Microwave-assisted synthesis of a pyrazolyl ketone library for evaluation as p38 MAPK inhibitors in Werner syndrome cells

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Microwave-assisted synthesis of a pyrazolyl ketone library for evaluation as p38 MAPK inhibitors in Werner syndrome cells

**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.

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 aluminium-backed plates coated with Merck thin layer chromatography was carried out using Kieselgel 60 H silica or Matrex silica 60. Analytical chromatography was carried out using Merck fractions with boiling point (bp) 40–60°C. Flash chromatography was carried out using Merck commercial reagents were used with.

Melting points were determined on a Kofer 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; values were recorded in Hz, unless 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 ([Cl], 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$_2$O. The precipitate was filtered and washed with Et$_2$O 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$_2$O) (found: MH$, 279.1129$. C$_{17}$H$_{15}$N$_2$O$_3$ [MH$^+$] requires 279.1128; IR (KBr) $\nu_{max}$ 3063, 3012, 2961, 2924, 1637, 1598, 1574, 1489, 1372, 1316, 1270, 1226, 1045, 991, 869, 832, 742, 683; $^1$H NMR (400 MHz, CDCl$_3$) $\delta$ 7.25 (H, d, J 13.0, NH), 8.06 (H, d, 13.0 Hz), 7.55 (H, d, J 7.4), 7.47–7.36 (4H), 7.28 (H, t, J 7.2), 7.21 (2H, d, J 8.0), 7.09 (H, d, J 8.4), 3.86 (3H, s), $^1^3$C NMR (100 MHz, CDCl$_3$) $\delta$ 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$, 90\%)$.
Using microwaves for the rapid synthesis of a pyrazolyl ketone library

A mixture of 3-methoxy-2-benzoyl-3-phenylaminocarboxylic acid (2A) or 2-benzoyl-3-phenylaminocarboxylic acid (2B) ([Rf = 0.108 mmol, 1 equivalent] and the hydrazine [Rf = 0.108 mmol, 1 equivalent], in the presence or absence of Et$_3$N (0.12 mmol, 1.1 equivalent), in ethanol (1.23 ml) was irradiated in a sealed tube at 140°C (measured using an 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-fluorophenyl)-1H-pyrazol-4-yl] 3-methoxyphenyl ketone (4C) ([Rf = 0.07] (light petroleum:EtOAc, 3:2)] was obtained as a colorless solid (17.2 mg, 76%) [5] mp 140°C (light petroleum) (found: MH$, ^+ 281.0957$; C$_{16}$H$_{13}$NO$_2$F [MH$^+$] requires 281.0964); IR (KBr) $\nu$$_{max}$ 3369, 3242, 3171, 1612, 1537, 1493, 1304, 1121, 1059, 875, 842, 822, 754, 733, 689, 590; $^1$H NMR (400 MHz, CDCl$_3$) $\delta$ 7.84–7.80 (2H, m), 7.78 (H, m, 3'-H), 7.64–7.60 (2H, m), 6.10 (2H, br s, NH$_2$), 3.87 (3H, s, OMe) ; $^1$C NMR (100 MHz, CDCl$_3$) $\delta$ 189.5 (C), 150.4 (C), 142.1 (CH), 139.3 (C), 133.2 (m, 3'-C), 131.6 (CH), 128.6 (CH), 128.2 (CH), 126.2 (d, $J$$\text{C}$,Br$\text{C}$ = 6.4, CH), 116.9 (d, $J$$\text{C}$,Br$\text{C}$ = 23.1, CH), 104.8 (C); m/z (EI) 204 (MH$^+$, 30%), 280 (100).

[5-amino-1-(4-bromophenyl)-1H-pyrazol-4-yl] phenyl ketone (4D) ([Rf = 0.07] (light petroleum:EtOAc, 3:2)] was obtained as a colorless solid (7.3 mg, 63%), mp 147–150°C (light petroleum) (found: MH$, ^+ 281.0957$; C$_{16}$H$_{13}$NO$_2$Br [MH$^+$] requires 282.0964); IR (KBr) $\nu$$_{max}$ 3369, 3242, 3171, 1612, 1537, 1493, 1304, 1121, 1059, 875, 842, 822, 754, 733, 689, 590; $^1$H NMR (400 MHz, CDCl$_3$) $\delta$ 7.84–7.80 (2H, m), 7.78 (H, m, 3'-H), 7.64–7.60 (2H, m), 6.10 (2H, br s, NH$_2$), 3.87 (3H, s, OMe) ; $^1$C NMR (100 MHz, CDCl$_3$) $\delta$ 189.8 (C), 150.5 (C), 142.1 (CH), 139.7 (C), 133.2 (d, $J$$\text{C}$,Br$\text{C}$ = 3.3, C), 131.6 (CH), 128.8 (CH), 128.2 (CH), 126.2 (d, $J$$\text{C}$,Br$\text{C}$ = 8.4, CH), 116.9 (d, $J$$\text{C}$,Br$\text{C}$ = 23.1, CH), 104.8 (C); m/z (EI) 204 (MH$^+$, 30%), 280 (100).
[5-amino-1-(2,6-dichlorophenyl)-1H-pyr- azol-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\(_{23}H\(_{16}\)N\(_2\)O\(_8\)Cl\(_2\) [MH\(^+\)] requires 332.0357); IR (KBr): \( \nu_{3393}, 3257, 3169, 1622, 1568, 1539, 1507, 1446, 1388, 1309, 1274, 1213, 1161, 1075, 899, 788, 743, 700, 674, 540, 524; \( \delta \) (H NMR (400 MHz, CDCl\(_3\)) \( \delta 7.90 \) (1H, s, 3\'-CH), 7.87–7.84 (2H, m), 7.56–7.49 (5H), 7.46–7.41 (1H, m), 5.86 (2H, br s, NH\(_2\)); \( ^{13}\)C NMR (100 MHz, CDCl\(_3\)) \( \delta 189.7 \) (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\(_{13}H\(_{16}\)N\(_2\)O\(_5\)Cl\(_2\), 70), 332 (C\(_{12}H\(_{12}\)N\(_2\)O\(_4\)Cl\(_2\), 100).

[5-amino-1-(2,4-difluorophenyl)-1H-pyr- azol-4-yl] phenyl ketone (4F) \( [R_f = 0.5] \) (light petroleum:EtOAc, 3:2) was obtained as a colourless solid (15.4 mg, 64\%), mp 153°C (light petroleum) (found: MH\(^+\), 299.0865. C\(_{22}H\(_{16}\)N\(_2\)OF\(_2\) [MH\(^+\)] requires 299.0870); IR (KBr): \( \nu_{3392}, 3248, 3166, 2923, 1624, 1602, 1544, 1501, 1441, 1403, 1320, 1272, 1146, 1118, 1076, 974, 954, 903, 876, 853, 800, 759, 734, 701, 619, 565; \( \delta \) (H NMR (400 MHz, CDCl\(_3\)) \( \delta 7.85–7.80 \) (2H, m), 7.58–7.48 (4H), 7.04–7.11 (2H, m), 6.02 (2H, br s, NH\(_2\)); \( ^{13}\)C NMR (100 MHz, CDCl\(_3\)) \( \delta 189.6 \) (C), 163.0 (dd, \( J_{CF} \) 254.7, \( J_{CF} \) 10.3, C), 156.9 (dd, \( J_{CF} \) 255.4, \( J_{CF} \) 12.0, C), 151.8 (C), 142.8 (CH), 139.6 (C), 131.6 (CH), 129.9 (dd, \( J_{CF} \) 10.2, 1.4, CH), 128.6 (CH), 128.2 (CH), 120.9 (dd, \( J_{CF} \) 12.4, \( J_{CF} \) 4.0, C), 118.2 (dd, \( J_{CF} \) 22.6, \( J_{CF} \) 3.6, CH), 105.6 (dd, \( J_{CF} \) 27.1, 23.0, CH), 104.3 (C); \( m/z \) (EI) 298 (MH\(^+\), 100%), 222 (30).

[5-amino-1-(pentfluorophenyl)-1H-pyr- azol-4-yl] 3-methoxyphenyl ketone (4G) \( [R_f = 0.66] \) (light petroleum:EtOAc, 3:2) was obtained as a colourless solid (21.5 mg, 78\%), mp 180–181°C (light petroleum) (found: MH\(^+\), 308.1394. C\(_{24}H\(_{16}\)N\(_2\)O\(_2\) [MH\(^+\)] requires 308.1394); IR (KBr): \( \nu_{3391}, 3254, 2961, 2917, 2853, 1614, 1572, 1543, 1502, 1483, 1429, 1311, 1285, 1247, 1053, 935, 811, 769, 708; \( \delta \) (H NMR (400 MHz, CDCl\(_3\)) \( \delta 7.79 \) (1H, s, 3\'-CH), 7.45–7.40 (4H), 7.37–7.32 (3H), 7.11–7.08 (1H, 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\(_3\)) \( \delta 189.4 \) (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 (C), 21.2 (CH); \( m/z \) (ApCl) 308 (MH\(^+\), 100%).

[5-amino-1-(4-methyl-1H-pyr- azol-4-yl] 3-methoxyphenyl ketone (4J) \( [R_f = 0.6] \) (light petroleum:EtOAc, 3:2) was obtained as a colourless solid (32 mg, 58\%), mp 165°C (light petroleum) (found: MH\(^+\), 213.0899. C\(_{24}H\(_{16}\)N\(_2\)O\(_2\) [MH\(^+\)] requires 213.0902); IR (KBr): \( \nu_{3207}, 3178, 3158, 2995, 2917, 2884, 2840, 1602, 1583, 1506, 1471, 1458, 1432, 1288, 1257, 1211, 1096, 1053, 932, 874, 781, 714, 687; \( \delta \) (H NMR (400 MHz, CDCl\(_3\)) \( \delta 7.85 \) (1H, s, 3\'-CH), 7.55 (H, d, \( J_{8,8} \) 7.8, 5–H), 6.98–6.95 (1H, 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\(_3\)) \( \delta 189.6 \) (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), 39.8 (CH); \( m/z \) (EI) 213 (MH\(^+\), 100%)

[5-amino-1-(4-chlorophenyl)-1H-pyr- azol-4-yl] phenyl ketone (4K) \( [R_f = 0.54] \) (light petroleum:EtOAc, 3:2) was obtained as a colourless solid (9.4 mg, 39\%), mp 168°C (light petroleum) (found: MH\(^+\), 298.0732. C\(_{29}H\(_{16}\)N\(_2\)O\(_2\)Cl\(_2\) [MH\(^+\)] requires 298.0747); IR (KBr): \( \nu_{3397}, \)
3253, 3060, 2923, 1615, 1598, 1539, 1498, 1395, 1307, 1279, 1211, 1096, 1012, 903, 845, 823, 802, 741, 732, 700, 679, 523; \( ^1H \) NMR (400 MHz, CDCl\(_3\)) \( \delta \) 7.83–7.78 (3H), 7.59–7.48 (7H), 6.10 (2H, br s, NH\(_2\)); \(^1^3C\) NMR (100 MHz, CDCl\(_3\)) \( \delta \) 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\(_{10}H_{13}N_3O_3Cl^+\), 30%); 298 (C\(_{14}H_{15}N_2O_3Cl^+\), 100).

5-aminoo-1-(4-chlorophenyl)-1H-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); IR (KBr) \( \nu_{\text{max}} \) 3367, 3249, 3171, 2955, 2834, 1614, 1575, 1540, 1497, 1483, 1454, 1433, 1307, 1287, 1248, 1210, 1095, 1012, 912, 846, 811, 796, 706, 683, 613; \(^1H\) NMR (400 MHz, CDCl\(_3\)) \( \delta \) 7.80 (H, s, 3-H), 7.53–7.50 (4H), 7.42–7.40 (2H, m), 7.33 (H, s, 2-H), 7.12–7.07 (H, m, 4-H), 6.09 (2H, br s, NH\(_2\)), 3.87 (3H, s, CH\(_3\)), \(^1^3C\) NMR (100 MHz, CDCl\(_3\)) \( \delta \) 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\(_{16}H_{15}N_2O_3Cl^-\), 30%), 328 (C\(_{14}H_{15}N_2O_3Cl^+\), 100).

5-aminoo-1-(2,4-difluorophenyl)-1H-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\(_{14}H_{15}N_2O_3F_2\) [MH\(^+\)] requires 330.1049; IR (KBr) \( \nu_{\text{max}} \) 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\(_3\)) \( \delta \) 7.85 (H, s, 3-H), 7.58–7.51 (H, m), 7.42–7.40 (2H, m), 7.32 (H, m, 2-H), 7.11–7.04 (3H), 6.00 (2H, br s, NH\(_2\)), 3.88 (3H, s, CH\(_3\)); \(^1^3C\) NMR (100 MHz, CDCl\(_3\)) \( \delta \) 189.4 (C), 163.0 (dd, \( ^J_{CF} \) 253.5, \( ^J_{CC} \) 10.9, C), 159.7 (C), 157.0 (dd, \( ^J_{CF} \) 256.8, \( ^J_{CC} \) 13.1, C), 151.8 (C), 142.9 (CH), 140.9 (C), 129.9 (dd, \( ^J_{CF} \) 10.2, \( ^J_{CC} \) 1.4, CH), 129.1 (CH), 120.9 (dd, \( ^J_{CF} \) 12.0, \( ^J_{CC} \) 3.6, CH), 120.7 (CH), 118.0 (CH), 112.8 (dd, \( ^J_{CF} \) 23.0, \( ^J_{CC} \) 4.0, CH), 112.7 (CH), 105.6 (dd, \( ^J_{CF} \) 26.3, 23.0, CH), 104.3 (C), 55.5 (CH\(_3\)); m/z (ES) 364 (C\(_{16}H_{15}N_2O_3Cl^-\), 30%), 362 (C\(_{14}H_{15}N_2O_3Cl^+\), 50).

5-aminoo-1-(2-fluorophenyl)-1H-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\(_{15}H_{15}N_2O_3F\) [MH\(^+\)] requires 312.1143; IR (KBr) \( \nu_{\text{max}} \) 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\(_3\)) \( \delta \) 8.06 (H, s, 3-H), 7.48–7.40 (3H), 7.29–7.23 (H, m), 7.15–6.91 (2H, m), 6.90–6.65 (4H), 3.70 (3H, s, CH\(_3\)); \(^1^3C\) NMR (100 MHz, CDCl\(_3\)) \( \delta \) 189.6 (C), 159.6 (C), 156.4 (d, \( ^J_{CF} \) 254.0, C), 149.4 (C), 143.4 (CH), 135.8 (C), 131.4 (d, \( ^J_{CF} \) 7.7, CH), 130.2 (d, \( ^J_{CF} \) 7.6, CH), 128.7 (CH), 126.8 (d, \( ^J_{CF} \) 11.7, C), 125.1 (d, \( ^J_{CF} \) 4.0, CH), 120.7 (CH), 116.9 (d, \( ^J_{CF} \) = 19.4 Hz, CH), 116.4 (CH), 113.8 (CH), 93.2 (C), 55.3 (CH\(_3\)); m/z (ES) 312 (MH\(^+\), 100%).
a colorless solid (24.4 mg, 92%), mp 125–126°C (light petroleum) (found: MH^+ 372.0339). C_{16}H_{15}N_{4}O_{2}^{59}Br [MH^+] requires 372.0342); IR (KBr) v_max 3369, 3242, 3171, 2955, 1614, 1582, 1541, 1490, 1461, 1397, 1324, 1308, 1280, 1245, 1210, 1146, 1039, 937, 874, 845, 821, 799, 770, 732, 613, 544; ^1H NMR (400 MHz, CDCl_3) δ 7.82 (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 (H, m, 2-H), 7.12–7.07 (H, m, 4-H), 6.09 (2H, br s, NH), 3.88 (3H, s, CH_3); ^13C 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 (C), 122.1 (C), 120.7 (CH), 118.0 (CH), 112.8 (CH), 105.0 (C), 55.5 (CH_3); m/z (ES) 374 (C_{16}H_{15}N_{4}O_{2}^{59}Br^+, 40%), 372 (C_{16}H_{15}N_{4}O_{2}^{35}Br^+, 35), 105 (100).

[5-amino-1-(pentafluoroaryl)-1H-pyrazol-4-yl] phenyl ketone (4R) [R = 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_{12}H_{12}F_{5}N_{2}O [MH^+] requires 354.0666); IR (KBr) v_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; ^1H NMR (400 MHz, CDCl_3) δ 7.89 (H, s, 3’-H), 7.81–7.78 (2H, m), 7.60–7.55 (H, m), 7.54–7.48 (2H, m), 6.13 (2H, br s, NH); ^13C NMR (100 MHz, CDCl_3) δ 189.6 (C), 152.8 (C), 144.1 (app d, J_C,F = 2579, C), 144.0 (CH), 139.1 (C), 138.3 (app d, J_C,F = 2543, C), 138.2 (app d, J_C,F = 2598, 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-fluoroaryl)-1H-pyrazol-4-yl] phenyl ketone (4S) [R = 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_{13}H_{11}F_{2}N_{2}O [MH^+] requires 281.0964); IR (KBr) v_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; ^1H 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); ^13C NMR (100 MHz, CDCl_3) δ 189.6 (C), 156.4 (d, J_C,F = 2517, C), 151.7 (C), 142.8 (CH), 139.7 (C), 131.6 (CH), 131.1 (d, J_C,F = 8.1, CH), 128.6 (d, J_C,F = 10.2, CH), 128.5 (CH), 128.2 (CH), 125.5 (d, J_C,F = 3.7, CH), 124.2 (d, J_C,F = 12.1, CH), 117.2 (d, J_C,F = 19.4, CH), 104.4 (C); m/z (EI) 280 (MH^+, 100%).

[5-amino-1-(2,5-dichlorophenyl)-1H-pyrazol-4-yl] 3-phenyl ketone (4T) [R = 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_{12}H_{9}N_{2}O_{2}Cl [MH^+] requires 332.0357); IR (KBr) v_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; ^1H NMR (400 MHz, CDCl_3) δ 7.85–7.80 (3H), 7.59–7.56 (5H), 7.47–7.42 (H, m), 5.90 (2H, br s, NH); ^13C 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%).
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colorless solid (4.0 mg, 82%), mp 93°C (light petroleum) (found: MH+ 274.1549, C15H12N2O2 [MH]+ requires 274.1550); IR (KBr) νmmax 3386, 3292, 2982, 2923, 1598, 1576, 1530, 1496, 1459, 1317, 1288, 1247, 1128, 1044, 923, 834, 764, 685; 1H NMR (400 MHz, CDCl3) δ 7.57 (1H, s, 3´-H), 7.42–7.31 (2H, m), 7.30–7.27 (2H, m), 7.08–7.04 (1H, m, 4-H), 6.10 (2H, br s, NH), 3.85 (3H, s, OCH3), 1.67 (9H, s, CMe3); 13C NMR (100 MHz, CDCl3) δ 189.0 (C), 159.0 (C), 150.6 (C), 139.5 (C), 136.0 (C), 129.4 (CH), 120.7 (CH), 117.7 (CH), 112.6 (CH), 105.8 (C), 55.5 (CH3) (28.9 (CH3); m/z (ES) 274 (MH+ 100%), 259 (70), 218 (60).

3-methoxyphenyl ketone (4X) [R1 = 0.5 (light petroleum):EtOAc, 3:2] was obtained as a colorless solid (2.5 mg, 41%), mp 190–191°C (light petroleum:EtOAc, 3:2)

Determinatio n 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 transcriptase (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 IC50 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 RO32901195.

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 Et₃N (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 R², identified as an important group in previous SAR studies using enzyme in vitro assays and lipopolysaccharide (LPS)-stimulated peripheral blood mononuclear cell-based assay. Comparing the activity of 4C (R² = 4-fluorophenyl) and 4H (R² = 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. 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, pentafluorophenyl 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>
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<th>Table 1. Efficiency of the microwave-assisted synthesis of pyrazolyl ketones 4A–X.</th>
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*Refers to isolated yield after purification by column chromatography.

*Et₃N (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.

**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
Microwave irradiation promotes both Knoevenagel condensation and heterocyclocondensation in the rapid synthesis of a 24-membered structure–activity relationships indicated that large four pyrazolyl ketones displayed activity, which compared favorably with, or even exceeded, that of RO3201195, with particular promise. Ann. N. Y.

For the most part, pyrazolyl ketones inhibited the anisomycin-induced activation of the p38 pathway in human hTERT-immortalized 60A, 1386–1393 (2005).

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.

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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No writing assistance was utilized in the production of this manuscript.

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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