-Ethyl-*N'*-(3-dimethylaminopropyl)carbodiimide hydrochloride; EtOAc, ethyl acetate; Hex, *n*-hexane. Ether refers to diethyl ether and brine to a saturated aqueous solution of sodium chloride.

All compounds were >95% purity as determined by LCMS (wavelengths 230 nm, 254 nm and 270 nm) and by ¹H nmr, and by TLC where R_f data given, unless otherwise indicated. Where Cl or Br was present, expected isotopic distribution patterns were observed.

Compounds **1** (CCT018159) and **2** are commercially available from ChemDiv¹ (Catalogue Nos. 1187-0530 and 1187-0523 respectively). The preparation of compound **3** has been previously described elsewhere.²

Table 2. Compound Characterisation Data Available

Compound Number	¹H NMR	HPLC*	Mass Spectrum	¹³C NMR	Micro analysis (CHN)
4	√	√	√	√	
5	√	√	√	√	√
6	√	√	√	√	
7	√	√	√		
8	√	√	√		
9	√	√	√		
10	√	√	√		
11	√	√	√	√	√
12	√	√	√		
15	√	√	√		
16	√	√	√		
17	√	√	√		
18	√	√	√		
19	√	√	√		
20	√	√	√		
22	√	√	√		
23	√	√	√		
24	√	√	√		

1.2 Synthetic Procedures

Scheme 1

Reagents and Conditions: a. 4-Methoxyphenylacetic acid, $BF_3 \cdot OEt_2$, 90 °C, 90 mins.; b. Ac_2O , K_2CO_3 , DMF, 115 °C, 90 mins.; c. Hydrazine hydrate, EtOH, 78 °C, 5 h.; d. $BnNMe_3^+Br_3^-$, DCM, rt, 90 mins.

2,4-Dihydroxyphenyl-4-methoxybenzylketone **15**

Resorcinol **13** (4.4 g, 40 mmol) and 4-methoxyphenylacetic acid (6.6 g, 40 mmol) in boron trifluoride etherate (25 mL, 0.2 mol) were heated, under a nitrogen atmosphere, at 90 °C for 90 mins to give a pale red solution. The solution was allowed to cool and poured into aqueous sodium acetate (10% aq.; 200 mL) and the mixture stirred to give a pale yellow precipitate. The solids were removed by filtration and washed with water (200 mL). The solids were taken up in ethyl acetate (250 mL) and washed with water (200 mL). The solution was dried over anhydrous magnesium sulfate and concentrated to a yellow semi-solid. Trituration with diethyl ether (100 mL) gave **15** (2.2 g, 21%) as a pale orange solid after drying *in vacuo*; R_f 0.19 (DCM), 0.84 (EtOAc) LC retention time 2.39 mins, m/z $[M+H]^+$ 259.2; δ_H (DMSO- d_6) 7.95 (1H, d, J 8.9 Hz, ArH), 7.2 (2H, d, J

8.7 Hz, 2 × ArH), 6.9 (2H, d, *J* 8.7 Hz, 2 × ArH), 6.4 (1H, d, *J* 9.9 Hz, ArH), 6.25 (1H, s, ArH), 4.2 (2H, s, CH₂), 3.75 (3H, s, OCH₃).

7-Hydroxy-3-(4-methoxyphenyl)-2-methylchromen-4(4H)-one **17**

Acetic anhydride (3 mL, 30 mmol) was added to a suspension of potassium carbonate (4.0 g, 29 mmol) and 2,4-dihydroxyphenyl-4-methoxybenzylketone **15** (1.95 g, 7.5 mmol) in DMF (10 mL), and the resulting suspension heated at 115 °C for 90 mins. The mixture was allowed to cool and poured into water (200 mL), to give an off-white precipitate. The solids were removed by filtration and washed with water (100 mL) and diethyl ether (2 × 40 mL), to give **17** (1.65 g, 78%) as an off-white powder after drying *in vacuo*; R_f 0.00 (DCM), 0.77 (EtOAc); LC retention time 2.26 mins, *m/z* [M+H]⁺ 283.2; δ_H(DMSO-*d*₆) 7.8 (1H, d, *J* 8.7 Hz, ArH), 7.2 (2H, d, *J* 8.8 Hz, 2 × ArH), 7.0 (2H, d, *J* 8.8 Hz, 2 × ArH), 6.9 (1H, d, *J* 8.7 Hz, ArH), 6.8 (1H, s, ArH), 3.8 (3H, s, OCH₃), 2.2 (3H, s, 3CH₃).

3-(2,4-Dihydroxyphenyl)-4-(4-methoxyphenyl)-5-methyl-1H-pyrazole **4**

Hydrazine hydrate (~55% aq.; 2mL) was added to a suspension of 7-hydroxy-3-(4-methoxyphenyl)-2-methylchromen-4-one **17** (0.57 g, 2 mmol) in ethanol (15 mL) and the mixture heated under reflux for 5 h to give a pale brown solution. The solution was allowed to cool and concentrated to a pale yellow solid. The solids were washed with water, to give **4** (0.45 g, 76%) as an off-white solid after drying *in vacuo*. A sample was recrystallised from boiling toluene, to give the pyrazole **4** as a pale brown crystalline solid, washed with hexane and dried *in vacuo*; R_f 0.12 (2 : 1 Hex : EtOAc); LC retention time 2.16 mins, *m/z* [M+H]⁺ 297.2; δ_H(Acetone-*d*₆) 7.2 (2H, d, *J* 8.8 Hz, 2 × ArH), 7.0 (2H, d, *J* 8.8 Hz, 2 × ArH), 6.9 (1H, d, *J* 8.6 Hz, ArH), 6.35 (1H, s, ArH), 6.1 (1H, d, *J* 8.7 Hz, ArH), 3.85 (3H, s, OCH₃), 2.2 (3H, s, CH₃); δ_C(Acetone-*d*₆) 159.8, 159.1, 158.7, 132.4, 129.1, 127.1, 117.1, 114.9, 110.6, 106.7, 104.0, 55.5, 9.7

Compound **6** was prepared as for **4** above, starting from 4-chlororesorcinol:

5-Chloro-2,4-dihydroxyphenyl-4-methoxybenzylketone 16

As **15**, starting from 4-chlororesorcinol **14** (10.0 g, 69.2 mmol) in boron trifluoride etherate (43 mL, 141.93 mmol) to give **16** (18.15 g, 90%) as a tan coloured solid; LC retention time 2.74 mins, m/z $[M+H]^+$ 293.2/295.2; δ_H (DMSO- d_6) 7.65 (1H, s, ArH), 7.0 (2H, d, J 8.7 Hz, $2 \times$ ArH), 6.7 (2H, d, J 8.7 Hz, $2 \times$ ArH), 5.45 (1H, s, ArH), 3.7 (2H, s, CH_2), 3.5 (3H, s, OCH_3).

6-Chloro-7-hydroxy-3-(4-methoxyphenyl)-2-methylchromen-4(1H)-one 18

As **17**, starting from 5-chloro-2,4-dihydroxyphenyl-4-methoxybenzylketone **16** (2.95 g, 10.0 mmol) to give **18** (2.20 g, 69%) as a pale brown powder; LC retention time 2.33 mins, m/z $[M+H]^+$ 317.2/319.2, δ_H (DMSO- d_6) 8.0 (1H, s, ArH), 7.3 (2H, d, J 8.7 Hz, $2 \times$ ArH), 7.15 (1H, s, ArH), 7.1 (2H, d, J 8.7 Hz, $2 \times$ ArH), 3.9 (3H, s, OCH_3), 2.35 (3H, s, CH_3).

3-(5-Chloro-2,4-dihydroxyphenyl)-4-(4-methoxyphenyl)-5-methyl-1H-pyrazole 6

As **4**, starting from 6-chloro-7-hydroxy-3-(4-methoxyphenyl)-2-methylchromen-4-one **18** (100 mg, 0.3 mmol) to give **6** (53 mg, 53%) as a white crystalline solid after recrystallisation from boiling 2-propanol; LC retention time 2.34 mins, m/z $[M+H]^+$ 331.2/333.2; δ_H (Acetone- d_6) 7.25 (2H, d, J 8.8 Hz, $2 \times$ ArH), 7.15 (2H, d, J 8.8 Hz, $2 \times$ ArH), 7.1 (1H, s, ArH), 6.5 (1H, s, ArH), 3.85 (3H, s, OCH_3), 2.2 (3H, s, CH_3); δ_C (Acetone- d_6) 160.1, 157.8, 153.7, 132.4, 128.7, 126.5, 117.4, 115.1, 111.9, 110.7, 105.2, 55.6, 9.6.

3-(5-Bromo-2,4-dihydroxyphenyl)-4-(4-methoxyphenyl)-5-methyl-1H-pyrazole 5

Benzyltrimethylammonium tribromide (4.0 g, 10.2 mmol) was added portionwise to a stirred suspension of 3-(2,4-dihydroxyphenyl)-4-(4-methoxyphenyl)-5-methyl(1H)pyrazole **4** (2.95 g, 10.0 mmol) in dichloromethane (50 mL) and the mixture stirred at room temperature for 90 mins. The mixture was washed with water ($3 \times$ 50 mL) and brine (25 mL). The solution was dried over anhydrous magnesium sulfate and concentrated to give a pale yellow solid. The crude product was purified by flash

chromatography (SiO₂) eluting with 2 : 1 hexane : ethyl acetate to give **5** (1.75 g, 47%) as an off-white powder; *R_f* 0.16 (2 : 1 Hex : EtOAc); LC retention time 2.37 mins, *m/z* [M+H]⁺ 375.1/377.1; δ_{H} (Acetone-*d*₆) 7.25 (2H, d, *J* 8.8 Hz, 2 × *ArH*), 7.2 (1H, s, *ArH*), 7.1 (2H, d, *J* 8.8 Hz, 2 × *ArH*), 6.55 (1H, s, *ArH*), 3.9 (3H, s, OCH₃), 2.2 (3H, s, CH₃); δ_{C} (Acetone-*d*₆) 160.1, 158.4, 154.7, 139.4, 132.3, 131.8, 126.4, 117.4, 115.1, 112.5, 105.0, 99.0, 55.6, 9.6; Anal. (C₁₇H₁₅BrN₂O₃) C, H, N.

Scheme 2

Reagents and conditions: a. i. ClC(=O)CO₂Me, pyr, 0 °C, 18 h; ii. HCl, MeOH-H₂O, reflux, 4 h; b. NaHCO₃(aq), MeOH, 65 °C, 5 h; d. RNH₂, EDCI, 1-HOBt, *N*-methylmorpholine, DCM, rt, 18 h; e. BnNMe₃Br₃, MeCN-DMF, rt, 90 mins.

Methyl 6-chloro-7-hydroxy-3-(4-methoxyphenyl)-4-oxo-4H-chromene-2-carboxylate **19**

5-Chloro-2,4-dihydroxyphenyl-4-methoxybenzylketone **16** (2.50 g, 8.55 mmol) was taken up in anhydrous pyridine (30 mL) and cooled on an ice bath to 0 °C. Methyl chlorooxoacetate (2.80 mL, 3.73 g, 25.64 mmol) was added dropwise and the solution was stoppered and allowed to stand in the refrigerator over night. The bright orange solution was added carefully to HCl (1.0 M aq; 100 mL) and extracted with dichloromethane (2 × 70 mL). The organic phases were combined and washed with brine (3 × 50 mL), dried over anhydrous magnesium sulfate, filtered and concentrated *in vacuo* to a yellow solid, which was suspended in a mixture of HCl (1.0 M aq; 20 mL) and methanol (20 mL) and heated at reflux for 4 h. The reaction mixture was cooled and concentrated *in vacuo* to give **19** (2.40 g, 78%) as a pale yellow solid; LC retention time 2.42 mins, m/z $[M+H]^+$ 361.2/363.2; δ_H (CDCl₃) 8.22 (1H, s, ArH), 7.21 (2H, d, J 8.8 Hz, 2 × ArH), 7.13 (1H, s, ArH), 6.95 (2H, d, J 8.8 Hz, 2 × ArH), 3.83 (3H, s, OCH₃), 3.72 (3H, s, OCH₃).

6-Chloro-7-hydroxy-3-(4-methoxyphenyl)-4-oxo-4H-chromene-2-carboxylic acid **20**

Methyl 6-chloro-7-hydroxy-3-(4-methoxyphenyl)-4-oxo-4H-chromene-2-carboxylate **19** (2.40 g, 6.67 mmol) was taken up in a mixture of NaHCO₃ (sat. aq; 35 mL) and methanol (18 mL) and was heated at 65 °C for 5 h. The solution was cooled to room temperature and concentrated *in vacuo* to remove the methanol. The residual aqueous solution was acidified with HCl (1.0 M aq) and a buff coloured precipitate formed. This was collected by vacuum filtration and washed with water and with diethyl ether to give **20** (902 mg, 39%) as an off-white powder; LC retention time 1.81 mins, m/z $[M+H]^+$ 347.2/349.2; δ_H (DMSO-*d*₆) 14.3 (1H, br s, COOH), 11.95 (1H, s, ArOH), 7.95 (1H, s, ArH), 7.18 (2H, d, J 8.8 Hz, 2 × ArH), 7.11 (1H, s, ArH), 6.95 (2H, d, J 8.8 Hz, 2 × ArH), 3.78 (3H, s, OCH₃).

3-(5-Chloro-2,4-dihydroxyphenyl)-4-(4-methoxyphenyl)-1H-pyrazole-5-carboxylic acid **7**

6-Chloro-7-hydroxy-3-(4-methoxyphenyl)-4-oxo-4H-chromene-2-carboxylic acid **20** (390 mg, 1.08 mmol) was taken up in ethanol (10 mL) and hydrazine hydrate (0.16 mL, 3.25 mmol) was added. To aid dissolution, NaHCO₃ (sat. aq; 2 mL) was added, and then

the mixture was heated at 70°C under nitrogen for 18 h. The solution was cooled to room temperature and concentrated *in vacuo* to a brown oil which was acidified with HCl (1.0 M aq; 20 mL) and extracted with diethyl ether (2 × 15 mL). The combined organic phases were washed with HCl (1.0 M aq; 20 mL), dried over MgSO₄, filtered and concentrated *in vacuo* to give **7** (366 mg, 94%) as a yellow foam; *R_f* 0.04 (10% MeOH-DCM), LC retention time 2.02 mins, *m/z* [M+H]⁺ 361.2/363.2; δ_H(Acetone-*d*₆) 10.5 (1H, br s, ArOH), 8.87 (1H, br s, ArOH), 7.27 (2H, d, *J* 8.8 Hz, 2 × ArH), 6.99 (2H, d, *J* 8.8 Hz, 2 × ArH), 6.90 (1H, s, ArH), 6.57 (1H, s, ArH), 3.84 (3H, s, OCH₃).

General procedure for amide synthesis

Amides **8**, **9**, **11** and **12** were prepared from the acid **7** according to the following general procedure:

3-(5-Chloro-2,4-dihydroxyphenyl)-4-(4-methoxyphenyl)-1*H*-pyrazole-5-carboxylic acid **7** (40 mg, 0.11 mmol) was taken up as a suspension in anhydrous dichloromethane (8 mL). The resulting solution was cooled to 0 °C under nitrogen. 1-Hydroxybenzotriazole hydrate (41 mg, 0.33 mmol) was added, followed by *N*-methylmorpholine (0.08 mL, 112 mg, 1.11 mmol), *N*-Ethyl-*N'*-(3-dimethylaminopropyl)carbodiimide hydrochloride (64 mg, 0.33 mmol) and a solution of the requisite amine (2.0 M in MeOH; 0.11 mL, 0.22 mmol). The resulting mixture was stirred to room temperature overnight. The solution was diluted with dichloromethane (20 mL) and washed with HCl (1.0 M aq; 20 mL), NaHCO₃ (sat. aq; 20 mL) and brine (20 mL), then dried over MgSO₄, filtered and concentrated *in vacuo*. The residue was purified by preparative LCMS to give the amide product (see Section 1.1 General points for column and gradient conditions).

3-(5-Chloro-2,4-dihydroxyphenyl)-4-(4-methoxyphenyl)-1H-pyrazole-5-carboxamide 8

Off-white powder; LC retention time 1.92 mins, *m/z* [M+H]⁺ 360.2/362.2; δ_H(Acetone-*d*₆) 13.1 (1H, br s, pyrazole NH), 10.9 (1H, br s, ArOH), 8.87 (1H, br s, ArOH), 7.36 (2H, br d, *J* 8.6 Hz, 2 × ArH), 7.10 (2H, br s, 2 × ArH), 6.84 (1H, s, ArH), 6.8 (1H, br s, CONHH), 6.58 (1H, s, ArH), 5.7 (1H, br s, CONHH), 3.87 (3H, s, OCH₃).

N-Methyl-3-(5-chloro-2,4-dihydroxyphenyl)-4-(4-methoxyphenyl)-1H-pyrazole-5-carboxamide **9**

Off-white powder; R_f 0.56 (10% MeOH-DCM); LC retention time 1.97 mins, m/z $[M+H]^+$ 374.3/376.2; δ_H (Acetone- d_6) 10.4 (1H, br s, ArOH), 8.85 (1H, br s, ArOH), 7.32 (2H, d, J 8.8 Hz, $2 \times$ ArH), 7.05 (2H, br d, app. J 8.4 Hz, $2 \times$ ArH), 6.4 (1H, br s, NHMe), 3.86 (3H, s, OCH₃), 2.73 (3H, d, J 4.8 Hz, NHCH₃).

N-Ethyl-3-(5-Chloro-2,4-dihydroxyphenyl)-4-(4-methoxyphenyl)-1H-pyrazole-5-carboxamide **11** (VER-49009)

Off-white powder; R_f 0.50 (10% MeOH-DCM); LC retention time 2.11 mins, m/z $[M+H]^+$ 388.3/390.2; δ_H (Acetone- d_6) 10.5 (1H, br s, ArOH), 8.9 (1H, br s, ArOH), 7.35 (2H, d, J 8.8 Hz, $2 \times$ ArH), 7.08 (2H, br d, app. J 8.6 Hz, $2 \times$ ArH), 6.86 (1H, s, ArH), 6.57 (1H, s, ArH), 6.3 (1H, br s, NHCH₂CH₃), 3.87 (3H, s, OCH₃), 3.22 (2H, qd, J 7.2, 5.8 Hz, NHCH₂CH₃), 0.97 (3H, t, J 7.2 Hz, NHCH₂CH₃); δ_H (DMSO- d_6) 13.04 (1H, s, Pyrazole NH), 10.25 (1H, s, ArOH), 9.84 (1H, s, ArOH), 8.02 (1H, t, J 5.7 Hz, NHCH₂CH₃), 7.11 (2H, d, J 8.7 Hz, $2 \times$ ArH), 6.83 (1H, s, ArH), 6.78 (2H, d, J 8.7 Hz, $2 \times$ ArH), 6.57 (1H, s, ArH), 3.71 (3H, s, OCH₃), 3.19 (2H, app. qn, app. J 7.0 Hz, NHCH₂CH₃), 1.06 (3H, t, J 7.2 Hz, NHCH₂CH₃); δ_C (DMSO- d_6) 162.5, 157.7, 155.4, 154.0, 143.5, 137.3, 131.3, 131.0, 125.2, 119.2, 112.9, 109.5, 108.9, 103.7, 54.9, 33.2, 15.0; Anal. (C₁₉H₁₈ClN₃O₄) C, H, N; requires C, 58.84; H 4.68; N 10.83 %. Found 59.11; H, 4.60; N, 10.75%

N-Isopropyl-3-(5-Chloro-2,4-dihydroxyphenyl)-4-(4-methoxyphenyl)-1H-pyrazole-5-carboxamide **12**

Off white powder; R_f 0.57 (10% MeOH-DCM); LC retention time 2.20 mins, m/z $[M+H]^+$ 402.3/404.3; δ_H (Acetone- d_6) 13.1 (1H, br s, pyrazole NH), 10.9 (1H, br s, ArOH), 8.88 (1H, br s, ArOH), 7.37 (2H, d, J 8.4 Hz, $2 \times$ ArH), 7.13 (2H, br s, $2 \times$ ArH), 6.90 (1H, s, ArH), 6.58 (1H, s, ArH), 5.75 (1H, br s, NHCH(CH₃)₂), 3.96 (1H, m, NHCH(CH₃)₂), 3.88 (3H, s, OCH₃), 0.97 (6H, br d, app. J 5.3 Hz, NHCH(CH₃)₂).

Compound **10** was prepared using the conditions described above from **15** as described:

Methyl 7-hydroxy-3-(4-methoxyphenyl)-4-oxo-4H-chromene-2-carboxylate **21**

As for **19**, starting from 2,4-dihydroxyphenyl-4-methoxybenzylketone **15** (1.50 g, 5.81 mmol). Crude material used in following step without purification or characterisation.

7-Hydroxy-3-(4-methoxyphenyl)-4-oxo-4H-chromene-2-carboxylic acid **22**

As for **20**, starting from crude **21** from above, to give **22** (1.59 g, 88% over 2 steps) as a yellow powder; LC retention time 1.56 mins, m/z $[M+H]^+$ 313.2; δ_H (DMSO- d_6) 14.0 (1H, br s, COOH), 11.00 (1H, br s, ArOH), 7.91 (1H, d, J 8.8 Hz, ArH), 7.19 – 7.17 (2H, m, 2 $\times$ ArH), 6.98 – 6.89 (4H, m, 4 $\times$ ArH), 3.78 (3H, s, OCH₃).

3-(2,4-Dihydroxyphenyl)-4-(4-methoxyphenyl)-1H-pyrazole-5-carboxylic acid **23**

As for **7**, starting from **22** (1.59 g, 5.08 mmol), to give **23** (1.22 g, 73%) as an amber oil; LC retention time 1.86 mins, m/z $[M+H]^+$ 327.2; δ_H (DMSO- d_6) 13.0 (2H, br s, COOH and pyrazole NH), 9.73 (1H, br s, ArOH), 9.43 (1H, br s, ArOH), 7.10 (2H, d, J 8.7 Hz, 2 $\times$ ArH), 6.81 (2H, d, J 8.7 Hz, 2 $\times$ ArH), 6.67 (1H, d, J 8.4 Hz, ArH), 6.31 (1H, m, ArH), 6.08 (1H, dd, J 8.4, 2.3 Hz, ArH), 3.73 (3H, s, OCH₃).

N-Ethyl-3-(2,4-dihydroxyphenyl)-4-(4-methoxyphenyl)-1H-pyrazole-5-carboxamide **24**

Following the general procedure for amide synthesis described above, from **23** (600 mg, 1.83 mmol). Purification was by trituration with ether, giving **24** (156 mg, 24%) as a buff coloured solid; LC retention time 1.98 mins, m/z $[M+H]^+$ 354.2; δ_H (MeOH- d_4) 7.18 (2H, d, J 8.6 Hz, 2 $\times$ ArH), 6.88 – 6.86 (2H, m, 2 $\times$ ArH), 6.71 (1H, d, J 8.5 Hz, ArH), 6.34 (1H, d, J 2.3 Hz, ArH), 6.09 (1H, dd, J 8.5, 2.3 Hz, ArH), 3.29 (2H, q, J 7.3 Hz, NHCH₂CH₃), 1.10 (3H, br t, app. J 6.7 Hz, NHCH₂CH₃).

N-Ethyl-3-(5-bromo-2,4-dihydroxyphenyl)-4-(4-methoxyphenyl)-1H-pyrazole-5-carboxamide **10**

N-Ethyl-3-(2,4-dihydroxyphenyl)-4-(4-methoxyphenyl)-1H-pyrazole-5-carboxamide **24** (156 mg, 0.441 mmol) was dissolved in MeCN (8 mL) and DMF (0.5 mL). Benzyltrimethylammonium tribromide (162 mg, 0.415 mmol) was added and the mixture

stirred at rt under nitrogen for 90 mins. The reaction mixture was evaporated *in vacuo* and the residue partitioned between EtOAc (25 mL) and water (25 mL). The organic layer was washed with brine (25 mL), dried over magnesium sulphate, filtered and concentrated *in vacuo* to give, after trituration with ether, **10** (65 mg, 34%) as a buff coloured powder; LC retention time 2.175 mins, m/z $[M+H]^+$ 432.2/434.2; δ_H (DMSO- d_6) 13.05 (1H, s, pyrazole NH), 10.32 (1H, s, ArOH), 9.87 (1H, s, ArOH), 8.03 (1H, t, J 5.8 Hz, NHCH₂CH₃), 7.11 (2H, d, J 8.6 Hz, $2 \times$ ArH), 6.97 (1H, s, ArH), 6.78 (2H, d, J 8.6 Hz, $2 \times$ ArH), 6.56 (1H, s, ArH), 3.22 – 3.15 (2H, m, NHCH₂CH₃), 1.06 (3H, t, J 7.2 Hz, NHCH₂CH₃).

2. X-Ray Crystallographic Data for Compounds 1 and 11

2.1 Protein Production & Crystallisation

Protein production and crystallisation was performed as previously reported.³

2.2 Structure Determination

The ligand-bound data sets were collected at cryo-temperature. Data were collected on an in-house RU-H3R rotating anode generator with R-Axis IV++ image plate detector and were subsequently processed using D*Trek, supplied by Rigaku, MSC. The crystals belong to one of two space groups; I222 or P21212. The structures were solved by molecular replacement using a previously solved Hsp90 α protein model (PDB code: 1UY6; PU3 ligand and solvent removed) and the program AMoRe.⁴ Twenty cycles of rigid-body then restrained refinement were carried out using the refinement program REFMAC5⁵ followed by model building using the molecular graphics program O.⁶ Crystallographic water molecules were added by cycling REFMAC5 with ARP/wARP.⁷ The progress of the refinement was assessed using R_{free} and the conventional R factor. Once refinement of the structure was completed the structures were validated using PROCHECK⁸ and various programs from the CCP4i package.⁹ Full data collection and refinement statistics are presented in Table 3.

Table 3 Data collection and refinement statistics for Hsp90 in complex with compounds **1** (CCT018159) and **11** (VER-49009)

<i>Data collection statistics</i>	CCT018159 (1)	VER-00049009 (11)
Resolution (Å)	30.0 – 2.00	30.0 – 2.05
Space group	P2 ₁ 2 ₁ 2	I222
Cell dimensions (Å)		
a =	64.19	65.40
b =	88.51	88.84
c =	98.55	99.27
No. molecules/asymmetric unit	2	1
Volume (Å ³)	5.6 x 10 ⁵	5.6 x 10 ⁵
Solvent content (%)	57.5	55.3
Measured reflections	65483	64517
Unique reflections	19136	18512
Completeness: Overall / in hrb* (%)	99.0 / 93.0	98.5 / 93.1
Average multiplicity / in hrb	3.42 / 2.44	2.1 / 1.7
Mean I/σI: Overall / in hrb	10.6 / 3.4	6.4 / 1.6
R _{merge} : Overall / in hrb (%)	8.0 / 29.6	7.8 / 37.0
<i>Refinement statistics</i>		
R _{free} (%)	24.4	24.4
R _{cryst} (%)	20.2	19.2
Rms Deviations:		
Bonds (Å)	0.011	0.013
Angles (°)	1.3	1.4
B Factor (Å ²)	1.6	1.8
PDB Code	2BT0	2BSM

R_{free} is the R factor calculated using 5% of the reflection data chosen randomly and omitted from the refinement process, whereas R_{cryst} is calculated with the remaining data used in the refinement. Rms bond lengths and angles are the deviations from ideal values; the rms deviation in B factors is calculated between covalently bonded atoms. *hrb: highest resolution bin

3. Biological Assay Procedures

Details of FP (Fluorescence Polarisation) IC₅₀ determinations using full length human Hsp90 β ,¹⁰ ATPase IC₅₀ determinations using yeast Hsp90,^{3,10b} GI₅₀ determinations on the HCT116 cell line^{3,10b,11} and Western blotting methods³ have been previously described.

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