Electronic Supplementary Information

Reactions of salicylaldehydes with activated terminal alkynes in aqueous media: Synthesis of 3-substituted 4-hydroxy chromenes as potential cytotoxic agents

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**General methods:** Unless stated otherwise, solvents and chemicals were obtained from commercial sources and were used without further purification. Reactions were monitored by thin layer chromatography (TLC) on silica gel plates (60 F254), visualizing with ultraviolet light or iodine spray. Flash chromatography was performed on silica gel (230-400 mesh) using hexane and ethyl acetate. $^1$H NMR and $^{13}$C NMR spectra were determined in DMSO-$d_6$ solution by using a 400 MHz spectrometer. Proton chemical shifts (δ) are relative to tetramethylsilane (TMS, δ = 0.00) as internal standard and expressed in ppm. Spin multiplicities are given as s (singlet), d (doublet), t (triplet) and m (multiplet) as well as b (broad). Coupling constants (J) are given in hertz. Infrared spectra were recorded on a FT-IR spectrometer. Melting points were determined using melting point B-540 apparatus and are uncorrected. HRMS was determined using waters LCT premier XETOF ARE-047 apparatus.

**General method for the preparation of 3:** To a mixture of salicyaldehyde 1 (1.0 mmol), and alkyne 2 (1.0 mmol) in 1:1 aqueous 1,4-dioxane (2 mL) [1,4-dioxane (1.0 mL) + water (1.0 mL)] was added DABCO (0.5 equiv) at room temperature and the mixture was stirred at 25-35 °C for 8-12 h. After completion of the reaction the mixture was diluted with cold water (10 mL) and extracted with ethyl acetate (3 x 20 mL). The organic layers were collected, combined, washed with cold water (2 x 10 mL), dried over anhydrous Na$_2$SO$_4$, filtered and concentrated under reduced pressure. The crude mass obtained was purified by column chromatography on silica gel using 30% hexane-ethyl acetate to give the desired compound.

**Ethyl 4-hydroxy-4$H$-chromene-3-carboxylate (3a)**

![Ethyl 4-hydroxy-4$H$-chromene-3-carboxylate (3a)](image)

Off white solid; Yield: 85%; mp: 131-133 °C; $^1$H NMR (400 MHz, DMSO-$d_6$): δ 7.69 (s, 1H, H-C=C-), 7.52 (d, J = 6.4 Hz, 1H, ArH), 7.39 -7.37 (m, 2H, ArH), 7.06 - 7.01 (m, 2H, ArH + OH), 6.15 (d, J = 6.8 Hz, 1H, CHO), 4.25 (q, J = 7.2 Hz, 2H, CH$_2$), 1.28 (t, J = 7.2 Hz, 3H, Me); $^{13}$C NMR (100 MHz, DMSO-$d_6$): δ 164.2 (C=O), 152.1, 132.6, 131.8, 129.2, 123.1, 121.5, 119.3, 116.7, 87.9, 60.4 (OCH$_2$), 14.1 (Me); IR (KBr): 3672, 3394, 2693, 1692 (C=O), 1645, 1457,
1378, 1299, 1048, 967 cm\(^{-1}\); m/z (ES): 243.06 (M+Na, 100 %); HRMS: m/z [M + Na] calcd for C\(_{12}\)H\(_{12}\)O\(_4\)Na: 243.0633; found: 243.0613.

**Ethyl 4-hydroxy-6-methoxy-4H-chromene-3-carboxylate (3b)**

Yellow solid; Yield: 84%; mp: 118-120 °C; \(^1\)H NMR (400 MHz, DMSO-\(d_6\)): \(\delta\) 7.66 (s, 1H, H-C=C-), 7.24 (d, \(J= 6.4\) Hz, 1H, ArH), 7.14 (s, 1H, ArH), 6.96-6.95 (m, 2H, ArH + OH), 6.10 (d, \(J= 6.4\) Hz, 1H, CHOH), 4.25 (q, \(J= 6.8\) Hz, 2H, OCH\(_2\)), 3.73 (s, 3H, OMe), 1.29 (t, \(J= 7.6\) Hz, 3H, Me); \(^{13}\)C NMR (100 MHz, DMSO-\(d_6\)): \(\delta\) 164.2 (C=O), 153.6, 146.0, 132.7, 123.5, 119.6, 118.3, 117.5, 112.7, 87.8, 60.3 (OCH\(_2\)), 55.5 (OMe), 14.1 (Me); IR (KBr): 3567, 3393, 2972, 1678 (C=O), 1638, 1581, 1493, 1461, 1371, 1237, 1034, 978 cm\(^{-1}\); m/z(ES): 233.08 (M-OH, 100 %); HRMS: m/z [M -OH] calcd for C\(_{13}\)H\(_{13}\)O\(_4\): 233.0814; found: 233.0820.

**Ethyl 4-hydroxy-8-methoxy-4H-chromene-3-carboxylate (3c)**

Light pink solid; Yield: 72%; mp: 153-155 °C; \(^1\)H NMR (400 MHz, DMSO-\(d_6\)): \(\delta\) 7.66 (s, 1H, H-C=C-), 7.39 (d, \(J= 6.4\) , 1H, ArH), 7.11-7.08 (m, 2H, ArH + OH), 6.99-6.95 (m, 1H, ArH), 6.18 (d, \(J= 6.4\) , 1H, CHOH), 4.25 (q, \(J= 6.8\) Hz, 2H, OCH\(_2\)), 3.80 (s, 3H, OMe), 1.26 (t, \(J= 7.6\) Hz, 3H, Me); \(^{13}\)C NMR (100 MHz, DMSO-\(d_6\)): \(\delta\) 164.2 (C=O), 148.2, 141.3, 132.7, 123.0, 121.2, 120.8, 119.8, 114.6, 87.8, 60.4 (OCH\(_2\)), 55.6 (OMe), 14.1 (Me); IR (KBr): 3471, 2980, 1703 (C=O), 1632, 1578, 1481, 1340, 1266, 1212, 1189, 1115, 1022, 962 cm\(^{-1}\); m/z (ES): 233.08 (M-OH, 100 %); HRMS: m/z [M -OH] calcd for C\(_{13}\)H\(_{13}\)O\(_4\): 233.0814; found: 233.0826.
Ethyl 4-hydroxy-7-methoxy-4H-chromene-3-carboxylate (3d)

![Chemical structure of ethyl 4-hydroxy-7-methoxy-4H-chromene-3-carboxylate (3d)]

Light orange solid; Yield: 68%; mp: 168-170 °C; ¹H NMR (400 MHz, DMSO-"d₆"): δ 7.64 (s, 1H, H-C=C-), 7.41 (d, J = 6.4 Hz, 1H, ArH), 7.32 (d, J = 6.4 Hz, 1H, ArH), 6.65-6.58 (m, 2H, ArH + OH), 6.13 (d, J = 6.4 Hz, 1H, CHOH), 4.20 (q, J = 7.2 Hz, 2H, OCH₂), 3.78 (s, 3H, OMe), 1.25 (t, J = 7.2 Hz, 3H, Me); ¹³C NMR (100 MHz, DMSO-"d₆"): δ 164.0 (C=O), 162.3, 153.6, 132.1, 129.7, 120.1, 112.4, 108.1, 101.6, 88.2, 59.6 (OCH₂), 55.1 (OMe), 13.7 (Me); IR (KBr): 3367, 3050, 2963, 2842, 1724 (C=O), 1677, 1561, 1506, 1296, 1157, 1032, 979 cm⁻¹; m/z (ES): 233.08 (M-OH, 100 %); HRMS: m/z [M-OH] calcd for C₁₃H₁₃O₄: 233.0814; found: 233.0805.

Methyl 4-hydroxy-4H-chromene-3-carboxylate (3e)

![Chemical structure of methyl 4-hydroxy-4H-chromene-3-carboxylate (3e)]

Off white solid; Yield: 82%; mp: 123-125 °C; ¹H NMR (400 MHz, DMSO-"d₆"): δ 7.72 (s, 1H, H-C=C-), 7.52 (d, J = 6.4 Hz, 1H, ArH), 7.40-7.36 (m, 2H, ArH), 7.06-7.01 (m, 2H, ArH + OH), 6.16 (d, J = 6.8 Hz, 1H, CHOH), 3.77 (s, 3H, OMe); ¹³C NMR (100 MHz, DMSO-"d₆"): δ 164.7 (C=O), 152.1, 132.9, 131.9, 129.2, 122.8, 121.5, 119.2, 116.7, 87.9, 51.7 (OMe); IR (KBr): 3385, 2953, 1680 (C=O), 1606, 1444, 1322, 1221, 1053, 991 cm⁻¹; m/z (ES): 189.05 (M-OH, 100 %); HRMS: m/z [M-OH] calcd for C₁₁H₉O₃: 189.0552; found: 189.0550.

Methyl 4-hydroxy-6-methoxy-4H-chromene-3-carboxylate (3f)
Orange solid; Yield: 81%; mp: 124-125 °C; 'H NMR (400 MHz, DMSO-\textit{d}_6): \delta 7.68 (s, 1H, H-C=C-), 7.26 (d, \textit{J} = 6.4 Hz, 1H, ArH), 7.13 (d, \textit{J} = 2.4 Hz, 1H, ArH), 6.96-6.97 (m, 2H, ArH + OH), 6.10 (d, \textit{J} = 6.4, 1H, CH\textsubscript{OH}), 3.77 (s, 3H, OMe), 3.73 (s, 3H, OMe); \textsuperscript{13}C NMR (100 MHz, DMSO \textit{d}_6): \delta 164.7 (C=O), 153.7, 146.0, 132.9, 123.3, 119.6, 118.3, 117.5, 112.7, 87.8, 55.5 (OMe), 51.7 (OMe); IR (KBr): 3411, 3009, 2950, 2839, 1679 (C=O), 1583, 1493, 1426, 1346, 1238, 1122, 1035, 996 cm\textsuperscript{-1}; m/z (ES): 219.06 (M-OH, 100%); HRMS: m/z [M -OH] calcd for C\textsubscript{12}H\textsubscript{11}O\textsubscript{4}: 219.0653; found: 219.0652.

**Methyl 4-hydroxy-8-methoxy-4H-chromene-3-carboxylate (3g)**

Orange solid; Yield: 69%; mp: 154-156 °C; 'H NMR (400 MHz, DMSO-\textit{d}_6): \delta 7.69 (s, 1H, H-C=C-), 7.42 (d, \textit{J} = 6.0, 1H, ArH), 7.11-7.08 (m, 2H, ArH + OH), 6.97-6.95 (m, 1H, ArH), 6.20 (d, \textit{J} = 6.0 Hz, 1H, CH\textsubscript{OH}), 3.84 (s, 3H, OMe), 3.80 (s, 3H, OMe); \textsuperscript{13}C NMR (DMSO-\textit{d}_6, 100 MHz): \delta 164.7 (C=O), 148.2, 141.4, 133.0, 122.7, 121.2, 120.8, 119.7, 114.7, 87.8, 55.6 (OMe), 55.8 (OMe); IR (KBr): 3483, 3009, 2941, 2670, 1702 (C=O), 1639, 1609, 1483, 1343, 1300, 1262, 1213, 1117, 1047, 984 cm\textsuperscript{-1}; m/z (ES): 219.06 (M-OH, 100%); HRMS: m/z [M -OH] calcd for C\textsubscript{12}H\textsubscript{11}O\textsubscript{4}: 219.0657; found: 219.0654.

**Ethyl 6-chloro-4-hydroxy-4H-chromene-3-carboxylate (3h)**

Orange solid; Yield: 69%; mp: 154-156 °C; 'H NMR (400 MHz, DMSO-\textit{d}_6): \delta 7.69 (s, 1H, H-C=C-), 7.42 (d, \textit{J} = 6.0, 1H, ArH), 7.11-7.08 (m, 2H, ArH + OH), 6.97-6.95 (m, 1H, ArH), 6.20 (d, \textit{J} = 6.0 Hz, 1H, CH\textsubscript{OH}), 3.84 (s, 3H, OMe), 3.80 (s, 3H, OMe); \textsuperscript{13}C NMR (DMSO-\textit{d}_6, 100 MHz): \delta 164.7 (C=O), 148.2, 141.4, 133.0, 122.7, 121.2, 120.8, 119.7, 114.7, 87.8, 55.6 (OMe), 55.8 (OMe); IR (KBr): 3483, 3009, 2941, 2670, 1702 (C=O), 1639, 1609, 1483, 1343, 1300, 1262, 1213, 1117, 1047, 984 cm\textsuperscript{-1}; m/z (ES): 219.06 (M-OH, 100%); HRMS: m/z [M -OH] calcd for C\textsubscript{12}H\textsubscript{11}O\textsubscript{4}: 219.0657; found: 219.0654.
Light yellow solid; Yield: 82%; mp: 93-94 °C; \(^1\)H NMR (400 MHz, DMSO-\(d_6\)): \(\delta\) 7.90 (s, 1H, H-\(\text{C} = \text{C}\)), 7.55 (d, \(J = 2.0\) Hz, 1H, ArH), 7.42-7.39 (m, 1H, ArH), 7.22 (d, \(J = 8.8\) Hz, 1H, ArH), 5.81 (d, \(J = 7.2\) Hz, 1H, CHOCH), 5.42 (d, \(J = 7.2\) Hz, 1H, OH), 4.24 (q, \(J = 6.4\) Hz, 2H, OCH\(_2\)), 1.29 (t, \(J = 6.4\) Hz, 3H, Me); \(^{13}\)C NMR (100 MHz, DMSO-\(d_6\)): \(\delta\) 164.1 (C=O), 150.9, 131.5, 131.3, 128.3, 125.2, 124.3, 120.9, 118.7, 88.3, 60.7 (OCH\(_2\)), 14.2 (Me); IR (KBr): 3404, 3059, 2977, 2903, 1707 (C=O), 1582, 1479, 1084, 912 cm\(^{-1}\); m/z (ES): 237.03 (M-OH 100 %); HRMS: m/z [M -OH] calcd for C\(_{12}\)H\(_{10}\)O\(_3\)Cl: 237.0318; found: 237.0322.

**1-(4-hydroxy-4\(^H\)-chromen-3-yl)ethan-1-one (3i)**

![Image of 1-(4-hydroxy-4\(^H\)-chromen-3-yl)ethan-1-one (3i)]

Light pink solid; Yield: 75%; mp 155-157 °C; \(^1\)H NMR (400 MHz, DMSO-\(d_6\)): 7.82 (s, 1H, H-\(\text{C} = \text{C}\)), 7.51 (d, \(J = 7.2\) Hz, 1H, ArH), 7.40-7.38 (m, 1H, ArH), 7.22 (d, \(J = 6.4\) Hz, 1H), 7.08-7.00 (m, 2H, ArH + OH), 6.20 (d, \(J = 6.4\) Hz, 1H, CHOCH), 2.40 (s, 3H, Me); \(^{13}\)C NMR (100 MHz, DMSO-\(d_6\)): \(\delta\) 196.1 (C=O), 152.4, 133.4, 132.1, 130.5, 129.5, 121.6, 119.7, 116.9, 87.5, 25.6 (Me); IR (KBr): 3348, 3070, 3005, 2966, 1650 (C=O), 1604, 1362, 1212, 1039, 981 cm\(^{-1}\); m/z (ES): 173.06 (M-OH, 100 %); HRMS: m/z [M -OH] calcd for C\(_{11}\)H\(_9\)O\(_2\): 173.0600; found 173.060.

**1-(4-Hydroxy-6-methoxy-4\(^H\)-chromen-3-yl)ethan-1-one (3j)**

![Image of 1-(4-Hydroxy-6-methoxy-4\(^H\)-chromen-3-yl)ethan-1-one (3j)]

Yellow solid; Yield: 78%; mp 144-146 °C; \(^1\)H NMR (400 MHz, DMSO-\(d_6\)): 7.78 (s, 1H, H-\(\text{C} = \text{C}\)), 7.10-7.12 (m, 2H, ArH), 6.99-6.93 (m, 2H, ArH + OH), 6.15 (d, \(J = 6.4\) Hz, 1H, CHOCH), 3.73 (s, 3H, OMe), 2.39 (s, 3H, Me); \(^{13}\)C NMR (100 MHz, DMSO-\(d_6\)): \(\delta\) 196.0 (C=O), 153.7,146.3, 133.4,130.9, 120.0,118.6, 117.6, 112.7, 87.3, 55.5 (OMe), 25.5 (Me); IR (KBr): 3348, 3012, 2955, 2946, 1650 (C=O), 1631, 1572, 1488, 1241, 1036 cm\(^{-1}\); m/z (ES): 203.07 (M-OH, 100 %); HRMS: m/z [M -OH] calcd for C\(_{12}\)H\(_{11}\)O\(_3\): 203.0708; found 203.0713.
1-(6-Chloro-4-hydroxy-4\textit{H}-chromen-3-yl)ethanone (3k)

![Chemical Structure Image]

Gummy solid; Yield: 72%; \textsuperscript{1}H NMR (400 MHz, DMSO-\textit{d}_6): 8.13 (s, 1H, H-C=C-), 7.54 (d, \textit{J}=2.4 Hz, 1H, ArH), 7.42-7.39 (m, 1H, ArH), 7.24 (d, \textit{J}= 8.8 Hz, 1H, ArH), 5.70 (d, \textit{J}=6.8 Hz, 1H, CHO\textsubscript{H}), 5.48 (d, \textit{J}=6.8 Hz, 1H, OH), 2.32 (s, 3H, Me); \textsuperscript{13}C NMR (100 MHz, DMSO-\textit{d}_6): \textit{\delta} 195.9 (C=O), 151.1, 132.0, 131.4, 131.3, 128.3, 125.1, 121.3, 118.7, 87.7, 25.6 (Me); IR (KBr): 3462, 3089, 2924, 1654 (C=O), 1642, 1603, 1478, 1434, 1332, 1255, 1231, 1078, 1034, 979 cm\textsuperscript{-1}; \textit{m/z} (ES): 207.02 (M-OH, 100 %); HRMS: \textit{m/z} [M -OH] calcd for C\textsubscript{11}H\textsubscript{8}O\textsubscript{2}Cl: 207.0213; found 207.0221

4-Hydroxy-4\textit{H}-chromene-3-carboxamide (3l)

![Chemical Structure Image]

Off white solid; Yield: 82%; mp 161-163 °C; \textsuperscript{1}H NMR (400 MHz, DMSO-\textit{d}_6): \textit{\delta} 7.64 (bs,1H, NH), 7.43 (s, 1H, H-C=C-), 7.33-7.28 (m, 2H), 7.20 (bs, 1H, NH), 7.12 (d, \textit{J}=6.0 Hz, 1H), 7.03-6.97 (m, 2H, ArH + OH), 6.20 (d, \textit{J}=6.4 Hz, 1H, CHO\textsubscript{H}); \textsuperscript{13}C NMR (100 MHz, DMSO-\textit{d}_6):\textit{\delta} 166.4 (C=O), 151.6, 130.7, 128.3, 127.3, 127.1, 121.4, 119.8, 116.6, 88.1; IR (KBr): 3375, 3195, 2983, 2923, 1664 (C=O), 1594, 1430, 1221, 1034, 969 cm\textsuperscript{-1}; \textit{m/z} (ES): 174.05 (M-OH, 100 %); HRMS: \textit{m/z} [M + H] calcd for C\textsubscript{10}H\textsubscript{8}NO\textsubscript{2}: 174.0555; found: 174.0548.

4-Hydroxy-6-methoxy-4\textit{H}-chromene-3-carboxamide (3m)

![Chemical Structure Image]
Light Orange solid; Yield: 80%; mp: 164-166 °C; ^1H NMR (400 MHz, DMSO-^d_6): δ 7.60 (bs, 1H, NH), 7.38 (s, 1H, H-C=C-), 7.20 (bs, 1H, NH), 7.03 (d, J=6.4 Hz, 1H, ArH), 6.90-6.88 (m, 3H, ArH+ OH), 6.15 (d, J=6.4 Hz, 1H, CHOH) 3.73 (s, 3H, OMe); ^13C NMR (100 MHz, DMSO d_6): 166.3 (C=O), 153.6, 145.4, 127.8, 127.1, 120.1, 117.2, 116.4, 112.0, 88.0, 55.4 (OMe); IR (KBr): 3362, 3006, 2958, 2829, 1691 (C=O), 1595, 1579, 1494, 1447, 1407, 1336, 1255, 1213, 1044 cm\(^{-1}\); m/z (ES): 204.06 (M-OH, 100 %); HRMS: m/z [M-OH] calcd for C\(_{11}\)H\(_{10}\)NO\(_3\): 204.0661; found: 204.0669.

4-Hydroxy-8-methoxy-4H-chromene-3-carboxamide (3n)

Off white solid; Yield: 70%; mp 179-181 °C; ^1H NMR (400 MHz, DMSO-^d_6): δ 7.65 (bs, 1H, NH), 7.41 (s, 1H, H-C=C-), 7.20 (bs, 1H, NH), 7.17 (d, J = 6.0 Hz, 1H, ArH), 7.03 (d, J=6.4 Hz, 1H, ArH), 6.99-6.90 (m, 2H, ArH + OH), 6.24 (d, J=6.4 Hz, 1H, CHOH) 3.79 (s, 3H, OMe); ^13C NMR (100 MHz, DMSO-d_6): δ 169.3 (C=O), 166.3, 148.2, 127.1, 127.0, 120.9, 120.2, 120.0, 113.6, 87.9, 55.6 (OMe); IR (KBr): 3369, 3174, 2839, 1685 (C=O), 1595, 1482, 1419, 1343, 1268, 1214, 1045 cm\(^{-1}\); m/z (ES): 204.06 (M-OH, 100 %); HRMS: m/z [M-OH] calcd for C\(_{11}\)H\(_{10}\)NO\(_3\): 204.0661; found: 204.0664.

4-Hydroxy-N-methyl-4H-chromene-3-carboxamide (3o)

Off white; Yield: 77%; mp 238-240 °C; ^1H NMR (400 MHz, DMSO-d_6): 8.10-8.06 (m, 1H, NH), 7.35 (s, 1H, H-C=C-), 7.26-7.21 (m, 2H, ArH), 7.06-6.88 (m, 3H, ArH + OH), 6.50 (d, J=7.2 Hz, 1H, CHOH), 2.59 (d, J=4.4 Hz, 3H, Me); ^13C NMR (100 MHz, DMSO-d_6): 170.5 (C=O), 149.5, 144.1, 129.1, 124.0, 119.5, 116.6, 109.0, 82.0, 58.5, 25.7 (Me); IR (KBr): 3108,
2938, 1677 (C=O), 1604, 1490, 1455, 1234, 1037, 966 cm$^{-1}$; m/z (ES): 188.07 (M-OH, 100 %); HRMS: m/z [M -OH] calcd for C$_{11}$H$_{10}$NO$_2$: 188.0712; found 188.0703.

4-hydroxy-6-methoxy-N-methyl-4$H$-chromene-3-carboxamide (3p)

![Chemical Structure](image)

Light orange; Yield: 78%; mp 170-172 °C; $^1$H NMR (400 MHz, DMSO-$d_6$): 8.09-8.05 (m, 1H, NH), 7.32 (s, 1H, H-C=C-), 7.07 (d, $J= 6.4$ Hz, 1H, ArH), 6.92-6.90 (m, 3H, ArH + OH), 6.17 (d, $J= 6.4$ Hz, 1H, CH$_{OH}$), 3.73 (s, 3H, OMe), 2.71 (d, $J=4.4$ Hz, 3H, Me); $^{13}$C NMR (100 MHz, DMSO-$d_6$): 164.9 (C=O), 153.6,145.4, 128.1, 126.4, 120.1, 117.2, 116.7, 112.1, 88.1, 55.5, 25.9 (Me); IR (KBr): 3362, 2895, 2839, 1652 (C=O), 1612,1556,1496, 1410, 1276, 1214, 1052 cm$^{-1}$; m/z (ES): 218.08 (M-OH, 100 %); HRMS: m/z [M -OH] calcd for C$_{12}$H$_{12}$NO$_3$: 218.0817; found 218.0826.

**Cell Proliferation Assay**

The anti-proliferative activity and cancer cell selectivity of the synthesized compounds on normal and cancer cells was evaluated using the SRB (Sulforhodamine B) cell proliferation assay. This assay was chosen because of its sensitivity, large dynamic range and the ability to measure cell proliferation over three days with normalization to initial cell number as well as to vehicle-treated cells. Further, this assay is the standardized assay of choice for anticancer compound screening at the National Cancer Institute (NIH). The SRB assay provides a colorimetric readout which can be spectrophotometrically measured and does not involve antibodies or toxic reagents. The assay is based on detection of total protein content of cells, which increases or decreases in proportion with cell number.

In brief, the assay was performed as follows: Cancer Cal 27 (oral cancer cell line) and MDA-MB231 (breast cancer cell line) and non-cancer (Human Embryonic Kidney (HEK) 293T cell line) cells were seeded in 96-well plates and incubated overnight. The optimum cell numbers
to be seeded were determined by a growth curve analysis for each cell line. In the initial (single
dose) screen, compounds (dissolved in 100% DMSO to a stock concentration of 100mM) were
added to the adhered cells at a final concentration of 10µM. After 72h of treatment, the cells
were washed with phosphate-buffered saline and ice-cold 10% trichloroacetic acid added to the
cells to precipitate all proteins for 1h at 4 °C. The cells were then washed with water and air-
dried. Cellular proteins were then stained using 0.4% SRB solution in 1% acetic acid for 10 min
at room temperature. The unbound dye was washed away by destaining with 1% acetic acid and
bound dye solubilized with 10mM Tris solution. Absorbance of solubilized dye was measured at
a wavelength of 590 nm. Percentage growth was determined by the formula \[\frac{(A_t-A_0/A_c-A_0)}{100}\], where
\(At=\) absorbance after 72h of test compound treatment, \(A_0=\) Absorbance at time 0,
\(A_c=\) Absorbance after 72h without treatment. (Compounds which resulted in <50% growth of
cancer cells were considered potentially anti-proliferative. Among such compounds, those which
retained >75% growth of non-cancerous cells were considered potentially selective to cancer
cells).

References

   with a tetrazolium assay versus a protein assay against a diverse panel of human tumor cell


   National Cancer Institute.