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Cell Culture Services

Report 8 – Final project report

2020/12/14

Client:	**WSU TEAM/BMM**
Quotation number:	**2019/WSU/01_v02**
Contact person:	**Wayiza S Masamba, PhD, CChem, MRSC**
Services:	**Assessment of cell survival and proliferation and Assessment of product toxicity**

1. Background

Anticancer potency and toxicity potential of WSU natural products was determined using MTT assay as a colorimetric assay. A yellow water-soluble tetrazolium (3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide (MTT) is reduced by metabolically active mitochondrial dehydrogenase enzymes to purple, water insoluble formazan crystals within the cell (Mosmann, 1983). The water insoluble formazan crystals can be dissolved to provide a colour change, which can be spectrophotometrically measured and provides an estimate of the number of metabolically active (viable) cells.

The relative percentage viable cells were calculated and were expressed as percentage cell viability relative to the untreated control (assumed to be 100% viable).

All MTT experiments are to be performed in six-fold (two biological repeats of three technical repeats each). SPSS statistical analysis software (IBM Analytics, Version 25), in conjunction with the Probit Analysis Method, are then used to calculate IC_{50} (50% viability inhibition) values and 95% confidence limit ranges from the MTT analyses.

Non-adherent cell lines are difficult to evaluate with the MTT assay, and was therefore evaluated with the L-Lactate dehydrogenase (LDH) assay. LDH is a cytoplasmic enzyme responsible for the conversion of pyruvate to L-lactate, as well as NADH to NAD^+ during glycolysis (Kaja *et al*., 2017). When cells are damaged, LDH is released into the extracellular environment from the cytoplasm, and therefore correlates with the presence of damage and toxicity in cells. LDH is also very stable in cell culture medium, making it a robust and reproducible assay for cell viability. The LDH-Glo™ cytotoxicity assay from Promega is used. This is a bioluminescent plate-based assay, and is very sensitive and accurate. The LDH that is release from damaged cells catalyses the oxidation of lactate to pyruvate, as well as the reduction of NAD+ to NADH. A reductase then uses the NADH to generate luciferin which allows generation of a luminescent signal, proportional to the amount of LDH present.

All LDH experiments are to be performed in three-fold (one biological repeat of three technical repeats).

2. Aim of services

The assessment of product toxicity, cell survival and proliferation *in vitro* in cell culture models, using standard cell viability assays such as the 3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyl tetrazolium bromide (MTT) and Lactate dehydrogenase (LDH) assays.

3. Agreed to service levels and fees

Nature of products:

Natural products – To be provided as dried powder, in suitable quantities needed for the assays, clearly labelled, soluble in Dimethyl sulfoxide (DMSO).

Number of samples:

20 maximum (16 received to date) – Temperature and light insensitive. To be returned to the Client on completion of the Services.

Expected time of exposure:

72 hours – Single treatment and incubated for 72 h before assaying

Expected concentrations for each sample:

1.5625 µg/ml; 3.125 µg/ml; 6.25 µg/ml; 12.5 µg/ml; 25.00 µg/ml; 50.00 µg/ml; 100.00 µg/ml; 200 µg/ml – starting series. To be discussed following first experiments if necessary to adjust.

Solvent:

DMSO

Timeline:

As soon as the samples are received, the work can be initiated.

Payment Terms:

Invoices will be prepared monthly with reports of the month's results, to be paid as stipulated by the service contract.

4. Materials and methodology

Cell lines used for screening natural products were hosted by NWU, Potchefstroom. Briefly, cells at different passage numbers were seeded at different density as indicated on Table 4.1 and incubated for 24 h in 5% CO_2 at 37ºC, to adhere to the surface area. After which they were treated at two-fold 8-serial dilutions at concentration ranging between 1.5625 and 200 µg/ml and incubated for 72 h without media replenishment. All test compounds were dissolved in 100% DMSO at about 200mg/ml stocks, from which working stocks were prepared in complete culture medium to a final concentration below 0.1% DMSO (v/v) prior to treatments. At the end of the incubation period, treated cells were rinsed twice with 100 µl PBS and 200 µl MTT solution at a final concentration of 0.5 mg/ml was added to each well according to the SOP (Pharmacen_CTC_SOP023_v01_MTT_Assay) (available on request). Treated cultures were then incubated for 4 h, after which spent MTT solution was removed and discarded appropriately. 150µl of DMSO was added to dissolve water insoluble formazan and plate incubated for an additional hour. Prior to measurement of absorbance, the plate was placed on the shaker for 15 minutes and absorbance was measured at two wavelengths, 560 nm cell signal and 630 nm background signal.

For the LDH assay, the LDH-Glo™ cytotoxicity assay from Promega is used according to the manufacturers guidelines. LDH is very sensitive, so is performed on only one biological repeat with 3 technical repeats.

Experimental groups for MTT:

Each sample was tested on 3 technical repeats and two biological replicates (unless indicated differently). 0.2% Triton X-100 (TX-100) known to induce cell-apoptosis (Ahn *et al*., 1997) was used as an experimental positive control. To measure the effect of TX-100, at the end of the experiment, untreated cells were rinsed following the same method as other experimental groups and exposed to TX-100 for 15 minutes prior to an addition of MTT solution.

Cell survival rate expressed relative to untreated cell control, was calculated according to the following equation:

$$\text{Cell survival rate } (\%) = \frac{\Delta \text{ Absorbance} - \Delta \text{ Blank}}{\Delta \text{ Control} - \Delta \text{ Blank}} \text{ X } 100$$

Where,

Δ Absorbance = Absorbance at 560 nm – Absorbance at 630 nm

Δ Blank = DMSO blank at 560 nm – DMSO blank 630 nm

Δ Control = Untreated cells at 560 nm – Untreated cells at 630 nm

The selectivity index (SI), which indicates the cytotoxic selectivity (i.e. safety of the product) of natural products measured against cancer cell lines versus normal cells (Vero), was calculated using the following formula:

$$\text{Selective Index} = \frac{\text{IC50 of normal cells}}{\text{IC50 of cancer cells}}$$

Table 4.1 Seeding information of different cell lines

No.	Cell line	Cell line description	Passage no.	Seeding density (cells/ well x 10^3)
1	H69V	Human small cell lung carcinoma	10-17	8
2	143B	Human bone osteosarcoma	11-25	4
3	C3A	Human hepatocellular carcinoma	11-17	8
4	A375	Human skin melanoma	23-30	4
5	HT29	Human colon adenocarcinoma	147-149	10
6	MCF7	Human breast adenocarcinoma	8-13	10
7	Vero	African green monkey, kidney, non-cancer	10-20	4
8a	84BR	Human skin fibroblast, non-cancer	5	10
8b	BJ-5ta	Human skin fibroblasts hTERT immortalized, non-cancer	12-14	3
9	AGS	Human stomach adenocarcinoma	7-8	10
10	Clone 15 HL-60	Human acute promyelocytic leukemia	14	25
11	DU145	Human prostate carcinoma	63-66	5
12	A-704	Kidney adenocarcinoma	73-77	6
~~13~~	~~HeLa~~	~~Cervix adenocarcinoma~~		
13	A549	Human non-small cell lung carcinoma	8-11	3
14	K-562	Chronic myelogenous Leukemia (CML)	4-8	20
15	U-87 MG	Human Likely glioblastoma	134-138	8
~~16~~	~~U937~~	~~Histiocytic lymphoma~~		
16	BT-20	Triple negative invasive ductal human carcinoma breast cancer	27-29	6
17	Panc 02.03	Pancreas adenocarcinoma	26-28	8

5. Results and discussion

All 16 samples received have been fully assayed (n=6) on 14 cell lines that form part of this report.

All analysed samples were completely dissolved in DMSO, however, some products precipitated when dissolved in cell culture medium (see Table 5.1, below).

Table 5.1 Sample ID and solubility profile

No.	ID	DSMO solubility	Media solubility
1	RBME	Complete	Soluble
2	RBAC	Complete	Soluble
3	RBET	Complete	Soluble
4	LUENT	Complete	Soluble
5	RBMW	Complete	Soluble
6	YS2	Complete	Soluble
7	F6A-F5ZS	Complete	Soluble
8	G4/MUC	Complete	Soluble
9	G3W	Complete	Soluble
10	MESC-INO	Complete	Precipitate, 80%
11	YS4	Complete	Soluble
12	G5/MUC	Complete	Soluble
13	G1/W	Complete	Soluble
14	IN1	Complete	Soluble
15	YS5	Complete	Precipitate, 80%
16	F7NN	Complete	Precipitate, 80%

New results not included in Report 7

The results presented in this report (Report 8) include data that was not previously assayed (n=6) for the Panc 02.03 (human pancreas adenocarcinoma), as well selectivity indexes (against two non-cancerous controls: Vero and BJ-5ta cell lines) results.

This is the final report for the first project, and data and graphs were checked and finalized. Some changes may be present from the initial reports, the data in this report is the correct and final data.

5.1. Results for compound RBME

5.1.1. Vero (African green monkey – non-cancer control)

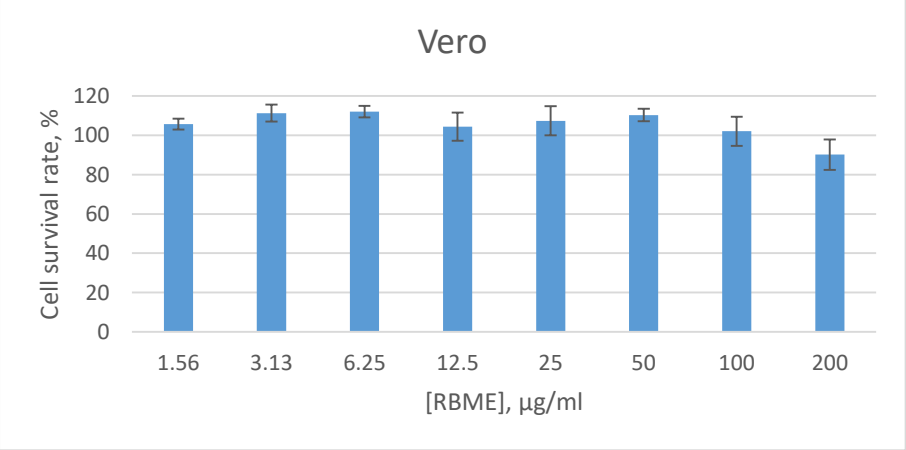

Figure 1.1: Cell survival following treatment of Vero cells for 72 h with RBME, as determined with the MTT assay (error bars = standard deviation, *n* = 6).

5.1.2 BJ-5ta (Human skin fibroblasts hTERT immortalized, non-cancer)

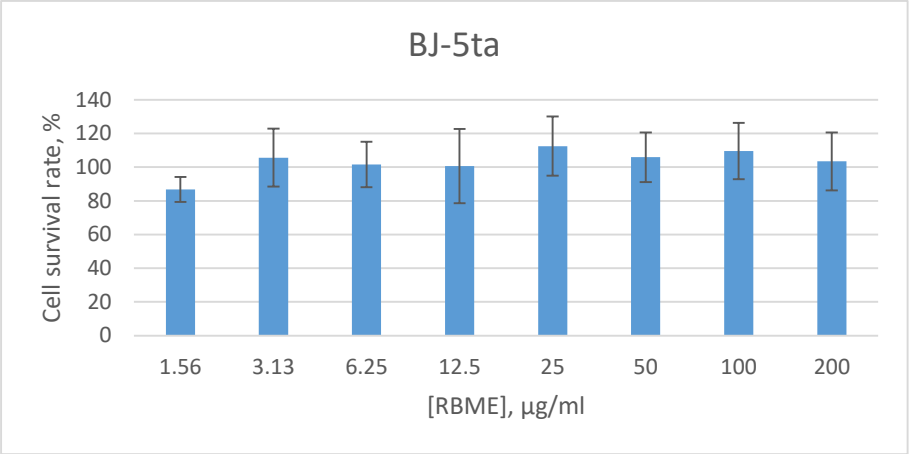

Figure 1.2: Cell survival following treatment of BJ-5ta cells for 72 h with RBME, as determined with the MTT assay (error bars = standard deviation, *n* = 6).

5.1.3. H69V (Human small cell lung carcinoma)

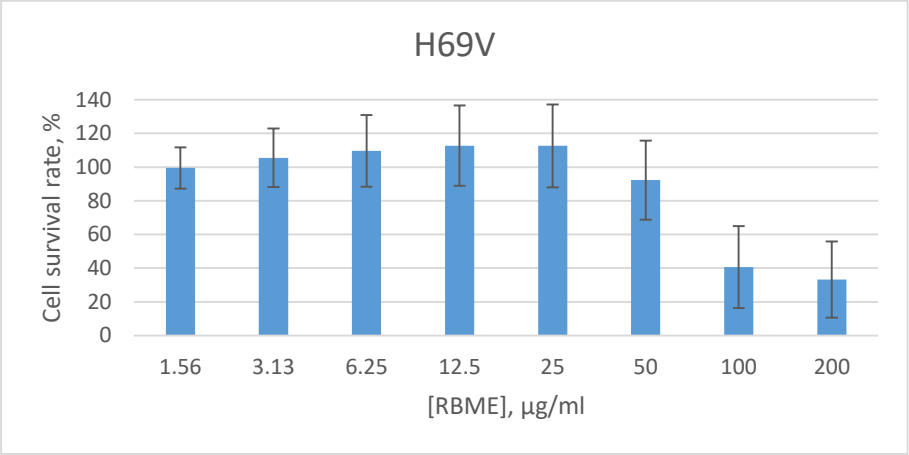

Figure 1.3: Cell survival following treatment of H69V cells for 72 h with RBME, as determined with the MTT assay (error bars = standard deviation, *n* = 6).

5.1.4. 143B (Human osteosarcoma)

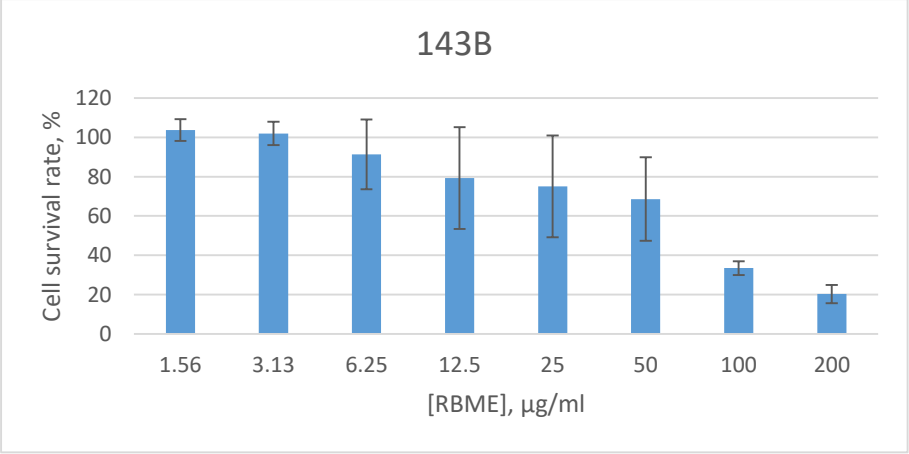

Figure 1.4: Cell survival following treatment 143B cells for 72 h with RBME, as determined with the MTT assay (error bars = standard deviation, *n* = 6).

5.1.5. HepG2/C3A (Human hepatocellular carcinoma)

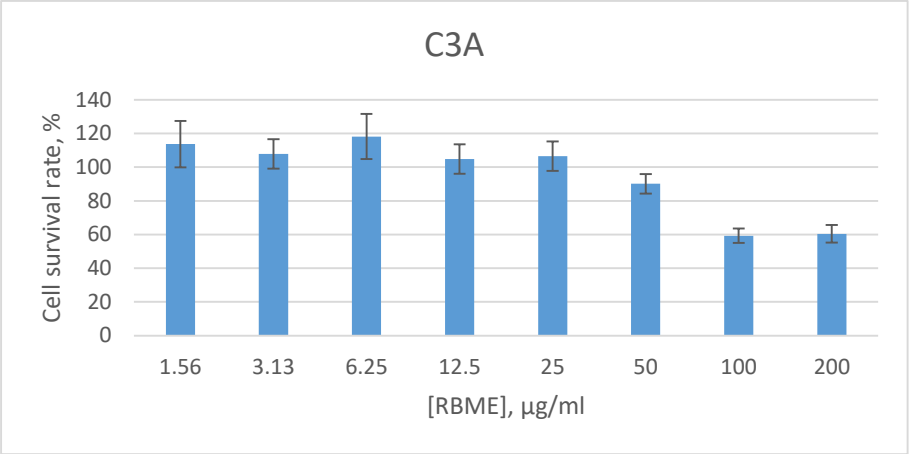

Figure 1.5: Cell survival following treatment of HepG2/C3A cells for 72 h with RBME, as determined with the MTT assay (error bars = standard deviation, *n* = 6).

5.1.6. A375 (Human melanoma)

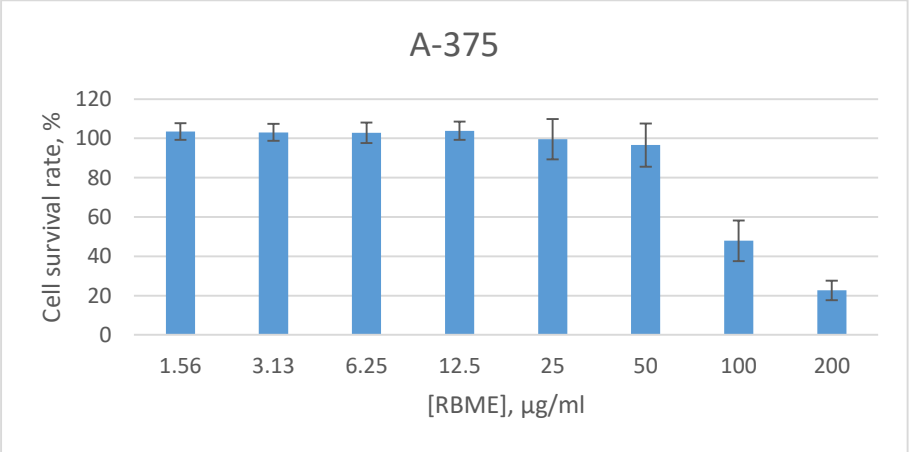

Figure 1.6: Cell survival following treatment of A375 cells for 72 h with RBME, as determined with the MTT assay (error bars = standard deviation, *n* = 6).

5.1.7. HT29 (Human colon carcinoma)

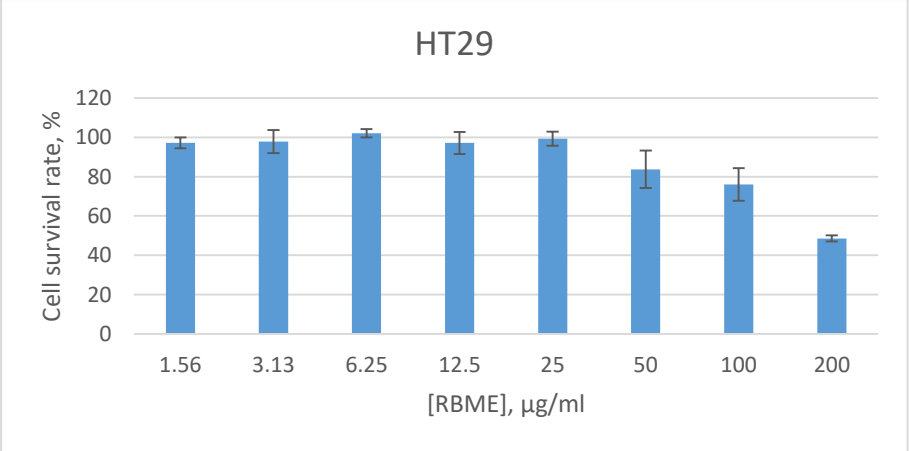

Figure 1.7: Cell survival following treatment of HT29 cells for 72 h with RBME, as determined with the MTT assay (error bars = standard deviation, *n* = 6).

5.1.8. MCF7 (Human breast adenocarcinoma)

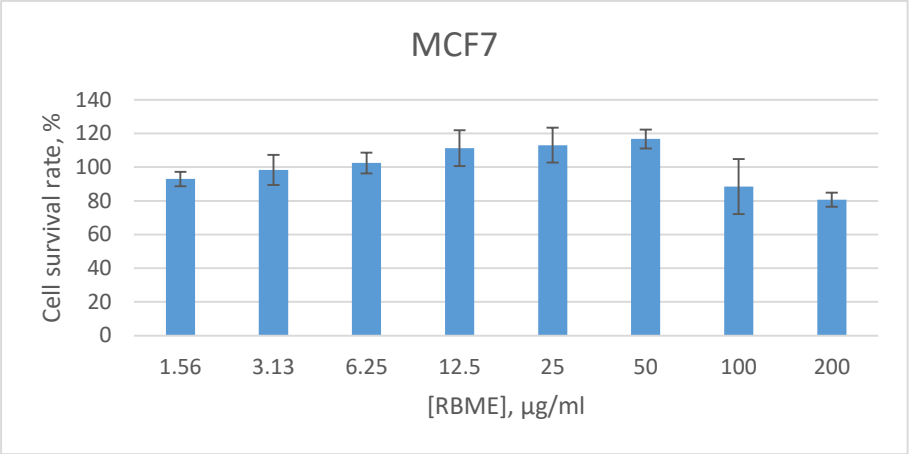

Figure 1.8: Cell survival following treatment of MCF7 cells for 72 h with RBME, as determined with the MTT assay (error bars = standard deviation, *n* = 6).

5.1.9 AGS (Human stomach adenocarcinoma)

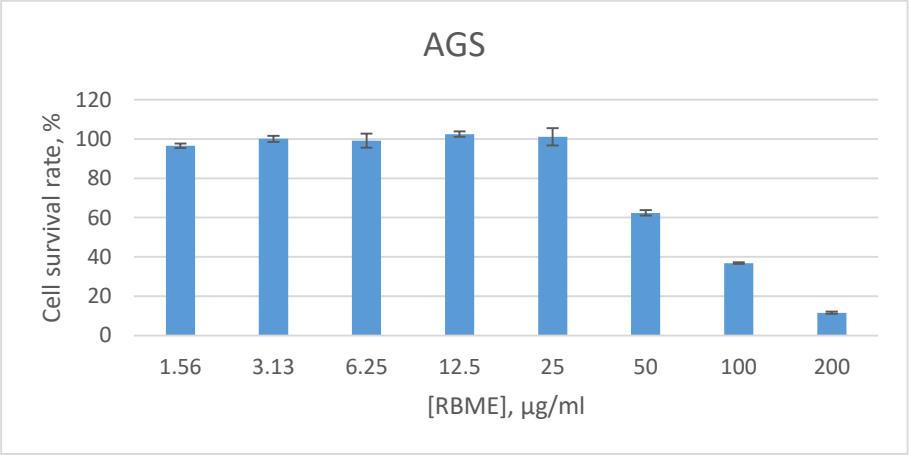

Figure 1.9: Cell survival following treatment of AGS cells for 72 h with RBME, as determined with the MTT assay (error bars = standard deviation, *n* = 6).

5.1.10. Clone 15 HL-60 (Human acute promyelocytic leukemia)

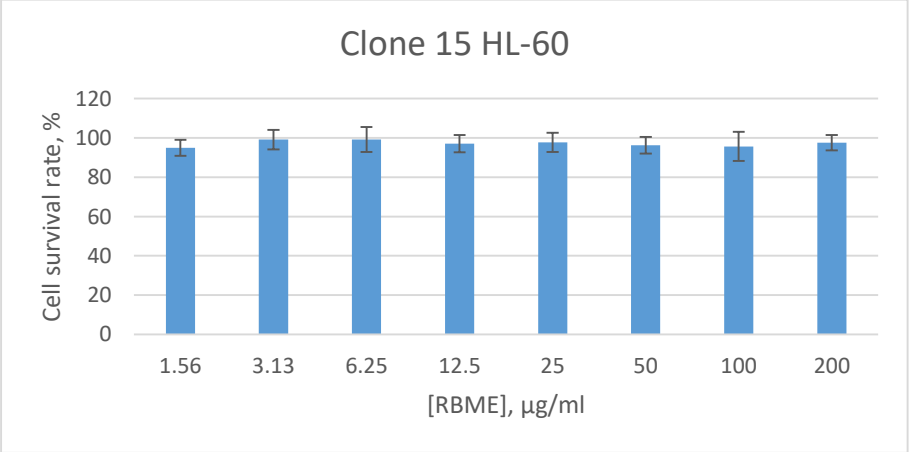

Figure 1.10: Cell survival following treatment of Clone 15 HL-60 cells for 72 h with RBME, as determined with the LDH assay (error bars = standard deviation, *n* = 3).

5.1.11. DU145 (Human prostate carcinoma)

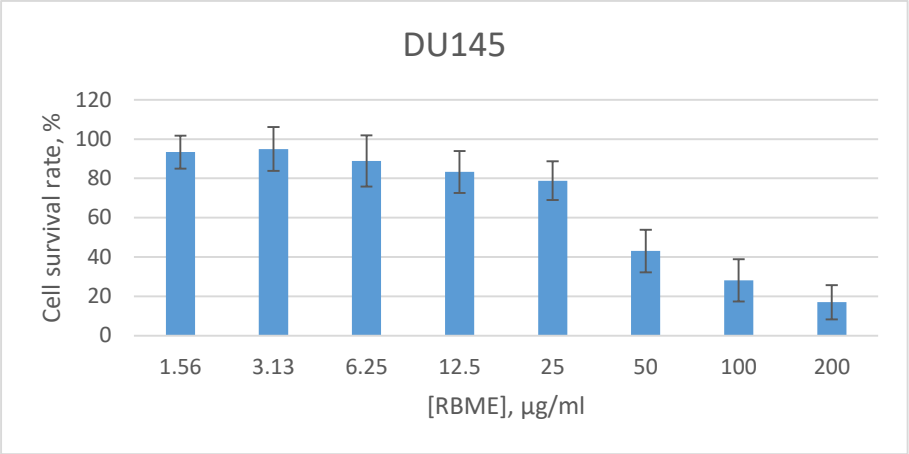

Figure 1.11: Cell survival following treatment of DU145 cells for 72 h with RBME, as determined with the MTT assay (error bars = standard deviation, *n* = 6).

5.1.12. A-704 (human kidney adenocarcinoma)

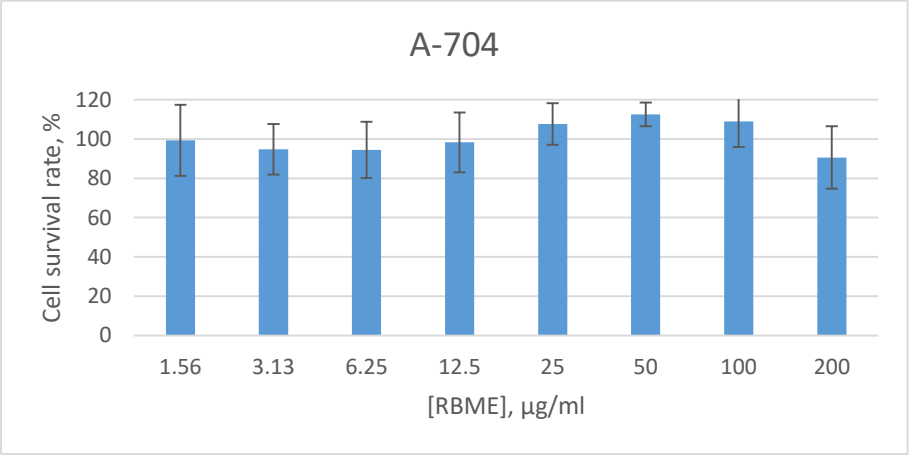

Figure 1.12: Cell survival following treatment of A-704 cells for 72 h with RBME, as determined with the MTT assay (error bars = standard deviation, *n* = 6).

5.1.13. K-562 (Human chronic myelogenous Leukemia)

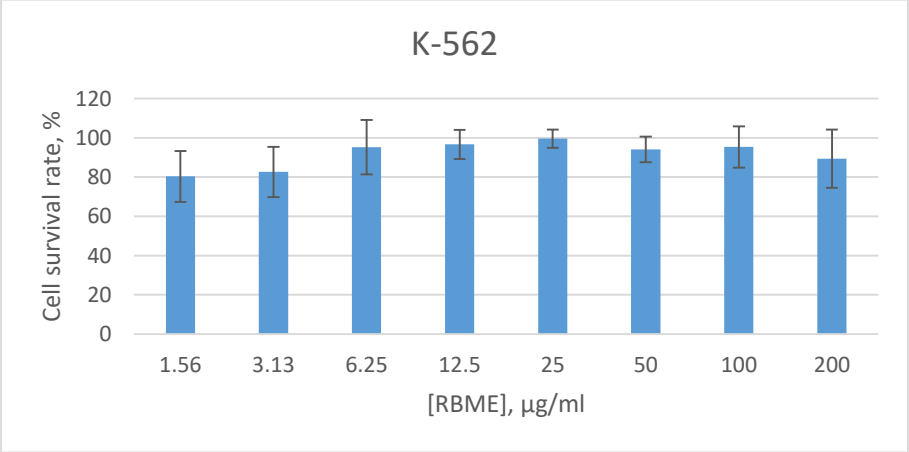

Figure 1.13A: Cell survival following treatment of K-562 cells for 72 h with RBME, as determined with the LDH assay (error bars = standard deviation, *n* = 3).

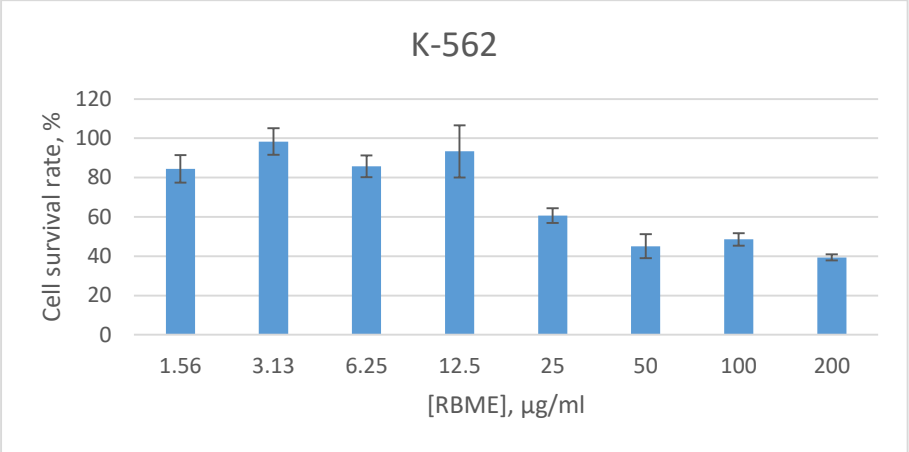

Figure 1.13B: Cell survival following treatment of K-562 cells for 72 h with RBME, as determined with the MTT assay (error bars = standard deviation, *n* = 3).

5.1.14. U-87 MG (Human glioblastoma)

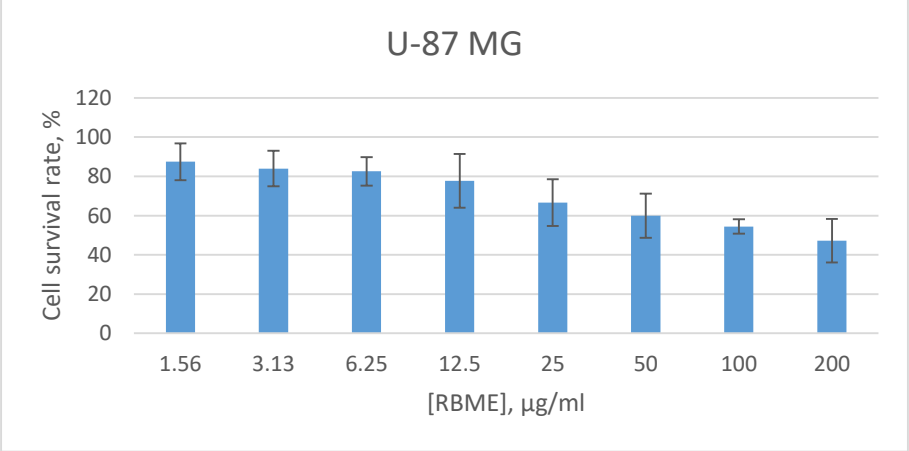

Figure 1.14: Cell survival following treatment of U-87 MG cells for 72 h with RBME, as determined with the MTT assay (error bars = standard deviation, *n* = 6).

5.1.15. A-549 (Human non-small cell lung carcinoma)

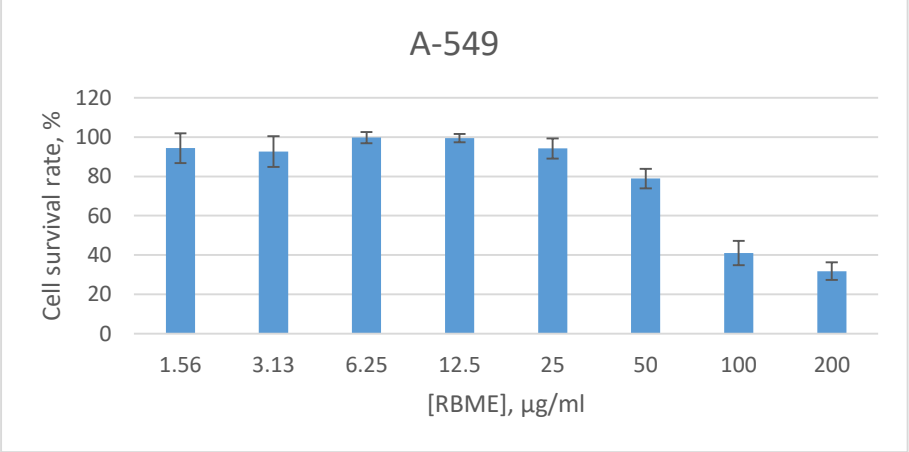

Figure 1.15: Cell survival following treatment of A-549 cells for 72 h with RBME, as determined with the MTT assay (error bars = standard deviation, *n* = 6).

5.1.16. BT-20 (Human triple negative invasive ductal human carcinoma breast cancer)

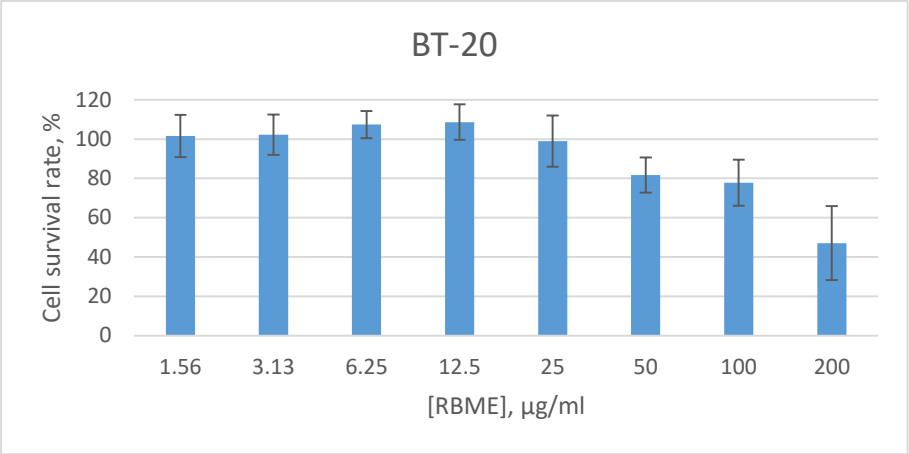

Figure 1.16: Cell survival following treatment of BT-20 cells for 72 h with RBME, as determined with the MTT assay (error bars = standard deviation, *n* = 6).

5.1.17. Panc 02.03 (Human pancreas adenocarcinoma)

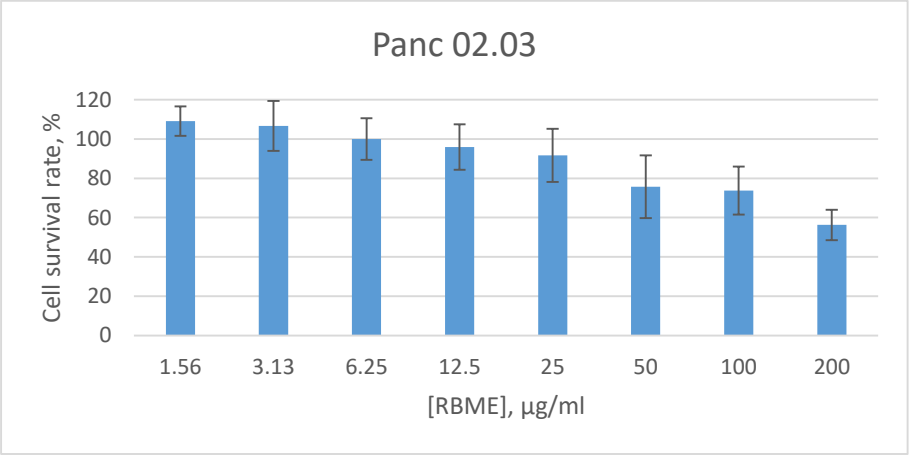

Figure 1.17: Cell survival following treatment of Panc 02.03 cells for 72 h with RBME, as determined with the MTT assay (error bars = standard deviation, *n* = 6).

Discussion for compound RBME results:

RBME does not appear to be toxic in the non-cancerous Vero or BJ-5ta cell lines, with almost no reduction in cell viability after 72 h exposure to RBME. Low concentrations seem to induce cell growth in several of the cancer cell lines tested, with viability increased to more than that of the untreated control. Inhibition of cell viability was observed at higher concentrations of RBME, with the most notable effects observed in H69V, 143B, A375, AGS, DU145, U-87 MG, A549, BT-20 and Panc 02.03 cell lines. Some activity was also observed in the K-562 cell line, although not completely trustworthy.

5.2. Results for compound RBAC

5.2.1. Vero (African green monkey – non-cancer control)

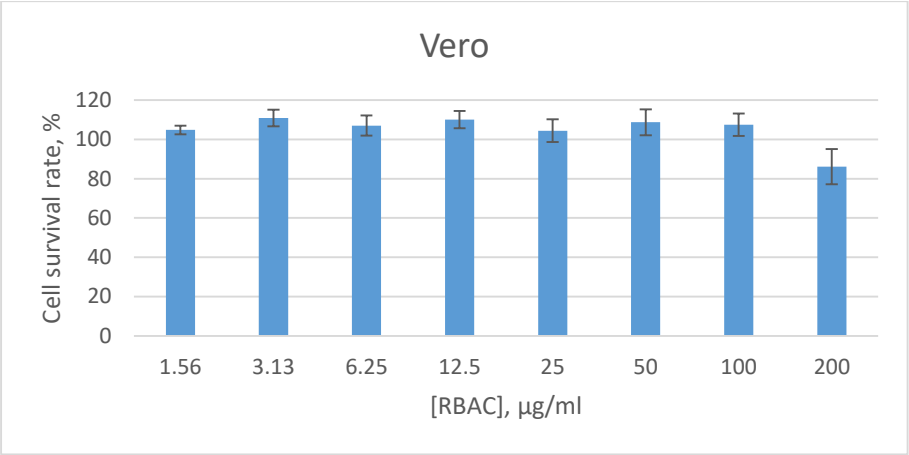

Figure 2.1: Cell survival following treatment of Vero cells for 72 h with RBAC, as determined with the MTT assay (error bars = standard deviation, *n* = 6).

5.2.2 BJ-5ta (Human skin fibroblasts hTERT immortalized, non-cancer)

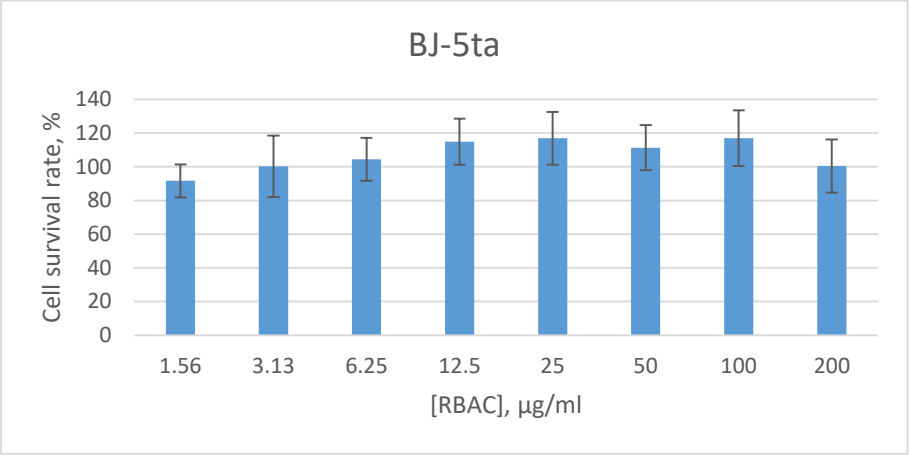

Figure 2.2: Cell survival following treatment of BJ-5ta cells for 72 h with RBAC, as determined with the MTT assay (error bars = standard deviation, *n* = 6).

5.2.3. H69V (Human small cell lung carcinoma)

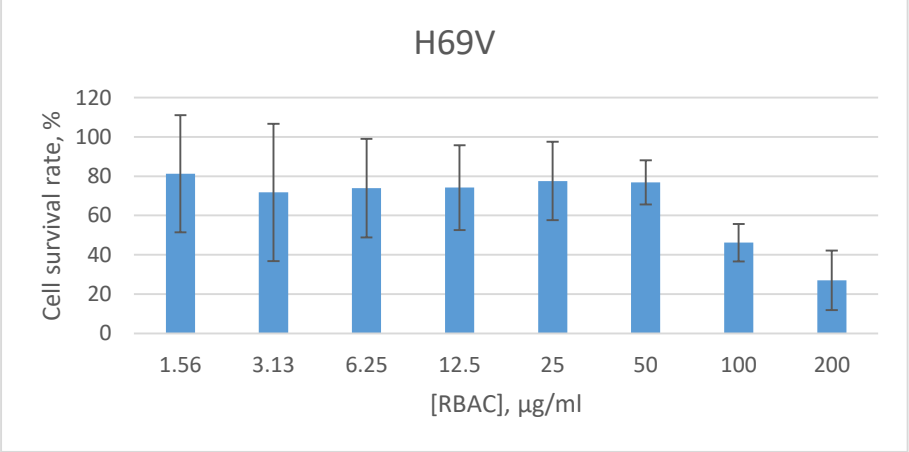

Figure 2.3: Cell survival following treatment of H69V cells for 72 h with RBAC, as determined with the MTT assay (error bars = standard deviation, *n* = 6).

5.2.4. 143B (Human osteosarcoma)

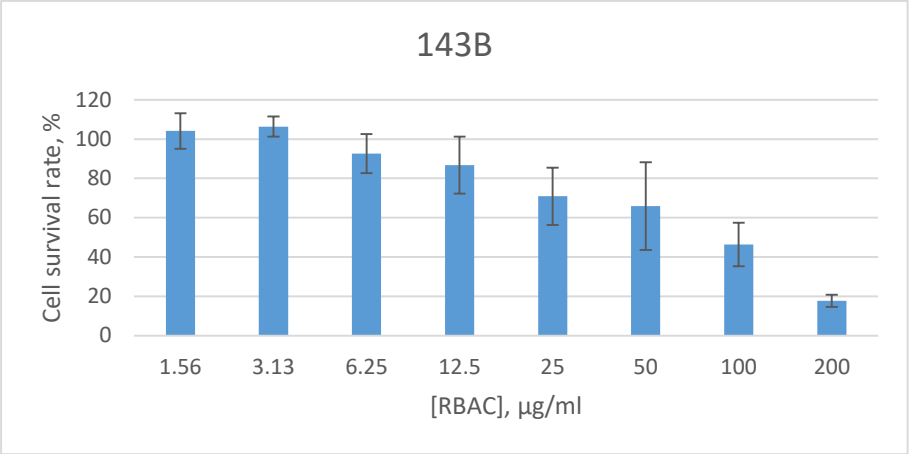

Figure 2.4: Cell survival following treatment of 143B cells for 72 h with RBAC, as determined with the MTT assay (error bars = standard deviation, *n* = 6).

5.2.5. HepG2/C3A (Human hepatocellular carcinoma)

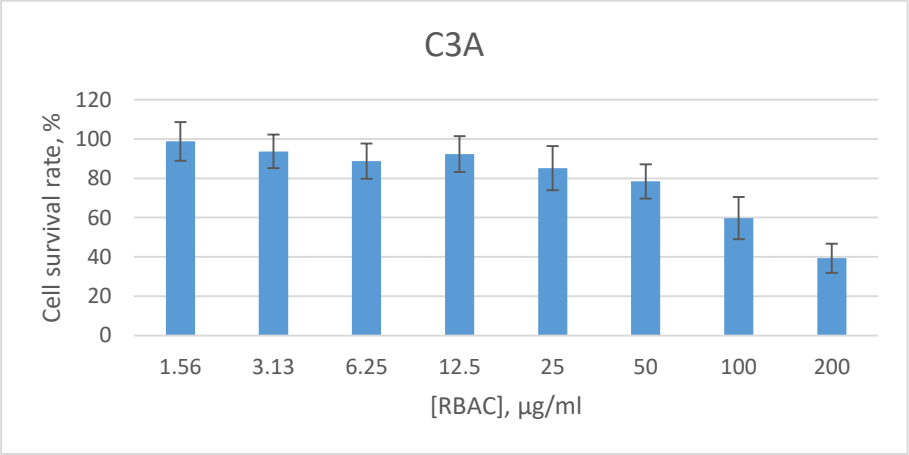

Figure 2.5: Cell survival following treatment of HepG2/C3A cells for 72 h with RBAC, as determined with the MTT assay (error bars = standard deviation, *n* = 6).

5.2.6. A375 (Human melanoma)

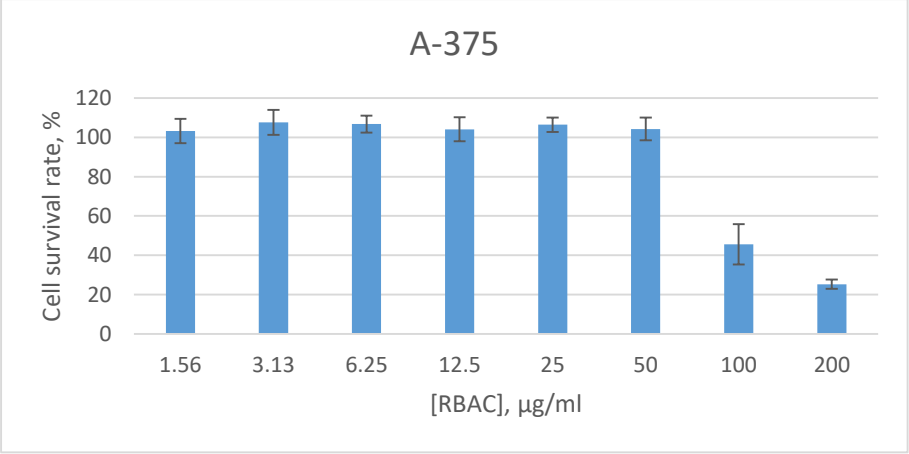

Figure 2.6: Cell survival following treatment of A375 cells for 72 h with RBAC, as determined with the MTT assay (error bars = standard deviation, *n* = 6).

5.2.7. HT29 (Human colon carcinoma)

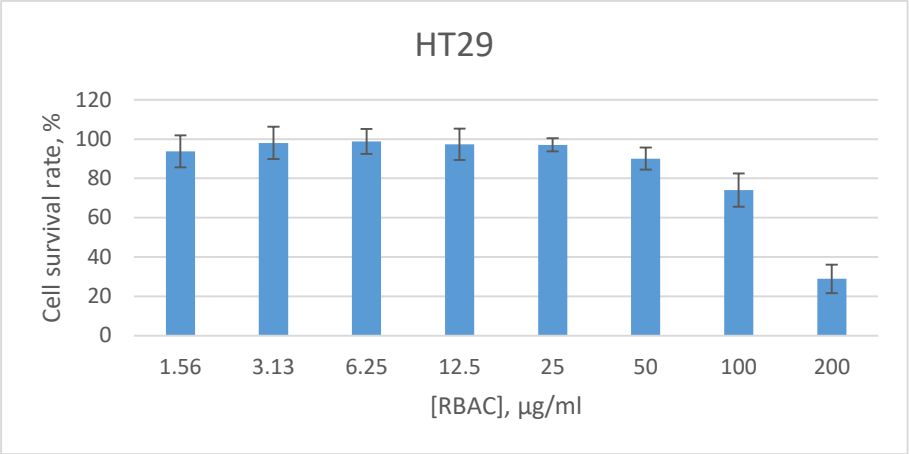

Figure 2.7: Cell survival following treatment of HT29 cells for 72 h with RBAC, as determined with the MTT assay (error bars = standard deviation, *n* = 6).

5.2.8. MCF7 (Human breast adenocarcinoma)

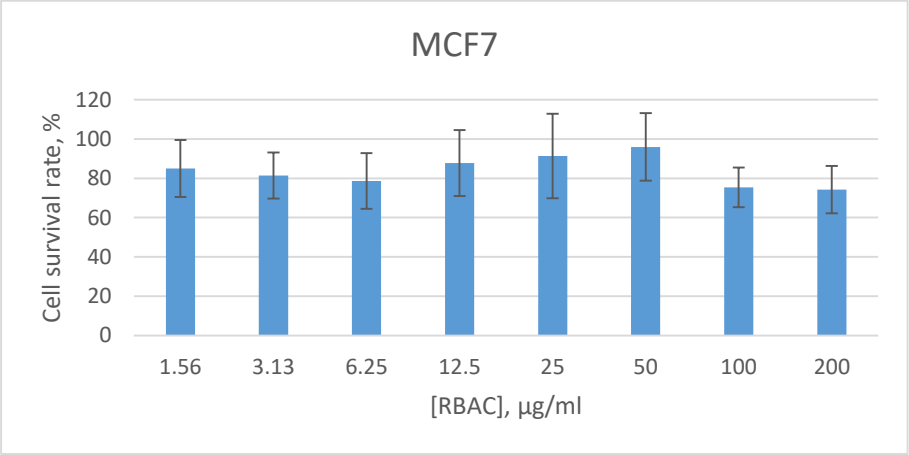

Figure 2.8: Cell survival following treatment of MCF7 cells for 72 h with RBAC, as determined with the MTT assay (error bars = standard deviation, *n* = 6)

5.2.9. AGS (Human stomach adenocarcinoma)

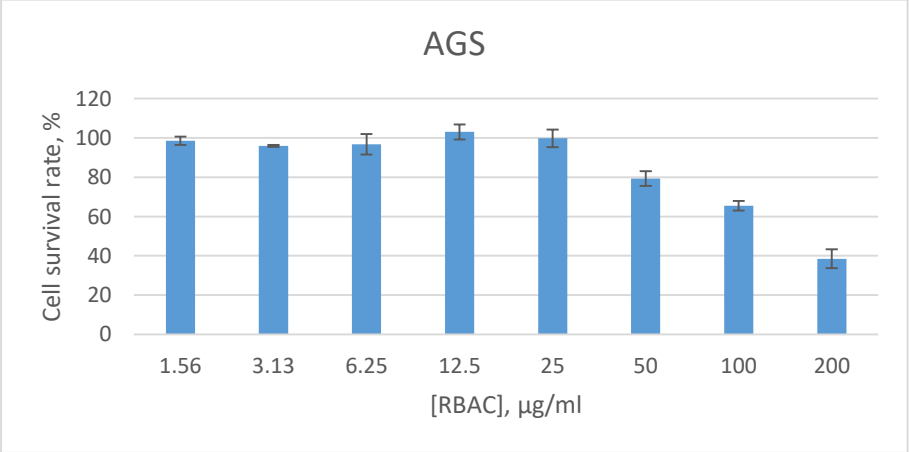

Figure 2.9: Cell survival following treatment of AGS cells for 72 h with RBAC, as determined with the MTT assay (error bars = standard deviation, *n* = 6).

5.2.10. Clone 15 HL-60 (Human acute promyelocytic leukemia)

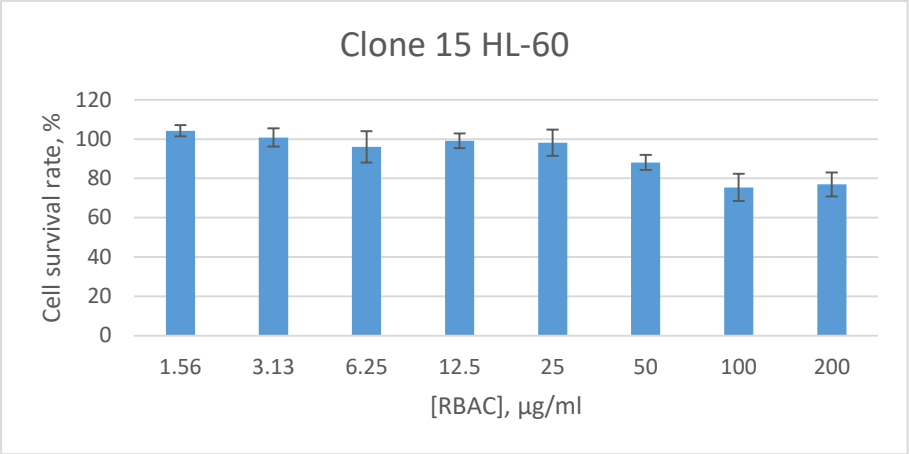

Figure 2.10: Cell survival following treatment of Clone 15 HL-60 cells for 72 h with RBAC, as determined with the LDH assay (error bars = standard deviation, *n* = 3).

5.2.11. DU145 (Human prostate carcinoma)

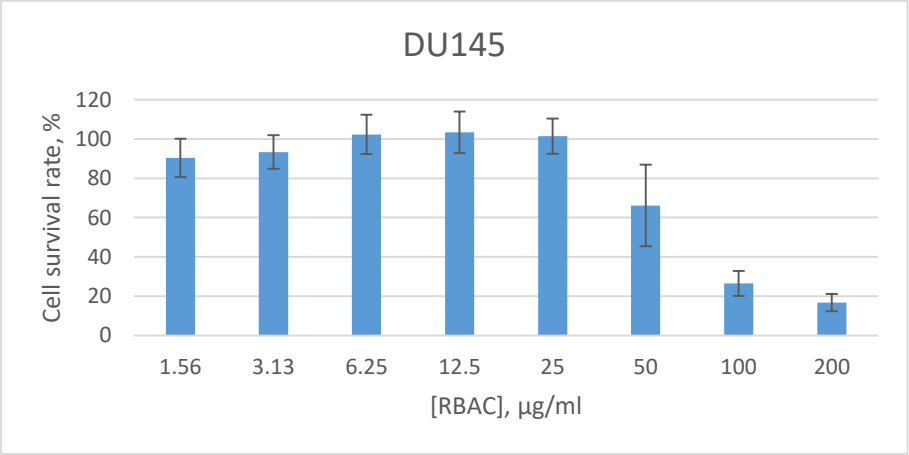

Figure 2.11: Cell survival following treatment of DU145 cells for 72 h with RBAC, as determined with the MTT assay (error bars = standard deviation, *n* = 6).

5.2.12. A-704 (human kidney adenocarcinoma)

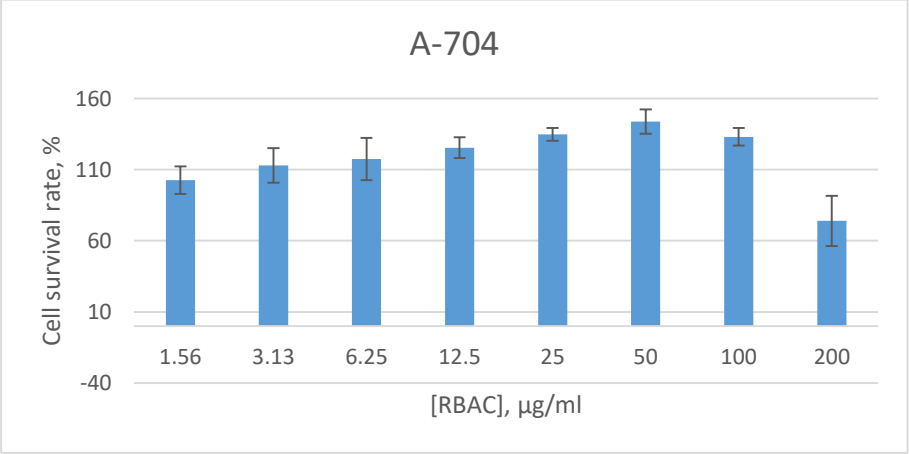

Figure 2.12: Cell survival following treatment of A-704 cells for 72 h with RBAC, as determined with the MTT assay (error bars = standard deviation, *n* = 6).

5.2.13. K-562 (Human chronic myelogenous Leukemia)

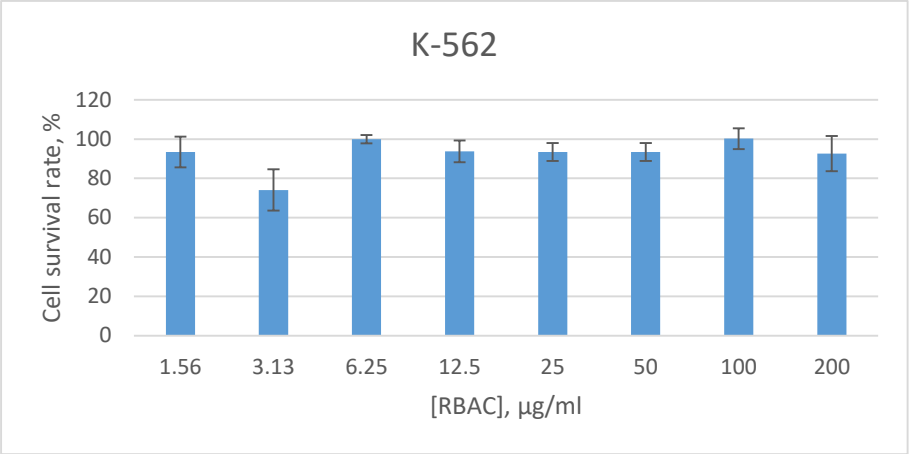

Figure 2.13A: Cell survival following treatment of K-562 cells for 72 h with RBAC, as determined with the LDH assay (error bars = standard deviation, *n* = 3).

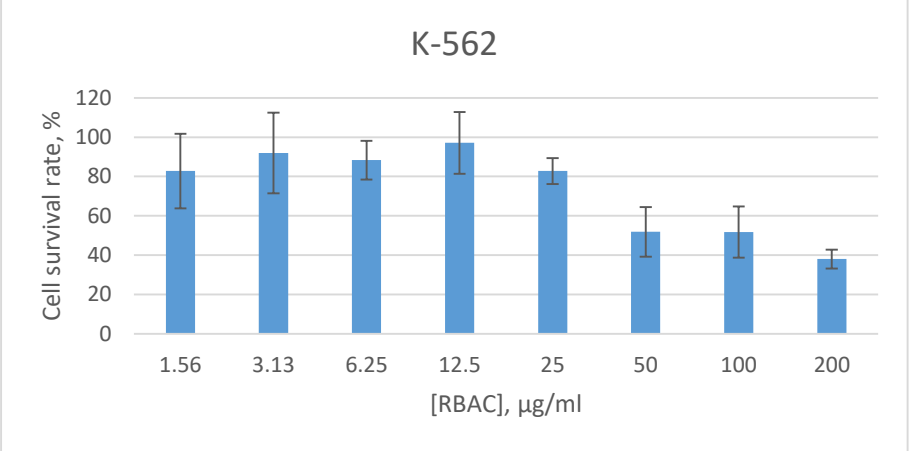

Figure 2.13B: Cell survival following treatment of K-562 cells for 72 h with RBAC, as determined with the MTT assay (error bars = standard deviation, *n* = 3).

5.2.14. U-87 MG (Human glioblastoma)

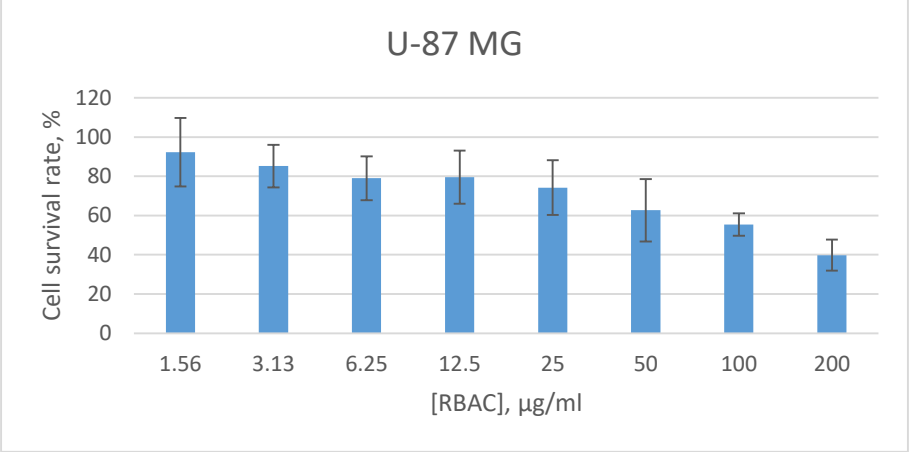

Figure 2.14: Cell survival following treatment of U-87 MG cells for 72 h with RBAC, as determined with the MTT assay (error bars = standard deviation, *n* = 6).

5.2.15. A-549 (Human non-small cell lung carcinoma)

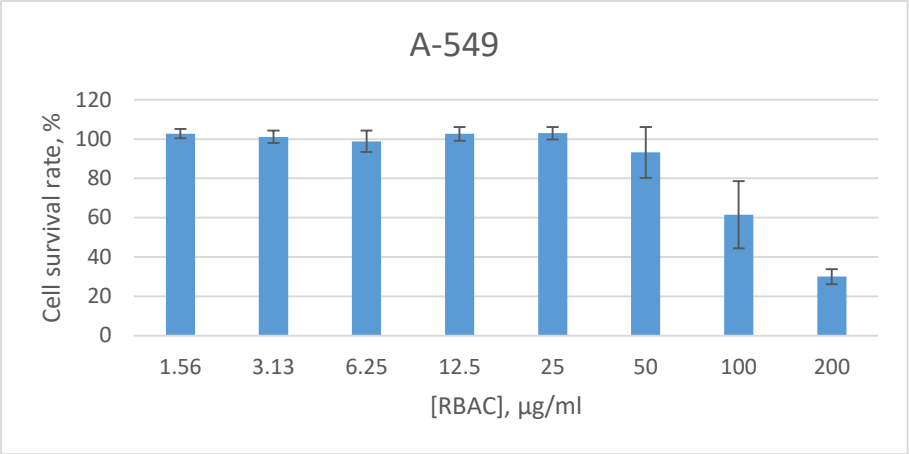

Figure 2.15: Cell survival following treatment of A-549 cells for 72 h with RBAC, as determined with the MTT assay (error bars = standard deviation, *n* = 6).

5.2.16. BT-20 (Human triple negative invasive ductal human carcinoma breast cancer)

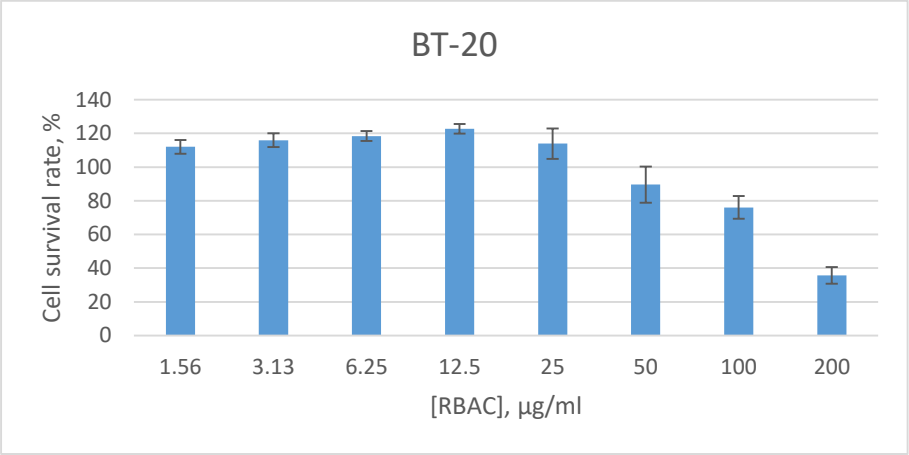

Figure 2.16: Cell survival following treatment of BT-20 cells for 72 h with RBAC, as determined with the MTT assay (error bars = standard deviation, *n* = 6).

5.2.17. Panc 02.03 (Human pancreas adenocarcinoma)

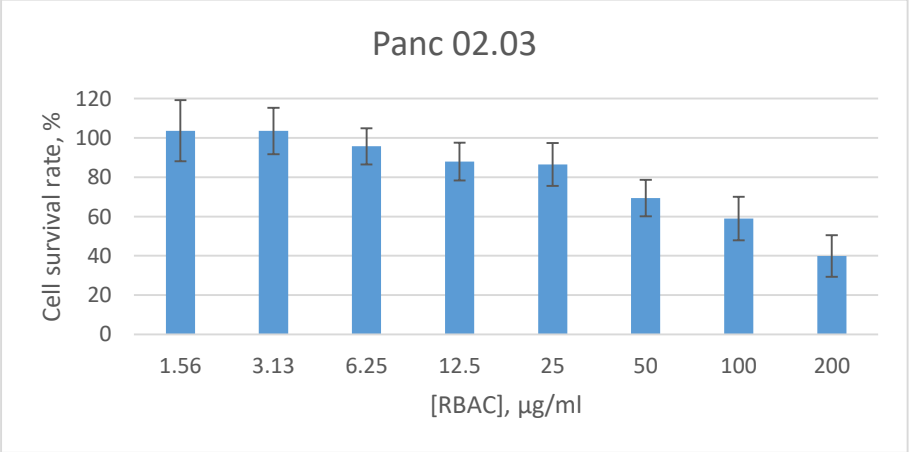

Figure 2.17: Cell survival following treatment of Panc 02.03 cells for 72 h with RBAC, as determined with the MTT assay (error bars = standard deviation, *n* = 6).

Discussion for compound RBAC results:

RBAC does not appear to be toxic in the non-cancerous Vero or BJ-5ta cell line, with almost no reduction in cell viability after 72 h exposure to RBAC, except at the highest dose of 200 ug/ml. Low concentrations seem to induce cell growth in several of the cancer cell lines tested, with viability increased to more than that of the untreated control. Inhibition of cell viability was observed at higher concentrations of RBME, with the most notable effects observed in H69V, 143B, HepG2/C3A, A375, DU145, U-87 MG, A549, BT-20 and Panc 02.03 cell lines. RBAC appears to have very good potential against the 143B osteosarcoma cancer line, as well as the DU145 prostate carcinoma cell line. Some activity was also observed in the K-562 cell line, although not completely trustworthy.

5.3. Results for compound RBET

5.3.1. Vero (African green monkey – non-cancer control)

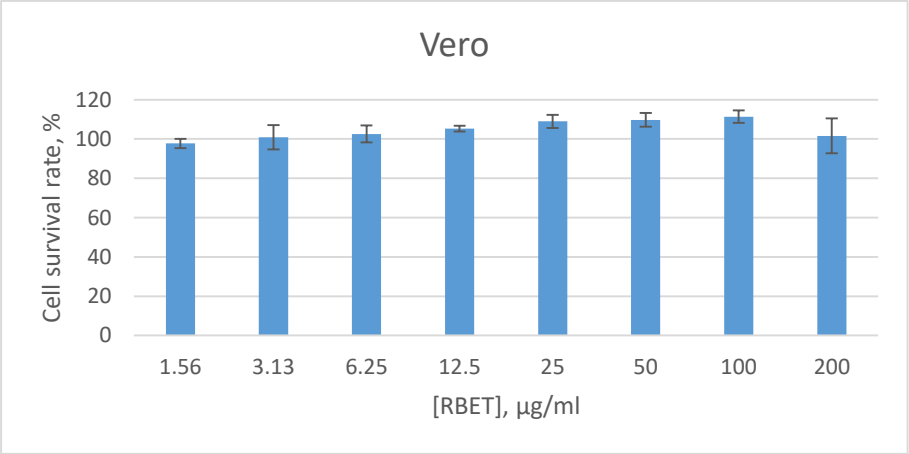

Figure 3.1: Cell survival following treatment of Vero cells for 72 h with RBET, as determined with the MTT assay (error bars = standard deviation, *n* = 6).

5.3.2 BJ-5ta (Human skin fibroblasts hTERT immortalized, non-cancer)

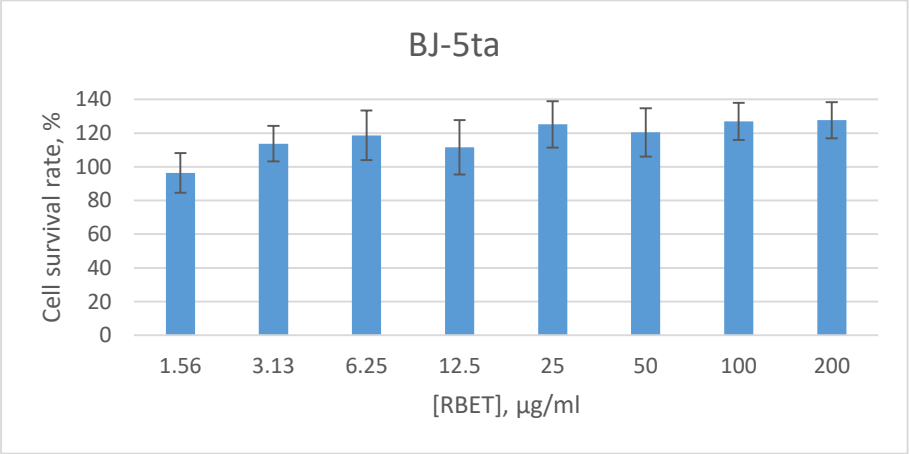

Figure 3.2: Cell survival following treatment of BJ-5ta cells for 72 h with RBET, as determined with the MTT assay (error bars = standard deviation, *n* = 6).

5.3.3. H69V (Human small cell lung carcinoma)

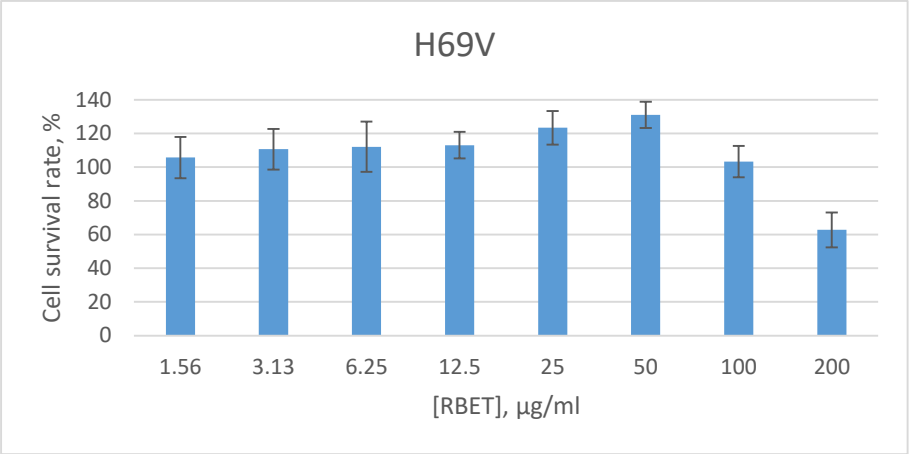

Figure 3.3: Cell survival following treatment of H69V cells for 72 h with RBET, as determined with the MTT assay (error bars = standard deviation, *n* = 6).

5.3.4. 143B (Human osteosarcoma)

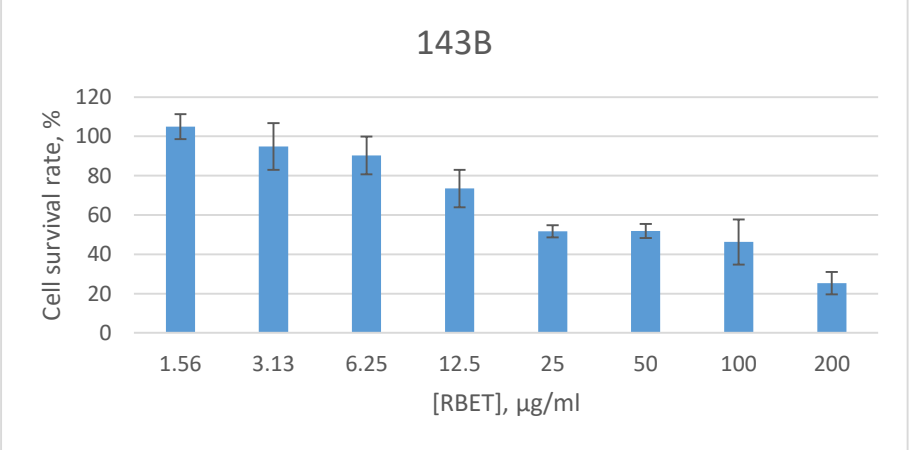

Figure 3.4: Cell survival following treatment of 143B cells for 72 h with RBET, as determined with the MTT assay (error bars = standard deviation, *n* = 6).

5.3.5. HepG2/C3A (Human hepatocellular carcinoma)

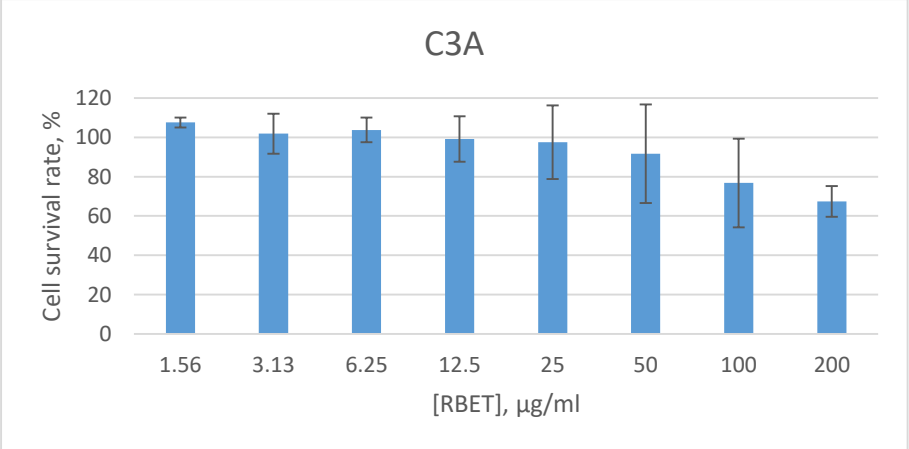

Figure 3.5: Cell survival following treatment of HepG2/C3A cells for 72 h with RBET, as determined with the MTT assay (error bars = standard deviation, *n* = 6).

5.3.6. A375 (Human melanoma)

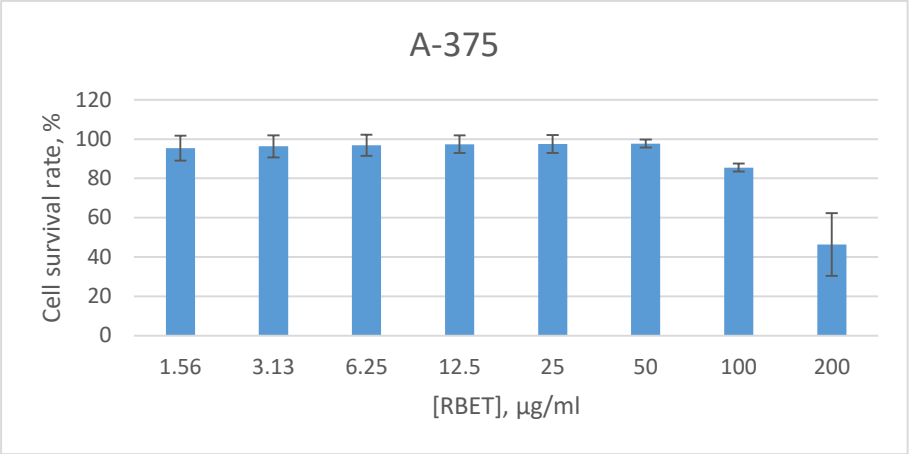

Figure 3.6: Cell survival following treatment of A375 cells for 72 h with RBET, as determined with the MTT assay (error bars = standard deviation, *n* = 6).

5.3.7. HT29 (Human colon carcinoma)

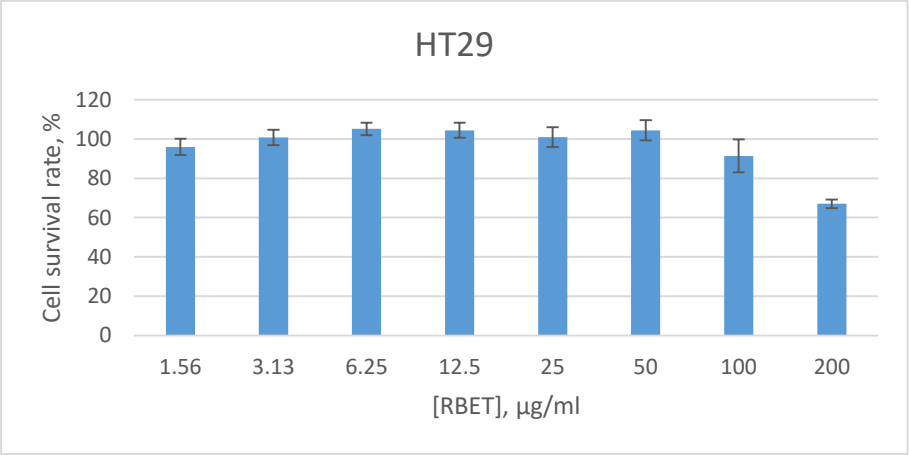

Figure 3.7: Cell survival following treatment of HT29 cells for 72 h with RBET, as determined with the MTT assay (error bars = standard deviation, *n* = 6).

5.3.8. MCF7 (Human breast adenocarcinoma)

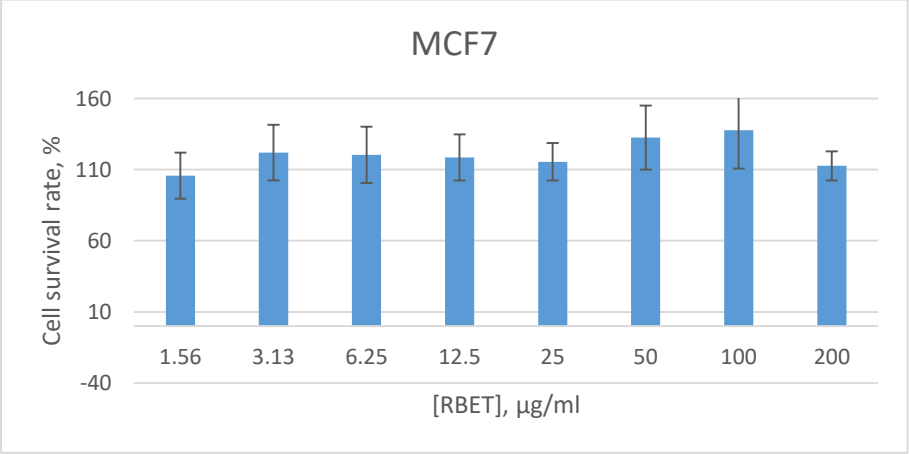

Figure 3.8: Cell survival following treatment of MCF7 cells for 72 h with RBET, as determined with the MTT assay (error bars = standard deviation, *n* = 6).

5.3.9. AGS (Human stomach adenocarcinoma)

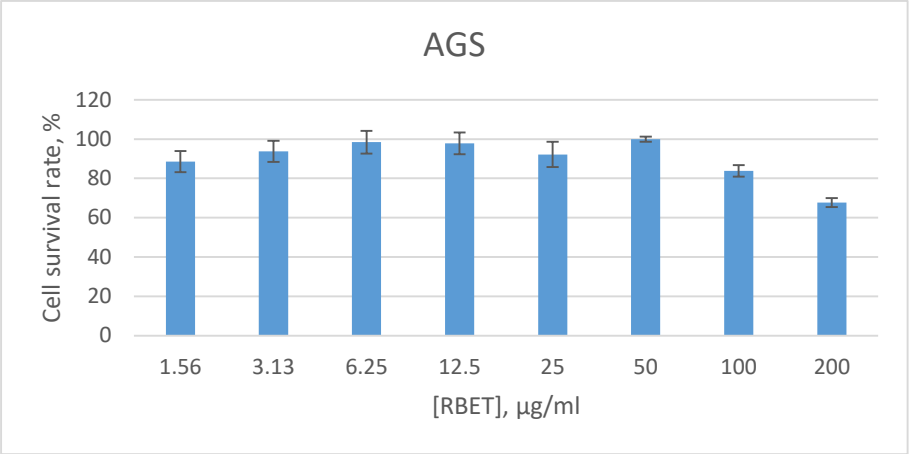

Figure 3.9: Cell survival following treatment of AGS cells for 72 h with RBET, as determined with the MTT assay (error bars = standard deviation, *n* = 6).

5.3.10. Clone 15 HL-60 (Human acute promyelocytic leukemia)

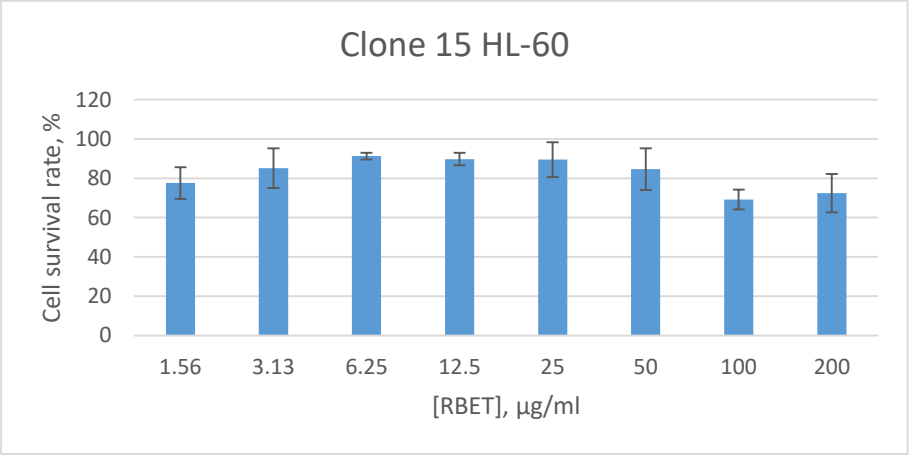

Figure 3.10: Cell survival following treatment of Clone 15 HL-60 cells for 72 h with RBET, as determined with the LDH assay (error bars = standard deviation, *n* = 3).

5.3.11. DU145 (Human prostate carcinoma)

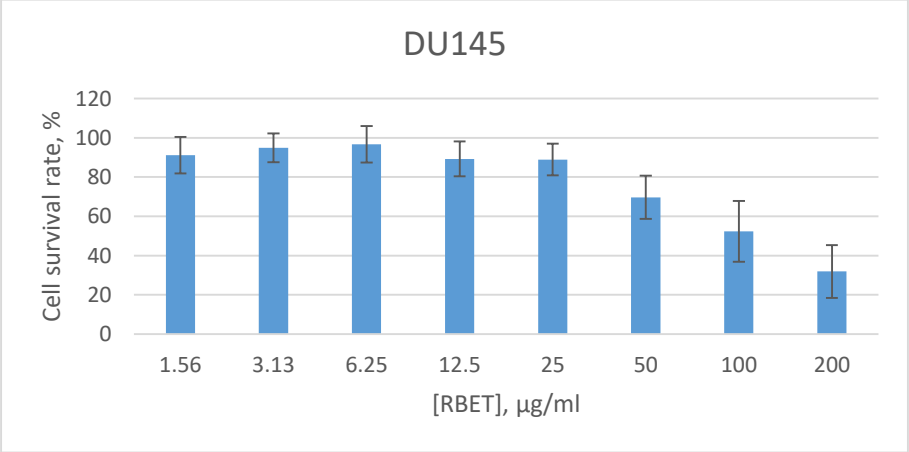

Figure 3.11: Cell survival following treatment of DU145 cells for 72 h with RBET, as determined with the MTT assay (error bars = standard deviation, *n* = 6).

5.3.12. A-704 (human kidney adenocarcinoma)

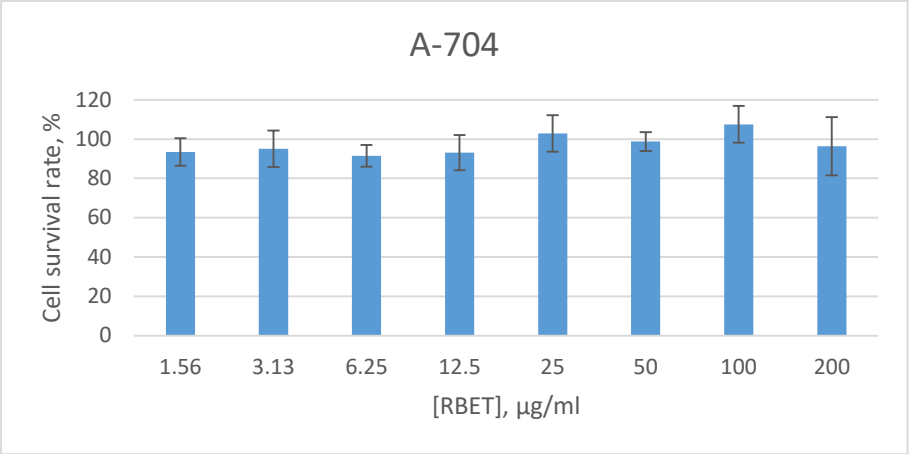

Figure 3.12: Cell survival following treatment of A-704 cells for 72 h with RBET, as determined with the MTT assay (error bars = standard deviation, *n* = 6).

5.3.13. K-562 (Human chronic myelogenous Leukemia)

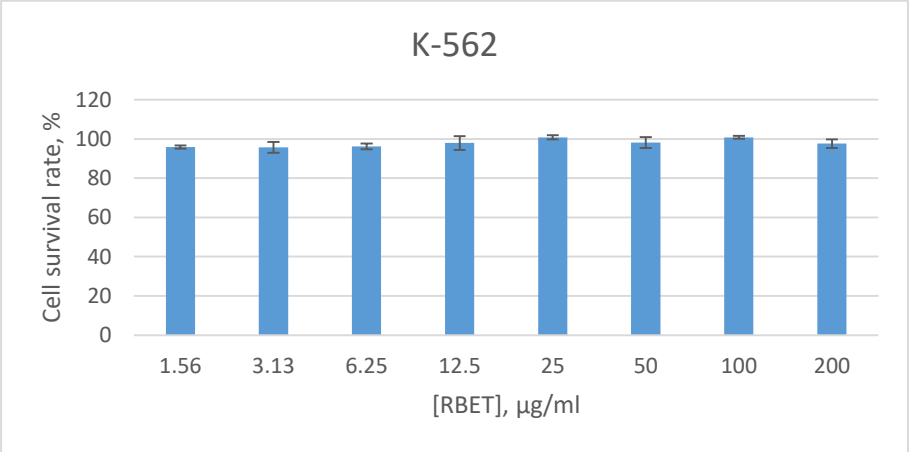

Figure 3.13A: Cell survival following treatment of K-562 cells for 72 h with RBET, as determined with the LDH assay (error bars = standard deviation, *n* = 3).

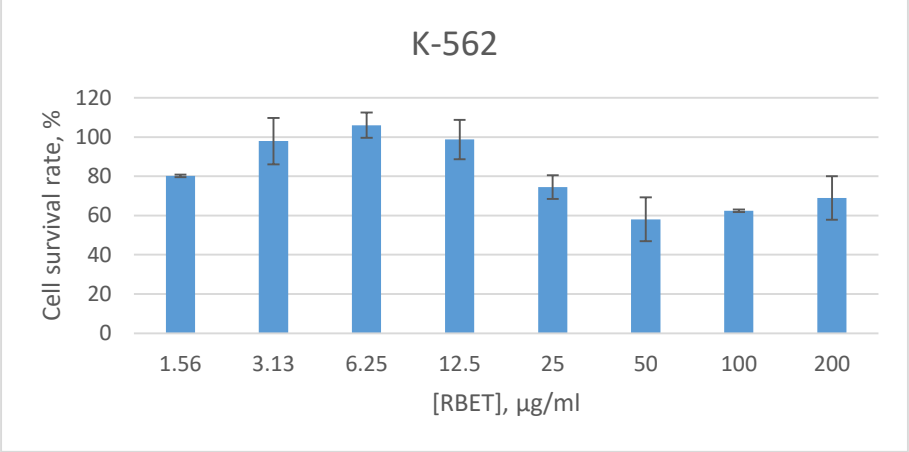

Figure 3.13B: Cell survival following treatment of K-562 cells for 72 h with RBET, as determined with the MTT assay (error bars = standard deviation, *n* = 3).

5.3.14. U-87 MG (Human glioblastoma)

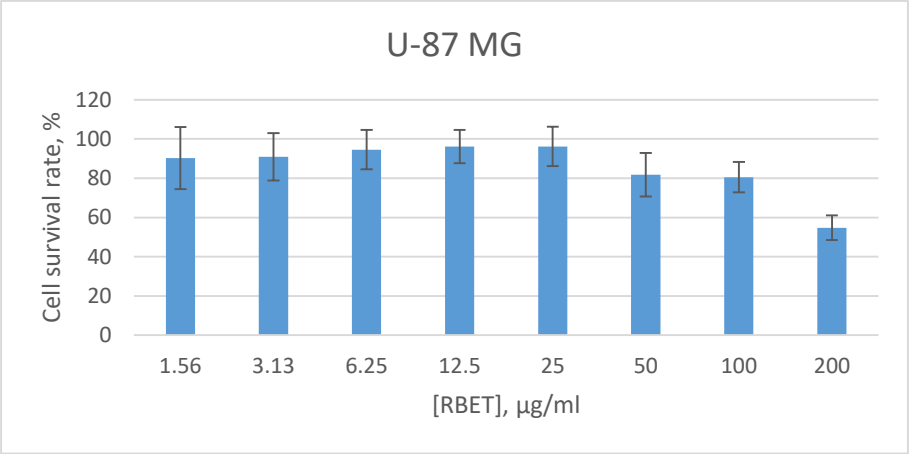

Figure 3.14: Cell survival following treatment of U-87 MG cells for 72 h with RBET, as determined with the MTT assay (error bars = standard deviation, *n* = 6).

5.3.15. A-549 (Human non-small cell lung carcinoma)

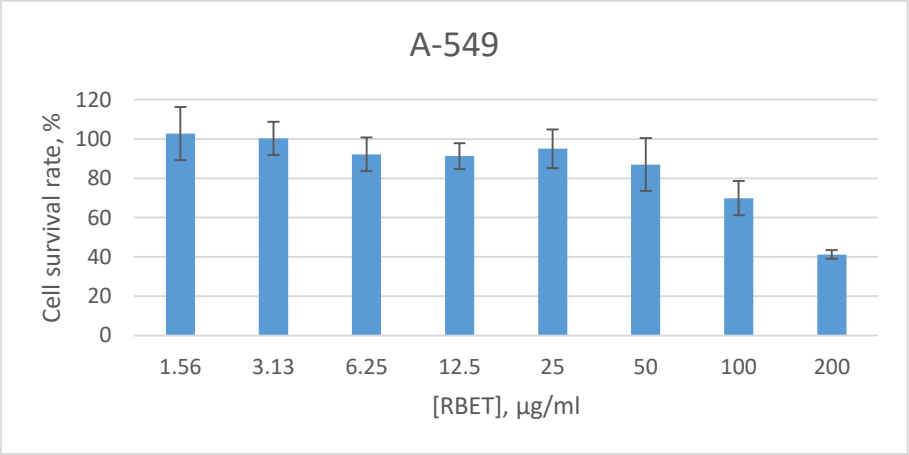

Figure 3.15: Cell survival following treatment of A-549 cells for 72 h with RBET, as determined with the MTT assay (error bars = standard deviation, *n* = 6).

5.3.16. BT-20 (Human triple negative invasive ductal human carcinoma breast cancer)

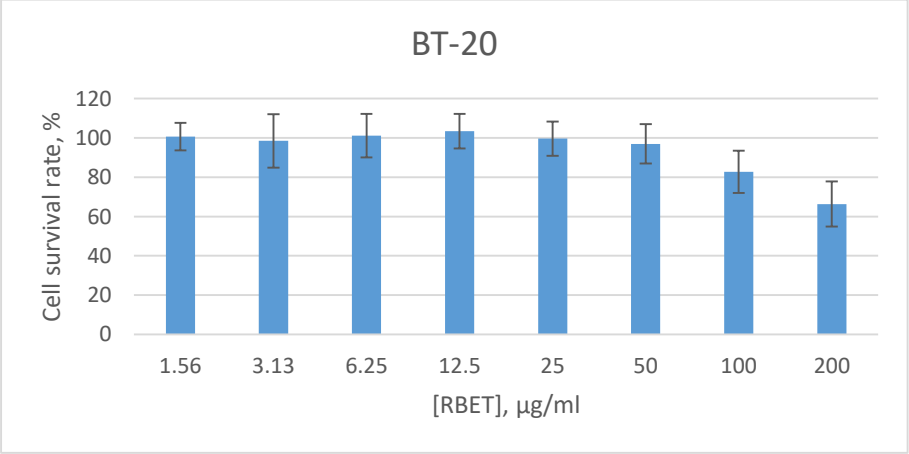

Figure 3.16: Cell survival following treatment of BT-20 cells for 72 h with RBET, as determined with the MTT assay (error bars = standard deviation, *n* = 6).

5.3.17. Panc 02.03 (Human pancreas adenocarcinoma)

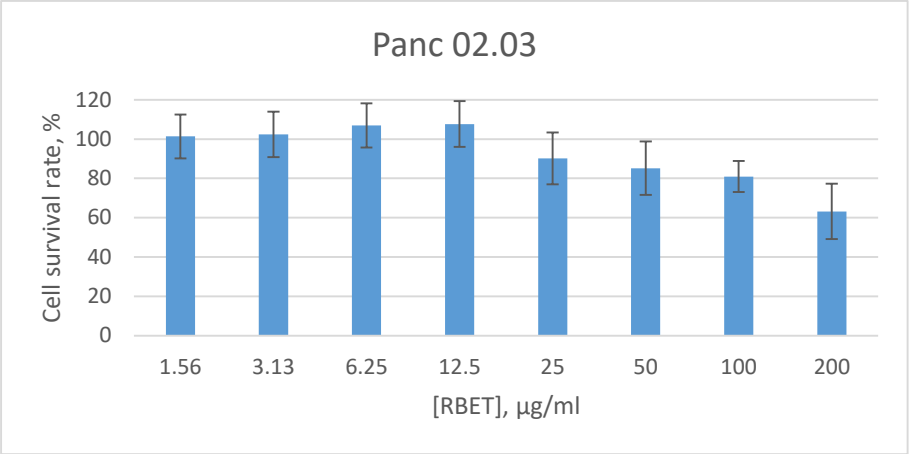

Figure 3.17: Cell survival following treatment of Panc 02.03 cells for 72 h with RBET, as determined with the MTT assay (error bars = standard deviation, *n* = 6).

Discussion for compound RBET results:

RBET does not appear to be toxic in the non-cancerous Vero and BJ-5ta cell lines, with no reduction in cell viability observed after 72 h exposure. Rather, the compound appeared to increase non-cancerous fibroblast proliferation. *Low concentrations seem to induce cell growth in several of the cancer cell lines tested, with viability increased to more than that of the untreated control. Inhibition of cell viability was observed at higher concentrations of RBET, A549, BT-20 and Panc 02.03, with the most notable effects observed in the 143B and DU145 cell lines. RBET appears to have very good potential against these cell lines. Some activity was also observed in the K-562 cell line, although not completely trustworthy.*

5.4. Results for compound LUENT

5.4.1. Vero (African green monkey – non-cancer control)

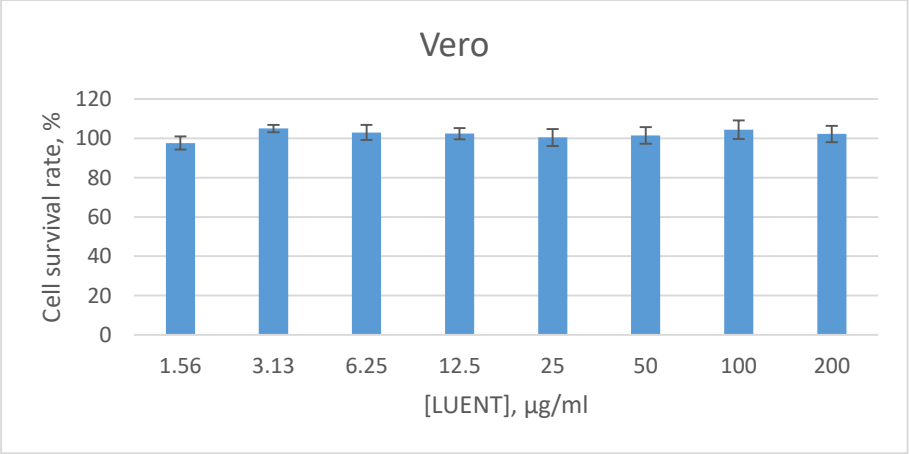

Figure 4.1: Cell survival following treatment of Vero cells for 72 h with LUENT, as determined with the MTT assay (error bars = standard deviation, *n* = 6).

5.4.2 BJ-5ta (Human skin fibroblasts hTERT immortalized, non-cancer)

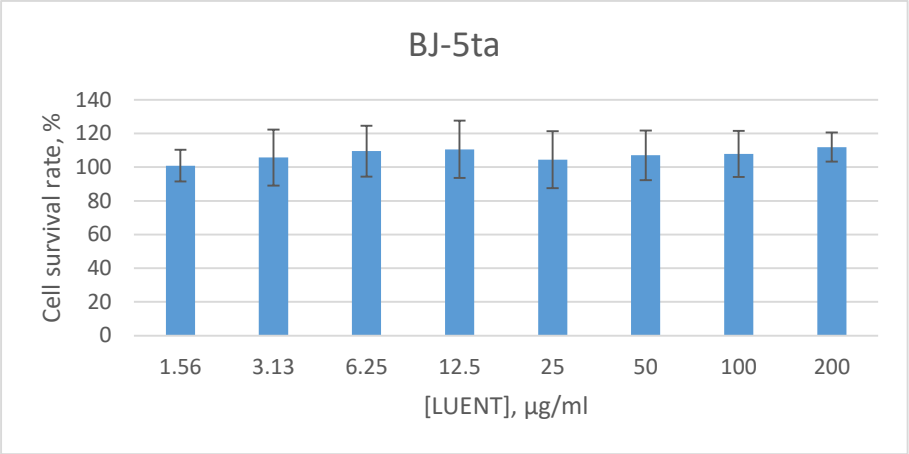

Figure 4.2: Cell survival following treatment of BJ-5ta cells for 72 h with LUENT, as determined with the MTT assay (error bars = standard deviation, *n* = 6).

5.4.3. H69V (Human small cell lung carcinoma)

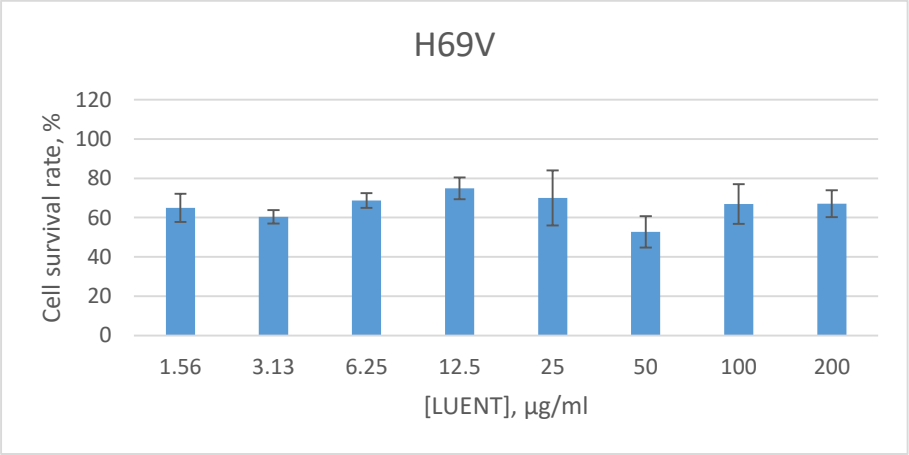

Figure 4.3: Cell survival following treatment of H69V cells for 72 h with LUENT, as determined with the MTT assay (error bars = standard deviation, *n* = 6).

5.4.4. 143B (Human osteosarcoma)

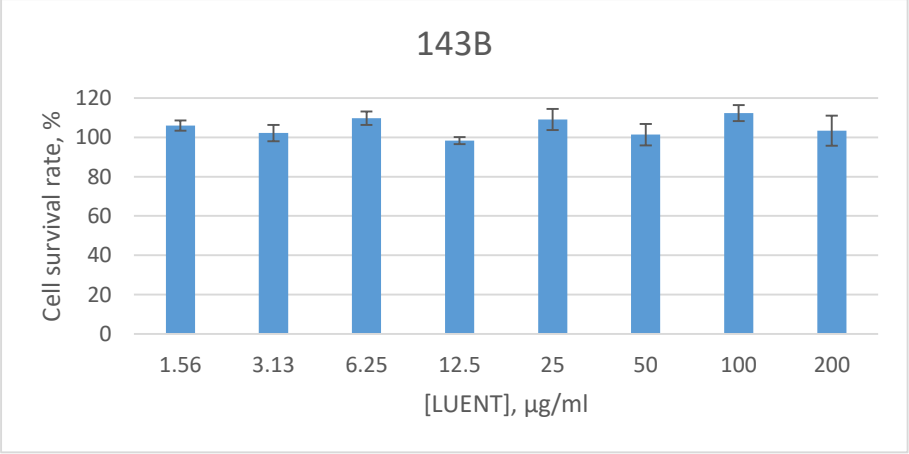

Figure 4.4: Cell survival following treatment of 143B cells for 72 h with LUENT, as determined with the MTT assay (error bars = standard deviation, *n* = 6).

5.4.5. HepG2/C3A (Human hepatocellular carcinoma)

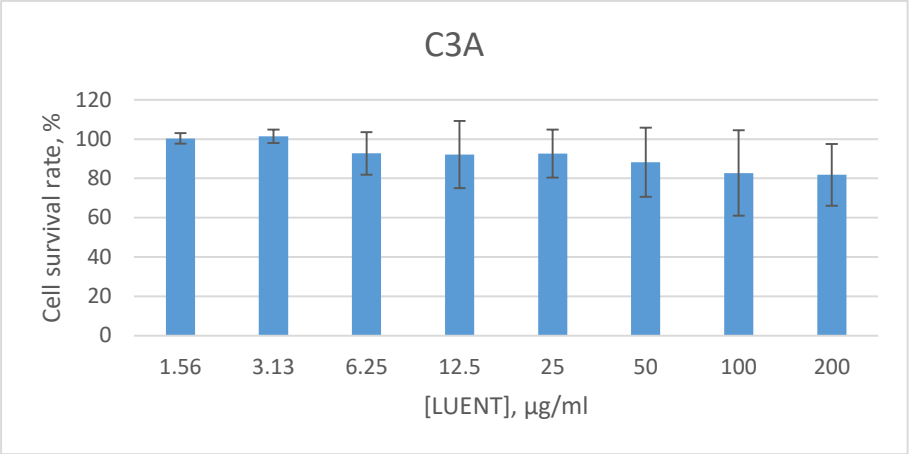

Figure 4.5: Cell survival following treatment of HepG2/C3A cells for 72 h with LUENT, as determined with the MTT assay (error bars = standard deviation, *n* = 6).

5.4.6. A375 (Human melanoma)

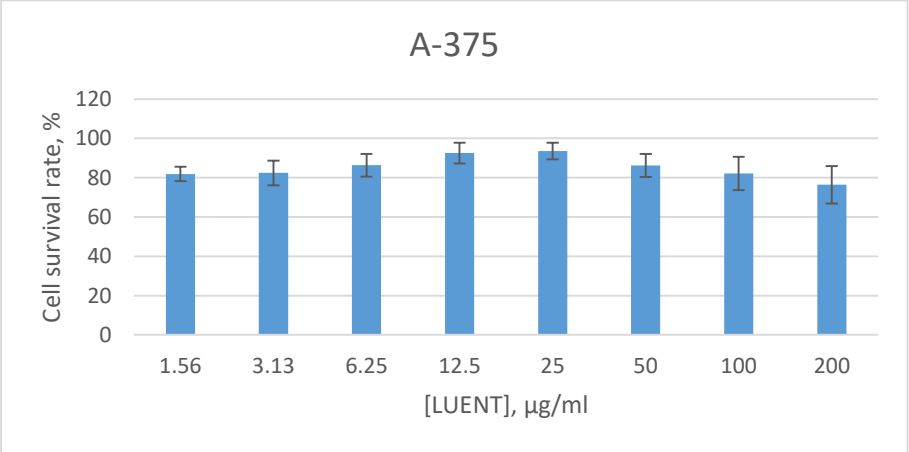

Figure 4.6: Cell survival following treatment of A375 cells for 72 h with LUENT, as determined with the MTT assay (error bars = standard deviation, *n* = 6).

5.4.7. HT29 (Human colon carcinoma)

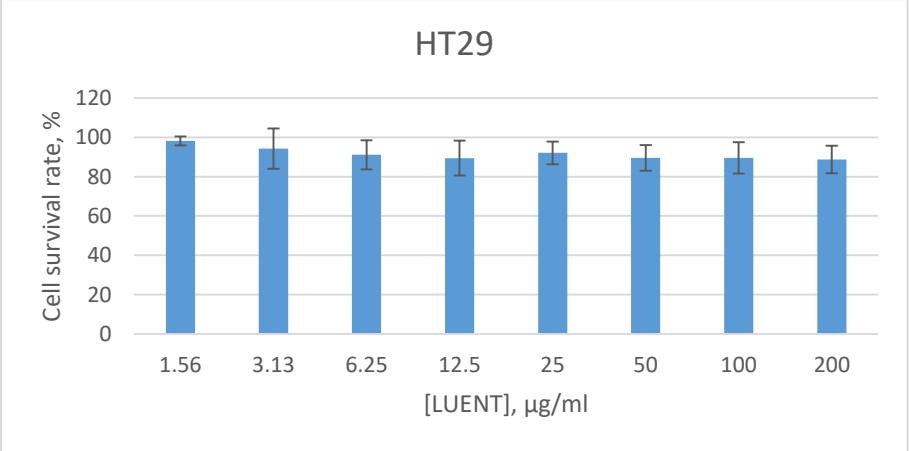

Figure 4.7: Cell survival following treatment of HT29 cells for 72 h with LUENT, as determined with the MTT assay (error bars = standard deviation, *n* = 6).

5.4.8. MCF7 (Human breast adenocarcinoma)

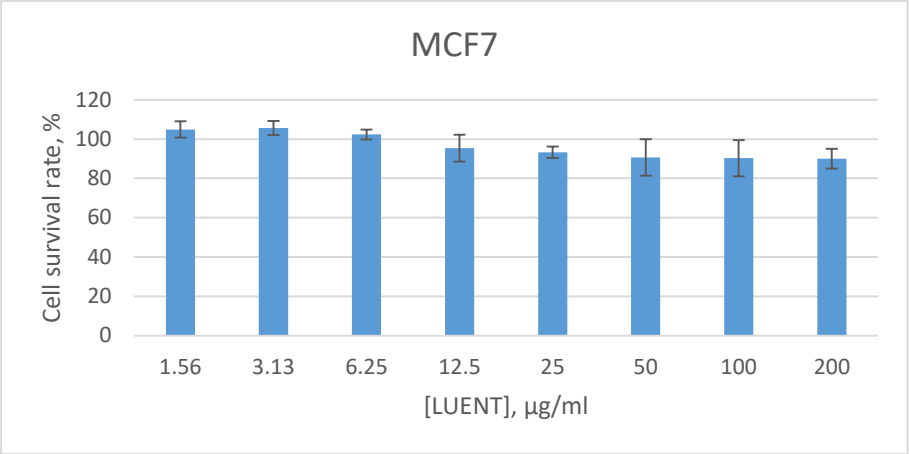

Figure 4.8: Cell survival following treatment of MCF7 cells for 72 h with LUENT, as determined with the MTT assay (error bars = standard deviation, *n* = 6).

5.4.9. AGS (Human stomach adenocarcinoma)

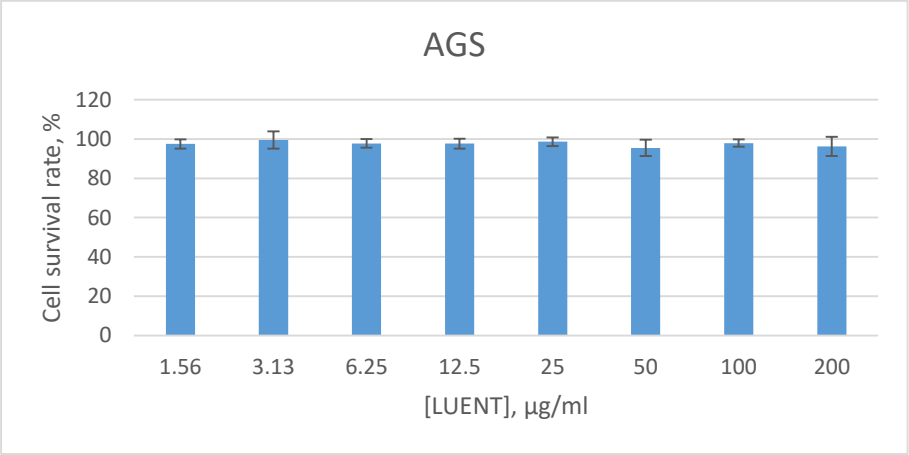

Figure 4.9: Cell survival following treatment of AGS cells for 72 h with LUENT, as determined with the MTT assay (error bars = standard deviation, *n* = 6).

5.4.10. Clone 15 HL-60 (Human acute promyelocytic leukemia)

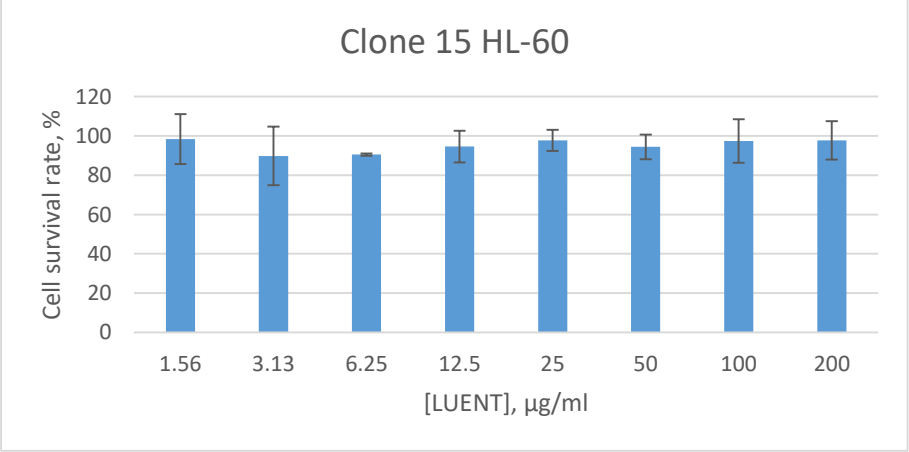

Figure 4.10: Cell survival following treatment of Clone 15 HL-60 cells for 72 h with LUENT, as determined with the LDH assay (error bars = standard deviation, *n* = 3).

5.4.11. DU145 (Human prostate carcinoma)

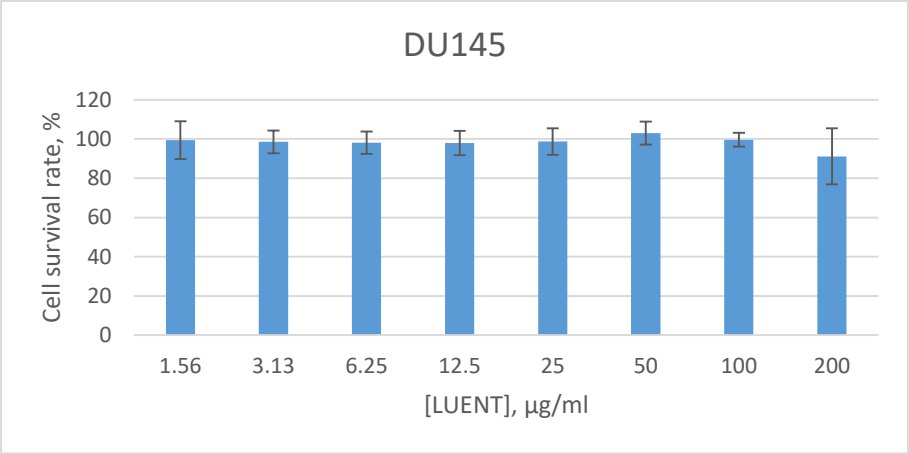

Figure 4.11: Cell survival following treatment of DU145 cells for 72 h with LUENT, as determined with the MTT assay (error bars = standard deviation, *n* = 6).

5.4.12. A-704 (human kidney adenocarcinoma)

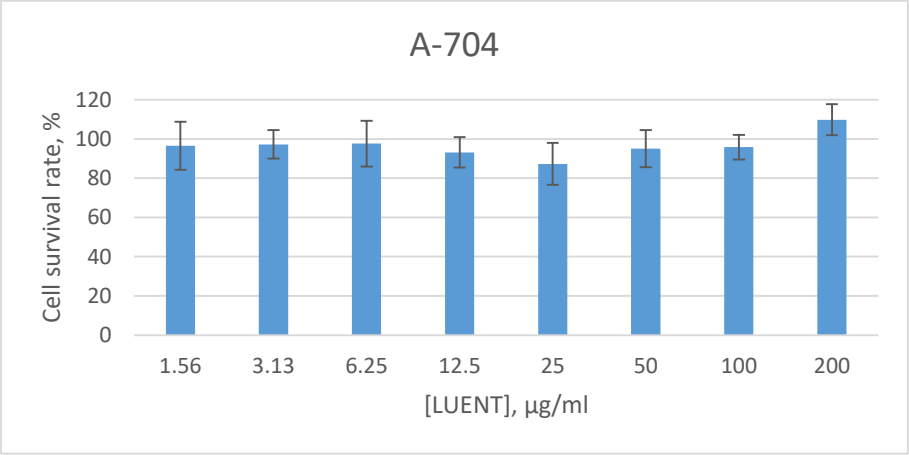

Figure 4.12: Cell survival following treatment of A-704 cells for 72 h with LUENT, as determined with the MTT assay (error bars = standard deviation, *n* = 6).

5.4.13. K-562 (Human chronic myelogenous Leukemia)

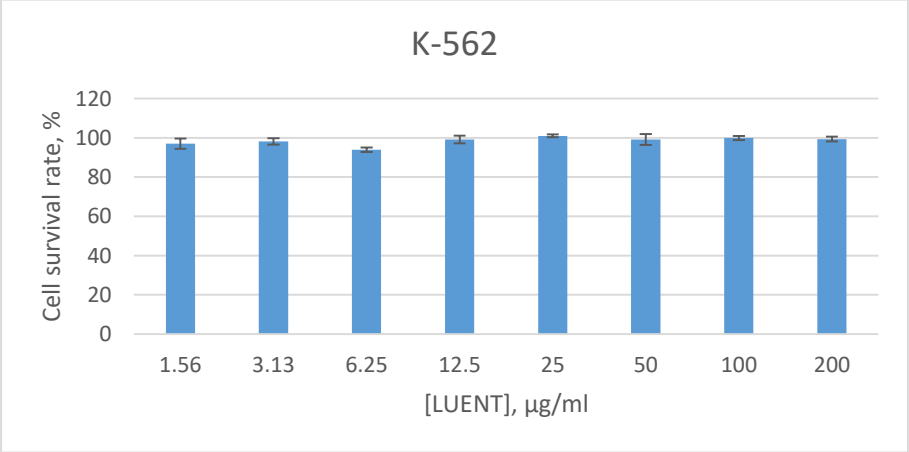

Figure 4.13A: Cell survival following treatment of K-562 cells for 72 h with LUENT, as determined with the LDH assay (error bars = standard deviation, *n* = 3).

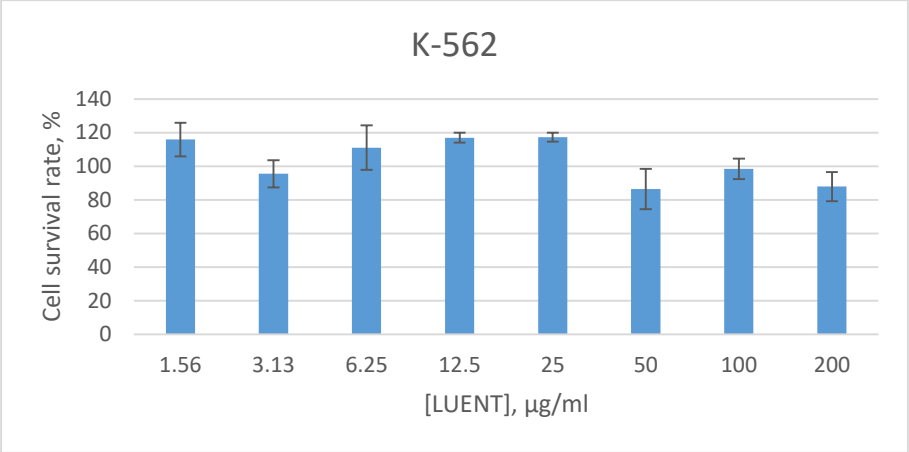

Figure 4.13B: Cell survival following treatment of K-562 cells for 72 h with LUENT, as determined with the MTT assay (error bars = standard deviation, *n* = 3).

5.4.14. U-87 MG (Human glioblastoma)

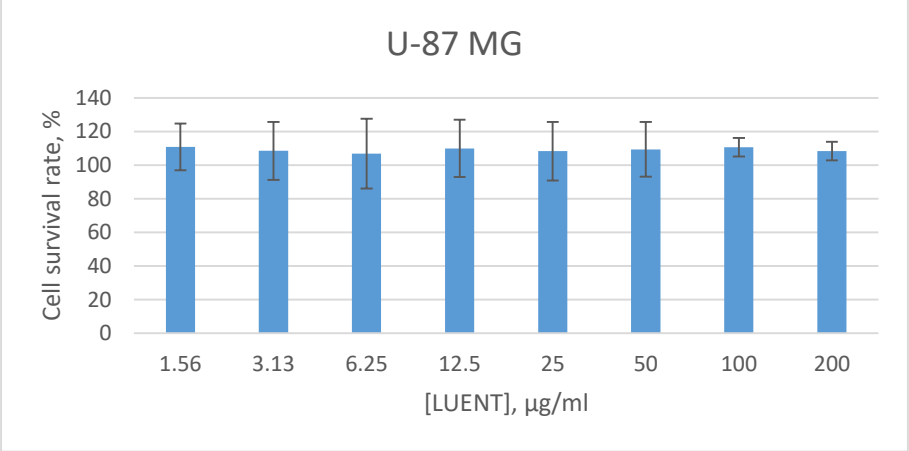

Figure 4.14: Cell survival following treatment of U-87 MG cells for 72 h with LUENT, as determined with the MTT assay (error bars = standard deviation, *n* = 6).

5.4.15. A-549 (Human non-small cell lung carcinoma)

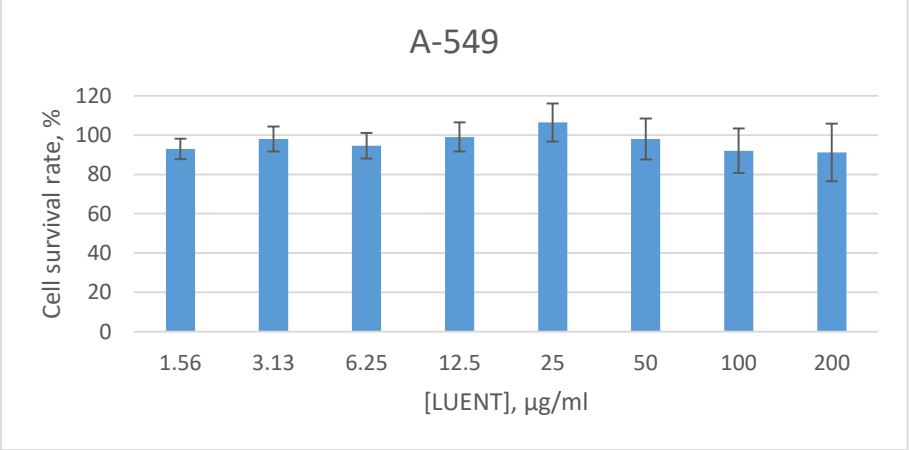

Figure 4.15: Cell survival following treatment of A-549 cells for 72 h with LUENT, as determined with the MTT assay (error bars = standard deviation, *n* = 6).

5.4.16. BT-20 (Human triple negative invasive ductal human carcinoma breast cancer)

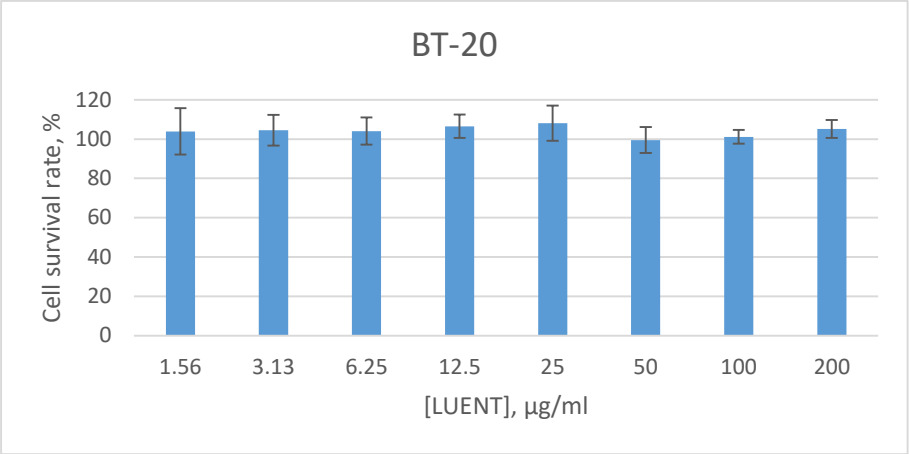

Figure 4.16: Cell survival following treatment of BT-20 cells for 72 h with LUENT, as determined with the MTT assay (error bars = standard deviation, *n* = 6).

5.4.17. Panc 02.03 (Human pancreas adenocarcinoma)

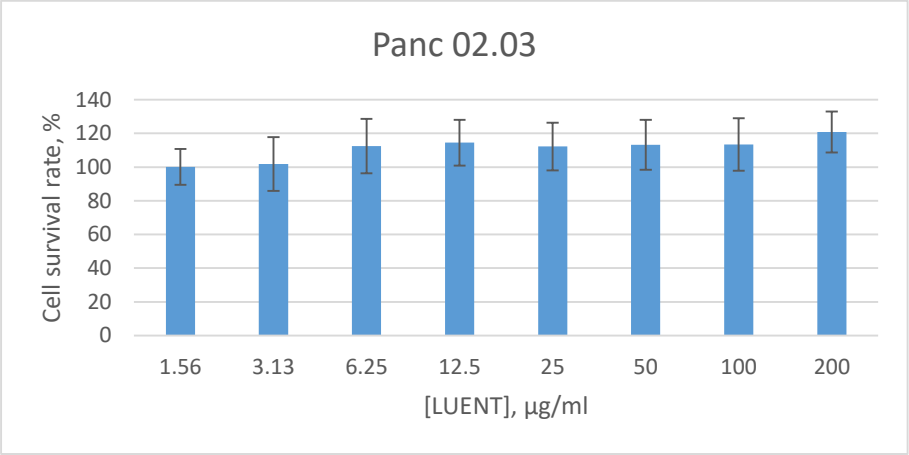

Figure 4.17: Cell survival following treatment of Panc 02.03 cells for 72 h with LUENT, as determined with the MTT assay (error bars = standard deviation, *n* = 6).

Discussion for compound LUENT results:

LUENT appears to be slightly toxic to the non-cancerous Vero cell line, with a 20 to 30% reduction in cell viability after 72 h exposure to LUENT. There was no apparent toxicity against the BJ-5at cell line. Inhibition of cell viability was limited in the cancer cell lines.

5.5. Results for compound RBMW

5.5.1. Vero (African green monkey – non-cancer control)

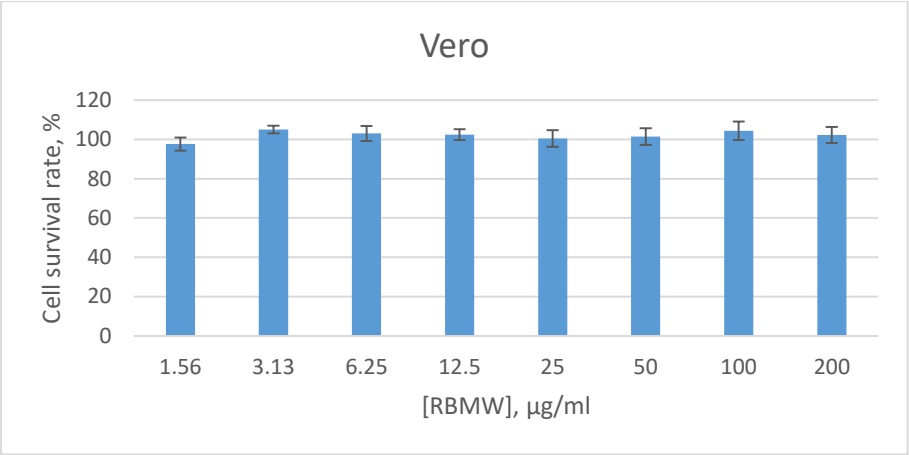

Figure 5.1: Cell survival following treatment of Vero cells for 72 h with RBMW, as determined with the MTT assay (error bars = standard deviation, *n* = 6).

5.5.2. BJ-5ta (Human skin fibroblasts hTERT immortalized, non-cancer)

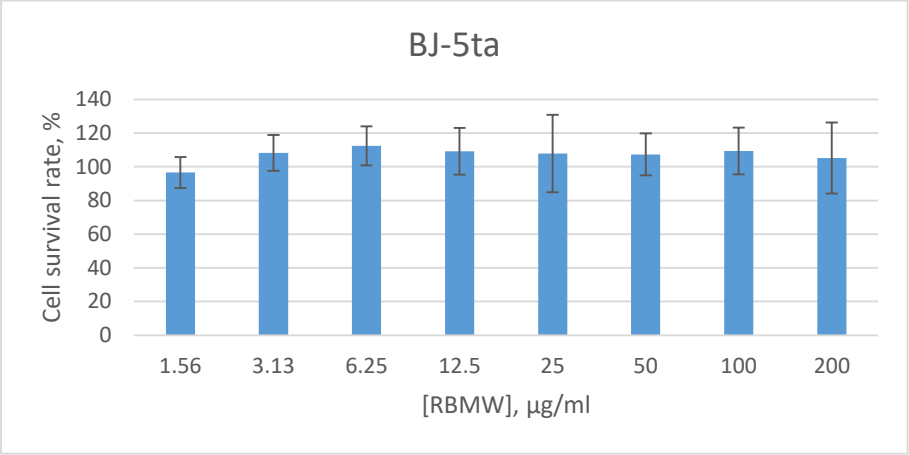

Figure 5.2: Cell survival following treatment of BJ-5ta cells for 72 h with RBMW, as determined with the MTT assay (error bars = standard deviation, *n* = 6).

5.5.3. H69V (Human small cell lung carcinoma)

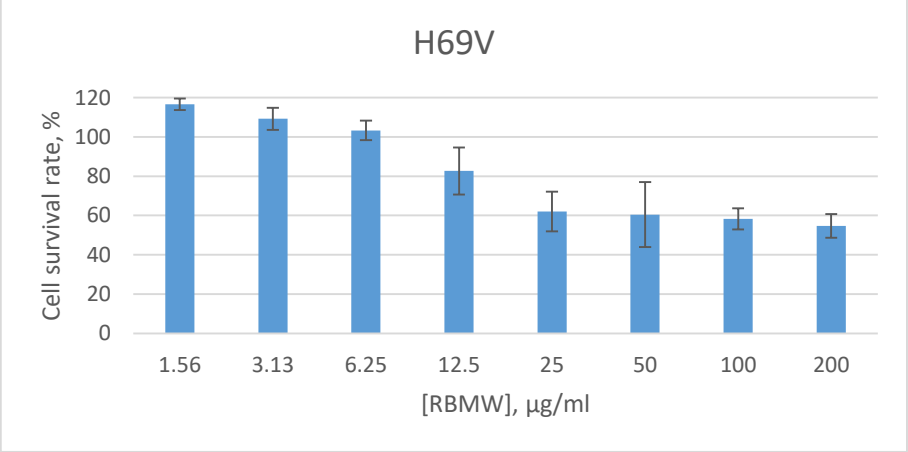

Figure 5.3: Cell survival following treatment of H69V cells for 72 h with RBMW, as determined with the MTT assay (error bars = standard deviation, *n* = 6).

5.5.4. 143B (Human osteosarcoma)

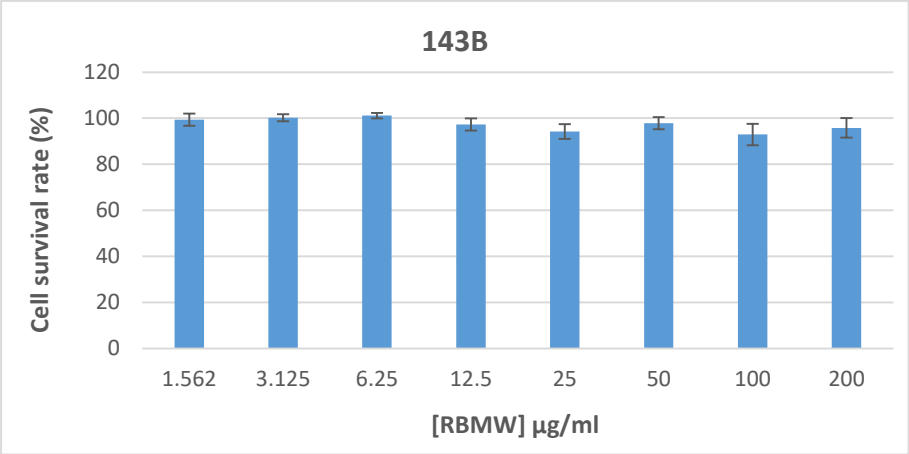

Figure 5.4: Cell survival following treatment of 143B cells for 72 h with RBMW, as determined with the MTT assay (error bars = standard deviation, *n* = 6).

5.5.5. HepG2/C3A (Human hepatocellular carcinoma)

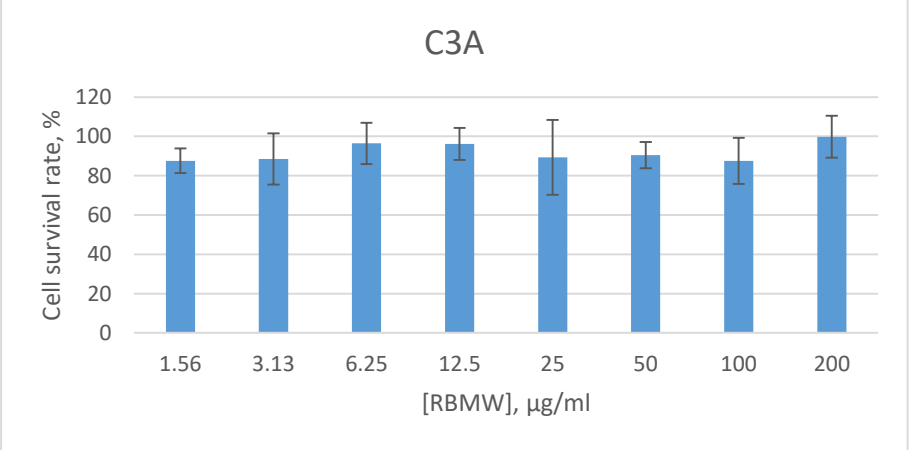

Figure 5.5: Cell survival following treatment of HepG2/C3A cells for 72 h with RBMW, as determined with the MTT assay (error bars = standard deviation, *n* = 6).

5.5.6. A375 (Human melanoma)

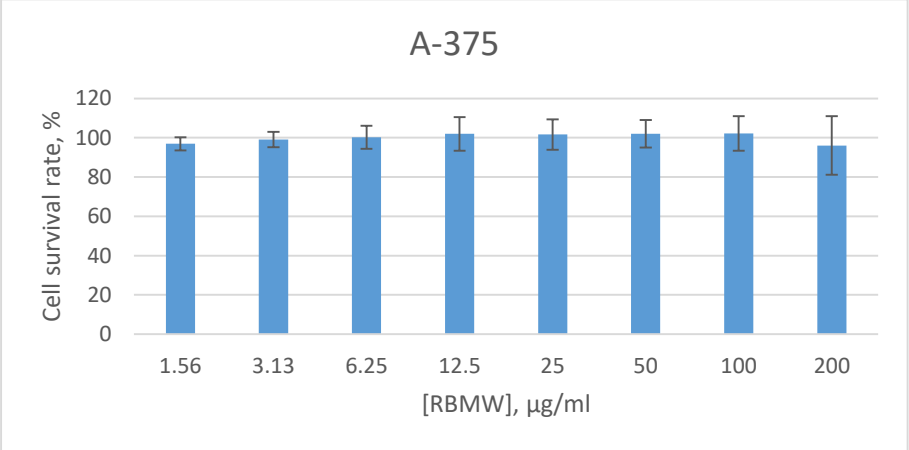

Figure 5.6: Cell survival following treatment of A375 cells for 72 h with RBMW, as determined with the MTT assay (error bars = standard deviation, *n* = 6).

5.5.7. HT29 (Human colon carcinoma)

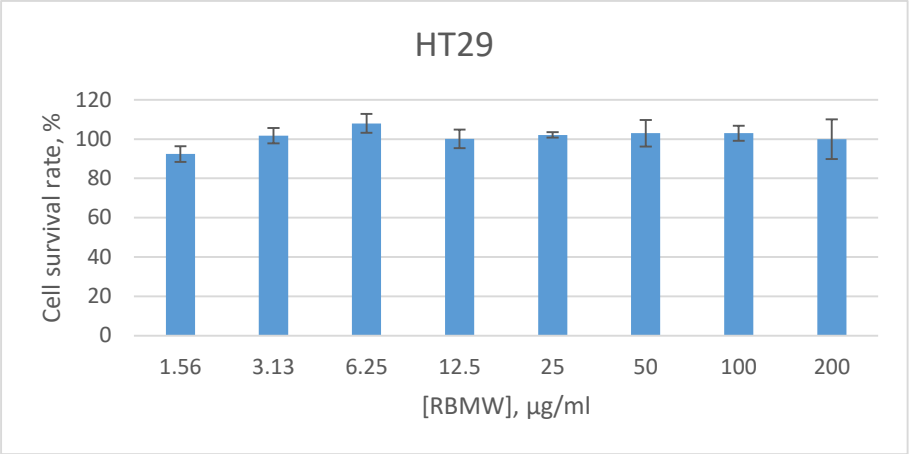

Figure 5.7: Cell survival following treatment of HT29 cells for 72 h with RBMW, as determined with the MTT assay (error bars = standard deviation, *n* = 6).

5.5.8. MCF7 (Human breast adenocarcinoma)

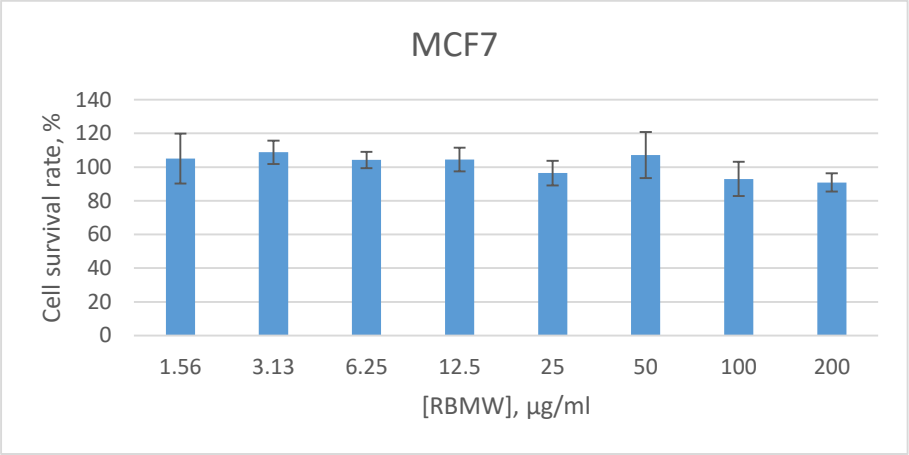

Figure 5.8: Cell survival following treatment of MCF7 cells for 72 h with RBMW, as determined with the MTT assay (error bars = standard deviation, *n* = 6).

5.5.9. AGS (Human stomach adenocarcinoma)

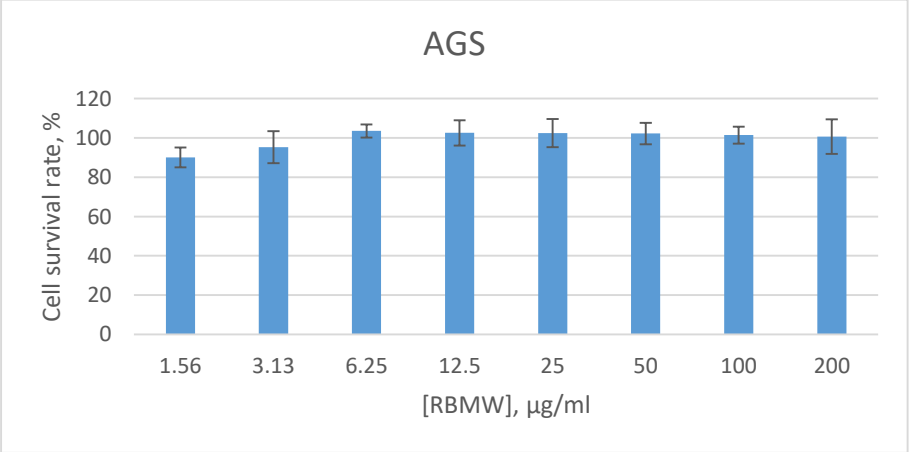

Figure 5.9: Cell survival following treatment of AGS cells for 72 h with RBMW, as determined with the MTT assay (error bars = standard deviation, *n* = 6).

5.5.10. Clone 15 HL-60 (Human acute promyelocytic leukemia)

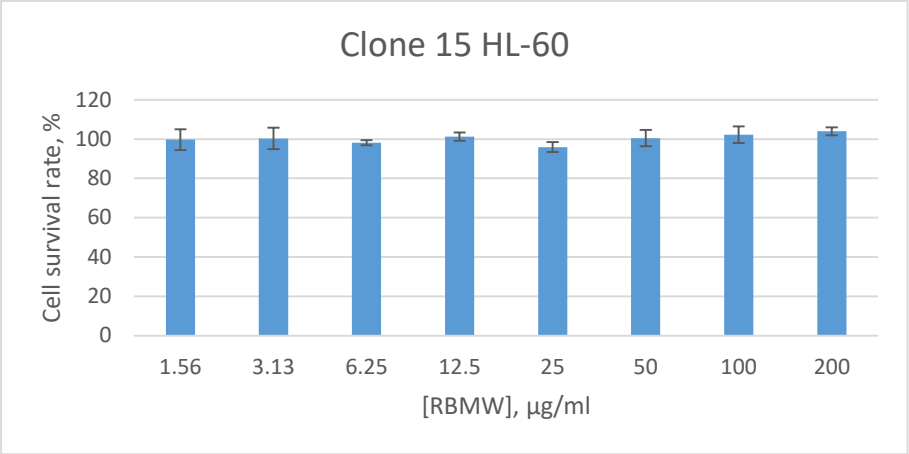

Figure 5.10: Cell survival following treatment of Clone 15 HL-60 cells for 72 h with RBMW, as determined with the LDH assay (error bars = standard deviation, *n* = 3).

5.5.11. DU145 (Human prostate carcinoma)

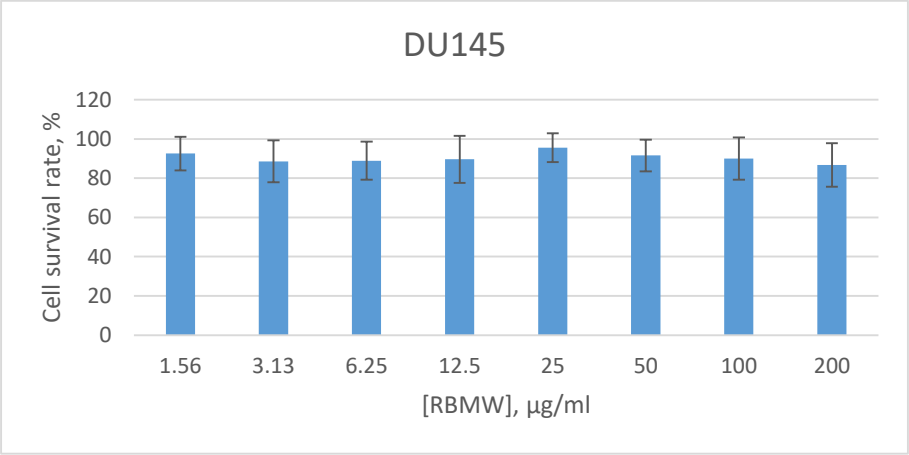

Figure 5.11: Cell survival following treatment DU145 cells for 72 h with RBMW, as determined with the MTT assay (error bars = standard deviation, *n* = 6).

5.5.12. A-704 (human kidney adenocarcinoma)

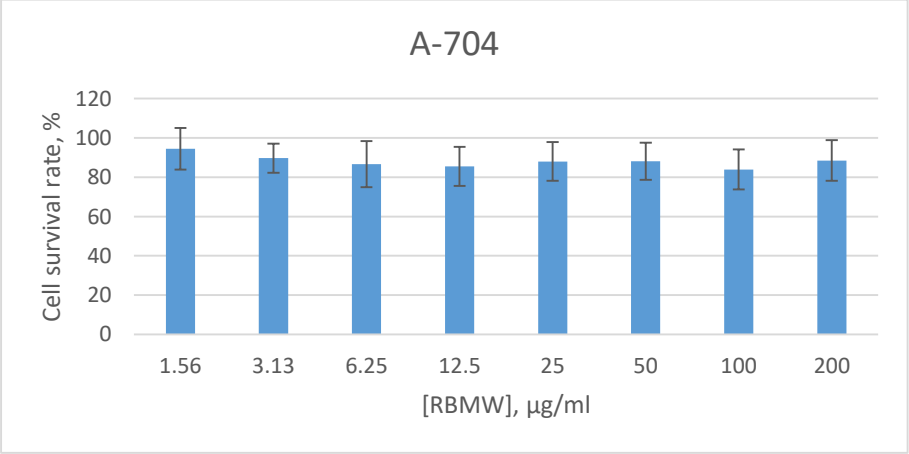

Figure 5.12: Cell survival following treatment of A-704 cells for 72 h with RBMW, as determined with the MTT assay (error bars = standard deviation, *n* = 6).

5.5.13. K-562 (Human chronic myelogenous Leukemia)

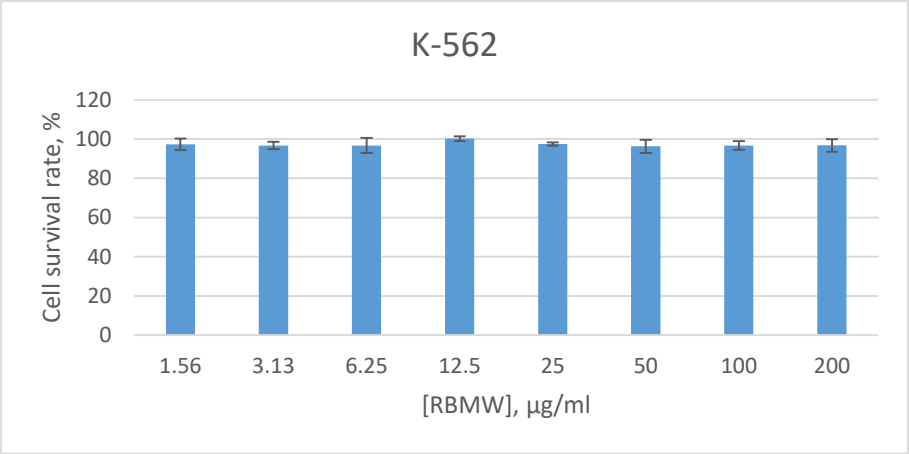

Figure 5.13A: Cell survival following treatment of K-562 cells for 72 h with RBMW, as determined with the LDH assay (error bars = standard deviation, *n* = 3).

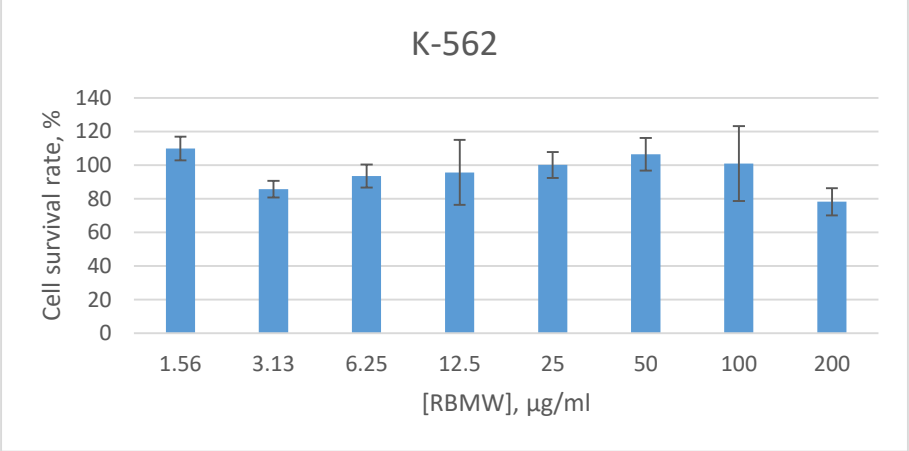

Figure 5.13B: Cell survival following treatment of K-562 cells for 72 h with RBMW, as determined with the MTT assay (error bars = standard deviation, *n* = 3).

5.5.14. U-87 MG (Human glioblastoma)

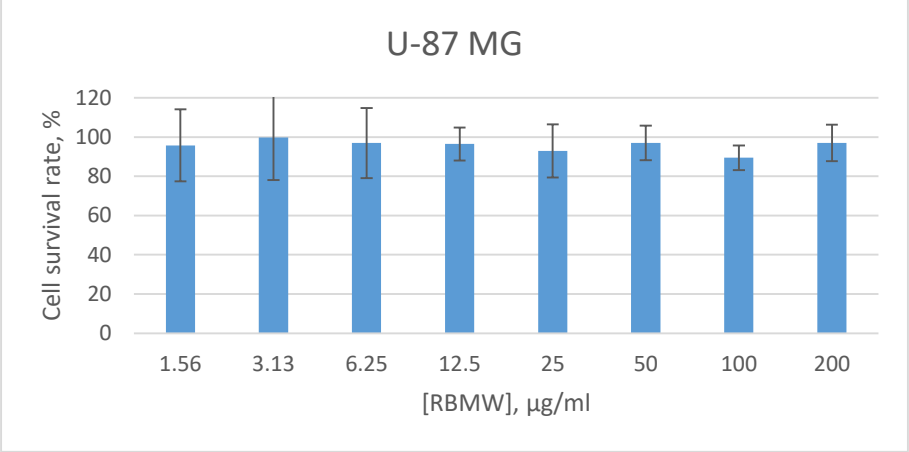

Figure 5.14: Cell survival following treatment of U-87 MG cells for 72 h with RBMW, as determined with the MTT assay (error bars = standard deviation, *n* = 6).

5.5.15. A-549 (Human non-small cell lung carcinoma)

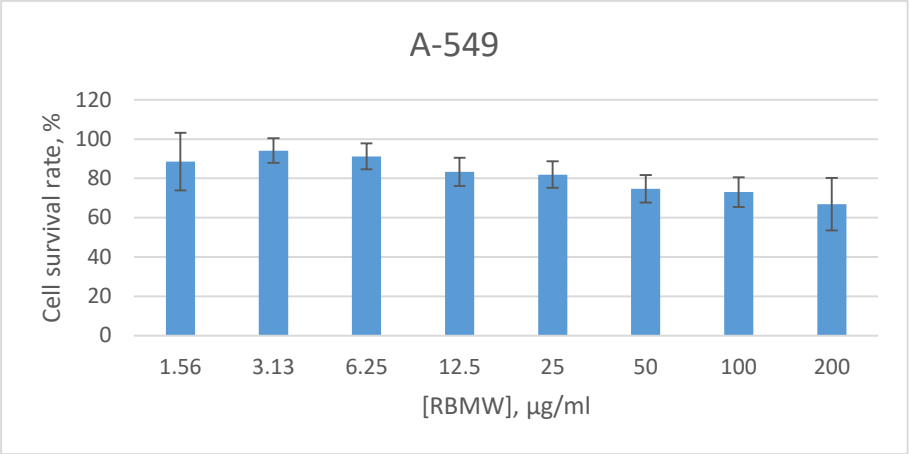

Figure 5.15: Cell survival following treatment of A-549 cells for 72 h with RBMW, as determined with the MTT assay (error bars = standard deviation, *n* = 6).

5.5.16. BT-20 (Human triple negative invasive ductal human carcinoma breast cancer)

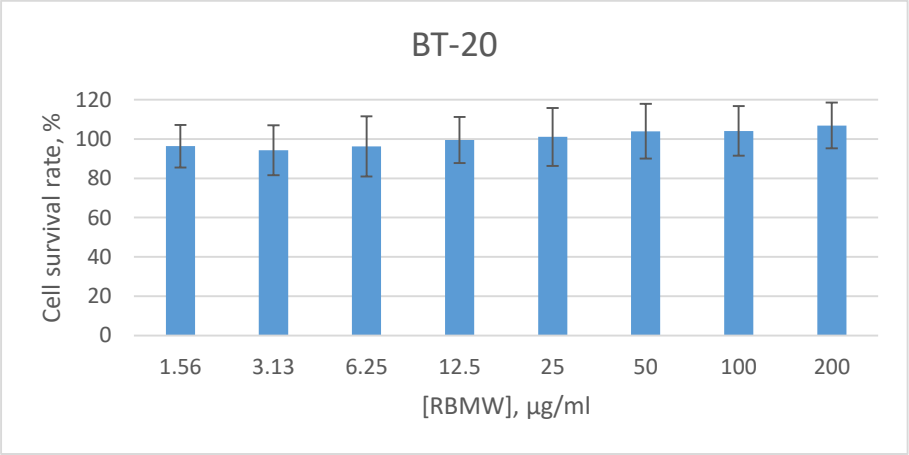

Figure 5.16: Cell survival following treatment of BT-20 cells for 72 h with RBMW, as determined with the MTT assay (error bars = standard deviation, *n* = 6).

5.5.17. Panc 02.03 (Human pancreas adenocarcinoma)

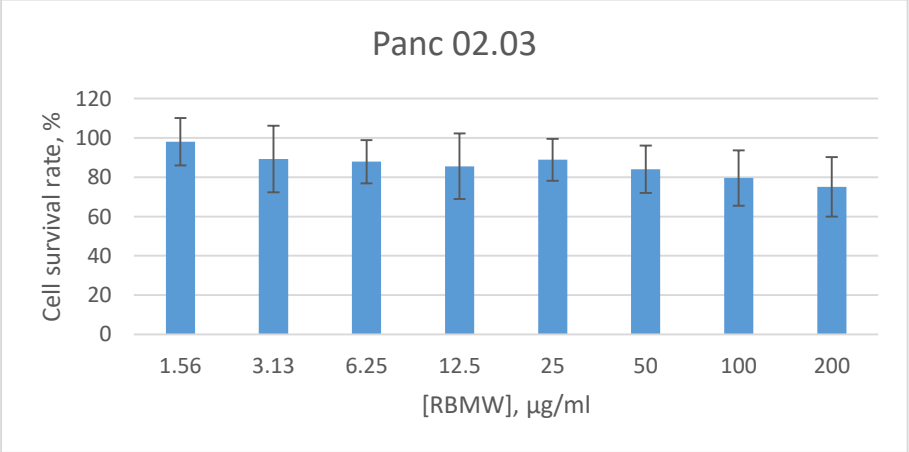

Figure 5.17: Cell survival following treatment of Panc 02.03 cells for 72 h with RBMW, as determined with the MTT assay (error bars = standard deviation, *n* = 6).

Discussion for compound RBMW results:

RBMW appears to be slightly toxic to the non-cancerous Vero cell line, with a 20 – 30% reduction in cell viability after 72 h exposure to RBMW. No toxicity was observed in the non-cancerous BJ-5ta cell line. Low concentrations seem to induce cell growth in several of the cancer cell lines tested, with viability increased to more than that of the untreated control. Inhibition of cell viability was observed at higher concentrations of RBMW, with the most notable effects observed in H69V cell line.

5.6. Results for compound YS2

5.6.1. Vero (African green monkey – non-cancer control)

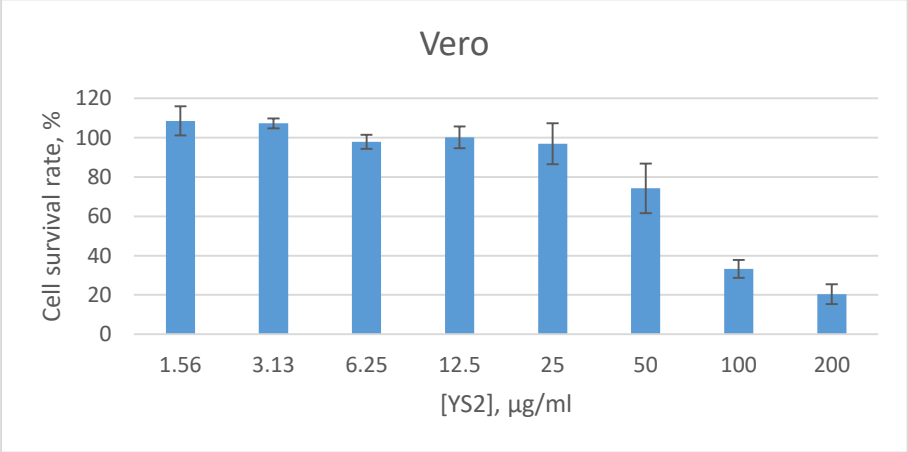

Figure 6.1: Cell survival following treatment of Vero cells for 72 h with YS2, as determined with the MTT assay (error bars = standard deviation, *n* = 6).

5.6.2a. 84BR (Human dermal fibroblasts – non-cancer control)

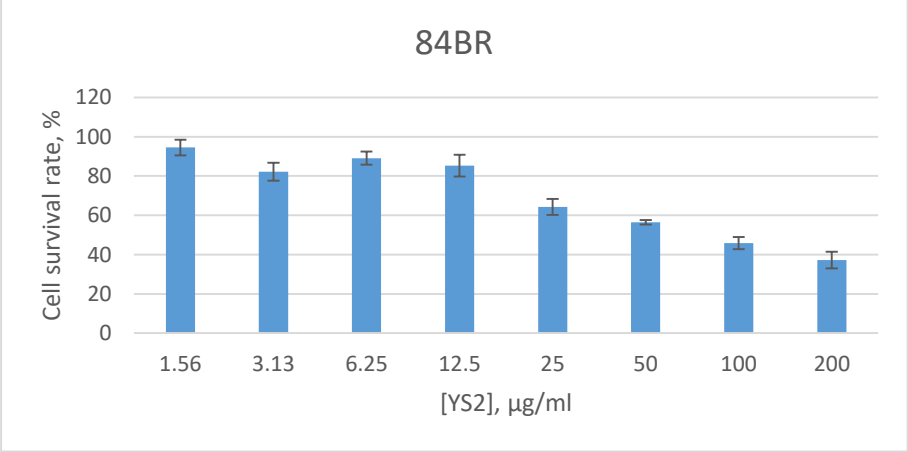

Figure 6.2a: Cell survival following treatment of 84BR cells for 72 h with YS2, as determined with the MTT assay (error bars = standard deviation, *n* = 3).

5.6.2b BJ-5ta (Human skin fibroblasts hTERT immortalized, non-cancer)

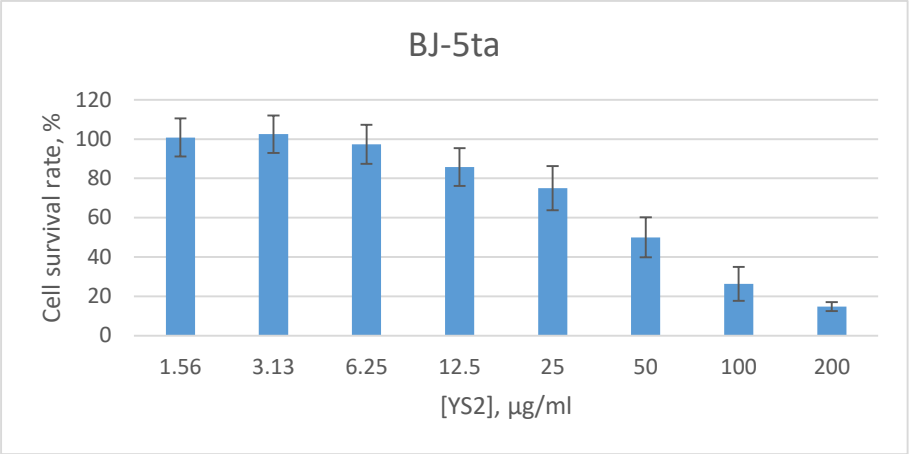

Figure 6.2b: Cell survival following treatment of BJ-5ta cells for 72 h with YS2, as determined with the MTT assay (error bars = standard deviation, *n* = 6).

5.6.3. H69V (Human small cell lung carcinoma)

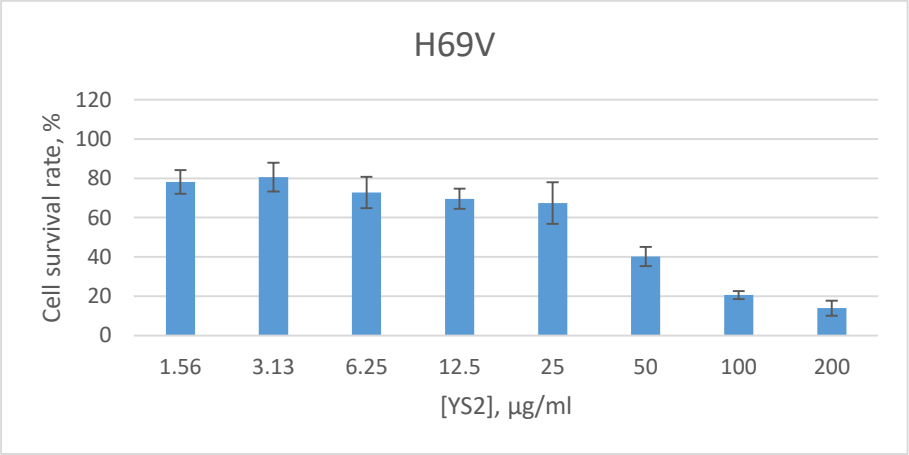

Figure 6.3: Cell survival following treatment of H69V cells for 72 h with YS2, as determined with the MTT assay (error bars = standard deviation, *n* = 6).

5.6.4. 143B (Human osteosarcoma)

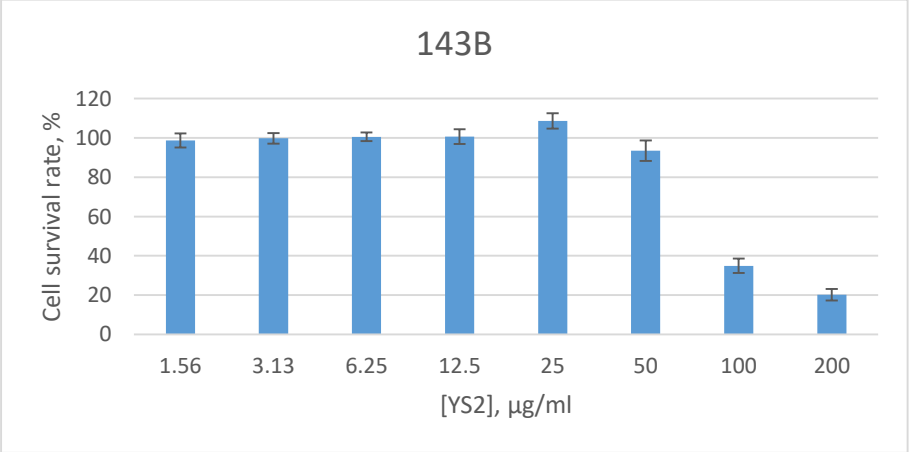

Figure 6.4: Cell survival following treatment of 143B cells for 72 h with YS2, as determined with the MTT assay (error bars = standard deviation, *n* = 6).

5.6.5. HepG2/C3A (Human hepatocellular carcinoma)

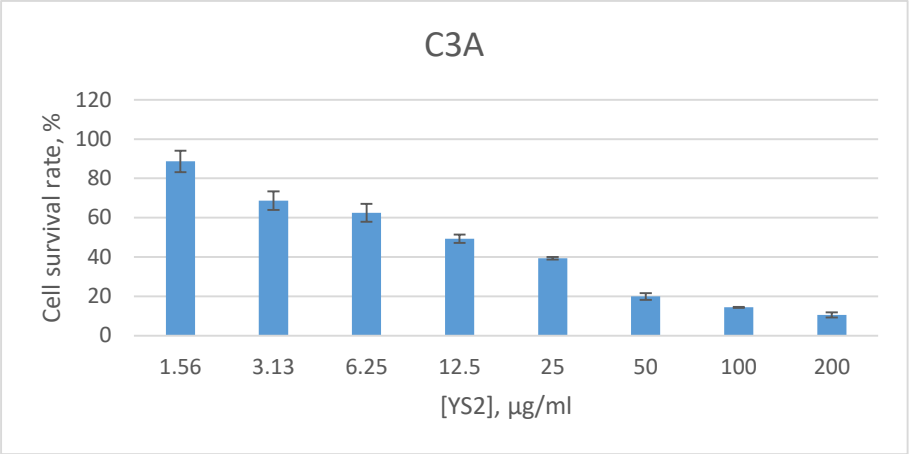

Figure 6.5: Cell survival following treatment of HepG2/C3A cells for 72 h with YS2, as determined with the MTT assay (error bars = standard deviation, *n* = 6).

5.6.6. A375 (Human melanoma)

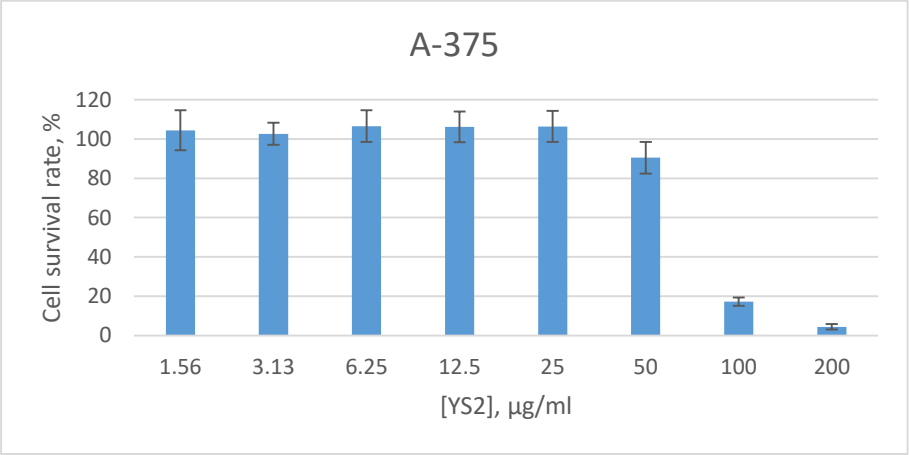

Figure 6.6: Cell survival following treatment of A375 cells for 72 h with YS2, as determined with the MTT assay (error bars = standard deviation, *n* = 6).

5.6.7. HT29 (Human colon carcinoma)

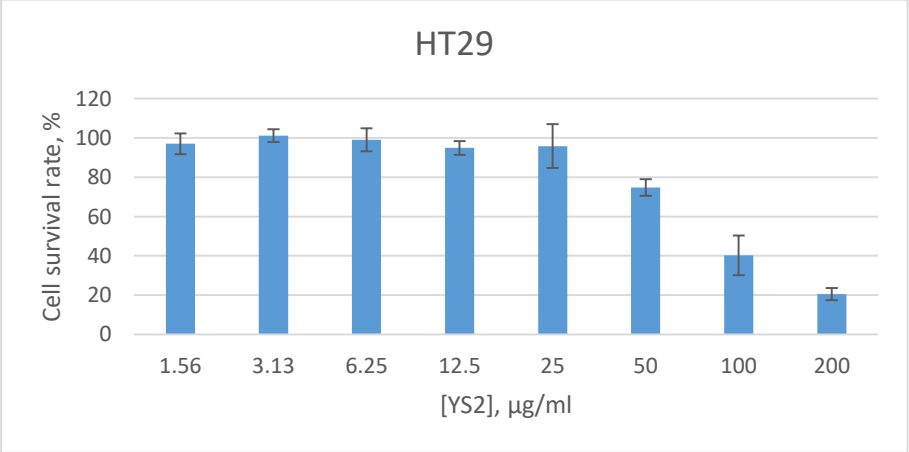

Figure 6.7: Cell survival following treatment of HT29 cells for 72 h with YS2, as determined with the MTT assay (error bars = standard deviation, *n* = 6).

5.6.8. MCF7 (Human breast adenocarcinoma)

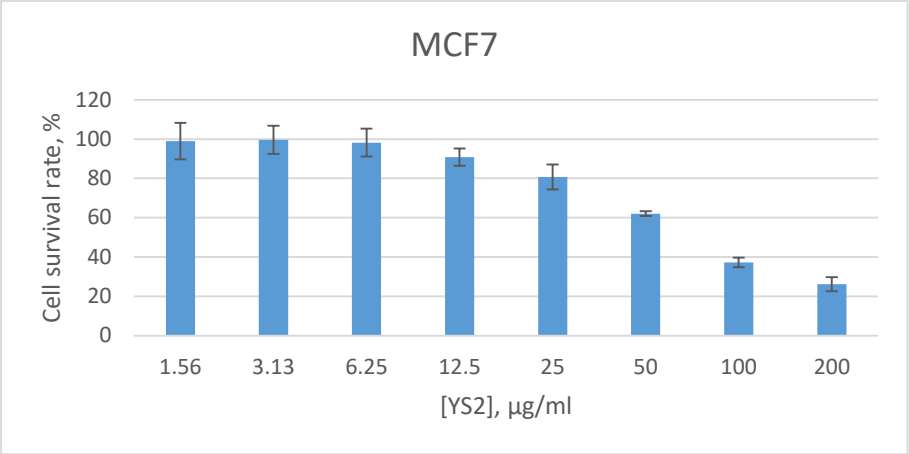

Figure 6.8: Cell survival following treatment of MCF7 cells for 72 h with YS2, as determined with the MTT assay (error bars = standard deviation, *n* = 6).

5.6.9. AGS (Human stomach adenocarcinoma)

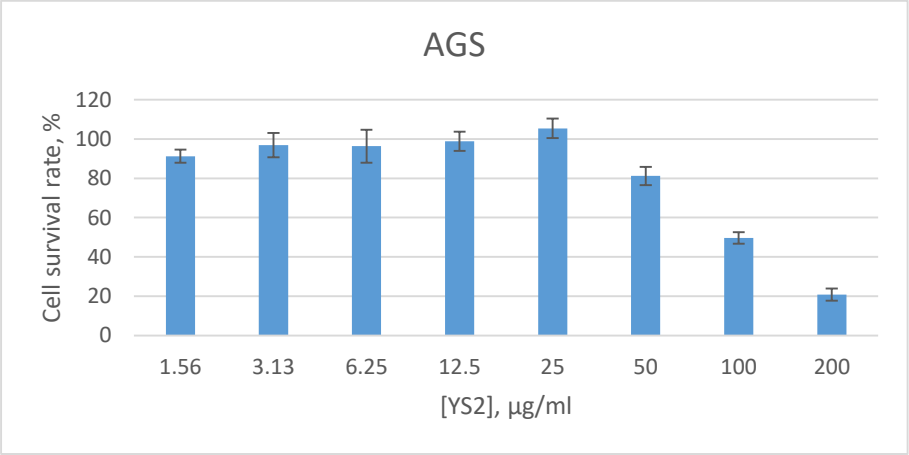

Figure 6.9: Cell survival following treatment of AGS cells for 72 h with YS2, as determined with the MTT assay (error bars = standard deviation, *n* = 6).

5.6.10. Clone 15 HL-60 (Human acute promyelocytic leukemia)

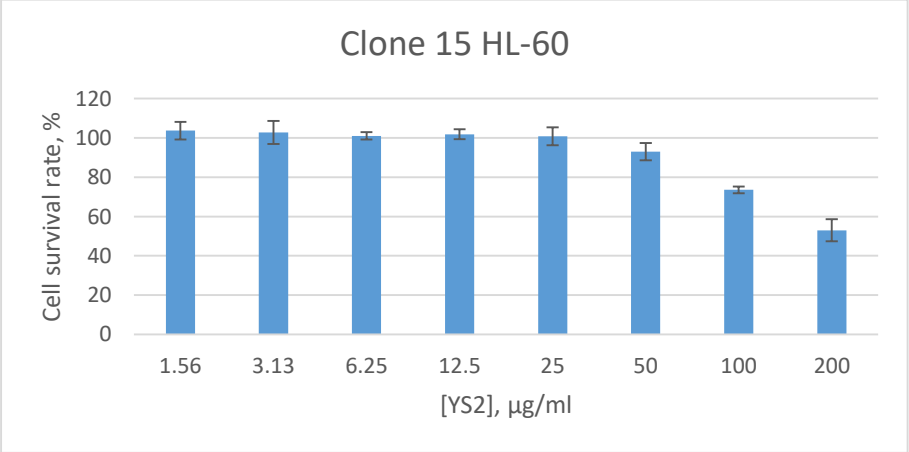

Figure 6.10: Cell survival following treatment of Clone 15 HL-60 cells for 72 h with YS2, as determined with the LDH assay (error bars = standard deviation, *n* = 3).

5.6.11. DU145 (Human prostate carcinoma)

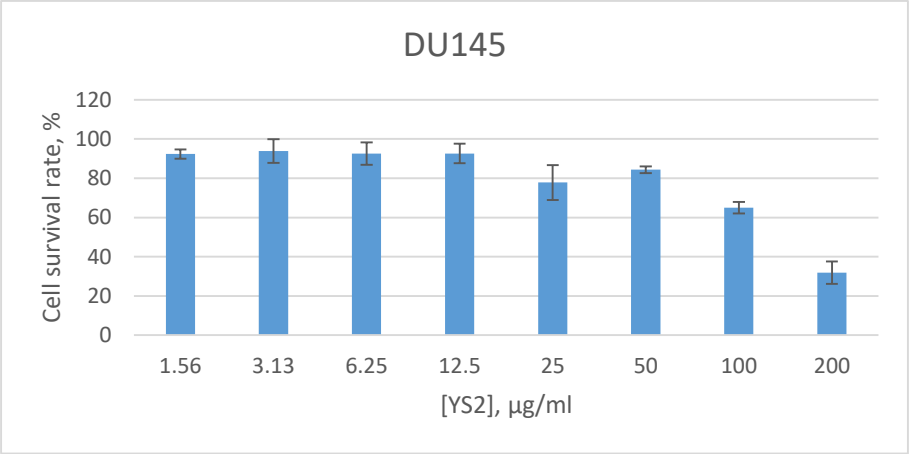

Figure 6.11: Cell survival following treatment DU145 cells for 72 h with YS2, as determined with the MTT assay (error bars = standard deviation, *n* = 6).

5.6.12. A-704 (human kidney adenocarcinoma)

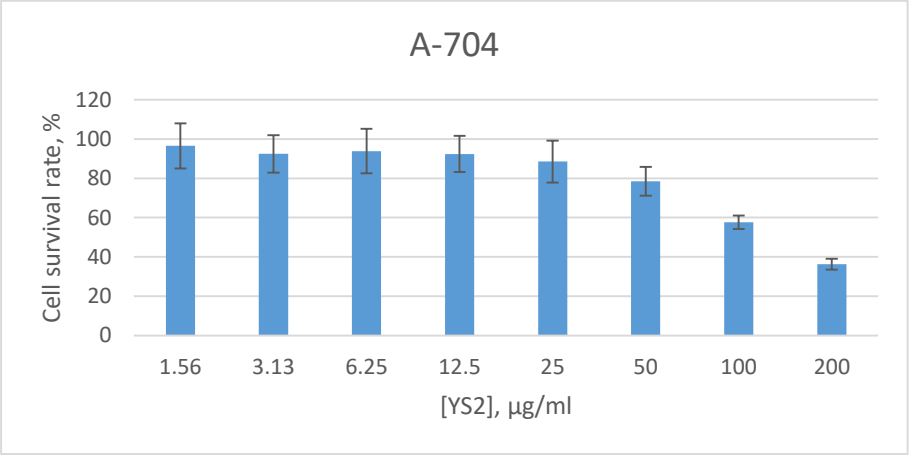

Figure 6.12: Cell survival following treatment of A-704 cells for 72 h with YS2, as determined with the MTT assay (error bars = standard deviation, *n* = 6).

5.6.13. K-562 (Human chronic myelogenous Leukemia)

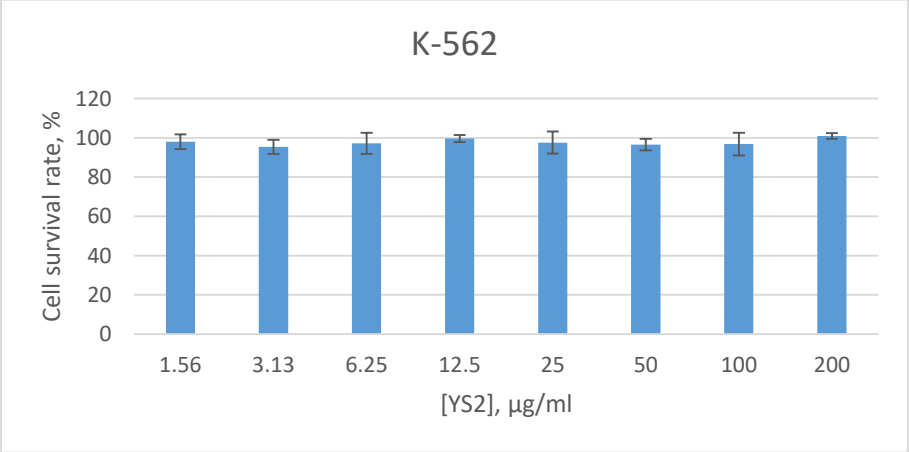

Figure 6.13A: Cell survival following treatment of K-562 cells for 72 h with YS2, as determined with the LDH assay (error bars = standard deviation, *n* = 3).

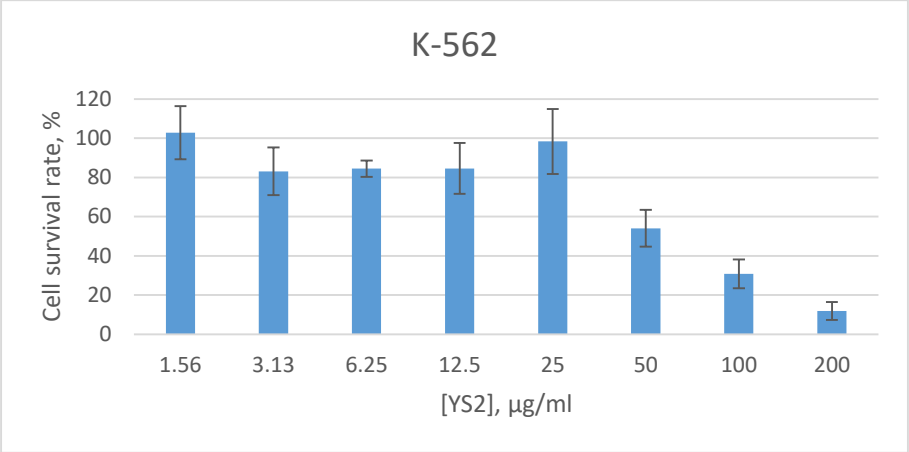

Figure 6.13B: Cell survival following treatment of K-562 cells for 72 h with YS2, as determined with the MTT assay (error bars = standard deviation, *n* = 3).

5.6.14. U-87 MG (Human glioblastoma)

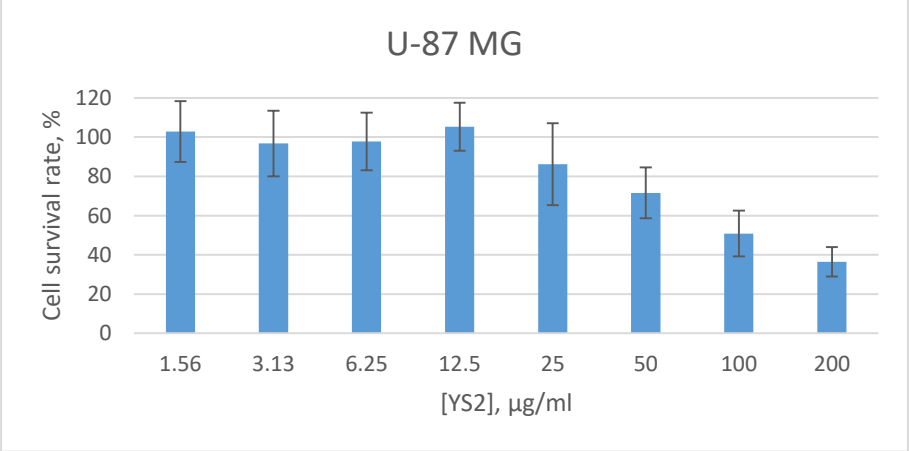

Figure 6.14: Cell survival following treatment of U-87 MG cells for 72 h with YS2, as determined with the MTT assay (error bars = standard deviation, *n* = 6).

5.6.15. A-549 (Human non-small cell lung carcinoma)

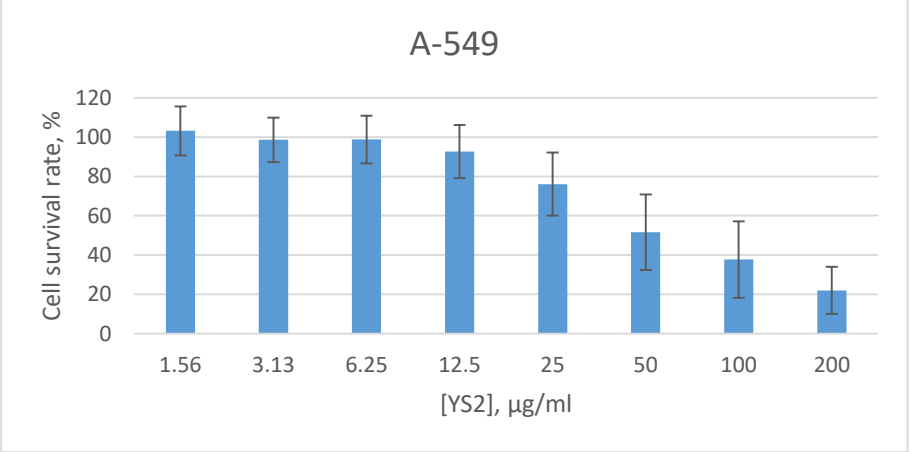

Figure 6.15: Cell survival following treatment of A-549 cells for 72 h with YS2, as determined with the MTT assay (error bars = standard deviation, *n* = 6).

5.6.16. BT-20 (Human triple negative invasive ductal human carcinoma breast cancer)

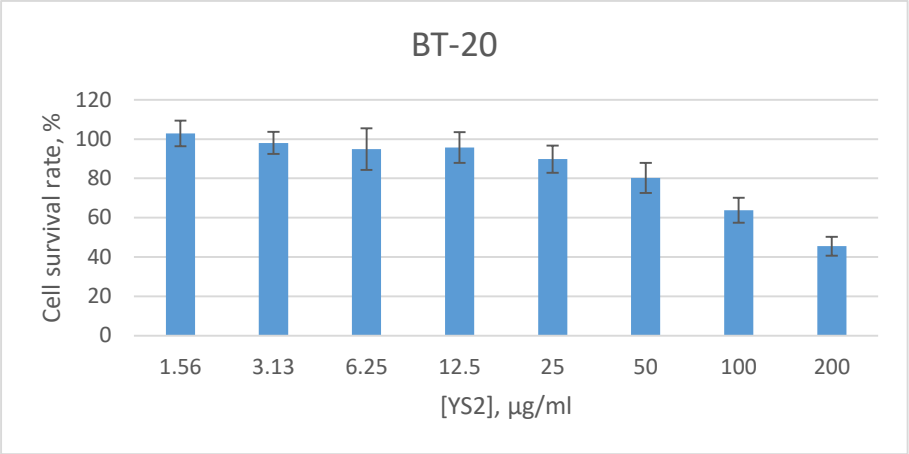

Figure 6.16: Cell survival following treatment of BT-20 cells for 72 h with YS2, as determined with the MTT assay (error bars = standard deviation, *n* = 6).

5.6.17. Panc 02.03 (Human pancreas adenocarcinoma)

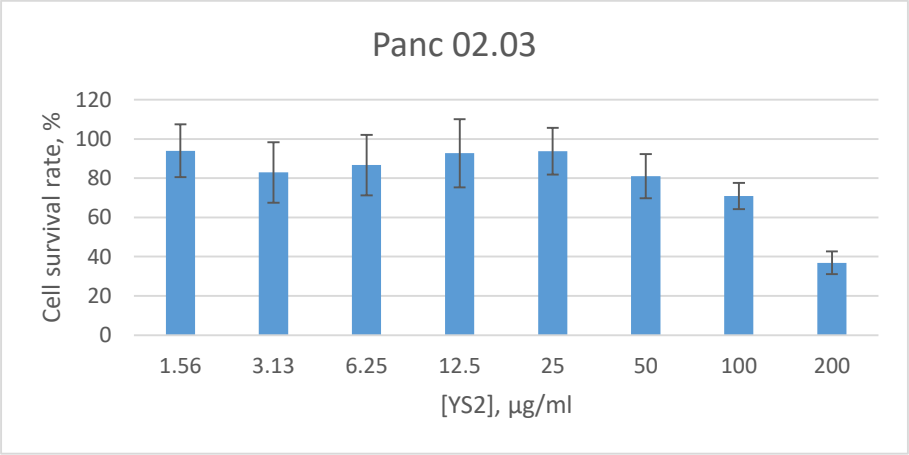

Figure 6.17: Cell survival following treatment of Panc 02.03 cells for 72 h with YS2, as determined with the MTT assay (error bars = standard deviation, *n* = 6).

Discussion for compound YS2 results:

YS2 appears to have dose-dependent toxicity in all the non-cancerous cell lines, with marked reduction in cell viability after 72 h exposure to 25, 50, 100 and 200 ug/ml YS2. Inhibition of cell viability was observed to some extent in a concentration dependent manner, with the most notable effects observed in the H69V and HepG2/C3A cell lines. YS2 seems to have some potential against the liver carcinoma HepG2/C3A cell line.

5.7. Results for compound F6A-F5ZS

5.7.1. Vero (African green monkey – non-cancer control)

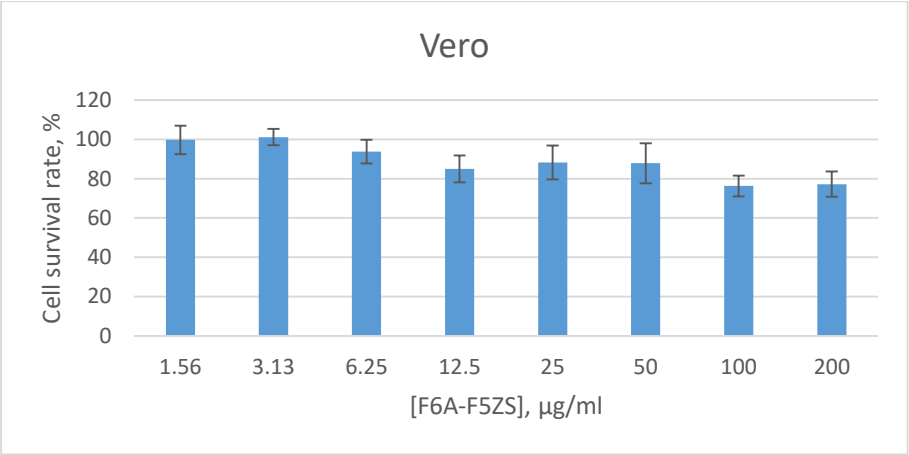

Figure 7.1: Cell survival following treatment of Vero cells for 72 h with F6A-F5ZS, as determined with the MTT assay (error bars = standard deviation, *n* = 6).

5.7.2a. 84BR (Human dermal fibroblasts – non-cancer control)

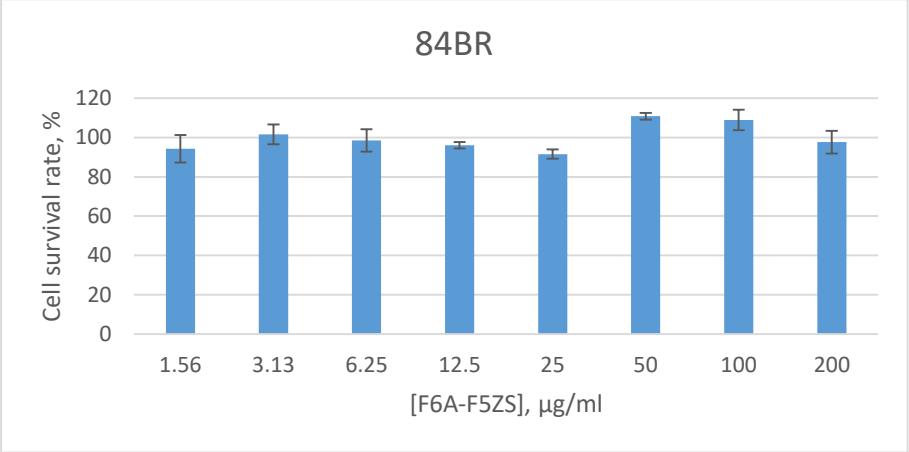

Figure 7.2a: Cell survival following treatment of 84BR cells for 72 h with F6A-F5ZS, as determined with the MTT assay (error bars = standard deviation, *n* = 3).

5.7.2b. BJ-5ta (Human skin fibroblasts hTERT immortalized, non-cancer)

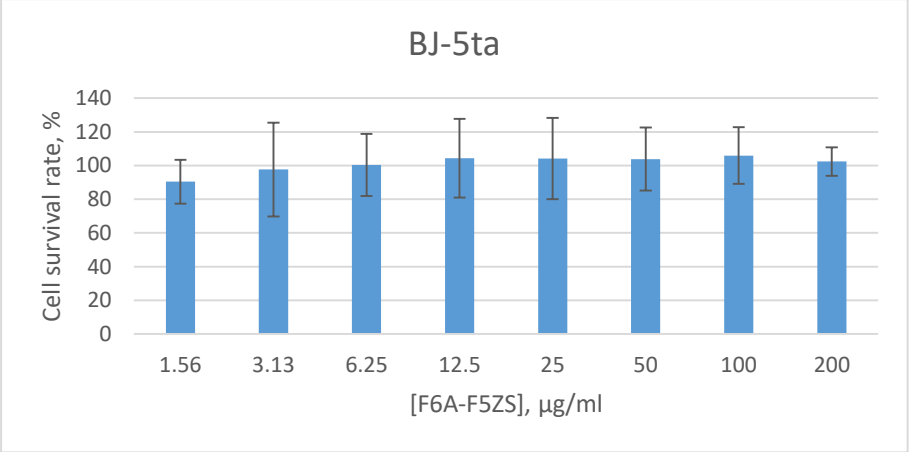

Figure 7.2b: Cell survival following treatment of BJ-5ta cells for 72 h with F6A-F5ZS, as determined with the MTT assay (error bars = standard deviation, *n* = 6).

5.7.3. H69V (Human small cell lung carcinoma)

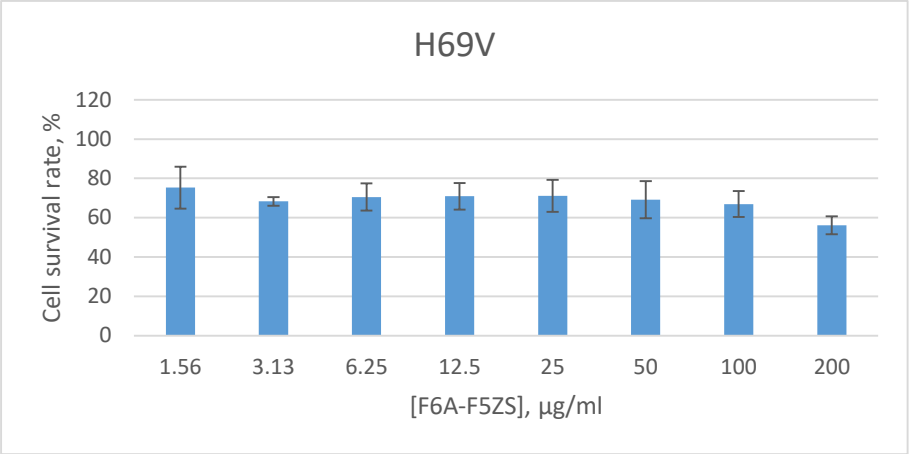

Figure 7.3: Cell survival following treatment of H69V cells for 72 h with F6A-F5ZS, as determined with the MTT assay (error bars = standard deviation, *n* = 6).

5.7.4. 143B (Human osteosarcoma)

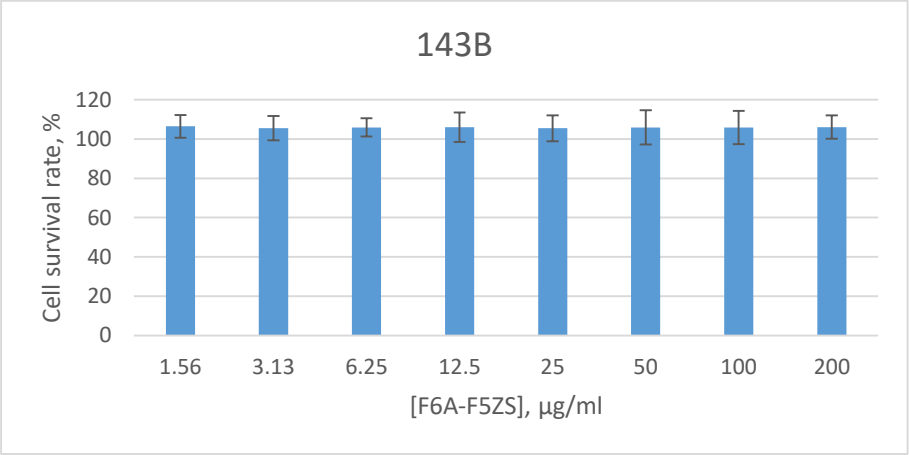

Figure 7.4: Cell survival following treatment of 143B cells for 72 h with F6A-F5ZS, as determined with the MTT assay (error bars = standard deviation, *n* = 6).

5.7.5. HepG2/C3A (Human hepatocellular carcinoma)

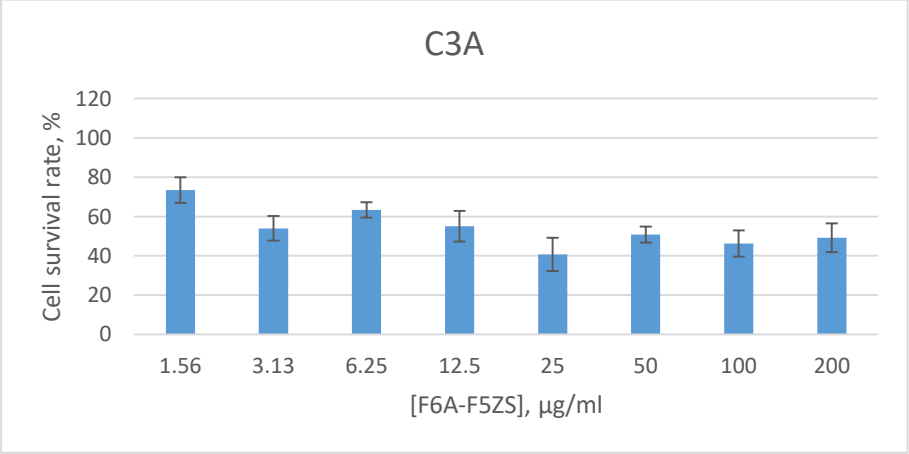

Figure 7.5: Cell survival following treatment of HepG2/C3A cells for 72 h with F6A-FZS, as determined with the MTT assay (error bars = standard deviation, *n* = 6).

5.7.6. A375 (Human melanoma)

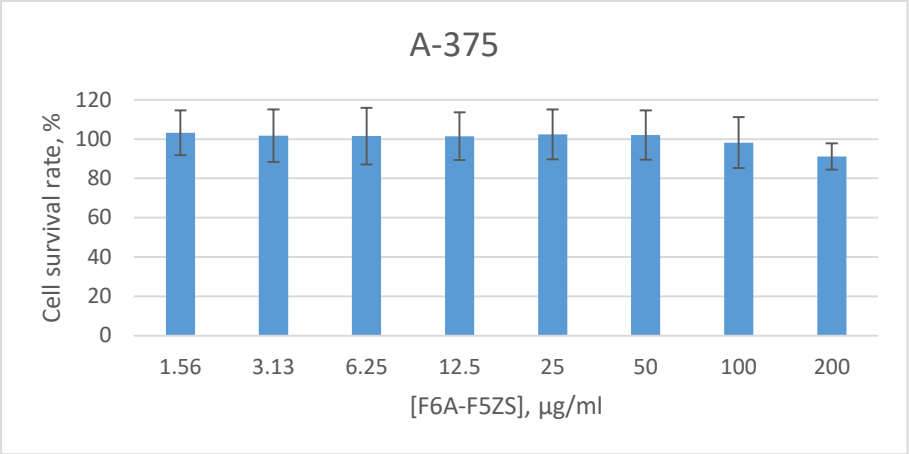

Figure 7.6: Cell survival following treatment of A375 cells for 72 h with F6A-FZS, as determined with the MTT assay (error bars = standard deviation, *n* = 6).

5.7.7. HT29 (Human colon carcinoma)

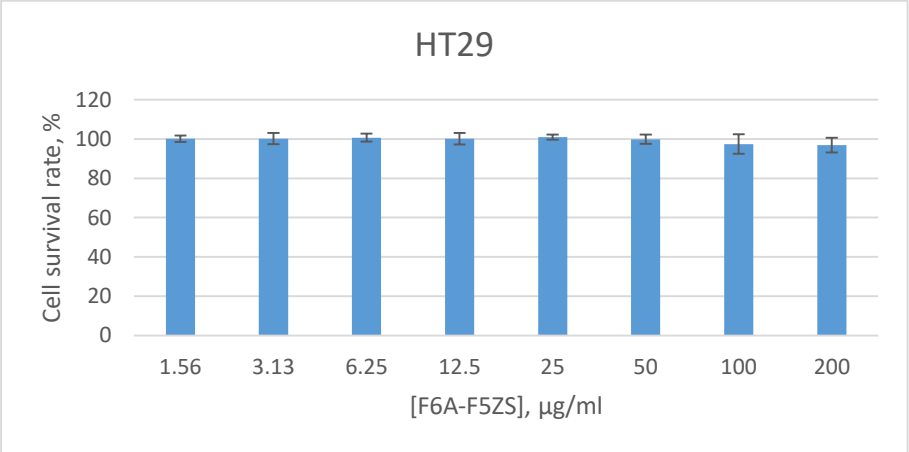

Figure 7.7: Cell survival following treatment of HT29 cells for 72 h with F6A-F5ZS, as determined with the MTT assay (error bars = standard deviation, *n* = 6).

5.7.8. MCF7 (Human breast adenocarcinoma)

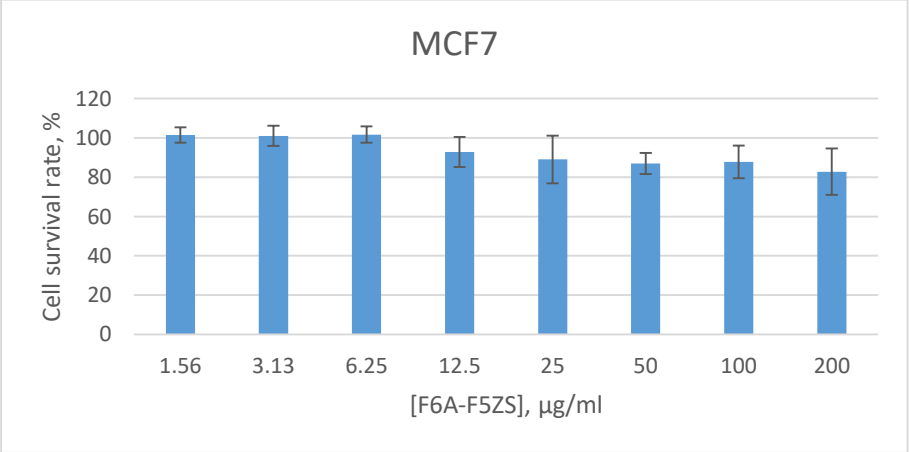

Figure 7.8: Cell survival following treatment of MCF7 cells for 72 h with F6A-F5ZS, as determined with the MTT assay (error bars = standard deviation, *n* = 6).

5.7.9. AGS (Human stomach adenocarcinoma)

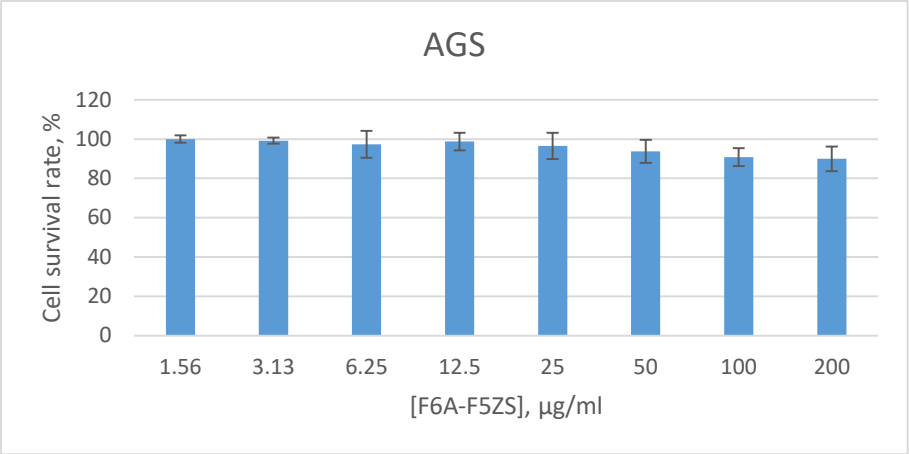

Figure 7.9: Cell survival following treatment of AGS cells for 72 h with F6A-F5ZS, as determined with the MTT assay (error bars = standard deviation, *n* = 6).

5.7.10. Clone 15 HL-60 (Human acute promyelocytic leukemia)

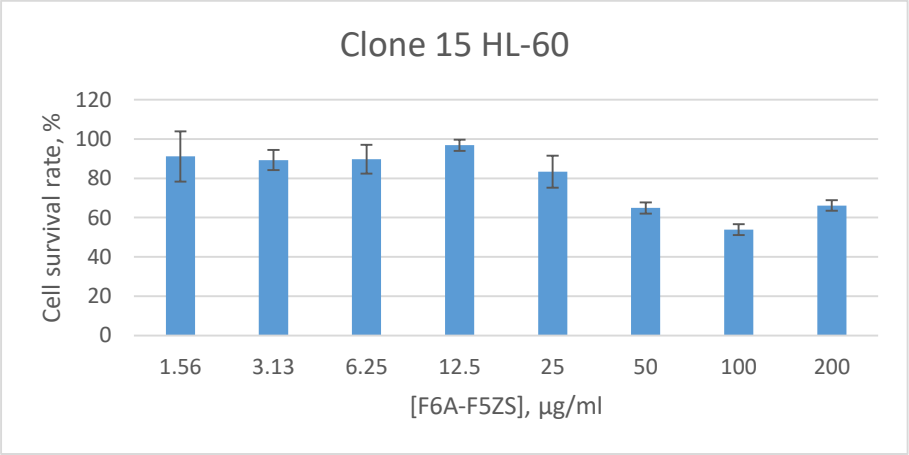

Figure 7.10: Cell survival following treatment of Clone 15 HL-60 cells for 72 h with F6A-F5ZS, as determined with the LDH assay (error bars = standard deviation, *n* = 3).

5.7.11. DU145 (Human prostate carcinoma)

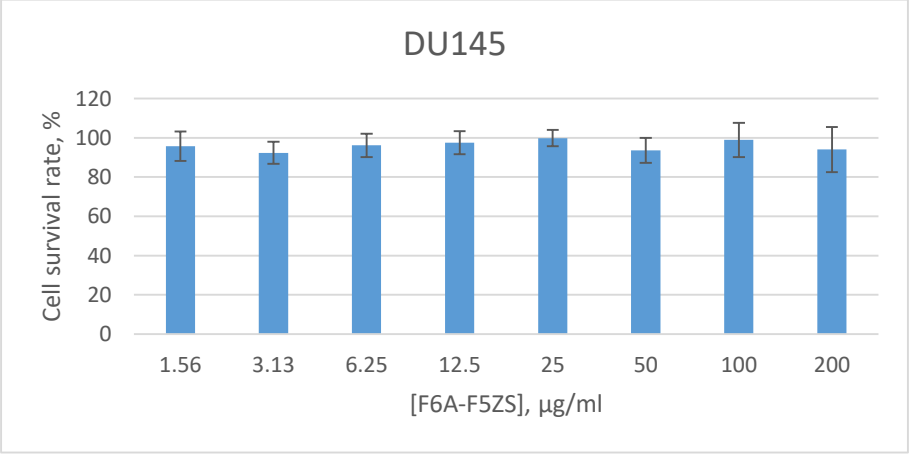

Figure 7.11: Cell survival following treatment of DU145 cells for 72 h with F6A-F5ZS, as determined with the MTT assay (error bars = standard deviation, *n* = 6).

5.7.12. A-704 (human kidney adenocarcinoma)

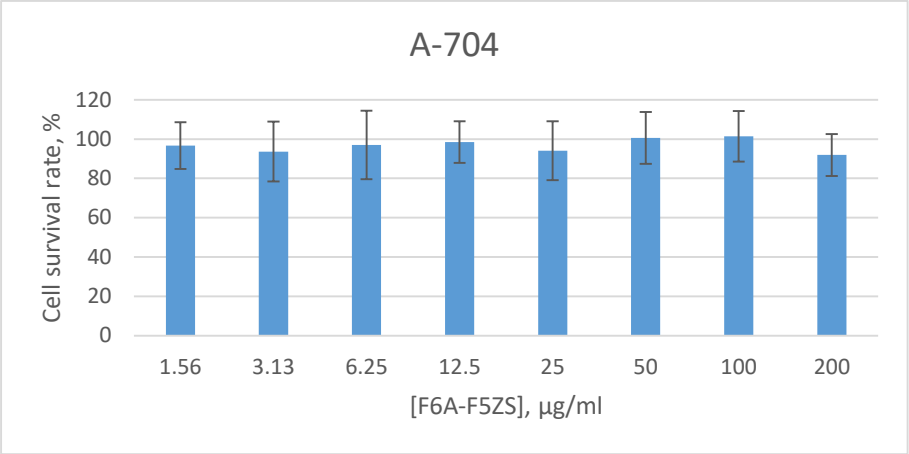

Figure 7.12: Cell survival following treatment of A-704 cells for 72 h with F6A-F5ZS, as determined with the MTT assay (error bars = standard deviation, *n* = 6).

5.7.13. K-562 (Human chronic myelogenous Leukemia)

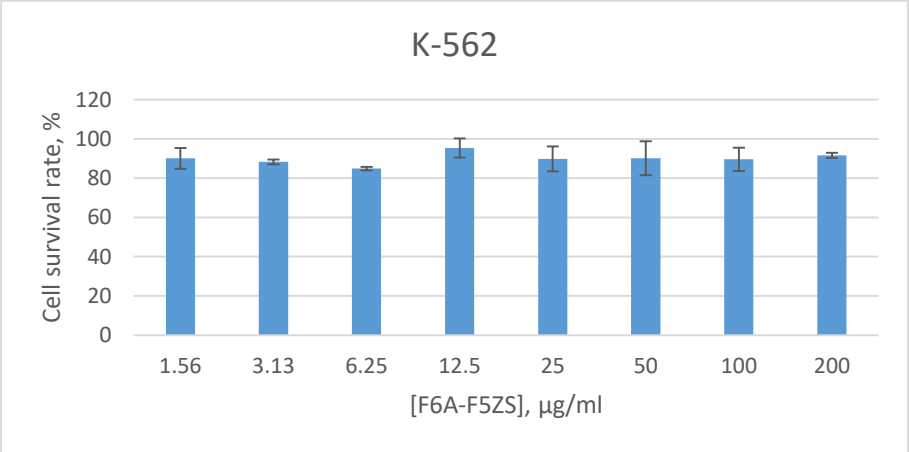

Figure 7.13A: Cell survival following treatment of K-562 cells for 72 h with F6A-F5ZS, as determined with the LDH assay (error bars = standard deviation, *n* = 3).

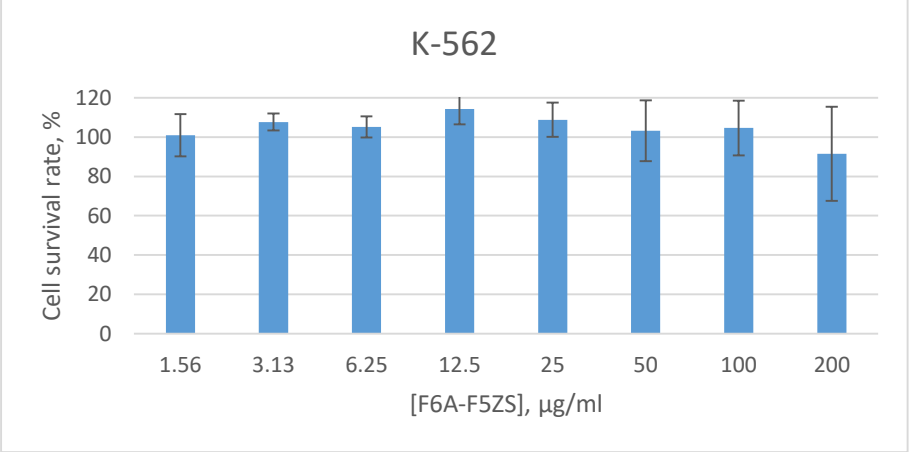

Figure 7.13B: Cell survival following treatment of K-562 cells for 72 h with F6A-F5ZS, as determined with the MTT assay (error bars = standard deviation, *n* = 3).

5.7.15. U-87 MG (Human glioblastoma)

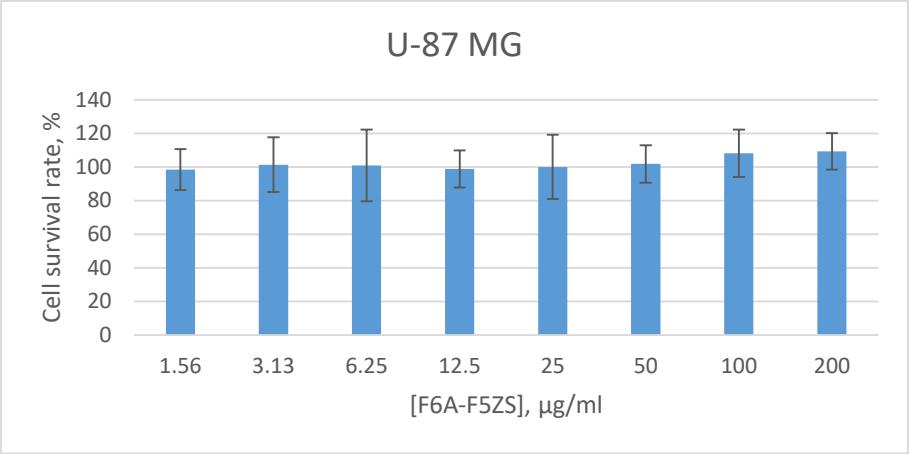

Figure 7.15: Cell survival following treatment of U-87 MG cells for 72 h with F6A-F5ZS, as determined with the MTT assay (error bars = standard deviation, *n* = 6).

5.7.15. A-549 (Human non-small cell lung carcinoma)

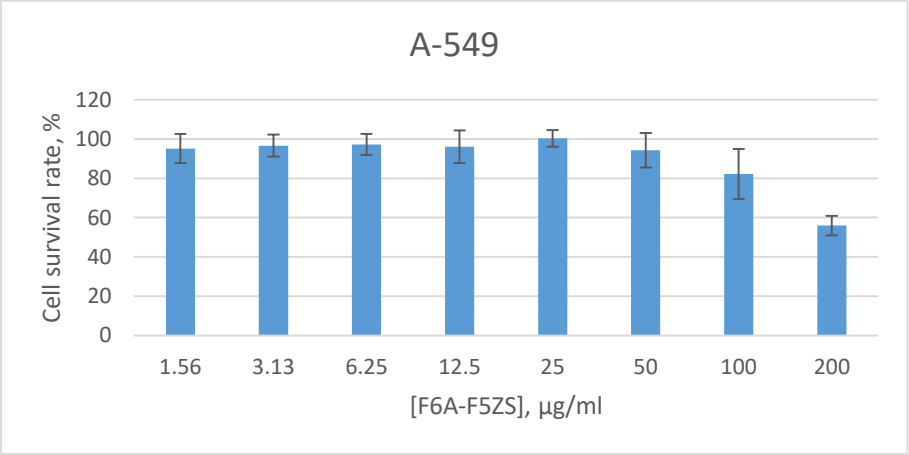

Figure 7.15: Cell survival following treatment of A-549 cells for 72 h with F6A-F5ZS, as determined with the MTT assay (error bars = standard deviation, *n* = 6).

5.7.16. BT-20 (Human triple negative invasive ductal human carcinoma breast cancer)

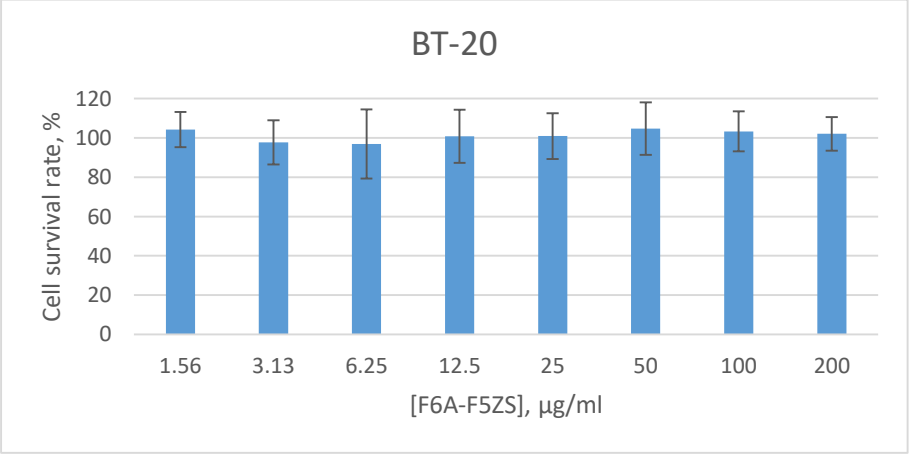

Figure 7.16: Cell survival following treatment of BT-20 cells for 72 h with F6A-F5ZS, as determined with the MTT assay (error bars = standard deviation, *n* = 6).

5.7.17. Panc 02.03 (Human pancreas adenocarcinoma)

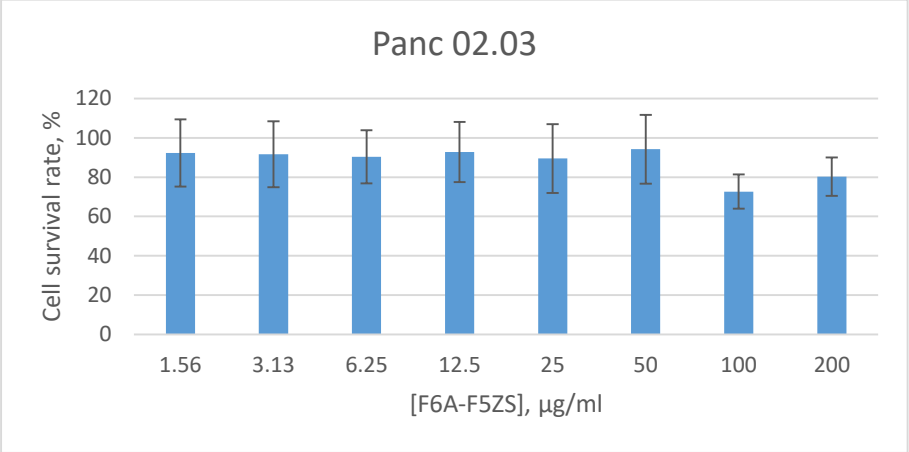

Figure 7.17: Cell survival following treatment of Panc 02.03 cells for 72 h with F6A-F5ZS, as determined with the MTT assay (error bars = standard deviation, *n* = 6).

Discussion for compound F6A-F5ZS results:

F6A-F5ZS does not appear to be toxic in the non-cancerous Vero cell line, with no more than 20% reduction in cell viability after 72 h exposure to F6A-F5ZS. The 84BR and BJ-5ta cell lines showed no toxicity to this compound at the concentrations tested. This compound seems to have potential against the small cell lung carcinoma H69V, acute promyelocytic leukemia Clone 15 HL-60 and non-small cell lung carcinoma A-549 cell lines at high concentrations. Although the exact concentration required is inconclusive, the compound does appear to have potentially good activity against the liver carcinoma HepG2/C3A cell line.

5.8. Results for compound G4/MUC

5.8.1. Vero (African green monkey – non-cancer control)

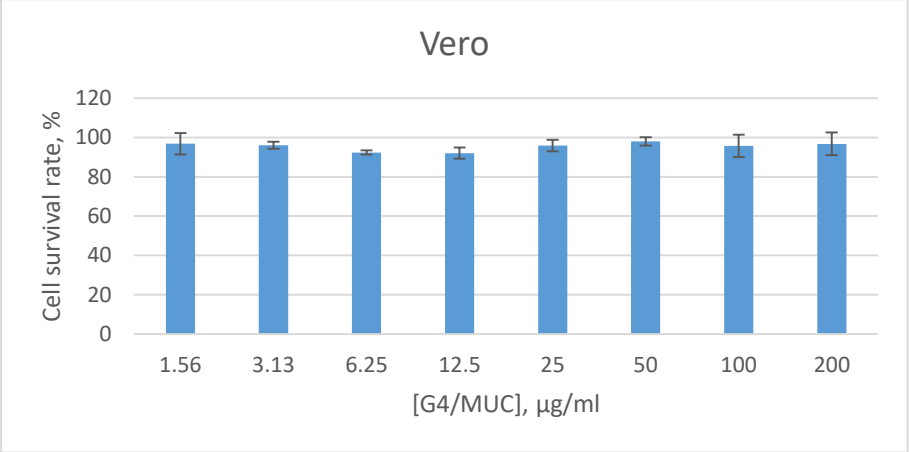

Figure 8.1: Cell survival following treatment of Vero cells for 72 h with G4/MUC, as determined with the MTT assay (error bars = standard deviation, *n* = 6).

5.8.2a. 84BR (Human dermal fibroblasts – non-cancer control)

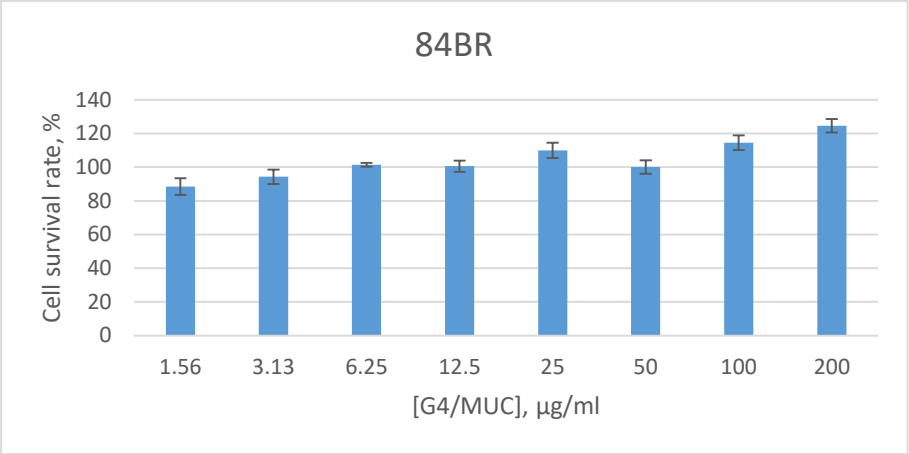

Figure 8.2a: Cell survival following treatment of 84BR cells for 72 h with G4/MUC, as determined with the MTT assay (error bars = standard deviation, *n* = 3).

5.8.2b. BJ-5ta (Human skin fibroblasts hTERT immortalized, non-cancer)

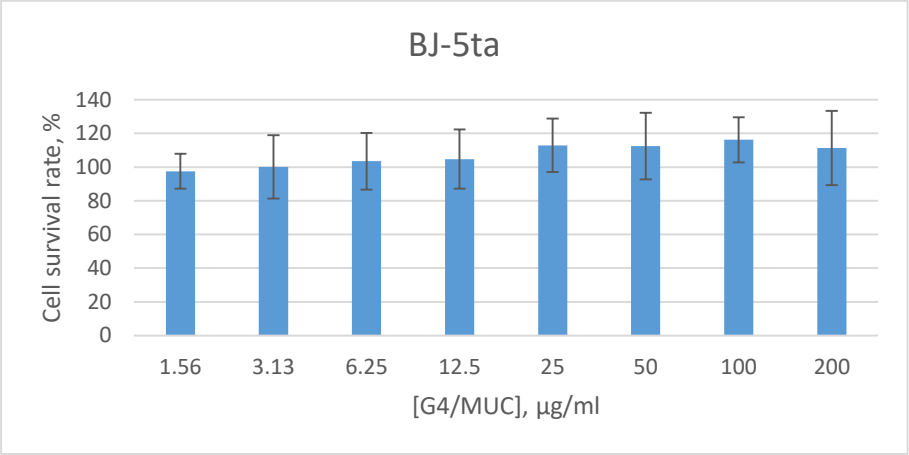

Figure 8.2b: Cell survival following treatment of BJ-5ta cells for 72 h with G4/MUC, as determined with the MTT assay (error bars = standard deviation, *n* = 6).

5.8.3. H69V (Human small cell lung carcinoma)

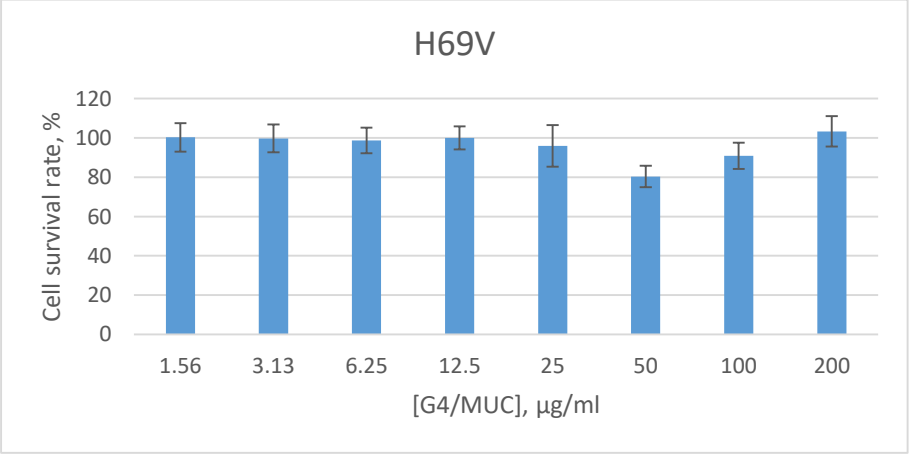

Figure 8.3: Cell survival following treatment of H69V cells for 72 h with G4/MUC, as determined with the MTT assay (error bars = standard deviation, *n* = 6).

5.8.4. 143B (Human osteosarcoma)

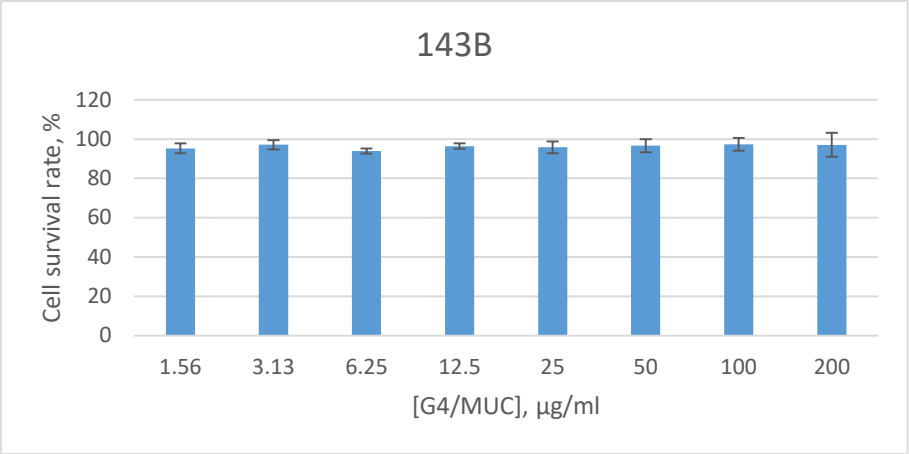

Figure 8.4: Cell survival following treatment of 143B cells for 72 h with G4/MUC, as determined with the MTT assay (error bars = standard deviation, *n* = 6).

5.8.5. HepG2/C3A (Human hepatocellular carcinoma)

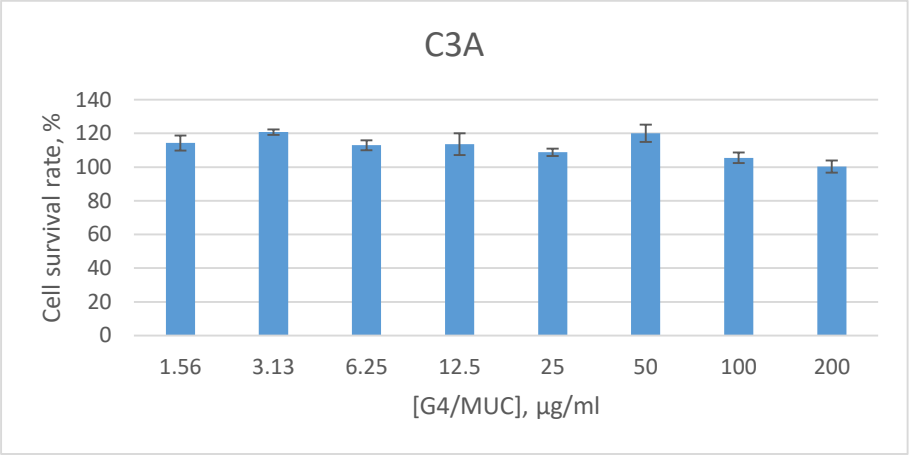

Figure 8.5: Cell survival following treatment of HepG2/C3A cells for 72 h with G4/MUC, as determined with the MTT assay (error bars = standard deviation, *n* = 6).

5.8.6. A375 (Human melanoma)

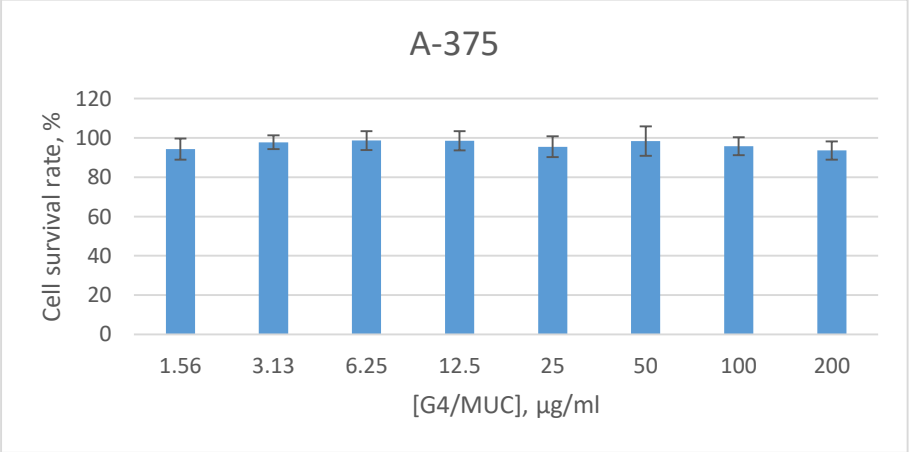

Figure 8.6: Cell survival following treatment of A375 cells for 72 h with G4/MUC, as determined with the MTT assay (error bars = standard deviation, *n* = 6).

5.8.7. HT29 (Human colon carcinoma)

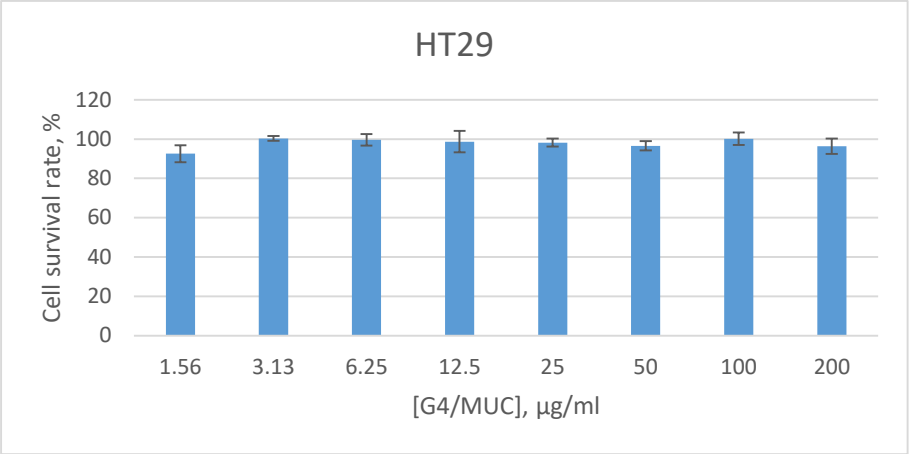

Figure 8.7: Cell survival following treatment of HT29 cells for 72 h with G4/MUC, as determined with the MTT assay (error bars = standard deviation, *n* = 6).

5.8.8. MCF7 (Human breast adenocarcinoma)

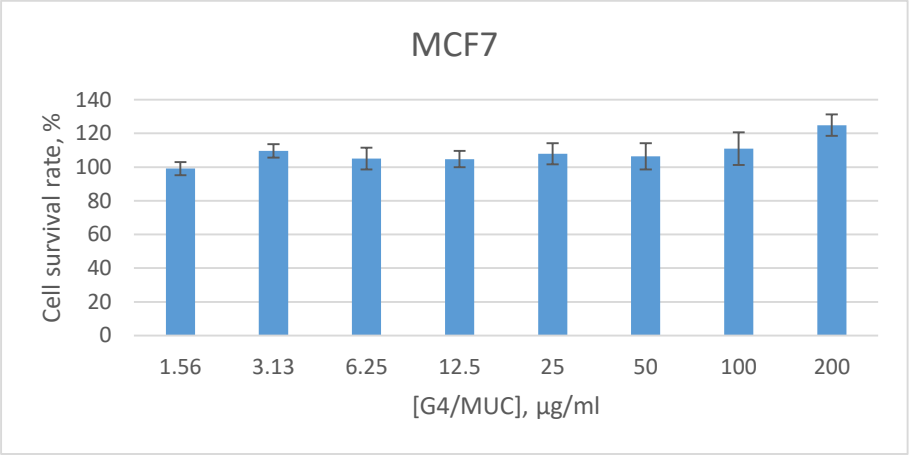

Figure 8.8: Cell survival following treatment of MCF7 cells for 72 h with G4/MUC, as determined with the MTT assay (error bars = standard deviation, *n* = 6).

5.8.9. AGS (Human stomach adenocarcinoma)

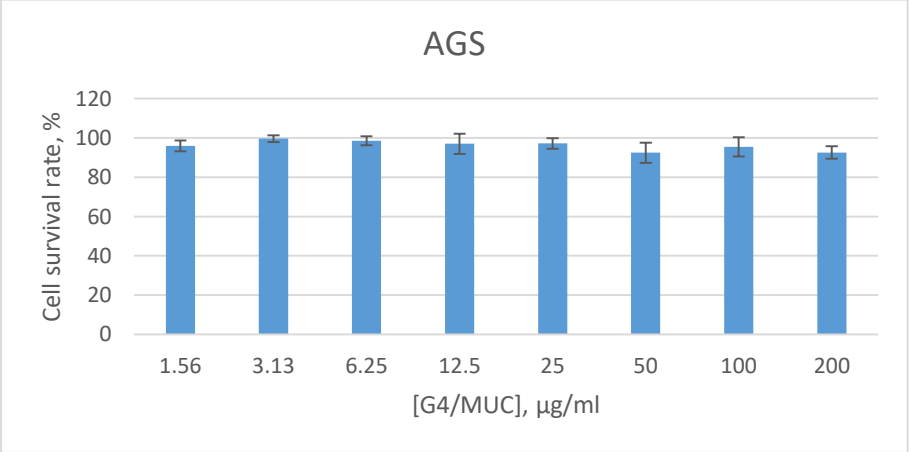

Figure 8.9: Cell survival following treatment of AGS cells for 72 h with G4/MUC, as determined with the MTT assay (error bars = standard deviation, *n* = 6).

5.8.10. Clone 15 HL-60 (Human acute promyelocytic leukemia)

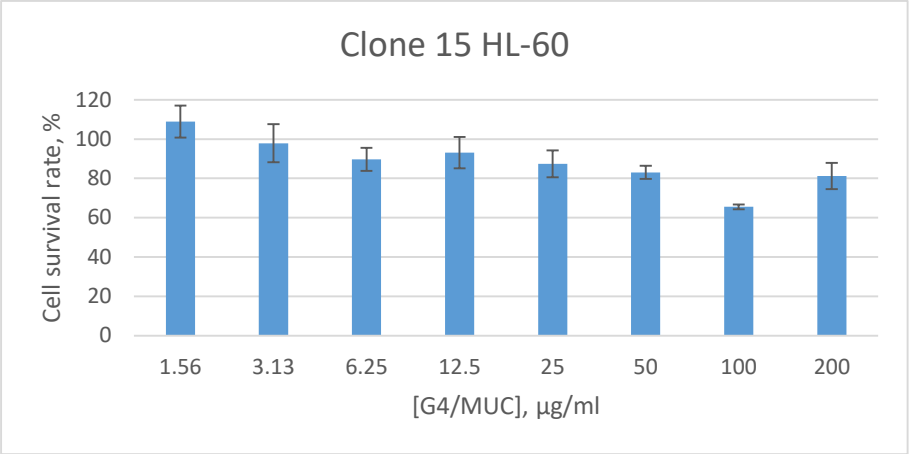

Figure 8.10: Cell survival following treatment of Clone 15 HL-60 cells for 72 h with G4/MUC, as determined with the LDH assay (error bars = standard deviation, *n* = 3).

5.8.11. DU145 (Human prostate carcinoma)

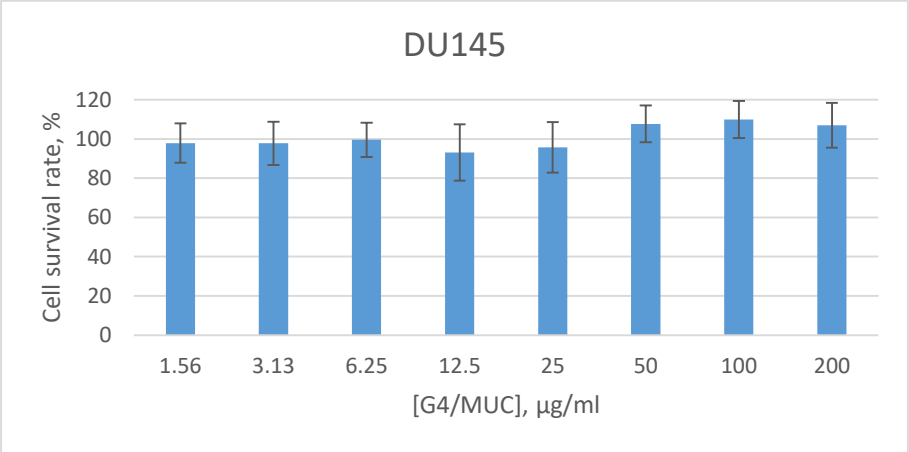

Figure 8.11: Cell survival following treatment of DU145 cells for 72 h with G4/MUC, as determined with the MTT assay (error bars = standard deviation, *n* = 6).

5.8.12. A-704 (human kidney adenocarcinoma)

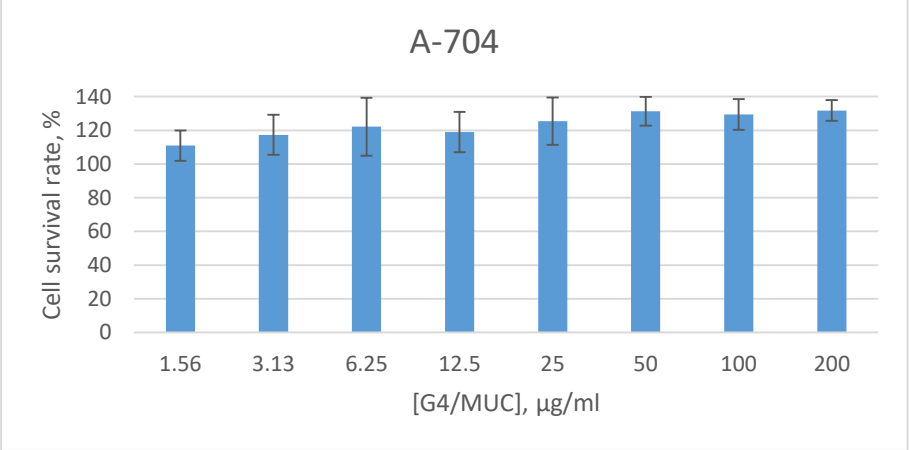

Figure 8.12: Cell survival following treatment of A-704 cells for 72 h with G4/MUC, as determined with the MTT assay (error bars = standard deviation, *n* = 6).

5.8.13. K-562 (Human chronic myelogenous Leukemia)

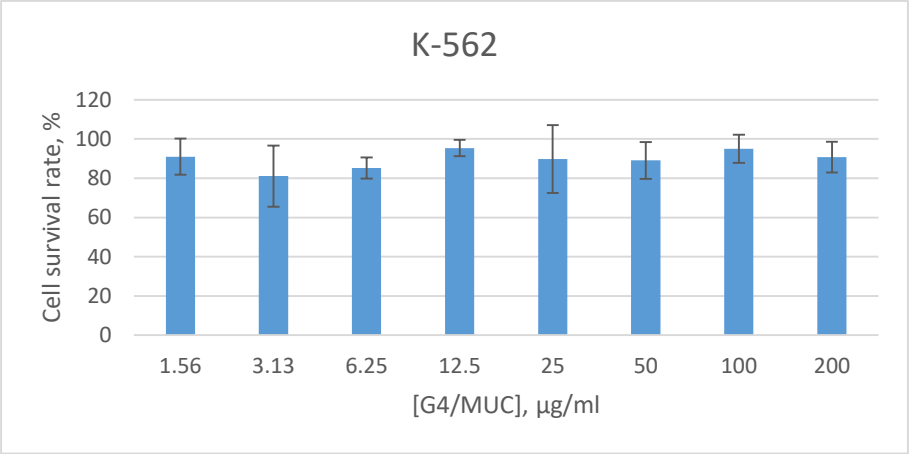

Figure 8.13A: Cell survival following treatment of K-562 cells for 72 h with G4/MUC, as determined with the LDH assay (error bars = standard deviation, *n* = 3).

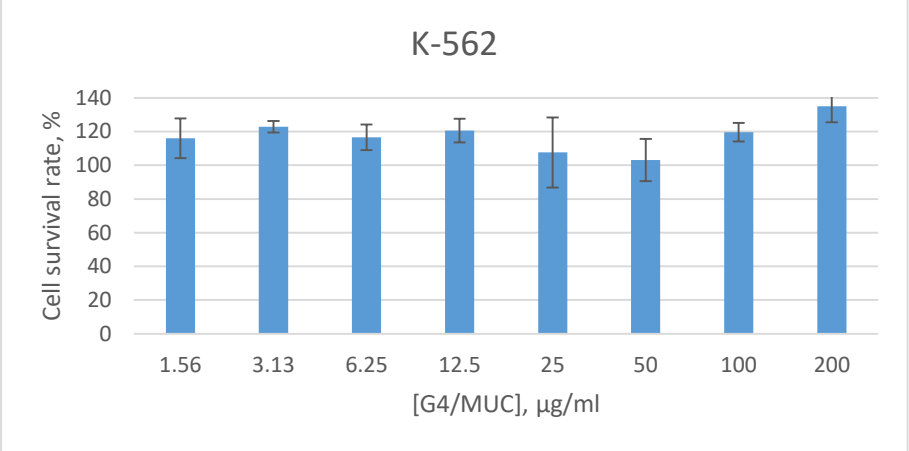

Figure 8.13B: Cell survival following treatment of K-562 cells for 72 h with G4/MUC, as determined with the MTT assay (error bars = standard deviation, *n* = 3).

5.8.14. U-87 MG (Human glioblastoma)

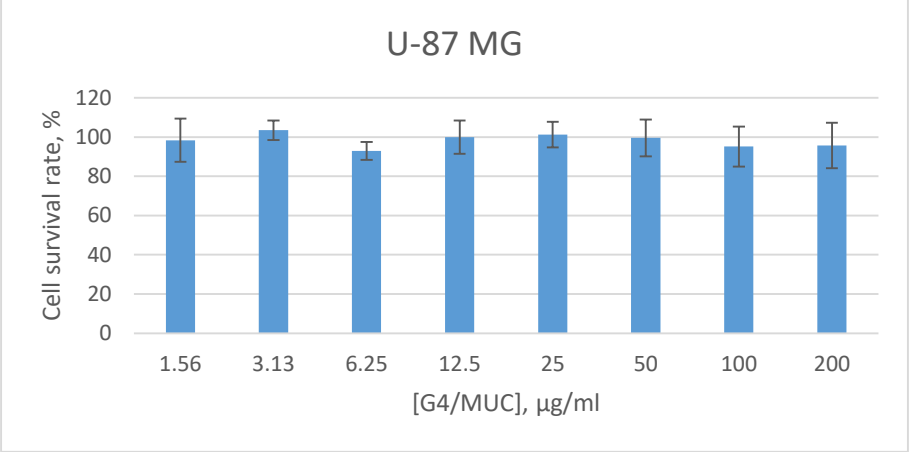

Figure 8.14: Cell survival following treatment of U-87 MG cells for 72 h with G4/MUC, as determined with the MTT assay (error bars = standard deviation, *n* = 6).

5.8.15. A-549 (Human non-small cell lung carcinoma)

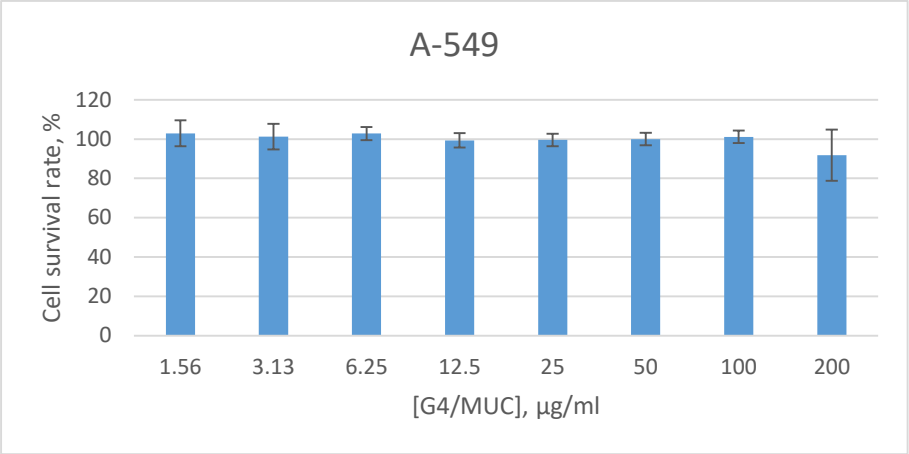

Figure 8.15: Cell survival following treatment of A-549 cells for 72 h with G4/MUC, as determined with the MTT assay (error bars = standard deviation, *n* = 6).

5.8.16. BT-20 (Human triple negative invasive ductal human carcinoma breast cancer)

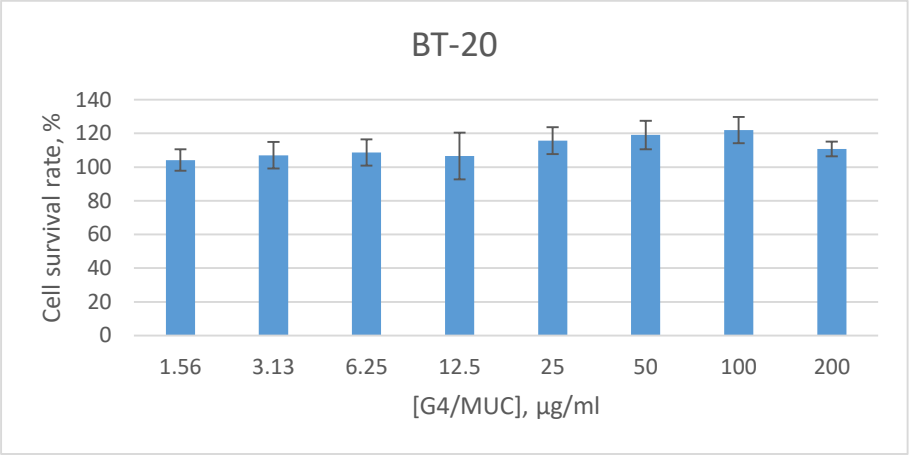

Figure 8.16: Cell survival following treatment of BT-20 cells for 72 h with G4/MUC, as determined with the MTT assay (error bars = standard deviation, *n* = 6).

5.8.17. Panc 02.03 (Human pancreas adenocarcinoma)

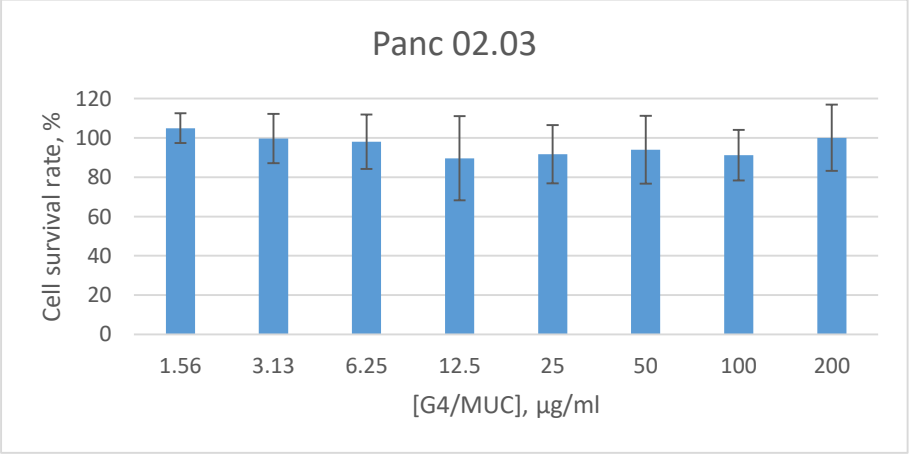

Figure 8.17: Cell survival following treatment of Panc 02.03 cells for 72 h with G4/MUC, as determined with the MTT assay (error bars = standard deviation, *n* = 6).

Discussion for compound G4/MUC results:

G4/MUC appears to not be toxic to the non-cancerous cell lines after 72 h exposure to G4/MUC, but rather seem to stimulate growth. Low concentrations seem to induce cell growth in some of the cancer cell lines tested, with viability increased to more than that of the untreated control. G4/MUC appears to have some potential against the acute promyelocytic leukemia Clone 15 HL-60 cell line.

5.9. Results for compound G3/W

5.9.1. Vero (African green monkey – non-cancer control)

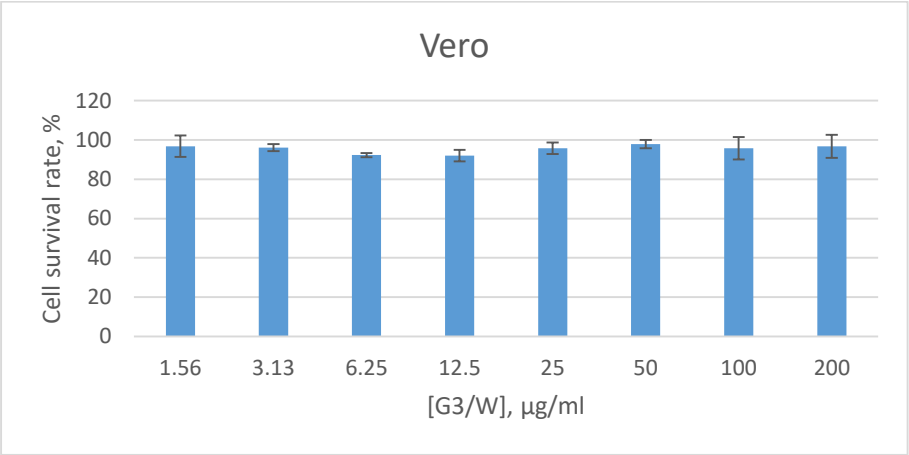

Figure 9.1: Cell survival following treatment of Vero cells for 72 h with G3/W, as determined with the MTT assay (error bars = standard deviation, *n* = 6).

5.9.2a. 84BR (Human dermal fibroblasts – non-cancer control)

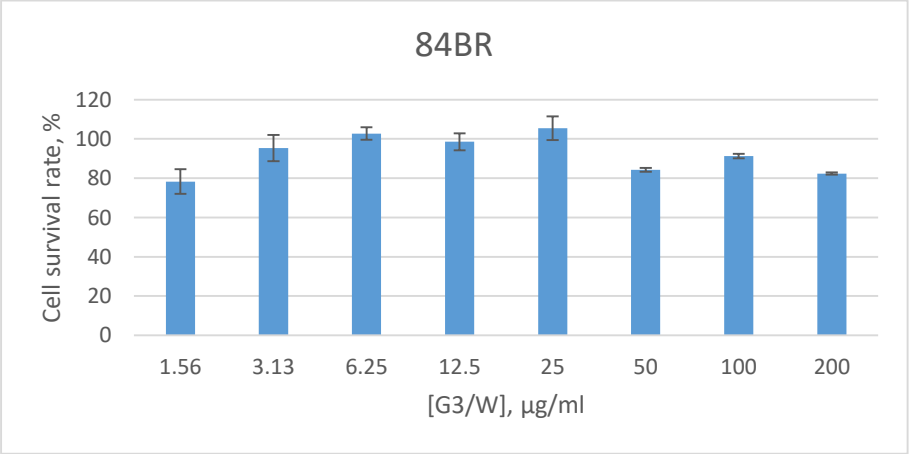

Figure 9.2a: Cell survival following treatment of 84BR cells for 72 h with G3/W, as determined with the MTT assay (error bars = standard deviation, *n* = 3).

5.9.2b. BJ-5ta (Human skin fibroblasts hTERT immortalized, non-cancer)

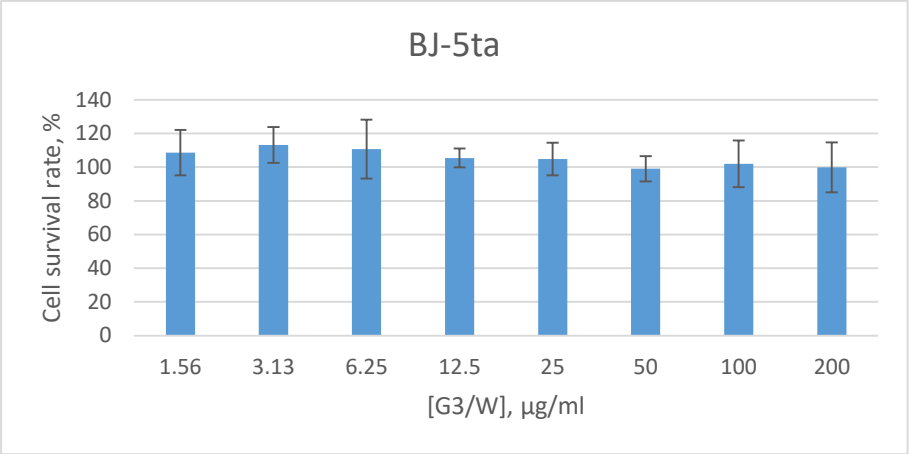

Figure 9.2b: Cell survival following treatment of BJ-5ta cells for 72 h with G3/W, as determined with the MTT assay (error bars = standard deviation, *n* = 6).

5.9.3. H69V (Human small cell lung carcinoma)

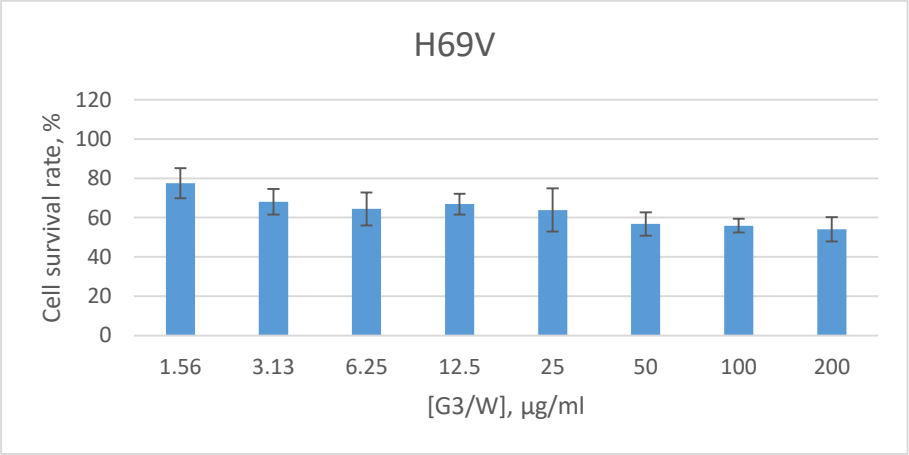

Figure 9.3: Cell survival following treatment of H69V cells for 72 h with G3W, as determined with the MTT assay (error bars = standard deviation, *n* = 6).

5.9.4. 143B (Human osteosarcoma)

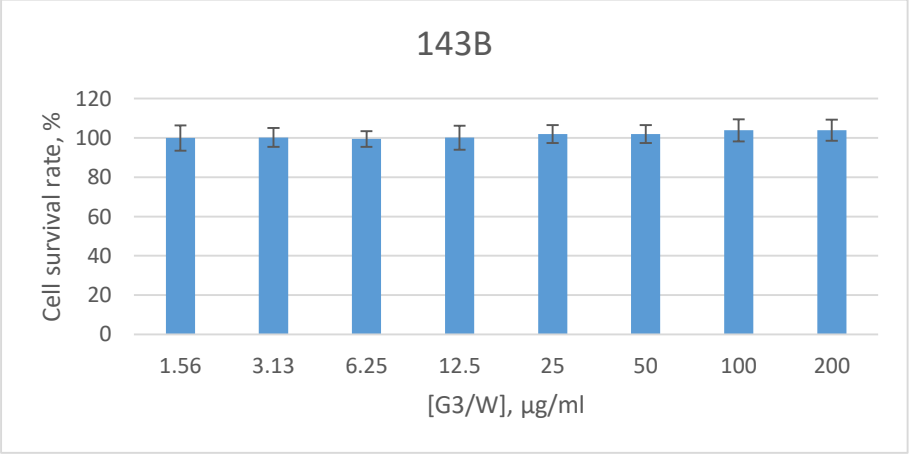

Figure 9.4: Cell survival following treatment of 143B cells for 72 h with G3/W, as determined with the MTT assay (error bars = standard deviation, *n* = 6).

5.9.5. HepG2/C3A (Human hepatocellular carcinoma)

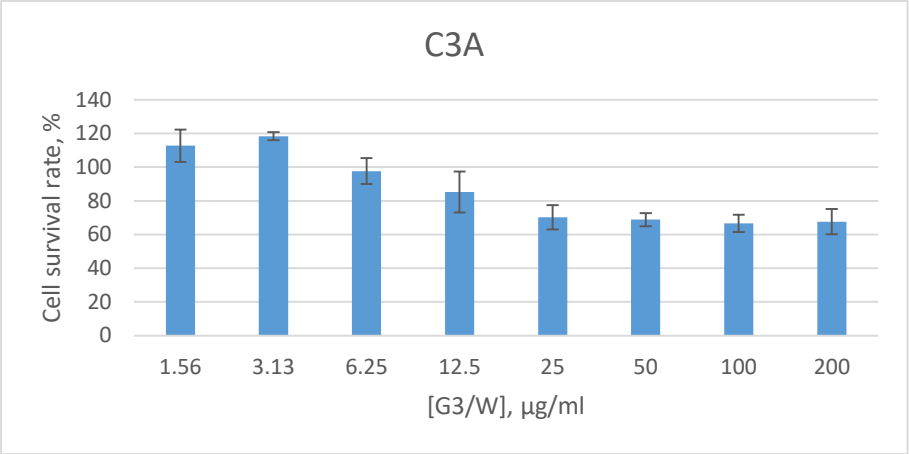

Figure 9.5: Cell survival following treatment of HepG2/C3A cells for 72 h with G3/W, as determined with the MTT assay (error bars = standard deviation, *n* = 6).

5.9.6. A375 (Human melanoma)

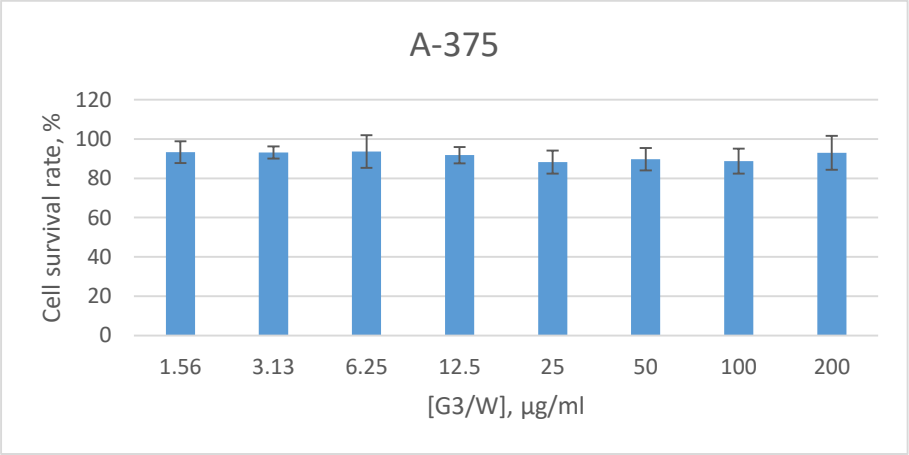

Figure 9.6: Cell survival following treatment of A375 cells for 72 h with G3/W, as determined with the MTT assay (error bars = standard deviation, *n* = 6).

5.9.7. HT29 (Human colon carcinoma)

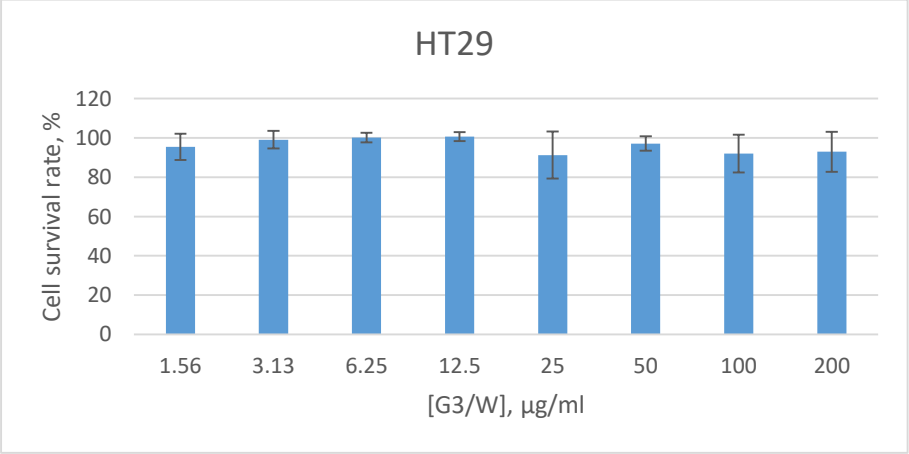

Figure 9.7: Cell survival following treatment of HT29 cells for 72 h with G3/W, as determined with the MTT assay (error bars = standard deviation, *n* = 6).

5.9.8. MCF7 (Human breast adenocarcinoma)

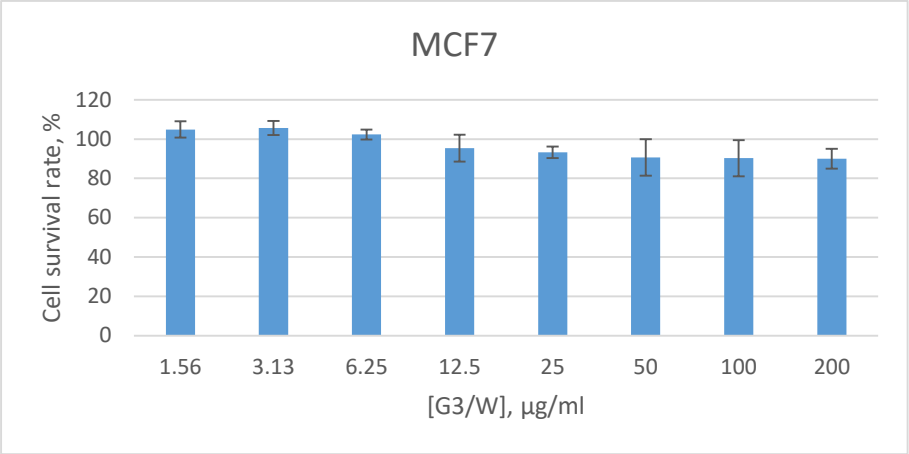

Figure 9.8: Cell survival following treatment of MCF7 cells for 72 h with G3W, as determined with the MTT assay (error bars = standard deviation, *n* = 6).

5.9.9. AGS (Human stomach adenocarcinoma)

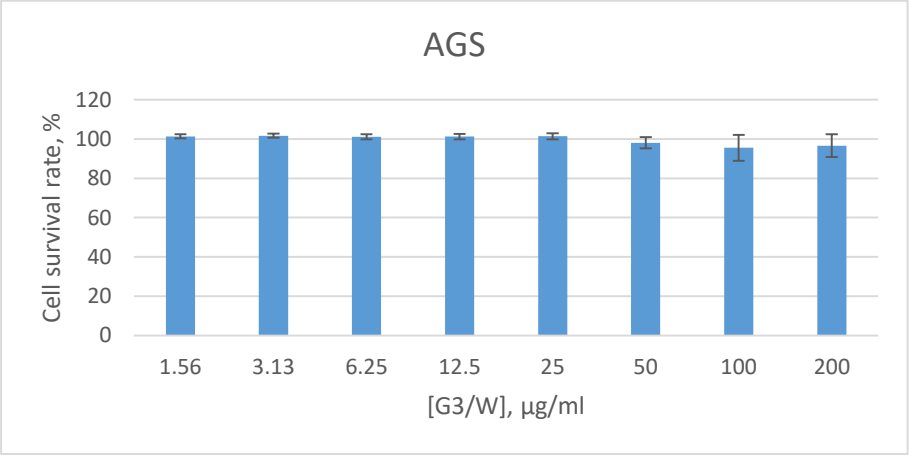

Figure 9.9: Cell survival following treatment of AGS cells for 72 h with G3/W, as determined with the MTT assay (error bars = standard deviation, *n* = 6).

5.9.10. Clone 15 HL-60 (Human acute promyelocytic leukemia)

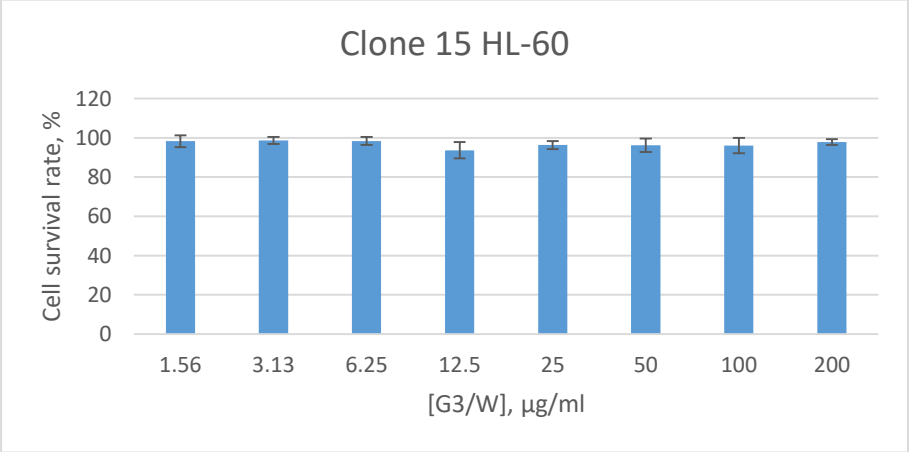

Figure 9.10: Cell survival following treatment of Clone 15 HL-60 cells for 72 h with G3/W, as determined with the LDH assay (error bars = standard deviation, *n* = 3).

5.9.11. DU145 (Human prostate carcinoma)

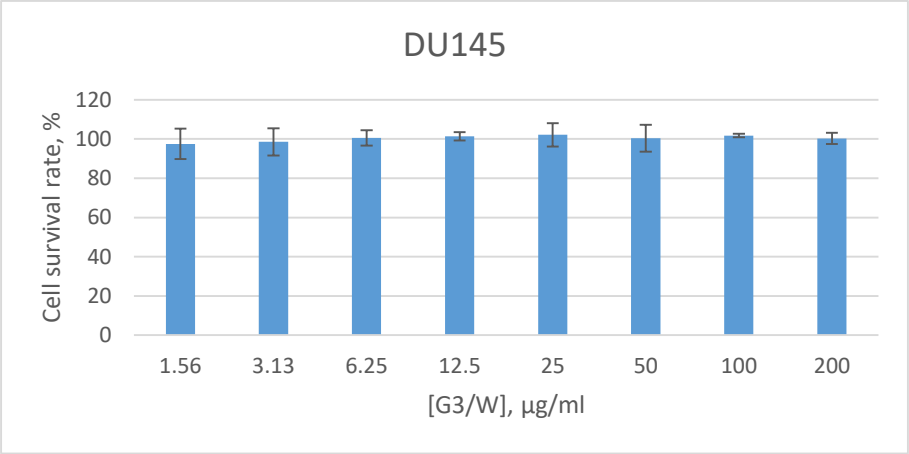

Figure 9.11: Cell survival following treatment of DU145 cells for 72 h with G3/W, as determined with the MTT assay (error bars = standard deviation, *n* = 6).

5.9.12. A-704 (human kidney adenocarcinoma)

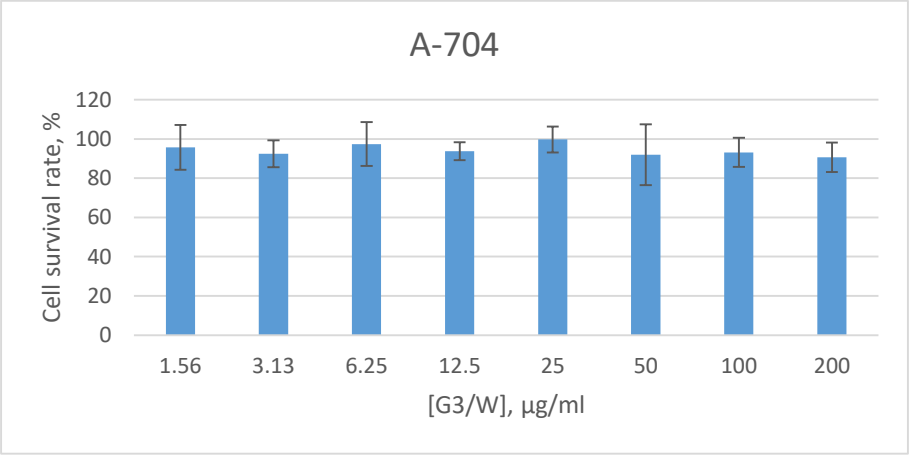

Figure 9.12: Cell survival following treatment of A-704 cells for 72 h with G3/W, as determined with the MTT assay (error bars = standard deviation, *n* = 6).

5.9.13. K-562 (Human chronic myelogenous Leukemia)

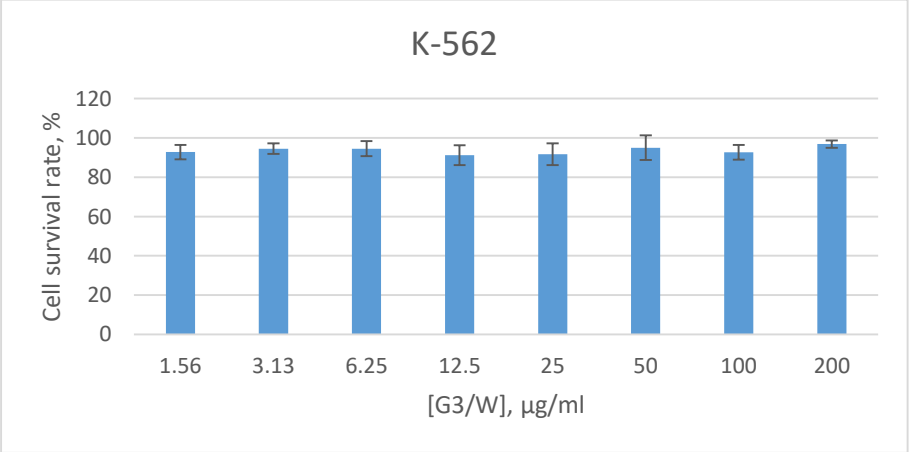

Figure 9.13A: Cell survival following treatment of K-562 cells for 72 h with G3/W, as determined with the LDH assay (error bars = standard deviation, *n* = 3).

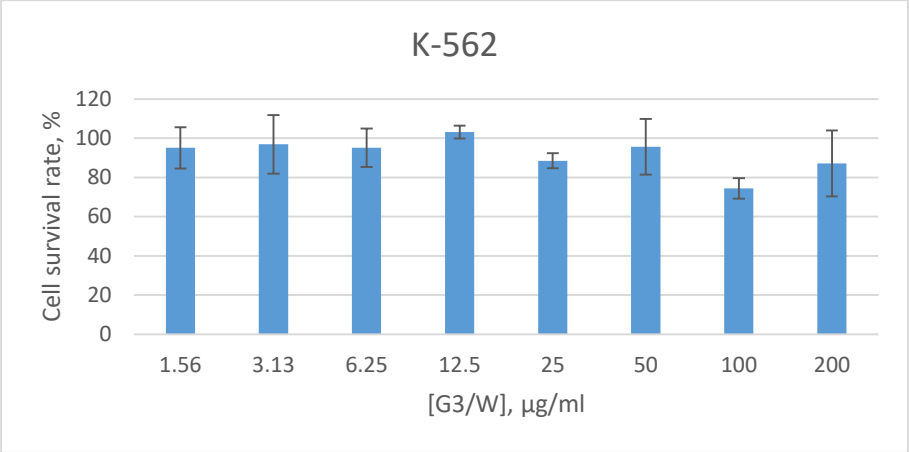

Figure 9.13B: Cell survival following treatment of K-562 cells for 72 h with G3/W, as determined with the MTT assay (error bars = standard deviation, *n* = 3).

5.9.14. U-87 MG (Human glioblastoma)

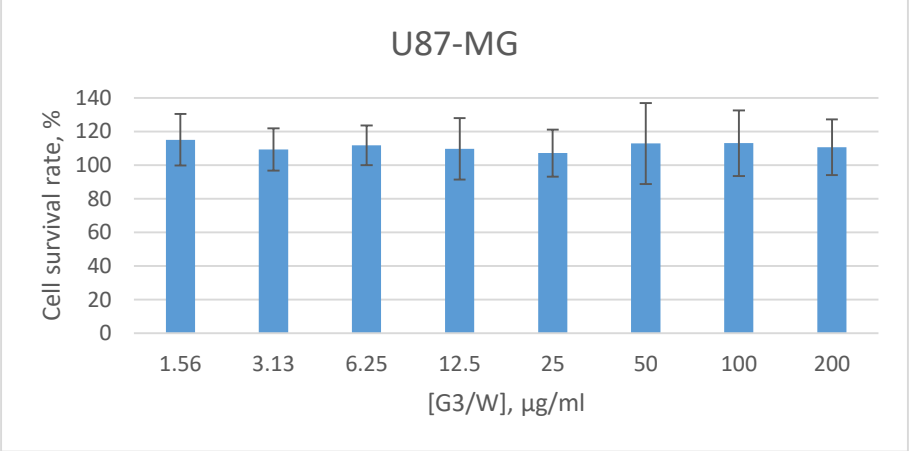

Figure 9.14: Cell survival following treatment of U-87 MG cells for 72 h with G3/W, as determined with the MTT assay (error bars = standard deviation, *n* = 6).

5.9.15. A-549 (Human non-small cell lung carcinoma)

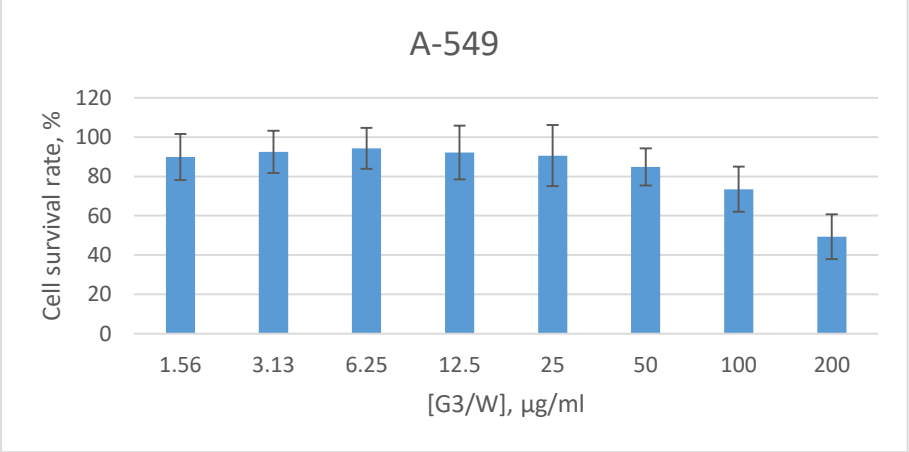

Figure 9.15: Cell survival following treatment of A-549 cells for 72 h with G3/W, as determined with the MTT assay (error bars = standard deviation, *n* = 6).

5.9.16. BT-20 (Human triple negative invasive ductal human carcinoma breast cancer)

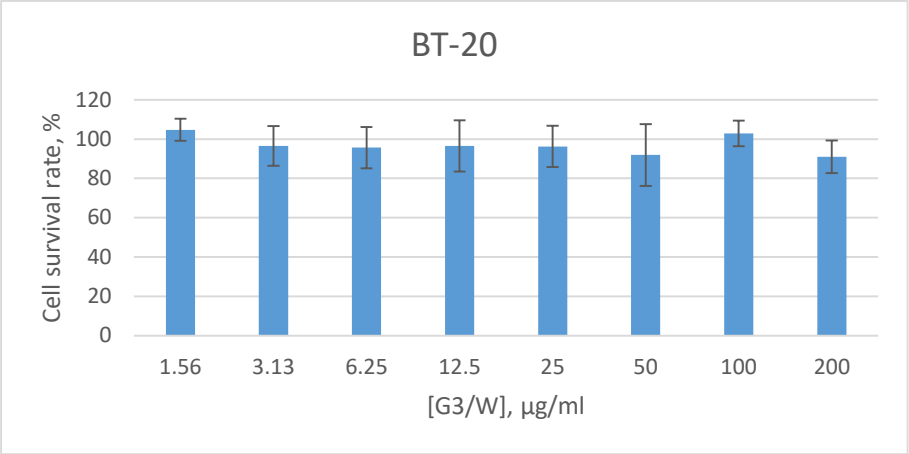

Figure 9.16: Cell survival following treatment of BT-20 cells for 72 h with G3/W, as determined with the MTT assay (error bars = standard deviation, *n* = 6).

5.9.17. Panc 02.03 (Human pancreas adenocarcinoma)

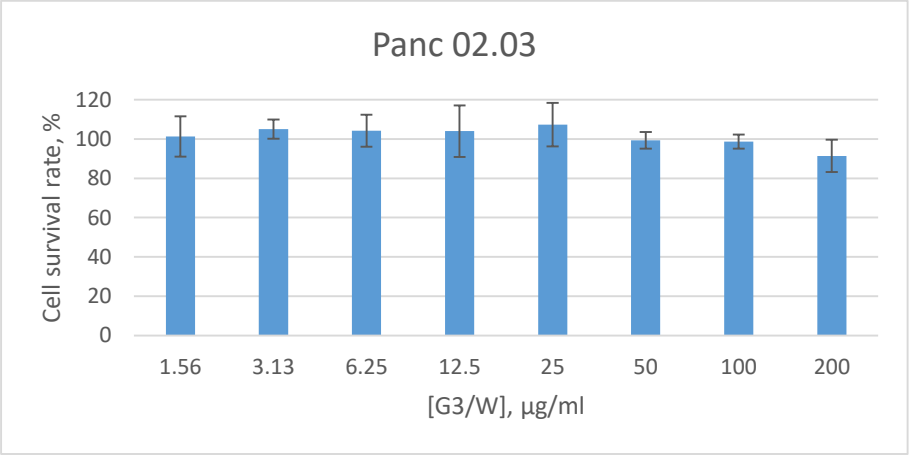

Figure 9.17: Cell survival following treatment of Panc 02.03 cells for 72 h with G3/W, as determined with the MTT assay (error bars = standard deviation, *n* = 6).

Discussion for compound G3/W results:

G3/W appears to be very slightly toxic to the non-cancerous Vero and 84BR cell lines at higher concentrations, with a 20–25% reduction in cell viability after 72 h exposure to G3/W. No toxicity was apparent in the non-cancerous BJ-5ta cell line. Inhibition of cell viability was only observed in the H69V and HepG2/C3A cell lines, but with good activity. Inhibition of the A549 cell line was also observed at a high concentration.

5.10. Results for compound MESC-INO

5.10.1. Vero (African green monkey – non-cancer control)

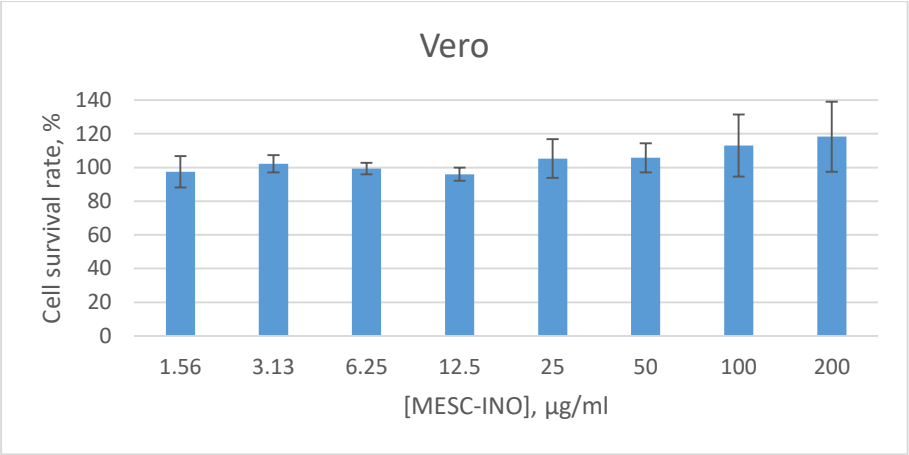

Figure 10.1: Cell survival following treatment of Vero cells for 72 h with MESC-INO, as determined with the MTT assay (error bars = standard deviation, *n* = 6).

5.10.2 BJ-5ta (Human skin fibroblasts hTERT immortalized, non-cancer)

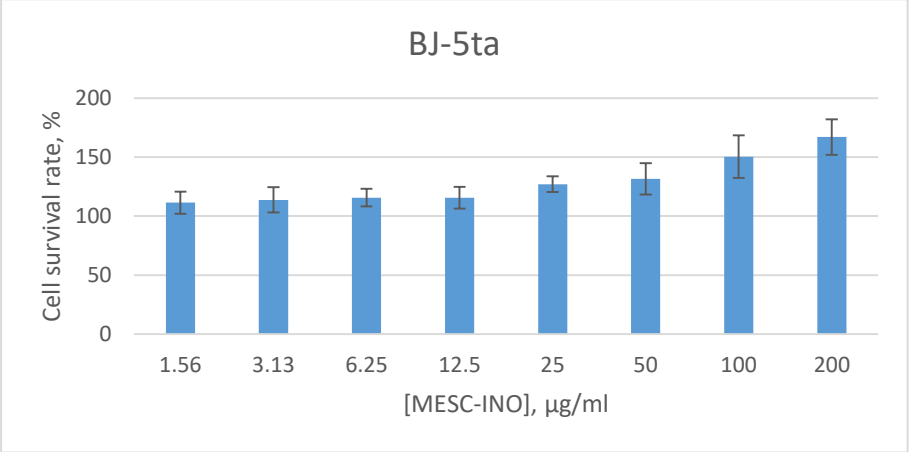

Figure 10.2: Cell survival following treatment of BJ-5ta cells for 72 h with MESC-INO, as determined with the MTT assay (error bars = standard deviation, *n* = 6).

5.10.3. H69V (Human small cell lung carcinoma)

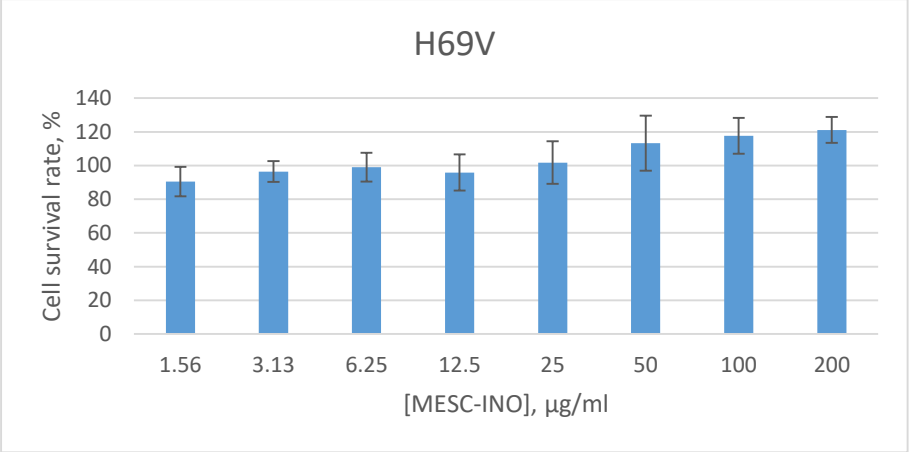

Figure 10.3: Cell survival following treatment of H69V cells for 72 h with MESC-INO, as determined with the MTT assay (error bars = standard deviation, *n* = 6).

5.10.4. 143B (Human osteosarcoma)

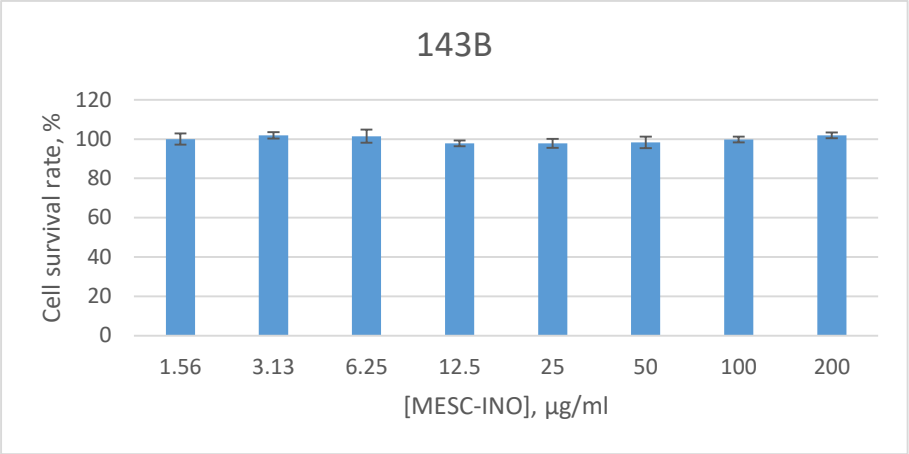

Figure 10.4: Cell survival following treatment of 143B cells for 72 h with MESC-INO, as determined with the MTT assay (error bars = standard deviation, *n* = 6).

5.10.5. HepG2/C3A (Human hepatocellular carcinoma)

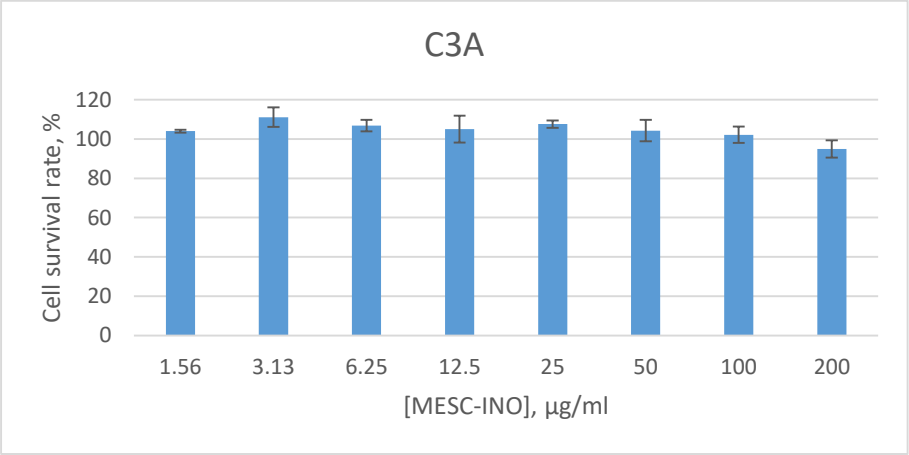

Figure 10.5: Cell survival following treatment of HepG2/C3A cells for 72 h with MESC-INO, as determined with the MTT assay (error bars = standard deviation, *n* = 6).

5.10.6. A375 (Human melanoma)

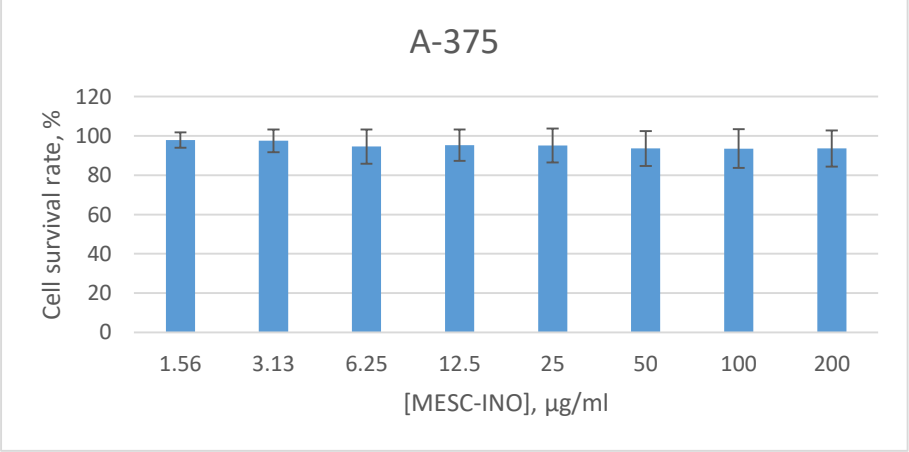

Figure 10.6: Cell survival following treatment of A375 cells for 72 h with MESC-INO, as determined with the MTT assay (error bars = standard deviation, *n* = 6).

5.10.7. HT29 (Human colon carcinoma)

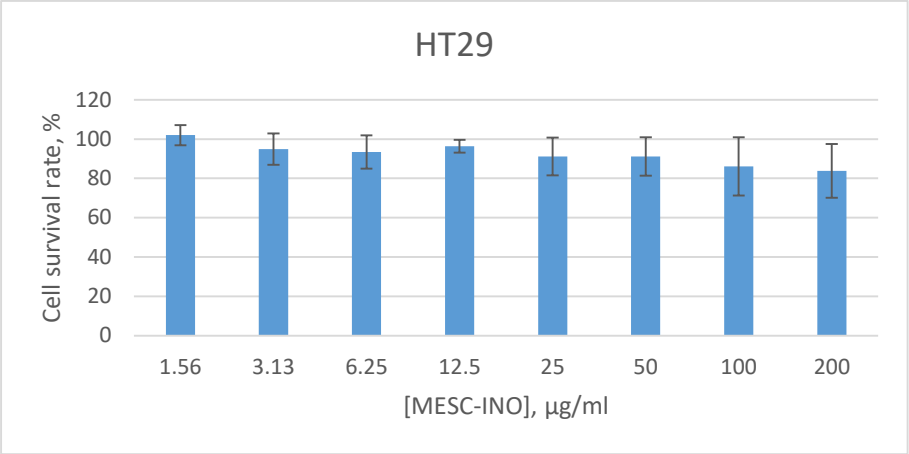

Figure 10.7: Cell survival following treatment of HT29 cells for 72 h with MESC-INO, as determined with the MTT assay (error bars = standard deviation, *n* = 6).

5.10.8. MCF7 (Human breast adenocarcinoma)

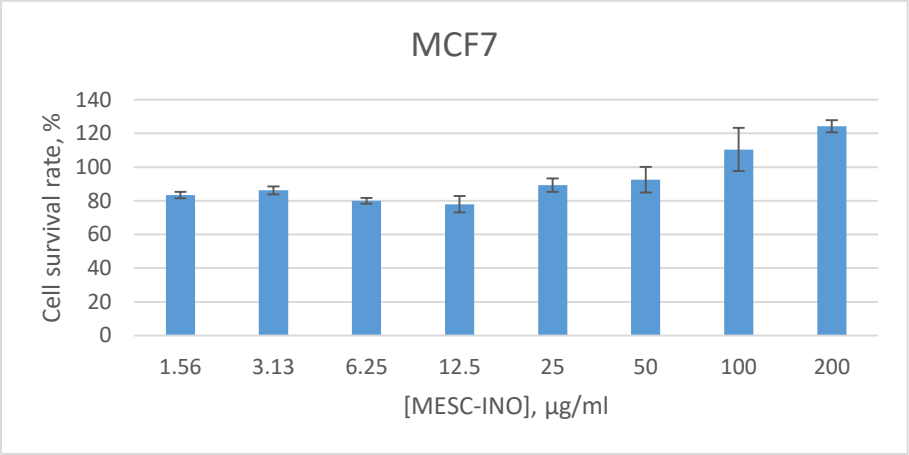

Figure 10.8: Cell survival following treatment of MCF7 cells for 72 h with MESC-INO as determined with the MTT assay (error bars = standard deviation, *n* = 6).

5.10.9. AGS (Human stomach carcinoma)

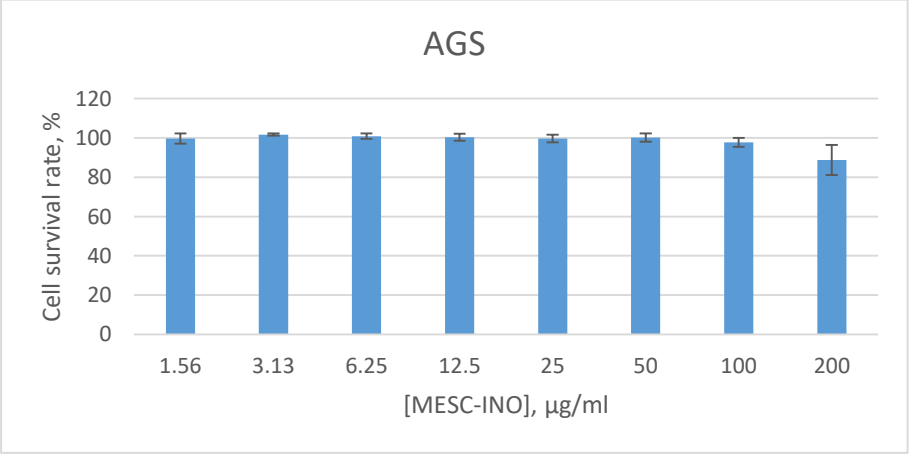

Figure 10.9: Cell survival following treatment of AGS cells for 72 h with MESC-INO, as determined with the MTT assay (error bars = standard deviation, *n* = 6).

5.10.10. Clone 15 HL-60 (Human acute promyelocytic leukemia)

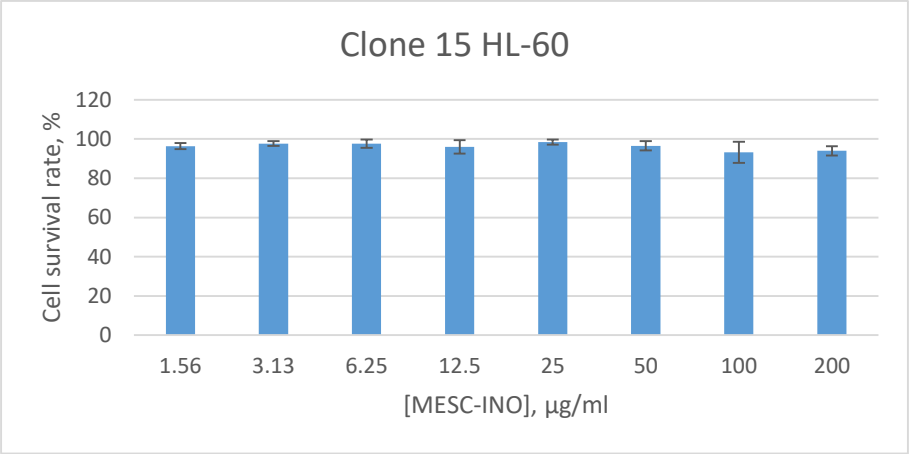

Figure 10.10: Cell survival following treatment of Clone 15 HL-60 cells for 72 h with MESC-INO, as determined with the LDH assay (error bars = standard deviation, *n* = 3).

5.10.11. DU145 (Human prostate carcinoma)

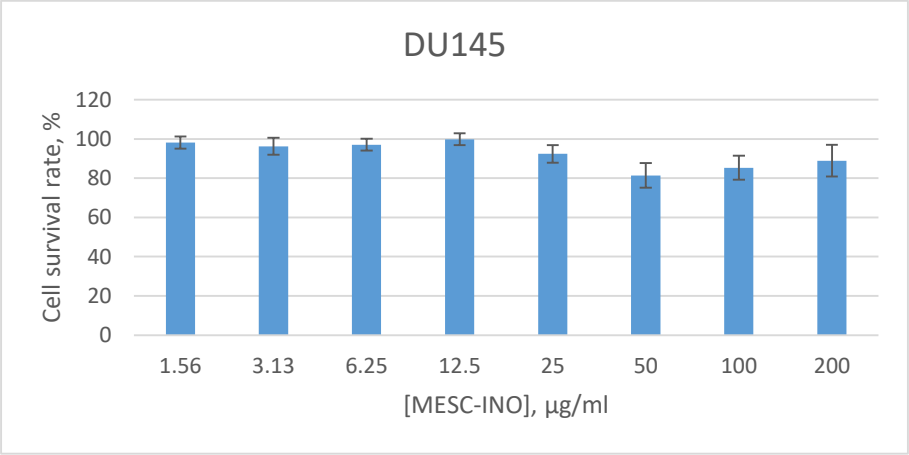

Figure 10.11: Cell survival following treatment of DU145 cells for 72 h with MESC-INO, as determined with the MTT assay (error bars = standard deviation, *n* = 6).

5.10.12. A-704 (human kidney adenocarcinoma)

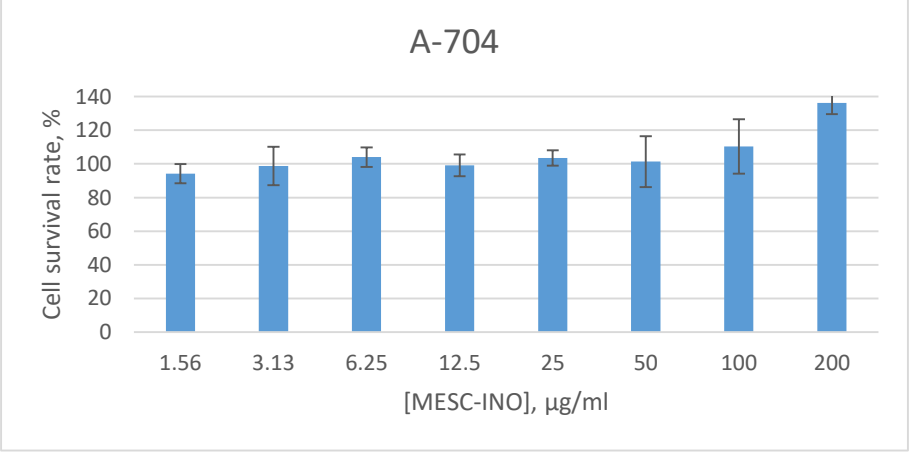

Figure 10.12: Cell survival following treatment of A-704 cells for 72 h with MESC-INO, as determined with the MTT assay (error bars = standard deviation, *n* = 6).

5.10.13. K-562 (Human chronic myelogenous Leukemia)

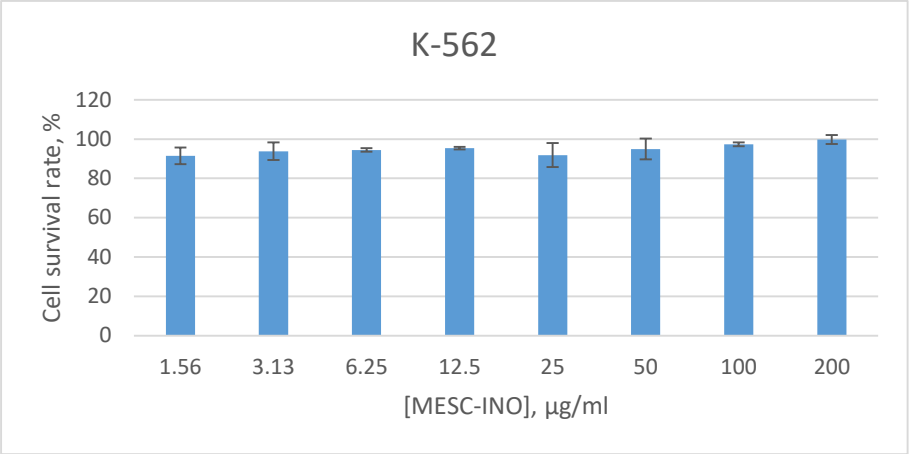

Figure 10.13A: Cell survival following treatment of K-562 cells for 72 h with MESC-INO, as determined with the LDH assay (error bars = standard deviation, *n* = 3).

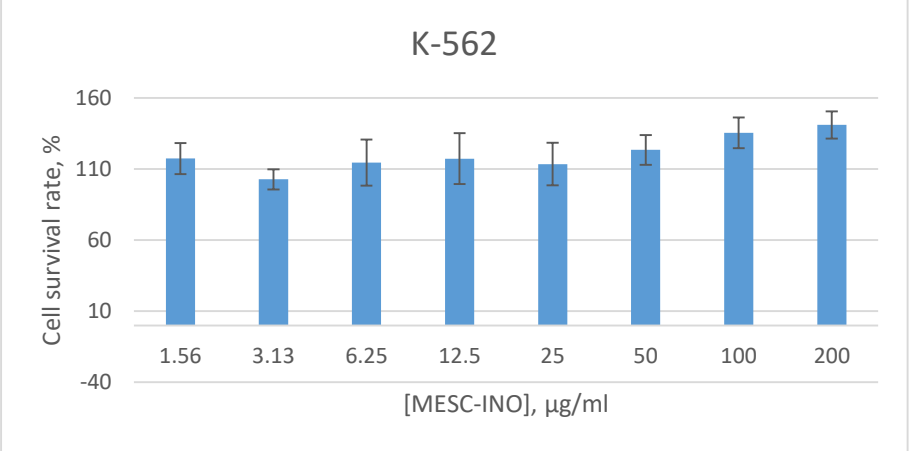

Figure 10.13B: Cell survival following treatment of K-562 cells for 72 h with MESC-INO, as determined with the LDH assay (error bars = standard deviation, *n* = 3).

5.10.14. U-87 MG (Human glioblastoma)

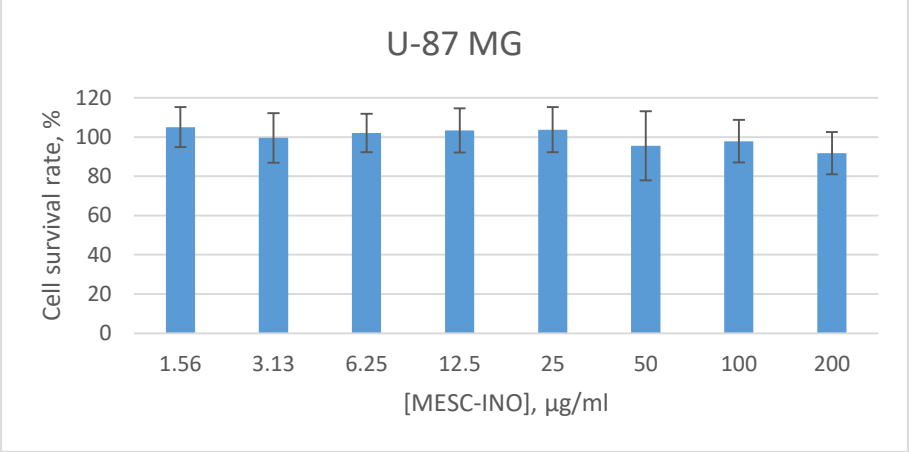

Figure 10.14: Cell survival following treatment of U-87 MG cells for 72 h with MESC-INO, as determined with the MTT assay (error bars = standard deviation, *n* = 6).

5.10.15. A-549 (Human non-small cell lung carcinoma)

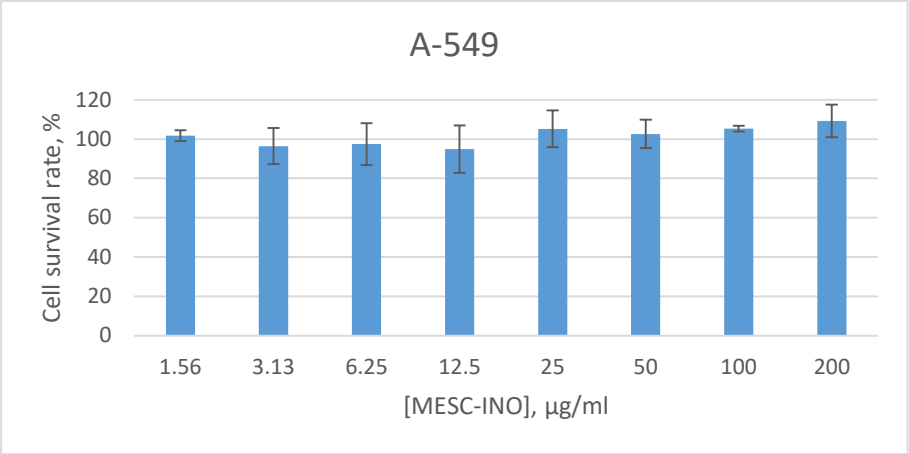

Figure 10.15: Cell survival following treatment of A-549 cells for 72 h with MESC-INO, as determined with the MTT assay (error bars = standard deviation, *n* = 6).

5.10.16. BT-20 (Human triple negative invasive ductal human carcinoma breast cancer)

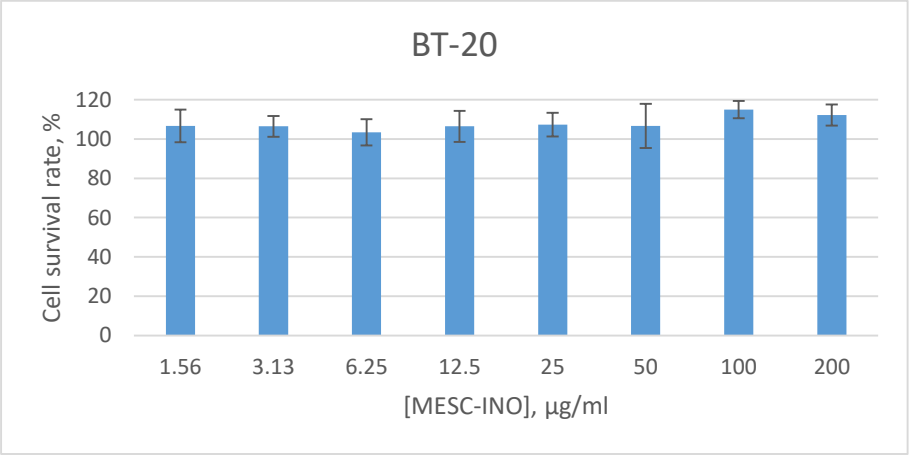

Figure 10.16: Cell survival following treatment of BT-20 cells for 72 h with MESC-INO, as determined with the MTT assay (error bars = standard deviation, *n* = 6).

5.10.17. Panc 02.03 (Human pancreas adenocarcinoma)

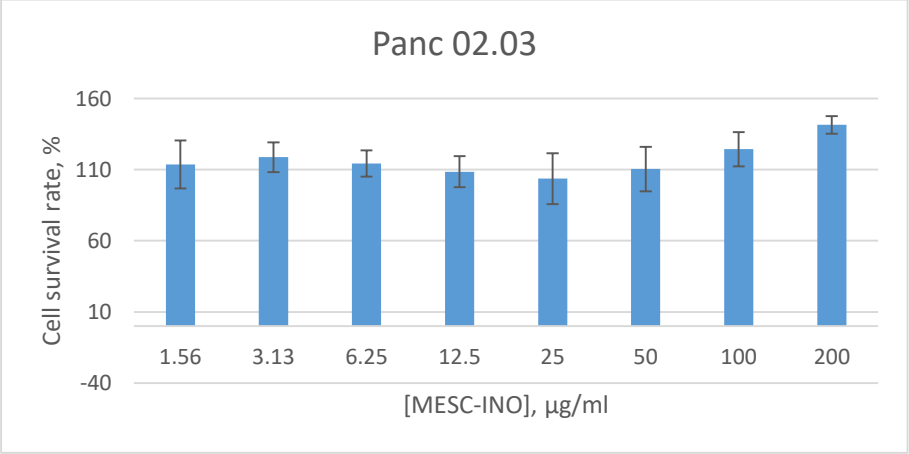

Figure 10.17: Cell survival following treatment of Panc 02.03 cells for 72 h with MESC-INO, as determined with the MTT assay (error bars = standard deviation, *n* = 6).

Discussion for compound MESC-INO results:

MESC-INO appears to not be toxic to the non-cancerous Vero or BJ-5ta cell lines, showing a potential increase in cell viability after 72 h exposure to higher concentrations of MESC-INO. This same induced cell growth at higher concentrations was also observed in several of the cancer cell lines tested, with viability increased to more than that of the untreated control.

Slight activity was observed in the MCF7 cell line, but at lower concentrations. This compound may need to be investigated further at even lower concentrations.

5.11. Results for compound YS4

5.11.1. Vero (African green monkey – non-cancer control)

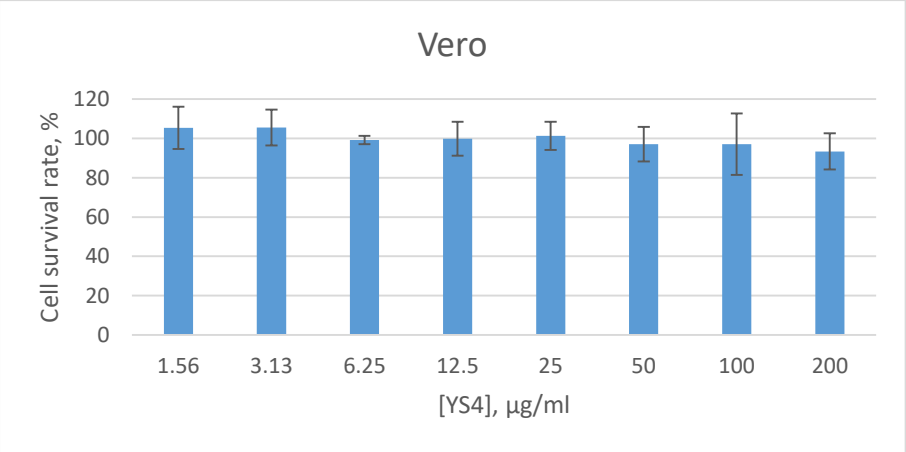

Figure 11.1: Cell survival following treatment of Vero cells for 72 h with YS4, as determined with the MTT assay (error bars = standard deviation, *n* = 6).

5.11.2. BJ-5ta (Human skin fibroblasts hTERT immortalized, non-cancer)

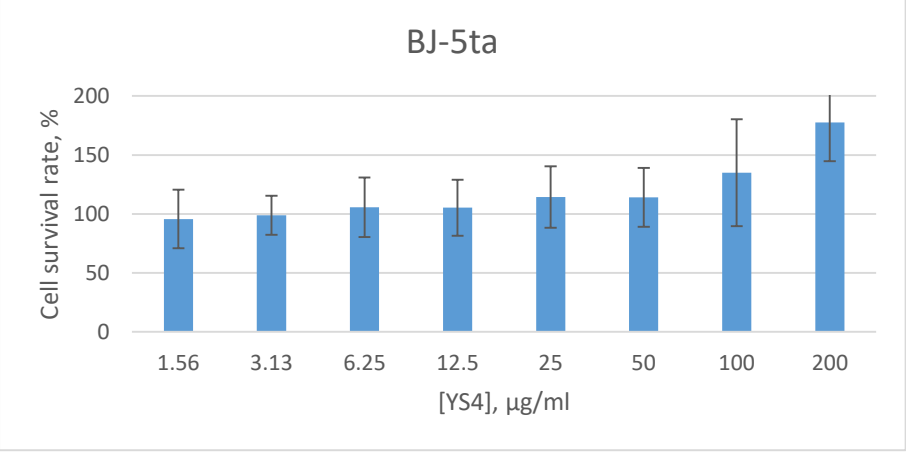

Figure 11.2: Cell survival following treatment of BJ-5ta cells for 72 h with YS4, as determined with the MTT assay (error bars = standard deviation, *n* = 6).

5.11.3. H69V (Human small cell lung carcinoma)

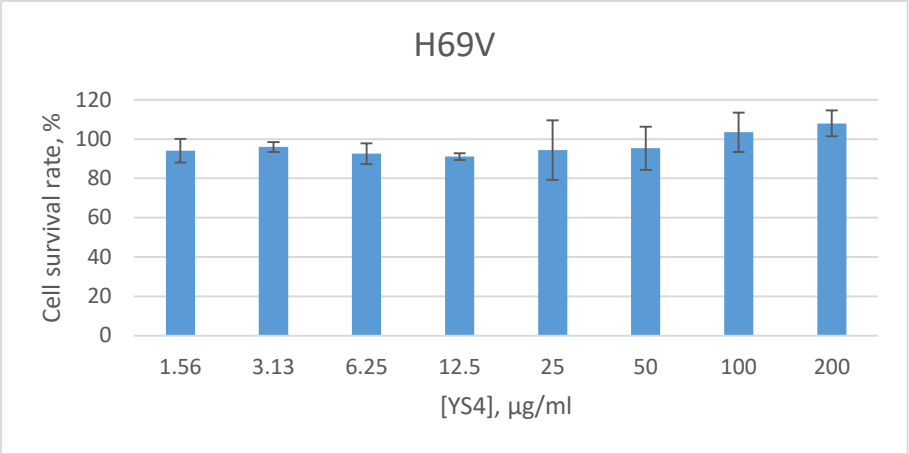

Figure 11.3: Cell survival following treatment of H69V cells for 72 h with YS4, as determined with the MTT assay (error bars = standard deviation, *n* = 6).

5.11.4. 143B (Human osteosarcoma)

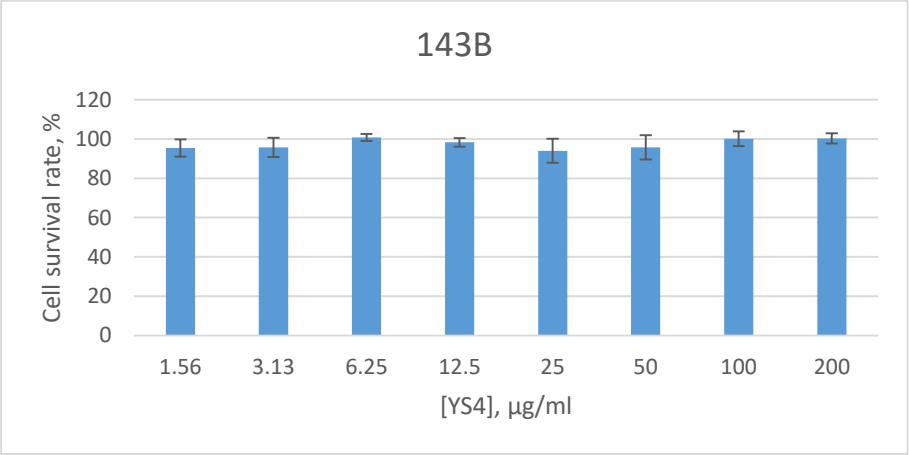

Figure 11.4: Cell survival following treatment of 143B cells for 72 h with YS4, as determined with the MTT assay (error bars = standard deviation, *n* = 6).

5.11.5. HepG2/C3A (Human hepatocellular carcinoma)

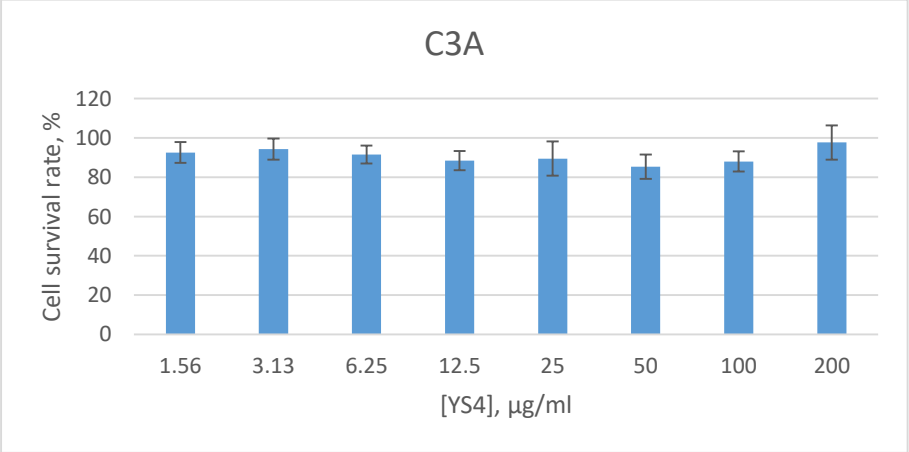

Figure 11.5: Cell survival following treatment of C3A cells for 72 h with YS4, as determined with the MTT assay (error bars = standard deviation, *n* = 6).

5.11.6. A375 (Human melanoma)

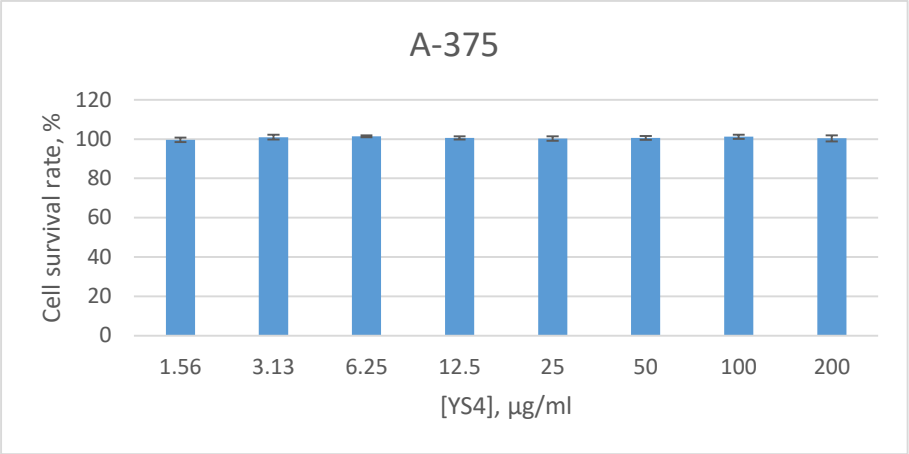

Figure 11.6: Cell survival following treatment of A375 cells for 72 h with YS4, as determined with the MTT assay (error bars = standard deviation, *n* = 6).

5.11.7. HT29 (Human colon carcinoma)

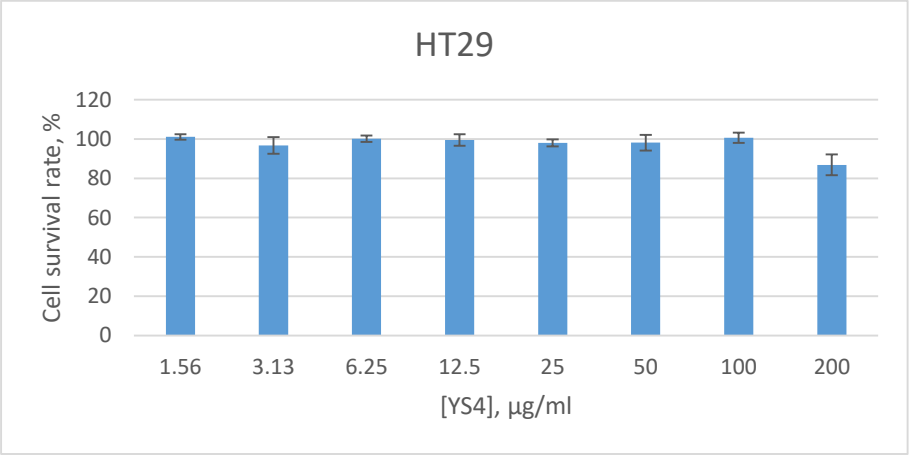

Figure 11.7: Cell survival following treatment of HT29 cells for 72 h with YS4, as determined with the MTT assay (error bars = standard deviation, *n* = 6).

5.11.8. MCF7 (Human breast adenocarcinoma)

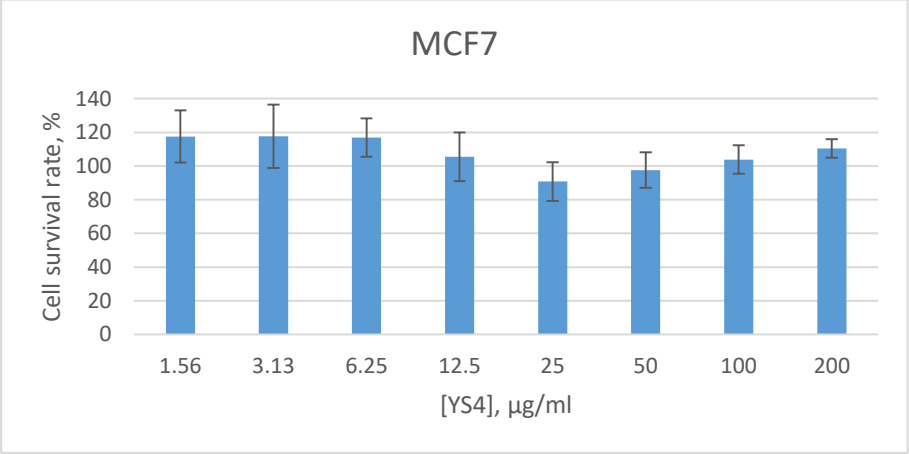

Figure 11.8: Cell survival following treatment of MCF7 cells for 72 h with YS4 as determined with the MTT assay (error bars = standard deviation, *n* = 6).

5.11.9. AGS (Human stomach carcinoma)

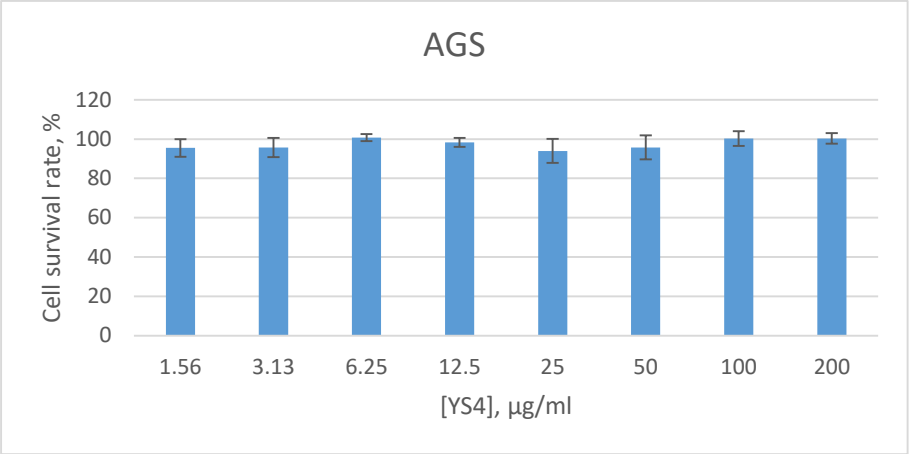

Figure 11.9: Cell survival following treatment of AGS cells for 72 h with YS4, as determined with the MTT assay (error bars = standard deviation, *n* = 6).

5.11.10. Clone 15 HL-60 (Human acute promyelocytic leukemia)

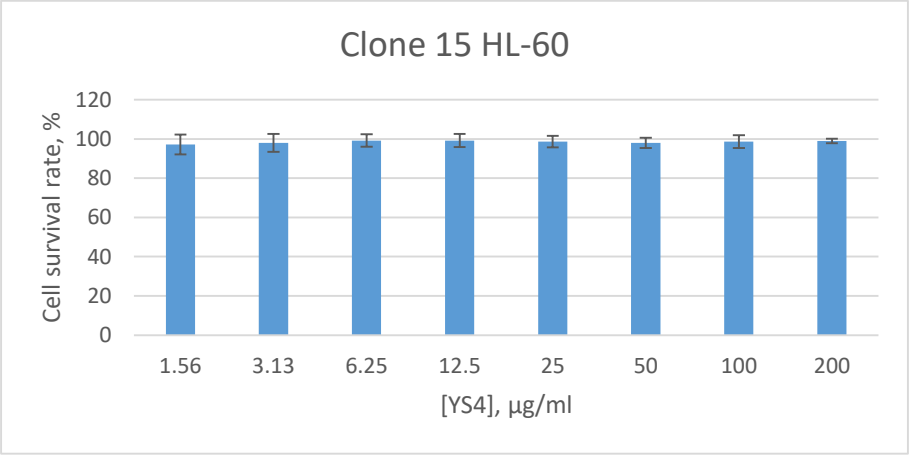

Figure 11.10: Cell survival following treatment of Clone 15 HL-60 cells for 72 h with YS4, as determined with the LDH assay (error bars = standard deviation, *n* = 3).

5.11.11. DU145 (Human prostate carcinoma)

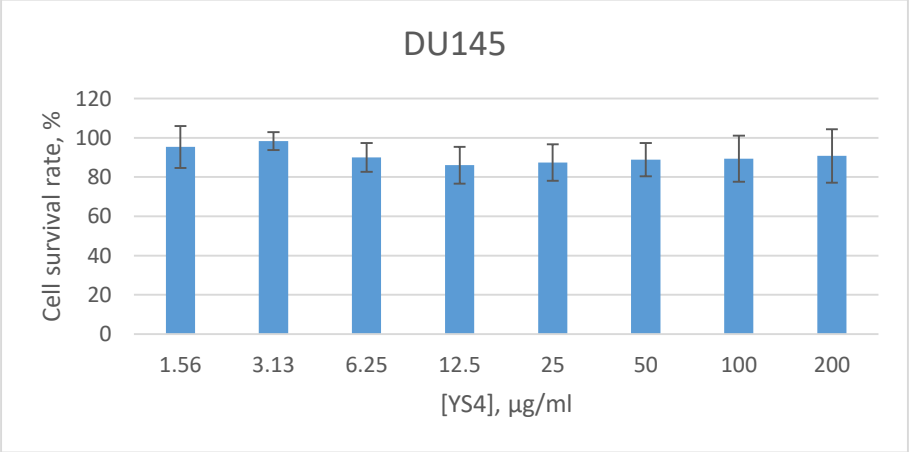

Figure 11.11: Cell survival following treatment of DU145 cells for 72 h with YS4, as determined with the MTT assay (error bars = standard deviation, *n* = 6).

5.11.12. A-704 (human kidney adenocarcinoma)

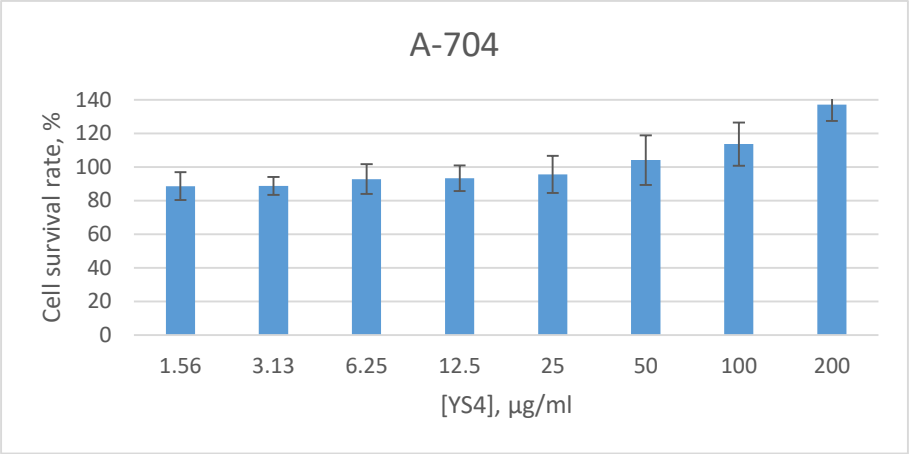

Figure 11.12: Cell survival following treatment of A-704 cells for 72 h with YS4, as determined with the MTT assay (error bars = standard deviation, *n* = 6).

5.11.13. K-562 (Human chronic myelogenous Leukemia)

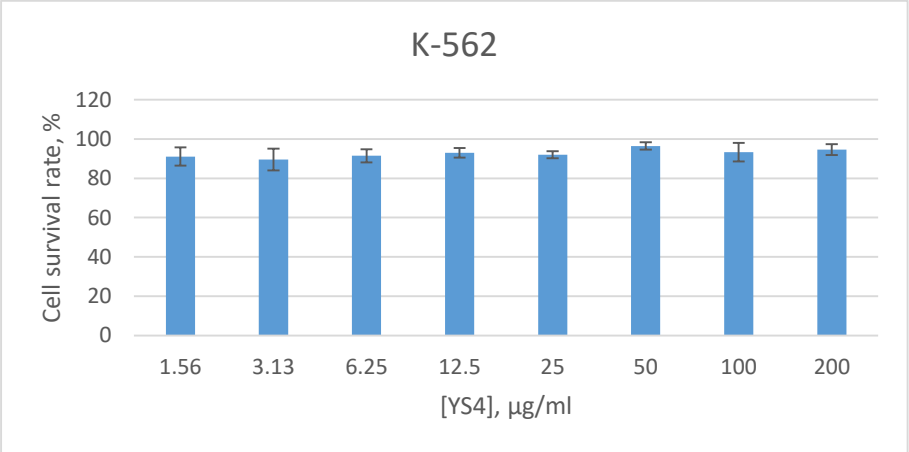

Figure 11.13A: Cell survival following treatment of K-562 cells for 72 h with YS4, as determined with the LDH assay (error bars = standard deviation, *n* = 3).

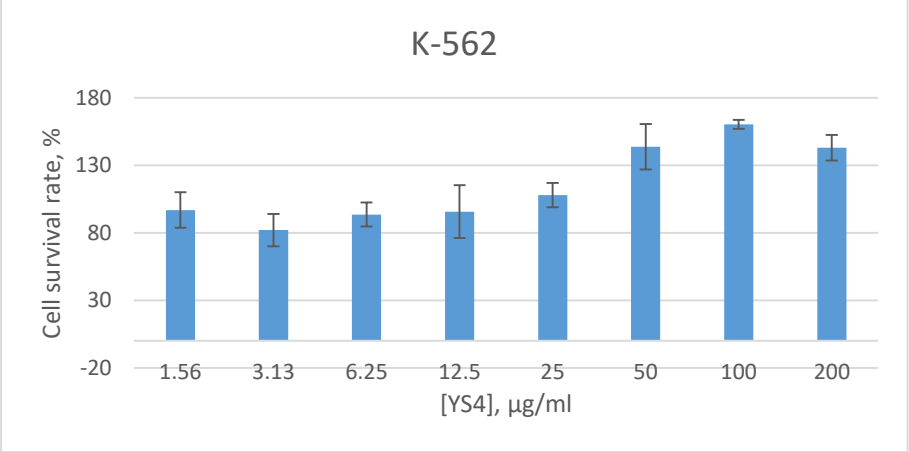

Figure 11.13B: Cell survival following treatment of K-562 cells for 72 h with YS4, as determined with the MTT assay (error bars = standard deviation, *n* = 3).

5.11.14. U-87 MG (Human glioblastoma)

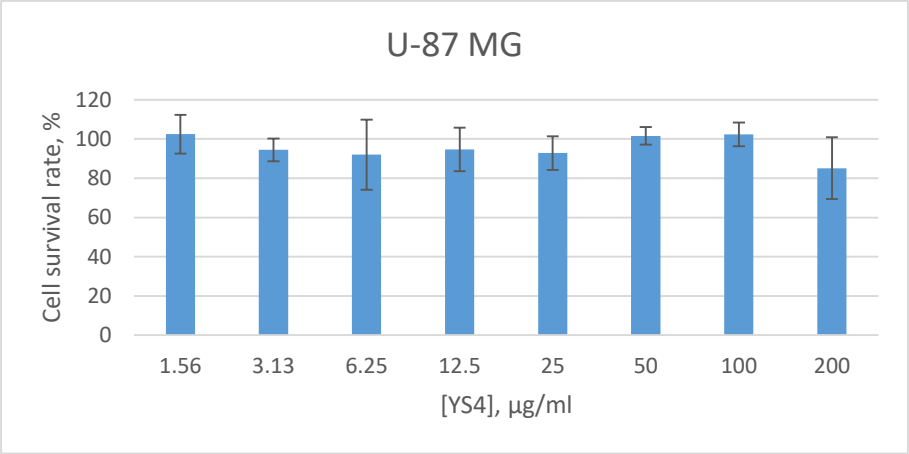

Figure 11.14: Cell survival following treatment of U-87 MG cells for 72 h with YS4, as determined with the MTT assay (error bars = standard deviation, *n* = 6).

5.11.15. A-549 (Human non-small cell lung carcinoma)

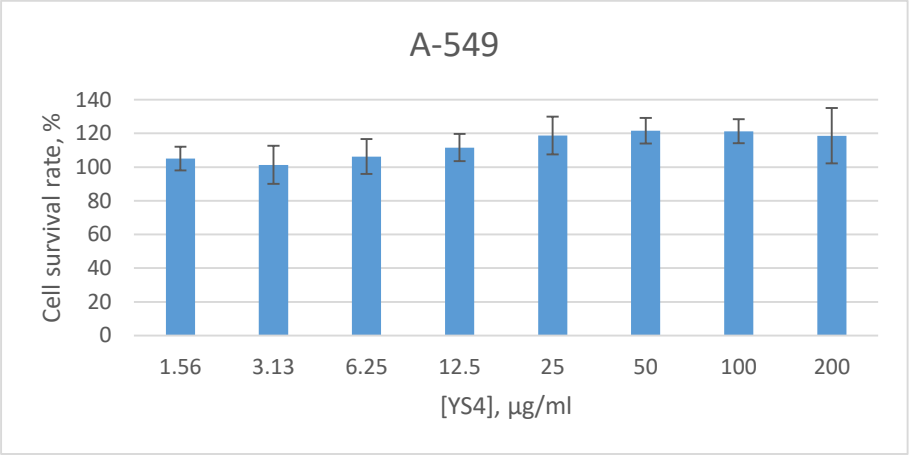

Figure 11.15: Cell survival following treatment of A-549 cells for 72 h with YS4, as determined with the MTT assay (error bars = standard deviation, *n* = 6).

5.11.16. BT-20 (Human triple negative invasive ductal human carcinoma breast cancer)

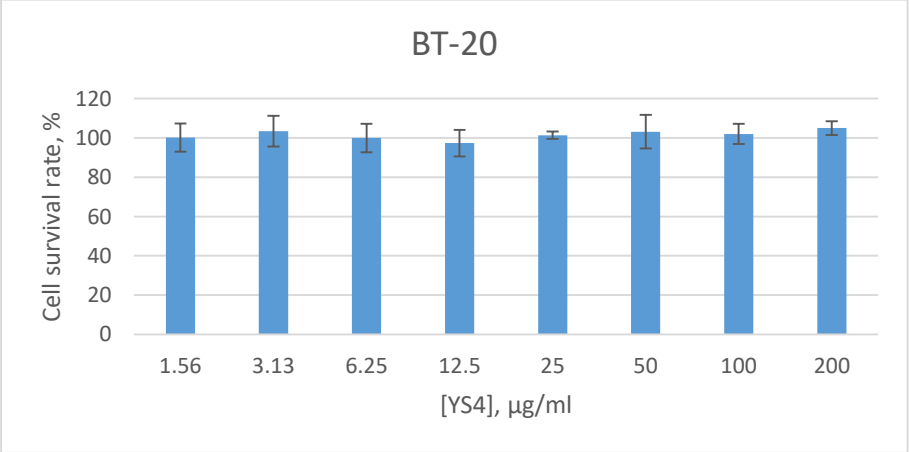

Figure 11.16: Cell survival following treatment of BT-20 cells for 72 h with YS4, as determined with the MTT assay (error bars = standard deviation, *n* = 6).

5.11.17. Panc 02.03 (Human pancreas adenocarcinoma)

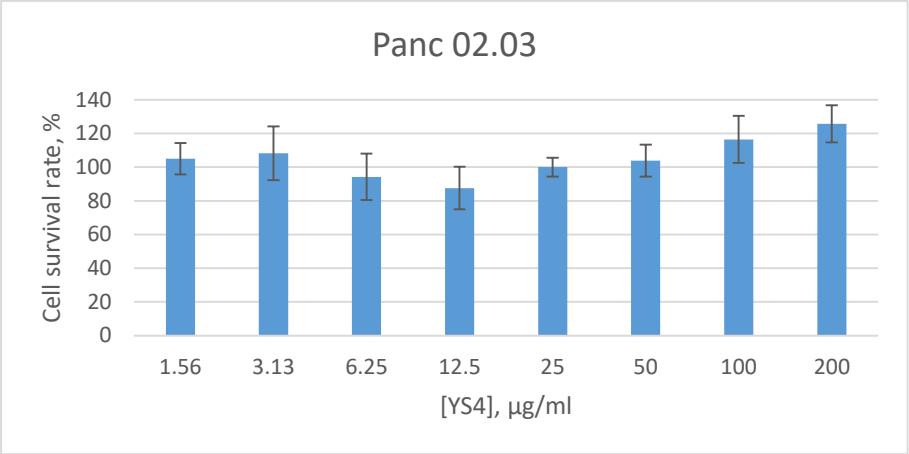

Figure 11.17: Cell survival following treatment of Panc 02.03 cells for 72 h with YS4, as determined with the MTT assay (error bars = standard deviation, *n* = 6).

Discussion for compound YS4 results:

YS4 appears to not be toxic in the non-cancerous cells, but rather seem to increase cell viability in a concentration dependent manner. YS4 does not appear to be active in any cancer cell lines assayed so far.

5.12. Results for compound G5/MUC

5.12.1. Vero (African green monkey – non-cancer control)

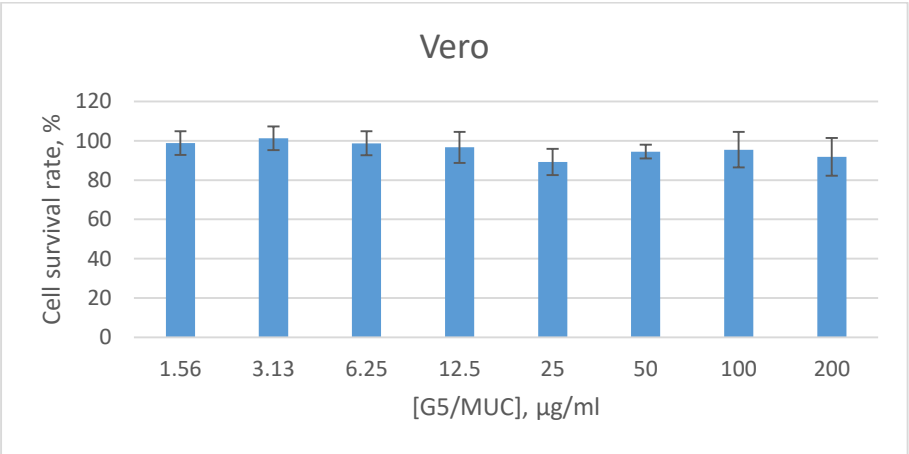

Figure 12.1: Cell survival following treatment of Vero cells for 72 h with G5/MUC, as determined with the MTT assay (error bars = standard deviation, *n* = 6).

5.12.2. BJ-5ta (Human skin fibroblasts hTERT immortalized, non-cancer)

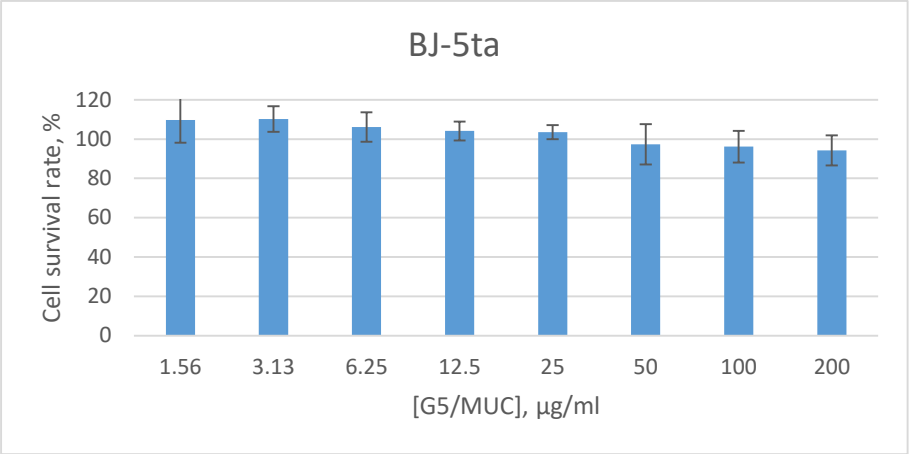

Figure 12.2: Cell survival following treatment of BJ-5ta cells for 72 h with G5/MUC, as determined with the MTT assay (error bars = standard deviation, *n* = 6).

5.12.3. H69V (Human small cell lung carcinoma)

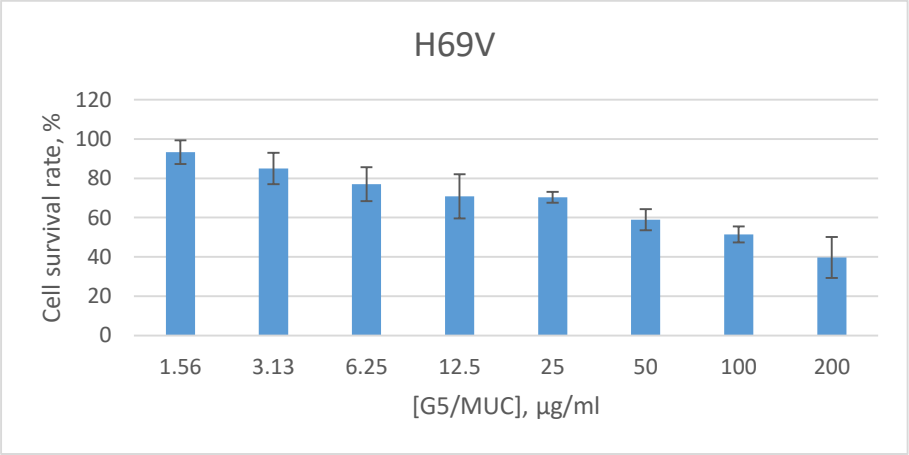

Figure 12.3: Cell survival following treatment of H69V cells for 72 h with G5/MUC, as determined with the MTT assay (error bars = standard deviation, *n* = 6).

5.12.4. 143B (Human osteosarcoma)

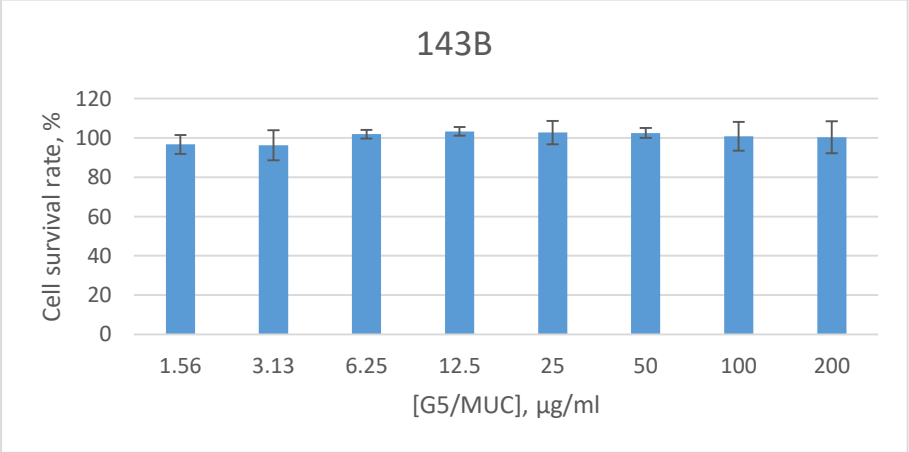

Figure 12.4: Cell survival following treatment of 143B cells for 72 h with G5/MUC, as determined with the MTT assay (error bars = standard deviation, *n* = 6).

5.12.5. HepG2/C3A (Human hepatocellular carcinoma)

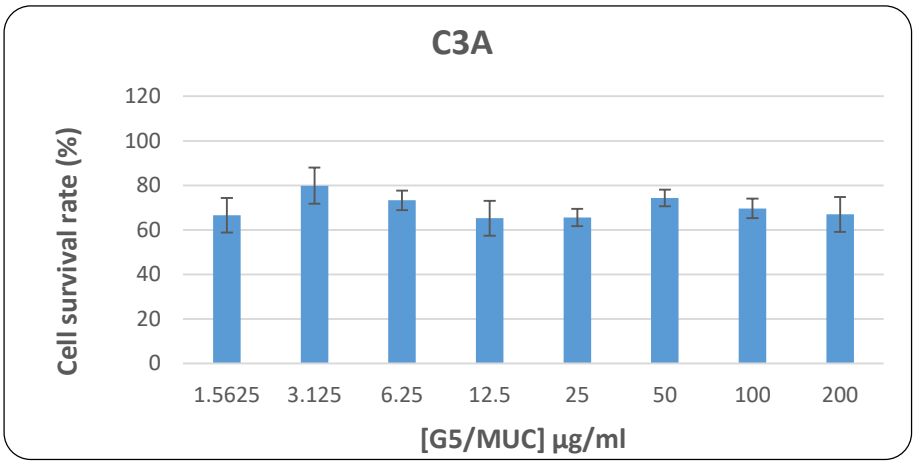

Figure 12.5: Cell survival following treatment of C3A cells for 72 h with G5/MUC, as determined with the MTT assay (error bars = standard deviation, *n* = 6).

5.12.6. A375 (Human melanoma)

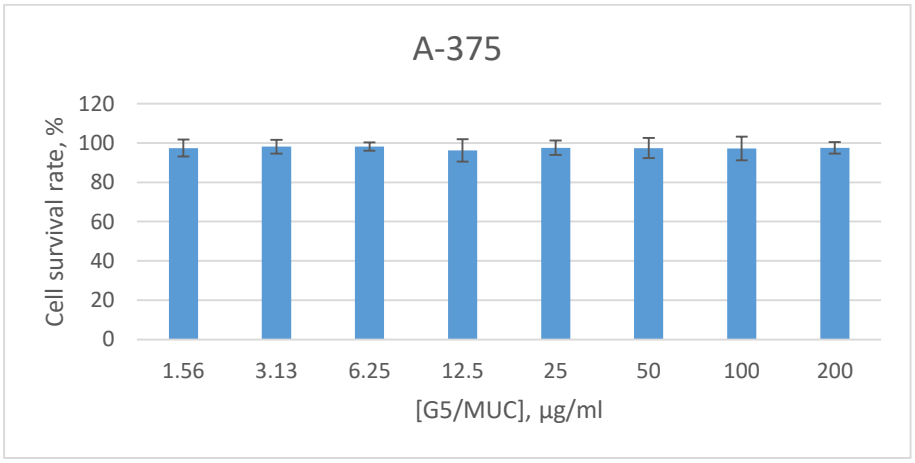

Figure 12.6: Cell survival following treatment of A375 cells for 72 h with G5/MUC, as determined with the MTT assay (error bars = standard deviation, *n* = 6).

5.12.7. HT29 (Human colon carcinoma)

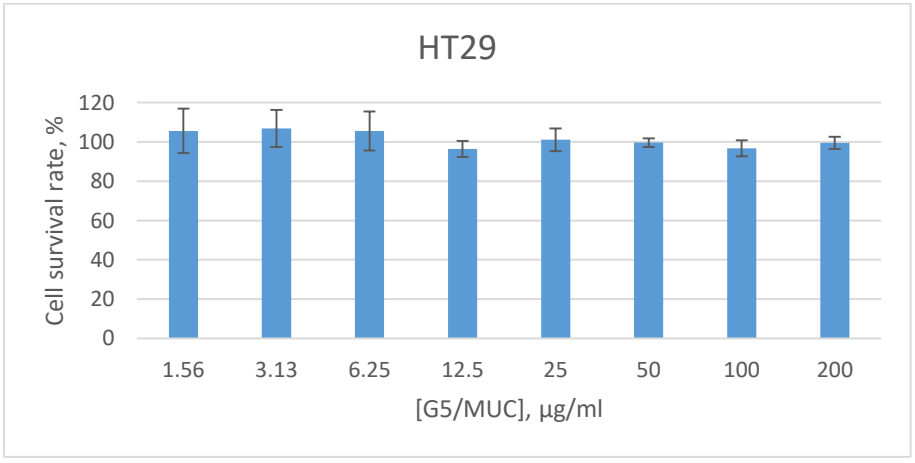

Figure 12.7: Cell survival following treatment of HT29 cells for 72 h with G5/MUC, as determined with the MTT assay (error bars = standard deviation, *n* = 6).

5.12.8. MCF7 (Human breast adenocarcinoma)

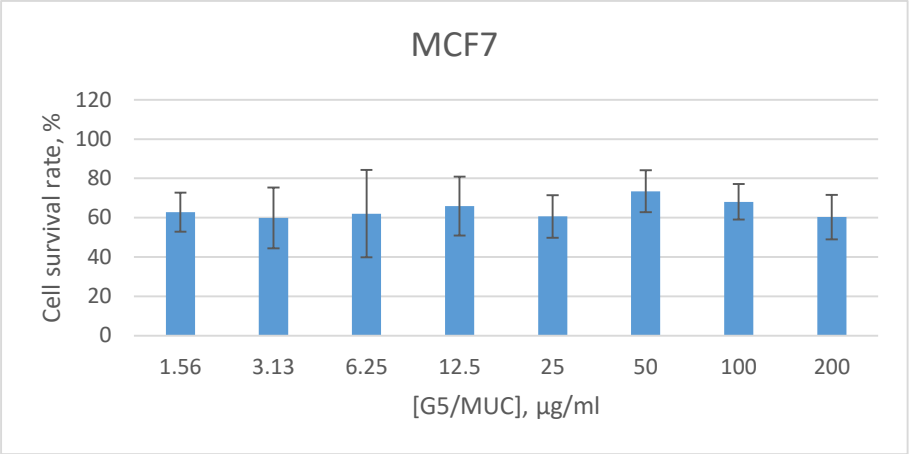

Figure 12.8: Cell survival following treatment of MCF7 cells for 72 h with G5/MUC as determined with the MTT assay (error bars = standard deviation, *n* = 6).

5.12.9. AGS (Human stomach carcinoma)

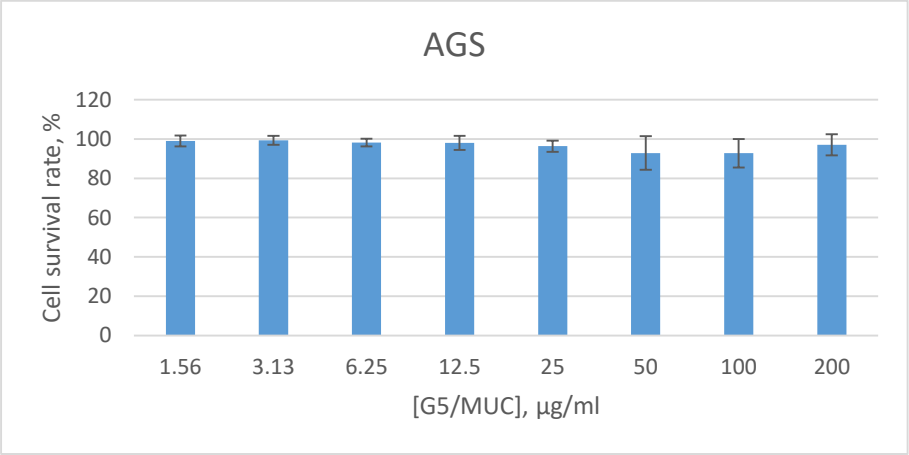

Figure 12.9: Cell survival following treatment of AGS cells for 72 h with G5/MUC, as determined with the MTT assay (error bars = standard deviation, *n* = 6).

5.12.10. Clone 15 HL-60 (Human acute promyelocytic leukemia)

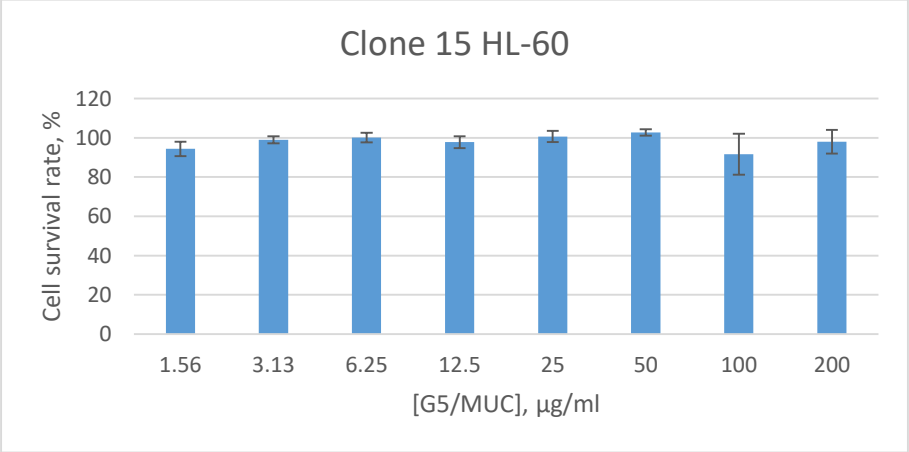

Figure 12.10: Cell survival following treatment of Clone 15 HL-60 cells for 72 h with G5/MUC, as determined with the LDH assay (error bars = standard deviation, *n* = 3).

5.12.11. DU145 (Human prostate carcinoma)

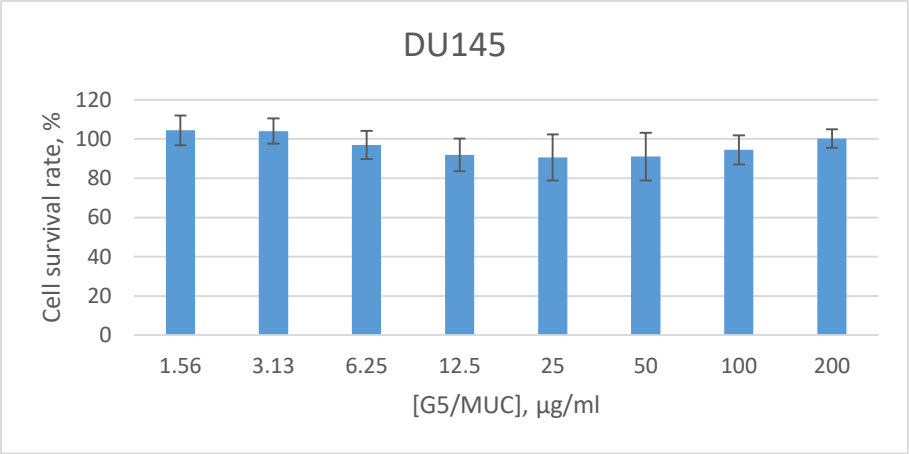

Figure 12.11: Cell survival following treatment of DU145 cells for 72 h with G5/MUC, as determined with the MTT assay (error bars = standard deviation, *n* = 6).

5.12.12. A-704 (human kidney adenocarcinoma)

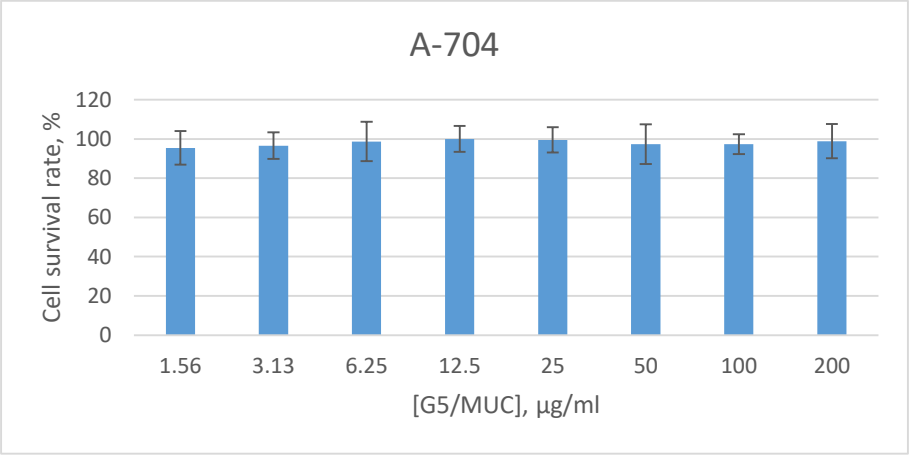

Figure 12.12: Cell survival following treatment of A-704 cells for 72 h with G5/MUC, as determined with the MTT assay (error bars = standard deviation, *n* = 6).

5.12.13. K-562 (Human chronic myelogenous Leukemia)

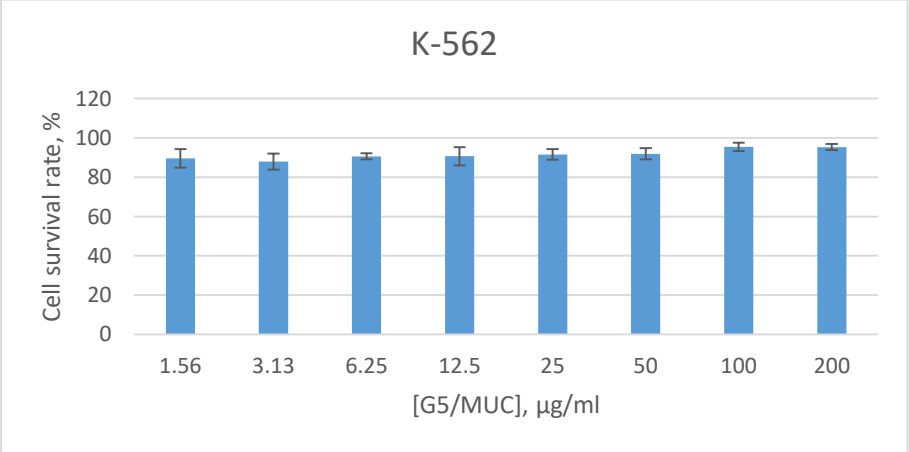

Figure 12.13A: Cell survival following treatment of K-562 cells for 72 h with G5/MUC, as determined with the LDH assay (error bars = standard deviation, *n* = 3).

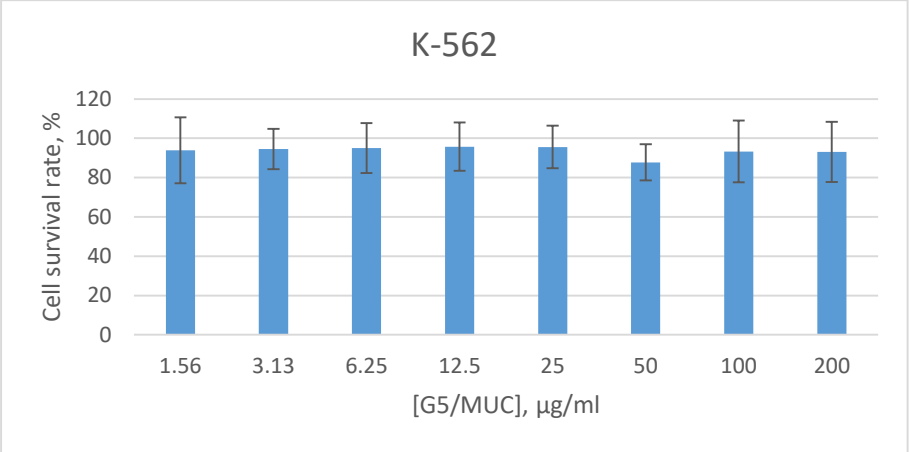

Figure 12.13B: Cell survival following treatment of K-562 cells for 72 h with G5/MUC, as determined with the MTT assay (error bars = standard deviation, *n* = 3).

5.12.14. U-87 MG (Human glioblastoma)

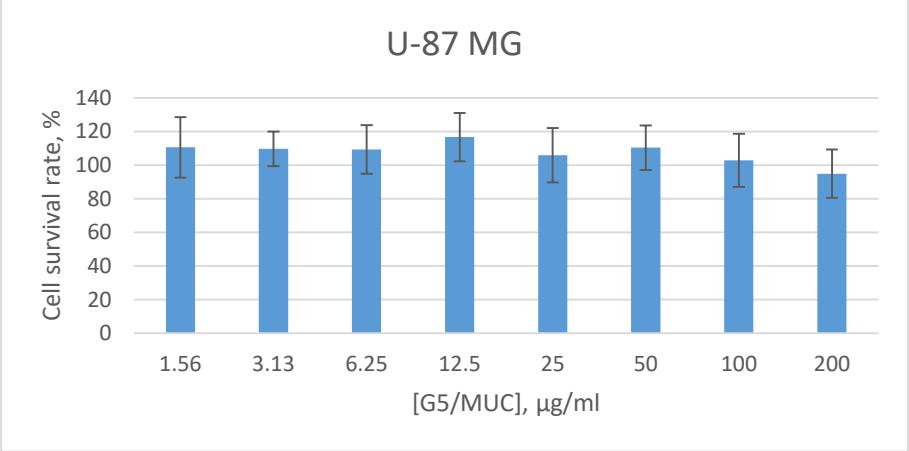

Figure 12.14: Cell survival following treatment of U-87 MG cells for 72 h with G5/MUC, as determined with the MTT assay (error bars = standard deviation, *n* = 6).

5.12.15. A-549 (Human non-small cell lung carcinoma)

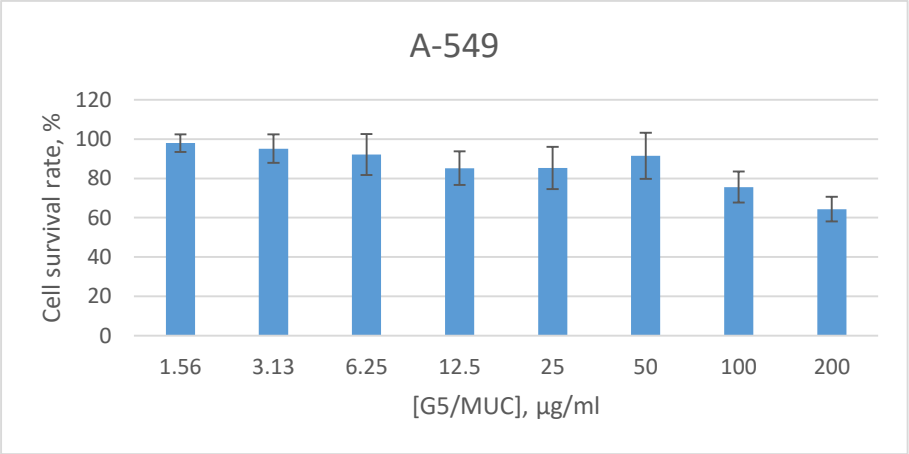

Figure 12.15: Cell survival following treatment of A-549 cells for 72 h with G5/MUC, as determined with the MTT assay (error bars = standard deviation, *n* = 6).

5.12.16. BT-20 (Human triple negative invasive ductal human carcinoma breast cancer)

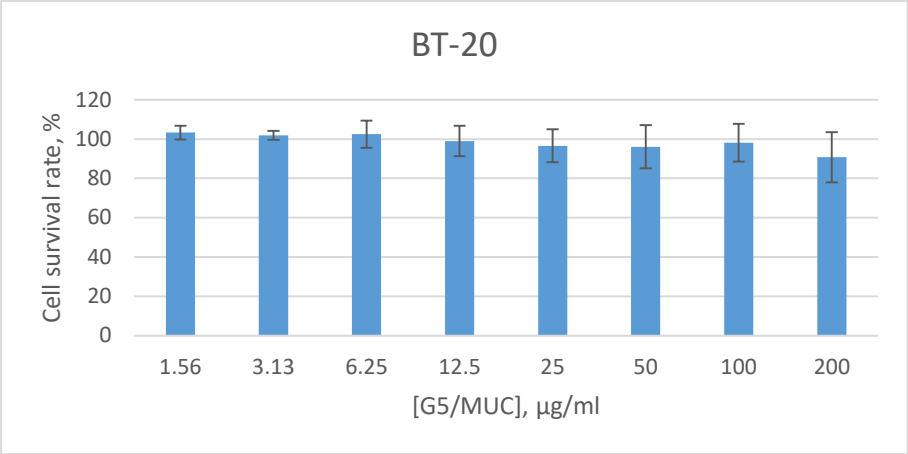

Figure 12.16: Cell survival following treatment of BT-20 cells for 72 h with G5/MUC, as determined with the MTT assay (error bars = standard deviation, *n* = 6).

5.12.17. Panc 02.03 (Human pancreas adenocarcinoma)

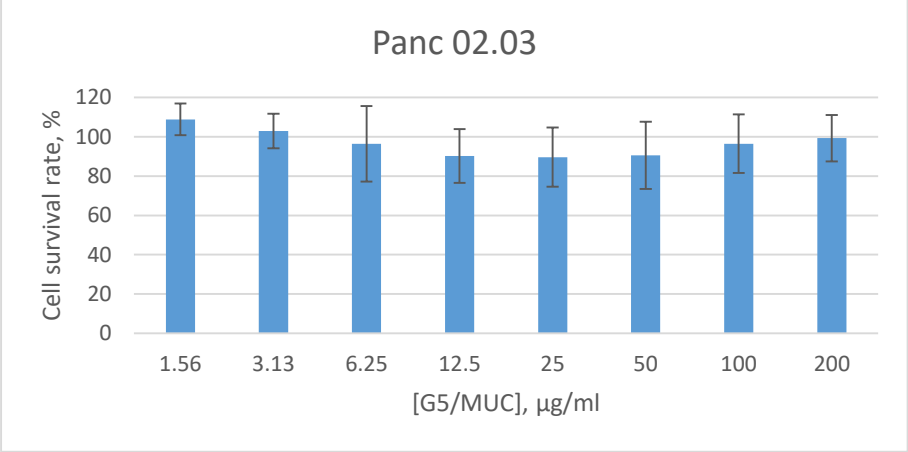

Figure 12.17: Cell survival following treatment of Panc 02.03 cells for 72 h with G5/MUC, as determined with the MTT assay (error bars = standard deviation, *n* = 6).

Discussion for compound G5/MUC results:

G5/MUC appears to not be toxic to the non-cancer Vero or BJ-5ta cell lines. The compound appears to have slight activity in the C3A and A-549 cell lines, with good activity in the MCF7 and H69V cell lines, whereby there was a decrease in cell survival with increasing concentrations.

5.13. Results for compound G1/W

5.13.1. Vero (African green monkey – non-cancer control)

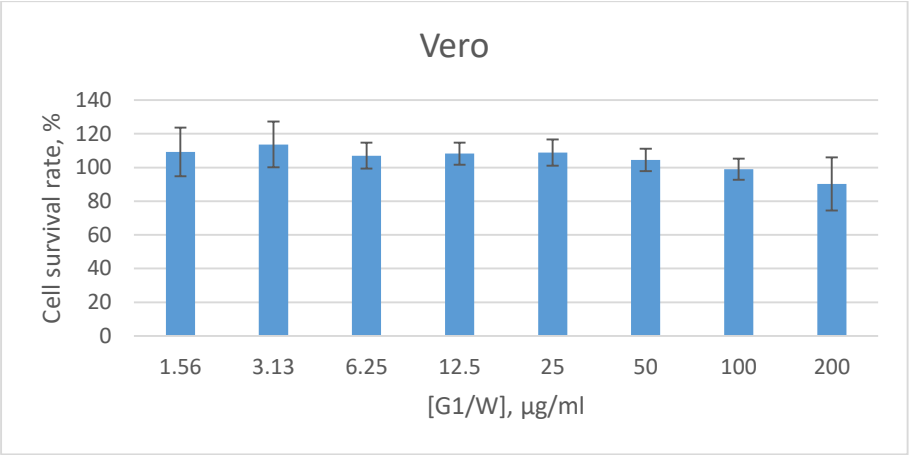

Figure 13.1: Cell survival following treatment of Vero cells for 72 h with G1/W, as determined with the MTT assay (error bars = standard deviation, *n* = 6).

5.13.2. BJ-5ta (Human skin fibroblasts hTERT immortalized, non-cancer)

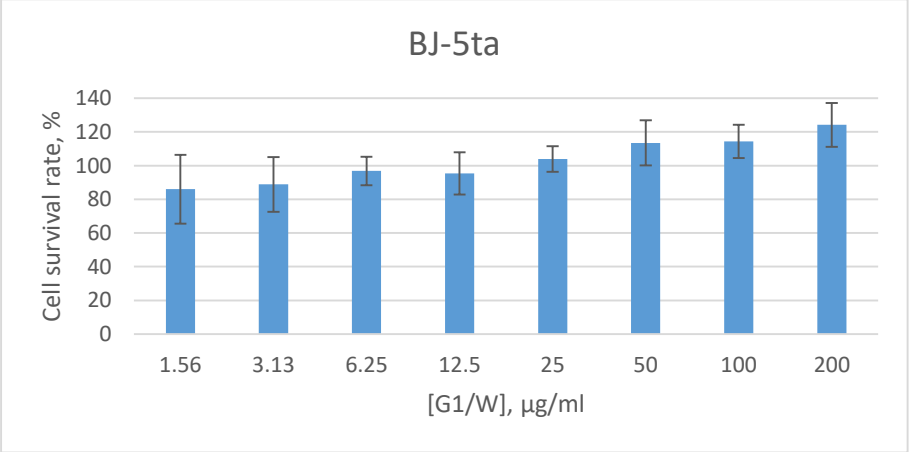

Figure 13.2: Cell survival following treatment of BJ-5ta cells for 72 h with G1/W, as determined with the MTT assay (error bars = standard deviation, *n* = 6).

5.13.3. H69V (Human small cell lung carcinoma)

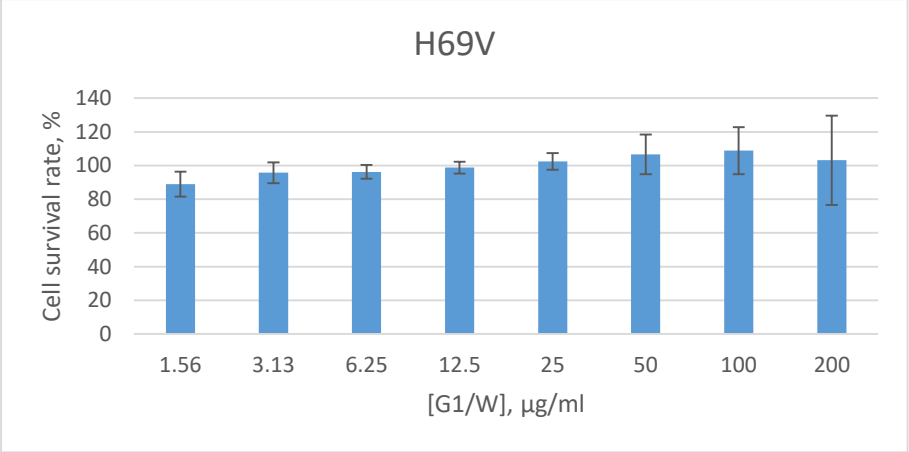

Figure 13.3: Cell survival following treatment of H69V cells for 72 h with G1/W, as determined with the MTT assay (error bars = standard deviation, *n* = 6).

5.13.4. 143B (Human osteosarcoma)

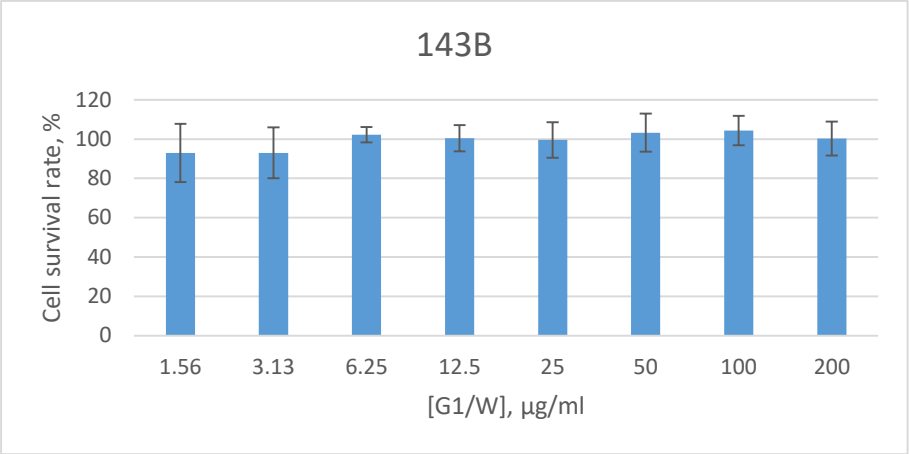

Figure 13.4: Cell survival following treatment of 143B cells for 72 h with G1/W, as determined with the MTT assay (error bars = standard deviation, *n* = 6).

5.13.5. HepG2/C3A (Human hepatocellular carcinoma)

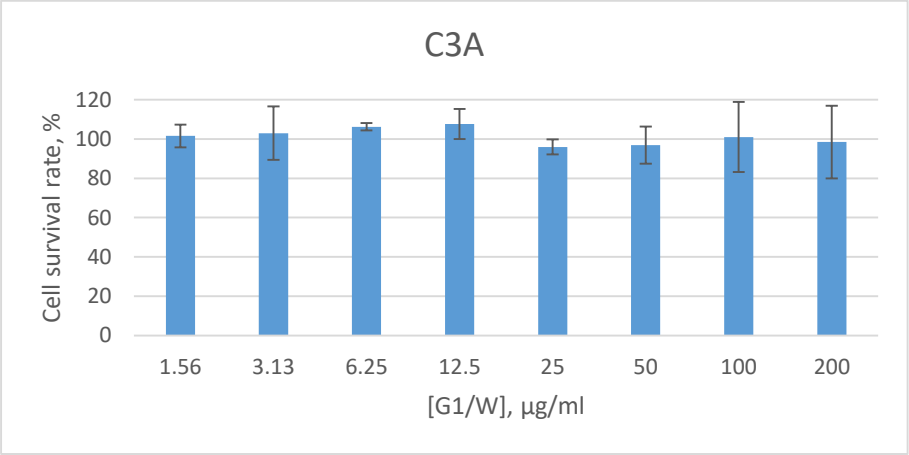

Figure 13.5: Cell survival following treatment of C3A cells for 72 h with G1/W, as determined with the MTT assay (error bars = standard deviation, *n* = 6).

5.13.6. A375 (Human melanoma)

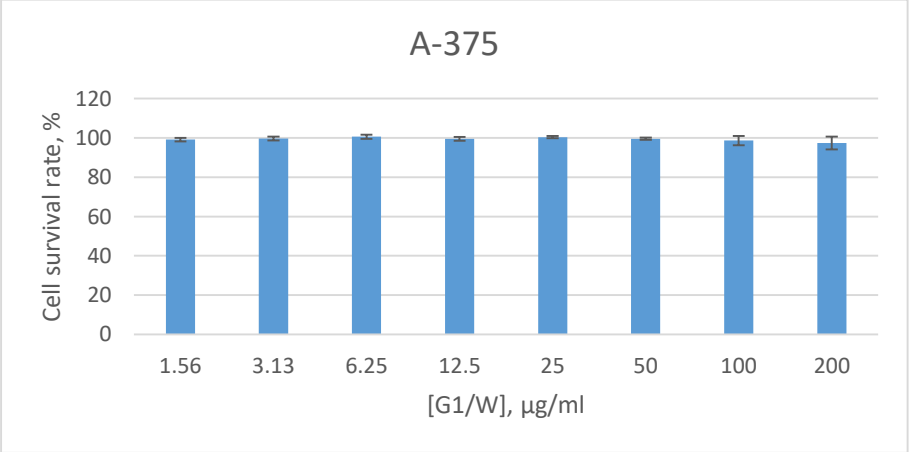

Figure 13.6: Cell survival following treatment of A375 cells for 72 h with G1/W, as determined with the MTT assay (error bars = standard deviation, *n* = 6).

5.13.7. HT29 (Human colon carcinoma)

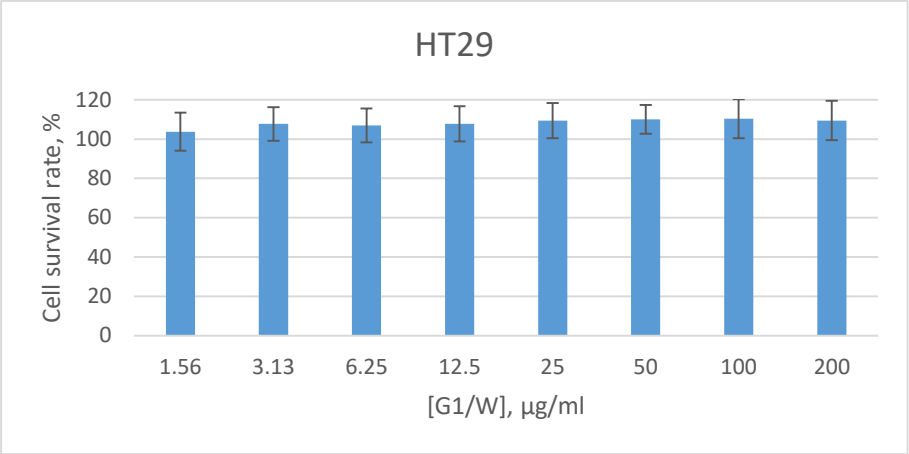

Figure 13.7: Cell survival following treatment of HT29 cells for 72 h with G1/W, as determined with the MTT assay (error bars = standard deviation, *n* = 6).

5.13.8. MCF7 (Human breast adenocarcinoma)

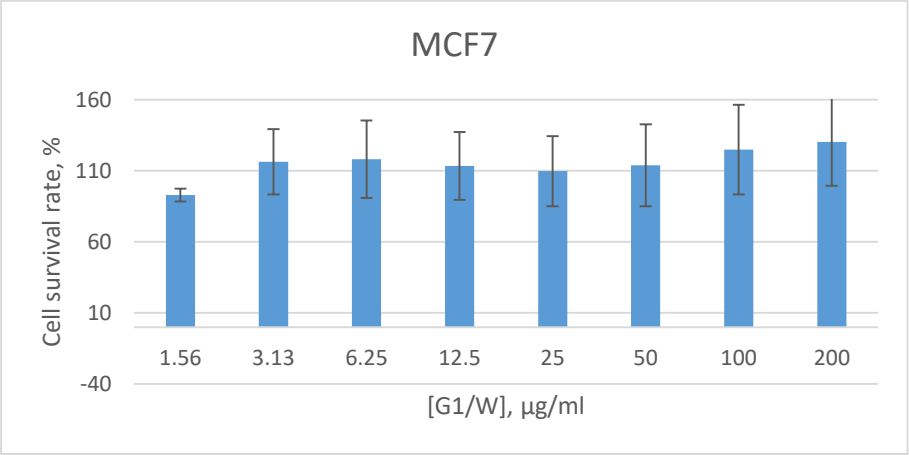

Figure 13.8: Cell survival following treatment of MCF7 cells for 72 h with G1/W as determined with the MTT assay (error bars = standard deviation, *n* = 6).

5.13.9. AGS (Human stomach carcinoma)

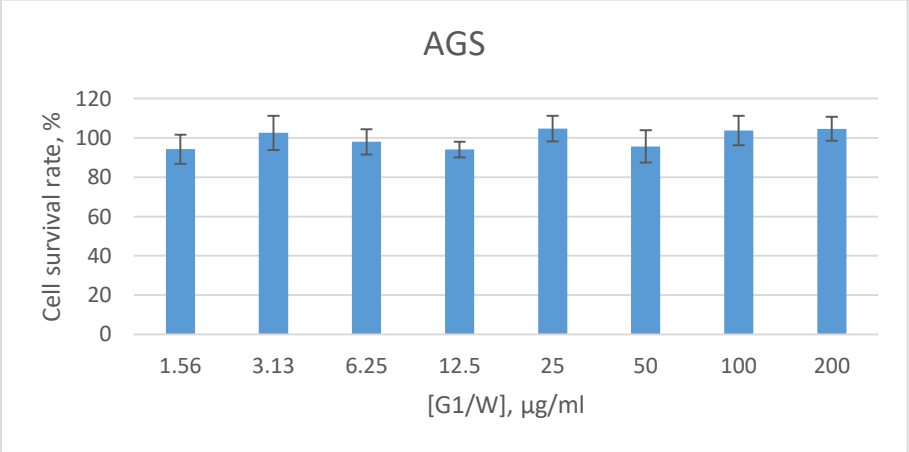

Figure 13.9: Cell survival following treatment of AGS cells for 72 h with G1/W, as determined with the MTT assay (error bars = standard deviation, *n* = 6).

5.13.10. Clone 15 HL-60 (Human acute promyelocytic leukemia)

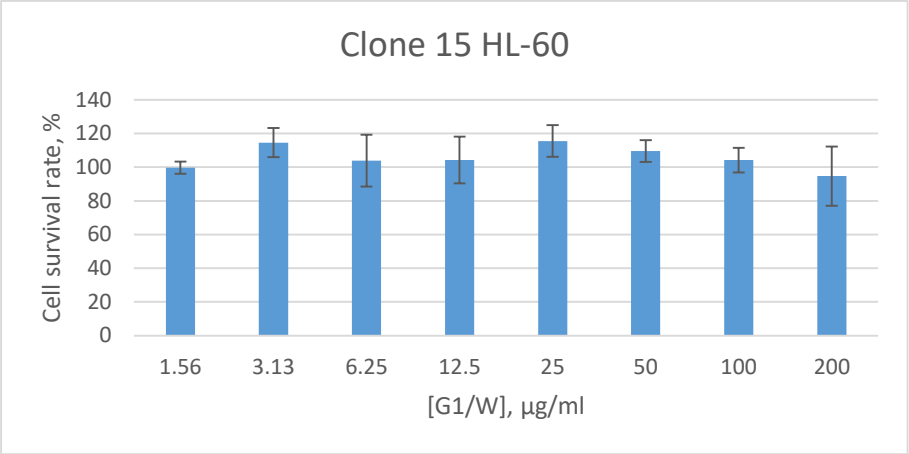

Figure 13.10: Cell survival following treatment of Clone 15 HL-60 cells for 72 h with G1/W, as determined with the LDH assay (error bars = standard deviation, *n* = 3).

5.13.11. DU145 (Human prostate carcinoma)

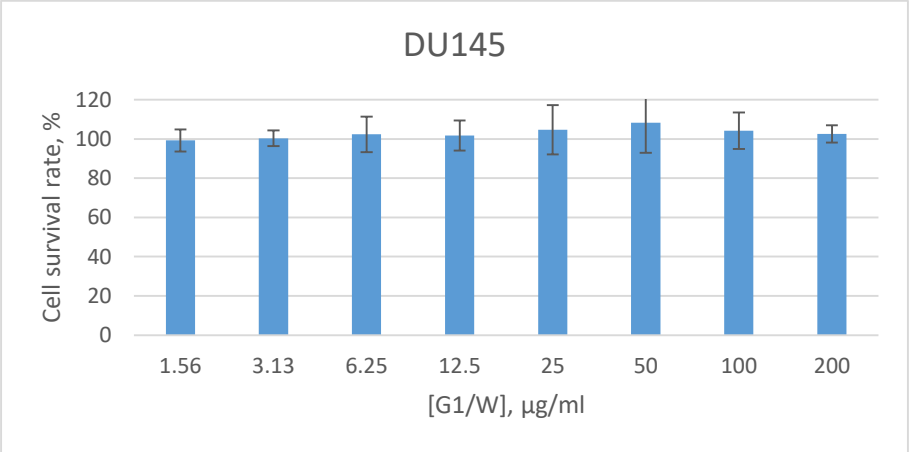

Figure 13.11: Cell survival following treatment of DU145 cells for 72 h with G1/W, as determined with the MTT assay (error bars = standard deviation, *n* = 6).

5.13.12. A-704 (human kidney adenocarcinoma)

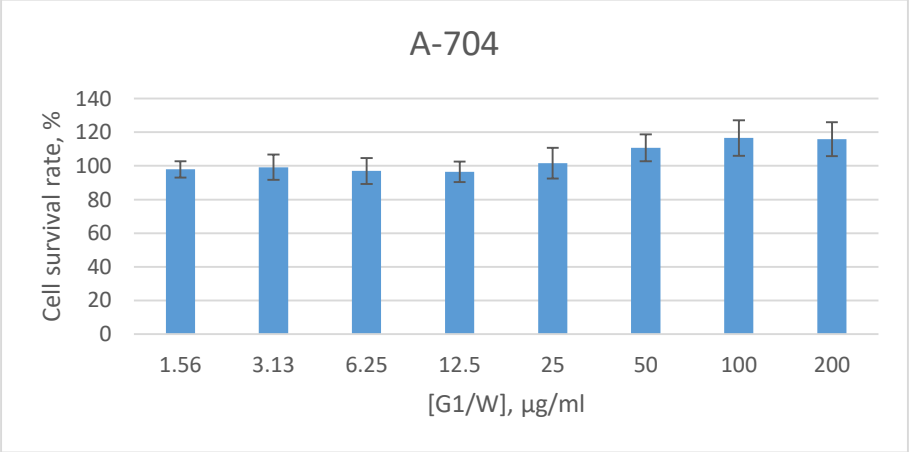

Figure 13.12: Cell survival following treatment of A-704 cells for 72 h with G1/W, as determined with the MTT assay (error bars = standard deviation, *n* = 6).

5.13.13. K-562 (Human chronic myelogenous Leukemia)

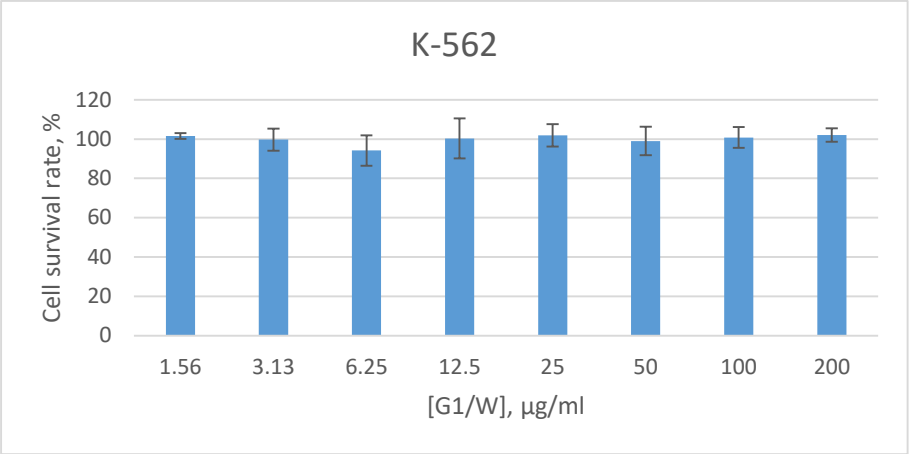

Figure 13.13A: Cell survival following treatment of K-562 cells for 72 h with G1/W, as determined with the LDH assay (error bars = standard deviation, *n* = 3).

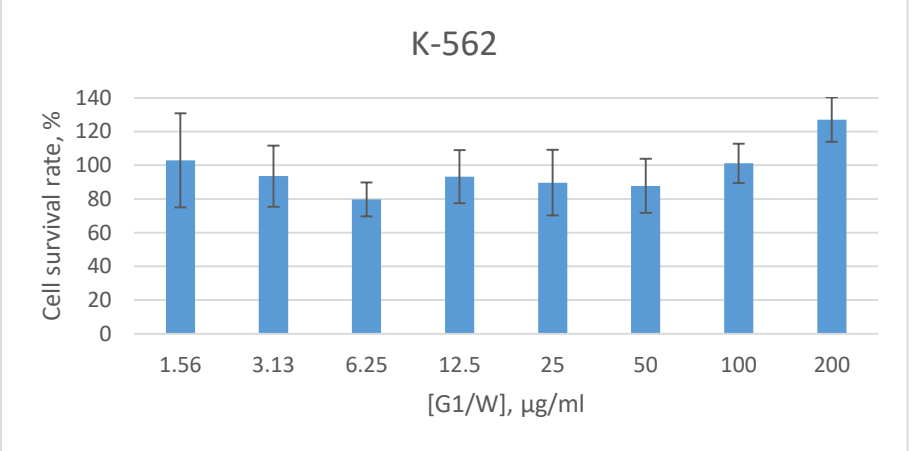

Figure 13.13B: Cell survival following treatment of K-562 cells for 72 h with G1/W, as determined with the MTT assay (error bars = standard deviation, *n* = 3).

5.13.14. U-87 MG (Human glioblastoma)

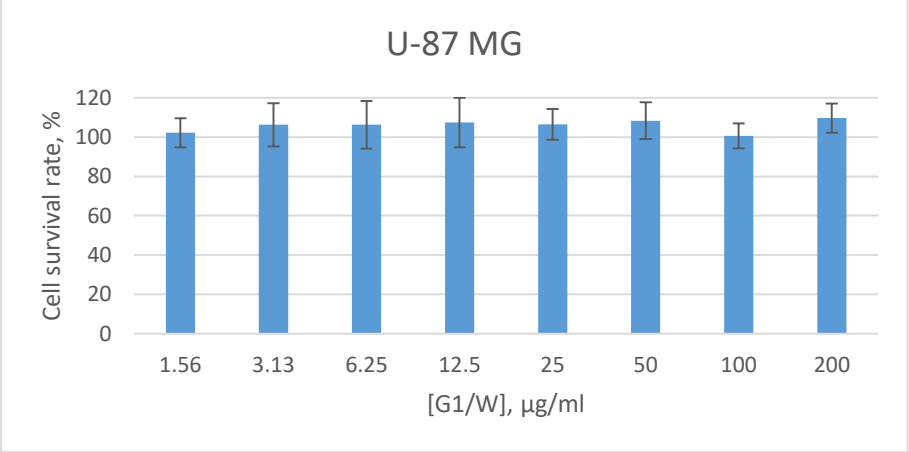

Figure 13.14: Cell survival following treatment of U-87 MG cells for 72 h with G1/W, as determined with the MTT assay (error bars = standard deviation, *n* = 6).

5.13.15. A-549 (Human non-small cell lung carcinoma)

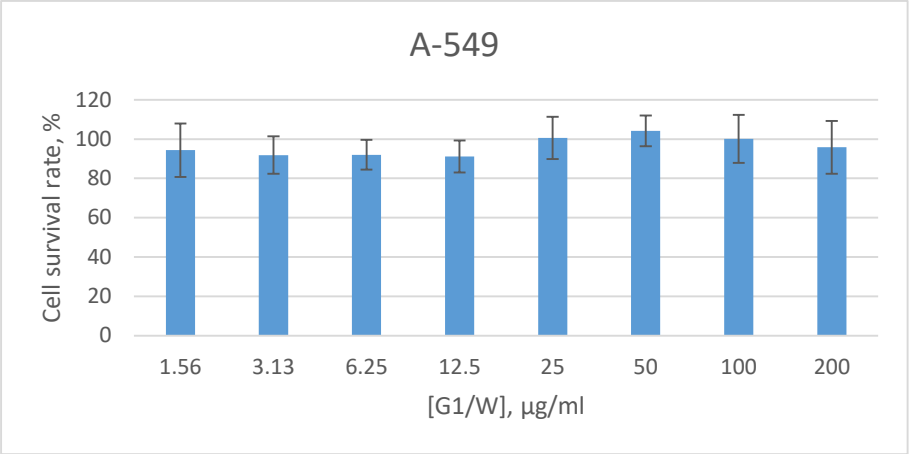

Figure 13.15: Cell survival following treatment of A-549 cells for 72 h with G1/W, as determined with the MTT assay (error bars = standard deviation, *n* = 6).

5.13.16. BT-20 (Human triple negative invasive ductal human carcinoma breast cancer)

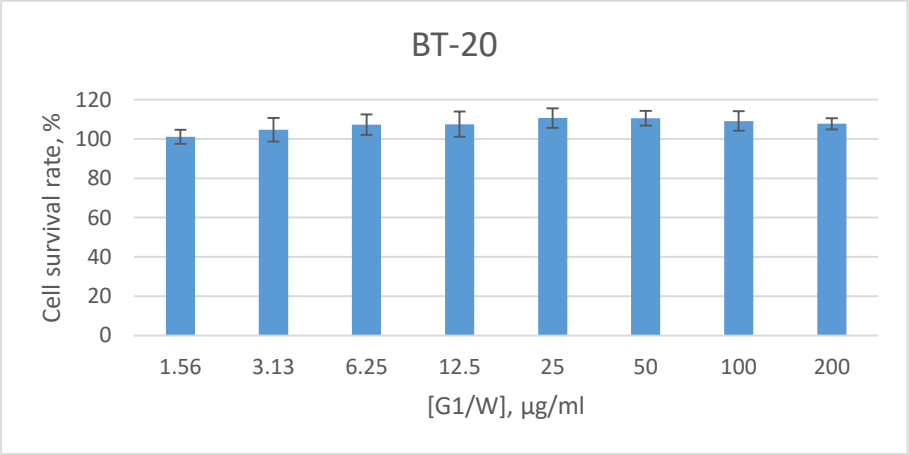

Figure 13.16: Cell survival following treatment of BT-20 cells for 72 h with G1/W, as determined with the MTT assay (error bars = standard deviation, *n* = 6).

5.13.17. Panc 02.03 (Human pancreas adenocarcinoma)

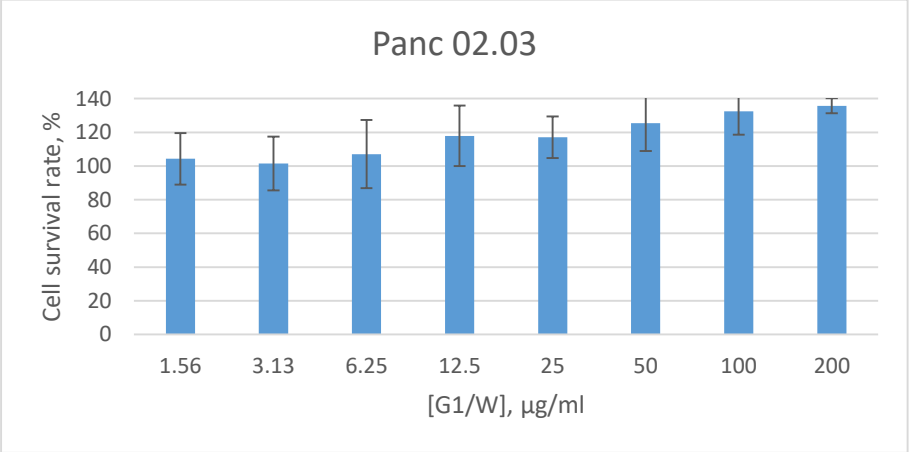

Figure 13.17: Cell survival following treatment of Panc 02.03 cells for 72 h with G1/W, as determined with the MTT assay (error bars = standard deviation, *n* = 6).

Discussion for compound G1/W results:

G1/W appears to not be toxic, but also had no activity in any of the cell lines assayed so far.

5.14. Results for compound IN1

5.14.1. Vero (African green monkey – non-cancer control)

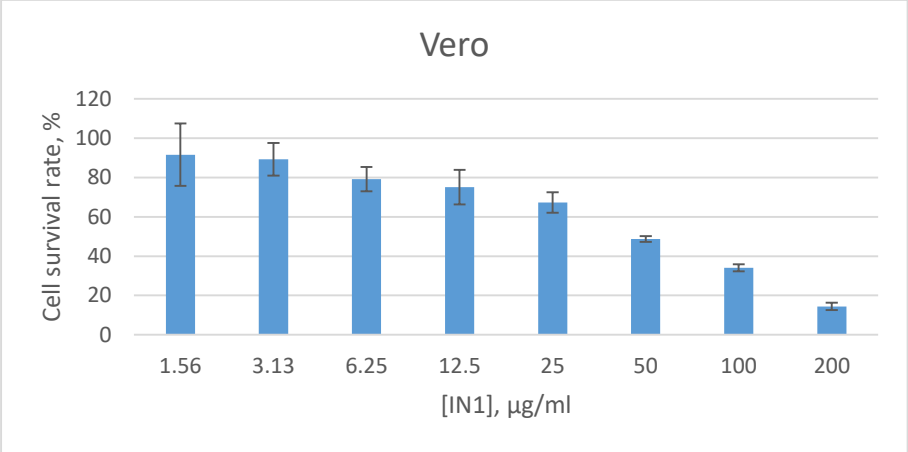

Figure 14.1: Cell survival following treatment of Vero cells for 72 h with IN1, as determined with the MTT assay (error bars = standard deviation, *n* = 6).

5.14.2. BJ-5ta (Human skin fibroblasts hTERT immortalized, non-cancer)

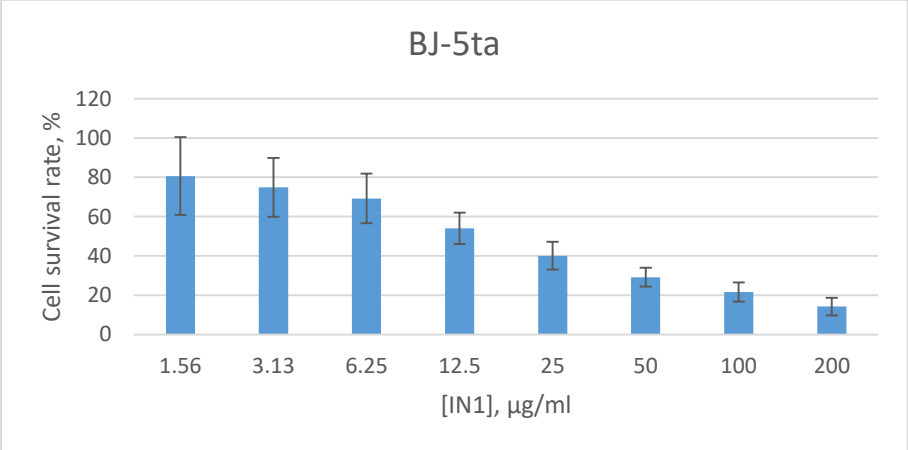

Figure 14.2: Cell survival following treatment of BJ-5ta cells for 72 h with IN1, as determined with the MTT assay (error bars = standard deviation, *n* = 6).

5.14.3. H69V (Human small cell lung carcinoma)

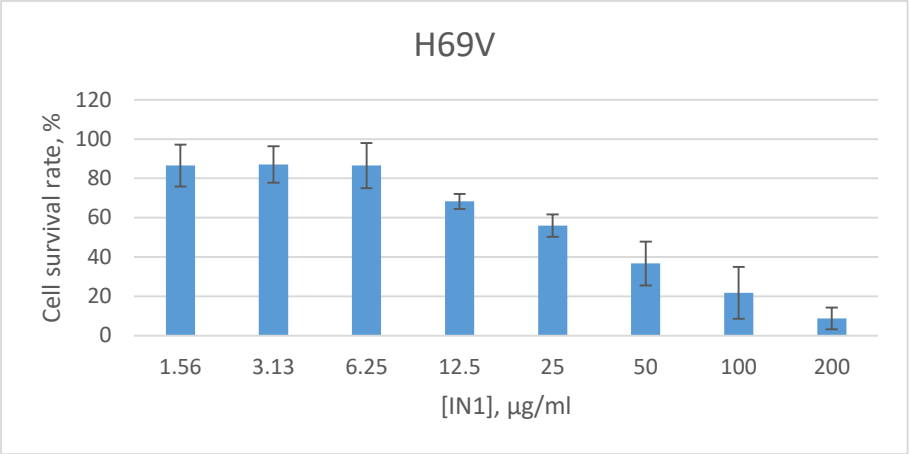

Figure 14.3: Cell survival following treatment of H69V cells for 72 h with IN1, as determined with the MTT assay (error bars = standard deviation, *n* = 6).

5.14.4. 143B (Human osteosarcoma)

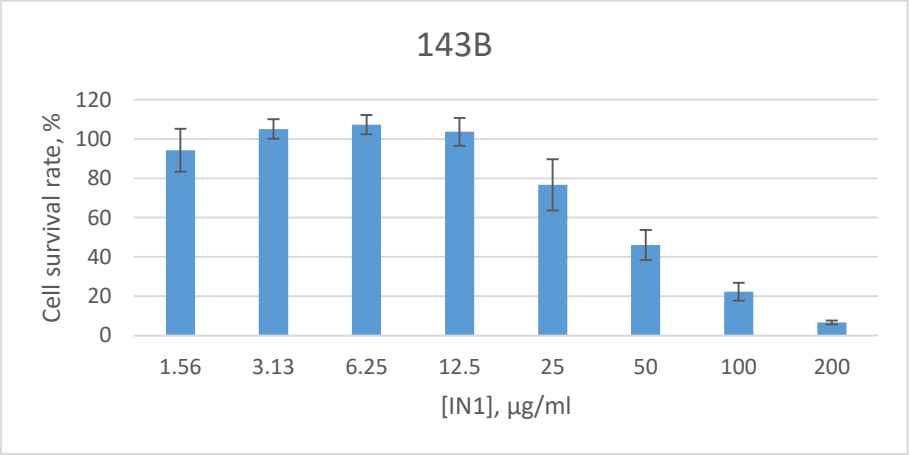

Figure 14.4: Cell survival following treatment of 143B cells for 72 h with IN1, as determined with the MTT assay (error bars = standard deviation, *n* = 6).

5.14.5. HepG2/C3A (Human hepatocellular carcinoma)

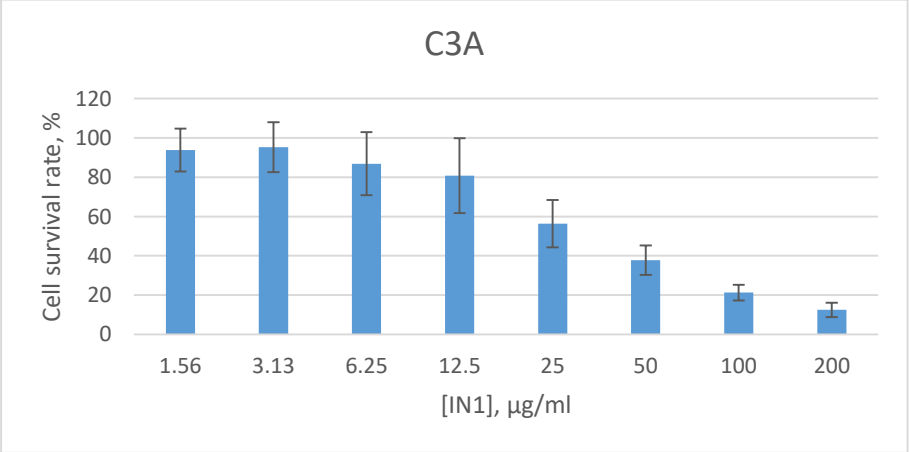

Figure 14.5: Cell survival following treatment of HepG2/C3A cells for 72 h with IN1, as determined with the MTT assay (error bars = standard deviation, *n* = 6).

5.14.6. A375 (Human melanoma)

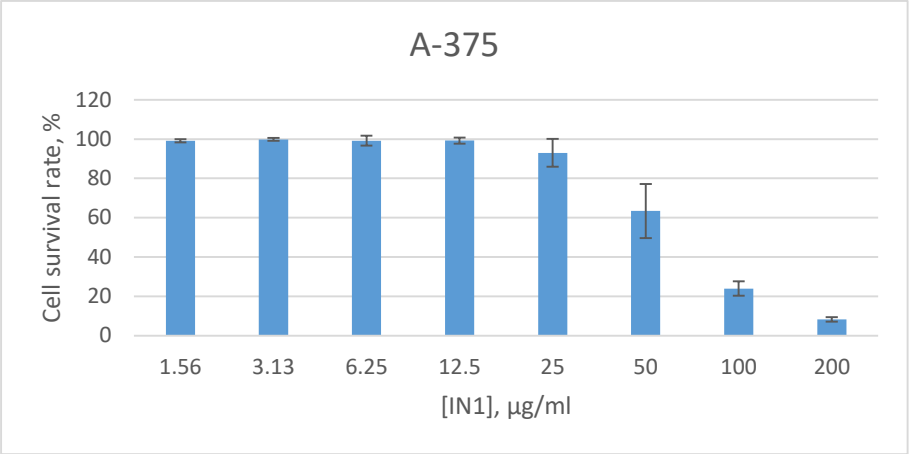

Figure 14.6: Cell survival following treatment of A375 cells for 72 h with IN1, as determined with the MTT assay (error bars = standard deviation, *n* = 3).

5.14.7. HT29 (Human colon carcinoma)

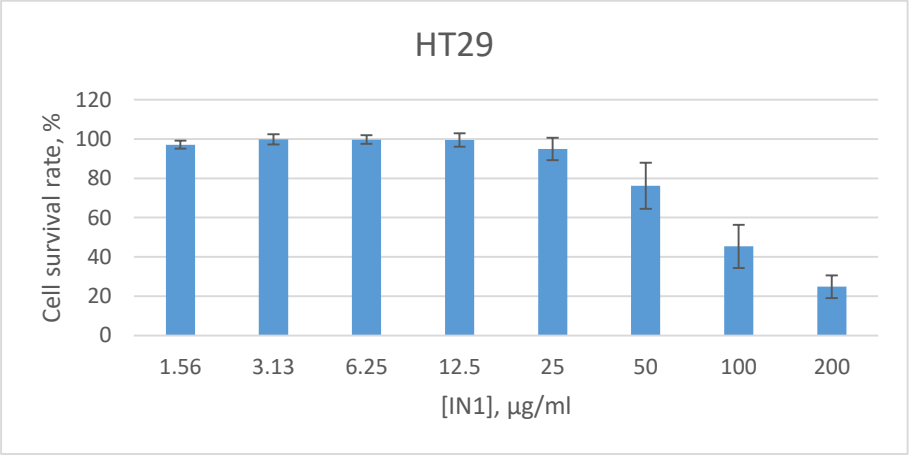

Figure 14.7: Cell survival following treatment of HT29 cells for 72 h with IN1, as determined with the MTT assay (error bars = standard deviation, *n* = 6).

5.14.8. MCF7 (Human breast adenocarcinoma)

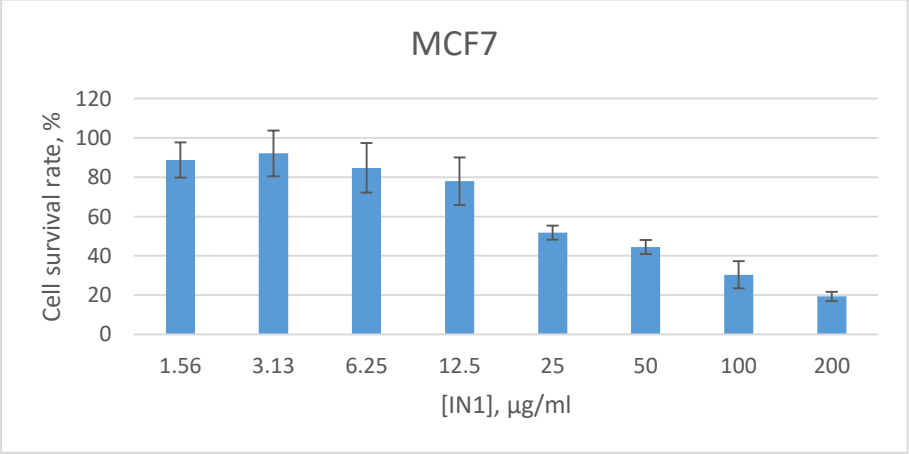

Figure 14.8: Cell survival following treatment of MCF7 cells for 72 h with IN1 as determined with the MTT assay (error bars = standard deviation, *n* = 6).

5.14.9. AGS (Human stomach carcinoma)

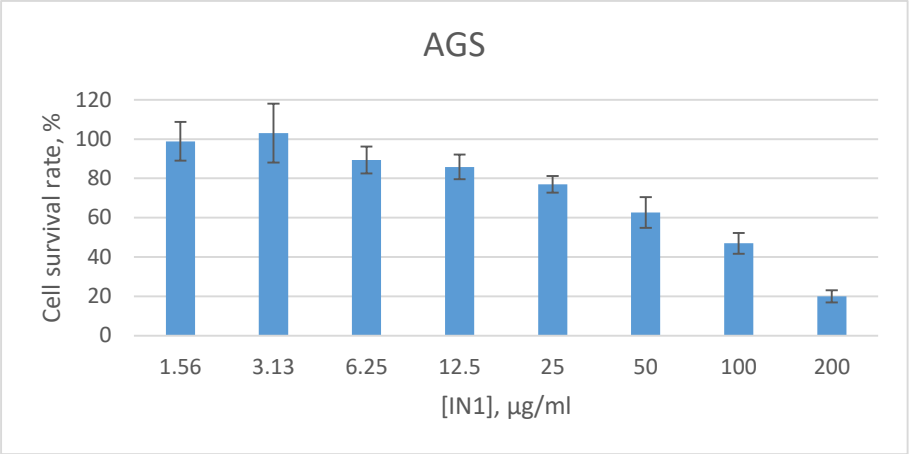

Figure 14.9: Cell survival following treatment of AGS cells for 72 h with IN1, as determined with the MTT assay (error bars = standard deviation, *n* = 6).

5.14.10. Clone 15 HL-60 (Human acute promyelocytic leukemia)

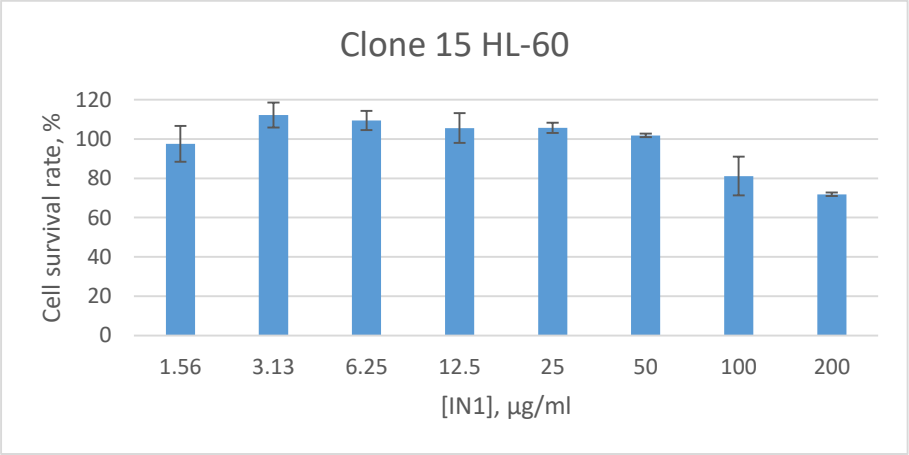

Figure 14.10: Cell survival following treatment of Clone 15 HL-60 cells for 72 h with IN1, as determined with the LDH assay (error bars = standard deviation, *n* = 3).

5.14.11. DU145 (Human prostate carcinoma)

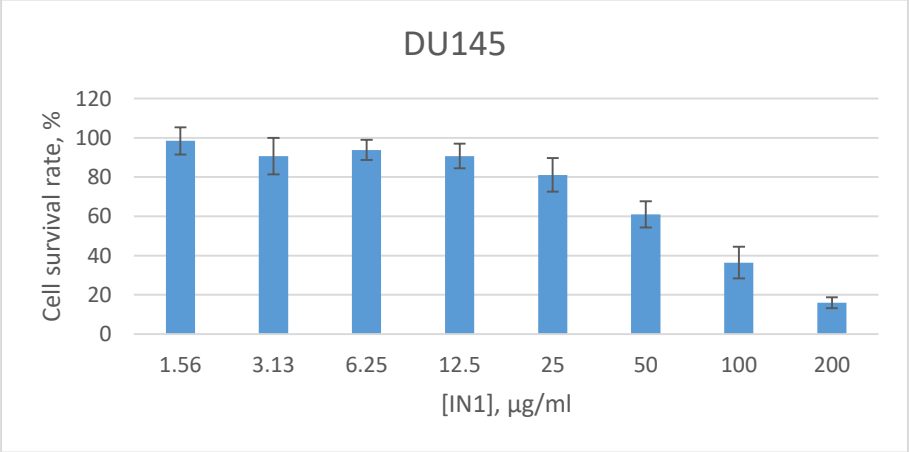

Figure 14.11: Cell survival following treatment of DU145 cells for 72 h with IN1, as determined with the MTT assay (error bars = standard deviation, *n* = 6).

5.14.12. A-704 (human kidney adenocarcinoma)

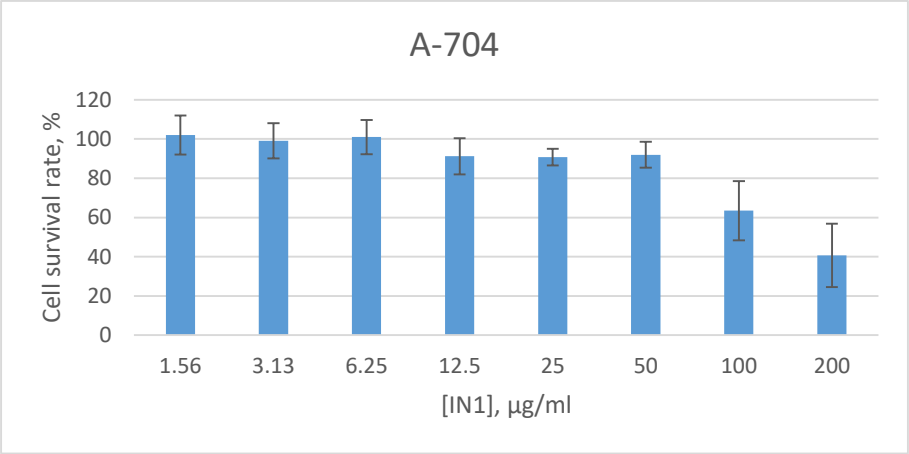

Figure 14.12: Cell survival following treatment of A-704 cells for 72 h with IN1, as determined with the MTT assay (error bars = standard deviation, *n* = 6).

5.14.13. K-562 (Human chronic myelogenous Leukemia)

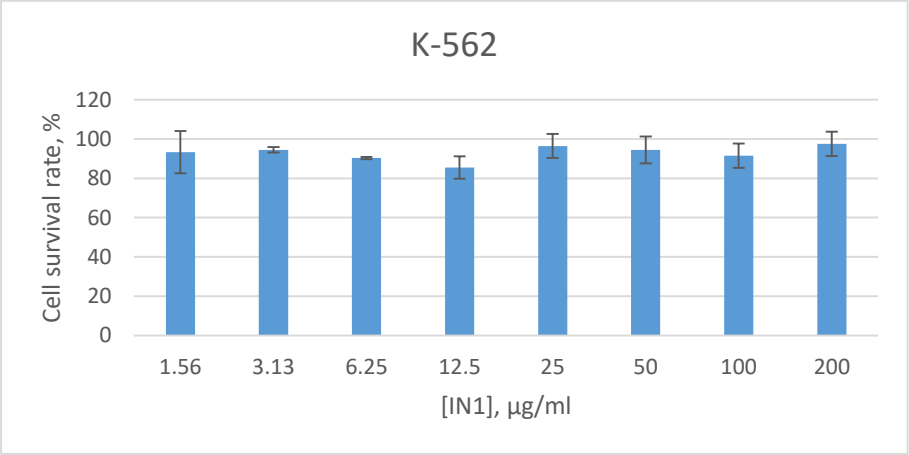

Figure 14.13A: Cell survival following treatment of K-562 cells for 72 h with IN1, as determined with the LDH assay (error bars = standard deviation, *n* = 3).

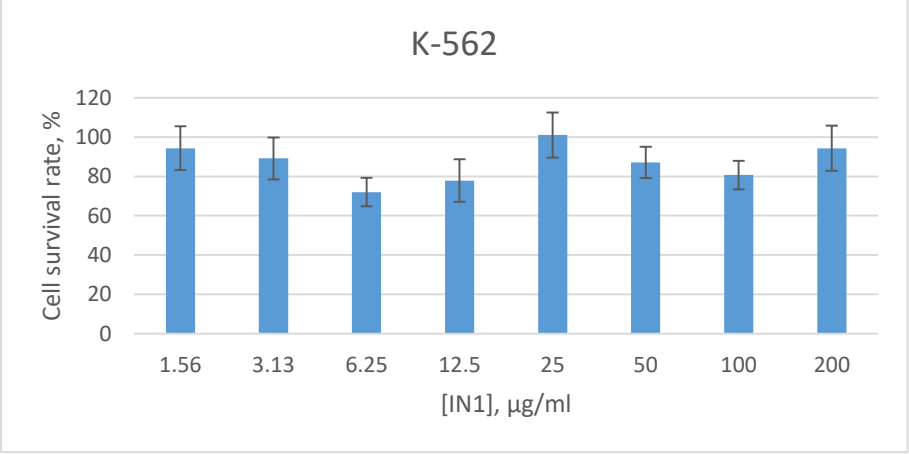

Figure 14.13B: Cell survival following treatment of K-562 cells for 72 h with IN1, as determined with the MTT assay (error bars = standard deviation, *n* = 3).

5.14.14. U-87 MG (Human glioblastoma)

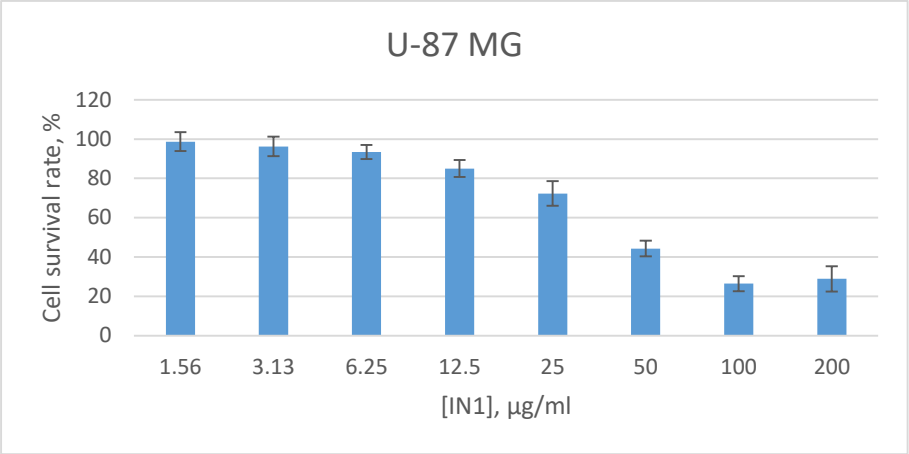

Figure 14.14: Cell survival following treatment of U-87 MG cells for 72 h with IN1, as determined with the MTT assay (error bars = standard deviation, *n* = 6).

5.14.15. A-549 (Human non-small cell lung carcinoma)

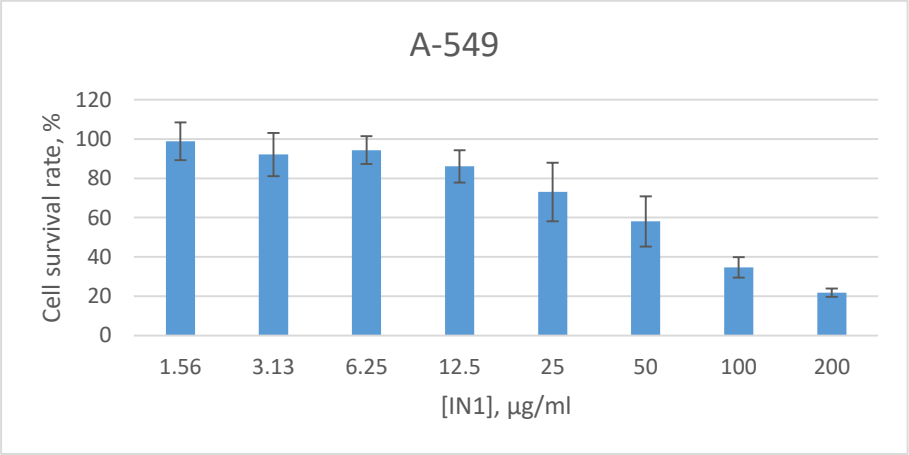

Figure 14.15: Cell survival following treatment of A-549 cells for 72 h with IN1, as determined with the MTT assay (error bars = standard deviation, *n* = 6).

5.14.16. BT-20 (Human triple negative invasive ductal human carcinoma breast cancer)

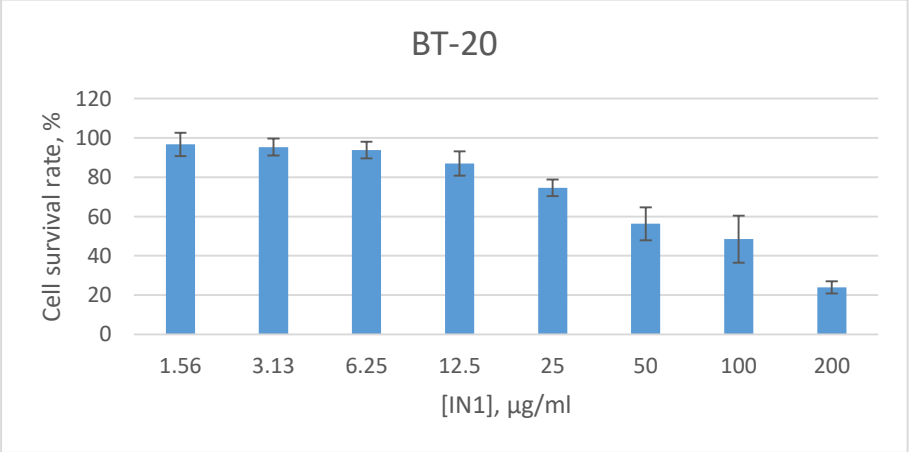

Figure 14.16: Cell survival following treatment of BT-20 cells for 72 h with IN1, as determined with the MTT assay (error bars = standard deviation, *n* = 6).

5.14.17. Panc 02.03 (Human pancreas adenocarcinoma)

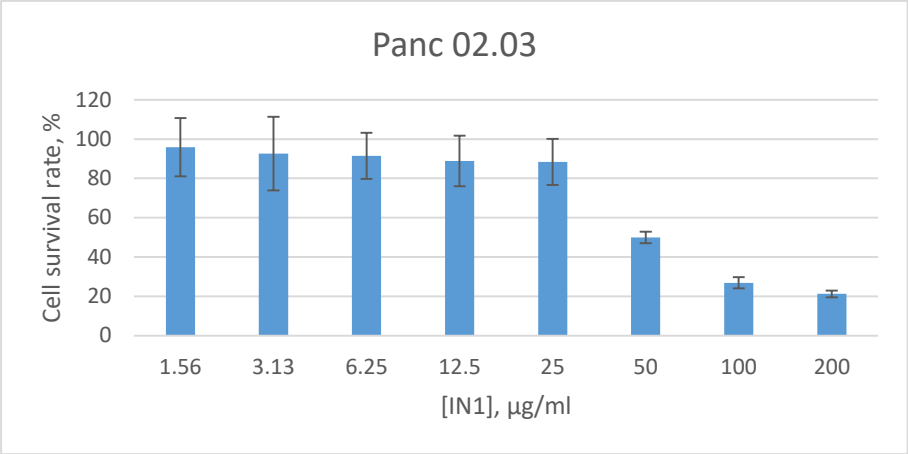

Figure 14.17: Cell survival following treatment of Panc 02.03 cells for 72 h with IN1, as determined with the MTT assay (error bars = standard deviation, *n* = 6).

Discussion for compound IN1 results:

IN1 appears to have toxic effects against the non-cancerous Vero and BJ-5ta cells, with more than 30% cell viability inhibition above 25 ug/ml. No marked activity more than this cytotoxicity could be observed.

5.15. Results for compound YS5

5.15.1. Vero (African green monkey – non-cancer control)

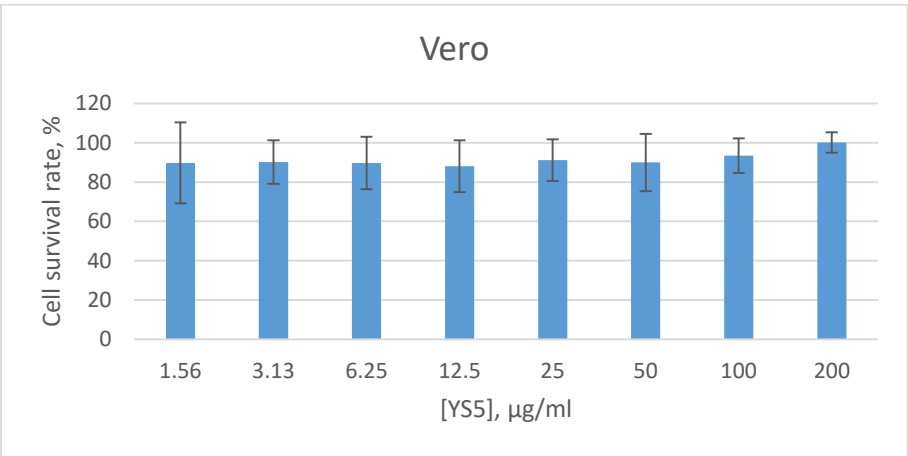

Figure 15.1: Cell survival following treatment of Vero cells for 72 h with YS5, as determined with the MTT assay (error bars = standard deviation, *n* = 6).

5.15.2. BJ-5ta (Human skin fibroblasts hTERT immortalized, non-cancer)

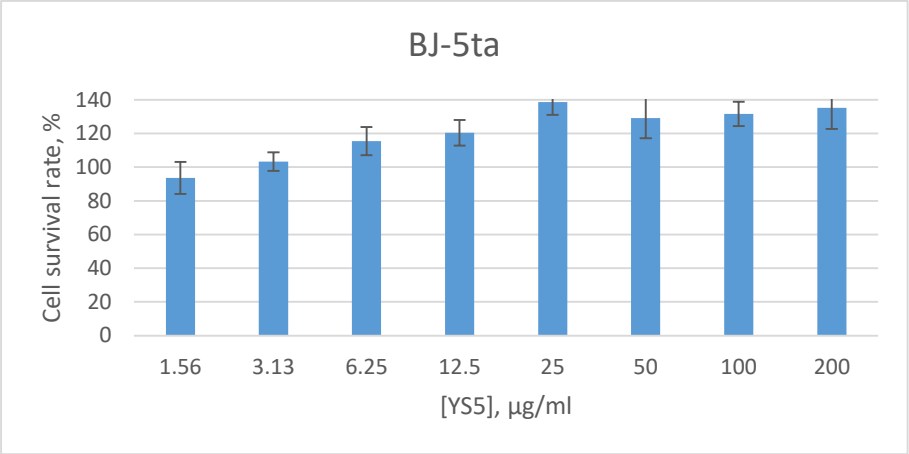

Figure 15.2: Cell survival following treatment of BJ-5ta cells for 72 h with YS5, as determined with the MTT assay (error bars = standard deviation, *n* = 6).

5.15.3. H69V (Human small cell lung carcinoma)

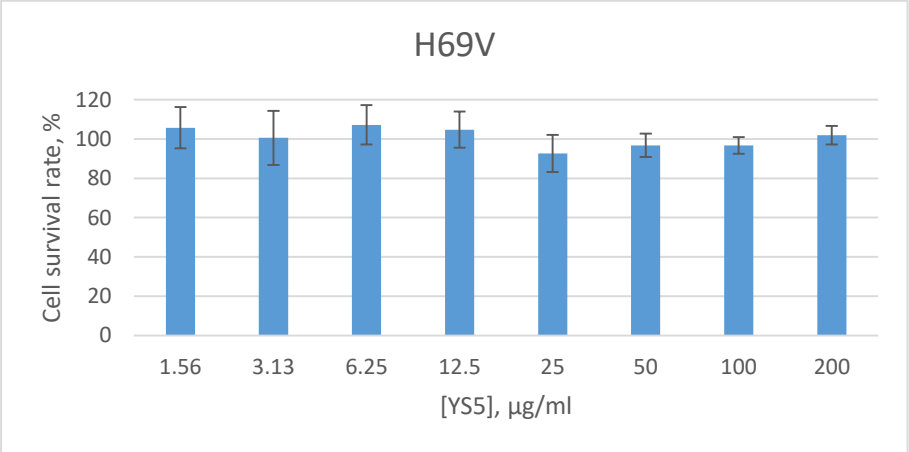

Figure 15.3: Cell survival following treatment of H69V cells for 72 h with YS5, as determined with the MTT assay (error bars = standard deviation, *n* = 6).

5.15.4. 143B (Human osteosarcoma)

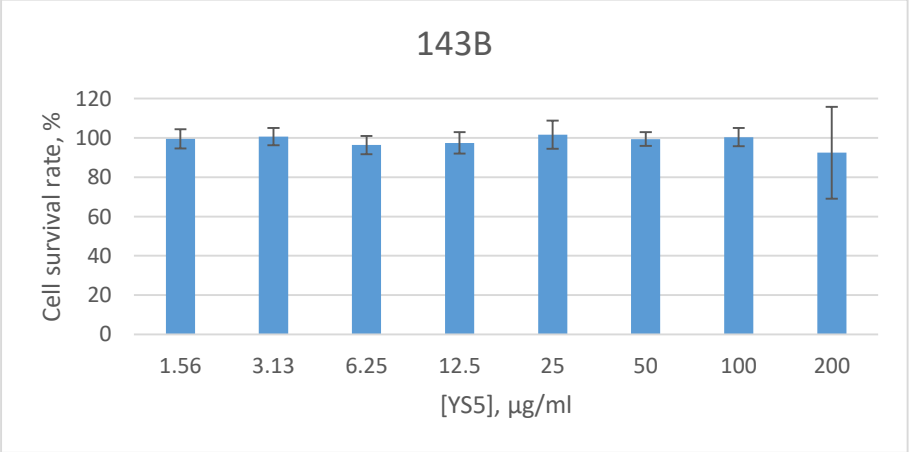

Figure 15.4: Cell survival following treatment of 143B cells for 72 h with YS5, as determined with the MTT assay (error bars = standard deviation, *n* = 6).

5.15.5. HepG2/C3A (Human hepatocellular carcinoma)

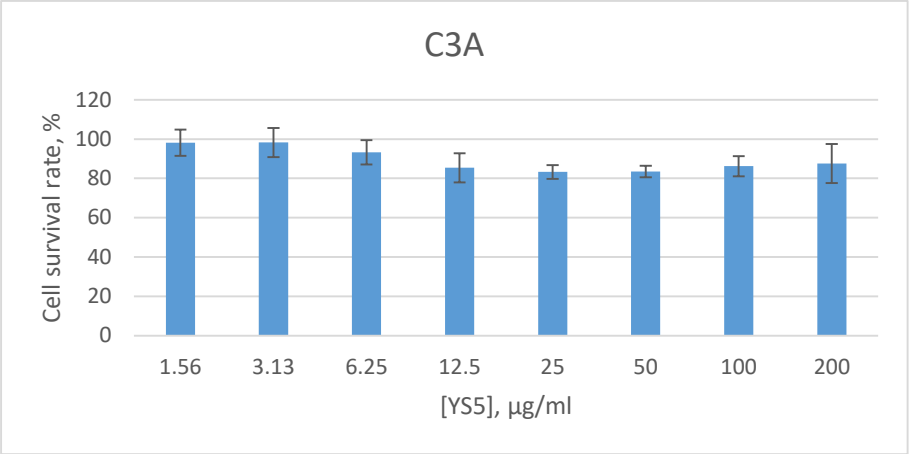

Figure 15.5: Cell survival following treatment of HepG2/C3A cells for 72 h with YS5, as determined with the MTT assay (error bars = standard deviation, *n* = 6).

5.15.6. A375 (Human melanoma)

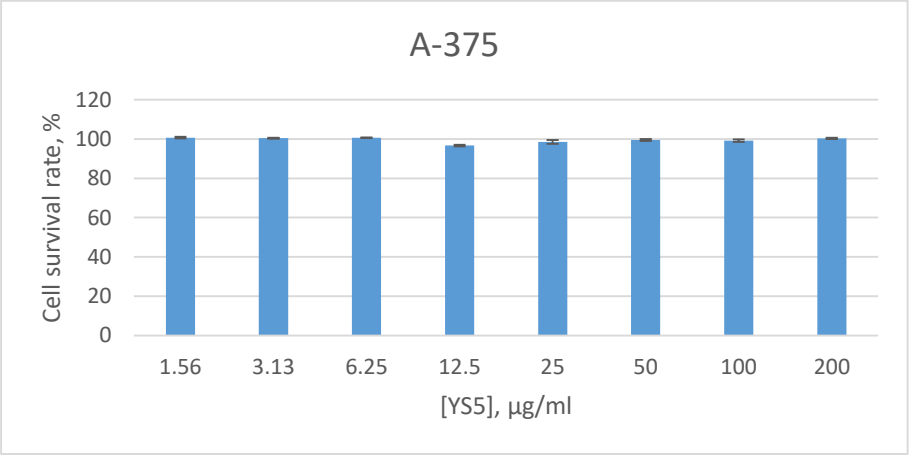

Figure 15.6: Cell survival following treatment of A375 cells for 72 h with YS5, as determined with the MTT assay (error bars = standard deviation, *n* = 6).

5.15.7. HT29 (Human colon carcinoma)

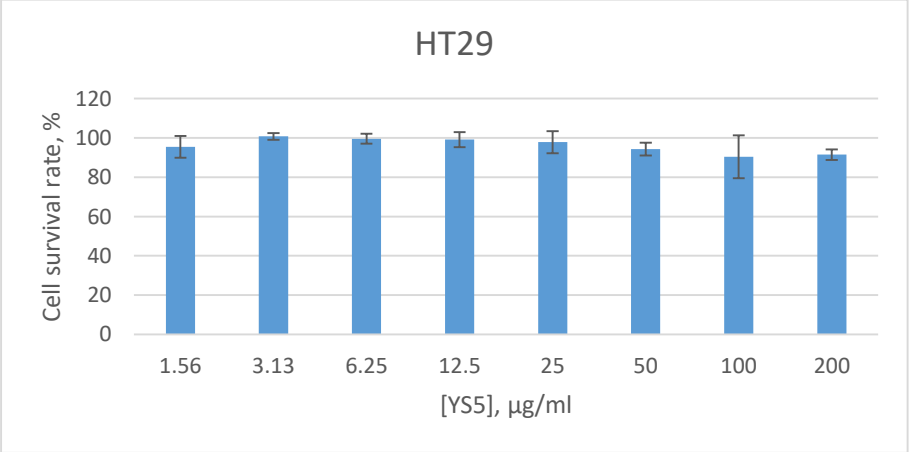

Figure 15.7: Cell survival following treatment of HT29 cells for 72 h with YS5, as determined with the MTT assay (error bars = standard deviation, *n* = 6).

5.15.8. MCF7 (Human breast adenocarcinoma)

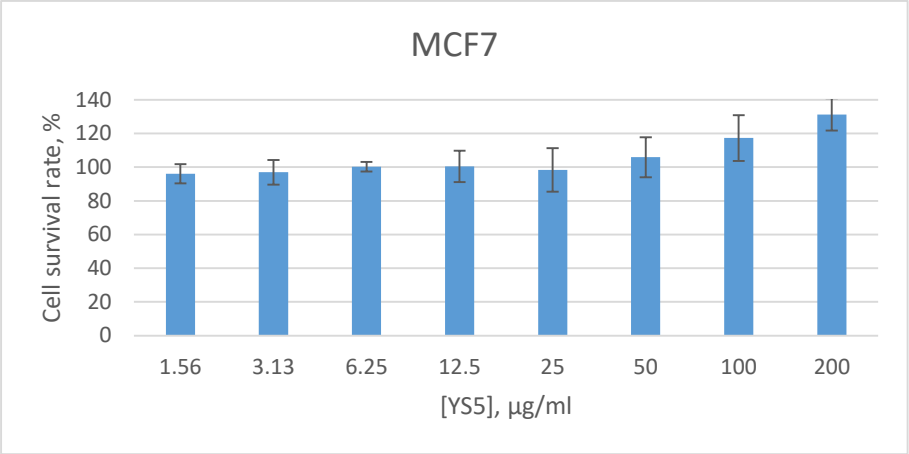

Figure 15.8: Cell survival following treatment of MCF7 cells for 72 h with YS5 as determined with the MTT assay (error bars = standard deviation, *n* = 6).

5.15.9. AGS (Human stomach carcinoma)

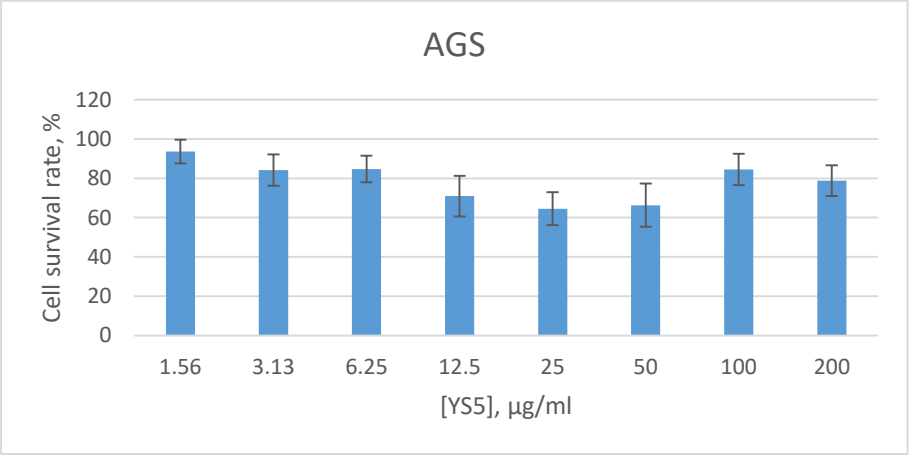

Figure 15.9: Cell survival following treatment of AGS cells for 72 h with YS5, as determined with the MTT assay (error bars = standard deviation, *n* = 6).

5.15.10. Clone 15 HL-60 (Human acute promyelocytic leukemia)

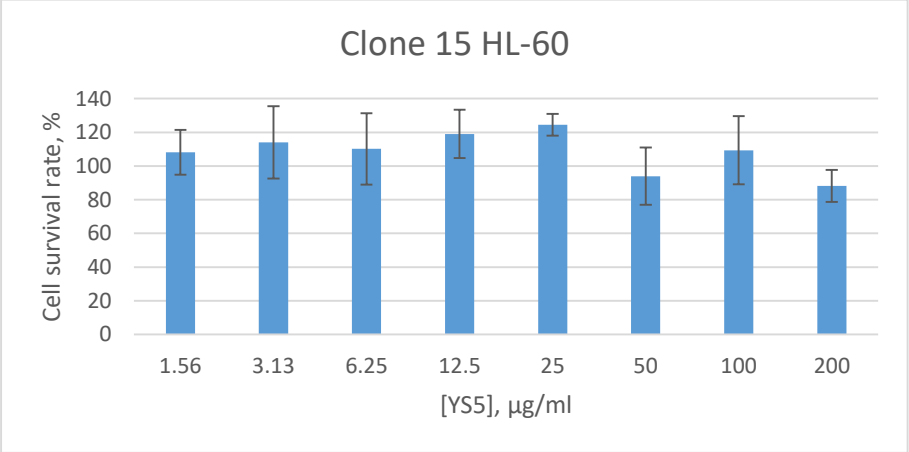

Figure 15.10: Cell survival following treatment of Clone 15 HL-60 cells for 72 h with YS2, as determined with the LDH assay (error bars = standard deviation, *n* = 3).

5.15.11. DU145 (Human prostate carcinoma)

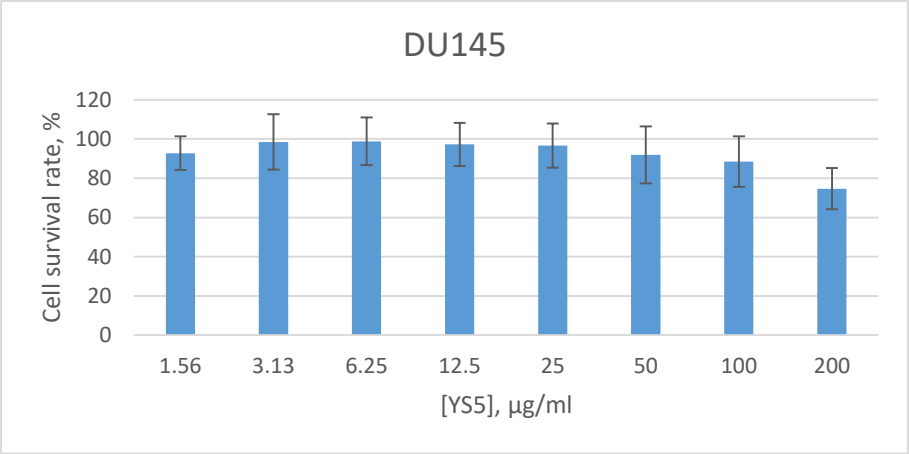

Figure 15.11: Cell survival following treatment DU145 cells for 72 h with YS5, as determined with the MTT assay (error bars = standard deviation, *n* = 6).

5.15.12. A-704 (human kidney adenocarcinoma)

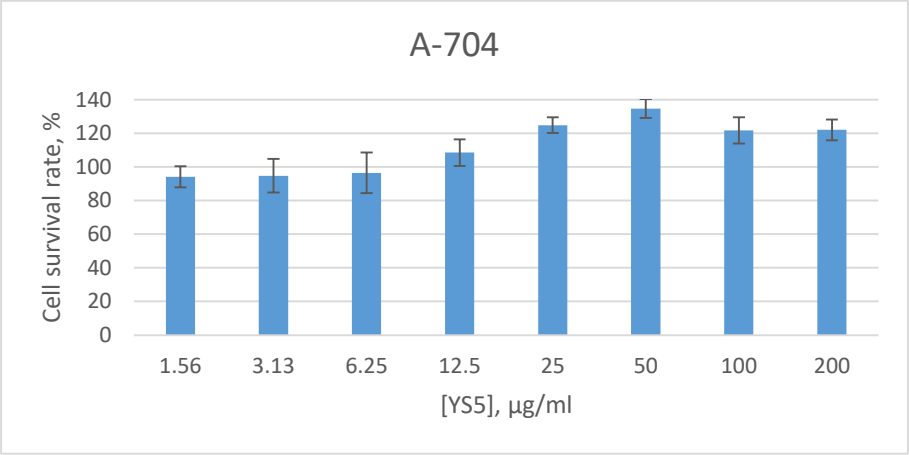

Figure 15.12: Cell survival following treatment of A-704 cells for 72 h with YS2, as determined with the MTT assay (error bars = standard deviation, *n* = 6).

5.15.13. K-562 (Human chronic myelogenous Leukemia)

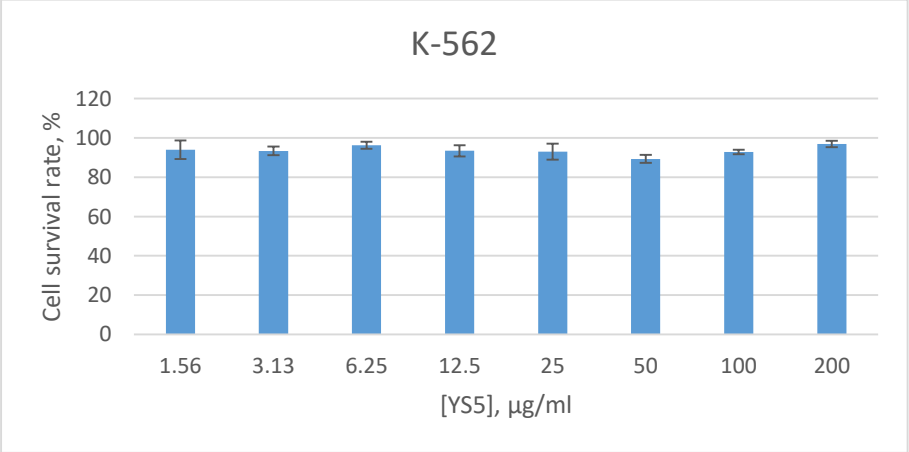

Figure 15.13A: Cell survival following treatment of K-562 cells for 72 h with YS2, as determined with the LDH assay (error bars = standard deviation, *n* = 3).

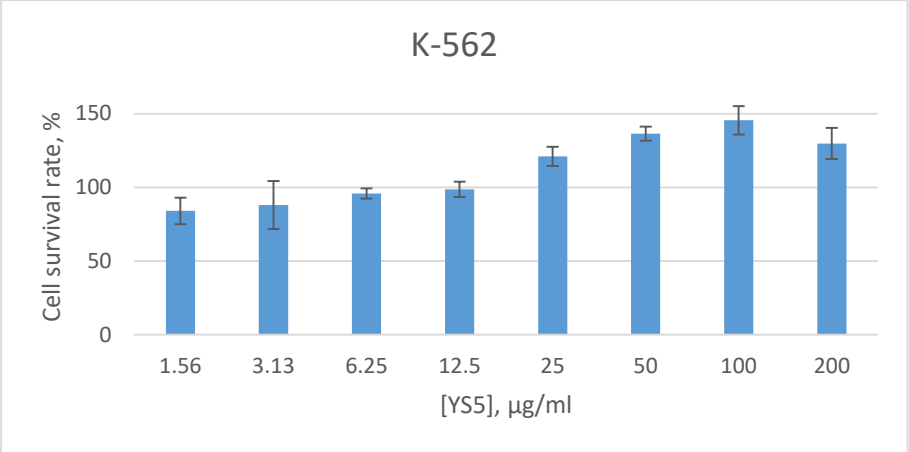

Figure 15.13B: Cell survival following treatment of K-562 cells for 72 h with YS2, as determined with the MTT assay (error bars = standard deviation, *n* = 3).

5.15.14. U-87 MG (Human glioblastoma)

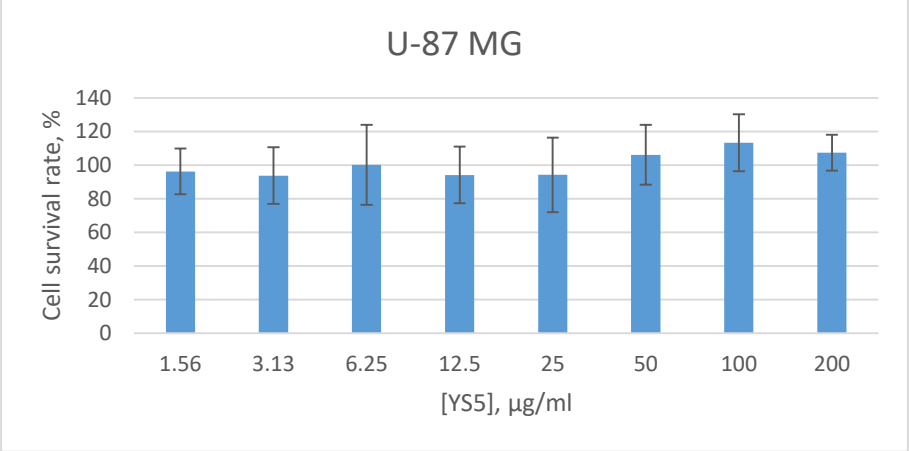

Figure 15.14: Cell survival following treatment of U-87 MG cells for 72 h with YS5, as determined with the MTT assay (error bars = standard deviation, *n* = 6).

5.15.15. A-549 (Human non-small cell lung carcinoma)

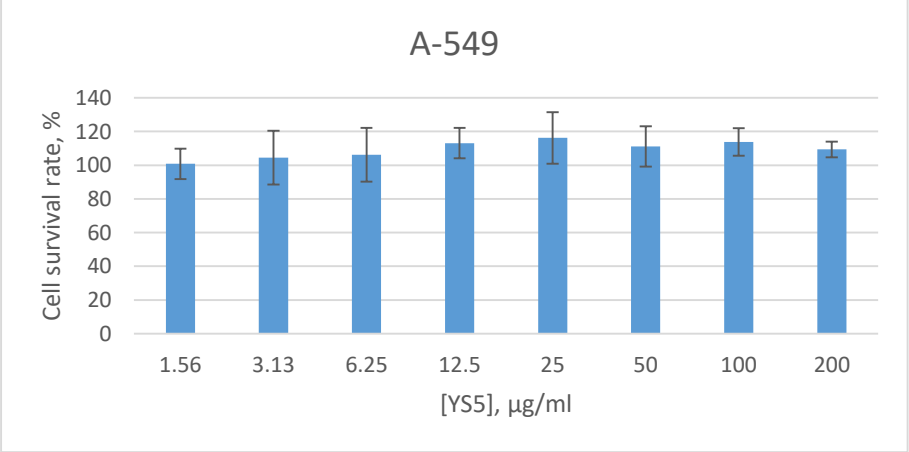

Figure 15.15: Cell survival following treatment of A-549 cells for 72 h with YS5, as determined with the MTT assay (error bars = standard deviation, *n* = 6).

5.15.16. BT-20 (Human triple negative invasive ductal human carcinoma breast cancer)

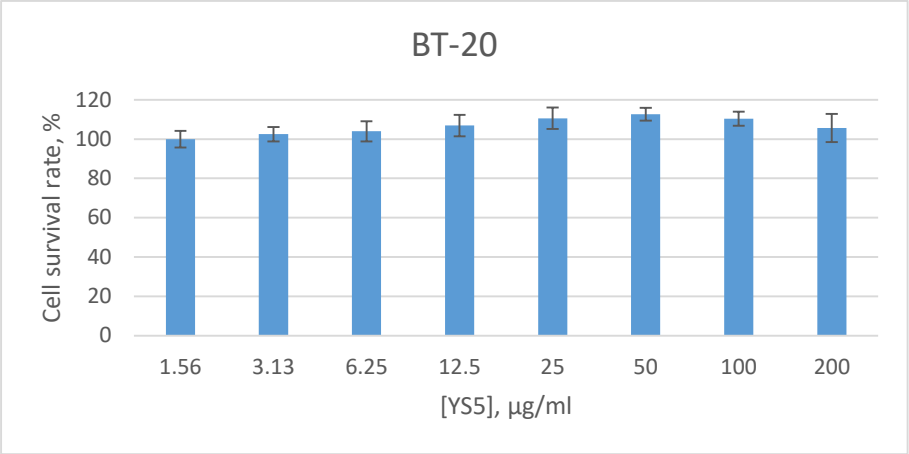

Figure 15.16: Cell survival following treatment of BT-20 cells for 72 h with YS5, as determined with the MTT assay (error bars = standard deviation, *n* = 6).

5.15.17. Panc 02.03 (Human pancreas adenocarcinoma)

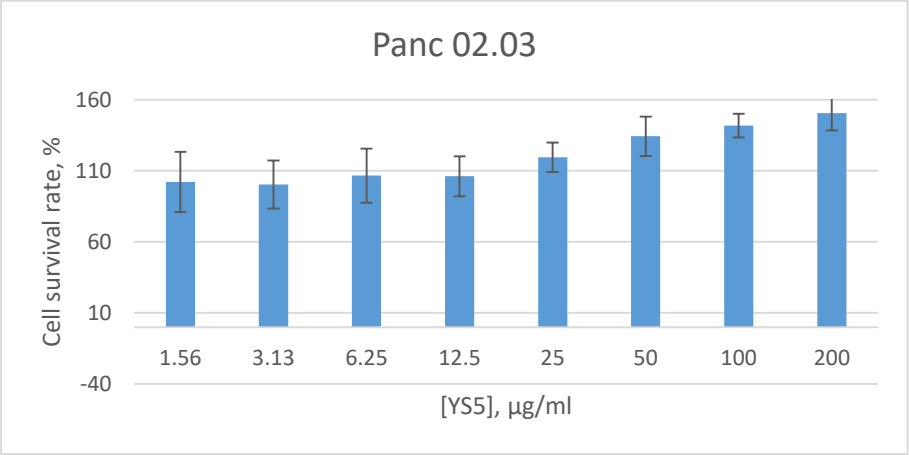

Figure 15.17: Cell survival following treatment of Panc 02.03 cells for 72 h with YS5, as determined with the MTT assay (error bars = standard deviation, *n* = 6).

Discussion for compound YS5 results:

YS5 seems to not toxic to the Vero or BJ-5ta non-cancerous cell lines, but rather increased viability in a concentration dependent manner. There was very slight activity against the HepG2/C3A cell line. However, there seems to be an increasing cell survival with increasing concentration observed with MCF7 cells. There was good activity observed against the stomach carcinoma AGS cell line.

5.16. Results for compound F7/NN

5.16.1. Vero (African green monkey – non-cancer control)

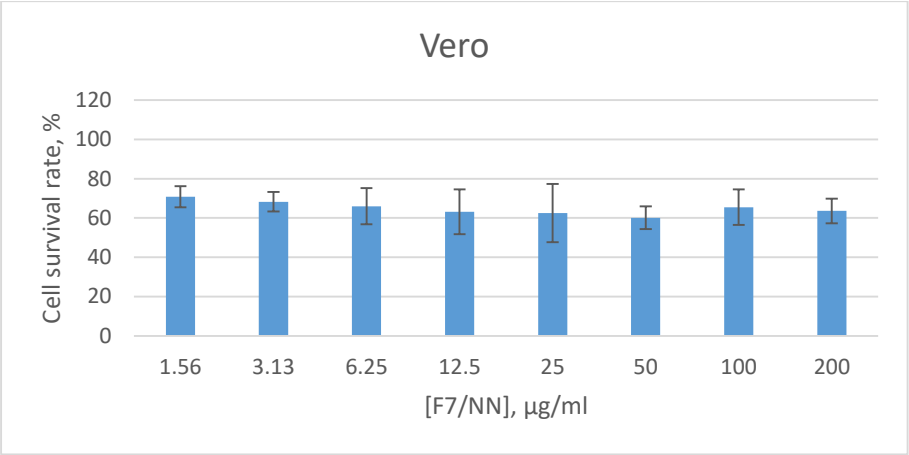

Figure 16.1: Cell survival following treatment of Vero cells for 72 h with F7/NN, as determined with the MTT assay (error bars = standard deviation, *n* = 6).

5.16.2. BJ-5ta (Human skin fibroblasts hTERT immortalized, non-cancer)

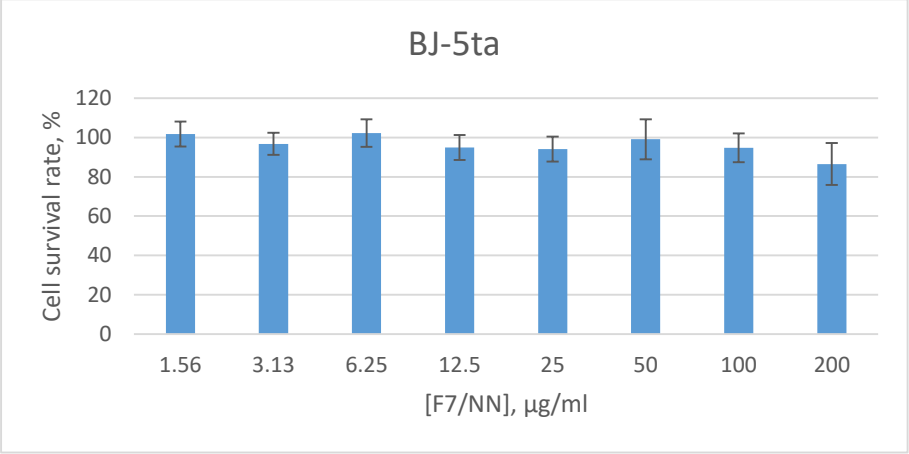

Figure 16.2: Cell survival following treatment of BJ-5ta cells for 72 h with F7/NN, as determined with the MTT assay (error bars = standard deviation, *n* = 6).

5.16.3. H69V (Human small cell lung carcinoma)

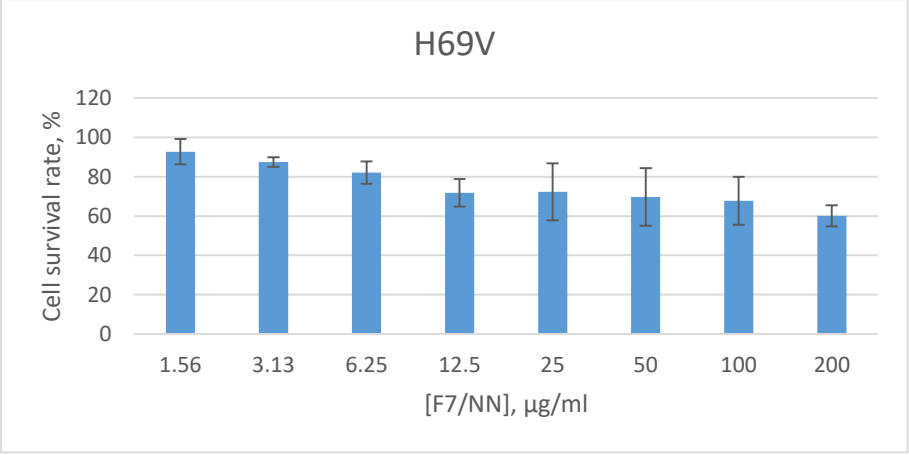

Figure 15.3: Cell survival following treatment of H69V cells for 72 h with F7/NN, as determined with the MTT assay (error bars = standard deviation, *n* = 6).

5.16.4. 143B (Human osteosarcoma)

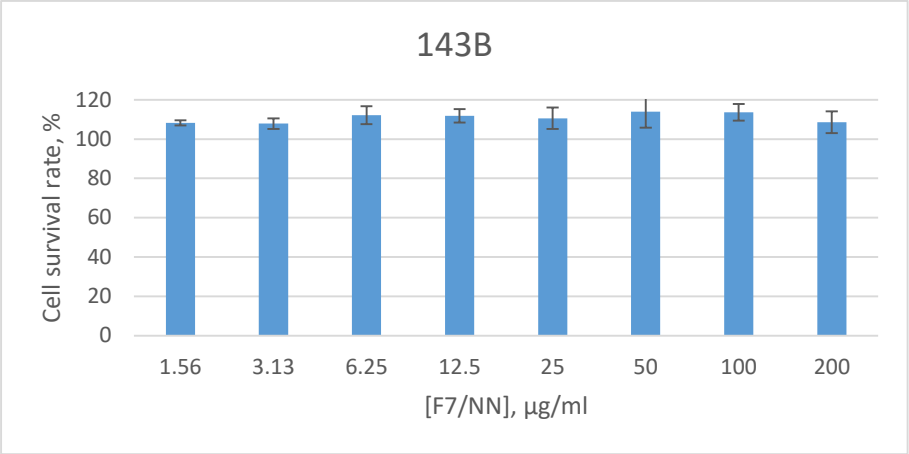

Figure 16.4: Cell survival following treatment of 143B cells for 72 h with F7/NN, as determined with the MTT assay (error bars = standard deviation, *n* = 6).

5.16.5. HepG2/C3A (Human hepatocellular carcinoma)

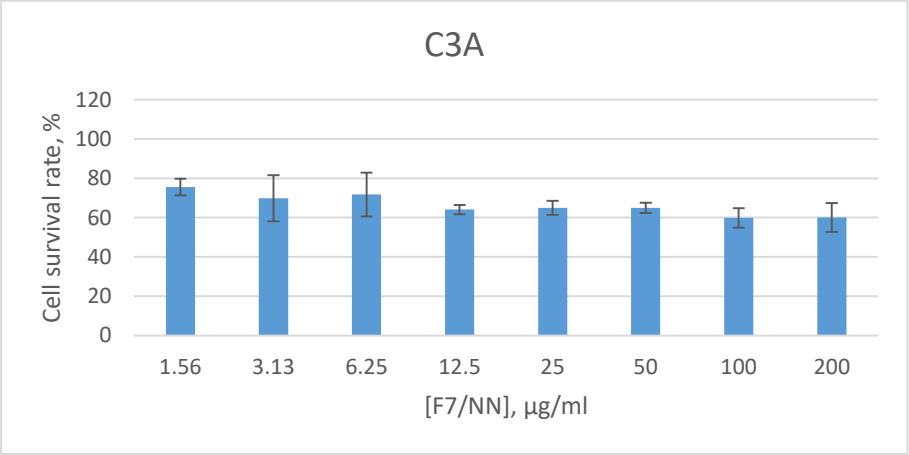

Figure 16.5: Cell survival following treatment of HepG2/C3A cells for 72 h with F7/NN, as determined with the MTT assay (error bars = standard deviation, *n* = 6).

5.16.6. A375 (Human melanoma)

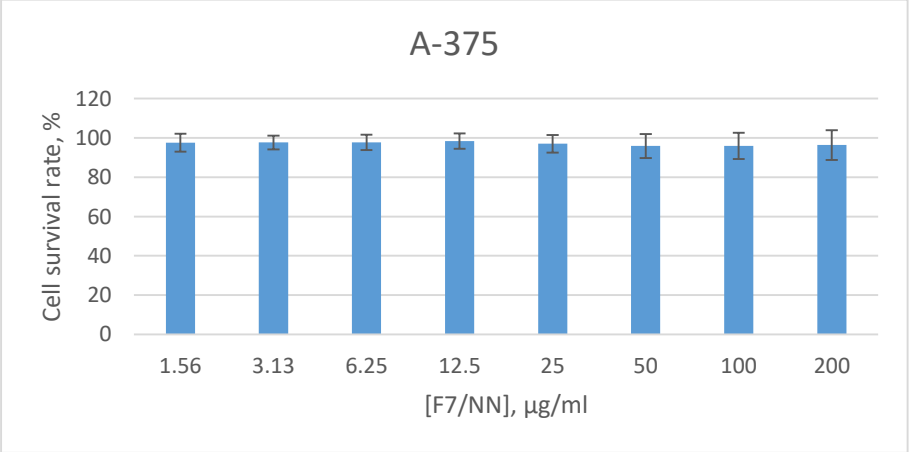

Figure 16.6: Cell survival following treatment of A375 cells for 72 h with F7/NN, as determined with the MTT assay (error bars = standard deviation, *n* = 6).

5.16.7. HT29 (Human colon carcinoma)

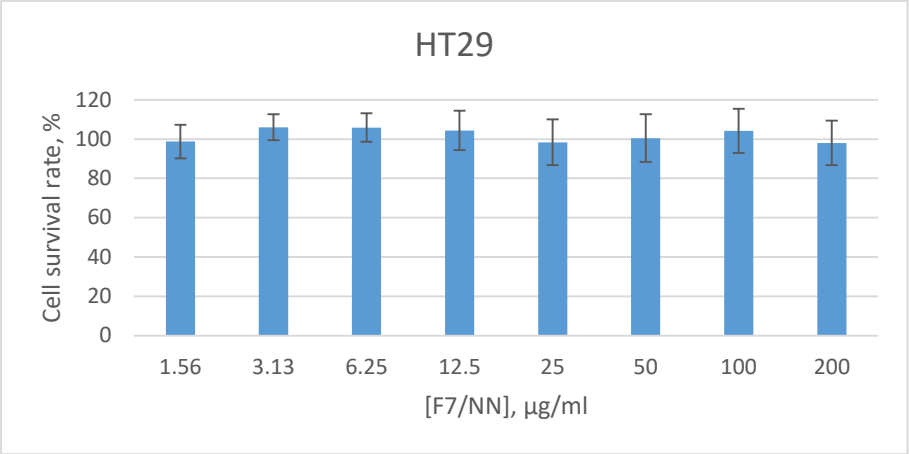

Figure 16.7: Cell survival following treatment of HT29 cells for 72 h with F7/NN, as determined with the MTT assay (error bars = standard deviation, *n* = 6).

5.16.8. MCF7 (Human breast adenocarcinoma)

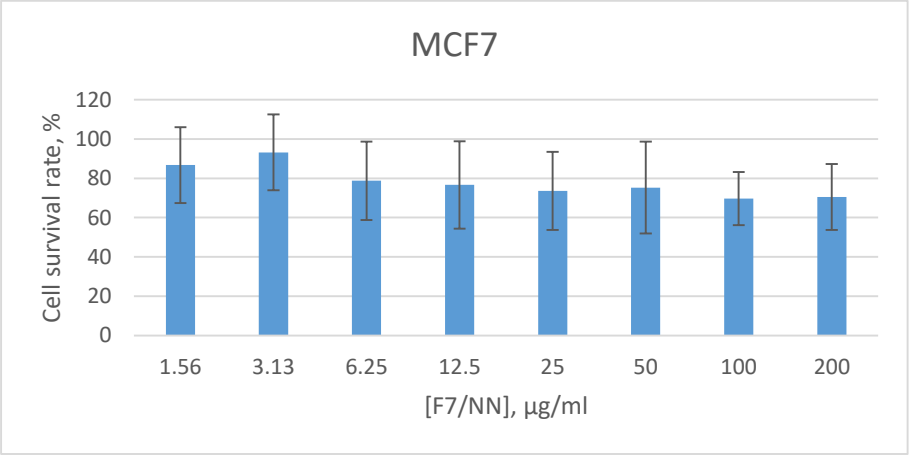

Figure 16.8: Cell survival following treatment of MCF7 cells for 72 h with F7/NN as determined with the MTT assay (error bars = standard deviation, *n* = 6).

5.16.9. AGS (Human stomach carcinoma)

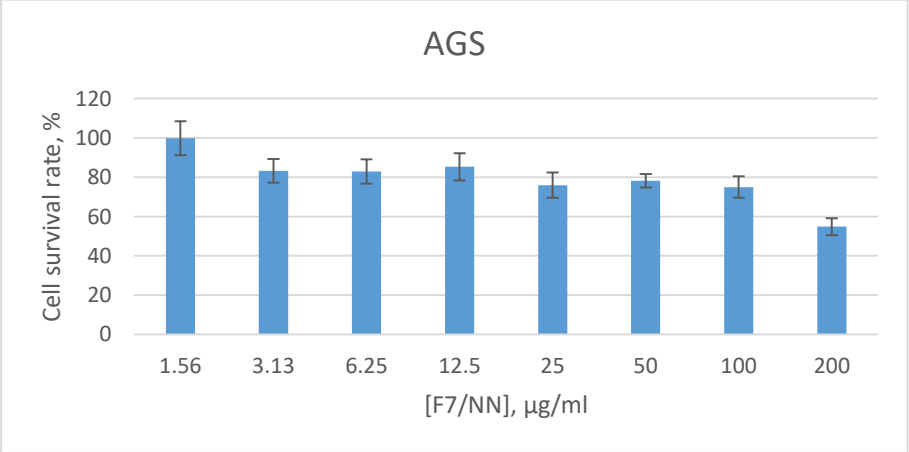

Figure 16.9: Cell survival following treatment of AGS cells for 72 h with F7/NN, as determined with the MTT assay (error bars = standard deviation, *n* = 6).

5.16.10. Clone 15 HL-60 (Human acute promyelocytic leukemia)

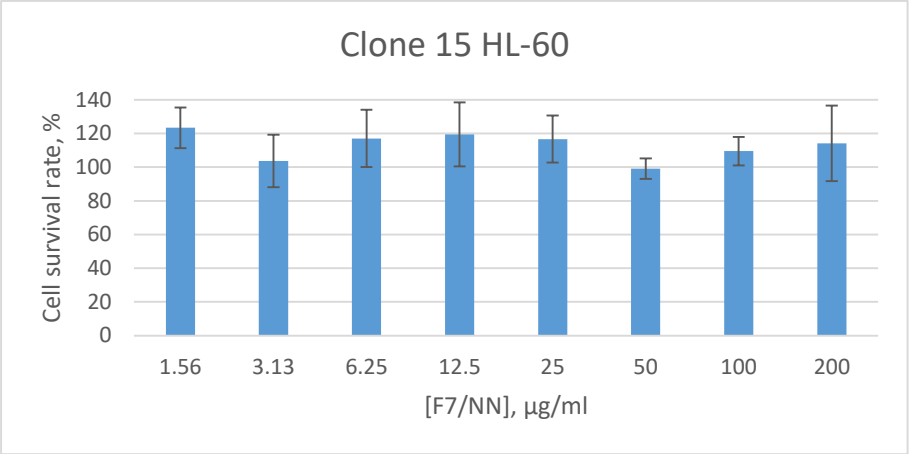

Figure 16.10: Cell survival following treatment of Clone 15 HL-60 cells for 72 h with F7/NN, as determined with the LDH assay (error bars = standard deviation, *n* = 3).

5.16.11. DU145 (Human prostate carcinoma)

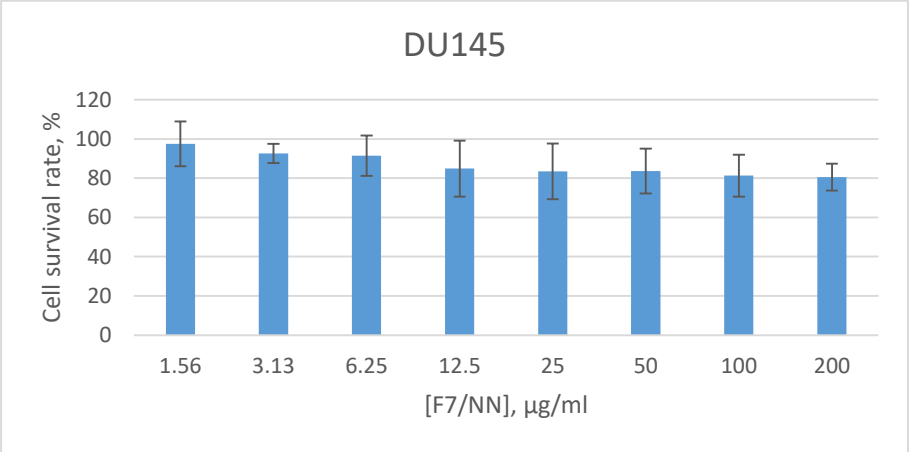

Figure 16.11: Cell survival following treatment of DU145 cells for 72 h with F7/NN, as determined with the MTT assay (error bars = standard deviation, *n* = 6).

5.16.12. A-704 (human kidney adenocarcinoma)

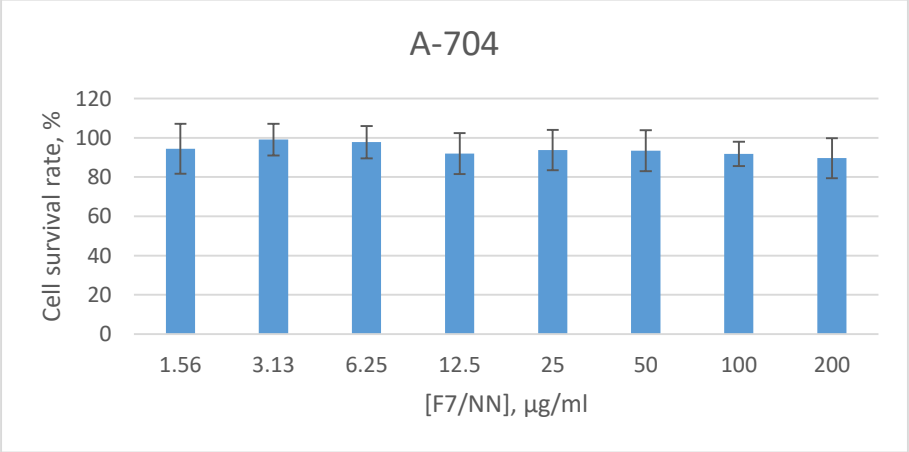

Figure 16.12: Cell survival following treatment of A-704 cells for 72 h with F7/NN, as determined with the MTT assay (error bars = standard deviation, *n* = 6).

5.16.13. K-562 (Human chronic myelogenous Leukemia)

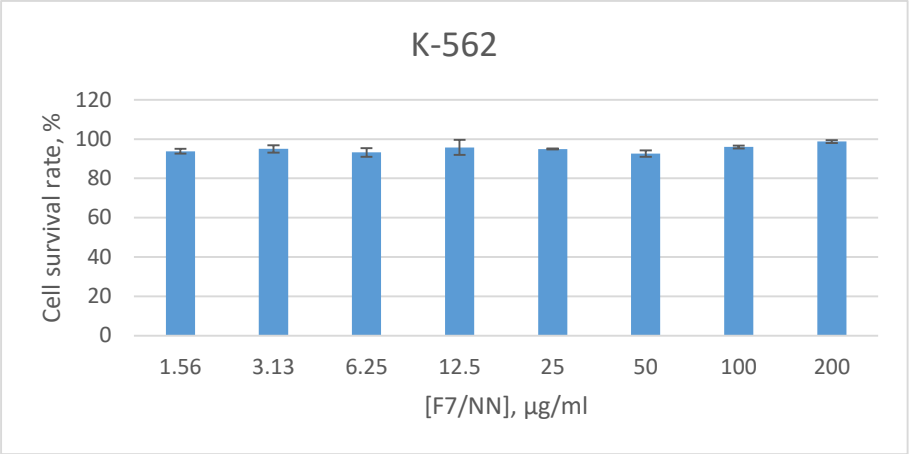

Figure 16.13A: Cell survival following treatment of K-562 cells for 72 h with F7/NN, as determined with the LDH assay (error bars = standard deviation, *n* = 3).

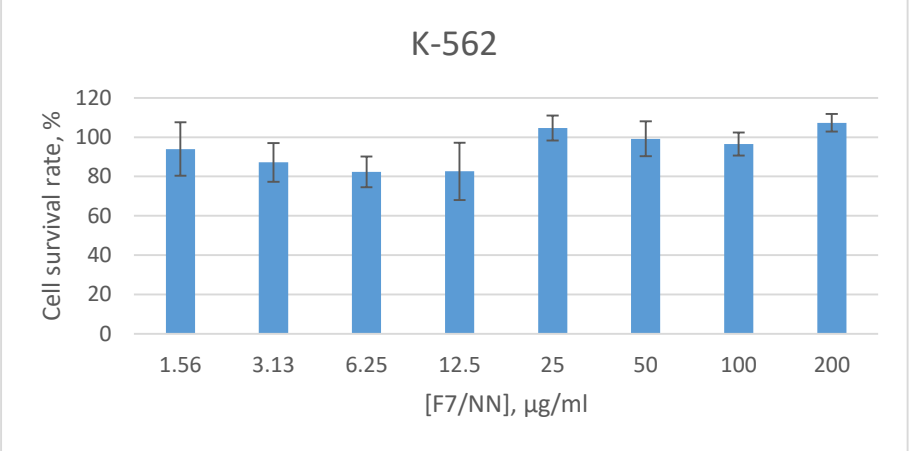

Figure 16.13B: Cell survival following treatment of K-562 cells for 72 h with F7/NN, as determined with the MTT assay (error bars = standard deviation, *n* = 3).

5.16.14. U-87 MG (Human glioblastoma)

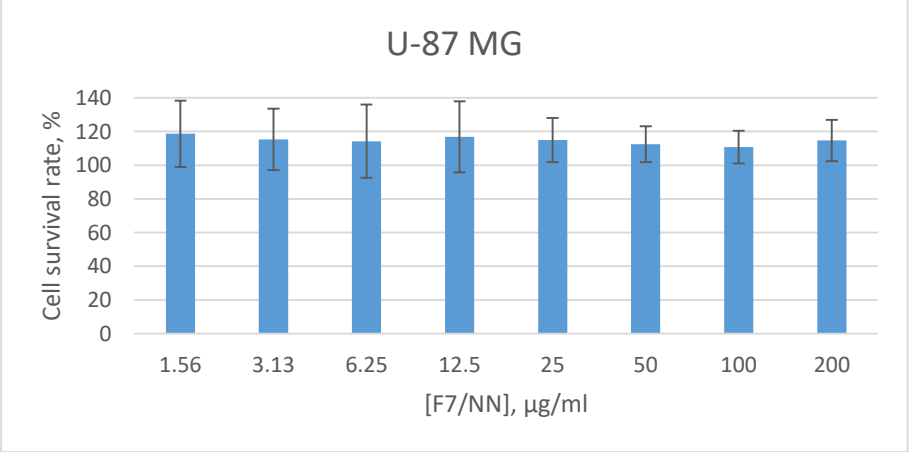

Figure 16.14: Cell survival following treatment of U-87 MG cells for 72 h with F7/NN, as determined with the MTT assay (error bars = standard deviation, *n* = 6).

5.16.15. A-549 (Human non-small cell lung carcinoma)

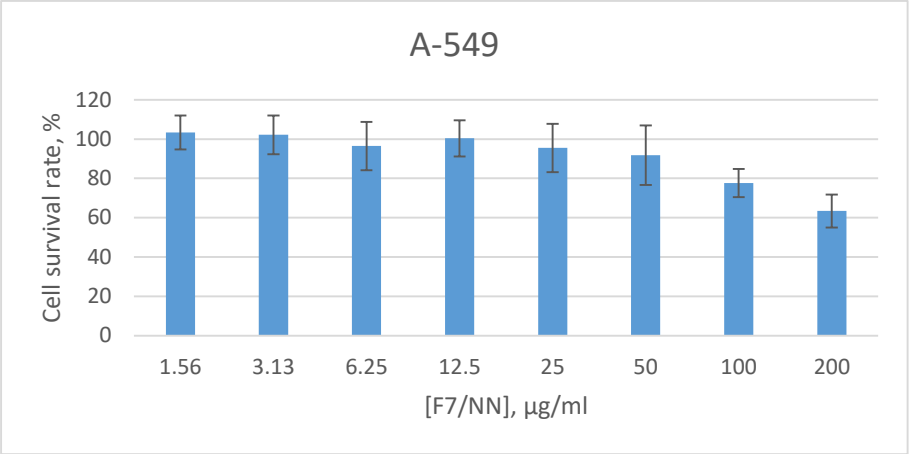

Figure 16.15: Cell survival following treatment of A-549 cells for 72 h with F7/NN, as determined with the MTT assay (error bars = standard deviation, *n* = 6).

5.16.16. BT-20 (Human triple negative invasive ductal human carcinoma breast cancer)

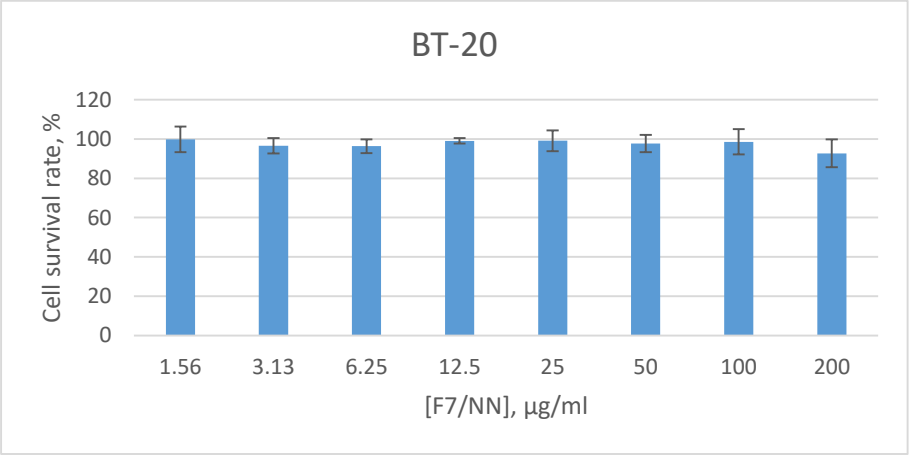

Figure 16.16: Cell survival following treatment of BT-20 cells for 72 h with F7/NN, as determined with the MTT assay (error bars = standard deviation, *n* = 6).

5.16.17. Panc 02.03 (Human pancreas adenocarcinoma)

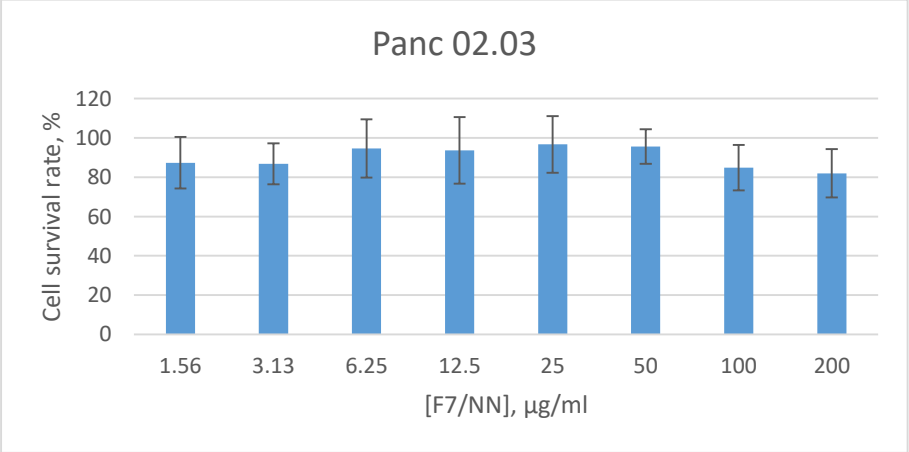

Figure 16.17: Cell survival following treatment of Panc 02.03 cells for 72 h with F7/NN, as determined with the MTT assay (error bars = standard deviation, *n* = 6).

Discussion for compound F7/NN results:

F7/NN seems to be slightly toxic to the Vero cell line at all concentration with a decrease in cell viability of 30 to 40%. No such toxicity was observed for the non-cancerous BJ-5ta cell line. Some potential activity was observed for the H69V, HepG2/C3A, MCF7 and AGS cell lines.

6. Discussion of results

All 16 compounds were tested against all 17 cell lines.

6.1. Final IC_{50} and Selective index results (*n* = 6)

Selective indices (SI) enable discernment between toxicity and anticancer activity.

Table 6.1 Cytotoxic activity (IC_{50}, µg/ml) and Selective index (SI) of WSU natural products.

Abbreviations:

NA= no activity;

NT= not toxic;

ND = not detectable, since toxicity concentration was not detected under tested conditions;

SI1 =Selective index measured against non-cancerous cell line, Vero cells;

SI2 =Selective index measured against non-cancerous cell line, BJ-5ta cells;

Grey highlight = above range tested, not accurate.

Red text = highly selective compounds

Cell line	RBME	RBAC	RBET	LUENT
Vero	NT	NT	NT	NT
84BR	-	-	-	-
BJ-5ta	NT	NT	NT	NT
H69V	IC50 = 88.1 (51.9-178.2) SI1, SI2 = ND	IC50 = 84.1 (54.4-161.8) SI1, SI2 = ND	NA	NA
143B	IC50 = 52.4 (34.5-83.3) SI1, SI2 = ND	IC50 = 68.5 (52.0-95.4) SI1, SI2 = ND	IC50 = 52.2 (43.3-64.3) SI1, SI2 = ND	NA
C3A	IC50 = 246.8 (187.6-373.7) SI1, SI2 = ND	IC50 = 201.9 (130.7-375.9) SI1, SI2 = ND	IC50 = 645.5 (287.4-3516.1) SI1, SI2 = ND	NA
A375	IC50 = 99.2 (61.8-176.1) SI1, SI2 = ND	IC50 = 91.1 (46.1-170.8) SI1, SI2 = ND	NA	NA
HT29	IC50 = 376.5 (225.9-844.8) SI1, SI2 = ND	IC50 = 142.3 (125.8-164.8) SI1, SI2 = ND	NA	NA
MCF7	NA	NA	NA	NA
AGS	IC50 = 62.0 (49.0-78.5) SI1, SI2 = ND	IC50 = 188.85 (143.2-269.1) SI1, SI2 = ND	NA	NA
Clone 15 HL-60	NA	NA	NA	NA

DU145	IC50 = 51.9 (38.4-74.2) SI1, SI2 = ND	IC50 = 59.6 (39.7-96.7) SI1, SI2 = ND	IC50 = 107.3 (84.3-145.8) SI1, SI2 = ND	NA
A-704	NA	NA	NA	NA
K-562 (LDH)	NA	NA	NA	NA
K-562 (MTT)	IC50 = 76.8 (50.3-136.3) SI1, SI2 = ND	IC50=99.1 (67.8-172.7) SI1, SI2 = ND	NA	NA
U-87 MG	IC50 = 151.5 (95.5-291.2) SI1, SI2 = ND	IC50 = 141.7 (85.2-303.2) SI1, SI2 = ND	NA	NA
A-549	IC50 = 103.0 (91.5-117.3) SI1, SI2 = ND	IC50 = 126.4 (97.8-172.9) SI1, SI2 = ND	IC50 = 178.9 (137.6-257.6) SI1, SI2 = ND	NA
BT-20	IC50 = 223.8 (154.4-458.6) SI1, SI2 = ND	IC50 = 156.6 (134.2-192.5) SI1, SI2 = ND	NA	NA
Panc 02.03	NA	IC50 = 137.2 (104.2-202.2) SI1, SI2 = ND	NA	NA

Cell line	**RBMW**	**YS2**	**F6A-F5ZS**	**G4/MUC**
Vero	NT	IC50 = 77.9 (66.7-91.6)	NT	NT
84BR	-	IC50 = 83.9 (62.7-120.5) N=3	NT N=3	NT N=3
BJ-5ta	NT	IC50 = 48.4 (39.5-60.3)	NT	NT
H69V	IC50 = 183.3 (107.0-545.5) SI1, SI2 = ND	IC50 = 25.8 (20.6-32.8) SI1 = 3.02 SI2 = 1.87	NA	NA
143B	NA	IC50 = 98.6 (82.0-118.4) SI1 = 0.79 SI2 = 0.49	NA	NA
C3A	NA	IC50 = 11.7 (10.9-13.1) SI1 = 6.66 SI2 =4.13	NA	NA
A375	NA	IC50 = 74.3 (55.8-94.4) SI1 = 1.04 SI2 = 0.65	NA	NA
HT29	NA	IC50 = 84.2 (60.4-127.9) SI1 = 0.92 SI2 = 0.57	NA	NA
MCF7	NA	IC50 = 76.4 (61.9-97.5) SI1 = 1.02 SI2 = 0.63	NA	NA

AGS	NA	IC50 = 100.0 (68.7-161.1) SI1 = 0.78 SI2 = 0.48	NA	NA
Clone 15 HL-60	NA	IC50 = 206.6 (183.3-239.6) SI1 = 0.37 SI2 = 0.26	NA	NA
DU145	NA	IC50 = 190.5 (122.9.-355.3) SI1 = 0.41 SI2 = 0.25	NA	NA
A-704	IC50=142.3 (111.1-196.0) SI1, SI2 = ND	NA	NA	NA
K-562 (LDH)	NA	NA	NA	NA
K-562 (MTT)	NA	IC50 = 42.9 (29.2-67.7) SI1 = 1.81 SI2 = 1.12	NA	NA
U-87 MG	NA	IC50 = 122.4 (77.8-236.1) SI1 = 0.63 SI2 = 0.39	NA	NA
A-549	NA	IC50 = 58.7 (44.4-81.0) SI1 = 1.32 SI2 = 0.82	NA	NA
BT-20	NA	IC50 = 232.1 (162.1-380.1) SI1 = 0.33 SI2 = 0.21	NA	NA
Panc 02.03	NA	IC50 = 151.2 (126.8-193.5) SI1 = 0.51 SI2 = 0.32	NA	NA

Cell line	**G3/W**	**MESC-INO**	**YS4**	**G5/MUC**
Vero	IC50 = 440.1 (228.2-1336.2)	NT	NT	NT
84BR	NT N=3	-	-	-
BJ-5ta	NT	NT	NT	NT
H69V	IC50 = 359.2 (158.4-1519.7) SI = 1.23	NA	NA	IC50 = 97.6 (72.8-140.2) SI1, SI2 = ND
143B	NA	NA	NA	NA
C3A	NA	NA	NA	NA
A375	NA	NA	NA	NA
HT29	NA	NA	NA	NA
MCF7	NA	NA	NA	NA
AGS	NA	NA	NA	NA
Clone 15 HL-60	NA	NA	NA	NA
DU145	NA	NA	NA	NA

A-704	NA	NA	NA	NA
K-562 (LDH)	NA	NA	NA	NA
K-562 (MTT)	NA	NA	NA	NA
U-87 MG	NA	NA	NA	NA
A-549				
BT-20	NA	NA	NA	NA
Panc 02.03	NA	NA	NA	NA

Cell line	**G1/W**	**IN1**	**YS5**	**F7NN**
Vero	NT	IC50 = 39.8 (32.5-49.7)	NT	NT
84BR	-	-	-	-
BJ-5ta	NT	IC50 = 25.4 (22.4-28.6)	NT	NT
H69V	NA	IC50 = 26.1 (21.0-32.9) SI1 = 1.52 SI2 = 0.97	NA	IC50 = 655.3 (282.6-2792.4) SI=can't be reached
143B	NA	IC50 = 36.3 (23.6-53.5) SI1 = 1.10 SI2 =0.69	NA	NA
C3A	NA	IC50 = 27.2 (22.0-33.6) SI1 = 1.46 SI2 = 0.93	NA	NA
A375	NA	IC50 = 63.3 (24.4-209.8) SI1 = 0.63 SI2 =0.40	NA	NA
HT29	NA	IC50 = 99.6 (73.9-144.9) SI1 = 0.39 SI2 = 0.25	NA	NA
MCF7	NA	IC50 = 34.8 (28.6-42.9) SI1 = 1.14 SI2 = 0.73	NA	NA
AGS	NA	IC50 = 73.5 (58.0-97.6) SI1 = 0.54 SI2 = 0.34	NA	NA
Clone 15 HL-60	NA	NA	NA	NA
DU145	NA	IC50 = 64.2 (49.2-88.3) SI1 = 0.62 SI2 = 0.39	NA	NA
A-704	NA	IC50 = 159.1 (126.8-220.9) SI1 = 0.25 SI2 = 0.16	NA	NA

K-562 (LDH)	NA	NA	NA	NA
K-562 (MTT)	NA	NA	NA	NA
U-87 MG	NA	IC50 = 53.8 (46.2-63.5) SI1 = 0.74 SI2 = 0.47	NA	NA
A-549	NA	IC50 = 60.1 (46.7-80.6) SI1 = 0.66 SI2 = 0.42	NA	NA
BT-20	NA	IC50 = 75.1 (61.4-94.5) SI1 = 0.53 SI2 = 0.34	NA	NA
Panc 02.03	NA	IC50 = 49.7 (37.4-68.9) SI1 = 0.80 SI2 = 0.51	NA	NA

Discussion on selectivity index:

Please note: To calculate an IC_{50} value, a minimum of 2 concentrations above and 2 concentrations below 50% cell viability must be present. For some compounds, the IC_{50} concentration exceeded the maximum concentration tested (200 µg/ml), and is therefore not a significant value and only an extrapolation.

The selectivity criteria used was based on the previous work, whereby an SI value higher than 2 was considered as highly selective (Prayong et al., 2008 & Machana et. al., 2011).

From the SI values presented in Table 6.1, YS2 is the compound that showed a promising anticancer effect against H69V and C3A cell lines with an SI >2. YS2 has some potency against K-562.

Although not as highly selective, RBME, RBAC and IN1 had potency and some selectivity against most cell lines. IN1 had most notable potency and selectivity against the H69V and HepG2/C3A cell lines.

RBET has potency and some selectivity against 143B and DU145 cell lines. RBMW has potency and some selectivity against H69V and A-704 cell lines. G5/MUC has potency and some selectivity against H69V cell line.

9. References

Ahn JM, Kim SJ, Kim H, Park C, Kim WH, Park JH. Triton X-100 induces apoptosis in human hepatoma cell lines, 1997. *Yonsei Med J*, 38(1):52-9.

Kaja S, Payne AJ, Naumchuk Y, Koulen P. Quantification of lactate dehydrogenase for cell viability testing using cell lines and primary cultured astrocytes, 2017. *Curr Protoc Toxicol*. 72:2.26.1-2.26.10.

Machana S, Weerapreeyakul N, Barusrux S, Nonpunya A, Sripanidkulchai B, Thitimetharoch T. Cytotoxic and apoptotic effects of six herbal plants against the human hepatocarcinoma (HepG2) cell line. *Chin Med.* 2011;**6**(1):39.

Mosmann T. Rapid colorimetric assay for cellular growth and survival: application to proliferation and cytotoxicity assays, 1983. *J Immunol Methods*. 16;65(1-2):55-63.

Prayong P, Barusrux S, Weerapreeyakul N. Cytotoxic activity screening of some indigenous Thai plants. *Fitoterapia.* 2008;**79**(7-8):598–601.

9. Report compilation

This report was compiled by Dr Hanna Svitina (PhD) and Dr Clarissa Willers (PhD), and approved by Prof Chrisna Gouws (PhD.; Pr.Sci.Nat.)

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