Supplementary data for article:

SUPPLEMENTARY DATA

Antiproliferative activity and QSAR studies of a series of new 4-aminomethylidene derivatives of some pyrazol-5-ones

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1. Experimental

1.1. Physical Measurements

Melting points were determined on a Mel-Temp capillary melting points apparatus, model 1001 and are uncorrected. Elemental (C, H, N, S) analysis of the samples was carried out by standard micromethods in the Center for Instrumental Analysis, Faculty of Chemistry, Belgrade. IR spectra were recorded on a Perkin Elmer Spectrum One FT-IR spectrometer with a KBr disc. All ^{1}H and ^{13}C NMR spectra were recorded on a Varian Gemini 200 spectrometer and Bruker Avance III 500 MHz spectrometer. The full assignments of all reported ^{1}H NMR signals were made by use of 1D and 2D NMR experiments such as COSY, HSQC and HMBC heteronuclear correlation techniques.

1.2. Spectral data for the synthesized compounds

1.2.1. **1a**: 0.37 g (75 %); light yellow powder; Mp=231–232°C. ^{1}H NMR (200 MHz, DMSO-d$_6$): δ = 1.36 (t, 3H, *J*=7.2 Hz, CH$_3$), 4.32 (q, 2H, *J*=7.2 Hz, CH$_2$), 7.49 (m, 3H, Ar–H), 7.67 (dd, 2H, *J*=7.8 and 1.6 Hz, Ar–H), 8.38 (s, 1H, Pz–H), 8.45 (s, 1H, CH=N), 11.62 (s, 1H, NH–C–O), 12.05 (s, 1H, OH), 13.36 (s, 1H, NH, Pz). ^{13}C NMR (50 MHz, DMSO-d$_6$): δ =14.38, 60.36, 100.84, 101.09, 127.21, 128.94, 129.06, 132.37, 134.26, 142.55, 147.81, 148.78, 162.44, 168.62. IR (KBr disc, cm^{-1}): 3422, 3314, 3110, 1710, 1679, 1626, 1574, 1295, 771. Anal. Calcd. for C$_{16}$H$_{15}$N$_5$O$_3$ (325.33 g/mol): C, 59.07; H, 4.65; N, 21.53; Found: C, 59.48; H, 4.75; N, 21.64.

1.2.2. **1b**: 0.27 g (56 %); light yellow powder; Mp=203–204°C. ^{1}H NMR (200 MHz, DMSO-d$_6$): δ = 6.63 (dd, 1H, *J*=3.2 and 1.8 Hz, Fur–H), 6.83 (d, 1H, *J*=3.2 Hz, Fur–H), 6.86 (d, 1H, *J*=3.2 Hz, Pz–H), 7.49 (m, 3H, Ar–H), 7.71 (dd, 2H, *J*=7.8 and 1.6 Hz, Ar–H), 7.80 (d, 1H, *J*=1.8 Hz, Fur–H), 8.45 (s, 1H, –CH=N), 11.59 (s, 1H, NH–C–O), 11.90 (bs, 1H, OH), 13.23 (s, 1H, NH, Pz). ^{13}C NMR (50 MHz, DMSO-d$_6$): δ = 90.95, 99.03, 107.55, 112.03, 127.34, 128.72, 128.95, 132.62, 135.64, 143.45, 144.37, 145.66, 148.73, 148.98, 168.77. IR (KBr disc, cm^{-1}): 3429, 3199, 3153, 1659, 1612, 1489, 1300, 1278, 955, 781. Anal. Calcd. for C$_{17}$H$_{13}$N$_5$O$_2$ (319.32 g/mol): C, 63.94; H, 4.10; N, 21.93; Found: C, 64.19; H, 4.16; N, 22.07.

1.2.3. **1c**: 0.21 g (52 %); light yellow powder; Mp=191–192°C (dec). ^{1}H NMR (200 MHz, DMSO-d$_6$): δ = 2.22 (s, 3H, CH$_3$), 6.25 (s, 1H, Pz–H), 7.48 (m, 3H, Ar–H), 7.68 (dd, 2H, *J*=7.8 and 1.6 Hz, Ar–H), 8.36 (s, 1H, –CH=N), 10.95 (bs, 1H, OH), 11.55 (s, 1H, NH–C–O), 12.39 (s, 1H, NH, Pz). ^{13}C NMR (50 MHz, DMSO-d$_6$): δ = 10.88, 93.35, 98.56, 127.28, 128.68, 128.96, 132.70, 140.76, 145.74, 148.31, 148.66, 168.76. IR (KBr disc, cm^{-1}): 3421, 3190, 3152, 1665,

1622, 1579, 1487, 1404, 1302, 753. Anal. Calcd. for $C_{14}H_{13}N_5O$ (267.29 g/mol): C, 62.91; H, 4.90; N, 26.20; Found: C, 63.24; H, 4.99; N, 26.44.

1.2.4. **1d**: 0.20 g (53 %); yellow powder; Mp=207–208°C (dec). ^{1}H NMR (200 MHz, DMSO-d_6): δ = 6.50 (d, 1H, *J*=2.4 Hz, Pz–H), 7.45 (m, 3H, Ar–H), 7.69 (dd, 2H, *J*=7.8 and 1.6 Hz, Ar–H), 7.74 (d, 1H, *J*=2.4 Hz, Pz–H), 8.43 (s, 1H, –CH=N), 11.40 (bs, 1H, OH), 11.57 (s, 1H, NH–C–O), 12.69 (bs, 1H, NH, Pz). ^{13}C NMR (50 MHz, DMSO-d_6): δ = 94.26, 98.71, 127.31, 128.71, 128.96, 130.97, 132.69, 145.67, 148.26, 148.74, 168.77. IR (KBr disc, cm^{-1}): 3415, 3306, 3189, 1673, 1614, 1596, 1399, 1385, 742. Anal. Calcd. for $C_{13}H_{11}N_5O$ (253.26 g/mol): C, 61.65; H, 4.38; N, 27.65; Found: C, 61.95; H, 4.43; N, 27.81.

1.2.5. **1e**: 0.38 g (76 %); light yellow powder; Mp=210–211°C (dec). ^{1}H NMR (200 MHz, DMSO-d_6): δ = 6.99 (s, 1H, Pz–H), 7.46 (m, 6H, Ar–H), 7.73 (m, 4H, Ar–H), 8.47 (s, 1H, –CH=N), 11.58 (bs, 1H, OH), 11.63 (s, 1H, NH–C–O), 13.21 (bs, 1H, NH, Pz). ^{13}C NMR (50 MHz, DMSO-d_6): δ = 91.70, 98.97, 124.95, 125.35, 127.39, 128.80, 128.83, 129.04, 129.26, 132.68, 144.22, 145.72, 148.80, 149.16, 168.88. IR (KBr disc, cm^{-1}): 3427, 3169, 1658, 1609, 1586, 1487, 1405, 1299, 1280, 954, 783. Anal. Calcd. for $C_{19}H_{15}N_5O$ (329.36 g/mol): C, 69.29; H, 4.59; N, 21.26; Found: C, 69.30; H, 4.59; N, 21.44.

1.2.6. **1f · H$_2$O**: 0.40 g (76 %); light yellow powder; Mp=137–138°C (dec). ^{1}H NMR (500 MHz, DMSO-d_6): δ = 3.08, (dd, 1H, J = 14.0 and 8.5 Hz, CH–C*H$_2$*), 3.29, (dd, 1H, J = 14.0 and 4.5 Hz, CH–C*H$_2$*), 4.71, (dd 1H, J = 8.5 and 4.5 Hz, C*H*–COO), 7.24–7.38 (m, 10H, Ar–H), 7.65 (s, 1H, –CH=N), 10.09 (bs, 1H, OH), 11.25 (s, 1H, NH–C–O), 12.79 (bs, 1H, COO*H*). ^{13}C NMR (125 MHz, DMSO-d_6): δ = 38.81, 61.43, 96.42, 126.91, 126.94, 128.24, 128.55, 128.63, 129.72, 132.75, 136.19, 148.25, 152.89, 168.61, 171.57. IR (KBr disc, cm^{-1}): 3413, 3352, 3152, 1710, 1659, 1590, 1507, 1400, 1306, 1283, 771. Anal. Calcd. for $C_{19}H_{19}N_3O_4$ (353.38 g/mol): C, 64.58; H, 5.42; N, 11.89; Found: C, 64.28; H, 5.60; N, 11.82.

1.2.7. **1g · H$_2$O**: 0.32 g (62 %); yellow powder; Mp=107–108°C (dec). ^{1}H NMR (500 MHz, DMSO-d_6): δ = 2.05, (s, 3H, CH$_3$); 2.07, (m, 1H, CH–C*H$_2$*); 2.15, (m, 1H, CH–C*H$_2$*); 2.50, (m, 2H, S–C*H$_2$*); 4.46, (dd, 1H, J = 8.0 and 5.0 Hz, C*H*–COO), 7.39 (t, 1H, *J*=7.0 Hz, Ar–H), 7.44 (t, 2H, *J*=7.0 Hz, Ar–H), 7.61 (dd, 2H, *J*=7.0 and 1.5 Hz, Ar–H), 8.10 (s, 1H, –CH=N), 10.23 (bs, 1H, OH), 11.30 (s, 1H, NH–C–O), 12.85 (bs, 1H, COO*H*). ^{13}C NMR (125 MHz, DMSO-d_6): δ = 14.54, 29.07, 32.20, 59.75, 96.68, 127.00, 128.25, 128.66, 132.91, 148.18, 153.33, 168.73, 172.02. IR (KBr disc, cm^{-1}): 3420, 3170, 1720, 1662, 1606, 1505, 1400, 1280, 769. Anal. Calcd. for $C_{15}H_{19}N_3O_4S$ (337.40 g/mol): C, 53.40; H, 5.68; N, 12.45; S, 9.50; Found: C, 53.27; H, 5.88; N, 12.41; S, 9.84.

1.2.8. **1h**: 0.20 g (44 %); light yellow powder; Mp=164–165°C (dec). ^{1}H NMR (500 MHz, DMSO-d_6): δ = 2.11, (s, 3H, CH_3), 3.03, (dd, 1H, J = 14.0 and 7.0 Hz, CH–CH_2), 3.08, (dd, 1H, J = 14.0 and 4.5 Hz, CH–CH_2), 4.66, (dd 1H, J = 7.0 and 4.5 Hz, CH–COO), 7.39 (t, 1H, *J*=7.0 Hz, Ar–H), 7.45 (t, 2H, *J*=7.0 Hz, Ar–H), 7.61 (d, 2H, *J*=7.0 Hz, Ar–H), 8.14 (s, 1H, –CH=N), 10.22 (bs, 1H, OH), 11.32 (s, 1H, NH–C–O), 12.84 (bs, 1H, COOH). ^{13}C NMR (125 MHz, DMSO-d_6): δ = 15.43, 37.06, 60.00, 96.73, 126.99, 128.32, 128.72, 132.89, 148.26, 153.43, 168.74, 171.02. IR (KBr disc, cm^{-1}): 3413, 3256, 1731, 1662, 1605, 1506, 1400, 1304, 771. Anal. Calcd. for $C_{14}H_{15}N_3O_3S$ (305.36 g/mol): C, 55.07; H, 4.95; N, 13.76; S, 10.50; Found: C, 54.85; H, 5.14; N, 13.62; S, 10.27.

1.2.9. **1i**: 0.18 g (44 %); yellow powder; Mp=178–179°C (dec). ^{1}H NMR (500 MHz, DMSO-d_6): δ = 3.79, (dd, 1H, J = 11.0 and 3.5 Hz, CH–CH_2), 3.84, (dd, 1H, J = 11.0 and 4.5 Hz, CH–CH_2), 4.47, (m, 1H, CH–COO), 5.45, (bs, 1H, CH_2–OH), 7.39 (t, 1H, *J*=7.0 Hz, Ar–H), 7.44 (t, 2H, *J*=7.0 Hz, Ar–H), 7.60 (d, 2H, *J*=7.0 Hz, Ar–H), 8.10 (s, 1H, –CH=N), 10.27 (bs, 1H, OH), 11.27 (s, 1H, NH–C–O), 12.89 (bs, 1H, COOH). ^{13}C NMR (125 MHz, DMSO-d_6): δ = 62.24, 62.31, 96.58, 126.96, 128.22, 128.72, 132.98, 148.19, 153.61, 168.69, 170.85. IR (KBr disc, cm^{-1}): 3421, 3226, 1715, 1661, 1616, 1603, 1400, 1255, 772. Anal. Calcd. for $C_{13}H_{13}N_3O_4$ (275.26 g/mol): C, 56.72; H, 4.76; N, 15.27; Found: C, 57.01; H, 4.67; N, 15.10.

1.2.10. **1j · H₂O**: 0.42 g (75 %); yellow powder; Mp=155–156°C (dec). ^{1}H NMR (500 MHz, DMSO-d_6): δ = 2.92, (dd, 1H, J = 13.5 and 9.0 Hz, CH–CH_2), 3.17, (dd, 1H, J = 13.5 and 4.5 Hz, CH–CH_2), 4.60, (dd 1H, J = 9.0 and 4.5 Hz, CH–COO), 6.72 (d, 2H, *J*=8.5 Hz, Ar–H), 7.02 (d, 2H, *J*=8.5 Hz, Ar–H), 7.41 (m, 3H, Ar–H), 7.53 (m, 2H, Ar–H), 7.55 (s, 1H, –CH=N), 9.38 (bs, 1H, Ar–OH), 10.09 (bs, 1H, OH), 11.22 (s, 1H, NH–C–O), 12.86 (bs, 1H, COOH). ^{13}C NMR (125 MHz, DMSO-d_6): δ = 38.45, 61.54, 96.27, 115.32, 126.91, 128.17, 128.63, 128.73, 130.75, 132.75, 148.25, 152.76, 156.44, 168.57, 171.64. IR (KBr disc, cm^{-1}): 3401, 3296, 3204, 1735, 1656, 1609, 1591, 1514, 1331, 1284, 776. Anal. Calcd. for $C_{19}H_{19}N_3O_5$ (369.38 g/mol): C, 61.78; H, 5.18; N, 11.38; Found: C, 61.48; H, 5.28; N, 11.16.

1.2.11. **1k**: 0.21 g (43 %); light yellow powder; Mp=232–233°C (dec). ^{1}H NMR (500 MHz, DMSO-d_6): δ = 3.04, (dd, 1H, J = 15.0 and 8.0 Hz, CH–CH_2), 3.18, (dd, 1H, J = 15.0 and 4.0 Hz, CH–CH_2), 4.63, (dd 1H, J = 8.0 and 4.0 Hz, CH–COO), 6.95, (s, 1H, His–H), 7.38, (m, 1H, NH, His), 7.43 (m, 5H, Ar–H), 7.70 (s, 1H, –CH=N), 7.78 (s, 1H, His–H), 10.17 (bs, 1H, OH), 11.21 (s, 1H, NH–C–O), 12.89 (bs, 1H, COOH). ^{13}C NMR (125 MHz, DMSO-d_6): δ = 31.14, 60.24, 96.22, 116.56, 126.85, 128.13, 128.62, 128.96, 132.86, 135.11, 148.15, 152.78, 168.51, 171.68. IR (KBr disc, cm^{-1}): 3235, 3151, 1711, 1670, 1635, 1591, 1350, 1300, 1273, 775. Anal. Calcd. for $C_{16}H_{15}N_5O_3$ (325.33 g/mol): C, 59.07; H, 4.65; N, 21.53; Found: C, 59.23; H, 4.55; N, 21.44.

1.2.12. **1l · H₂O**: 0.49 g (84 %); light yellow powder; Mp=163–164°C (dec). ^{1}H NMR (500 MHz, DMSO-d_6): δ = 3.19, (dd, 1H, J = 14.5 and 9.0 Hz, CH–CH_2), 3.43, (dd, 1H, J = 14.5 and 4.0 Hz, CH–CH_2), 4.66, (dd 1H, J = 9.0 and 4.0 Hz, CH–COO), 7.00 (t, 1H, *J* = 7.0 Hz, Ar–H),

7.13 (m, 3H, Ar–H), 7.20 (d, 1H, J = 2.0 Hz, Trp–H), 7.30 (m, 3H, Ar–H), 7.41 (s, 1H, –CH=N), 7.43 (m, 1H, Ar–H), 7.58 (d, 1H, J = 8.0 Hz, Ar–H), 10.22 (bs, 1H, OH), 11.05 (d, 1H, J = 2.0 Hz, N–H, Trp), 11.20 (s, 1H, NH–C–O), 12.77 (bs, 1H, COO*H*). ^{13}C NMR (125 MHz, DMSO-d_6): δ = 29.77, 60.83, 96.04, 108.45, 111.56, 118.41, 118.73, 121.23, 124.79, 126.71, 126.90, 128.00, 128.56, 132.77, 136.33, 148.14, 152.48, 168.55, 172.04. IR (KBr disc, cm^{-1}): 3399, 3220, 1723, 1657, 1595, 1503, 1340, 1284, 744. Anal. Calcd. for $C_{21}H_{20}N_4O_4$ (392.41 g/mol): C, 64.28; H, 5.14; N, 14.28; Found: C, 64.17; H, 5.08; N, 14.44.

1.2.13. **1m**: 0.45 g (74 %); yellow powder; Mp=244–245°C (dec). ^{1}H NMR (200 MHz, DMSO-d_6): δ = 1.37 (t, 3H, J=7.2 Hz, CH$_3$), 4.34 (q, 2H, J=7.2 Hz, CH$_2$), 7.21 (t, 1H, J=7.4 Hz, Ar–H), 7.53 (m, 5H, Ar–H), 7.79 (m, 2H, Ar–H), 8.07 (d, 2H, J=7.8 Hz, Ar–H), 8.41 (d, 1H, J=1.8 Hz, Pz–H), 8.54 (d, 1H, J=13.0 Hz, =CH–N), 12.14 (d, 1H, J=13.0 Hz, NH), 13.44 (s, 1H, NH, Pz). ^{13}C NMR (50 MHz, DMSO-d_6): δ = 14.37, 60.45, 101.17, 101.27, 118.38, 124.60, 127.67, 128.99, 129.16, 129.59, 131.43, 134.10, 138.84, 143.91, 147.62, 149.66, 162.58, 165.19. IR (KBr disc, cm^{-1}): 3421, 3377, 3234, 3156, 1672, 1614, 1595, 1490, 1340, 1283, 776. Anal. Calcd. for $C_{22}H_{19}N_5O_3$ (401.42 g/mol): C, 65.83; H, 4.77; N, 17.45; Found: C, 65.98; H, 4.82; N, 17.54.

1.2.14. **1n**: 0.42 g (71 %); light yellow powder; Mp=247–248°C (dec). ^{1}H NMR (200 MHz, DMSO-d_6): δ = 6.65 (dd, 1H, J=3.6 and 1.8 Hz, Fur–H), 6.85 (d, 1H, J=3.6 Hz, Fur–H), 6.87 (d, 1H, J=3.2 Hz, Pz–H), 7.21 (t, 1H, J=7.4 Hz, Ar–H), 7.52 (m, 5H, Ar–H), 7.81 (d, 1H, J=1.8 Hz, Fur–H), 7.83 (m, 2H, Ar–H), 8.10 (d, 2H, J=7.8 Hz, Ar–H), 8.54 (s, 1H, –CH=N), 11.94 (bs, 1H, OH), 13.31 (s, 1H, NH, Pz). ^{13}C NMR (50 MHz, DMSO-d_6): δ = 91.53, 99.54, 107.62, 112.02, 118.45, 124.55, 127.84, 128.99, 129.07, 129.39, 131.69, 135.57, 138.99, 143.49, 144.23, 146.58, 148.74, 149.71, 165.26. IR (KBr disc, cm^{-1}): 3436, 3250, 3117, 1677, 1620, 1485, 1399, 1327, 752. Anal. Calcd. for $C_{23}H_{17}N_5O_2$ (395.42 g/mol): C, 69.86; H, 4.33; N, 17.71; Found: C, 70.01; H, 4.40; N, 17.77.

1.2.15. **1o**: 0.31 g (61 %); light yellow crystals ; Mp=221–222°C (dec). ^{1}H NMR (200 MHz, DMSO-d_6): δ = 2.23 (s, 3H, CH$_3$), 6.29 (s, 1H, Pz–H), 7.19 (t, 1H, J=7.4 Hz, Ar–H), 7.50 (m, 5H, Ar–H), 7.79 (m, 2H, Ar–H), 8.09 (d, 2H, J=7.8 Hz, Ar–H), 8.45 (s, 1H, –CH=N), 11.83 (bs, 1H, OH), 12.47 (s, 1H, NH, Pz). ^{13}C NMR (50 MHz, DMSO-d_6): δ = 10.84, 93.95, 99.09, 118.42, 124.49, 127.77, 128.97, 129.07, 129.36, 131.77, 139.05, 140.78, 146.35, 148.05, 149.65, 165.28. IR (KBr disc, cm^{-1}): 3420, 3269, 1680, 1617, 1602, 1522, 1482, 1402, 1331, 754. Anal. Calcd. for $C_{20}H_{17}N_5O$ (343.39 g/mol): C, 69.96; H, 4.99; N, 20.39; Found: C, 70.14; H, 5.03; N, 20.65.

1.2.16. **1p**: 0.30 g (61 %); light yellow powder; Mp=214–215°C (dec). ^{1}H NMR (200 MHz, DMSO-d_6): δ = 6.55 (s, 1H, Pz–H), 7.19 (t, 1H, J=7.4 Hz, Ar–H), 7.51 (m, 5H, Ar–H), 7.79 (s, 1H, Pz–H), 7.80 (m, 2H, Ar–H), 8.09 (d, 2H, J=7.8 Hz, Ar–H), 8.52 (s, 1H, –CH=N), 11.94 (bs, 1H, OH), 12.79 (s, 1H, NH, Pz). ^{13}C NMR (50 MHz, DMSO-d_6): δ = 94.88, 99.24, 118.43,

124.51, 127.80, 128.99, 129.08, 129.37, 130.96, 131.76, 139.05, 146.59, 148.03, 149.71, 165.28. IR (KBr disc, cm^{-1}): 3435, 3232, 1656, 1629, 1597, 1481, 1401, 1344, 756. Anal. Calcd. for $C_{19}H_{15}N_5O$ (329.36 g/mol): C, 69.29; H, 4.59; N, 21.26; Found: C, 69.55; H, 4.63; N, 21.33.

1.2.17. **1q**: 0.44 g (73 %); light yellow powder; Mp=287–288°C (dec). ^{1}H NMR (200 MHz, DMSO-d$_6$): δ = 7.02 (s, 1H, Pz–H), 7.21 (t, 1H, *J*=7.4 Hz, Ar–H), 7.47 (m, 8H, Ar–H), 7.40 (d, 2H, *J*=7.0 Hz, Ar–H), 7.83 (m, 2H, Ar–H), 8.09 (d, 2H, *J*=7.8 Hz, Ar–H), 8.55 (s, 1H, –CH=N), 11.37 (bs, 1H, OH), 13.29 (s, 1H, NH, Pz). ^{13}C NMR (50 MHz, DMSO-d$_6$): δ = 92.25, 99.44, 118.52, 124.62, 125.35, 127.85, 128.86, 129.04, 129.13, 129.19, 129.25, 129.45, 131.73 139.02, 144.16, 146.64, 148.93, 149.71, 165.35. IR (KBr disc, cm^{-1}): 3434, 3212, 1672, 1623, 1598, 1488, 1481, 1403, 1333, 755. Anal. Calcd. for $C_{25}H_{19}N_5O$ (405.46 g/mol): C, 74.06; H, 4.72; N, 17.27; Found: C, 74.19; H, 4.81; N, 17.44.

1.2.18. **1r** and **1s**: 0.55 g for **1r** and 0.43 g for **1s** (89 % for 1r and 69 % for 1s); yellow powder; Mp=235–236°C (dec). ^{1}H NMR (500 MHz, DMSO-d$_6$): δ = 3.15, (dd, 1H, J = 14.0 and 8.5 Hz, CH–C*H*$_2$), 3.34, (dd, 1H, J = 14.0 and 4.5 Hz, CH–C*H*$_2$), 4.83, (ddd, 1H, J = 9.0, 8.5 and 4.5 Hz, C*H*–COO), 7.16 (t, 1H, *J*=7.5 Hz, Ar–H), 7.28 (m, 3H, Ar–H), 7.35 (t, 2H, *J*=7.5 Hz, Ar–H), 7.40–7.45, (m, 7H, Ar–H), 7.77, (d, 1H, J = 13.5 Hz, =CH–N), 8.04, (d, 2H, *J*=7.5 Hz, Ar–H), 10.21 (dd, 1H, *J* = 13.5 and 9.0 Hz, NH), 13.63 (bs, 1H, COO*H*). ^{13}C NMR (125 MHz, DMSO-d$_6$): δ = 38.73, 61.50, 96.92, 118.08, 124.05, 126.98, 127.34, 128.56, 128.74, 128.82, 128.94, 129.71, 131.69, 135.96, 139.09, 149.08, 153.97, 165.29, 171.29. IR (KBr disc, cm^{-1}): 3445, 1731, 1656, 1597, 1574,1494, 1353, 1258, 1188, 758, 698. Anal. Calcd. for $C_{25}H_{21}N_3O_3$ (411.46 g/mol): C, 72.98; H, 5.14; N, 10.21; Found: C, 72.96; H, 5.18; N, 10.29.

1.2.19. **1t**: 0.43 g (73 %); yellow powder; Mp=167–168°C (dec). ^{1}H NMR (500 MHz, DMSO-d$_6$): δ = 2.06, (s, 3H, CH$_3$); 2.18, (m, 2H, CH–C*H*$_2$); 2.54, (m, 2H, S–C*H*$_2$); 4.62, (ddd, 1H, J = 9.0, 8.0 and 3.5 Hz, C*H*–COO), 7.17 (t, 1H, *J*=7.5 Hz, Ar–H), 7.43 (t, 2H, *J*=7.5 Hz, Ar–H), 7.48–7.53, (m, 3H, Ar–H), 7.75 (dd, 2H, *J*=8.0 and 1.5 Hz, Ar–H), 8.07, (d, 2H, *J*=8.0 Hz, Ar–H), 8.26, (d, 1H, J = 13.5 Hz, =CH–N), 10.31 (dd, 1H, *J* = 13.5 and 9.0 Hz, NH), 13.48 (bs, 1H, COO*H*). ^{13}C NMR (125 MHz, DMSO-d$_6$): δ = 14.50, 29.01, 31.67, 59.75, 97.32, 118.14, 124.09, 127.50, 128.76, 128.80, 129.01, 131.87, 139.10, 149.10, 154.62, 165.44, 171.75. IR (KBr disc, cm^{-1}): 3436, 3285, 1731, 1655, 1598, 1572,1494, 1351, 1281, 758. Anal. Calcd. for $C_{21}H_{21}N_3O_3S$ (395.48 g/mol): C, 63.78; H, 5.35; N, 10.63; S, 8.11; Found: C, 64.02; H, 5.25; N, 10.79; S, 8.26.

1.2.20. **1u**: 0.37 g (64 %); yellow powder; Mp=149–150°C (dec). ^{1}H NMR (500 MHz, DMSO-d$_6$): δ = 2.13, (s, 3H, CH$_3$); 3.10, (dd, 1H, J = 14.0 and 7.0 Hz, CH–C*H*$_2$), 3.14, (dd, 1H, J = 14.0 and 4.5 Hz, CH–C*H*$_2$), 4.76, (m, 1H, C*H*–COO), 7.17 (t, 1H, *J*=7.0 Hz, Ar–H), 7.43 (t, 2H, *J*=8.0 Hz, Ar–H), 7.47–7.54, (m, 3H, Ar–H), 7.74 (d, 2H, *J*=7.0 Hz, Ar–H), 8.07, (d, 2H, *J*=8.0 Hz, Ar–H), 8.27, (d, 1H, J = 13.5 Hz, =CH–N), 10.37 (dd, 1H, *J* = 13.5 and 9.0 Hz, NH), 13.62 (bs, 1H, COO*H*). ^{13}C NMR (125 MHz, DMSO-d$_6$): δ = 15.36, 36.82, 60.07, 97.24, 118.13, 124.10, 127.46, 128.76, 128.85, 129.04, 131.86, 139.11, 149.11, 154.56, 165.44, 170.81. IR

(KBr disc, cm^{-1}): 3421, 3116, 1736, 1650, 1598, 1574,1496, 1349, 1183, 767. Anal. Calcd. for C$_{20}$H$_{19}$N$_3$O$_3$S (381.46 g/mol): C, 62.97; H, 5.02; N, 11.02; S, 8.41; Found: C, 63.05; H, 4.95; N, 10.98; S, 8.35.

1.2.21. **1v**: 0.32 g (61 %); yellow powder; Mp=106–107°C (dec). ^{1}H NMR (500 MHz, DMSO-d$_6$): δ = 3.83, (m, 1H, CH–C*H*$_2$), 3.88, (dd, 1H, J = 10.5 and 3.5 Hz, CH–C*H*$_2$), 4.56, (m, 1H, C*H*–COO), 5.45, (bs, 1H, CH$_2$–O*H*), 7.17 (t, 1H, *J*=7.5 Hz, Ar–H), 7.43 (t, 2H, *J*=7.5 Hz, Ar–H), 7.48 (m, 3H, Ar–H), 7.73 (d, 2H, *J*=7.0 Hz, Ar–H), 8.08, (d, 2H, *J*=8.0 Hz, Ar–H), 8.25, (d, 1H, J = 13.5 Hz, =CH–N), 10.39 (dd, 1H, *J* = 13.5 and 8.5 Hz, NH), 13.40 (bs, 1H, COO*H*). ^{13}C NMR (125 MHz, DMSO-d$_6$): δ = 62.18, 62.42, 97.14, 118.10, 124.03, 127.43, 128.76, 128.83, 128.99, 131.96, 139.20, 149.09, 154.73, 165.45, 170.64. IR (KBr disc, cm^{-1}): 3410, 3232, 1734, 1657, 1598, 1495, 1348, 758. Anal. Calcd. for C$_{19}$H$_{17}$N$_3$O$_4$ (351.23 g/mol): C, 64.95; H, 4.88; N, 11.96; Found: C, 65.25; H, 4.82; N, 11.69.

1.2.22. **1w**: 0.51 g (80 %); yellow powder; Mp=209–210°C (dec). ^{1}H NMR (500 MHz, DMSO-d$_6$): δ = 2.99, (dd, 1H, J = 14.0 and 9.0 Hz, CH–C*H*$_2$), 3.23, (dd, 1H, J = 14.0 and 4.0 Hz, CH–C*H*$_2$), 4.83, (ddd, 1H, J = 9.0, 9.0 and 4.0 Hz, C*H*–COO), 6.75, (d, 2H, *J*=8.5 Hz, Ar–H), 7.06 (d, 2H, *J*=8.5 Hz, Ar–H), 7.16 (t, 1H, *J*=7.5 Hz, Ar–H), 7.40–7.47 (m, 7H, Ar–H), 7.66, (d, 1H, J = 13.0 Hz, =CH–N), 8.05, (d, 2H, *J*=8.0 Hz, Ar–H), 9.41 (bs, 1H, OH, Tyr), 10.19, (dd, 1H, *J* = 13.0 and 9.0 Hz, NH), 13.61 (bs, 1H, COO*H*). ^{13}C NMR (125 MHz, DMSO-d$_6$): δ = 38.28, 61.75, 96.77, 115.37, 118.08, 124.03, 125.79, 127.36, 128.74, 128.78, 128.92, 130.82, 131.71, 139.13, 149.14, 153.80, 156.50, 165.30, 171.40. IR (KBr disc, cm^{-1}): 3517, 3420, 3204, 1732, 1655, 1596, 1516, 1494, 1351, 1175, 760. Anal. Calcd. for C$_{25}$H$_{21}$N$_3$O$_4$ (427.46 g/mol): C, 70.25; H, 4.95; N, 9.83; Found: C, 69.95; H, 5.01; N, 9.79;

1.2.23. **1x**: 0.42 g (70 %); yellow powder; Mp=214–215°C (dec). ^{1}H NMR (500 MHz, DMSO-d$_6$): δ = 3.12, (dd, 1H, J = 15.0 and 8.5 Hz, CH–C*H*$_2$), 3.23, (dd, 1H, J = 15.0 and 4.0 Hz, CH–C*H*$_2$), 4.73, (m, 1H, C*H*–COO), 7.02, (s, 1H, His–H), 7.16 (t, 1H, *J*=7.5 Hz, Ar–H), 7.42 (t, 2H, *J*=8.0 Hz, Ar–H), 7.46–7.52 (m, 4H; 3H, Ar–H and 1H, NH, His), 7.57, (d, 2H, *J*=7.0 Hz, Ar–H), 7.86, (m, 2H, =CH–N and 1h, His), 8.05, (d, 2H, *J*=8.0 Hz, Ar–H), 10.31, (m, 1H, NH), 13.54 (bs, 1H, COO*H*). ^{13}C NMR (125 MHz, DMSO-d$_6$): δ = 30.77, 60.47, 96.83, 116.43, 118.07, 123.97, 127.36, 128.72, 128.80, 128.91, 131.86, 132.70, 135.17, 139.18, 149.09, 153.93, 165.27, 171.44. IR (KBr disc, cm^{-1}): 3420, 3155, 1715, 1653, 1598, 1570,1499, 1347, 1333, 762. Anal. Calcd. for C$_{22}$H$_{19}$N$_5$O$_3$ (401.42 g/mol): C, 65.83; H, 4.77; N, 17.45; Found: C, 65.52; H, 4.80; N, 17.33;

1.2.24. **1y**: 0.50 g (74 %); yellow powder; Mp=234–235°C (dec). ^{1}H NMR (500 MHz, DMSO-d$_6$): δ = 3.28, (dd, 1H, J = 14.5 and 8.5 Hz, CH–C*H*$_2$), 3.45, (dd, 1H, J = 14.5 and 4.5 Hz, CH–C*H*$_2$), 4.79, (ddd 1H, J = 9.0, 8.5 and 4.0 Hz, C*H*–COO), 7.00 (t, 1H, *J* = 7.5 Hz, Ar–H), 7.12, (t, 1H, *J* = 7.5 Hz, Ar–H), 7.16, (t, 1H, *J* = 7.5 Hz, Ar–H), 7.25 (m, 3H; 2H, Ar–H and 1H, Trp–H), 7.35–7.43 (m, 6H; 5H, Ar–H and 1H, =CH–N), 7.58 (m, 2H, Ar–H), 8.04 (d, 2H, *J* = 7.5 Hz,

Ar–H), 10.28 (dd, 1H, J = 13.5 and 9.0 Hz, NH), 11.07 (s, 1H, N–H, Trp), 13.58 (bs, 1H, COOH). ^{13}C NMR (125 MHz, DMSO-d$_6$): δ = 29.37, 60.72, 96.70, 108.03, 111.56, 118.08, 118.38, 118.74, 121.27, 124.01, 124.91, 126.84, 127.17, 128.66, 128.72, 128.79, 131.69, 136.31, 139.13, 149.02, 153.70, 165.31, 171.74. IR (KBr disc, cm^{-1}): 3399, 3220, 1723, 1657, 1595, 1503, 1340, 1284, 744. Anal. Calcd. for $C_{27}H_{22}N_4O_3$ (450.50 g/mol): C, 71.99; H, 4.92; N, 12.44; Found: C, 71.77; H, 4.92; N, 12.30.

1.3. Treatment of tumor cell lines

Stock solutions (10mM) of compounds were made in dimethylsulfoxide (DMSO), and dissolved in corresponding medium to the required working concentrations. Human breast cancer MDA-MB-361 and MDA-MB-453 cells were cultured as a monolayer, while were grown in a suspension in the complete nutrient medium, at 37ºC in humidified air atmosphere with 5% CO_2. For the growth of MDA-MB-361 and MDA-MB-453 cells complete medium was enriched with 1.11 g/L glucose. Then, MDA-MB-361 cells (7000 cells per well) and MDA-MB-453 cells (3000 cells per well), were seeded into 96-well microtiter plates. Twenty-four hours later, after the cell adherence, five different, double diluted, concentrations of investigated compounds were added to the wells, except for the control cells to which a nutrient medium only was added. Nutrient medium was RPMI-1640, supplemented with L-glutamine (3 mM), streptomycin (100 lg/mL), and penicillin (100 IU/mL), 10% heat inactivated (56ºC) FBS and 25 mM Hepes, and the pH of the medium was adjusted to 7.2 by bicarbonate solution. The cultures were incubated for 72 h. At the end of this incubation period, antiproliferative activity *in vitro* was determined by the MTT test[1] modified by Ohno and Abe.[2] Results are presented as the mean ± SD of three independent experiments. IC$_{50}$ is used as the measure of the toxic agents action and is determined from the graph S(%)=f(c), as the concentration of the agent which induces decrease in cell survival to 50%.

1.4. QSAR analysis

The set of 25 compounds synthesized in this study (Scheme 1) was used for QSAR (quantitative structure-activity relationships) analysis. All structures were constructed using Spartan software.[3] Geometry optimization was performed by the AM1 semi-empirical method implemented in the Spartan software. Calculation of descriptors was performed using Codessa software (Comprehensive Descriptors for Structural and Statistical Analysis).[4] A total of 450 descriptors were calculated and divided into five groups: constitutional, topological, geometrical, electrostatic and quantum-chemical. The heuristic method (HM) implemented in Codessa software was used for the selection of the most significant descriptors for antiproliferative activity of investigated derivatives on MDA-MB-361 and MDA-MB-453 cancer cells. HM, as an advanced algorithm based on MLR, is suitable for preliminary studies of structural features important for activity of ligands, when mechanism of action of new compounds is not discovered yet. The advantage of HM is based on its unique strategy of selecting descriptors on the basis of

their statistical significance: a) first of all, all descriptors are checked to ensure that values of each descriptor are available for each structure, otherwise descriptors for which values are not available in data set are discarded; b) descriptors having constant value for all structures in the data set are also discarded; c) thereafter all possible one-parameter regression models are tested and insignificant descriptors are removed; d) the program calculates the pair correlation matrix of descriptors and further reduces the descriptor pool by eliminating highly correlated descriptors. Therefore, HM represents an excellent tool for descriptor selection in preliminary analysis of structural features affecting the activity of drugs.[5] For the purpose of heuristic analysis in this work, whole set of 25 compounds was divided in two subsets: subset 1, composed of compounds without substituent at position 1 (**1a-1l**), and subset 2, containing compounds with phenyl ring at position 1 of pyrazol-5-one (**1m-1y**). We first performed one-parameter heuristic analysis in order to investigate the structural features that are most significant for antiproliferative activity of compounds synthesized. Heuristic analysis of the most significant descriptors was applied on whole set, subset 1 and subset 2, regarding to activity on MDA-MB-361 and MDA-MB-453 cancer cells.

1.5. Flow cytometry analysis

Cellular DNA content and cell distribution were quantified by flow cytometry using propidium iodide (PI). Cells (3×10^5 cells/well) were seeded in 6-well plates and incubated with or without $2xIC_{50}$ concentration of investigated compounds for 24 h. After treatment, the cells were collected by trypsinization, and fixed in ice-cold 70% ethanol at -20ºC overnight. After fixation, the cells were washed in PBS and pellets obtained by centrifugation was treated with RNase (100 lg/mL) at 37ºC temperature for 30 min and then incubated with propidium iodide (PI) (40 lg/mL) for at least 30 min. DNA content and cell cycle distribution were analyzed using a Becton Dickinson FAC-Scan flow cytometer. Flow cytometry analysis was performed using a CellQuestR (Becton Dickinson, San Jose, CA, USA), on a minimum of 10,000 cells per sample.[6]

1.6. Tube formation assay (in vitro angiogenesis assay)

Potential of investigated pyrazolones to inhibit angiogenesis in vitro was analyzed by tube formation assay in mouse endothelial cells (MS1-murineendothelialcells immortalized by infection encoding SV40 large T antigen). MS1 cells plated into gel of basement membrane proteins, rapidly organize into multicellular tube-like structures (R), while anti-angigenic effect of tested compounds is observed as reduction of tube formation.[7] Briefly, 24-well plates were coated with collagen and allowed to solidify at 37°C 1 h.

MS1 cells were seeded 1x105 cells/well in medium. 2 h after cells settled treatment was added. Compounds were applied in sub-toxic concentration being 30 μM (for group of compounds **1c, 1d, 1g, 1h, 1i, 1k, 1l, 1x**), 10 μM (for group of compounds **1b, 1e, 1f, 1j, 1p, 1y, 1v**) and 3 μM (for group of compounds **1r, 1s, 1m, 1o, 1n, 1q, 1a, 1w, 1t, 1u**), separated according to their cytotoxic potential. Tube formation was observed periodically over the time under microscope and representative pictures were taken after 12 h incubation**.**

2. Antiproliferative activity

Fig. S1. and Fig. S2. depict the cytotoxic curves from MTT assay showing the survival of MDA-MB-361 and MDA-MB-453 cell grown for 72 h in the presence of increasing concentrations of **1o** and **1q**, respectively.
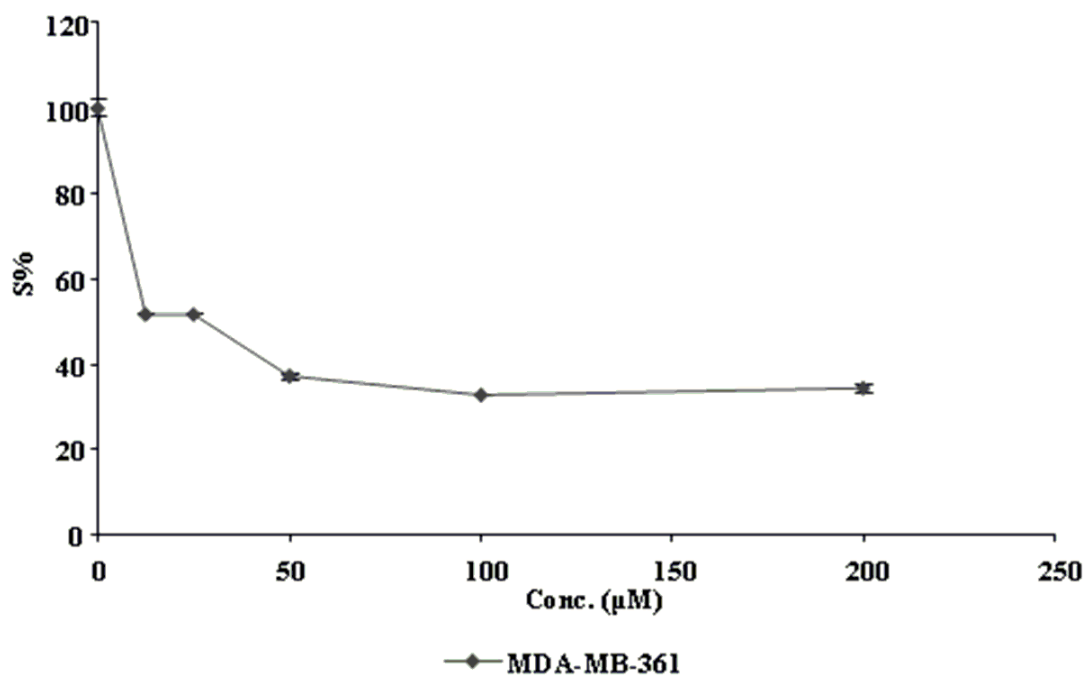

Fig. S1. Representative graph shows survival of MDA-MB-361 cell grown for 72 h in the presence of increasing concentrations of **1o**.
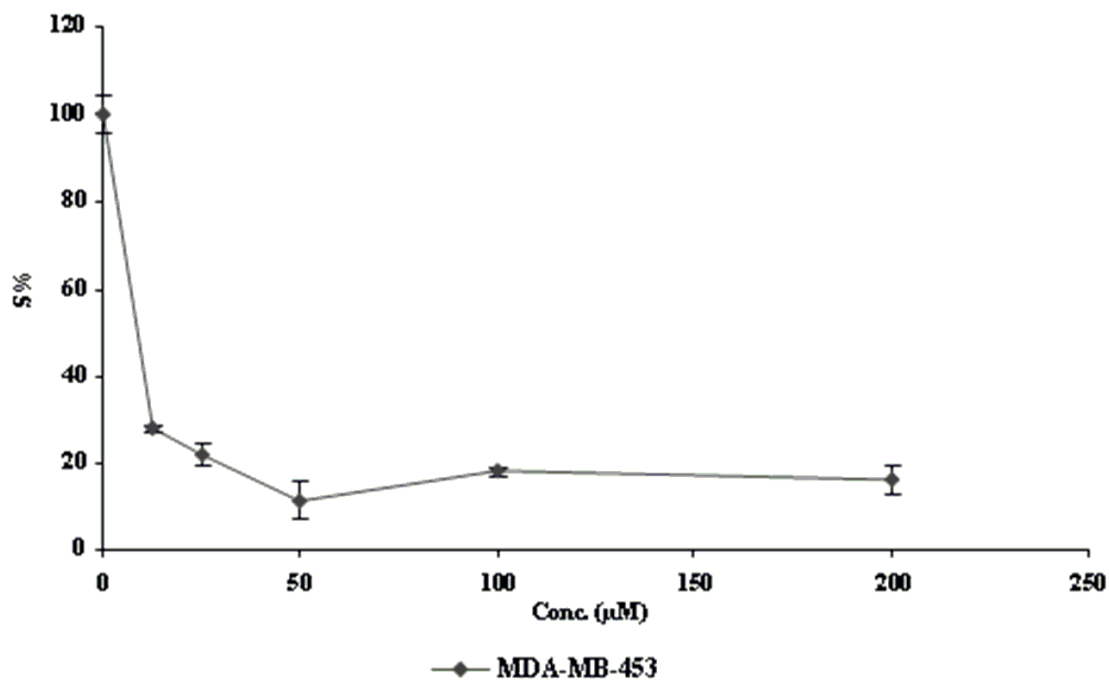
Fig. S2. Representative graph shows survival of MDA-MB-453 cell grown for 72 h in the presence of increasing concentrations of **1q**.

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