Microwave-assisted synthesis of *N*-pyrazole ureas and the p38α inhibitor BIRB 796 for study into accelerated cell ageing

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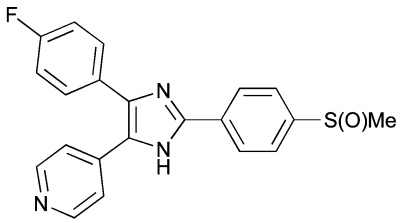
Microwave irradiation of substituted hydrazines and β-ketoesters gives 5-aminopyrazoles in excellent yield, which can be transformed to the corresponding *N*-carbonyl derivatives by treatment with an isocyanate or chloroformate. Derivatization of 4-nitronaphth-1-ol using predominantly microwave heating methods and reaction with an *N*-pyrazole carbamate provides a rapid route to the *N*-pyrazole urea BIRB 796 in high purity, as a potent and selective inhibitor of p38α mitogen-activated protein kinase for the study of accelerated ageing in Werner syndrome cells.

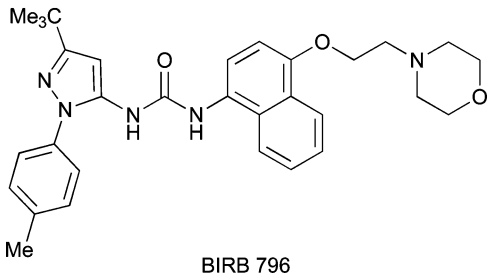
Introduction

P38α is one isoform of the mitogen-activated protein kinase (MAPK) intracellular enzymes, which are central to the regulation of cytokine biosynthesis and inflammatory cell signalling.[1,2] When activated, p38α MAPK is phosphorylated in an activation loop by dual specificity kinase MKK3 and 6 in response to extracellular stimuli and phosphorylates other kinases, leading to the regulation of target genes.[3] The reduction of pro-inflammatory cytokine levels offers a means for the treatment of inflammatory disorders such as rheumatoid arthritis and so the design of safe and efficacious p38α inhibitors suitable for clinical investigation remains a compelling therapeutic target.[2,4] Following the discovery that pyridinylimidazole p38α inhibitors such as SB203580 mediate multiple cellular responses,[1] including the production of inflammatory cytokines, a wide variety of structurally-distinct chemotypes[5,6] have been discovered to inhibit this enzyme with notable differences in binding motif.[7] Urea-based inhibitors, including Boehringer Ingelheim's p38 MAPK candidate BIRB 796[8] which was advanced to clinical trials,[9] are one such chemotype, adopting a unique binding mode that is distinct from adenosine 5′-triphosphate competitive binders.[10]

Werner syndrome (WS) is a rare autosomal recessive disorder.[11] The mutated gene (*WRN*) encodes for a RecQ helicase involved in DNA replication, recombination and repair.[12] Individuals living with the syndrome display the premature onset of many of the clinical features of old age, show early susceptibility to a number of major age-related diseases and have a greatly abbreviated median life expectancy (47 years).[11] Consequently, WS is widely used as a model disease to investigate the mechanisms underlying normal human ageing.[13] As part of our interest in mechanisms of premature cell senescence, we showed that by administering the p38α inhibitor SB203580 to WS cells all of the features of accelerated replicative decline were rescued, including growth rate and cell morphology.[14] This observation suggested that the abbreviated life span of WS cells is due to stress-induced growth arrest mediated by p38α MAPK, which we speculate is transduced from frequently stalled replication forks. It also offers a means to clinically regulate this process and, by chemotherapeutic means, intervene in a premature ageing syndrome. This manuscript describes our rapid microwave-assisted route to BIRB 796 to corroborate these findings on the role of signal transduction in replicative senescence.



SB203580



BIRB 796

Results and discussion

The synthesis of pyrazole-derived inhibitors of p38α, leading to the discovery of BIRB 796,[8] has been described by Boehringer Ingelheim and utilizes, as the key step, the reaction of a 5-aminopyrazole and 4-aminonaphthol with phosgene (Fig. 1)[8c] to give the *N*-pyrazole urea (22% yield for this, the final step in the synthesis). These procedures use traditional conductive heating methods and in our hands proceeded in very disappointing yield when carried out on a small scale. Given our previous success in microwave-mediated heteroannelation processes,[15] we set out to realize the rapid and efficient synthesis of BIRB 796 using microwave irradiation, to facilitate its biological study.

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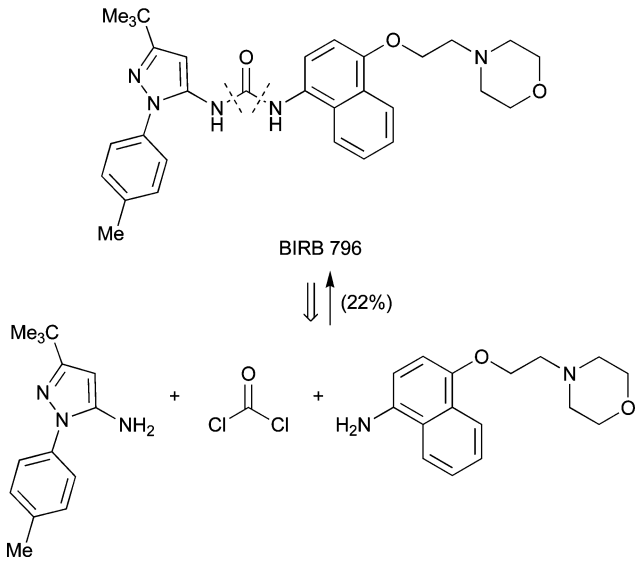


Fig. 1 The Boehringer Ingelheim synthesis of BIRB 796.[8c]

Taking the Boehringer Ingelheim approach as the first premise of study,[8c] microwave irradiation of a β-ketoester and substituted hydrazine should enable rapid access to the heteroaromatic amine for elaboration of BIRB 796. To test this hypothesis in a model microwave reaction, a mixture of phenylhydrazine **1a** and pivaloylacetonitrile **2a** was irradiated in a range of solvents at 110–120 °C to give 5-aminopyrazole **3a** (R^1 = Ph, R^2 = CMe_3) in 57–93% yield after 30–60 min (Table 1). Although the yields for experiments carried out in methanol (entry 3) and toluene–acetic acid (entry 7) were comparable to those obtained using conductive heating (entry 9), the reaction times were dramatically reduced and so the scope of this process was further explored.

A range of 5-aminopyrazoles **3a–i** was generated by irradiation of a subset of β-ketonitriles **2** and hydrazines **1** (Scheme 1, Table 2) in methanol. This solvent was chosen as the method of choice because of the ease of product purification simply by evaporation and trituration with light petroleum. Notably, the use of hydrazines either as the free base or the corresponding hydrochloride salt seemed to have little effect on the yield of product (compare entries 1–2 and 8–9). Subsequent derivatization, by reaction with phenyl isocyanate in CH_2Cl_2 (Scheme 1, step ii) gave urea **4a**, which has been reported as a p38α inhibitor in its own right.[8c] For this model urea, the yield using microwave dielectric heating (CH_2Cl_2, 80 °C, 30 min) was lower (79%) than the reaction under ambient conditions (93%). Alternatively, reaction of aminopyrazole **3b**, employed as its hydrochloride salt, with 2,2,2-trichloroethyl chloroformate[8d] in aqueous sodium hydroxide gave carbamate **4b** in 49% yield after rigorous purification by recrystallization, ready for combination with a suitable aminonaphthol component.

Synthesis of the naphthalene scaffold of BIRB 796 (Scheme 2) was investigated using a combination of microwave-mediated and traditional heating methods[8d] which were likewise

Table 1 Investigating conditions for microwave irradiation of phenylhydrazine **1a** (R^1 = Ph) and pivaloylacetonitrile **2a** (R^2 = CMe_3)

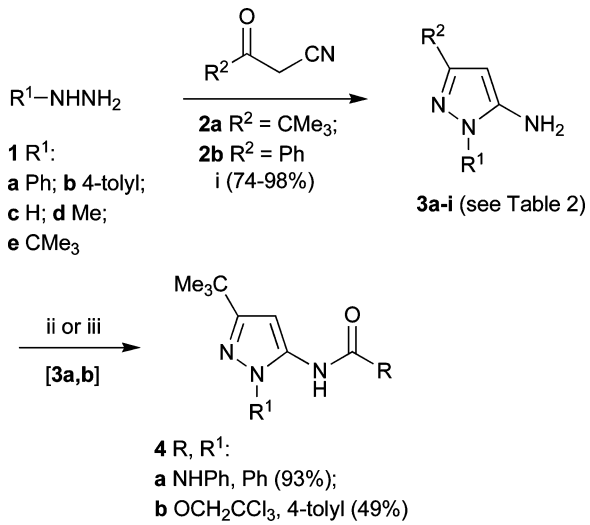
Entry	Solvent	°C[a]	Min	Yield%[b]
1	MeOH	120	15	32
2	MeOH	120	30	68
3	MeOH	120	40	93
4	EtOH	110	30	57
5	EtOH	120	40	78
6	PhMe	110	15	66
7	PhMe–AcOH (5 : 1)	110	40	93
8	DMSO	120	30	57
9[c]	PhMe (conductive heating)	Reflux	18 h	88 (89)[d]

[a] A constant temperature is maintained by the moderation of the initial microwave power (100 W or 150 W for entry 5). [b] Isolated yield of 5-aminopyrazole **3a** after evaporation and purification by trituration with light petroleum. [c] A conductive heating procedure was investigated according to literature conditions (reference 8*c*) for comparison. [d] The isolated yield for this transformation, as taken from the literature (reference 8*c*), is given in parentheses. In the literature experiment, pyrazole **3a** was obtained after purification by column chromatography on silica. Our alternative and more expedient purification method has little effect on yield (88 *vs.* 89%) and so variations can be attributed to differences in the reaction conditions.

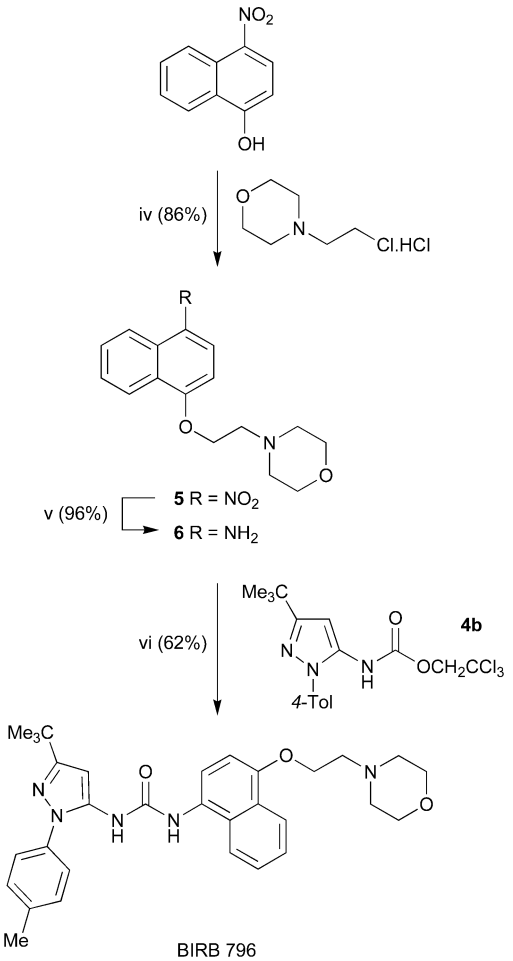
Table 2 Synthesis of pyrazoles **3a–i** at 120 °C for 40 min in MeOH under microwave-assisted conditions

Entry	1	2	3	R^1	R^2	Yield%[a]
1	**a**	**a**	**a**	Ph	CMe_3	93
2	**a**·HCl	**a**	**a**·HCl	Ph	CMe_3	89
3	**b**·HCl	**a**	**b**·HCl	4-Tolyl	CMe_3	90
4[b]	**b**·HCl	**a**	**b**·HCl	4-Tolyl	CMe_3	98
5	**c**	**a**	**c**	H	CMe_3	97
6	**d**	**a**	**d**	Me	CMe_3	91
7	**e**·HCl	**a**	—	CMe_3	CMe_3	[c]
8	**a**	**b**	**e**	Ph	Ph	80
9	**a**·HCl	**b**	**e**·HCl	Ph	Ph	88
10	**b**·HCl	**b**	**f**·HCl	4-Tolyl	Ph	74
11	**c**	**b**	**g**	H	Ph	84
12	**d**	**b**	**h**	Me	Ph	89
13	**e**·HCl	**b**	**i**·HCl	CMe_3	Ph	[d]

[a] Isolated yield of the corresponding aminopyrazole **3** after evaporation and purification by trituration with light petroleum. [b] Irradiated at 120 °C for 60 min. [c] Irradiation of **1e**·HCl and **2a** (entry 7) resulted in explosive tube rupture and should not be attempted. [d] Pyrazole **3i**·HCl (entry 13) was formed but could not be separated from side products.



Scheme 1 *Reagents and conditions*: (i) MeOH, microwaves, 120 °C, 40–60 min, 74–98%; (ii) PhNCO, CH_2Cl_2, rt, 20 min, 93% (from **3a**); (iii) 2,2,2-trichloroethyl chloroformate, EtOAc–H_2O, NaOH, 5–15 °C to rt, 49% (from **3b**·HCl); rt = room temperature.



Scheme 2 *Reagents and conditions*: (iv) NaOH, K_2CO_3, NMP, 100 °C, 2 h, 86%; (v) 10% Pd–C, HCO_2NH_4, EtOH, microwaves, 120 °C, 15 min, 96%; (vi) DMSO, microwaves, 100 °C, 30 min, 62%.

compared in terms of expediency and reaction efficiency (Table 3). Base-catalyzed alkylation of 4-nitronaphth-1-ol with (chloroethyl)morpholine in 1-methyl-2-pyrrolidinone (NMP) was carried out with conductive heating at 100 °C for 2 h to give naphtholic ether **5** in 86% yield. Efforts to develop a robust and reliable method for microwave-assisted alkylation (step iv) were hindered by difficulties with the agitation of the $NaOH/K_2CO_3$/NMP reaction mixture in the microwave synthesizer, the unreactivity of 4-nitronaphth-1-ol and problems with vessel pressurization through CO_2 release in sealed tube experiments. All of these problems were not overcome by the use of alternative bases and solvents or open vessel microwave reactions and so the conductive heating procedure was adopted as the method of choice for this step. However, microwave-assisted transfer hydrogenation (step v) of nitronaphthol **5** with Pd–C in the presence of ammonium formate at 120 °C furnished 4-aminonaphthalene **6** in 96% yield after only 15 min (Scheme 2) and provided the product in much higher purity than hydrogenation under ambient conditions (Table 3). Finally, the naphthalene and pyrazole building blocks were coupled by irradiating a mixture of amine **6** and trichloroethyl carbamate **4b** in DMSO for 30 min at 100 °C to give the urea, BIRB 796, with spectroscopic properties which agreed with published data.[16] The yield for the microwave-assisted transformation (Table 3, step vi) was notably higher than the reported conductive heating procedure,[8c] and so this approach was adopted as the method of choice.

The ability of BIRB 796 to inhibit the p38α signalling pathway was tested using a cell based technique in telomerase-immortalized WS cells.[18] This assay utilizes the immuno-detection of activated versions of p38α and its downstream signalling target HSP27 (Fig. 2). In control WS cells there was a low level of phosphorylated p38α (pp38), associated with moderate phosphorylation levels of its downstream target HSP27 (pHSP27) (lane 1). Treatment of cells with anisomycin greatly increased the activation of p38α causing an increase in pHSP27 levels (lane 2). BIRB 796 at 10 μM prevented the anisomycin-induced activation of p38α, as indicated by the much-reduced levels of pp38 and pHSP27 (lane 3). This

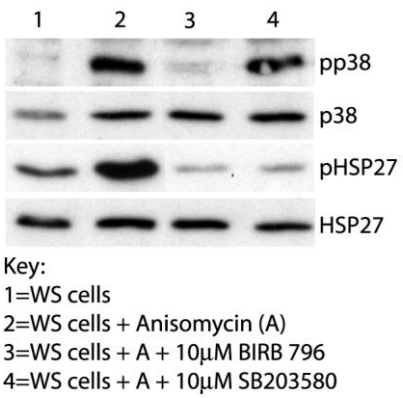


Fig. 2 Activation of p38α and its downstream target HSP27 in telomerase-immortalized WS cells treated with BIRB 796 or SB203580.

Table 3 The comparison of microwave irradiation and conductive heating for steps in the synthesis of *N*-pyrazole ureas and BIRB 796

	Conductive heating		Microwave irradiation	
Step	Conditions[a]	Yield%	Conditions	Yield%
i	PhMe, reflux, 18 h	88	MeOH, 120 °C, 60 min	98
ii	CH_2Cl_2, rt, 20 min	93	CH_2Cl_2, 80 °C, 30 min	79
iv	NaOH, K_2CO_3, NMP, 100 °C, 2 h	86	NaOH, K_2CO_3, NMP, 100 °C, 1 h	[b]
v	Pd–C, H_2, MeOH, rt, 24 h	85[c]	Pd–C, HCO_2NH_4, EtOH, 120 °C, 15 min	96
vi	DMSO, $^iPr_2NEt$, 60 °C, 3 h	24	DMSO, 100 °C, 30 min	62

[a] Conductive heating conditions (rt = room temperature) were based on published routes to BIRB 796[8] (see for example reference 8*c*) and represent standard procedures for comparison rather than identical thermal profiles (for probing microwave effects). [b] Incomplete reaction (see text). [c] The purity of the product of hydrogenation under ambient conditions was notably poorer by ^1H NMR spectroscopic analysis.

prevention of p38α activation was expected as BIRB 796 is known to stabilise p38α in a DFG-out mode that prevents the interaction of p38α with its upstream activating kinases MKK3 and MKK6.[17] As the level of pHSP27 is reduced in the BIRB 796-treated cells compared to the control (compare lanes 1 and 3), BIRB 796 not only prevents the anisomycin-induced activation of p38α, but also the p38α activation that is believed to be caused by genomic stress in the WS cells.[18]

In contrast, SB203580 at 10 μM inhibits p38α activity but not its activation (lane 4). From these experiments, BIRB 796 is confirmed as an inhibitor of the p38α signalling pathway in this cell-based system, thus validating its use in the investigation of p38α induced accelerated ageing in WS.

Conclusions

Microwave heating has been employed to accelerate multiple transformations in the rapid synthesis of BIRB 796, including the condensation of hydrazines and β-ketonitriles, transfer hydrogenation and urea formation. This p38α inhibitor shows promising *in vivo* behaviour and inhibits cell signalling in telomerase-immortalized WS cells. These observations support further study to rescue the accelerated-replicative decline of WS cells by regular treatment with BIRB 796. These experiments are now underway and offer a promising opportunity for drug intervention in a premature ageing syndrome.

Experimental

Commercially available reagents were used without further purification; solvents were dried by standard procedures. Light petroleum refers to the fraction with bp 40–60 °C and ether refers to diethyl ether. Unless otherwise stated, reactions were performed under an atmosphere of dry nitrogen. Flash chromatography was carried out using Merck Kieselgel 60 H silica or Matrex silica 60. Analytical thin layer chromatography was carried out using aluminium-backed plates coated with Merck Kieselgel 60 GF_{254} that were visualised under UV light (at 254 and/or 360 nm).

Fully characterized compounds were chromatographically homogeneous. Melting points were determined on a Kofler hot stage apparatus and are uncorrected. Infra-red spectra were recorded in the range 4000–600 cm^{-1} on a Perkin-Elmer 1600 series FTIR spectrometer using KBr disks for solid samples and thin films between NaCl plates for liquid samples and are reported in cm^{-1}. NMR spectra were recorded using a Bruker DPX 400 instrument or 500 Avance instrument operating at 400 MHz for ^1H spectra and 100 or 125 MHz for ^{13}C spectra; *J* values were recorded in Hz and multiplicities were expressed by the usual conventions. Low resolution mass spectra were determined using a Fisons VG Platform II Quadrupole instrument using atmospheric pressure chemical ionization (APcI) unless otherwise stated. ES refers to electrospray ionization, CI refers to chemical ionization (ammonia) and EI refers to electron ionization. High resolution mass spectra were obtained courtesy of the EPSRC Mass Spectrometry Service at University College of Wales, Swansea, UK using the ionization methods specified. Microanalyses were recorded using a Perkin-Elmer 240C Elemental Analyzer.

Typical experimental procedure for the microwave-assisted synthesis of aminopyrazoles 3

A solution of 4-tolylhydrazine hydrochloride (**1b**·HCl) (0.63 g, 4.0 mmol) and pivaloylacetonitrile (**2a**) (0.50 g, 4.0 mmol) in MeOH (2 mL) was irradiated (without concurrent cooling in an air stream) in a sealed tube at 120 °C for 40 min using a CEM Discover™ single-mode microwave synthesizer, by moderating the initial microwave power (100 W). After cooling in a stream of compressed air, the solution was evaporated *in vacuo* and the resulting crude mixture triturated with light petroleum to give *5-aminopyrazole* **3b**·HCl (0.96 g, 90%).

5-Amino-3-*tert*-butyl-1-phenyl-1*H*-pyrazole (3a). Compound **3a** (1.68 g, 93%) was prepared according to the above procedure using phenylhydrazine (**1a**) (0.83 mL, 8.4 mmol) and pivaloylacetonitrile (**2a**) (1.05 g, 8.4 mmol) and was obtained as a colourless solid, mp 64–66 °C (light petroleum) (Found C, 72.1; H, 7.95; N, 19.3. Calc. for $C_{13}H_{17}N_3$: C, 72.5; H, 7.95; N, 19.5%) (Found: MH^+, 216.1496. $C_{13}H_{18}N_3$ [MH^+] requires 216.1495); ν_{max}(KBr)/cm^{-1} 3280, 3144, 2962, 1628, 1596, 1555, 1508, 1478, 1452, 1376, 1241, 988, 772, 700; δ_H(400 MHz; CD_3OD) 7.50 (2H, m, 2′,6′-H), 7.38 (2H, m, 3′,5′-H), 7.23 (1H, m, 4′-H), 5.34 (1H, s, 4-H), 3.54 (2H, br s, NH_2), 1.12 (9H, s, CMe_3); δ_C(125 MHz; CD_3OD) 162.6 (C), 147.0 (C), 138.7 (C), 129.0 (CH), 127.0 (CH), 124.1 (CH), 86.7 (CH), 31.7 (C), 29.3 (Me); *m*/*z* 216 (MH^+, 100%).

5-Amino-3-*tert*-butyl-1-phenyl-1*H*-pyrazole hydrochloride (3a·HCl). Compound **3a**·HCl (0.89 g, 89%) was prepared according to the given procedure using phenylhydrazine hydrochloride (**1a**·HCl) (0.58 g, 4.0 mmol) and was obtained as an off-white solid, mp 156–157 °C (EtOAc) (Found: MH^+, 216.1501. $C_{13}H_{18}N_3$ [MH^+] requires 216.1495); ν_{max}(KBr)/cm^{-1} 3277, 3117, 2962, 2664, 2600, 1649, 1636, 1621, 1573, 1503, 1457, 1369, 1304, 1242, 1052; δ_H(400 MHz; $CDCl_3$) 7.71 (3H, m, 2′,4′,6′-H), 7.62 (2H, m, 3′,5′-H), 4.92 (3H, br s, NH_2 and 4-H), 1.41 (9H, s, CMe_3); δ_C(125 MHz; $CDCl_3$) 161.1 (C), 151.9 (C), 132.7 (C), 130.9 (CH), 130.2 (CH), 126.4 (CH), 87.4 (CH), 31.6 (C), 28.3 (Me); *m*/*z* (ES) 216 (MH^+, 100%).

5-Amino-3-*tert*-butyl-1-*p*-tolyl-1*H*-pyrazole hydrochloride (3b·HCl). Compound **3b**·HCl (0.96 g, 90%) was obtained as an off-white solid, mp 116–119 °C (light petroleum–MeOH) (Found: MH^+, 230.1653. $C_{14}H_{20}N_3$ [MH^+] requires 230.1652); ν_{max}(KBr)/cm^{-1} 3464, 3280, 3139, 2952, 1634, 1558, 1518, 1490, 1381, 1245; δ_H(400 MHz; $CDCl_3$) 7.44 (2H, d, *J* 8.3, 2′,6′-H), 7.26 (2H, d, *J* 8.3, 3′,5′-H), 5.53 (1H, s, 4-H), 3.72 (2H, br s, NH_2), 2.39 (3H, s, Me), 1.33 (9H, s, CMe_3); δ_C(125 MHz; $CDCl_3$) 162.1 (C), 144.8 (C), 136.9 (C), 136.3 (C), 129.9 (CH), 124.1 (CH), 87.4 (CH), 32.3 (C), 30.4 (Me), 21.1 (Me); *m*/*z* 230 (MH^+, 100%).

5-Amino-3-*tert*-butyl-1*H*-pyrazole (3c). Compound **3c** (0.54 g, 97%) was prepared according to the given procedure using hydrazine monohydrate (**1c**·H_2O) (0.20 mL, 4.1 mmol) and was obtained as a light orange solid, mp 78–79 °C (light petroleum–MeOH) (lit.,[19] 80 °C) (Found: MH^+, 140.1181. $C_7H_{14}N_3$ [MH^+] requires 140.1182); ν_{max}(KBr)/cm^{-1} 3327, 1591, 1504, 1364, 1318, 1242, 1206, 1128, 1090, 984; δ_H(400 MHz; $CDCl_3$) 5.26 (1H, s, 4-H), 3.29 (2H, s, NH_2), 1.08 (9H, s, CMe_3); δ_C(125 MHz; $CDCl_3$) 155.1 (C), 154.0 (C), 89.35 (CH), 31.04 (C), 30.1 (Me); *m*/*z* (ES) 140 (MH^+, 100%).

5-Amino-3-*tert*-butyl-1-methyl-1*H*-pyrazole (3d). Compound **3d** (0.55 g, 91%) was prepared according to the given procedure using methylhydrazine (**1a**) (0.21 mL, 3.9 mmol) and was obtained as a colourless solid, mp 156–157 °C (light petroleum) (Found: MH^+, 154.1340. $C_8H_{16}N_3$ [MH^+] requires 154.1339); ν_{max}(KBr)/cm^{-1} 2964, 1628, 1560, 1418, 1382, 1360, 1255; δ_H(400 MHz; $CDCl_3$) 5.34 (1H, s, 4-H), 3.56 (3H, s, Me), 3.47 (2H, br s, NH_2), 1.20 (9H, s, CMe_3); δ_C(125 MHz; $CDCl_3$) 160.6 (C), 144.5 (C), 87.5 (CH), 34.0 (Me), 32.0 (C), 30.9 (Me); *m*/*z* 154 (MH^+, 100%).

5-Amino-1,3-diphenyl-1*H*-pyrazole (3e). Compound **3e** (0.75 g, 80%) was prepared according to the given procedure using phenylhydrazine (**1a**) (0.39 mL, 4.0 mmol) and benzoylacetonitrile (**2b**) (0.58 g, 4.0 mmol) and was obtained as a light brown solid, mp 128–129 °C (light petroleum–EtOAc) (lit.,[20] 129–130 °C) (Found: MH^+, 236.1183. $C_{15}H_{14}N_3$ [MH^+] requires 236.1182); ν_{max}(KBr)/cm^{-1} 3308, 3208, 3046, 1633, 1596, 1560, 1503, 1463, 1375, 1068, 950, 918, 751; δ_H(400 MHz; $CDCl_3$) 7.74 (2H, m, 2,6-PhH), 7.56 (2H, m, *N*-2,6-PhH), 7.42 (2H, m, *N*-3,5-PhH), 7.31 (2H, m, 3,5-PhH), 7.28 (1H, m, *N*-4-PhH), 7.23 (1H, m, 4-PhH), 5.76 (1H, s, 4-H); δ_C(125 MHz; $CDCl_3$) 151.5 (C), 145.9 (C), 138.7 (C), 133.5 (C), 129.5 (CH), 128.5 (CH), 127.8 (CH), 127.5 (CH), 125.7 (CH), 124.2 (CH), 88.2 (CH); *m*/*z* (ES) 236 (MH^+, 100%).

5-Amino-1,3-diphenyl-1*H*-pyrazole hydrochloride (3e·HCl). Compound **3e**·HCl (0.95 g, 88%) was prepared according to the given procedure using phenylhydrazine hydrochloride (**1a**·HCl) (0.58 g, 4.0 mmol) and benzoylacetonitrile (**2b**) (0.58 g, 4.0 mmol) and was obtained as a colourless solid, mp 145–147 °C (EtOAc) (Found: MH^+, 236.1185. $C_{15}H_{14}N_3$ [MH^+] requires 236.1182); ν_{max}(KBr)/cm^{-1} 3246, 3100, 1634, 1574, 1510, 1289, 1103, 946, 918; δ_H(400 MHz; $CDCl_3$) 7.89 (2H, m, 2,6-PhH), 7.58 (2H, m, *N*-2,6-PhH), 7.53 (2H, m, *N*-3,5-PhH), 7.47 (2H, m, 3,5-PhH), 7.41 (1H, m, *N*-4-PhH), 7.39 (1H, m, 4-PhH), 6.09 (1H, s, 4-H); δ_C(125 MHz; $CDCl_3$) 150.6 (C), 149.1 (C), 140.9 (C), 137.8 (C), 134.7 (CH), 130.7 (CH), 129.4 (CH), 128.9 (CH), 127.4 (CH), 125.6 (CH), 89.5 (CH); *m*/*z* (ES) 236 (MH^+, 100%).

5-Amino-3-phenyl-1-*p*-tolyl-1*H*-pyrazole[21] hydrochloride (3f·HCl). Compound **3f**·HCl (0.81 g, 74%) was prepared according to the given procedure using benzoylacetonitrile (**2b**) (0.58 g, 4.0 mmol) and was obtained as a brown solid, mp 66–67 °C (Found: MH^+, 250.1340. $C_{16}H_{16}N_3$ [MH^+] requires 250.1344); ν_{max}(KBr)/cm^{-1} 3283, 3150, 1650, 1635, 1620, 1558, 1515, 1459, 1374, 1312, 1281, 1025, 814, 758; δ_H(400 MHz; CD_3OD) 7.78 (2H, m, 2,6-PhH), 7.54–7.43 (8H), 2.47 (3H, s, Me); δ_C(125 MHz; $CDCl_3$) 150.8 (C), 150.3 (C), 140.3 (C), 134.1 (C), 132.2 (C), 130.4 (CH), 129.9 (CH), 128.8 (CH), 126.1 (CH), 125.5 (CH), 87.8 (CH), 19.9 (Me); *m*/*z* 250 (MH^+, 100%).

5-Amino-3-phenyl-1*H*-pyrazole (3g). Compound **3g** (0.75 g, 80%) was prepared according to the given procedure using hydrazine monohydrate (**1c**·H_2O) (0.20 mL, 4.1 mmol) and benzoylacetonitrile (**2b**) (0.58 g, 4.0 mmol) and was obtained as a light brown solid, mp 94–95 °C (light petroleum) (Found: MH^+, 160.0868. $C_9H_{10}N_3$ [MH^+] requires 160.0869); ν_{max}(KBr)/cm^{-1} 3171, 1651, 1516, 1102, 1072, 1008, 956; δ_H(400 MHz; CD_3OD) 7.65 (2H, d, *J* 7.5, 2′,6′-H), 7.40 (2H, m, 3′,5′-H), 7.33 (1H, m, 4′-H), 5.95 (1H, s, 4-H); δ_C(125 MHz; CD_3OD) 154.3 (C), 145.7 (C), 130.3 (C), 128.9 (CH), 128.3 (CH), 125.4 (CH), 90.4 (CH); *m*/*z* 160 (MH^+, 100%).

5-Amino-3-phenyl-1-methyl-1*H*-pyrazole (3h). Compound **3h** (0.60 g, 89%) was prepared according to the given procedure using methylhydrazine (**1a**) (0.21 mL, 3.9 mmol) and benzoylacetonitrile (**2b**) (0.58 g, 4.0 mmol) and was obtained as an off-white solid, mp 128–130 °C (EtOAc) (lit.,[22] 129 °C) (Found: MH^+, 174.1023. $C_{10}H_{12}N_3$ [MH^+] requires 174.1026); ν_{max}(KBr)/cm^{-1} 3420, 3151, 1623, 1562, 1511, 1446, 1373, 1270, 1025, 959, 908; δ_H(400 MHz; $CDCl_3$) 7.75 (2H, m, 2′,6′-H), 7.39 (2H, m, 3′,5′-H), 7.29 (1H, m, 4′-H), 5.88 (1H, s, 4-H), 3.73 (3H, s, Me), 3.48 (2H, br s, NH_2); δ_C(125 MHz; $CDCl_3$) 149.7 (C), 145.6 (C), 133.9 (C), 128.5 (CH), 127.5 (CH), 125.3 (CH), 88.6 (CH), 34.4 (Me); *m*/*z* 174 (MH^+, 100%).

5-Amino-3-phenyl-1-*tert*-butyl-1*H*-pyrazole hydrochloride (3i·HCl). Compound **3i**·HCl (0.94 g, 94%) was prepared according to the given procedure using *tert*-butylhydrazine hydrochloride (**1e**·HCl) (0.50 g, 4.0 mmol) and benzoylacetonitrile (**2b**) (0.58 g, 4.0 mmol) and was obtained as an orange oil (Found: MH^+, 216.1496. $C_{13}H_{18}N_3$ [MH^+] requires 216.1495); δ_H(400 MHz; $CDCl_3$) 7.92 (2H, m, 2′,6′-H), 7.66 (1H, m, 4′-H), 7.52 (2H, m, 3′,5′-H), 6.28 (1H, s, 4-H), 1.47 (9H, s, CMe_3); δ_C(125 MHz; $CDCl_3$) 152.4 (C), 149.8 (C), 130.8 (C), 129.2 (CH), 128.7 (CH), 127.9 (CH), 93.5 (CH), 31.42 (C), 28.7 (Me); *m*/*z* 216 (MH^+, 100%), 160 (95), 129 (43).

1-(3-*tert*-Butyl-1-phenyl-1*H*-pyrazol-5-yl)-3-phenylurea (4a)

Phenyl isocyanate (0.33 mL, 3.0 mmol) was added to a stirred solution of 5-aminopyrazole **1a** (0.65 g, 3.0 mmol) in CH_2Cl_2 (6 mL). The mixture was stirred for 20 min and evaporated *in vacuo.* After triturating with EtOAc–light petroleum (1 : 1), purification by recrystallization (MeOH) gave the *title compound* (0.93 g, 93%) as colourless crystals, mp 197–200 °C (MeOH) (lit.,[8c] 211 °C) (Found C, 71.7; H, 6.65; N, 16.6. Calc. for $C_{20}H_{22}N_4O$: C, 71.8; H, 6.65; N, 16.8%) (Found: MH^+, 335.1863. $C_{20}H_{23}N_4O$ [MH^+] requires 335.1866); ν_{max}(KBr)/cm^{-1} 3380, 3278, 3141, 3082, 2957, 2865, 1665, 1599, 1550, 1500, 1449, 1372, 1312, 1224; δ_H(400 MHz; CD_3OD) 7.61–7.48 (5H, PhH), 7.37 (2H, m, *N*-2,6-PhH), 7.28 (2H, m, *N*-3,5-PhH), 7.04 (1H, m, *N*-4-PhH), 6.68 (1H, s, 4′-H), 1.36 (9H, s, CMe_3); δ_C(100 MHz; CD_3OD) 162.3 (C), 152.7 (C), 138.6 (C), 138.0 (C), 137.5 (C), 129.2 (CH), 128.5 (CH), 128.2 (CH), 125.6 (CH), 122.8 (CH), 118.9 (CH), 95.1 (CH), 31.9 (C), 29.4 (Me); *m*/*z* 335 (MH^+, 100%), 216 (47).

5-(2,2,2-Trichloroethoxycarbonyl)amino-3-*tert*-butyl-1-*p*-tolyl-1*H*-pyrazole (4b)

A mixture of 5-amino-3-*tert*-butyl-1-*p*-tolylpyrazole hydrochloride (**3b**·HCl) (3.0 g, 11.3 mmol), water (15 mL), EtOAc (30 mL) and NaOH (1.14 g, 28.5 mmol) was stirred at 0 °C for 30 min. 2,2,2-Trichloroethyl chloroformate (3.89 g, 18.4 mmol) was added over a period of 1 h at this temperature and then the mixture was stirred at RT for 2 h. The organic layer was decanted and the aqueous layer further extracted with EtOAc (2 × 20 ml). The combined organic layers were washed with brine (20 mL), dried (Na_2SO_4) and evaporated *in vacuo* to give the *title compound* (2.41 g, 47%) as a colourless solid, mp 151–153 °C (heptane) (Found: MH^+, 404.0694. $C_{17}H_{21}{}^{35}Cl_3N_3O_2$ [MH^+] requires 404.0694); ν_{max}(KBr)/cm^{-1} 3126, 2964, 1749, 1598, 1521, 1464, 1364, 1228; δ_H(400 MHz; $CDCl_3$) 7.36 (2H, d, *J* 8.3, 2′,6′-H),

7.31 (2H, d, *J* 8.3, 3′,5′-H), 6.94 (1H, br s, NH), 6.42 (1H, s, 4-H), 4.82 (2H, s, CH_2), 2.43 (3H, s, Me), 1.36 (9H, s, CMe_3); δ_C(125 MHz; $CDCl_3$) 162.2 (C), 151.0 (C), 138.3 (C), 135.4 (C), 134.8 (C), 130.3 (CH), 124.9 (CH), 95.1 (CH), 94.9 (C), 74.8 (CH_2), 32.4 (C), 30.4 (Me), 21.2 (Me); *m/z* 408 ($M[^{35}Cl^{37}Cl_2]H^+$, 35%), 406 ($M[^{35}Cl_2{}^{37}Cl]H^+$, 98), 404 ($M[^{35}Cl_3]H^+$, 96), 230 (100), 149 (18).

4-Nitro-1-(2-morpholinethoxy)naphthalene (5)

A mixture of 4-nitro-1-hydroxynaphthalene (3.00 g, 15.9 mmol), 4-(2-chloroethyl)morpholine hydrochloride (4.13 g, 22.2 mmol), NaOH (0.89 g, 22.2 mmol), K_2CO_3 (5.27 g, 38.2 mmol) and 1-methyl-2-pyrrolidinone (20 mL) was stirred at 100 °C for 2 h. After cooling to 5 °C, the mixture was stood at this temperature for 4 h and then filtered to give the *title compound* (4.13 g, 86%) as a brown solid, mp 98–99 °C (EtOH) (Found: MH^+, 303.1342. $C_{16}H_{19}N_2O_4$ [MH^+] requires 303.1345); ν_{max}(KBr)/cm^{-1} 2951, 2858, 2792, 1648, 1569, 1503, 1450, 1423, 1311, 1265, 1139, 1106, 1079, 1000; δ_H(400 MHz; CD_3OD) 8.72 (1H, d, *J* 8.5, 5-H or 8-H), 8.34 (1H, d, *J* 8.7, 3-H), 8.29 (1H, d, *J* 8.5, 8-H or 5-H), 7.69 (1H, m, 6-H or 7-H), 7.54 (1H, m, 7-H or 6-H), 6.76 (2H, d, *J* 8.7, 2-H), 4.49 (2H, m, 1′-H), 3.76 (4H, m, 2″,6″-H), 3.04 (2H, m, 2′-H), 2.71 (4H, m, 3″,5″-H); δ_C(125 MHz; CD_3OD) 159.6 (C), 139.2 (C), 130.1 (CH), 127.2 (CH), 126.9 (C), 126.6 (CH), 125.6 (C), 123.5 (CH), 122.7 (CH), 102.7 (CH), 67.3 (CH_2), 67.0 (CH_2), 57.3 (CH_2), 54.1 (CH_2); *m/z* 303 (MH^+, 100%).

4-Amino-1-(2-morpholinethoxy)naphthalene (6)

A mixture of 4-nitro-1-(2-morpholinethoxy)naphthalene (**5**) (0.30 g, 0.99 mmol), ammonium formate (0.38 g, 6.0 mmol), EtOH (4 mL) and Pd–C (10%; 50 mg) was irradiated (without concurrent cooling in an air stream) in a sealed tube at 100 °C for 15 min using a CEM Discover™ single-mode microwave synthesizer, by moderating the initial microwave power (120 W). After cooling in a stream of compressed air, the mixture was filtered through Celite® and evaporated *in vacuo* to give the *title compound* (0.26 g, 96%) as a purple residue, which was used without further purification (Found: MH^+, 273.1599. $C_{16}H_{21}N_2O_2$ [MH^+] requires 273.1598); ν_{max}(KBr)/cm^{-1} 3418, 2939, 1651, 1458, 1397, 1284, 1246, 1063, 984, 920; δ_H(400 MHz; CD_3OD) 8.52 (1H, d, *J* 8.5, 5-H or 8-H), 8.01 (1H, d, *J* 8.5, 8-H or 5-H), 7.82 (1H, m, 6-H or 7-H), 7.75 (1H, m, 7-H or 6-H), 7.62 (1H, d, *J* 8.3, 3-H), 7.13 (1H, d, *J* 8.3, 2-H), 4.19 (2H, m, 1′-H), 3.70 (4H, m, 2″,6″-H), 2.84 (2H, m, 2′-H), 2.60 (4H, m, 3″,5″-H); δ_C(125 MHz; CD_3OD) 144.2 (C), 131.6 (C), 122.4 (C), 121.7 (CH), 121.4 (CH), 121.1 (C), 188.6 (CH), 177.1 (CH), 105.8 (CH), 102.1 (CH), 63.0 (CH_2), 57.7 (CH_2), 34.6 (CH_2), 30.2 (CH_2); *m/z* 273 (MH^+, 100%), 132 (30).

1-[3-*tert*-Butyl-1-*p*-tolyl-1*H*-pyrazol-5-yl]-3-[4-(2-morphol-in-4-yl-ethoxy)naphthalen-1-yl]urea (BIRB 796)

A solution of 5-(2,2,2-trichloroethoxycarbonyl)amino-3-*tert*-butyl-1-*p*-tolylpyrazole (**4b**) (83 mg, 0.21 mmol) and 4-amino-1-(2-morpholinethoxy)naphthalene (**6**) (56 mg, 0.21 mmol) in DMSO (3 mL) was irradiated (without concurrent cooling in an air stream) in a sealed tube at 100 °C for 30 min using a CEM Discover™ single-mode microwave synthesizer, by moderating the initial microwave power (100 W). After cooling in a stream of compressed air, the mixture was partitioned between EtOAc (10 mL) and water (10 mL). The organic layer was washed successively with water (3 × 10 mL) and brine (10 mL), dried (Na_2SO_4) and evaporated *in vacuo.* Purification by column chromatography on silica, eluting with EtOAc, gave the *title compound* (67 mg, 62%) as an off-white solid, mp 117–119 °C (light petroleum–MeOH–EtOAc) (lit.,[8c] 142–143 °C) (Found: MH^+, 528.2970. $C_{31}H_{38}N_5O_3$ [MH^+] requires 528.2969); ν_{max}(KBr)/cm^{-1} 3302, 2959, 1654, 1545, 1516, 1458, 1376, 1262, 1093, 820; δ_H(400 MHz; $CDCl_3$) 8.19 (1H, m, 5-H), 7.77 (1H, m, 8-H), 7.47 (2H, 7,6-H), 7.26 (1H, d, *J* 8.1, 2-H), 6.90 (2H, m, 2,6-PhH), 6.87 (2H, m, 3,5-PhH), 6.63 (1H, d, *J* 8.1, 3-H), 6.43 (1H, br s, N′H), 6.37 (1H, s, 4-pyzH), 6.30 (1H, br s, NH), 4.28 (2H, m, 1′-H), 3.75 (4H, m, 2″,6″-H), 3.00 (2H, m, 2′-H), 2.71 (4H, m, 3″,5″-H) 2.22 (3H, s, Me), 1.26 (9H, s, CMe_3); δ_C(125 MHz; $CDCl_3$) 185.5 (C), 170.9 (C), 162.2 (C), 153.6 (C), 137.3 (C), 136.1 (C), 135.5 (C), 130.0 (C), 129.8 (CH), 127.5 (CH), 126.0 (CH), 124.2 (CH), 122.38 (CH), 122.36 (CH), 122.1 (CH), 122.0 (CH), 104.2 (CH), 73.9 (C), 57.3 (CH_2), 53.8 (CH_2), 50.7 (CH_2), 45.5 (CH_2), 32.4 (C), 30.35 (Me), 21.1 (Me); *m/z* 528 (MH^+, 100%), 273 (20), 230 (100), 188 (30).

Immuno-detection of activated p38α and its downstream target HSP27 in telomerase-immortalized WS cells treated with SB203580 or BIRB 796

The ability of BIRB 796 to inhibit the p38α signalling pathway was tested using a cell based technique involving the immuno-detection of activated versions of p38α and its downstream signalling target HSP27. The cells used were telomerase-immortalized WS cells that, despite being immortalized, maintain activation of the p38α pathway. WS cells in dishes were pre-incubated at 37 °C for 2 h in growth medium supplemented with either SB203580 or BIRB 796 at 10 μM. Then anisoymcin was added to the medium at 30 μM and the cells harvested 45 min later. In this system, the p38α pathway is induced by treatment of WS cells with anisomycin, and p38α activation is detected using antibodies specific for the activated (phosphorylated) forms of p38α and HSP27 immobilized on Western blots. Cells were harvested, proteins isolated, separated on polyacrylamide gels, and immunoblotted as previously described.[14] P38α and HSP27 were detected using antibodies against the activated (phosphorylated) and non-activated forms of the proteins.[14]

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