

***p*-Nitrobenzenesulfonamides and their Fluorescent Dansylsulfonamides derived from *N*-Alkylated *o*-(Purine-Methyl)Anilines as Novel Anti-Cancer Agents**

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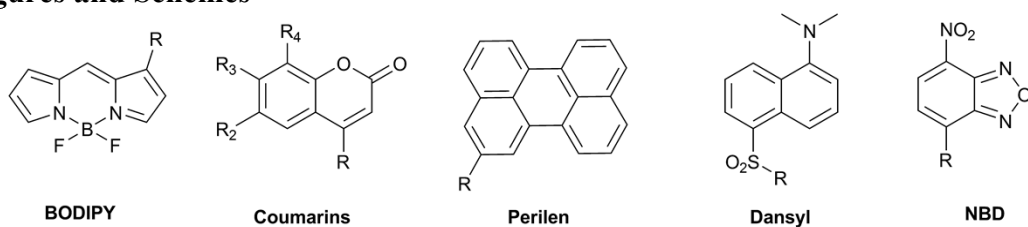
†J. A. M. and J. M. C. contributed equally.

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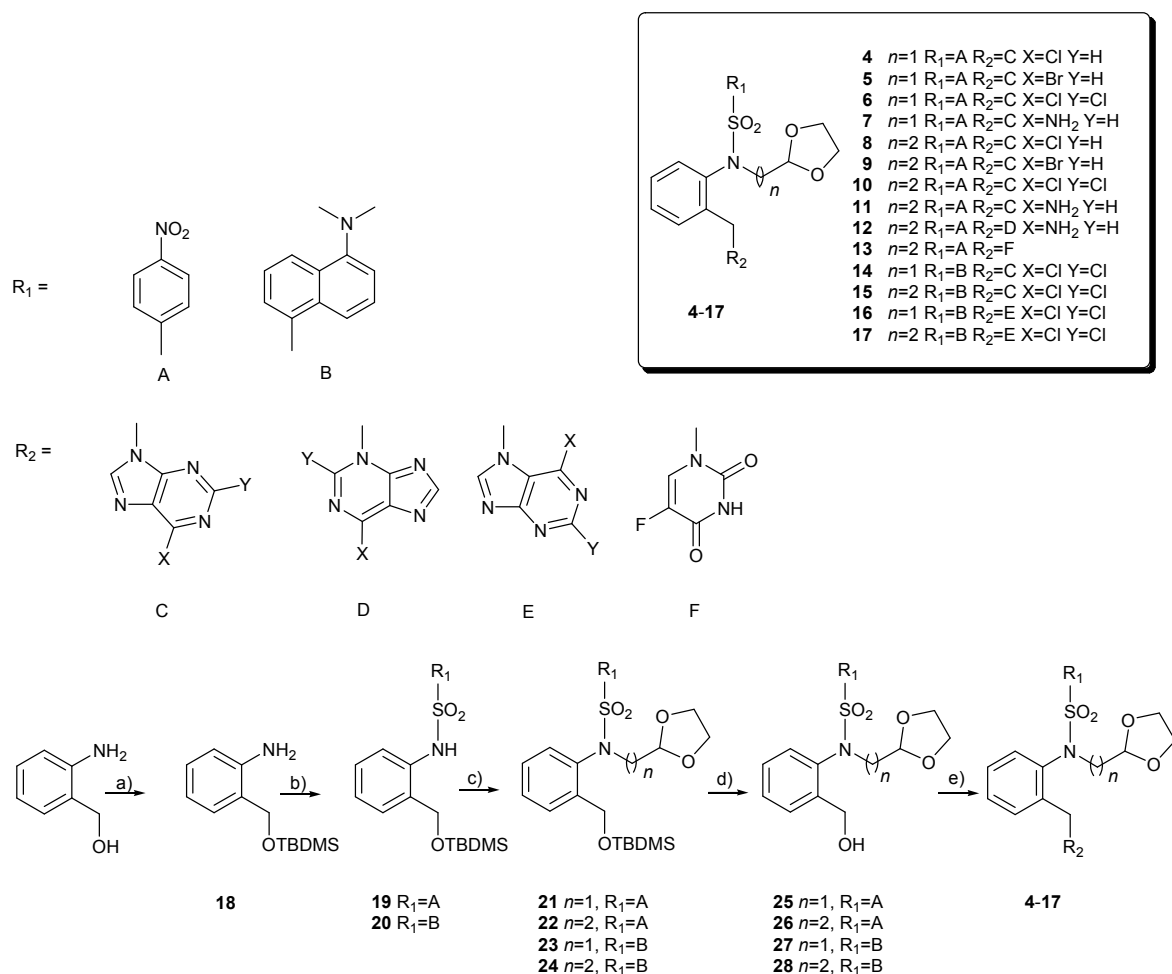
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1. Figures and Schemes

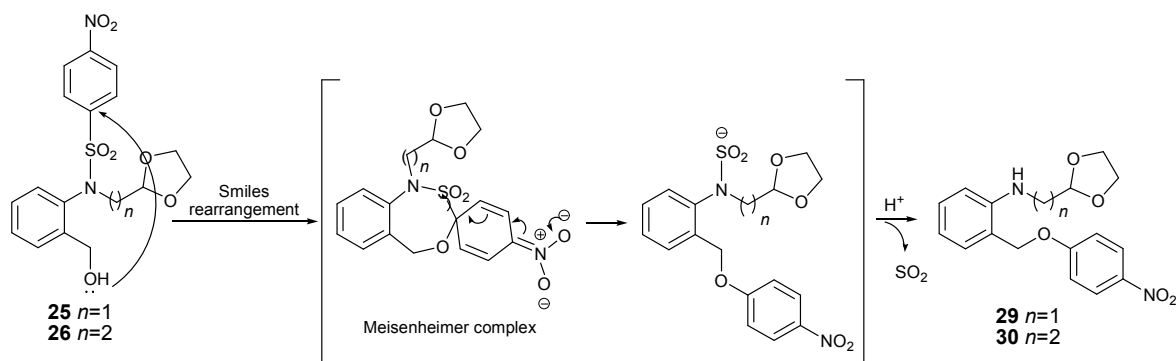


1.1. Figure S1. Some chromophores commonly used as labels for drug delivery with fluorescence techniques.



1.2. Scheme S1. Reagents and conditions: a) TBDMS-Cl, DMAP, Et₃N, anhydrous CH₂Cl₂, rt, 6h (100%). b) *p*-NO₂-Ph-SO₂Cl, anhydrous CH₂Cl₂, rt, 3h (100% for **19**); Ds-Cl, Et₃N, anhydrous CH₂Cl₂, 30 °C, 24h (100% for **20**). c) 2-Hydroxymethyl-1,3-dioxolane (for **21** and **23**) or 2-(2-hydroxyethyl)-1,3-dioxolane (for **22** and **24**), DIAD, PPh₃, anhydrous THF, 30 °C, 21h (84% for **21**, 71% for **22**, 68%, for **23** and 91% for **24**). d) TBAF, anhydrous THF, rt, 3h (48% for **25**, 78% for **26**, 100% for **27** and **28**). e) DIAD (1.1 eq for **8**, 2.2 eq for **4-7**, **9-17**), PPh₃ (1.1 eq for **8**, 2.2 eq for **4-7**, **9-17**), anhydrous THF, 140 °C (for **4-9,11-13**) or 160 °C (for **10, 14-17**), MW, 25 min (for **4-9,11-17**) or 1 h (for **10**) (53% for **4**, 44% for **5**, 53% for

6, 18% for **7**, 14% for **8**, 32% for **9**, 32% for **10**, 48% for **11**, 25% for **12**, 17% for **13**, 62% for **14**, 13% for **16**, 75% for **15**, and 7% for **17**).



1.3. Scheme S2. Proposed mechanism for the formation of by-products **29** and **30**.

2. Synthesis

Compounds **18**¹ and **19**² have been previously reported. Synthetic procedures have not been described for **4-17**, **20-18**, **29** and **30**. We herein therefore report the synthetic procedure and characterization data for the compounds that were employed.

2.1. Materials and methods

Melting points were taken in open capillaries on an Electrothermal melting point apparatus and are uncorrected. Analytical thin layer chromatography was performed using Merck Kieselgel 60 F254 aluminum sheets, the spots being developed with UV light ($\lambda = 254$ nm). All evaporation was carried out *in vacuo* with a Büchi rotary evaporator and the pressure controlled by a Vacuubrand CVCII apparatus. For flash chromatography, Merck silica gel 60 with a particle size of 0.040-0.063 mm (230-400 mesh ASTM) was used. Nuclear magnetic resonance spectra have been carried out at the Centro de Instrumentación Científica (CIC)/Universidad de Granada, and recorded on a 300 MHz ^1H and 75 MHz ^{13}C NMR Varian Inova-TM spectrometers at room temperature. Chemical shifts (δ) are quoted in parts per million (ppm) and are referenced to the residual solvent peak. Signals are designated as follows: s, singlet; d, doublet; dd, double doublet; ddd, double double doublet; pst, pseudotriplet; t, triplet; m, multiplet. High-resolution Nano-Assisted Laser Desorption/Ionization (NALDI-TOF) or Electrospray Ionization (ESITOF) mass spectra were carried out on a Bruker Autoflex or a Waters LCT Premier Mass Spectrometer, respectively. Anhydrous CH_2Cl_2 was purchased from VWR International EuroLab. Anhydrous conditions were performed under argon. All remaining reagents were purchased from Sigma-Aldrich.

2.2. *N*-[2-(*tert*-Butyldimethylsilyloxymethyl)phenyl]-5-dansylsulfonamide (**20**)

Dansyl chloride (3.42 g, 12.67 mmol) and Et₃N (1.76 g, 17.28 mmol) was added dropwise to a solution of **18** (2.74 g, 11.52 mmol) in anhydrous CH₂Cl₂ (35 mL). The reaction mixture was heated for 24 h at 30 °C, washed (H₂O), and dried (Na₂SO₄). The solvent was evaporated and the residue was purified by flash chromatography using EtOAc:hexane (0.5:10 → 1:10) as eluent to obtain **20** as a yellowish syrup (4.04 g, 100%). ¹H NMR (300 MHz, CDCl₃): δ 8.66 (s, 1H, NH), 8.49 (d, *J* = 8.5, 1H), 8.39 (d, *J* = 8.7, 1H), 8.22 (dd, *J* = 7.3, *J* = 1.2), 7.53 (dd, *J* = 8.7, *J* = 7.7, 1H), 7.47 – 7.42 (m, 2H), 7.18 – 7.14 (m, 2H), 6.93 (ddd, *J* = 7.5, *J* = 7.4, *J* = 1.1, 1H), 6.88 (dd, *J* = 7.5, *J* = 1.4, 1H), 4.30 (s, 2H), 2.86 (s, 6H), 0.94 (s, 9H), 0.06 (s, 6H). ¹³C NMR (75 MHz, CDCl₃): δ 152.03, 137.26, 135.57, 130.68, 130.38, 129.97, 129.95, 129.74, 128.78, 128.36, 128.00, 124.22, 123.26, 121.46, 118.95, 115.31, 65.40, 45.56, 25.94, 18.33, -5.26. HRMS (*m/z*): [M + H]⁺ calcd. for C₂₅H₃₅N₂O₃SSi 471.2059; found 471.2138. Anal. calcd. for C₂₅H₃₄N₂O₃SSi: C 63.79; H 7.28, N 5.95; found: C 63.76, H 7.22, N 5.92.

2.3. 2-(Hydroxymethyl)-1,3-dioxolane

A solution of 27% NaOH (22 mL) was added to a solution of Na₂CO₃·10H₂O (23.46 g, 0.082 mol) in H₂O (60 mL). 2-(2-Bromomethyl)-1,3-dioxolane (16.70 g, 0.100 mol) was added dropwise afterwards. The reaction mixture was heated for 6 h at 160 °C in a sealed tube, filtered, extracted (CH₂Cl₂) and dried (Na₂SO₄). The solvent was evaporated and the residue was purified by distillation in a Kugelrohr apparatus as a colourless syrup (2.43 g, 24%). ¹H NMR (300 MHz, CDCl₃): δ 5.06 (t, *J* = 4.0, 1H), 3.93 (m, 4H), 3.62 (d, *J* = 4.0, 2H). HRMS (*m/z*): [M + H]⁺ calcd. for C₄H₉O₃ 105.0473; found 105.0493.

2.4. 2-(Hydroxyethyl)-1,3-dioxolane

A solution of 27% NaOH (22 mL) was added to a solution of Na₂CO₃·10H₂O (23.46 g, 0.082 mol) in H₂O (60 mL). 2-(2-Bromoethyl)-1,3-dioxolane (18.10 g, 0.100 mol) was added dropwise afterwards. The reaction mixture was heated for 6 h at 160 °C in a sealed tube, filtered, extracted (CH₂Cl₂) and dried (Na₂SO₄). The solvent was evaporated and the residue was purified by distillation in a Kugelrohr apparatus as a colourless syrup (4.18 g, 36%). ¹H NMR (300 MHz, CDCl₃): δ 5.02 (t, *J* = 4.23, 1H), 3.98 (m, 4H), 3.78 (m, 2H), 2.32 (s, 1H), 1.96 (m, 2H). HRMS (*m/z*): [M + H]⁺ calcd. for C₅H₁₀O₃ 118.0630; found 118.0642.

2.5. *N*-[2-(*tert*-Butyldimethylsilyloxymethyl)phenyl]-*N*-[2-(1,3-dioxolan-2-yl)methyl]-*p*-nitrobenzenesulfonamide (21**)**

A solution of **19** (2.00 g, 4.73 mmol), 2-(hydroxymethyl)-1,3-dioxolane (492.4 mg, 4.73 mmol) and PPh₃ (1.49 g, 5.68 mmol) in anhydrous THF (25 mL) was cooled at -20 °C under argon atmosphere in a bath with acetone and CO₂. DIAD (1.05 g, 5.20 mmol) was added dropwise at -20 °C and the reaction mixture was left to rise up to 5 °C by adding acetone slowly to the bath. The reaction mixture was heated for 21 h at 30 °C. The solvent was evaporated and the residue was purified by flash chromatography using EtOAc:hexane (1:8) as eluent to yield **21** as a yellowish syrup (2.03 g, 84%). ¹H NMR (300 MHz, CDCl₃): δ 8.33 (d, *J*_I=*J*₂= 9.0, 2H), 7.85 (d, *J*_I=*J*₂= 9.0, 2H), 7.69 (dd, *J*_I= 7.7, *J*₂= 1.1, 1H), 7.39 (ddd, *J*_I= 7.7, *J*₂= 7.4, *J*₃=1.1, 1H), 7.11 (ddd, *J*_I= 7.8, *J*₂= 7.4, *J*₃=1.1, 1H), 6.47 (dd, *J*_I= 7.8, *J*₂= 1.1, 1H), 5.02 (d, *J*_{gem}= 14.4, 1H), 4.96 (pst, *J*= 4.7, 1H), 4.94 (d, *J*_{gem}= 14.4, 1H), 3.91 (dd, *J*_{gem}= 14.1, *J*_{vic}=5.1, 1H), 3.89 – 3.77 (m, 4H), 3.48 (dd, *J*_{gem}= 14.1, *J*_{vic}=5.1, 1H), 0.97 (s, 9H), 0.12 (s, 6H). ¹³C NMR (75 MHz, CDCl₃): δ 150.32, 144.27, 143.35, 135.88, 129.55, 129.40, 128.46, 127.57, 127.37, 124.05, 101.70, 65.14, 61.21, 54.76, 26.06, 18.71, -5.06. HRMS (*m/z*): [M + Na]⁺ calcd. for C₂₃H₃₂N₂O₇SSiNa 531.1699; found 531.1585. Anal. calcd. C₂₃H₃₂N₂O₇SSi: C 54.31, H 6.34, N 5.51; found: C 54.38; H 6.32, N 5.53.

2.6. *N*-[2-(*tert*-Butyldimethylsilyloxymethyl)phenyl]-*N*-[2-(1,3-dioxolan-2-yl)ethyl]-*p*-nitrobenzenesulfonamide (22**)**

A solution of **19** (2.00 g, 4.73 mmol), 2-(hydroxyethyl)-1,3-dioxolane (559.1 mg, 4.73 mmol) and PPh₃ (1.49 g, 5.68 mmol) in anhydrous THF (25 mL) was cooled at -20 °C under argon atmosphere in a bath with acetone and CO₂. DIAD (1.05 g, 5.20 mmol) was added dropwise at -20 °C and the reaction mixture was left to rise up to 5 °C by adding acetone slowly to the bath. The reaction mixture was heated for 21 h at 30 °C. The solvent was evaporated and the residue was purified by flash chromatography using EtOAc:hexane (1:7) as eluent to yield **22** as a white solid (1.75 g, 71%). Mp: 107-108 °C. ¹H NMR (300 MHz, CDCl₃): δ 8.32 (d, *J*₂₋₃=*J*₅₋₆= 8.6, 2H), 7.84 (d, *J*_I=*J*₂= 8.6, 2H), 7.66 (d, *J*= 7.5, 1H), 7.35 (pst, *J*_I= *J*₂= 7.5, 1H), 7.10 (pst, *J*_I= *J*₂= 7.5, 1H), 6.43 (d, *J*= 7.5, 1H), 4.94 (d, *J*_{gem}= 14.3, 1H), 4.87 (d, *J*_{gem}= 14.3, 1H), 4.80 (pst, *J*= 4.4, 1H), 4.03 – 3.92 (m, 1H), 3.92 – 3.75 (m, 4H), 3.43 – 3.30 (m, 1H), 2.03 – 1.89 (m, 1H), 1.80 – 1.65 (m, 1H), 0.92 (s, 9H), 0.02 (s, 6H). ¹³C NMR (75 MHz, CDCl₃): δ 150.41, 144.08, 143.43, 135.38, 129.51, 129.38, 128.77, 127.64, 126.93, 124.31, 102.11, 65.18, 61.37, 47.66, 32.77, 26.20, 18.62, -5.07. HRMS (*m/z*): [M + Na]⁺ calcd. for C₂₄H₃₄N₂O₇SSiNa 545.1856; found 545.1752. Anal. calcd. C₂₄H₃₄N₂O₇SSi: C 55.15, H 6.56, N 5.36; found: C 55.20; H 6.58, N 5.39.

**2.7. *N*-[2-(*tert*-Butyldimethylsilyloxymethyl)phenyl]-*N*-[2-(1,3-dioxolan-2-yl)methyl]-5-dansylsulfonamide
(23)**

A solution of **20** (2.00 g, 4.67 mmol), 2-(hydroxymethyl)-1,3-dioxolane (486.2 mg, 4.67 mmol) and PPh₃ (1.47 g, 5.60 mmol) in anhydrous THF (25 mL) was cooled at - 20 °C under argon atmosphere in a bath with acetone and CO₂. DIAD (1.04 g, 5.14 mmol) was added dropwise at - 20 °C and the reaction mixture was left to rise up to 5 °C by adding acetone slowly to the bath. The reaction mixture was heated for 21 h at 30 °C. The solvent was evaporated and the residue was purified by flash chromatography using EtOAc:hexane (1:9) as eluent to yield **23** as a yellowish syrup (1.76 g, 68%). ¹H NMR (300 MHz, CDCl₃): δ 8.54 (d, *J*= 8.4, 1H), 8.15 (d, *J*= 8.7, 1H), 8.02 (dd, *J*₁= 7.5, *J*₂= 1.1), 7.56 (d, *J*= 7.7, 1H), 7.42 (dd, *J*₁= 8.4, *J*₂= 7.5, 1H), 7.36 (dd, *J*₁= 8.7, *J*₂= 7.6, 1H), 7.30 – 7.26 (m, 1H), 7.14 (d, *J*= 7.6, 1H), 6.95 (ddd, *J*₁= 7.9, *J*₂= 7.5, *J*₃= 1.2, 1H), 6.64 (d, *J*= 7.9, 1H), 5.02 (pst, *J*= 4.8, 1H), 4.85 (d, *J*_{gem}= 14.4, 1H), 4.57 (d, *J*_{gem}= 14.4, 1H), 3.95 (dd, *J*_{gem}= 14.1, *J*_{vic}= 4.9, 1H), 3.93 – 3.70 (m, 4H), 3.51 (dd, *J*_{gem}= 14.1, *J*_{vic}= 4.6, 1H), 2.88 (s, 6H), 0.92 (s, 9H), 0.02 (s, 6H). ¹³C NMR (75 MHz, CDCl₃): δ 151.47, 143.00, 136.22, 134.41, 131.14, 130.67, 130.55, 129.98, 129.51, 128.63, 127.88, 127.64, 126.93, 123.21, 120.46, 115.32, 102.20, 64.98, 61.10, 54.58, 45.54, 26.08, 18.43, - 5.31. HRMS (m/z): [M + H]⁺ calcd. for C₂₉H₄₁N₂O₅SSi 557.2427; found 557.2514. Anal. calcd. C₂₉H₄₀N₂O₅SSi: C 62.56, H 7.24, N 5.03; found: C 62.60, H 7.28, N 5.08.

**2.8. *N*-[2-(*tert*-Butyldimethylsilyloxymethyl)phenyl]-*N*-[2-(1,3-dioxolan-2-yl)ethyl]-5-dansylsulfonamide
(24)**

A solution of **20** (2.00 g, 4.67 mmol), 2-(hydroxyethyl)-1,3-dioxolane (551.7 mg, 4.67 mmol) and PPh₃ (1.47 g, 5.60 mmol) in anhydrous THF (25 mL) was cooled at - 20 °C under argon atmosphere in a bath with acetone and CO₂. DIAD (1.04 g, 5.14 mmol) was added dropwise at - 20 °C and the reaction mixture was left to rise up to 5 °C by adding acetone slowly to the bath. The reaction mixture was heated for 21 h at 30 °C. The solvent was evaporated and the residue was purified by flash chromatography using EtOAc:hexane (1:9) as eluent to yield **24** as a yellowish syrup (2.43 g, 91%). ¹H NMR (300 MHz, CDCl₃): δ 8.54 (d, *J*= 8.5, 1H), 8.17 (d, *J*= 8.7, 1H), 8.05 (dd, *J*₁= 7.5, *J*₂= 1.2, 1H), 7.56 (d, *J*= 7.4, 1H), 7.44 (dd, *J*₁= 8.5, *J*₂= 7.5, 1H), 7.38 (dd, *J*₁= 8.7, *J*₂= 7.5, 1H), 7.29 (ddd, *J*₁= 7.6, *J*₂= 7.4, *J*₃= 1.1, 1H), 7.14 (d, *J*= 7.5, 1H), 7.00 (ddd, *J*₁= 8.0, *J*₂= 7.6, *J*₃= 1.5, 1H), 6.67 (dd, *J*₁= 8.0, *J*₂= 0.8, 1H), 4.81 (pst, *J*= 4.6, 1H), 4.71 (d, *J*_{gem}= 14.1, 1H), 4.58 (d, *J*_{gem}= 14.1, 1H), 3.95 – 3.88 (m, 1H), 3.89 – 3.71 (m, 4H), 3.64 – 3.53 (m, 1H), 2.88 (s, 6H), 1.95 – 1.86 (m, 1H), 1.81 – 1.73 (m, 1H), 0.90 (s, 9H), 0.02 (s, 6H). ¹³C NMR (75 MHz, CDCl₃): δ 151.60, 142.90, 135.53, 134.66, 131.13, 130.60, 130.52, 130.06, 129.49, 128.66, 128.07, 127.88, 127.15, 123.30, 120.50, 115.34, 102.34, 65.01, 61.22, 47.20, 45.59, 33.01, 26.10, 16.46, -5.26. HRMS (m/z): [M + H]⁺ calcd. for C₃₀H₄₃N₂O₅SSi 571.2584; found 571.2662. Anal. calcd. C₃₀H₄₂N₂O₅SSi: C 63.12, H 7.42, N 4.91; found: C 63.18, H 7.48, N 5.01.

2.9. *N*-[2-(1,3-Dioxolan-2-yl)methyl]-*N*-(2-hydroxymethylphenyl)-*p*-nitrobenzenesulfonamide (**25**) and *N*-[2-(1,3-dioxolan-2-yl)methyl]-2-(*p*-nitrophenoxymethyl)aniline (**29**)

TBAF (1.07 g, 3.40 mmol) was added to a solution of **21** (1.73 g, 3.40 mmol) in anhydrous THF (20 mL) and stirred for 3 h at rt under argon atmosphere. The reaction mixture was evaporated, washed (H₂O), dried (Na₂SO₄) and purified by flash chromatography using EtOAc:hexane (1:3 →1:1) as eluent gradient to allow the separation of **25** and **29**.

25: White solid (643.0 mg, 48%). Mp: 135-136 °C. ¹H NMR (300 MHz, CDCl₃): δ 8.34 (d, *J*₁=*J*₂= 9.0, 2H), 7.83 (d, *J*₁=*J*₂= 9.0, 2H), 7.64 (dd, *J*₁= 7.6, *J*₂= 1.5, 1H), 7.40 (ddd, *J*₁= 7.6, *J*₂= 7.5, *J*₃=1.2, 1H), 7.17 (ddd, *J*₁= 7.6, *J*₂= 7.5, *J*₃=1.5, 1H), 6.40 (dd, *J*₁= 7.6, *J*₂= 1.2, 1H), 5.07 (dd, *J*= 5.6, 4.1, 1H), 4.93 (d, *J*_{gem}= 12.5, 1H), 4.75 (d, *J*_{gem}= 12.5, 1H), 3.98 (dd, *J*_{gem}= 14.0, *J*_{vic}= 4.1, 1H), 3.95 – 3.80 (m, 4H), 3.32 (dd, *J*_{gem}= 14.0, *J*_{vic}= 5.7, 1H). ¹³C NMR (75 MHz, CDCl₃): δ 150.50, 143.36, 142.81, 137.50, 131.99, 129.90, 129.58, 128.86, 127.26, 124.27, 102.06, 65.18, 61.30, 55.17. HRMS (m/z): [M + Na]⁺ calcd. for C₁₇H₁₈N₂O₇SNa 417.0835; found 417.0733. Anal. calcd. C₁₇H₁₈N₂O₇S: C 51.77, H 4.60, N 7.10; found: C 51.80; H 4.65, N 7.06.

29: White solid (240.3 mg, 22%). Mp: 125-126 °C. ¹H NMR (300 MHz, CDCl₃): δ 8.21 (d, *J*₁=*J*₂= 9.3, 2H), 7.28 (ddd, *J*₁= 7.8, *J*₂= 7.6, *J*₃=1.6, 1H), 7.21 (dd, *J*₁= 7.7, *J*₂= 1.6, 1H), 7.08 (d, *J*₁=*J*₂= 9.3, 2H), 6.80 – 6.72 (m, 2H), 5.15 – 5.11 (m, 1H), 5.14 (s, 2H), 4.62 (s, 1H), 3.97 – 3.86 (m, 4H), 3.39 (d, *J*= 3.8, 2H). ¹³C NMR (75 MHz, CDCl₃): δ 163.44, 147.45, 142.02, 130.68, 130.41, 126.02, 119.83, 117.56, 115.23, 111.65, 102.41, 70.46, 65.44, 46.44. HRMS (m/z): [M + H]⁺ calcd. for C₁₇H₁₉N₂O₅ 331.1216; found 331.1293. Anal. calcd. for C₁₇H₁₈N₂O₅: C 61.81; H 5.49, N 8.48; found: C 61.98, H 5.27, N 8.52.

2.10. *N*-[2-(1,3-Dioxolan-2-yl)ethyl]-*N*-(2-hydroxymethylphenyl)-*p*-nitrobenzenesulfonamide (**26**) and *N*-[2-(1,3-dioxolan-2-yl)ethyl]-2-(*p*-nitrophenoxymethyl)aniline (**30**)

TBAF (371.7 mg, 1.18 mmol) was added to a solution of **22** (615.7 mg, 1.18 mmol) in anhydrous THF (20 mL) and stirred for 3 h at rt under argon atmosphere. The reaction mixture was evaporated, washed (H₂O), dried (Na₂SO₄) and purified by flash chromatography using EtOAc:hexane (1:3 →1:1) as eluent gradient to allow the separation of compounds **26** and **30**.

26: White solid (303.8 mg, 78%). Mp: 63-64 °C. ¹H NMR (300 MHz, CDCl₃): δ 8.35 (d, *J*₁=*J*₂= 8.8, 2H), 7.84 (d, *J*₁=*J*₂= 8.8, 2H), 7.67 (d, *J*= 7.7, 1H), 7.41 (pst, *J*₁= *J*₂= 7.7, 1H), 7.18 (pst, *J*₁= *J*₂= 7.7, 1H), 6.41 (d, *J*= 7.7, 1H), 4.84 (d, *J*_{gem}= 12.6, 1H), 4.80 (pst, *J*= 4.2, 1H), 4.63 (dd, *J*_{gem}= 12.6, *J*_{vic}= 7.4, 1H), 4.05 – 3.96 (m, 1H), 3.91 – 3.71 (m, 4H), 3.35 – 3.23 (m, 1H), 1.96 – 1.83 (m, 1H), 1.72 – 1.60 (m, 1H). ¹³C NMR (75 MHz, CDCl₃): δ 150.28, 143.28, 142.78, 136.38, 131.73, 129.75, 129.50, 128.87, 126.75, 124.32, 102.02, 65.16, 61.08, 47.63, 32.42. HRMS (m/z): [M - H]⁻ calcd. for C₁₈H₁₉N₂O₇S 407.0991; found 407.0922. Anal. calcd. C₁₈H₂₀N₂O₇S: C 52.93, H 4.94, N 6.86; found: C 52.80; H 4.90, N 6.88.

30: Yellowish solid (60.9 mg, 15%). Mp: 106-107 °C. ¹H NMR (300 MHz, CDCl₃) ¹H NMR (300 MHz, CDCl₃): δ 8.22 (d, *J*₁=*J*₂= 9.2, 2H), 7.30 (ddd, *J*₁= 7.9, *J*₂= 7.6, *J*₃=1.3, 1H), 7.21 (dd, *J*₁= 7.3, *J*₂= 1.3, 1H),

7.08 (d, $J_1=J_2=9.2$, 2H), 6.75 – 6.72 (m, 2H), 5.14 (s, 2H), 4.97 (s, 1H), 4.95 (t, $J=4.4$, 1H), 3.91 – 3.63 (m, 4H), 3.31 (t, $J=6.2$, 2H), 2.07 – 1.99 (m, 2H). ^{13}C NMR (75 MHz, CDCl_3): δ 163.84, 147.65, 141.87, 130.76, 130.51, 125.99, 119.35, 116.89, 115.09, 111.05, 104.14, 70.15, 64.96, 39.09, 33.00. HRMS (m/z): $[\text{M} + \text{H}]^+$ calcd. for $\text{C}_{18}\text{H}_{21}\text{N}_2\text{O}_7\text{S}$ 345.1372; found 345.1444. Anal. calcd. for $\text{C}_{18}\text{H}_{20}\text{N}_2\text{O}_5$: C 62.78; H 5.85, N 8.13; found: C 63.00, H 5.78, N 8.39.

2.11. *N*-[2-(1,3-Dioxolan-2-yl)methyl]-*N*-(2-hydroxymethylphenyl)-5-dansylsulfonamide (**27**)

TBAF (949.7 mg, 3.01 mmol) was added to a solution of **23** (1.67 g, 3.01 mmol) in anhydrous THF (18 mL) and stirred for 3 h at rt under argon atmosphere. The reaction mixture was evaporated, washed (H_2O), dried (Na_2SO_4) and purified by flash chromatography using EtOAc:hexane (1:3 \rightarrow 1:1) as eluent gradient to obtain **27** as a yellowish solid (1.33 g, 100%). Mp: 58-59 °C. ^1H NMR (300 MHz, CDCl_3): δ 8.57 (d, $J=8.4$, 1H), 8.05 – 8.02 (m, 2H), 7.57 (dd, $J_1=7.7$, $J_2=1.5$, 1H), 7.45 (dd, $J_1=8.4$, $J_2=7.5$, 1H), 7.32 (dd, $J_1=8.6$, $J_2=7.6$, 1H), 7.29 (ddd, $J_1=7.7$, $J_2=7.5$, $J_3=0.8$, 1H), 7.14 (d, $J=7.6$, 1H), 6.92 (ddd, $J_1=8.0$, $J_2=7.5$, $J_3=1.7$, 1H), 6.31 (dd, $J_1=8.0$, $J_2=0.8$, 1H), 5.11 (pst, $J=4.8$, 1H), 4.92 (dd, $J_{\text{gem}}=12.4$, $J_{\text{vic}}=5.2$, 1H), 4.70 (dd, $J_{\text{gem}}=12.4$, $J_{\text{vic}}=5.3$, 1H), 4.15 (dd, $J_{\text{gem}}=14.1$, $J_{\text{vic}}=4.3$, 1H), 3.94 – 3.76 (m, 4H), 3.25 (dd, $J_{\text{gem}}=14.1$, $J_{\text{vic}}=5.5$, 1H), 2.88 (s, 6H). ^{13}C NMR (75 MHz, CDCl_3): δ 151.59, 142.86, 138.03, 133.64, 131.42, 131.39, 130.99, 130.61, 130.05, 129.17, 128.80, 128.36, 128.00, 123.22, 120.34, 115.40, 102.54, 65.03, 61.53, 55.07, 45.57. HRMS (m/z): $[\text{M} + \text{H}]^+$ calcd. for $\text{C}_{23}\text{H}_{27}\text{N}_2\text{O}_5\text{S}$ 443.1562; found 443.1637. Anal. calcd. $\text{C}_{23}\text{H}_{26}\text{N}_2\text{O}_5\text{S}$: C 62.42, H 5.92, N 6.33; found: C 62.54, H 5.98, N 6.36.

2.12. *N*-[2-(1,3-Dioxolan-2-yl)ethyl]-*N*-(2-hydroxymethylphenyl)-5-dansylsulfonamide (**28**)

TBAF (1.33 g, 4.20 mmol) was added to a solution of **24** (2.40 g, 4.20 mmol) in anhydrous THF (25 mL) and stirred for 3 h at rt under argon atmosphere. The reaction mixture was evaporated, washed with H_2O , dried (Na_2SO_4) and purified by flash chromatography using EtOAc:hexane (1:3 \rightarrow 1:1) as eluent gradient to obtain **28** as a yellowish solid (1.58 g, 100%). Mp: 64-65 °C. ^1H NMR (300 MHz, CDCl_3): δ 8.56 (d, $J=8.4$, 1H), 8.08 (d, $J=8.7$, 1H), 8.05 (dd, $J_1=7.5$, $J_2=1.1$, 1H), 7.60 (dd, $J_1=7.6$, $J_2=1.5$, 1H), 7.46 (dd, $J_1=8.4$, $J_2=7.5$, 1H), 7.34 (dd, $J_1=8.7$, $J_2=7.6$, 1H), 7.30 (ddd, $J_1=7.6$, $J_2=7.5$, $J_3=0.8$, 1H), 7.14 (d, $J=7.6$, 1H), 6.95 (ddd, $J_1=7.9$, $J_2=7.5$, $J_3=1.5$, 1H), 6.37 (dd, $J_1=7.9$, $J_2=0.8$, 1H), 4.89 (d, $J_{\text{gem}}=12.3$, 1H), 4.82 (pst, $J=4.4$, 1H), 4.62 – 4.53 (m, 1H), 4.14 – 4.02 (m, 1H), 3.93 – 3.73 (m, 4H), 3.45 – 3.36 (m, 1H), 2.89 (s, 6H), 1.95 – 1.85 (m, 1H), 1.73 – 1.62 (m, 1H). ^{13}C NMR (75 MHz, CDCl_3): δ 151.63, 142.62, 136.69, 133.87, 131.29, 131.27, 130.85, 130.52, 130.06, 129.11, 128.82, 128.36, 127.94, 123.27, 120.33, 115.37, 102.26, 65.05, 61.22, 47.44, 45.57, 32.75. HRMS (m/z): $[\text{M} + \text{H}]^+$ calcd. for $\text{C}_{24}\text{H}_{29}\text{N}_2\text{O}_5\text{S}$ 457.1719; found 457.1797. Anal. calcd. $\text{C}_{24}\text{H}_{28}\text{N}_2\text{O}_5\text{S}$: C 63.14, H 6.18, N 6.14; found: C 63.18, H 6.20, N 6.19.

2.13. General Procedure for the Microwave-Assisted Synthesis of 4-7

A solution of **25** (1 mmol), PPh₃ (2.2 mmol) and the corresponding purine derivative of 5-FU (1 mmol) in anhydrous THF (5 mL) was cooled at -20 °C under argon atmosphere in a bath with acetone and CO₂. DIAD (2.2 equiv.) was added dropwise at -20 °C and the reaction mixture was left to rise up to 5 °C by adding acetone slowly to the bath. The microwave vial was sealed and irradiated at 140 °C for 25 min. After completion of irradiation time and cooling to room temperature through rapid pressurized air supply gas-jet, the solvent was evaporated and the residue was purified by flash chromatography using EtOAc:hexane (1:1) as eluent.

2.13.1. 6-Chloro-9-(2-{*N*-[(1,3-dioxolan-2-yl)methyl]-*N*-(*p*-nitrophenylsulfonyl)amine}phenylmethyl)-9*H*-purine (**4**)

White solid (71.6 mg, 53%). Mp: 220-221 °C. ¹H NMR (300 MHz, CDCl₃): δ 8.80 (s, 1H), 8.38 (s, 1H), 8.35 (d, *J*₁=*J*₂= 8.8, 2H), 7.84 (d, *J*₁=*J*₂= 8.8, 2H), 7.29 – 7.25 (m, 1H), 7.21 – 7.17 (m, 1H), 7.05 (d, *J*= 7.3, 1H), 6.49 (d, *J*= 7.3, 1H), 5.92 (d, *J*_{gem}= 16.2, 1H), 5.85 (d, *J*_{gem}= 16.2, 1H), 5.18 – 5.15 (m, 1H), 4.16 – 4.04 (m, 1H), 4.01 – 3.82 (m, 4H), 3.30 (dd, *J*_{gem}= 13.9, *J*_{vic}= 5.6, 1H). ¹³C NMR (75 MHz, CDCl₃): δ 152.69, 152.35, 151.23, 150.64, 146.49, 142.77, 137.98, 137.64, 131.47, 129.92, 129.56, 129.30, 129.14, 127.31, 124.41, 101.88, 65.33, 54.92, 43.41. HRMS (m/z): [M + H]⁺ calcd. for C₂₂H₂₀ClN₆O₆S 531.0775; found 531.0845. Anal. calcd. C₂₂H₁₉ClN₆O₆S: C 49.77, H 3.61, N 15.83; found: C 49.81, H 3.65, N 15.85.

2.13.2. 6-Bromo-9-(2-{*N*-[(1,3-dioxolan-2-yl)methyl]-*N*-(*p*-nitrophenylsulfonyl)amine}phenylmethyl)-9*H*-purine (**5**)

Yellowish solid (46.7 mg, 44%). Mp: 197-198 °C. ¹H NMR (300 MHz, CDCl₃): δ 8.75 (s, 1H), 8.38 (s, 1H), 8.35 (d, *J*₁=*J*₂= 8.7, 2H), 7.84 (d, *J*₁=*J*₂=8.7, 2H), 7.28 – 7.25 (m, 1H), 7.20 – 7.17 (m, 1H), 7.05 (d, *J*= 7.7, 1H), 6.45 (d, *J*= 7.7, 1H), 5.92 (d, *J*_{gem}= 16.1, 1H), 5.84 (d, *J*_{gem}= 16.1), 5.16 (dd, *J*= 5.6, 4.3, 1H), 4.09 (dd, *J*_{gem}= 14.1, *J*_{vic}= 4.0, 1H), 4.00 – 3.83 (m, 4H), 3.30 (dd, *J*_{gem}= 14.1, *J*_{vic}= 5.6, 1H). ¹³C NMR (75 MHz, CDCl₃): δ 152.34, 151.48, 150.41, 146.37, 153.37, 142.77, 138.00, 137.66, 134.09, 129.95, 129.58, 129.33, 129.19, 127.30, 124.43, 101.96, 65.25, 55.16, 43.59. HRMS (m/z): [M + Na]⁺ calcd. for C₂₂H₁₉BrN₆O₆SNa 597.0270; found 597.0159. Anal. calcd. C₂₂H₁₉BrN₆O₆S: C 45.92, H 3.33, N 14.61; found: C 45.98, H 3.38, N 14.69.

2.13.3. 2,6-Dichloro-9-(2-{*N*-[(1,3-dioxolan-2-yl)methyl]-*N*-(*p*-nitrophenylsulfonyl)amine}phenylmethyl)-9*H*-purine(**6**)

Yellowish solid (91.5 mg, 53%). Mp: 212-213 °C. ¹H NMR (300 MHz, CDCl₃): δ 8.38 (d, *J*₁=*J*₂= 8.9, 2H), 8.34 (s, 1H), 7.84 (d, *J*₁=*J*₂= 8.9, 2H), 7.30 (ddd, *J*₁= 7.8, *J*₂= 7.6, *J*₃=1.3, 1H), 7.20 (ddd, *J*₁= 7.8, *J*₂= 7.6, *J*₃=1.4, 1H), 6.80 (dd, *J*₁= 7.6, *J*₂= 1.4, 1H), 6.47 (dd, *J*₁= 7.6, *J*₂= 1.3, 1H), 5.90 (d, *J*_{gem}= 16.2, 1H), 5.80 (d,

$J_{gem} = 16.2$, 1H), 5.15 (dd, $J = 6.0, 4.1$, 1H), 4.09 (dd, $J_{gem} = 13.8, J_{vic} = 4.4$, 1H), 4.00 – 3.86 (m, 4H), 3.28 (dd, $J_{gem} = 13.8, J_{vic} = 5.6$, 1H). ^{13}C NMR (75 MHz, CDCl_3): δ 153.97, 153.36, 151.99, 150.68, 147.21, 142.71, 137.71, 137.56, 130.67, 130.01, 129.56, 129.49, 129.10, 127.32, 124.46, 101.85, 65.28, 55.19, 43.75. HRMS (m/z): $[\text{M} + \text{Na}]^+$ calcd. for $\text{C}_{22}\text{H}_{18}\text{Cl}_2\text{N}_6\text{O}_6\text{SNa}$ 587.0386; found 587.0276. Anal. calcd. $\text{C}_{22}\text{H}_{18}\text{Cl}_2\text{N}_6\text{O}_6\text{S}$: C 46.74, H 3.21, N 14.86; found: C 46.81, H 3.25, N 14.92.

2.13.4. 9-(2-{*N*-[(1,3-Dioxolan-2-yl)methyl]-*N*-(*p*-nitrophenylsulfonyl)amine}phenylmethyl)-9*H*-adenine (7)

Yellowish solid (28.5 mg, 18%). Mp: 227-228 °C. ^1H NMR (300 MHz, CDCl_3): δ 8.42 (s, 1H), 8.37 (d, $J_1 = J_2 = 8.8$, 2H), 8.04 (s, 1H), 7.86 (d, $J_1 = J_2 = 8.8$, 2H), 7.28 – 7.25 (m, 1H), 7.17 – 7.14 (m, 1H), 7.07 (d, $J = 7.9$, 1H), 6.44 (d, $J = 7.9$, 1H), 5.98 (s, 2H), 5.82 (d, $J_{gem} = 16.2$, 1H), 5.77 (d, $J_{gem} = 16.2$, 1H), 5.17 (pst, $J = 4.9$, 1H), 4.09 (dd, $J_{gem} = 13.8, J_{vic} = 4.3$, 1H), 4.01 – 3.85 (m, 4H), 3.33 (dd, $J_{gem} = 13.8, J_{vic} = 5.5$, 1H). ^{13}C NMR (75 MHz, CDCl_3): δ 155.08, 152.41, 152.40, 150.65, 150.64, 142.13, 138.76, 138.13, 137.37, 129.91, 129.58, 129.25, 128.97, 127.30, 124.39, 101.89, 65.28, 55.13, 43.09. HRMS (m/z): $[\text{M} + \text{H}]^+$ calcd. for $\text{C}_{22}\text{H}_{22}\text{N}_7\text{O}_6\text{S}$ 512.1274; found 512.1358. Anal. calcd. $\text{C}_{22}\text{H}_{22}\text{N}_7\text{O}_6\text{S}$: C 51.66, H 4.14, N 19.17; found: C 51.83, H 3.98, N 19.36.

2.14. General Procedure for the Microwave-Assisted Synthesis of 8-13

A solution of **26** (1 mmol), PPh_3 (1.1 mmol for **8**, 2.2 mmol for **9-13**) and the corresponding purine or 5-FU derivative (1 mmol) in anhydrous THF (5 mL) was cooled at -20 °C under argon atmosphere in a bath with acetone and CO_2 . DIAD (1.1 equiv. for **8**, 2.2 equiv. for **9-13**) was added dropwise at -20 °C and the reaction mixture was left to rise up to 5 °C by adding acetone slowly to the bath. The microwave vial was sealed and irradiated at: 140 °C for 25 min (**8, 9, 11, 12**), 160 °C for 1 h (**10**), 100 °C for 10 min (**13**). After completion of irradiation time and cooling to room temperature through rapid pressurized air supply gas-jet, the solvent was evaporated and the residue was purified by flash chromatography using EtOAc:hexane (1:1) as eluent.

2.14.1. 6-Chloro-9-(2-{*N*-[(1,3-dioxolan-2-yl)ethyl]-*N*-(*p*-nitrophenylsulfonyl)amine}phenylmethyl)-9*H*-purine (**8**)

Yellowish solid (18.5 mg, 14%). Mp: 80-81 °C. ^1H NMR (300 MHz, CDCl_3): δ 8.78 (s, 1H), 8.38 (s, 1H), 8.38 (d, $J_1 = J_2 = 8.9$, 2H), 7.85 (d, $J_1 = J_2 = 8.9$, 2H), 7.31 – 7.25 (m, 1H), 7.21 (ddd, $J_1 = 7.7, J_2 = 7.7, J_3 = 1.3$, 1H), 6.49 (dd, $J_1 = 7.7, J_2 = 1.3$, 1H), 6.41 (dd, $J_1 = 7.7, J_2 = 1.3$, 1H), 5.89 (d, $J_{gem} = 16.2$, 1H), 5.79 (d, $J_{gem} = 16.2$, 1H), 4.92 (pst, $J = 4.1$, 1H), 4.26 – 4.07 (m, 1H), 3.99 – 3.77 (m, 4H), 3.41 – 3.28 (m, 1H), 2.07 – 1.95 (m, 1H), 1.89 – 1.75 (m, 1H). ^{13}C NMR (75 MHz, CDCl_3): δ 152.52, 152.48, 151.35, 150.67, 146.28, 142.73, 137.70, 136.93, 131.12, 129.86, 129.65, 129.44, 129.23, 126.99, 124.42, 102.06, 65.25, 47.58, 43.93, 32.44.

HRMS (m/z): [M + H]⁺ calcd. for C₂₃H₂₂ClN₆O₆S 545.0932; found 545.1012. Anal. calcd. C₂₃H₂₁ClN₆O₆S: C 50.69, H 3.88, N 15.42; found: C 50.73, H 3.92, N 15.47.

2.14.2. 6-Bromo-9-(2-{*N*-[(1,3-dioxolan-2-yl)ethyl]-*N*-(*p*-nitrophenylsulfonyl)amine}phenylmethyl)-9*H*-purine (**9**)

Yellowish solid (45.4 mg, 32%). Mp: 80-81 °C. ¹H NMR (300 MHz, CDCl₃): δ 8.72 (s, 1H), 8.42 (s, 1H), 8.37 (d, *J*₁=*J*₂= 8.8, 2H), 7.84 (d, *J*₁=*J*₂= 8.8, 2H), 7.27 (pst, *J*₁=*J*₂= 7.3, 1H), 7.19 (pst, *J*₁=*J*₂= 7.3, 1H), 7.05 (d, *J*= 7.3, 1H), 6.48 (d, *J*= 7.3, 1H), 5.87 (d, *J*_{gem}= 16.1, 1H), 5.78 (d, *J*_{gem}= 16.1, 1H), 4.90 (pst, *J*= 4.0, 1H), 4.24 – 4.08 (m, 1H), 4.00 – 3.75 (m, 4H), 3.40 – 3.25 (m, 1H), 2.08 – 1.94 (m, 1H), 1.87 – 1.74 (m, 1H). ¹³C NMR (75 MHz, CDCl₃): δ 152.52, 151.28, 151.20, 150.64, 146.13, 142.60, 137.35, 136.99, 134.11, 129.86, 129.70, 129.65, 129.59, 126.98, 124.43, 102.00, 65.24, 47.47, 44.12, 32.38. HRMS (m/z): [M + H]⁺ calcd. for C₂₃H₂₂BrN₆O₆S 589.0427; found 589.0497. Anal. calcd. C₂₃H₂₁BrN₆O₆S: C 45.92, H 3.33, N 14.61; found: C 45.98, H 3.38, N 14.69.

2.14.3. 2,6-Dichloro-9-(2-{*N*-[(1,3-dioxolan-2-yl)ethyl]-*N*-(*p*-nitrophenylsulfonyl)amine}phenylmethyl)-9*H*-purine (**10**)

White solid (67.2 mg, 32%). Mp: 80-81 °C. ¹H NMR (300 MHz, CDCl₃): δ 8.39 (s, 1H), 8.36 (d, *J*₁=*J*₂= 8.5, 2H), 7.85 (d, *J*₁=*J*₂= 8.5, 2H), 7.30 (pst, *J*₁=*J*₂= 7.5, 1H), 7.22 (pst, *J*₁=*J*₂= 7.5, 1H), 7.06 (d, *J*= 7.5, 1H), 6.48 (d, *J*= 7.5, 1H), 5.80 (d, *J*_{gem}= 16.0, 1H), 5.70 (d, *J*_{gem}= 16.0, 1H), 4.93 (pst, *J*= 3.8, 1H), 4.21 – 4.09 (m, 1H), 3.97 – 3.72 (m, 4H), 3.33 – 3.21 (m, 1H), 2.04 – 1.93 (m, 1H), 1.84 – 1.71 (m, 1H). ¹³C NMR (75 MHz, CDCl₃): δ 153.86, 153.39, 152.05, 150.64, 146.95, 142.62, 137.31, 136.90, 131.51, 130.71, 129.90, 129.61, 129.19, 126.98, 124.43, 101.98, 65.25, 47.33, 43.90, 32.22. HRMS (m/z): [M + H]⁺ calcd. for C₂₃H₂₁Cl₂N₆O₆S 579.0542; found 579.0616. Anal. calcd. C₂₃H₂₀Cl₂N₆O₆S: C 47.68; H 3.48, N 14.50; found: C 47.72, H 3.53, N 14.54.

2.14.4. 9-(2-{*N*-[(1,3-Dioxolan-2-yl)ethyl]-*N*-(*p*-nitrophenylsulfonyl)amine}phenylmethyl)-9*H*-adenine (**11**)

White solid (93.0 mg, 48%). Mp: 172-173 °C. ¹H NMR (300 MHz, CDCl₃): δ 8.38 (s, 1H), 8.36 (d, *J*₁=*J*₂= 8.8, 2H), 8.04 (s, 1H), 7.86 (d, *J*₁=*J*₂= 8.8, 2H), 7.28 – 7.25 (m, 1H), 7.19 – 7.15 (m, 1H), 7.07 (d, *J*= 7.9, 1H), 6.45 (d, *J*= 7.9, 1H), 5.75 (d, *J*_{gem}= 16.2, 1H), 5.71 (d, *J*_{gem}= 16.2, 1H), 4.91 (pst, *J*= 4.1, 1H), 4.21 – 4.14 (m, 1H), 3.99 – 3.77 (m, 4H), 3.40 – 3.28 (m, 1H), 2.07 – 1.99 (m, 1H), 1.86 – 1.75 (m, 1H). ¹³C NMR (75 MHz, CDCl₃): δ 155.56, 152.95, 150.72, 150.54, 142.93, 141.57, 138.51, 136.55, 129.79, 129.59, 129.28, 129.05, 126.88, 124.38, 119.15, 102.00, 65.20, 47.54, 43.19, 32.42. HRMS (m/z): [M + H]⁺ calcd. for C₂₃H₂₄N₇O₆S 526.1431; found 526.1501. Anal. calcd. C₂₃H₂₃N₇O₆S: C 52.56; H 4.41, N 18.66; found: C 52.29, H 4.58, N 18.65.

2.14.5. 3-(2-{*N*-[(1,3-Dioxolan-2-yl)ethyl]-*N*-(*p*-nitrophenylsulfonyl)amine}phenylmethyl)-3*H*-adenine (**12**)

Yellowish solid (47.4 mg, 25%). Mp: 155-156 °C. ¹H NMR (300 MHz, CDCl₃): δ 8.44 (s, 1H), 8.38 (d, *J*₁=*J*₂= 8.8, 2H), 8.13 (s, 1H), 7.85 (d, *J*₁=*J*₂= 8.8, 2H), 7.33 – 7.29 (m, 1H), 7.25 – 7.20 (m, 1H), 7.13 (d, *J*= 7.7, 1H), 6.47 (d, *J*= 7.7, 1H), 6.05 (d, *J*_{gem}= 15.8, 1H), 5.88 (d, *J*_{gem}= 15.8, 1H), 4.90 (pst, *J*= 3.9, 1H), 4.29 – 4.11 (m, 1H), 4.03 – 3.73 (m, 4H), 3.41 – 3.24 (m, 1H), 2.04 – 1.90 (m, 1H), 1.90 – 1.74 (m, 1H). ¹³C NMR (75 MHz, CDCl₃): δ 154.43, 150.80, 149.80, 148.90, 146.09, 142.42, 137.11, 136.62, 129.98, 129.74, 129.67, 129.20, 127.03, 124.45, 119.15, 101.96, 65.24, 49.36, 47.47, 32.21. HRMS (m/z): [M + H]⁺ calcd. for C₂₃H₂₄N₇O₆S 526.1431; found 526.1505. Anal. calcd. C₂₃H₂₃N₇O₆S: C 52.56; H 4.41, N 18.66; found: C 52.32, H 4.30, N 18.78.

2.14.6. 1-(2-{*N*-[(1,3-Dioxolan-2-yl)ethyl]-*N*-(*p*-nitrophenylsulfonyl)amine}phenylmethyl)-5-fluorouracil (**13**)

White solid (35.5 mg, 17%). Mp: 128-129 °C. ¹H NMR (300 MHz, CDCl₃): δ 8.85 (d, *J*= 5.0 Hz, 1H), 8.38 (d, *J*₁=*J*₂= 8.8 Hz, 2H), 7.86 (d, *J*₁=*J*₂= 8.8 Hz, 2H), 7.62 (d, *J*= 5.0 Hz, 1H), 7.43 – 7.38 (m, 1H), 7.28 (d, *J*= 7.2 Hz, 1H), 7.24 – 7.19 (m, 1H), 6.44 (d, *J*= 7.9, 1H), 5.45 (d, *J*_{gem}= 16.0, 1H), 5.09 (d, *J*_{gem}= 16.0, 1H), 4.88 (pst, *J*= 4.1, 1H), 4.20 – 4.02 (m, 1H), 3.99 – 3.74 (m, 4H), 3.35 – 3.23 (m, 1H), 2.04 – 1.87 (m, 1H), 1.79 – 1.72 (m, 1H). ¹³C NMR (75 MHz, CDCl₃): δ 157.05 (d, *J*= 26.5 Hz), 150.61, 150.13, 142.79, 140.79 (d, *J*= 238.2 Hz), 137.66, 137.13, 130.02, 129.41, 129.29, 129.12 (d, *J*= 32.9 Hz), 128.87, 127.11, 124.44, 101.90, 65.19, 47.55, 47.46, 32.32. HRMS (m/z): [M + Na]⁺ calcd. for C₂₂H₂₁FN₄O₈SNa 543.1064; found 543.0961. Anal. calcd. C₂₂H₂₁FN₄O₈S: C 50.77; H 4.07, N 10.76; found: C 51.00, H 4.09, N 10.43.

2.15. General Procedure for the Microwave-Assisted Synthesis of **14-17**

A solution of **27** (for **14** and **15**) or **28** (for **16** and **17**) (1.00 mmol), PPh₃ (2.20 mmol) and 2,6-dichloropurine (1.00 mmol) in anhydrous THF (5 mL) was cooled at - 20 °C under argon atmosphere in a bath with acetone and CO₂. DIAD (2.20 mmol) was added dropwise at - 20 °C and the reaction mixture was left to rise up to 5 °C by adding acetone slowly to the bath. The microwave vial was sealed and irradiated at 160 °C for 25 min. After completion of irradiation time and cooling to room temperature through rapid pressurized air supply gas-jet, the solvent was evaporated and the residue was purified by flash chromatography using EtOAc:hexane (1:1) as eluent to yield regioisomers **14** and **16** in one reaction, and derivatives **15** and **17** in the other.

2.15.1. 2,6-Dichloro-9-(2-*N*-[(1,3-dioxolan-2-yl)methyl]-*N*-(5-dansyl)amine}phenylmethyl)-9*H*-purine
(14)

Yellowish solid (256.8 mg, 62%). Mp: 82-83 °C. ¹H NMR (300 MHz, CDCl₃): δ 8.60 (d, *J* = 7.6, 1H), 8.34 (s, 1H), 8.06 (dd, *J*₁ = 7.6, *J*₂ = 1.2, 1H), 8.01 (d, *J* = 7.6, 1H), 7.48 (pst, *J*₁ = *J*₂ = 7.6, 1H), 7.32 (pst, *J*₁ = *J*₂ = 7.6, 1H), 7.19 – 7.15 (m, 1H), 7.14 (d, *J* = 7.6, 1H), 6.99 (d, *J* = 7.8, 1H), 6.95 – 6.91 (m, 1H), 6.36 (d, *J* = 7.8, 1H), 5.92 (d, *J*_{gem} = 16.1, 1H), 5.82 (d, *J*_{gem} = 16.1, 1H), 5.18 (pst, *J* = 5.0, 1H), 4.24 (dd, *J*_{gem} = 13.9, *J*_{vic} = 4.5, 1H), 4.03 – 3.82 (m, 4H), 3.26 (dd, *J*_{gem} = 13.9, *J*_{vic} = 5.5, 1H), 2.89 (s, 6H). ¹³C NMR (75 MHz, CDCl₃): δ 156.33, 154.07, 153.24, 151.80, 147.51, 138.18, 137.52, 133.19, 131.50, 131.34, 130.65, 130.59, 130.11, 129.26, 128.91, 128.47, 128.19, 127.89, 123.25, 120.03, 115.51, 102.46, 65.14, 55.04, 45.57, 43.90. HRMS (m/z): [M + H]⁺ calcd. for C₂₈H₂₇Cl₂N₆O₄S 613.1113; found 613.1202. Anal. calcd. C₂₈H₂₆Cl₂N₆O₄S: C 54.82, H 4.27, N 13.70; found: C 54.89, H 4.32, N 13.78.

2.15.2. 2,6-Dichloro-7-(2-*N*-[(1,3-dioxolan-2-yl)methyl]-*N*-(5-dansyl)amine}phenylmethyl)-7*H*-purine
(16)

Yellowish solid (76.3 mg, 13%). Mp: 89-90 °C. ¹H NMR (300 MHz, CDCl₃): δ 8.60 (d, *J* = 8.4, 1H), 8.31 (s, 1H), 8.05 (dd, *J*₁ = 7.5, *J*₂ = 1.1, 1H), 8.01 (d, *J* = 8.7, 1H), 7.49 (dd, *J*₁ = 8.4, *J*₂ = 7.5, 1H), 7.33 (dd, *J*₁ = 8.7, *J*₂ = 7.6, 1H), 7.22 – 7.14 (m, 1H), 7.14 (d, *J* = 7.6, 1H), 7.01 – 6.95 (m, 1H), 6.74 (d, *J* = 8.0, 1H), 6.43 (d, *J* = 8.0, 1H), 6.12 (s, 2H), 5.17 (dd, *J* = 5.8, 4.3, 1H), 4.20 (dd, *J*_{gem} = 14.0, *J*_{vic} = 4.3, 1H), 4.02 – 3.76 (m, 4H), 3.22 (dd, *J*_{gem} = 14.0, *J*_{vic} = 5.9, 1H), 2.89 (s, 6H). ¹³C NMR (75 MHz, CDCl₃): δ 163.52, 153.29, 151.76, 151.65, 144.00, 138.08, 137.75, 132.94, 131.62, 131.48, 130.60, 130.12, 129.40, 129.14, 128.97, 128.26, 127.30, 123.25, 122.58, 119.94, 115.54, 101.96, 65.25, 55.16, 46.98, 45.56. HRMS (m/z): [M + H]⁺ calcd. for C₂₈H₂₇Cl₂N₆O₄S 613.1113; found 613.1184. Anal. calcd. C₂₈H₂₆Cl₂N₆O₄S: C 54.82, H 4.27, N 13.70; found: C 54.79, H 4.31, N 13.80.

2.15.3. 2,6-Dichloro-9-(2-*N*-[(1,3-dioxolan-2-yl)ethyl]-*N*-(5-dansyl)amine}phenylmethyl)-9*H*-purine (15)

Yellowish solid (317.1 mg, 75%). Mp: 106-107 °C. ¹H NMR (300 MHz, CDCl₃): δ 8.58 (d, *J* = 8.5, 1H), 8.27 (s, 1H), 8.09 – 8.06 (m, 2H), 7.49 (dd, *J*₁ = 8.5, *J*₂ = 7.5, 1H), 7.33 (dd, *J*₁ = 8.7, *J*₂ = 7.5, 1H), 7.22 – 7.17 (m, 1H), 7.13 (d, *J* = 7.5, 1H), 7.01 – 6.97 (m, 2H), 6.49 (dd, *J*₁ = 8.4, *J*₂ = 1.0, 1H), 5.79 (d, *J*_{gem} = 15.9, 1H), 5.73 (d, *J*_{gem} = 15.9, 1H), 4.89 (pst, *J* = 4.1, 1H), 4.21 – 4.09 (m, 1H), 3.95 – 3.76 (m, 4H), 3.47 – 3.39 (m, 1H), 2.88 (s, 6H), 1.99 – 1.90 (m, 1H), 1.82 – 1.73 (m, 1H). ¹³C NMR (75 MHz, CDCl₃): δ 153.92, 153.22, 151.85, 151.74, 147.12, 137.21, 137.00, 133.34, 131.50, 131.22, 130.66, 130.54, 130.08, 129.24, 129.12, 129.04, 128.63, 128.10, 123.28, 120.01, 115.45, 102.20, 65.16, 47.14, 45.55, 43.93, 32.66. HRMS (m/z): [M + H]⁺ calcd. for C₂₉H₂₉Cl₂N₆O₄S 627.1270; found 627.1346. Anal. calcd. C₂₉H₂₈Cl₂N₆O₄S: C 55.50, H 4.50, N 13.39; found: C 55.59, H 4.58, N 13.42.

2.15.4. 2,6-Dichloro-7-(2-(*N*-[(1,3-dioxolan-2-yl)ethyl]-*N*-(5-dansyl)amine}phenylmethyl)-7*H*-purine (**17**)

Yellowish solid (30.0 mg, 7%). Mp: 111-112 °C. ¹H NMR (300 MHz, CDCl₃): δ 8.59 (d, *J*= 8.4, 1H), 8.28 (s, 1H), 8.06 (d, *J*= 7.9, 1H), 8.06 (d, *J*= 8.5, 1H), 7.49 (dd, *J*₁= 8.4, *J*₂= 7.9, 1H), 7.34 (dd, *J*₁= 8.5, *J*₂= 7.6, 1H), 7.21 (pst, *J*₁=*J*₂= 7.6, 1H), 7.13 (d, *J*= 7.6, 1H), 7.03 (pst, *J*₁=*J*₂= 7.6, 1H), 6.68 (d, *J*= 7.6, 1H), 6.56 (d, *J*= 7.6, 1H), 6.07 (d, *J*_{gem}= 16.7, 1H), 5.94 (d, *J*_{gem}= 16.7, 1H), 4.88 (pst, *J*= 4.1, 1H), 4.15 – 4.04 (m, 1H), 3.94 – 3.74 (m, 4H), 3.51 – 3.41 (m, 1H), 2.89 (s, 6H), 1.96 – 1.85 (m, 1H), 1.84 – 1.74 (m, 1H). ¹³C NMR (75 MHz, CDCl₃): δ 163.67, 153.44, 151.84, 151.47, 144.00, 137.24, 137.14, 133.18, 131.59, 131.37, 130.62, 130.13, 129.45, 129.41, 129.07, 128.18, 127.30, 123.29, 122.40, 119.93, 115.50, 102.16, 65.20, 47.32, 47.15, 45.56, 32.94. HRMS (*m/z*): [*M* + *H*]⁺ calcd. for C₂₉H₂₉Cl₂N₆O₄S 627.1270; found 627.1348. Anal. calcd. C₂₉H₂₈Cl₂N₆O₄S: C 55.50, H 4.50, N 13.39; found: C 55.61, H 4.53, N 13.29.

3. Biological assays

3.1. Tables

3.1.1. Table S1. Anti-proliferative activities of the *p*-nitrobenzenesulfonyl (**4-13**) and dansyl (**14-17**) derivatives against the MCF-7, HCT-116 and A-375 cell lines, using the sulforhodamine-B, colorimetric assay (3 days).

Compound	MCF-7 (IC ₅₀ μM)	HCT-116 (IC ₅₀ μM)	A-375 (IC ₅₀ μM)
4	12.20 ± 0.080	3.100 ± 0.030	0.663 ± 0.018
5	12.10 ± 0.030	3.060 ± 0.020	1.371 ± 0.039
6	1.170 ± 0.028	15.87 ± 0.030	1.329 ± 0.016
7	15.15 ± 0.040	13.68 ± 0.040	6.549 ± 0.058
8	2.200 ± 0.050	2.620 ± 0.050	0.340 ± 0.009
9	3.260 ± 0.040	2.690 ± 0.060	0.557 ± 0.090
10	3.010 ± 0.008	4.110 ± 0.001	0.134 ± 0.033
11	17.22 ± 0.020	21.3 ± 0.040	6.823 ± 0.045
12	12.57 ± 0.030	13.6 ± 0.040	5.349 ± 0.014
13	27.30 ± 0.080	20.28 ± 0.100	11.23 ± 0.020
14	10.40 ± 0.030	2.910 ± 0.013	0.411 ± 0.030
15	2.610 ± 0.040	2.290 ± 0.020	0.338 ± 0.045
16	2.170 ± 0.070	1.280 ± 0.001	0.566 ± 0.017
17	1.930 ± 0.030	3.300 ± 0.005	0.566 ± 0.017

3.1.2. Table S2. Anti-proliferative activities by FACS for **8** and **10** against A-375 metastatic melanoma cell line depending on the ALDH activity after incubation during 40 min at 37°C. S+, for positive ALDH activity cells; S-, for negative ALDH activity cells and NS for cells growing in a sphere forming medium without sorter enrichment process (called no sorter).

	IC ₅₀ (μM)		
	A-375 S-	A-375 S+	A-375 NS
8	23.822±0.054	23.410±0.050	8.165±0.017
10	5.242±0.031	6.970±0.026	2.430±0.031

3.1.3. Table S3. Percentage of cell cycle distribution (FACS analysis was performed after 48 h of treatment) in the A-375 cancer cell line after treatment for 24 and 48 h for the ten most active compounds **4-6**, **8-10** and **14-17** as anti-proliferative agents ($3 \times IC_{50}$). All experiments were conducted in triplicate and gave similar results. The data are means \pm SEM of three independent determinations.

	Control	4	5	6	8	9	10
G₀/G₁	40.55 \pm 0.70	47.42 \pm 3.46	33.69 \pm 4.34	40.22 \pm 1.28	28.25 \pm 3.02	26.38 \pm 1.80	38.68 \pm 2.55
G₂/M	8.71 \pm 0.93	10.62 \pm 1.23	26.60 \pm 1.15	9.93 \pm 0.93	34.40 \pm 1.84	37.27 \pm 1.94	21.36 \pm 1.53
S	50.75 \pm 0.23	41.96 \pm 2.23	39.72 \pm 5.49	49.86 \pm 0.35	37.26 \pm 1.33	36.35 \pm 3.73	40.16 \pm 0.73

*24 h of treatment

	Control	14	15	16	17
G₀/G₁	65.75 \pm 1.33	66.69 \pm 0.59	62.36 \pm 0.06	67.34 \pm 0.33	69.82 \pm 3.11
G₂/M	12.42 \pm 0.87	11.06 \pm 0.49	12.31 \pm 0.01	9.85 \pm 0.21	8.49 \pm 0.05
S	21.84 \pm 0.47	22.26 \pm 0.10	25.34 \pm 0.05	22.83 \pm 0.53	21.70 \pm 3.16

*24 h of treatment

	Control	4	5	6	8	9	10
G₀/G₁	40.86 \pm 1.14	34.23 \pm 0.69	39.88 \pm 4.62	42.79 \pm 0.91	41.19 \pm 2.43	41.09 \pm 0.50	32.78 \pm 2.87
G₂/M	19.75 \pm 0.67	24.32 \pm 0.38	20.34 \pm 17.40	19.80 \pm 0.88	38.00 \pm 4.04	32.88 \pm 10.89	15.31 \pm 2.14
S	39.37 \pm 0.47	41.46 \pm 1.07	39.78 \pm 12.77	37.42 \pm 0.03	20.82 \pm 6.47	26.03 \pm 10.39	49.43 \pm 1.49

*48 h of treatment

	Control	14	15	16	17
G₀/G₁	68.02 \pm 3.01	60.02 \pm 0.54	59.33 \pm 2.93	58.56 \pm 0.55	67.80 \pm 0.82
G₂/M	10.49 \pm 1.66	13.73 \pm 0.26	13.37 \pm 1.80	10.92 \pm 0.97	7.46 \pm 0.28
S	21.50 \pm 1.35	26.26 \pm 0.80	27.30 \pm 1.13	30.53 \pm 0.42	27.75 \pm 3.13

*48 h of treatment

Compounds **5** (especially) and **10** (in lesser extent) did not alter the cell cycle phases after 48 h. Although we do not have an accurate explanation, it may be due to the cell cycle arrest and low rate of replication of the remaining cells in culture.

3.1.4. Table S4. Apoptosis induction in the A-375 cell line after treatment for 24 and 48 h ($3 \times IC_{50}$). The annexin V-FITC apoptosis detection kit I was used to detect apoptosis by flow cytometry. The data indicate the percentage of cells undergoing apoptosis in each sample. All experiments were conducted in triplicate and gave similar results. The data are means \pm SEM of three independent determinations. N= Necrosis, ESA= Early-stage apoptosis, LSA= Late-stage apoptosis.

Control	4	5	6	8	9	10
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N	0.45 ± 0.21	0.15 ± 0.07	1.45 ± 0.35	0.15 ± 0.07	0.10 ± 0.00	26.38 ± 1.80	0.45 ± 0.07
ESA	2.85 ± 0.35	30.30 ± 3.82	35.35 ± 4.17	8.85 ± 1.63	67.10 ± 1.41	71.25 ± 4.03	31.42 ± 0.59
LSA	4.30 ± 0.14	4.20 ± 0.71	36.45 ± 5.44	2.45 ± 0.21	8.80 ± 2.12	13.30 ± 6.22	30.35 ± 0.35

*24 h of treatment

	Control	14	15	16	17
N	0.70 ± 0.14	0.80 ± 0.28	0.90 ± 0.14	0.80 ± 0.42	0.60 ± 0.00
ESA	2.30 ± 0.42	4.00 ± 0.85	5.05 ± 1.34	3.75 ± 0.64	5.15 ± 0.49
LSA	1.15 ± 0.07	2.60 ± 0.14	5.35 ± 0.92	2.85 ± 0.78	2.25 ± 0.49

*24 h of treatment

	Control	4	5	6	8	9	10
N	0.40 ± 0.14	0.95 ± 0.21	4.50 ± 0.14	0.35 ± 0.07	2.40 ± 0.14	2.25 ± 0.49	1.60 ± 0.28
ESA	2.80 ± 0.42	13.20 ± 1.41	5.85 ± 0.49	2.15 ± 0.07	26.00 ± 0.14	25.45 ± 1.34	5.50 ± 0.57
LSA	2.70 ± 0.28	7.30 ± 0.99	65.25 ± 0.21	1.70 ± 0.28	56.95 ± 4.88	58.05 ± 2.75	25.2 ± 1.13

*48 h of treatment

	Control	14	15	16	17
N	0.70 ± 0.04	1.35 ± 0.78	0.90 ± 0.28	0.75 ± 0.21	0.65 ± 0.07
ESA	2.30 ± 0.42	2.95 ± 0.92	14.05 ± 0.21	3.30 ± 0.00	5.70 ± 0.71
LSA	2.15 ± 0.07	2.40 ± 0.57	10.25 ± 0.78	2.60 ± 0.99	2.45 ± 0.07

*48 h of treatment

Compound **15** increases the rate of apoptosis from 5.05 ± 1.34 (ESA) and 5.35 ± 0.92 (LSA) at 24 h to 14.05 ± 0.21 (ESA) and 10.25 ± 0.78 (LSA) at 48 h, respectively.

3.2. Cell culture

MCF-7, HCT-116 and A-375 cells were grown at 37 °C in an atmosphere containing 5% CO₂, with Dubelcco's modified Eagle Medium (DMEM) (Gibco, Grand Island, NY, USA) supplemented with 10% heat-inactivated fetal bovine serum (FBS) (Gibco), 2% L-glutamine, 2.7% sodium bicarbonate, 1% HEPES buffer, 40 mg/L gentamicin and 500 mg/L ampicillin.

3.2.1. Aldehyde dehydrogenase assay and separation of ALDH-positive cell subpopulation by FACS

The isolation of CSCs from A-375 melanoma cell line was carried out using the Aldefluor kit (STEMCELL Technologies, Vancouver, BC, Canada), which detects the activity of ALDH. Experiments were undertaken according to the manufacturer's instructions. Cells were suspended in an ALDEFLUOR assay buffer containing ALDH substrate (BAAA, 1 µmol/L per 1×10⁶ cells) and incubated during 40 min at 37°C. As a negative control, for each sample, an aliquot was treated with 50 mmol/L of diethylaminobenzaldehyde

(DEAB), a specific ALDH inhibitor. The brightly fluorescent ALDH-expressing cells (ALDH⁺ cells) were detected in the green fluorescence channel (520-540 nm) of a FACScan Aria III. The sorting gates were established using as negative controls the cells stained with propidium iodide (PI, Sigma-Aldrich Inc., St Louis, MO, USA) only. Each experiment was repeated three times.

All cells were washed with PBS and re-suspended in a sphere culture medium (DMEM:F12, 1% Penicillin/Streptomycin, B27, 10 µg/mL ITS, 1 µg/mL Hydrocortisone, 4 ng/mL Heparin, 10 ng/mL EGF, 20 ng/mL FGF) in ultra-low adherence 6-wells plates (Corning Inc., Corning, NY, USA) at 37°C in a humidified incubator with 5% CO₂.²

3.3. Drug treatment

Compounds were dissolved in DMSO and stored at -20 °C. For each experiment, the stock solutions were further diluted in medium to obtain the desired concentrations. The final solvent concentration in cell culture was ≤ 0.1% v/v of DMSO, a concentration without any effect on cell replication. Parallel cultures of MCF-7, HCT-116 and A375 cells in medium with DMSO were used as controls.

3.4. Proliferation assays

The effect of the compounds on cell viability was assessed using the sulforhodamine-B (SRB) colorimetric assay. Cells suspension (1×10^3 cells/well) were seeded onto 24-well plates and incubated for 24 h. The cells were then treated with different concentrations of drugs in their respective culture medium and maintained with the treatment for 3 days. Thereafter, cells were treated with 300 µL per well of cold 10% trichloroacetic acid and incubated at 4°C for 20 min. Then the cells were washed three times with water and left to dry. The fixed cells were stained for 20 min with 500 µL of 0.4% (w/v) SRB dissolved in 1% acetic acid in a shaker. Wells were rinsed with 1% acetic acid and air-dried. Bound dye was solubilized with 500 µL of 10 mM Tris base (pH 10.5) in a shaker during 10 min. Finally, three aliquots of 100 µL of each well were transferred to a 96-well plate to be read in a Titertek Multiscan apparatus (Flow, Irvine, California, USA) at 492 nm. We evaluated linearity of the SRB assay with a cell number for each cell stock before each cell growth experiment. The inhibitory concentration 50 (IC₅₀) values were calculated from semi-logarithmic dose-response curves by linear interpolation. All of the experiments were plated in triplicate wells and were carried out twice.

3.4.1. MTS cell viability assay

Enriched subpopulation of A-375 CSCs was seeded in a concentration of 3000 cells/well in ultra-low adherence 96-well plates in sphere cultured medium and treated with different compounds concentrations. After 72 h, 10 µL of CellTiter 96® AQueous One Solution Cell Proliferation Assay, MTS (10 mg/mL) (Promega Corporation, Madison, USA) was added to each well and incubated at 37°C for 2-4 h. Plates were read at 570 nm on a Bio-Rad plate reader.

3.5. Cell cycle distribution analysis

The cells at 70% confluence were treated with either DMSO alone or with concentrations of the compounds determined at their $3 \times IC_{50}$ dose values). Analysis was performed after 24 and 48 h of treatment as described.³ Briefly, cells were detached and washed with PBS. Then 700 μ L of cold ethanol at 70%, stored at -20°C , were added to the cells in shake. Cells were incubated at -20°C at least 20 minutes. Then ethanol was eliminated spinning down at 2500 rpm 5 minutes. Then, cells were washed with PBS and 250 μ L of a solution with 40 μ L/mL of PI and 100 μ L/mL of RNAsa was added. Cells were incubated at 37°C 30 min in dark and analyzed by flow cytometry using a FACS CANTO II and data obtained were analyzed with FACS DIVA software. All experiments were performed in triplicate and yielded similar results.

3.6. Apoptosis detection by staining with annexin V-FITC and propidium iodide

The annexin V-FITC apoptosis detection kit I (Pharmingen, San Diego, CA, USA) was used to detect apoptosis by flow cytometry according to our previous published protocol.³ Apoptosis inductions in the MCF-7, HCT-116 and A-375 human cancer cell lines after treatment for 24 and 48 h were determined for the compounds at doses of their corresponding $3 \times IC_{50}$ values. All experiments were performed in triplicate and yielded similar results.

3.7. Fluorescence detection and confocal imaging

MCF-7 breast cancer cells, HCT-116 colon cancer cells and A-375 melanoma cells were seeded on 96 wells plates (1×10^3 cells/well) and treated with 10 μ M of **15** for 30 min, 1h and 2h. After that, 96 wells plates were read on PowerWave X (Biotek Instruments Inc., Winooski, USA) ($\lambda_{\text{ex}}=340/30$; $\lambda_{\text{em}}=450/450$). A-375 melanoma cells were harvested on 13 mm \varnothing coverslips in a 24 well plate, and were treated for 30 minutes, 1 h and 2 h with a concentration of 10 μ M of **15**. Imaging experiments were conducted with a Zeiss LSM 710 laser-scanning microscope using a tissue culture chamber (5% CO_2 , 37°C) with a Plan-Apochromat 63 \times /1.40 Oil DIC m27. Images were processed with Zen Lite 2012 software.

3.8. *In vivo* acute toxicity

Acute toxicity was determined in six weeks old BALB/c mice. Compounds **10** and **15** dissolved in methylcellulose 1% were administered in a single oral bolus (n = 40) at dose levels of 50, 75, 100, 150 and 250 mg/kg every day for seven days. Control mice were (n = 10) inoculated with the same volume of methylcellulose. Mice were maintained under standard conditions and for each treatment schedule, were

weighed and assessed twice weekly for systemic toxicity (listlessness, weight loss) and local toxicity (alopecia, skin reaction, and leg motility) for 14 days.

3.9. *In vivo* distribution assay

For the *in vivo* distribution assay mice were treated with **10** and **15** at a dose of 100 mg/kg by oral administration. After six hours mice were sacrificed by cervical dislocation. Immediately, organs (kidney, pancreas, heart, lung and liver) were extracted by necropsy and images were acquired by an IVIS Spectrum imaging system (Caliper Life Sciences, Madison, USA) using the 465/520 nm, 430/520 nm and 430/540 nm excitation/emission filters.

3.10. Statistical analyses

All the quantitative data in the present study are reported as means \pm standard derivation from at least three independent experiments. Two-way ANOVA was used for grouped analysis of differences followed by Bonferroni post-tests.

4. References

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