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Inhibiting the cancer cell growth by maleopimarate amino imide bis-tetrazoles synthesized *via* the azido-Ugi reaction

Anna A. Smirnova, Elena V. Tretyakova and Oxana B. Kazakova

Experimental Section

General. The spectra were recorded at the Center for the Collective Use "Chemistry" of the Ufa Institute of Chemistry of the UFRC RAS and RCCU "Agidel" of the UFRC RAS. ^{1}H and ^{13}C NMR spectra were recorded on a "Bruker Avance-III" (Bruker, 500 and 125.5 MHz, respectively, δ, ppm, Hz) in $CDCl_3$, internal standard-tetramethylsilane. Mass spectra were obtained on a liquid chromatograph–mass spectrometer LCMS-2010 EV (Shimadzu). Melting points were detected on a microtable "Rapido PHMK05" (Nagema). Optical rotations were measured on a polarimeter Perkin-Elmer 241 MC (PerkinElmer) in a tube length of 1 dm. Elemental analysis was performed on a Euro EA-3000 CHNS analyzer (Eurovector), the main standard is acetanilide. Thin-layer chromatography analyses were performed on Sorbfil plates (Sorbpolimer), using the solvent system chloroform–ethyl acetate, 40:1. Substances were detected by a 10% solution of sulfuric acid solution with subsequent heating at 100-120°C for 2-3 min. All chemicals were of reagent grade (Sigma-Aldrich). Compound **1** was synthesized as described previously.[S1]

References

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S3 A. Monks, D. A. Scudiero, G. S. Johnson, K. D. Paull and E. A. Sausville, *Anticancer Drug Des.*, 1997, **12**, 533.

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Chemistry

Methyl 2-{2-[bis(1-tert-butyl-1H-tetrazol-5-ylmethyl)amino]ethyl}-12-isopropyl-6,9a-dimethyl-1,3-dioxo-1,2,3,3a,4,5,5a,6,7,8,9,9a,9b,10,11,11a-hexadecahydro-3b,11-ethenonaphtho[2,1-e]isoindole-6-carboxylate **2:** white solid, yield 89% (0.53 g), mp 54 °C, R_f 0.4 ($CHCl_3$-EtOAc, 40:3), $[\alpha]_{20}^{D}$ -23°(c 0.05, $CHCl_3$). ^{1}H NMR (δ, ppm, $CDCl_3$, 500 MHz): 0.55 (3H, s, CH_3), 0.81-0.99 (2H, m, CH_2), 0.98 (3H, d, J = 6.9 Hz, CH_3), 1.00 (3H, d, J = 6.9 Hz, CH_3), 1.10 (3H, s, CH_3), 1.14-1.45 (6H, m, CH, CH_2), 1.50 (18H, s, 6CH_3), 1.60-2.20 (6H, m, CH, CH_2), 2.55 (1H, d, J = 6.8 Hz, H-1a), 2.65-2.70 (2H, m, CH_2), 2.95-3.10 (2H, m, CH_2), 3.15 (1H, s, H-12), 3.30-3.40 (2H, m, CH_2), 3.65 (3H, s, CH_3), 3.95 (4H, s, CH_2), 5.35 (1H, br. s, H-14); ^{13}C NMR (δ, ppm, $CDCl_3$, 125.5 MHz): 179.4 (C-22), 179.2 (C-24), 178.4 (C-23), 152.7 (C=N), 147.0 (C-13), 124.6 (C-14), 68.0, 60.8, 59.4, 53.7, 52.8, 51.9, 49.3, 47.1, 45.3, 40.5, 38.6, 38.1, 37.6, 36.7, 35.8, 32.7, 30.3, 29.6, 29.5, 27.7, 23.7, 22.9, 21.8, 20.2, 17.0, 16.7, 15.6. Analysis calculated for $C_{39}H_{60}N_{10}O_4$: C, 63.91; H, 8.25; N, 19.11. Found: C, 63.95; H, 8.21; N, 19.06. MS(APCI) m/z 732.6 $[M]^+$ (calculated for $C_{39}H_{60}N_{10}O_4$, 732.98).

Methyl 2-{2-[bis(1-cyclohexyl-1H-tetrazol-5-ylmethyl)amino]ethyl}-12-isopropyl-6,9a-dimethyl-1,3-dioxo-1,2,3,3a,4,5,5a,6,7,8,9,9a,9b,10,11,11a-hexadecahydro-3b,11-ethenonaphtho[2,1-e]isoindole-6-carboxylate **3:** white solid, yield 91% (0.56 g), mp 58 °C, R_f 0.4 ($CHCl_3$-EtOAc, 40:3), $[\alpha]_{20}^{D}$ -43 °(c 0.05, $CHCl_3$). ^{1}H NMR (δ, ppm, $CDCl_3$, 500 MHz): 0.55 (3H, s, CH_3), 0.81-0.99 (2H, m, CH_2), 0.98 (3H, d, J = 6.9 Hz, CH_3), 1.00 (3H, d, J = 6.9 Hz, CH_3), 1.10 (3H, s, CH_3), 1.14-1.45 (11H, m, CH, CH_2), 1.60-2.20 (24H, m, CH, CH_2), 2.01-2.10 (1H, m, H-15), 2.45-2.55 (2H, m, CH_2), 2.75 (1H, d, J = 6.8 Hz, H-1a), 2.95 (1H, s, H-12), 3.40-3.50 (2H, m, CH_2), 3.60 (3H, s, CH_3), 4.00 (4H, s., CH_2), 5.30 (1H, br. s, H-14); ^{13}C NMR (δ, ppm, $CDCl_3$, 125.5 MHz): 179.1 (C-22), 178.5 (C-24), 177.5 (C-23), 150.6 (C=N), 147.0 (C-13), 124.5 (C-14), 57.8, 53.9, 52.5, 51.9, 51.7, 49.4, 47.1, 45.7, 45.0, 40.6, 38.1, 37.6, 36.6, 35.6, 35.5, 35.2, 32.9, 32.7, 31.9, 29.6, 27.6, 25.1, 24.8, 22.6, 21.7, 20.7, 17.0, 16.7, 15.6. Analysis calculated for $C_{43}H_{64}N_{10}O_4$: C, 65.79; H, 8.22; N, 17.84. Found: C, 66.01; H, 8.15; N, 17.90. MS(APCI) m/z 785.6 $[M]^+$ (calculated for $C_{43}H_{64}N_{10}O_4$, 785.05).

Methyl 2-{2-[bis(1-benzyl-1H-tetrazol-5-ylmethyl)amino]ethyl}-12-isopropyl-6,9a-dimethyl-1,3-dioxo-1,2,3,3a,4,5,5a,6,7,8,9,9a,9b,10,11,11a-hexadecahydro-3b,11-ethenonaphtho[2,1-e]isoindole-6-carboxylate **4**: white solid, yield 90% (0.56 g), mp 60 °C, R_f 0.4 ($CHCl_3$-EtOAc, 40:3), $[\alpha]_{20}^{D}$ -35 °(c 0.05, $CHCl_3$). ^{1}H NMR (δ, ppm, $CDCl_3$, 500 MHz): 0.55 (3H, s, CH_3), 0.81-0.99 (2H, m, CH_2), 0.98 (3H, d, J = 6.9 Hz, CH_3), 1.00 (3H, d, J = 6.9 Hz, CH_3), 1.10 (3H, s, CH_3), 1.14-1.45 (6H, m, CH, CH_2), 1.60-2.20 (7H, m, CH, CH_2), 2.01-2.10 (1H, m, H-15), 2.45 (1H, d, J = 6.8 Hz, H-1a), 2.65-2.70 (2H, m, CH_2), 3.05 (1H, s, H-12), 3.30-3.40 (2H, m, CH_2), 3.65 (3H, s, CH_3), 3.90 (4H, s., CH_2), 5.35 (1H, br. s, H-14), 5.40-5.60 (4H, m, CH_2), 7.10-7.40 (10H, m, H-Ar); ^{13}C NMR (δ, ppm, $CDCl_3$, 125.5 MHz): 179.2 (C-22), 178.7 (C-24), 177.5 (C-23), 151.6 (C=N), 147.1 (C-13), 133.3 (C-Ar), 129.2 (C-Ar), 129.1 (C-Ar), 128.9 (C-Ar), 127.7 (C-Ar), 127.5 (C-Ar), 124.6 (C-14), 53.9, 52.5, 51.9, 50.8, 49.7, 47.1, 45.7, 44.3, 41.5, 40.6, 38.1, 37.7, 37.4, 36.7, 35.5, 35.4, 35.2, 32.7, 27.6, 21.8, 20.2, 17.0, 16.8, 15.6. Analysis calculated for $C_{45}H_{56}N_{10}O_4$: C, 67.48; H, 7.05; N, 17.49. Found: C, 67.40; H, 6.99; N, 17.51. MS(APCI) m/z 801.6 $[M]^+$ (calculated for $C_{45}H_{56}N_{10}O_4$, 801.01).

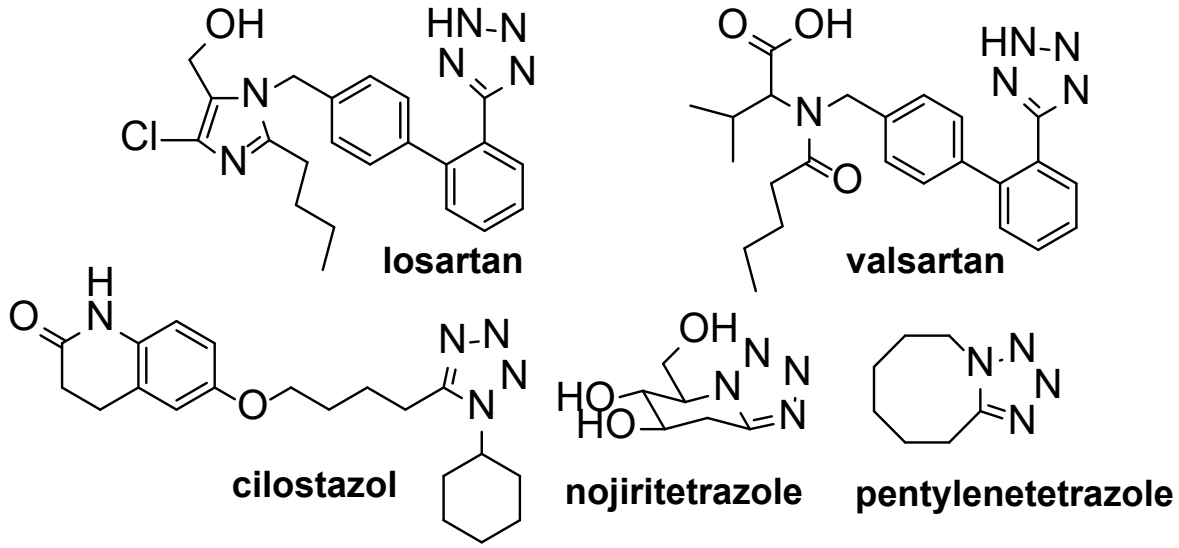

Figure S1. Some representative tetrazoles having biological activity**.**

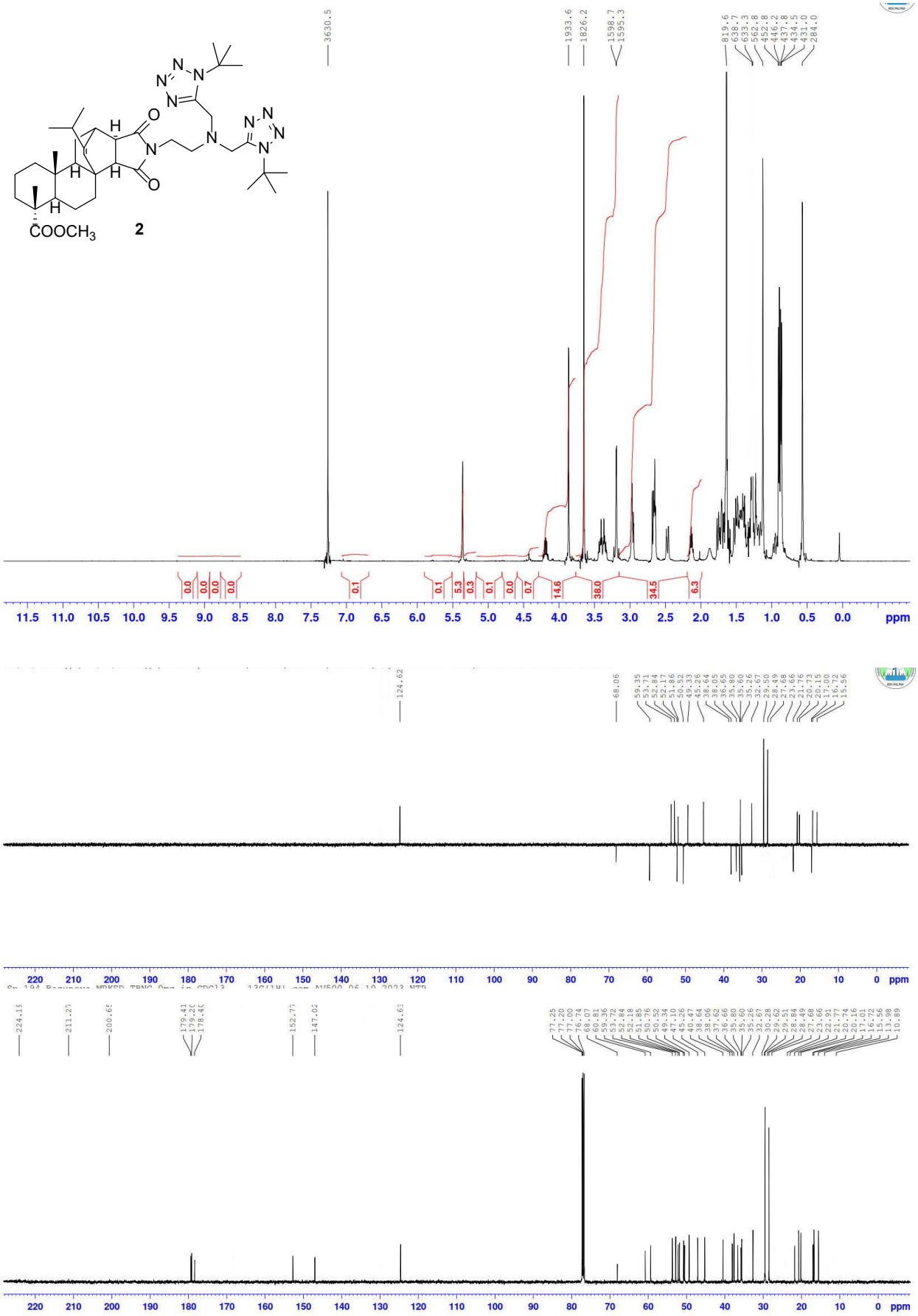

Figure S2. ^{1}H NMR and ^{13}C NMR spectra of compound **2** ($CDCl_3$).

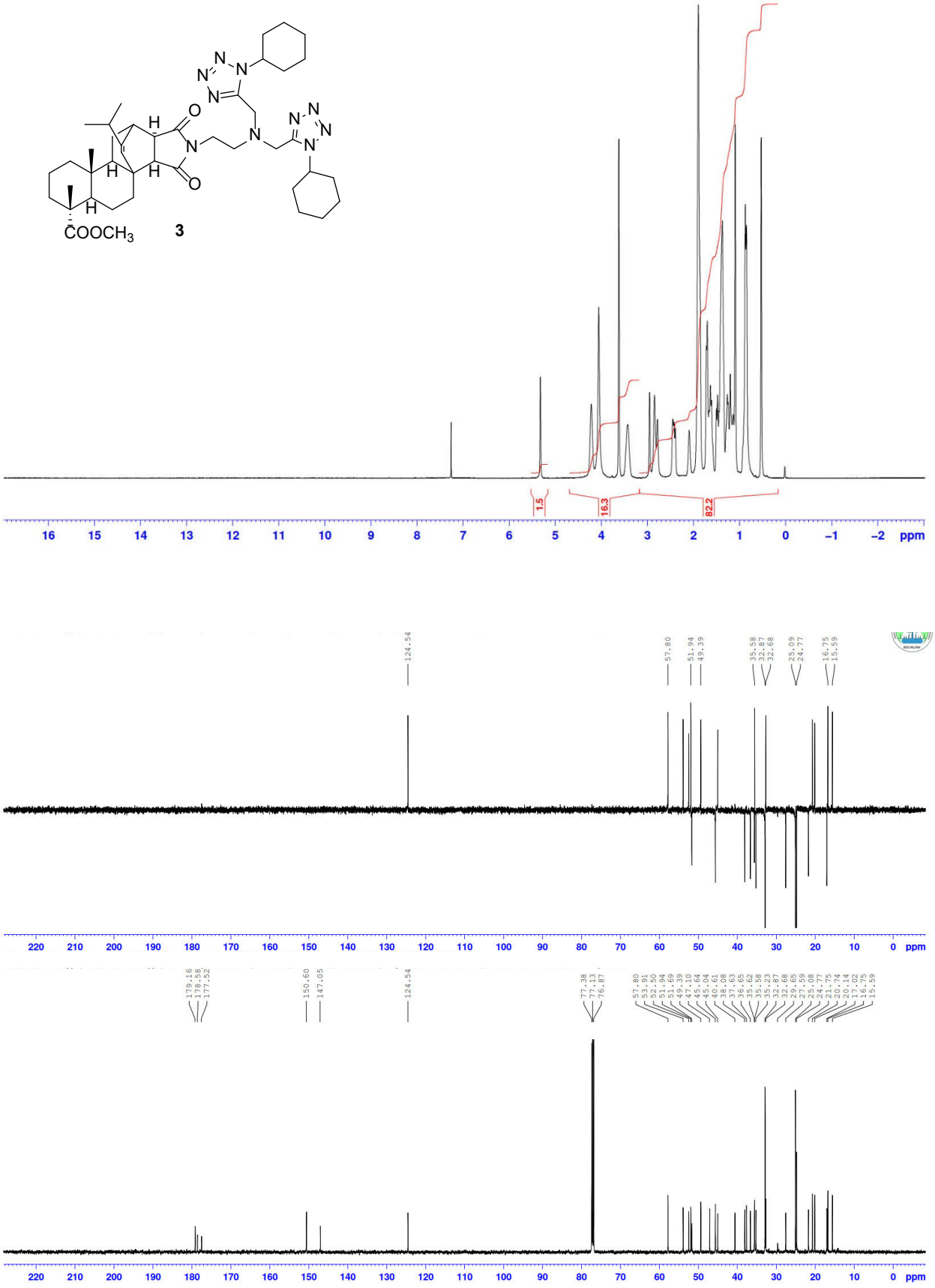

Figure S3. ^{1}H NMR and ^{13}C NMR spectra of compound **3** ($CDCl_3$).

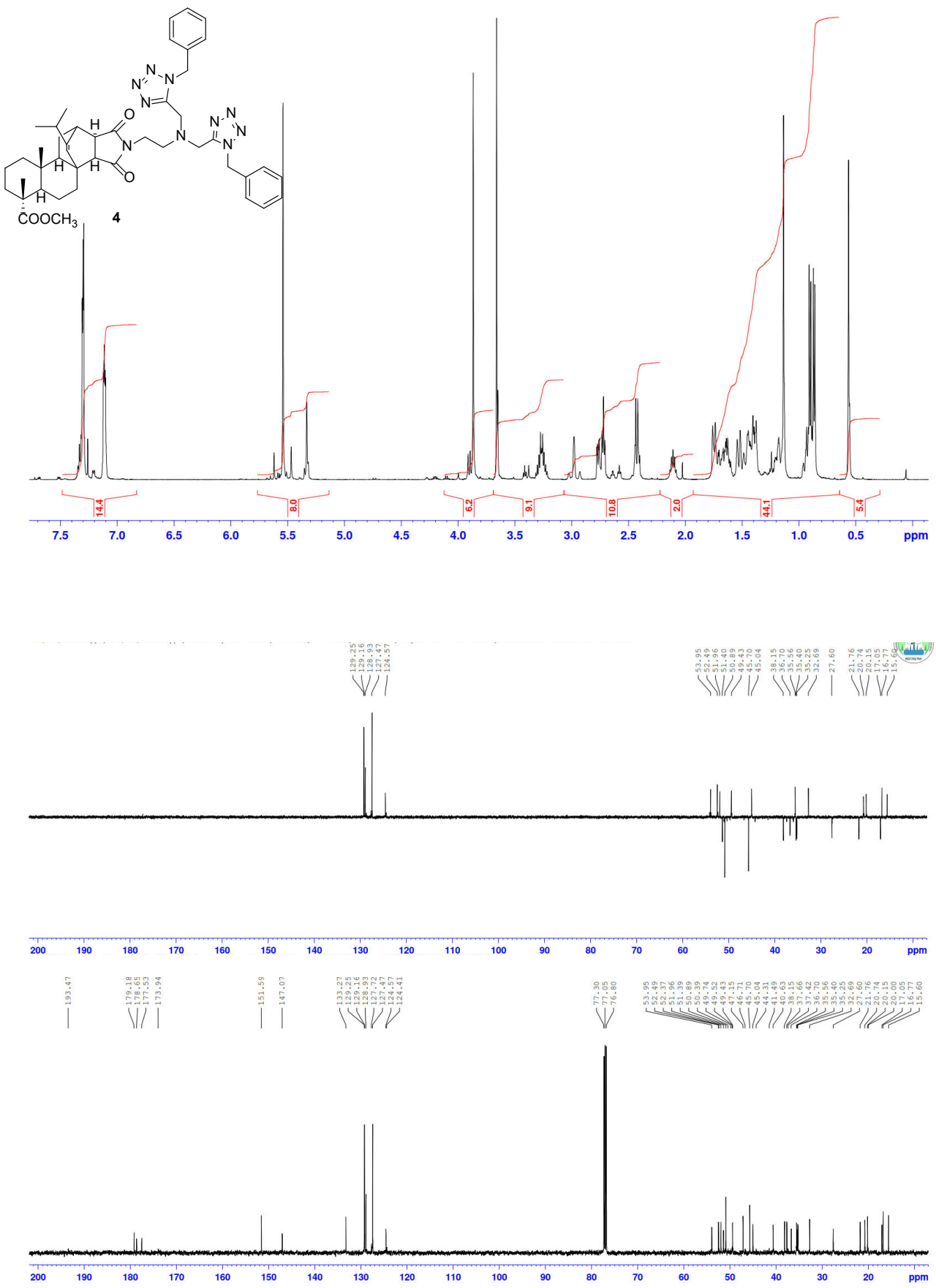

Figure S4. ^{1}H NMR and ^{13}C NMR spectra of compound **4** ($CDCl_3$).

Pharmacological studies

Anticancer assay

In vitro cancer screen in NCI, USA

Anticancer assay

The newly synthesized diterpene derivatives were exposed to comprehensive *in vitro* screening at the National Cancer Institute (NCI) 60-cancer cell lines panel at Bethesda, MD, USA, representing different types cancer, including leukemia, non-small cell lung cancer, colon cancer, melanoma, ovarian cancer, renal carcinoma, prostate cancer, and breast cancer. A single dose (10 μM) of the tested analogs was employed in the full NCI 60 cell lines panel assay.[S2-S4] The data reported as mean-graph of the percent growth of the treated cells, and presented as percentage growth inhibition (GI %) for the tested analogs. Primary anticancer assays were performed according to the NCI protocol as described elsewhere (see *e.g.*, http://dtp.nci.nih.gov). The compounds were added at a single concentration and the cell cultures were incubated for 48 h. The end point determinations were made with a protein binding dye, sulforhodamine B (SRB). The results for each compound are reported as the percent growth (GP %) of treated cells compared to untreated control cells (negative numbers indicate cell kill). The range of percent growth shows the lowest and the highest percent growth found among the different cancer cell lines.

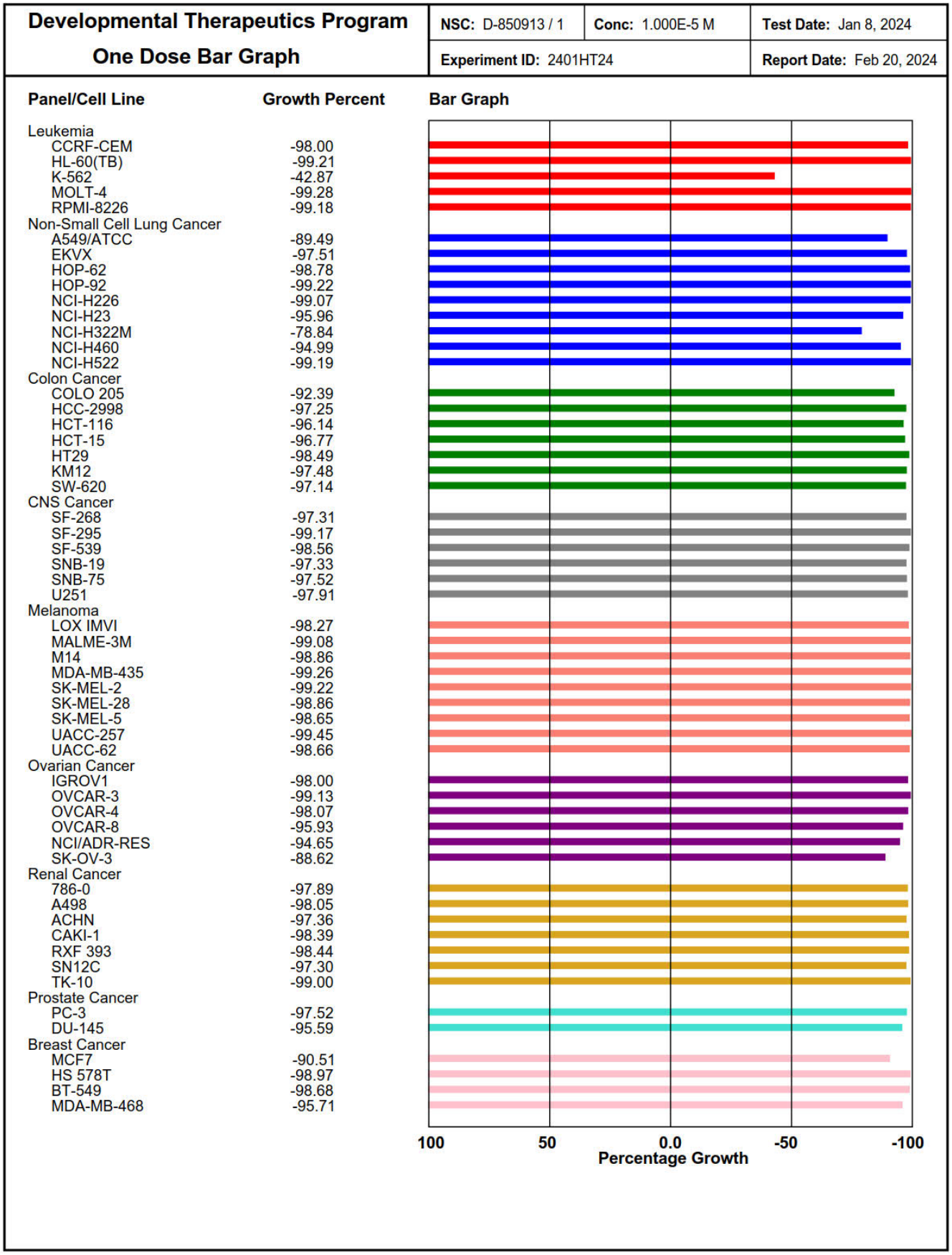

Figure S5. Anticancer screening data of compound **2** at single dose assay

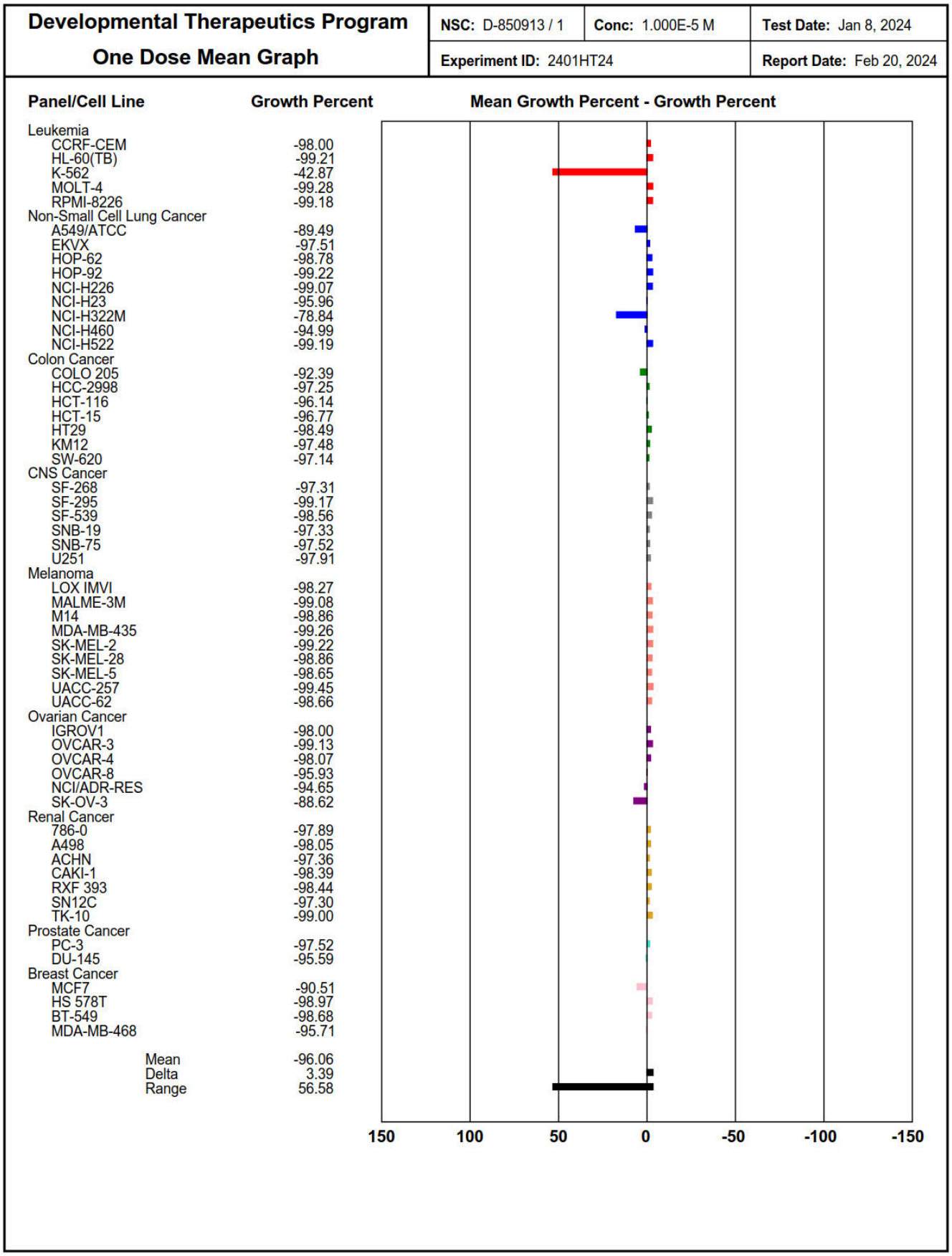

Figure S6. Anticancer screening data of compound **2** at single dose assay

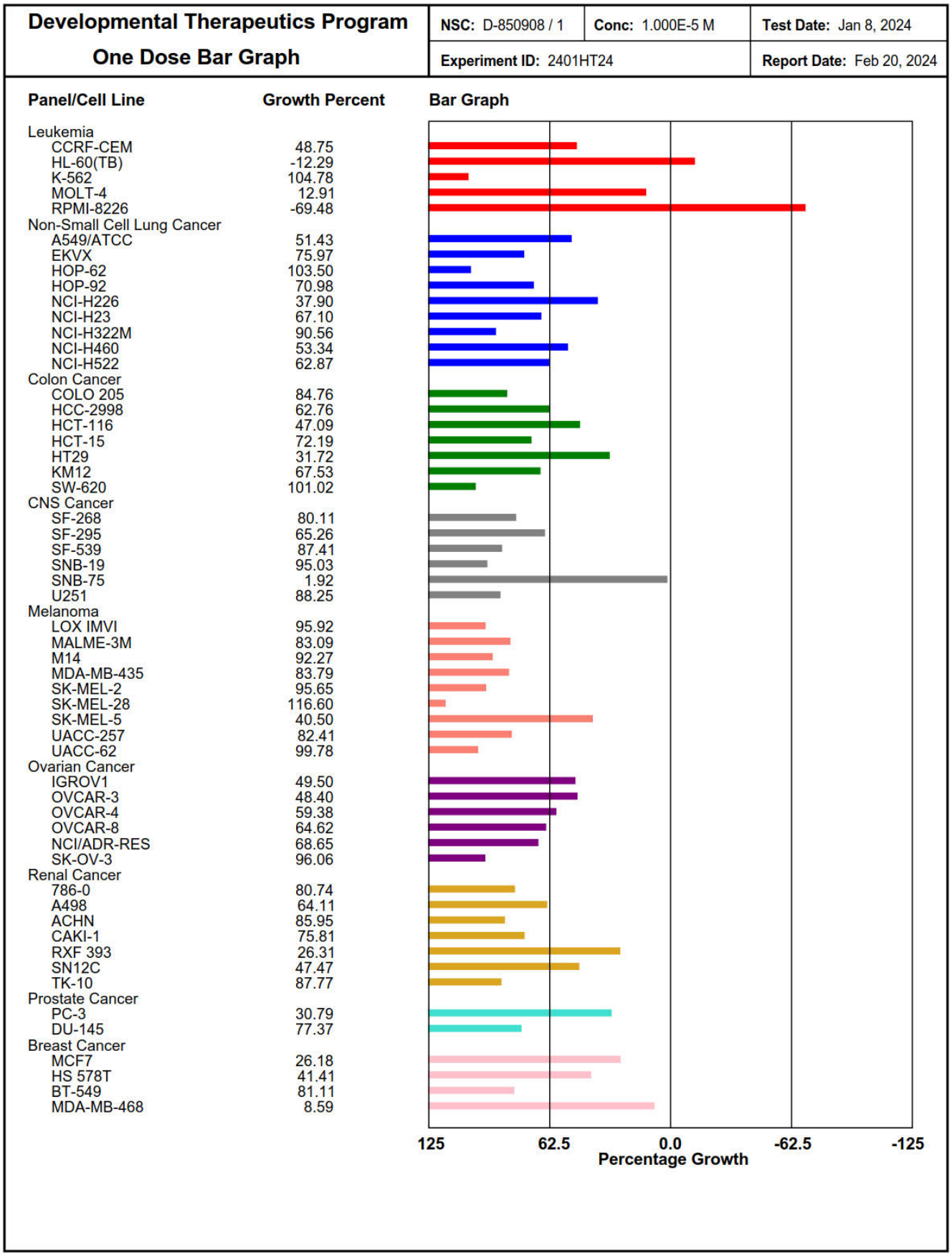

Figure S7. Anticancer screening data of compound **3** at single dose assay

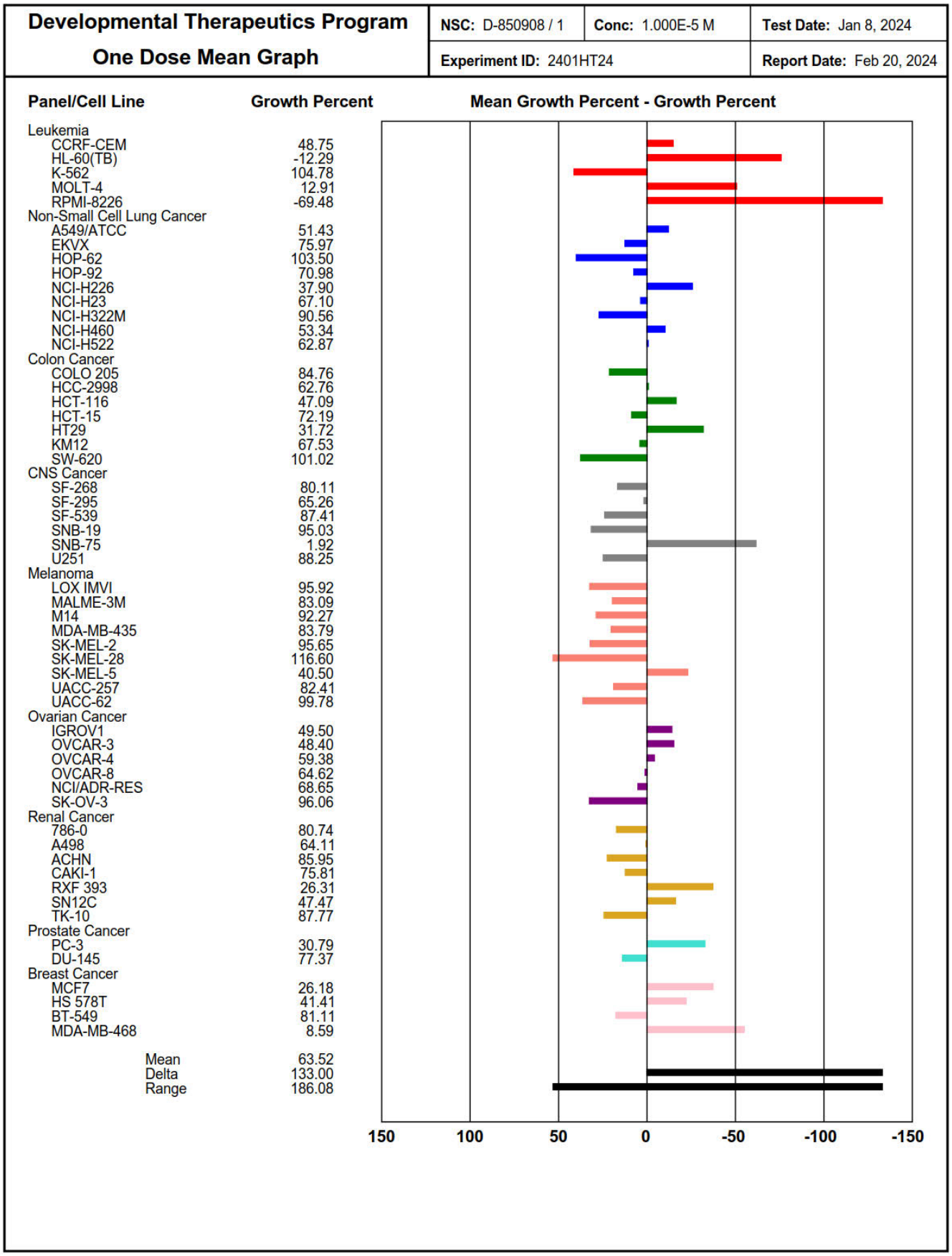

Figure S8. Anticancer screening data of compound **3** at single dose assay Compound 3

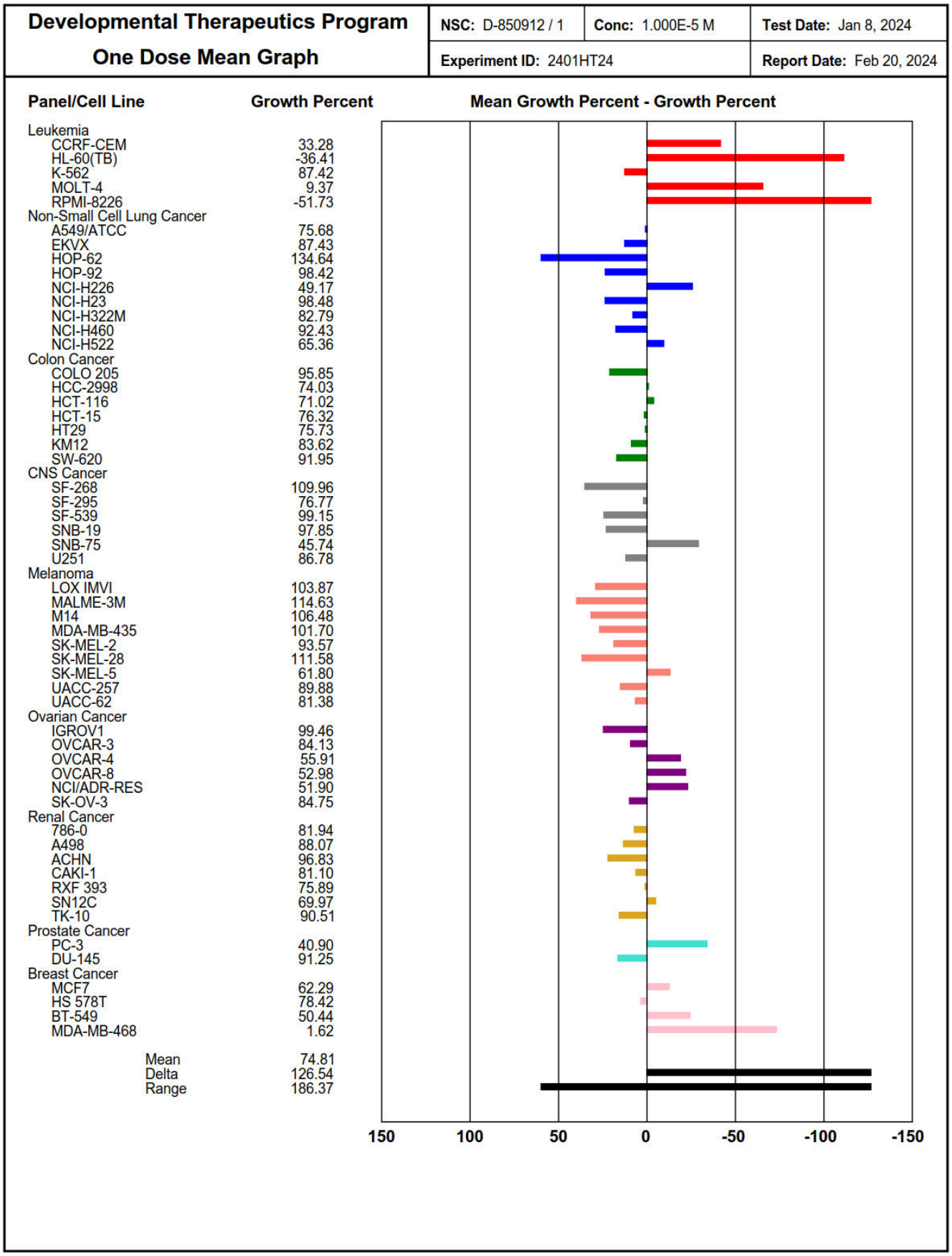

Figure S9. Anticancer screening data of compound **4** at single dose assay

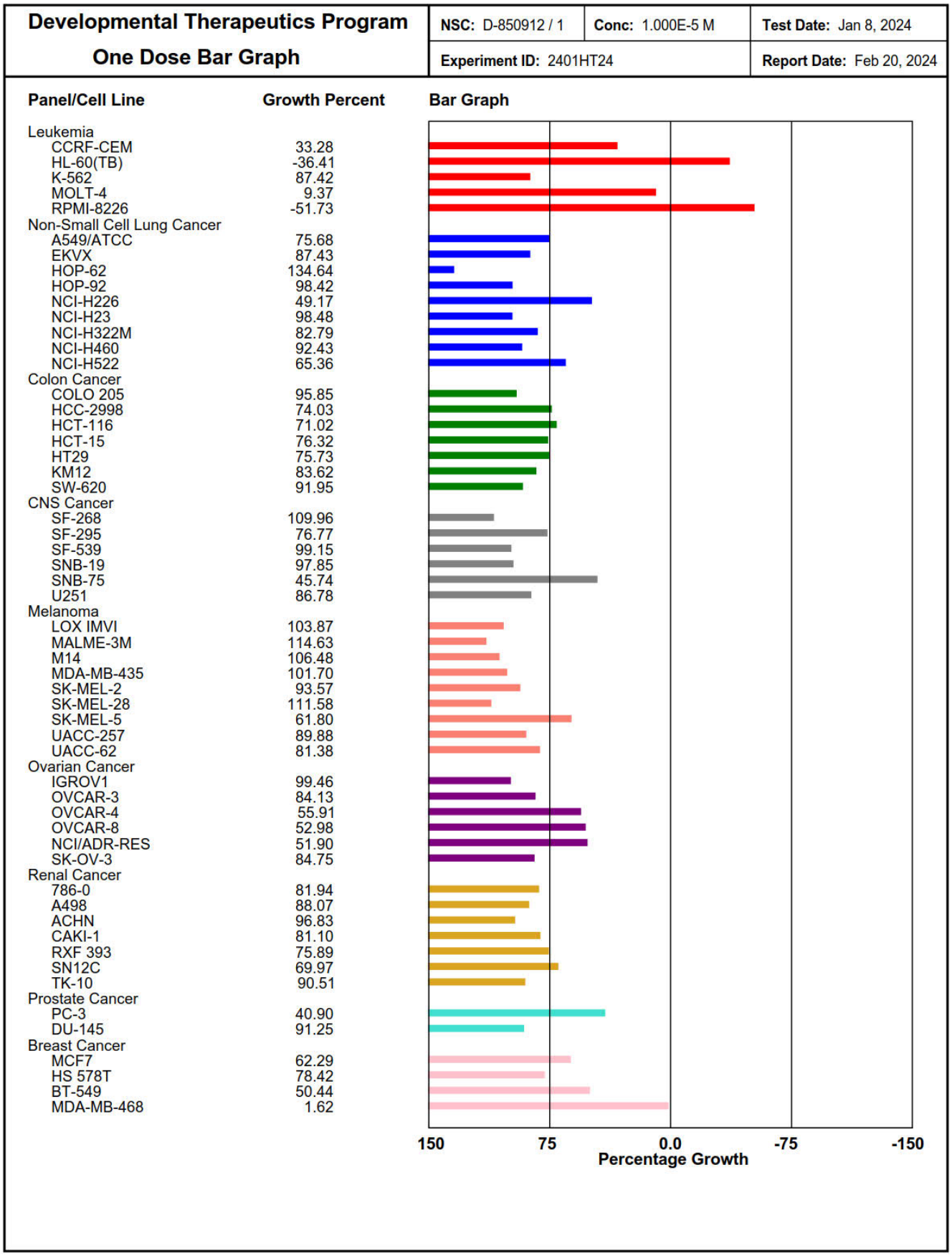

Figure S10. Anticancer screening data of compound **4** at single dose assay