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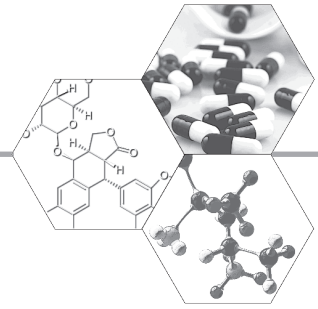

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Background: The pyrazolyl ketone motif of RO3201195, which exhibits good oral bioavailability and high selectivity for p38 MAPK over other kinases, is a key pharmacophore that could find application in the treatment of Werner syndrome. **Results and discussion:** Microwave irradiation promotes Knoevenagel condensation of a β-ketonitrile and formamidine, to give β-aminovinyl ketones, and their subsequent cyclocondensation with a subset of hydrazines to provide rapid access to a 24-membered library of pyrazolyl ketones. The library was evaluated in human hTERT-immortalized HCA2 dermal cells and Werner syndrome cells. **Conclusion:** Four compounds display comparable, if not slightly improved, potency over RO3201195.

Individuals with the rare genetic disorder Werner syndrome (WS) show the premature onset of many of the clinical features of old age, with early susceptibility to several major age-related diseases and a shortened median life expectancy (47 years). Mutation in the *WRN* gene [1,2] results in accelerated *in vivo* aging and premature senescence of cells *in vitro* that may underlie several features of WS clinical pathophysiology [3–5]. Senescent cells display deleterious biochemical features and a proinflammatory phenotype, suggesting a link between replicative senescence and tissue degeneration [6,7]. Unlike normal human fibroblasts, proliferating WS cells contain high levels of phosphorylated p38α, a stress-activated mitogen-activated protein kinase (MAPK), suggesting that WS cells undergo premature senescence by a telomere-independent mechanism [8–10], which synergizes with telomere-erosion processes [11]. The treatment of WS cells with the p38 inhibitor SB203580 resulted in the rescue of all of the features of accelerated replicative decline, including the short replicative lifespan, slow growth rate and altered cell morphology [8], indicating that the abbreviated life span of WS cells is linked to a stress-induced growth arrest mediated by p38α MAPK. If accelerated aging in WS is related to p38 activation and to accelerated cell aging, WS may provide a powerful model system to link cellular signaling events to the aging of mitotic tissues *in vivo* [12] and, thus, provides the opportunity for therapeutic intervention in the pathology of WS.

Given the poor kinase selectivity profile of the prototypical inhibitor SB203580, as well as reported toxicity issues, we set out to prepare a number of other p38α inhibitor chemotypes **(Figure 1)** for study in WS cells. Our recent reports on successful routes to BIRB 796 [13], VX-745 [14,15], UR-13756 [16] and RO3201195 [17] have all utilized microwave dielectric heating to accelerate access to compounds for study and have resulted in dramatic improvements in both the facility and efficiency of synthetic routes. The use of microwave irradiation has received increasing attention in recent years as a valuable alternative to the use of conductive heating for accelerating transformations in synthetic chemistry, medicinal chemistry and the biosciences [18–24]. In our recent route to RO3201195 **(Figure 1D)** [17], we demonstrated that microwave irradiation can facilitate the rapid synthesis of a pyrazolyl ketone building block for elaboration to the target inhibitor, which exhibits oral bioavailability and high selectivity for p38 over other kinases [25]. This central motif contains the key pharmacophore of the chemotype, with a unique hydrogen bond between an exocyclic N and Thr-106 in the ATP-binding pocket.

This manuscript describes experimental procedures for the rapid synthesis of a small library of pyrazolyl ketones and a key intermediate in the synthesis of RO3201195 and evaluates a series of related compounds for comparison in WS cells. Previous studies have indicated that *meta*-substitution of the benzoyl group is preferred, offering greater tolerability to a wide range of substituents, as well as being a facile point of installation for a solubilizing group, such as the 2,3-dihydroxypropoxy function in RO3201195. Preliminary structural–activity relationship (SAR) studies

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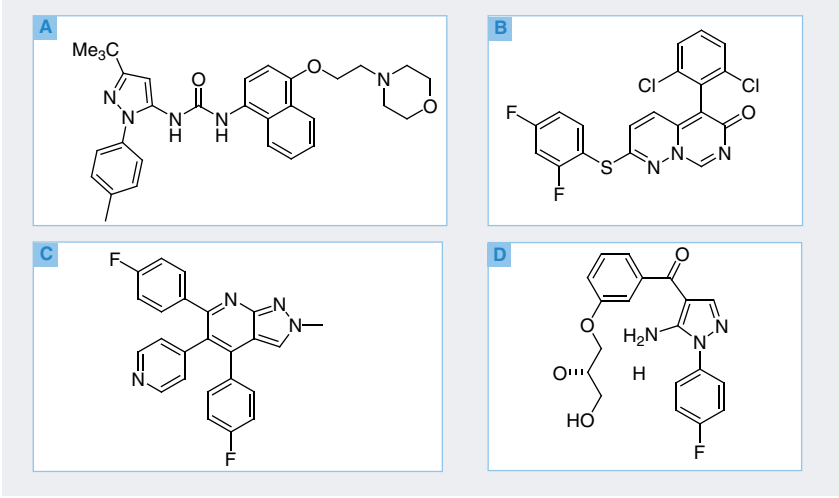

Figure 1. Microwave irradiation has been used to accelerate the synthesis of a number of p38 MAPK inhibitors, including (A) BIRB 796 [13]**; (B) VX-745** [14,15]**; (C) UR-13756** [16] **and (D) RO3201195** [17]**.**

on the *N*-phenyl function of this chemotype, on the other hand, indicated that only small hydrophobic groups were tolerated and that *para*-substitution with relatively less polar groups gave rise to higher activities. Keeping the core pharmacophore intact (exocyclic amine, benzoyl group and pyrazole), we set out to establish whether the trends in library activity described in the original report were also in evidence in WS cells. In so doing, it was hoped that it would also be possible to explore how the size of the substituted *N*-phenyl moiety influenced the activity for a series of compounds bearing functionality that could be readily converted to the solubilizing dihydroxypropoxy group.

Experimental section

■ General procedures

Commercially available reagents were used without further purification; solvents were dried by standard procedures. Light petroleum refers to the fraction with boiling point (bp) 40–60°C. 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 GF254 that were visualized under UV light (at 254 and/or 360 nm). Microwave irradiation experiments were performed using a self-tunable CEM Discover-focused monomodal microwave synthesizer at the given temperature, measured using the instrument's in-built IR sensor, by varying the irradiation power (initial power given in parentheses). Fully characterized compounds were chromatographically homogeneous. Melting points were determined on a Kofler hot stage apparatus and are uncorrected. IR spectra were recorded on a Perkin–Elmer 1600 series Fourier transform (FT)IR spectrometer in the range 4000–600 cm^{-1} using KBr disks for solid samples and thin films between NaCl plates for liquid samples or as a nujol mull 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 ^{1}H spectra and 100 or 125 MHz for ^{13}C spectra in $CDCl_3$ at 25°C unless stated otherwise and were reported in ppm; *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 stated otherwise as electrospray ionization (ES), chemical ionization ([CI], ammonia) or electron ionization (EI). *In vacuo* refers to evaporation at reduced pressure using a rotary evaporator and diaphragm pump, followed by the removal of trace volatiles using a vacuum (oil) pump.

■ General procedure for the microwave-assisted synthesis of aminoacrylonitriles **2**

A solution of benzoylacetonitrile **1B** (0.10 g, 0.69 mmol) and *N,N′*-diphenylformamidine (0.135 g, 0.69 mmol) in dry xylenes (0.5 ml) was irradiated in a sealed tube at 180°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 diluted with Et_2O. The precipitate was filtered and washed with Et_2O to give aminoacrylonitrile **2B**.

2-(3-methoxybenzoyl)-3-phenylaminoacrylonitrile **(2A)** [R_f = 0.46 (light petroleum:EtOAc, 4:1)] was obtained as a colourless solid [25] mp 105°C (Et_2O) (found: MH^+, 279.1129. $C_{17}H_{15}N_2O_2$ [MH^+] requires 279.1128); IR (KBr) ν_{max} 3063, 3012, 2961, 2840, 1637, 1598, 1574, 1489, 1394, 1372, 1316, 1270, 1226, 1045, 991, 869, 832, 742, 683; ^{1}H NMR (400 MHz, $CDCl_3$) δ 12.75 (^{1}H, d, *J* 13.0, NH), 8.06 (^{1}H, d, *J* 13.0 Hz), 7.55 (^{1}H, d, *J* 7.4), 7.47–7.36 (4H), 7.28 (^{1}H, t, *J* 7.2), 7.21 (2H, d, *J* 8.0), 7.09 (^{1}H, d, *J* 8.4), 3.86 (3H, s); ^{13}C NMR (100 MHz, $CDCl_3$) δ 192.2 (C), 159.5 (C), 154.1 (CH), 139.1 (C), 138.0 (C), 130.2 (CH), 129.5 (CH), 126.7 (CH), 120.5 (CH), 120.4 (C), 118.9 (CH), 117.9 (CH), 112.5 (CH), 83.4 (C), 55.5 (CH_3); *m/z* (APcI) 279 (MH^+, 90%).

2-benzoyl-3-phenylaminoacrylonitrile **(2B)** [R_f = 0.47 (light petroleum:EtOAc, 7:3)] was obtained as a colourless solid [25] melting point (mp) 158–159°C (light petroleum) (found: MH^+, 249.1022. $C_{16}H_{13}N_2O$ [MH^+] requires 249.1022); IR (KBr) ν_{max} 3185, 2361, 2205, 1697, 1665, 1606, 1577, 1559, 1542, 1474, 1422, 1396, 1314, 1269, 1145, 1076, 992, 924; ^{1}H NMR (400 MHz, $CDCl_3$) δ 12.70 (^{1}H, br s, NH), 8.08–8.03 (^{1}H, m, CH), 7.95–7.90 (2H, m), 7.58–7.41 (5H), 7.31–7.10 (3H); ^{13}C NMR (100 MHz, $CDCl_3$) δ 192.5 (C), 154.1 (CH), 138.0 (C), 132.3 (CH), 130.2 (CH), 129.7 (C), 128.4 (CH), 128.0 (CH), 126.7 (CH), 120.5 (C), 117.8 (CH), 117.9 (CH), 83.3 (C); *m/z* (APcI) 249 (MH^+, 100%).

■ General procedure for the microwave-assisted synthesis of pyrazolyl ketones 4A–X

A mixture of 3-methoxy-2-benzoyl-3-phenylaminoacrylonitrile **(2A)** or 2-benzoyl-3-phenylaminoacrylonitrile **(2B)** (0.108 mmol, 1 equivalent) and the hydrazine **3A-P** (0.108 mmol, 1 equivalent), in the presence or absence of Et_3N (0.12 mmol, 1.1 equivalent), in ethanol (1.23 ml) was irradiated in a sealed tube at 140°C (measured using the in-built IR sensor) for 1 h 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 concentrated and evaporated *in vacuo*. Purification by column chromatography on SiO_2 gel, eluting with light petroleum:EtOAc, gave the desired pyrazole.

[5-amino-1-(4-iodophenyl)-1*H*-pyrazol-4-yl] 3-methoxyphenyl ketone **(4A)** [R_f = 0.48 (light petroleum:EtOAc, 3:2)] was obtained as a colorless solid (22.6 mg, 75%), mp 142°C (light petroleum) (found: MH^+, 420.0207. $C_{17}H_{15}N_3O_2I$ [MH^+] requires 420.0203); IR (KBr) ν_{max} 3366, 3242, 3171, 2955, 1615, 1595, 1582, 1539, 1488, 1430, 1396, 1323, 1308, 1282, 1246, 1210, 1145, 1040, 1007, 938, 843, 817, 770, 684, 639, 613, 562; ^{1}H NMR (400 MHz, $CDCl_3$): δ 7.87–7.85 (2H, m), 7.81 (^{1}H, s, 3′-H), 7.42–7.38 (2H, m), 7.36–7.31 (3H), 7.11–7.08 (^{1}H, m, 4-H), 6.10 (2H, br s, NH_2), 3.87 (3H, s, OMe); ^{13}C NMR (100 MHz, $CDCl_3$) δ 189.5 (C), 159.7 (C), 150.5 (C), 142.4 (CH), 140.9 (C), 139.0 (CH), 136.9 (C), 129.6 (CH), 125.5 (CH), 120.7 (CH), 117.9 (CH), 112.8 (CH), 105.0 (C), 93.4 (C), 55.5 (CH_3); *m/z* (ES) 420 (MH^+, 100%).

[5-amino-1-(4-fluorophenyl)-1*H*-pyrazol-4-yl] 3-methoxyphenyl ketone **(4B)** [R_f = 0.57 (light petroleum:EtOAc, 3:2)] was obtained as a colorless solid (19 mg, 86%) [25] mp 114°C (light petroleum) (found: MH^+, 312.1143. $C_{17}H_{15}N_3O_2F$ [MH^+] requires 312.1143); IR (KBr) ν_{max} 3437, 3292, 2923, 1636, 1614, 1596, 1575, 1539, 1496, 1451, 1398, 1308, 1286, 1241, 1223, 1158, 1051, 929, 838, 759,678, 619; ^{1}H NMR (400 MHz, $CDCl_3$) δ 7.82 (^{1}H, s, 3′-H), 7.59–7.55 (2H, m), 7.44–7.43 (2H, m), 7.36–7.35 (^{1}H, m), 7.29–7.24 (2H, m), 7.14–7.11 (^{1}H, m), 6.07 (2H, br s, NH_2), 3.90 (3H, s, OCH_3); ^{13}C NMR (100 MHz, $CDCl_3$) δ 189.5 (C), 162.1 (d, $^1J_{C-F}$ 248.0, C), 159.7 (C), 150.5 (C), 142.0 (CH), 140.9 (C), 133.1 (d, $^4J_{C-F}$ 3.3, C), 129.5 (CH), 126.1 (d, $^3J_{C-F}$ 8.8, CH), 120.6 (CH), 117.8 (CH), 116.9 (d, $^2J_{C-F}$ 23.0, CH), 112.9 (CH), 104.8 (C), 55.4 (CH_3); *m/z* (APcI) 312 (MH^+, 100%).

[5-amino-1-(4-fluorophenyl)-1*H*-pyrazol-4-yl] phenyl ketone **(4C)** [R_f = 0.7 (light petroleum:EtOAc, 3:2)] was obtained as a colorless solid (17.2 mg, 76%) [25] mp 164°C (light petroleum) (found: MH^+, 281.0957. $C_{16}H_{13}N_3OF$ [MH^+] requires 281.0964); IR (KBr) ν_{max} 3447, 3315, 3067, 2923, 1633, 1614, 1594, 1541, 1516, 1485, 1442, 1402, 1307, 1228, 1156, 1020, 904, 875, 842, 822, 754, 733, 689, 619, 590; ^{1}H NMR (400 MHz, $CDCl_3$) δ 7.84–7.80 (2H, m), 7.78 (^{1}H, s, 3′-H), 7.59–7.47 (5H), 7.27–7.20 (2H, m), 6.05 (2H, br s, NH_2); ^{13}C NMR (100 MHz, $CDCl_3$) δ 189.8 (C), 162.1 (d, $^1J_{C-F}$ 250.6, C), 150.5 (C), 142.1 (CH), 139.7 (C), 133.2 (d, $^4J_{C-F}$ 3.3, C), 131.6 (CH), 128.6 (CH), 128.2 (CH), 126.2 (d, $^3J_{C-F}$ 8.4, CH), 116.9 (d, $^2J_{C-F}$ 23.1, CH), 104.8 (C); *m/z* (EI) 204 (MH^+, 30%), 280 (100).

[5-amino-1-(4-bromophenyl)-1*H*-pyrazol-4-yl] phenyl ketone **(4D)** [R_f = 0.75 (light petroleum:EtOAc, 3:2)] was obtained as a colorless solid (17.3 mg, 63%), mp 147–150°C (light petroleum) (found: MH^+, 342.0242. $C_{16}H_{13}N_3O^{79}Br$ [MH^+] requires 342.0242); IR (KBr) ν_{max} 3369, 3242, 3171, 1612, 1537, 1493, 1396, 1306, 1279, 1210, 1007, 904, 840, 822, 765, 734, 695, 671, 613, 527; ^{1}H NMR (400 MHz, $CDCl_3$) δ 7.87 (2H, d, *J* 8.8), 7.82–7.78 (3H), 7.49–7.59 (3H), 7.34 (2H, d, *J* 8.8), 6.10 (2H, br s, NH_2); ^{13}C NMR (100 MHz, $CDCl_3$) δ 189.8 (C), 150.4 (C), 142.4 (CH), 139.6 (C), 139.1 (CH), 137.0 (C), 131.6 (CH), 128.6 (CH), 128.0 (CH), 125.5 (CH), 122.0 (C), 105.0 (C); *m/z* (ES) 344 ($C_{16}H_{13}N_3O^{81}Br^+$, 90%), 342 ($C_{16}H_{13}N_3O^{79}Br^+$, 100).

[5-amino-1-(2,6-dichlorophenyl)-1*H*-pyrazol-4-yl] phenyl ketone **(4E)** [R_f = 0.37 (light petroleum:EtOAc, 3:2)] was obtained as a colourless solid (22.4 mg, 84%) [25], mp 220°C (light petroleum) (found: MH^+, 332.0342. $C_{16}H_{12}N_3O^{35}Cl_2$ [MH^+] requires 332.0357); IR (KBr) ν_{max} 3393, 3257, 3169, 1622, 1568, 1539, 1507, 1446, 1388, 1309, 1274, 1213, 1161, 1075, 899, 788, 743, 700, 674, 540, 524; ^{1}H NMR (400 MHz, $CDCl_3$) δ 7.90 (^{1}H, s, 3′-CH), 7.87–7.84 (2H, m), 7.56–7.49 (5H), 7.46–7.41 (^{1}H, m), 5.86 (2H, br s, NH_2); ^{13}C NMR (100 MHz, $CDCl_3$) δ 189.7 (C), 151.9 (C), 142.9 (CH), 139.6 (C), 135.8 (C), 135.7 (C), 132.0 (CH), 131.6 (CH), 129.2 (CH), 128.5 (CH), 128.3 (CH), 104.0 (C); *m/z* (ES) 334 ($C_{16}H_{12}N_3O^{37}Cl^{35}Cl^+$, 70), 332 ($C_{16}H_{12}N_3O^{35}Cl_2^+$, 100).

[5-amino-1-(2,4-difluorophenyl)-1H-pyrazol-4-yl] phenyl ketone **(4F)** [R_f = 0.5 (light petroleum:EtOAc, 3:2)] was obtained as a colorless solid (15.4 mg, 64%), mp 153°C (light petroleum) (found: MH^+, 299.0865. $C_{16}H_{12}N_3OF_2$ [MH^+] requires 299.0870); IR (KBr) ν_{max} 3392, 3248, 3166, 2923, 1624, 1602, 1546, 1515, 1502, 1441, 1403, 1320, 1272, 1146, 1118, 1076, 974, 954, 903, 876, 853, 800, 759, 734, 701, 619, 565; ^{1}H NMR (400 MHz, $CDCl_3$) δ 7.85–7.80 (3H), 7.58–7.48 (4H), 7.04–7.11 (2H, m), 6.02 (2H, br s, NH_2); ^{13}C NMR (100 MHz, $CDCl_3$) δ 189.6 (C), 163.0 (dd, $^1J_{C-F}$ 254.7, $^3J_{C-F}$ 10.3, C), 156.9 (dd, $^1J_{C-F}$ 255.4, $^3J_{C-F}$ 12.0, C), 151.8 (C), 142.8 (CH), 139.6 (C), 131.6 (CH), 129.9 (dd, $^3J_{C-F}$ 10.2, 1.4, CH), 128.6 (CH), 128.2 (CH), 120.9 (dd, $^2J_{C-F}$ 12.4, $^4J_{C-F}$ 4.0, C), 112.8 (dd, $^2J_{C-F}$ 22.6, $^4J_{C-F}$ 3.6, CH), 105.6 (dd, $^2J_{C-F}$ 27.1, 23.0, CH), 104.3 (C); *m/z* (EI) 298 (MH^+, 100%), 222 (30).

[5-amino-1-(pentafluorophenyl)-1H-pyrazol-4-yl] 3-methoxyphenyl ketone **(4G)** [R_f = 0.66 (light petroleum:EtOAc, 3:2)] was obtained as a colorless solid (21.5 mg, 78%), mp 180–181°C (light petroleum) (found: MH^+, 384.0761. $C_{17}H_{11}N_3O_2F_5$ [MH^+] requires 384.0766); IR (KBr) ν_{max} 3428, 3333, 2942, 1633, 1541, 1514, 1487, 1461, 1426, 1321, 1307, 1255, 1217, 1139, 1066, 1034, 991, 928, 848, 816, 792, 770; ^{1}H NMR (400 MHz, $CDCl_3$) δ 7.90 (^{1}H, s, 3′-H), 7.42–7.41 (2H, m), 7.32 (^{1}H, s, 2-H), 7.13–7.10 (^{1}H, m, 4-H), 6.30 (2H, br s, NH_2), 3.88 (3H, s, CH_3); ^{13}C NMR (125 MHz, $CDCl_3$) δ 189.3 (C), 159.8 (C), 152.8 (C), 144.1 (app d, $^1J_{C-F}$ 255.0, C), 144.0 (CH), 140.7 (app d, $^1J_{C-F}$ 216.0, C), 140.5 (C), 138.2 (app d, $^1J_{C-F}$ 247.0, C), 129.6 (CH), 120.6 (CH), 118.9 (CH), 112.9 (CH), 111.8 (m, C), 104.2 (C), 55.5 (CH_3); *m/z* (APcI) 384 (MH^+, 100%).

(5-amino-1-phenyl-1*H*-pyrazol-4-yl) phenyl ketone **(4H)** [R_f = 0.45 (light petroleum:EtOAc, 3:2)] was obtained as a colorless solid (40 mg, 95%) [25] mp 153°C (light petroleum) (found: MH^+, 264.1133. $C_{16}H_{14}N_3O$ [MH^+] requires 264.1131); IR (KBr) ν_{max} 3200, 2911, 2681, 2520, 2359, 1614, 1505, 1401, 1309, 1240, 1170, 1118, 906, 767, 732; ^{1}H NMR (400 MHz, $CDCl_3$) δ 7.70–7.60 (3H), 7.45–7.20 (8H), 5.90 (2H); ^{13}C NMR (100 MHz, $CDCl_3$) δ 189.9 (C), 150.4 (C), 142.0 (CH), 139.8 (C), 137.1 (C), 131.5 (CH), 130.0 (CH), 128.5 (CH), 128.4 (CH), 128.2 (CH), 124.0 (CH), 104.8 (C); *m/z* (APcI) 264 (MH^+, 100%).

[5-amino-1-(4-tolyl)-1*H*-pyrazol-4-yl] 3-methoxyphenyl ketone **(4I)** [R_f = 0.57 (light petroleum:EtOAc, 3:2)] was obtained as a colorless solid (32 mg, 58%), mp 165°C (light petroleum) (found: MH^+, 308.1394. $C_{18}H_{18}N_3O_2$ [MH^+] requires 308.1394); IR (KBr) ν_{max} 3381, 3254, 2961, 2917, 2853, 1614, 1572, 1543, 1502, 1483, 1429, 1311, 1285, 1247, 1053, 935, 811, 769, 708; ^{1}H NMR (400 MHz, $CDCl_3$) δ 7.79 (^{1}H, s, 3′-H), 7.45–7.40 (4H), 7.37–7.32 (3H), 7.11–7.08 (^{1}H, m, 4-H), 6.04 (2H, br s, NH_2), 3.87 (3H, s, OCH_3), 2.43 (3H, s, CH_3); ^{13}C NMR (100 MHz, $CDCl_3$) δ 189.4 (C), 159.7 (C), 150.4 (C), 141.9 (CH), 141.1 (C), 138.7 (C), 134.5 (C), 130.5 (CH), 129.5 (CH), 124.0 (CH), 120.7 (CH), 117.8 (CH), 112.8 (CH), 104.7(C), 55.5 (CH_3), 21.2 (CH_3); *m/z* (APcI) 308 (MH^+, 100%).

(5-amino-1-methyl-1*H*-pyrazol-4-yl) 3-methoxyphenyl ketone **(4J)** [R_f = 0.6 (light petroleum:EtOAc, 3:2)] was obtained as a colorless solid (46%) (found: MH^+, 213.0899. $C_{12}H_{14}N_3O_2$ [MH^+] 213.0902); IR (KBr) ν_{max} 3178, 3158, 2955, 2917, 2884, 2840, 1602, 1583, 1506, 1471, 1458, 1432, 1288, 1257, 1211, 1096, 1053, 1029, 957, 874, 851, 776, 714, 687; ^{1}H NMR (400 MHz, $CDCl_3$) δ 7.85 (^{1}H, s, 3′-CH), 7.55 (^{1}H, d, *J* 7.8, 6-H), 7.50 (^{1}H, s, 2-H), 7.37 (^{1}H, t, *J* 7.8, 5-H), 6.98–6.95 (^{1}H, m, 4-H), 6.10 (2H, br s, NH_2), 4.00 (3H, s, OCH_3), 3.87 (3H, s, CH_3); ^{13}C NMR (100 MHz, $CDCl_3$) δ 189.6 (C), 160.0 (C), 153.2 (C), 141.0 (CH), 136.7 (C), 130.0 (CH), 119.0 (CH), 115.8 (CH), 114.5 (C), 111.3 (CH), 55.4 (CH_3), 39.8 (CH_3); *m/z* (EI) 213 (MH^+, 100%).

[5-amino-1-(4-chlorophenyl)-1*H*-pyrazol-4-yl] phenyl ketone **(4K)** [R_f = 0.54 (light petroleum:EtOAc, 3:2)] was obtained as a colorless solid (9.4 mg, 39%), mp 168°C (light petroleum) (found: MH^+, 298.0732. $C_{16}H_{13}N_3O^{35}Cl$ [MH^+] requires 298.0747); IR (KBr) ν_{max} 3379,

3253, 3060, 2923, 1615, 1598, 1539, 1498, 1395, 1307, 1279, 1211, 1096, 1012, 903, 845, 823, 802, 741, 732, 700, 679, 523; ^{1}H NMR (400 MHz, $CDCl_3$) δ 7.83–7.78 (3H), 7.59–7.48 (7H), 6.10 (2H, br s, NH_2); ^{13}C NMR (100 MHz, $CDCl_3$) δ 189.8 (C), 150.5 (C), 142.3 (CH), 139.6 (C), 135.7 (C), 134.2 (C), 131.6 (CH), 130.2 (CH), 128.6 (CH), 128.2 (CH), 125.2 (CH), 104.9 (C); *m/z* (ES) 300 ($C_{16}H_{13}N_3O^{37}Cl^+$, 30%), 298 ($C_{16}H_{13}N_3O^{35}Cl^+$, 100).

5-amino-1-(4-chlorophenyl)-1*H*-pyrazol-4-yl] 3-methoxyphenyl ketone **(4L)** [R_f = 0.7 (light petroleum:EtOAc, 3:2)] was obtained as a colorless solid (13.5 mg, 57%), mp 129–130°C (light petroleum) (found: MH^+, 328.0868. $C_{17}H_{15}N_3O_2{}^{35}Cl$ [MH^+] requires 328.0853); IR (KBr) ν_{max} 3367, 3249, 3171, 2995, 2834, 1614, 1575, 1540, 1497, 1483, 1454, 1433, 1307, 1287, 1248, 1210, 1095, 1052, 1012, 934, 846, 811, 769, 706, 683, 613; ^{1}H NMR (400 MHz, $CDCl_3$) δ 7.80 (^{1}H, s, 3′-H), 7.53–7.50 (4H), 7.42–7.40 (2H, m), 7.33 (^{1}H, s, 2-H), 7.12–7.07 (^{1}H, m, 4-H), 6.09 (2H, br s, NH_2), 3.87 (3H, s, CH_3); ^{13}C NMR (100 MHz, $CDCl_3$) δ 189.5 (C), 159.7 (C), 150.5 (C), 142.3 (CH), 140.9 (C), 135.7 (C), 134.2 (C), 130.2 (CH), 129.5 (CH), 125.1 (CH), 120.7 (CH), 117.9 (CH), 112.5 (CH), 104.9 (C), 55.5 (CH_3); *m/z* (APcI) 330 ($C_{17}H_{15}N_3O_2{}^{37}Cl^+$, 30%), 328 ($C_{17}H_{15}N_3O_2{}^{35}Cl^+$, 100).

[5-amino-1-(2,4-difluorophenyl)-1*H*-pyrazol-4-yl] 3-methoxyphenyl ketone **(4M)** [R_f = 0.46 (light petroleum:EtOAc, 3:2)] was obtained as a colorless solid (26.6 mg, >98%), mp 90°C (light petroleum) (found: MH^+, 330.1048. $C_{17}H_{14}N_3O_2F_2$ [MH^+] requires 330.1049); IR (KBr) ν_{max} 3459, 3408, 3323, 3069, 2923, 1633, 1624, 1611, 1575, 1541, 1493, 1436, 1400, 1311, 1263, 1248, 1225, 1145, 1110, 1033, 961, 923, 855, 811, 760, 601; ^{1}H NMR (400 MHz, $CDCl_3$) δ 7.85 (^{1}H, s, 3′-H), 7.58–7.51 (^{1}H, m), 7.42–7.40 (2H, m), 7.32 (^{1}H, m, 2-H), 7.11–7.04 (3H), 6.00 (2H, br s, NH_2), 3.88 (3H, s, CH_3); ^{13}C NMR (100 MHz, $CDCl_3$) δ 189.4 (C), 163.0 (dd, $^1J_{C\text{-}F}$ 253.5, $^3J_{C\text{-}F}$ 10.9, C), 159.7 (C), 157.0 (dd, $^1J_{C\text{-}F}$ 256.8, $^3J_{C\text{-}F}$ 13.1, C), 151.8 (C), 142.9 (CH), 140.9 (C), 129.9 (dd, $^3J_{C\text{-}F}$ 10.2, $^4J_{C\text{-}F}$ 1.4, CH), 129.1 (CH), 120.9 (dd, $^2J_{C\text{-}F}$ 12.0, $^4J_{C\text{-}F}$ 3.6, C), 120.7 (CH), 118.0 (CH), 112.8 (dd, $^2J_{C\text{-}F}$ 23.0, ^4J 4.0, CH), 112.7 (CH), 105.6 (dd, $^2J_{C\text{-}F}$ 26.3, 23.0, CH), 104.3 (C), 55.5 (CH_3); *m/z* (APcI) 330 (MH^+, 100%).

[5-amino-1-(2-fluorophenyl)-1*H*-pyrazol-4-yl] 3-methoxyphenyl ketone **(4N)** [R_f = 0.36 (light petroleum:EtOAc, 3:2)] was obtained as a colorless solid (10 mg, 90%), mp 133–134°C (light petroleum) (found: MH^+, 312.1137. $C_{17}H_{15}N_3O_2F$ [MH^+] requires 312.1143); IR (KBr) ν_{max} 3356, 3261, 3178, 3051, 3005, 2968, 1627, 1612, 1581, 1545, 1510, 1458, 1397, 1294, 1243, 1054, 976, 871, 834, 774, 686; ^{1}H NMR (400 MHz, $CDCl_3$) δ 8.06 (^{1}H, s, 3′-H), 7.48–7.40 (3H), 7.29–7.23 (^{1}H, m), 7.15–6.91 (2H, m), 6.90–6.45 (4H), 3.70 (3H, s, CH_3); ^{13}C NMR (100 MHz, $CDCl_3$) δ 189.6 (C), 159.6 (C), 156.4 (d, $^1J_{C\text{-}F}$ 254.0, C), 149.4 (C), 143.4 (CH), 135.8 (C), 131.4 (d, $^3J_{C\text{-}F}$ 7.7, CH), 130.2 (d, $^3J_{C\text{-}F}$ 7.6, CH), 128.7 (CH), 126.8 (d, $^2J_{C\text{-}F}$ 11.7, C), 125.1 (d, $^4J_{C\text{-}F}$ 4.0, CH), 120.7 (CH), 116.9 (d, $^2J_{C\text{-}F}$ = 19.4 Hz, CH), 116.4 (CH), 113.8 (CH), 93.2 (C), 55.3 (CH_3); *m/z* (ES) 312 (MH^+, 100%).

[5-amino-1-(2,5-dichlorophenyl)-1H-pyrazol-4-yl] 3-methoxyphenyl ketone **(4O)** [R_f = 0.53 (light petroleum:EtOAc, 3:2)] was obtained as a colorless oil (23.3 mg, 78%); IR (KBr) ν_{max} 3406, 3287, 3178, 2961, 2917, 1622, 1578, 1536, 1480, 1429, 1373, 1307, 1285, 1251, 1209, 1094, 1039, 926, 875, 819, 766, 730, 580; ^{1}H NMR (400 MHz, $CDCl_3$) δ 7.85 (^{1}H, s, 3′-H), 7.55–7.52 (2H, m), 7.47–7.44 (^{1}H, m), 7.43–7.40 (2H, m), 7.34 (^{1}H, s, 2-H), 7.12–7.07 (^{1}H, m, 4-H), 5.95 (2H, br s, NH_2), 3.88 (3H, s, CH_3); ^{13}C NMR (100 MHz, $CDCl_3$) δ 189.4 (C), 159.7 (C), 151.7 (C), 142.7 (CH), 140.9 (C), 135.1 (C), 133.8 (C), 131.7 (CH), 131.4 (CH), 130.4 (C), 130.0 (CH), 129.6 (CH), 120.7 (CH), 117.9 (CH), 112.8 (CH), 104.1 (C), 55.5 (CH_3).

[5-amino-1-(2,6-dichlorophenyl)-1*H*-pyrazol-4-yl] 3-methoxyphenyl ketone **(4P)** [R_f = 0.3 (light petroleum:EtOAc, 3:2)] was obtained as a colorless oil (5.8 mg, 45%) (found: MH^+, 362.0460. $C_{17}H_{14}N_3O_2{}^{35}Cl_2$ [MH^+] requires 362.0458); IR (KBr) ν_{max} 3408, 3283, 3184, 2961, 2936, 1623, 1575, 1538, 1503, 1439, 1387, 1308, 1285, 1241, 1200, 1041, 926, 814, 793, 732, 642; ^{1}H NMR (400 MHz, $CDCl_3$) δ 7.90 (^{1}H, s, 3′-H), 7.54–7.50 (2H, m), 7.46–7.40 (3H), 7.35 (^{1}H, s, 2-H), 7.11–7.07 (^{1}H, m, 4-H), 5.88 (2H, br s, NH_2), 3.88 (3H, s, CH_3); ^{13}C NMR (100 MHz, $CDCl_3$) δ 189.3 (C), 159.7 (C), 152.0 (C), 142.8 (CH), 140.9 (C), 135.8 (C), 135.7 (C), 132.0 (CH), 129.5 (CH), 129.2 (CH), 120.7 (CH), 117.9 (CH), 112.8 (CH), 104.0 (C), 55.5 (CH_3); *m/z* (ES) 364 ($C_{17}H_{14}N_3O_2{}^{37}Cl^{35}Cl^+$, 30%), 362 ($C_{17}H_{14}N_3O_2{}^{35}Cl_2{}^+$, 50).

[5-amino-1-(4-bromophenyl)-1*H*-pyrazol-4-yl] 3-methoxyphenyl ketone **(4Q)** [R_f = 0.63 (light petroleum:EtOAc, 3:2)] was obtained as

a colorless solid (24.4 mg, 92%), mp 125–126°C (light petroleum) (found: MH^+, 372.0339. $C_{17}H_{15}N_3O_2{}^{79}Br$ [MH^+] requires 372.0342); IR (KBr) ν_{max} 3369, 3242, 3171, 2955, 1614, 1582, 1541, 1490, 1461, 1426, 1397, 1324, 1308, 1280, 1245, 1210, 1146, 1039, 937, 874, 845, 821, 799, 770, 732, 613, 544; ^{1}H NMR (400 MHz, $CDCl_3$) δ 7.82 (^{1}H, s, 3′-H), 7.67 (2H, d, *J* 8.8), 7.47 (2H, d, *J* 8.8), 7.42–7.40 (2H, m), 7.34–7.32 (^{1}H, m, 2-H), 7.12–7.07 (^{1}H, m, 4-H), 6.09 (2H, br s, NH_2), 3.88 (3H, s, CH_3); ^{13}C NMR (100 MHz, $CDCl_3$) δ 189.5 (C), 159.7 (C), 150.5 (C), 142.4 (CH), 140.9 (C), 136.2 (C), 133.1 (CH), 129.6 (CH), 125.4 (CH), 122.1 (C), 120.7 (CH), 118.0 (CH), 112.8 (CH), 105.0 (C), 55.5 (CH_3); *m/z* (ES) 374 ($C_{17}H_{15}N_3O_2{}^{81}Br^+$, 40%), 372 ($C_{17}H_{15}N_3O_2{}^{79}Br^+$, 35), 105 (100).

[5-amino-1-(pentafluorophenyl)-1*H*-pyrazol-4-yl] phenyl ketone **(4R)** [R_f = 0.74 (light petroleum:EtOAc, 3:2)] was obtained as a colorless solid (25 mg, 89%), mp 130–135°C (light petroleum) (found: MH^+, 354.0663. $C_{16}H_9N_3OF_5$ [MH^+] requires 354.0666); IR (KBr) ν_{max} 3413, 3381, 3298, 3184, 2923, 1621, 1605, 1544, 1505, 1480, 1432, 1393, 1306, 1223, 1162, 1114, 1073, 993, 899, 846, 802, 738, 724, 700, 675, 578, 527; ^{1}H NMR (400 MHz, $CDCl_3$) δ 7.89 (^{1}H, s, 3′-H), 7.81–7.78 (2H, m), 7.60–7.55 (^{1}H, m), 7.54–7.48 (2H, m), 6.13 (2H, br s, NH_2); ^{13}C NMR (100 MHz, $CDCl_3$) δ 189.6 (C), 152.8(C), 144.1 (app d, $^1J_{C\text{-}F}$ 257.9, C), 144.0 (CH), 139.1 (C), 138.3 (app d, $^1J_{C\text{-}F}$ 254.3, C), 138.2 (app d, $^1J_{C\text{-}F}$ 259.8, C), 131.9 (CH), 128.6 (CH), 128.4 (CH), 128.1 (CH), 128.0 (CH), 111.8 (m, C), 104.1 (C); *m/z* (ES) 354 (MH^+, 100%).

[5-amino-1-(2-fluorophenyl)-1*H*-pyrazol-4-yl] phenyl ketone **(4S)** [R_f = 0.46 (light petroleum:EtOAc, 3:2)] was obtained as a colorless solid (1.8 mg, 8%), mp 162°C (light petroleum) (found: MH^+, 281.0964. $C_{16}H_{13}N_3OF$ [MH^+] requires 281.0964); IR (KBr) ν_{max} 3398, 3249, 3162, 2923, 1623, 1574, 1547, 1505, 1459, 1402, 1313, 1264, 1228, 1212, 1126, 902, 877, 820, 770, 757, 741, 703, 635, 610, 549, 523; ^{1}H NMR (400 MHz, $CDCl_3$) δ 7.86–7.80 (3H), 7.59–7.46 (5H), 7.36–7.28 (2H, m), 6.04 (2H, br s, NH_2); ^{13}C NMR (100 MHz, $CDCl_3$) δ 189.6 (C), 156.4 (d, $^1J_{C\text{-}F}$ 251.7, C), 151.7 (C), 142.8 (CH), 139.7 (C), 131.6 (CH), 131.1 (d, $^3J_{C\text{-}F}$ 8.1, CH), 128.6 (d, $^3J_{C\text{-}F}$ 10.2, CH), 128.5 (CH), 128.2 (CH), 125.5 (d, $^4J_{C\text{-}F}$ 3.7, CH), 124.2 (d, $^2J_{C\text{-}F}$ 12.1, C), 117.2 (d, $^2J_{C\text{-}F}$ 19.4, CH), 104.4 (C); *m/z* (EI) 280 (MH^+, 100%).

[5-amino-1-(2,5-dichlorophenyl)-1*H*-pyrazol-4-yl] 3-phenyl ketone **(4T)** [R_f = 0.76 (light petroleum:EtOAc, 3:2)] was obtained as a colorless solid (25.3 mg, 95%), mp 154°C (light petroleum) (found: MH^+, 332.0342. $C_{16}H_{12}N_3O^{35}Cl_2$ [MH^+] requires 332.0357); IR (KBr) ν_{max} 3395, 3266, 3190, 2923, 1621, 1539, 1506, 1480, 1414, 1375, 1310, 1285, 1247, 1211, 1098, 1075, 1031, 971, 901, 878, 816, 752, 737, 701, 614, 578; ^{1}H NMR (400 MHz, $CDCl_3$) δ 7.85–7.80 (3H), 7.59–7.56 (5H), 7.47–7.42 (^{1}H, m), 5.90 (2H, br s, NH_2); ^{13}C NMR (100 MHz, $CDCl_3$) δ 189.6 (C), 151.7 (C), 142.7 (CH), 139.6 (C), 135.1 (C), 133.8 (C), 131.7 (CH), 131.6 (CH), 131.4 (C), 130.0 (CH), 129.3 (CH), 128.6 (CH), 128.2 (CH), 105.0 (C); *m/z* (APcI) 332 (MH^+, 100%).

[5-amino-1-(4-iodophenyl)-1*H*-pyrazol-4-yl] 3-phenyl ketone **(4U)** [R_f = 0.6 (light petroleum:EtOAc, 3:2)] was obtained as a colorless solid (1.4 mg, 5%), mp 170°C (light petroleum) (found: MH^+, 390.0093. $C_{16}H_{13}N_3OI$ [MH^+] requires 390.0103); IR (KBr) ν_{max} 3380, 3261, 3058, 2920, 2853, 1614, 1598, 1573, 1539, 1495, 1444, 1398, 1306, 1276, 1210, 1072, 1010, 904, 843, 822, 735, 698, 675, 614, 527, 507; ^{1}H NMR (400 MHz, $CDCl_3$) δ 7.83–7.80 (3H), 7.68 (2H, d, *J* 8.8), 7.57–7.50 (3H), 7.47 (2H, d, *J* 8.8), 6.10 (2H, br s, NH_2); ^{13}C NMR (100 MHz, $CDCl_3$) δ 189.3 (C), 150.0 (C), 142.4 (CH), 139.6 (C), 136.0 (C), 133.1 (CH), 131.6 (CH), 128.5 (CH), 128.2 (CH), 125.4 (CH), 104.9 (C), 90.6 (C); *m/z* (ES) 390 (MH^+, 100%).

[5-amino-1-(4-methoxyphenyl)-1*H*-pyrazol-4-yl] 3-methoxyphenyl ketone **(4V)** [R_f = 0.38 (light petroleum:EtOAc, 3:2)] was obtained as a colorless solid (4.1 mg, 71%), mp 137°C (light petroleum) (found: MH^+, 324.1343. $C_{18}H_{18}N_3O_3$ [MH^+] requires 324.1339); IR (KBr) ν_{max} 3383, 3253, 3176, 2957, 2924, 2848, 1614, 1581, 1541, 1504, 1462, 1397, 1310, 1249, 1029, 935, 838, 769; ^{1}H NMR (400 MHz, $CDCl_3$) δ 7.78 (^{1}H, s, 3′-H), 7.46 (2H, d, *J* 9.0), 7.42–7.38 (2H, m), 7.36–7.32 (^{1}H, m), 7.11–7.07 (^{1}H, m), 7.04 (2H, d, *J* 9.0), 5.60 (2H, br s, NH_2), 3.87 (3H, s, CH_3), 3.86 (3H, s, CH_3); ^{13}C NMR (125 MHz, $CDCl_3$) δ 189.3 (C), 159.7 (C), 159.6 (C), 150.6 (C), 141.7 (CH), 141.2 (C), 129.9 (C), 129.5 (CH), 125.9 (CH), 120.7 (CH), 117.8 (CH), 115.1 (CH), 112.9 (CH), 104.7 (C), 55.6 (CH_3), 55.4 (CH_3); *m/z* (APcI) 324 (MH^+, 100%).

(5-amino-1-*tert*-butyl-1*H*-pyrazol-4-yl) 3-methoxyphenyl ketone **(4W)** [R_f = 0.54 (light petroleum:EtOAc, 3:2)] was obtained as a

colorless solid (4.0 mg, 82%), mp 93°C (light petroleum) (found: MH^+, 274.1549. $C_{15}H_{20}N_3O_2$ [MH^+] requires 274.1550); IR (KBr) ν_{max} 3386, 3292, 2982, 2923, 1598, 1576, 1530, 1496, 1459, 1317, 1288, 1247, 1218, 1044, 923, 834, 764, 685; ^{1}H NMR (400 MHz, $CDCl_3$) δ 7.57 (^{1}H, s, 3′-H), 7.42–7.31 (2H, m), 7.30–7.27 (^{1}H, m, 2-H), 7.08–7.04 (^{1}H, m, 4-H), 6.10 (2H, br s, NH_2), 3.85 (3H, s, OCH_3), 1.67 (9H, s, CMe_3); ^{13}C NMR (100 MHz, $CDCl_3$) δ 189.0 (C), 159.0 (C), 150.6 (C), 139.5 (CH), 136.0 (C), 129.4 (CH), 120.7 (CH), 117.7 (CH), 112.6 (CH), 105.8 (C), 55.5 (C), 55.4 (CH_3), 28.9 (CH_3); *m/z* (ES) 274 (MH^+, 100%), 259 (70), 218 (60).

[5-amino-1-(4-nitrophenyl)-1*H*-pyrazol-4-yl] 3-methoxyphenyl ketone **(4X)** [R_f = 0.5 (light petroleum:EtOAc, 3:2)] was obtained as a colorless solid (2.5 mg, 41%), mp 190–191°C (light petroleum) (found: MH^+, 339.1086. $C_{17}H_{15}N_4O_4$ [MH^+] requires 339.1088); IR (KBr) ν_{max} 3450, 3304, 3066, 2936, 1636, 1613, 1575, 1538, 1524, 1486, 1450, 1347, 1308, 1290, 1055, 928, 855, 755; ^{1}H NMR (400 MHz, $CDCl_3$) δ 8.41 (2H, d, *J* 8.8), 7.88 (^{1}H, s, 3′-H), 7.84 (2H, d, *J* 8.8), 7.45–7.37 (2H, m), 7.33 (^{1}H, s, 2-H), 7.14–7.08 (^{1}H, m, 4-H), 6.30 (2H, br s, NH_2), 3.88 (3H, s, CH_3); ^{13}C NMR (100 MHz, $CDCl_3$) δ 189.6 (C), 159.8 (C), 150.9 (C), 146.4 (C), 143.3 (CH), 142.7 (C), 140.7 (C), 129.7 (CH), 125.5 (CH), 123.2 (CH), 120.6 (CH), 118.0 (CH), 112.9 (CH), 105.4 (C), 55.5 (CH_3); *m/z* (ES) 339 (MH^+, 100%).

■ Determination of the ability of the pyrazolyl ketone library to inhibit p38

The ability of the members of the pyrazolyl ketone library to inhibit the p38 stress-signaling pathway was tested in human telomerase reverse trancriptase (hTERT)-immortalized HCA2 dermal cells, as described previously, using an ELISA system (Cell Signaling, NEB, UK) [14]. Cells were seeded in 100-mm dishes in Earle's modification of Eagle medium (EMEM) and incubated at 37°C for 48 h. The medium was supplemented with a solution of the pyrazolyl ketone **4** in dimethyl sulfoxide (DMSO) at final concentrations of 150 nM and 1.50 µM, and the cells incubated for a further 2 h. Then anisomycin was added to the medium at 30 µM and the cells harvested 45 min later. Samples using DMSO only and DMSO plus anisomycin were used as controls. Cells were harvested, proteins isolated and the ELISAs carried out according to the manufacturer's instructions. Kinase activity was detected using antibodies specific for the phosphorylated form of the small heat shock protein (HSP27) and antibodies that detect the total levels of HSP27, the degree of activation being measured as the ratio of phosphoprotein/total protein. In this system, activation of p38 by anisomycin activates MAPK-activated kinase 2 (MK2) that then phosphorylates the HSP27. As MK2 is the major HSP27 kinase, the activity of p38 can be assessed by the phosphorylation status of HSP27 [26]. The concentrations of 150 nM and 1.5 µM were chosen because the IC_{50} for RO32901195 in this system was previously determined at approximately 190 nM [17] and we were interested in compounds that may be more efficacious than the lead compound RO3201195.

The ability of several of the pyrazolyl ketones to inhibit the p38 signaling pathway in WS cells was tested by preincubation with the selected compound at 1.5 µM at 37°C for 2 h, as described for HCA2 cells (see above). In this system, the p38 pathway is induced by treatment of WS cells with anisomycin and p38 activation is detected using antibodies specific for the activated (phosphorylated) forms of HSP27 immobilized on Western blots. Cells were harvested and proteins isolated, separated on polyacrylamide gels and immunoblotted as described previously [8]. In addition, the samples were quantified using an ELISA system as described above.

Results & discussion

Following our microwave-mediated route **(FIGURE 2)**, two benzoylacetonitriles **1A** and **1B**, the former readily available from methyl 4-methoxybenzoate by reaction with acetonitrile under basic conditions [27], were reacted with *N,N′*–diphenylformamidine in a microwave-assisted Knoevenagel condensation reaction at

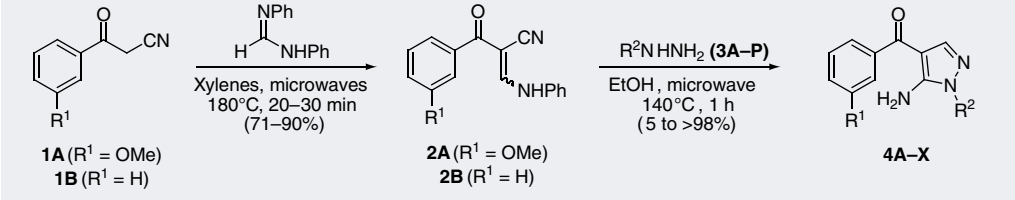

Figure 2. Microwave-assisted synthesis of pyrazolyl ketone library 4A–X.

180°C to give the two β-aminovinyl ketone precursors [28] **2A & B** in good yield after 20–30 min. Heterocyclocondensation was then effected, also under microwave dielectric heating, by irradiating **2A & B** with a range of hydrazines **3A–P** in ethanol at 140°C in the presence or absence of Et_3N (depending upon whether the hydrazine was obtained as the free base or HCl salt) to give a 24-membered pyrazolyl ketone library **4A–X (TABLE 1)**. The efficiency of this reaction proved variable, in particular when using benzoylacrylonitrile **2B**, although difficulties in isolation of the pure product could not be ruled out as the overriding factor, especially when considering the small scale. Gratifyingly, reactions of (3-methoxybenzoyl)acrylonitrile **2A** were much more predictable in terms of the isolated yield and all of the reactions investigated gave the desired product, following chromatographic purification on silica.

Biological evaluation of the library was carried out by testing the effects of preincubation of human HCA2 dermal fibroblasts with library members prior to induction of the p38 signaling pathway by treatment with anisomycin. The degree of inhibition was analyzed using an ELISA system (see methods) **(FIGURE 3)** at two different concentrations (150 nM and 1.5 μM) and the results compared with inhibition by the known pyrazolyl ketone RO3201195 (ROCHE). For the most part, pyrazolyl ketone library members inhibited the anisomycin-induced activation of the p38 pathway in human hTERT-immortalized HCA2 dermal cells. Of particular interest was the role of the *N*-aryl moiety R^2, identified as an important group in previous SAR studies using enzyme *in vitro* assays and lipopolysaccharide (LPS)-stimulated peripheral blood mononuclear cell-based assays [25]. Comparing the activity of **4C** (R^2 = 4-fluorophenyl) and **4H** (R^2 = phenyl) against anisomycin-induced p38 activation **(FIGURE 3)**, it was clear that the *para*-substituted fluorophenyl moiety was important – a finding supported by previous data [25]. Of the pyrazolyl ketones examined, a number of the library members displayed activity that compared favourably with, or even exceeded, that of RO3201195, including two *N*-(4-fluorophenyl)pyrazoles, **4B & C**, the *N*-(2,4-difluorophenyl)pyrazole **4F** and the *N*-methylpyrazole **4J**. RO3201195 contains an additional diol solubilizing group that, although it might cause some minor reduction in potency, enhances important physical properties including its bioavailability and pharmacokinetic profile and, so, the discovery of more potent inhibitors, while welcome, was not altogether surprising.

From our library, two of the compounds, **4C & H**, had been evaluated previously [25] for enzyme inhibition of p38 MAPK and on assay for inhibition of TNFα biosynthesis in THP-1 cells and the same activity order for these inhibitors was observed in our system. In accordance with previous findings, as the size of the *N*-aryl group increased, from 4-fluorophenyl **(4B & C)** to 4-chlorophenyl **(4K & L)**, 4-bromophenyl **(4D)** and 4-iodophenyl **(4A)**, the activity sharply declined. This phenomenon was also felt to be responsible for the poor activity of dichloride **4E**, pentafluoride **4G** and tolyl **4I**. It was unexpected that the inhibitor bearing a *N*-methyl group **4J** would be the most potent, but it could be anticipated that such a gain would be outweighed by a dramatic reduction in kinase selectivity (not tested). What was most gratifying was that, for the most part, the incorporation of a 3-methoxybenzoyl moiety caused no loss in potency with respect to the unsubstituted analogue (compare **4B & C** and **4K & L**), providing means for subsequent introduction of a

Table 1. Efficiency of the microwave-assisted synthesis of pyrazolyl ketones 4A–X.

Entry	2	R^1	3	R^2	4	Yield (%)*
1	**2A**	MeO	**3A**	4-iodophenyl	**4A**	75
2	**2A**	MeO	**3B**‡	4-fluorophenyl	**4B**	86
3	**2B**	H	**3B**‡	4-fluorophenyl	**4C**	76
4	**2B**	H	**3C**‡	4-bromophenyl	**4D**	63
5	**2B**	H	**3D**‡	2,6-dichlorophenyl	**4E**	84
6	**2B**	H	**3E**‡	2,4-difluorophenyl	**4F**	64
7	**2A**	MeO	**3F**	Pentafluorophenyl	**4G**	78
8	**2B**	H	**3G**	Phenyl	**4H**	95
9	**2A**	MeO	**3H**‡	4-tolyl	**4I**	58
10	**2A**	MeO	**3I**	Me	**4J**	46
11	**2B**	H	**3J**‡	4-chlorophenyl	**4K**	39
12	**2A**	MeO	**3J**‡	4-chlorophenyl	**4L**	57
13	**2A**	MeO	**3E**‡	2,4-difluorophenyl	**4M**	>98
14	**2A**	MeO	**3K**‡	2-fluorophenyl	**4N**	90
15	**2A**	MeO	**3L**	2,5-dichlorophenyl	**4O**	78
16	**2A**	MeO	**3D**‡	2,6-dichlorophenyl	**4P**	45
17	**2A**	MeO	**3C**‡	4-bromophenyl	**4Q**	92
18	**2B**	H	**3F**	pentafluorophenyl	**4R**	89
19	**2B**	H	**3K**‡	2-fluorophenyl	**4S**	8
20	**2B**	H	**3M**	2,5-dichlorophenyl	**4T**	95
21	**2B**	H	**3A**	4-iodophenyl	**4U**	5
22	**2A**	MeO	**3N**‡	4-methoxyphenyl	**4V**	71
23	**2A**	MeO	**3O**‡	*t*-butyl	**4W**	82
24	**2A**	MeO	**3P**	4-nitrophenyl	**4X**	41

*Refers to isolated yield after purification by column chromatography.
‡Et_3N (1.1 equivalent) was added to the reaction mixture in order to liberate the hydrazine free base.

solubilizing group. It was also unexpected that the 2,4-difluorophenyl-substituted analogue **4F** would have an improved activity profile over the 4-fluorophenyl compound **4C**. Although this phenomenon has been observed before in the enzyme activity of two similarly substituted pyrazolyl ketones [25], it apparently has not been pursued further.

The most effective of the pyrazolyl ketones in HCA2 cells were tested for their efficacy to inhibit p38 in WS cells by immunoblot detection of activated HSP27 **(FIGURE 4)**. In control WS cells, there was a low level of pHSP27 that was greatly increased by anisomycin treatment. The most effective inhibitors in HCA2 cells were equally as effective as inhibitors in WS cells, with difluoride **4F** again being the most effective and **4E** used as a negative control, as it showed little inhibition in HCA2 cells. As these are different cell systems (HCA2 are neonatal foreskin dermal cells while WS cells are adult dermal skin cells) that have different growth and morphological characteristics [9,10], and two different assay systems have been used, these data indicate a high degree of biological reproducibility. Interestingly, however, RO3201195 appears to exhibit a slight increase in potency in WS cells than in HCA2 cells.

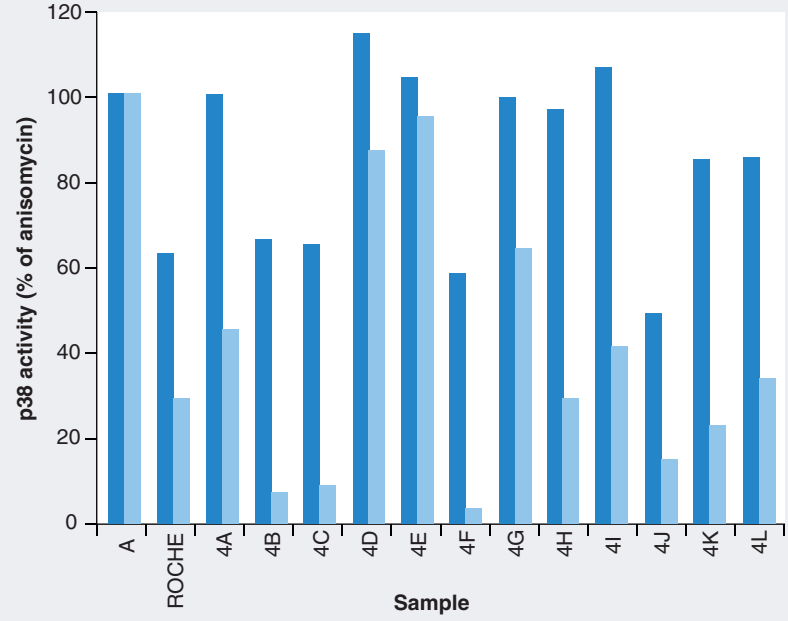

Figure 3. Inhibition of p38 MAPK by pyrazolyl ketones 4A–L and comparison with RO3201195 as shown by ELISA. Only the ratio of phosphorylated HSP27 to total HSP27 is indicated. A are cells treated with anisomycin, ROCHE are cells pretreated for 2 h with RO3201195 followed by treatment with anisomycin and **4A–L** are cells pretreated for 2 h with compounds **4A–L** followed by treatment with anisomycin. Dark bars are pyrazolyl ketones at 150 nM and lighter bars are pyrazolyl ketones at 1.5 μM.

Conclusion

In conclusion, a library of pyrazolyl ketones can be prepared rapidly in essentially a two-step process using microwave dielectric heating for evaluation as inhibitors of p38 MAPK signal transduction in WS cells. On biological evaluation, a number of inhibitors demonstrated similar, if not improved, potency in cell-based ELISA assays over RO2101195. Some SAR, in particular regarding the pyrazole *N*-aryl moiety, have been confirmed and promising candidates were evaluated further in WS cells, where a slight increase in activity may well be evident for RO3201195. Given the ease and speed of formation of these pyrazolyl ketones using microwave dielectric heating and their activity in WS cells, this library would appear to be ideal for further study to rescue premature senescence and the accelerated aging of WS cells in culture and correlate these observations with p38 activity. These studies are now underway in our laboratories and will be reported in due course.

Future perspective

Microwave dielectric heating has found widespread acceptance as an important means to accelerate chemical reactions. Nowhere has this been more prolific than in medicinal chemistry, where safe, effective, rapid, reliable and efficient processes are vital in order to accelerate and facilitate the drug-discovery process. There are many reaction types

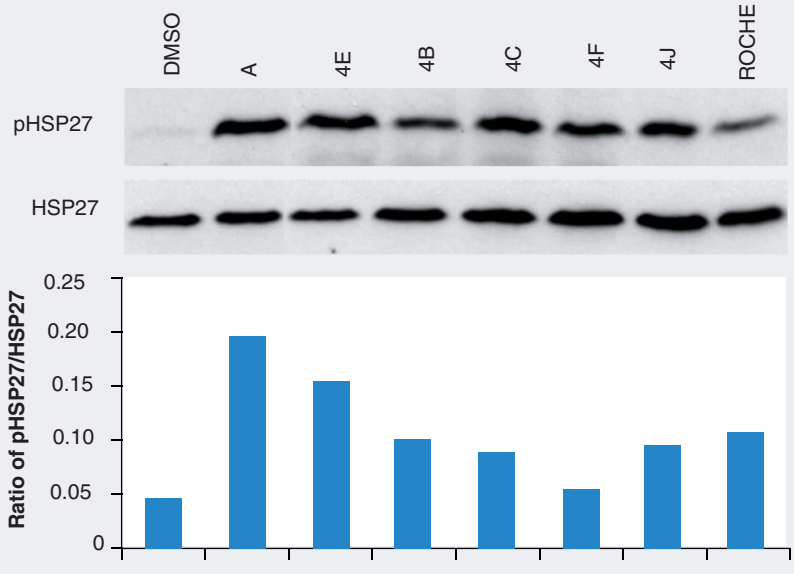

Figure 4. Effects of selected pyrazolyl ketones on the activity of p38 in WS cells. Top panels are immunoblots probed with antibodies for pHSP27 and HSP27. Bottom panel is an ELISA showing the ratio of pHSP27/HSP27 using the same samples for quantification.

that are highly suitable for investigation under microwave irradiation: transition metal-mediated processes, S_NAr reactions and heterocycle-forming processes, typified by the heterocyclocondensation reaction for pyrazole formation described in this report, being just a select few. The facility to operate under sealed-tube conditions above the boiling point of the solvent can simplify and accelerate chemical transformations and provides the means to carry out the synthesis of chemical libraries in an incredibly short time, especially when using an automated system. It is our expectation that the coming 5–10 years will see many more reports on the use and development of this technology, establishing its place as the principal synthetic tool of the medicinal chemist.

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Ethical conduct of research

The authors state that they have obtained appropriate institutional review board approval or have followed the principles outlined in the Declaration of Helsinki for all human or animal experimental investigations. In addition, for investigations involving human subjects, informed consent has been obtained from the participants involved.

Financial & competing interests disclosure

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Executive summary

- Microwave irradiation promotes both Knoevenagel condensation and heterocyclocondensation in the rapid synthesis of a 24-membered pyrazolyl ketone library.
- For the most part, pyrazolyl ketones inhibited the anisomycin-induced activation of the p38 pathway in human hTERT-immortalized HCA2 dermal cells.
- Structure–activity relationships indicated that large *N*-aryl groups were not tolerated by the p38 enzyme, in agreement with known data, whereas the addition of a 3-methoxy substituent in the benzoyl group caused no appreciable loss in activity.
- Four pyrazolyl ketones displayed activity, which compared favorably with, or even exceeded, that of RO3201195, with particular promise shown by a *N*-(2,4-difluorophenyl) analogue.
- The potency of promising pyrazolyl ketones was confirmed in WS cells, where a slight increase in activity may well be evident for RO3201195.

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