The Anticancer Effect of Two Unique Proteasome Inhibitors in Breast Cancer Cells

Hatice Özkan [1], Vildan Betül Yenigün [2]

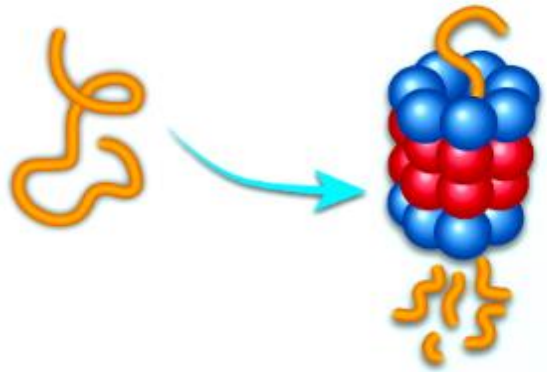
[1] Bezmialem Vakif University, Faculty of Medicine

[2] Bezmialem Vakif University, Faculty of Medicine, Department of Medical Biochemistry

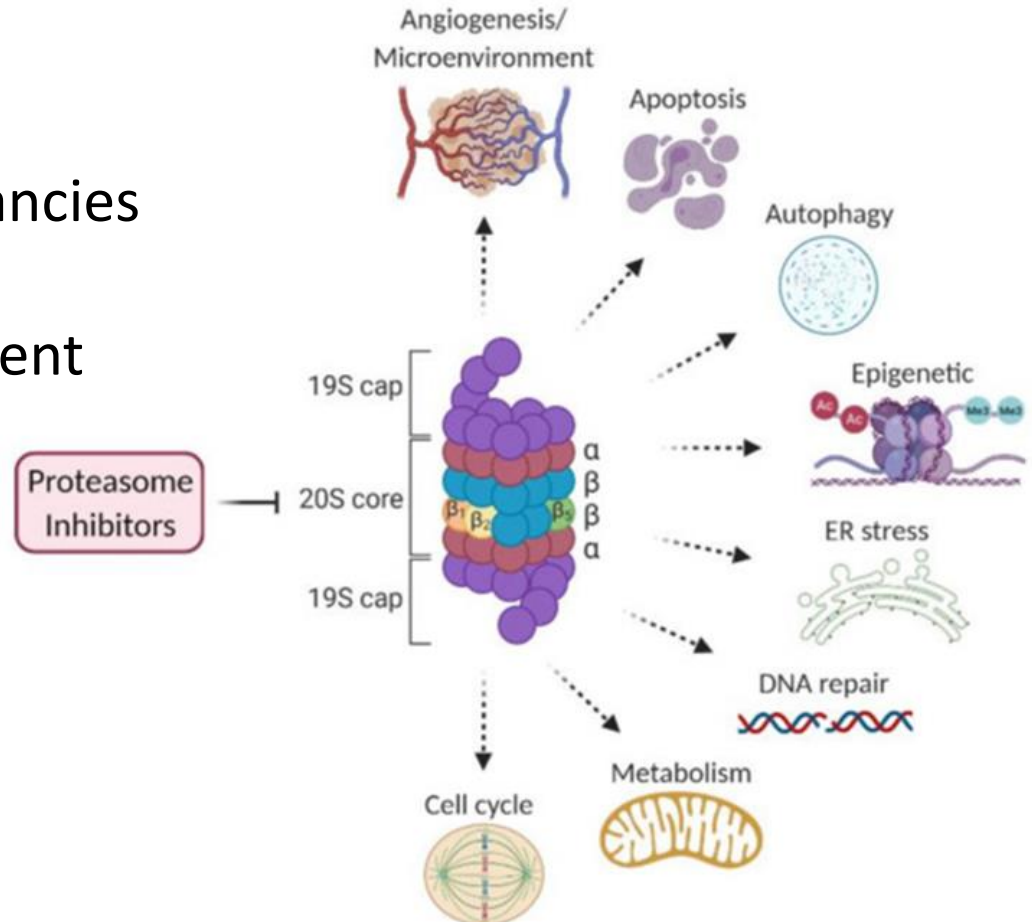
Plan

- Proteasome System
- Aim of Research
- Method
- Results
- Conclusion

What is Proteasome?

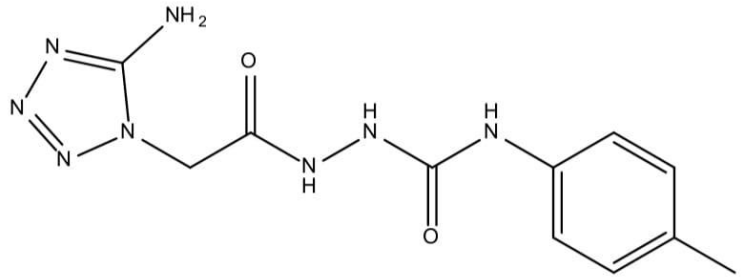


- degrades up to %80 of cellular proteins
- regulates molecular reactions
- abnormal proteasome activity common in malignancies
- Proteasome inhibition is a target for cancer treatment



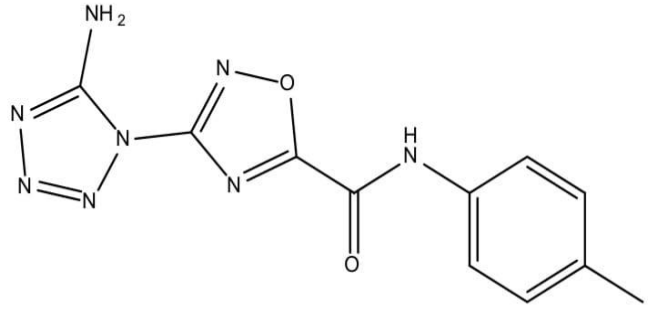
Aim

- The aim of this study is to investigate the anticancer activity of two newly synthesized, unique proteasome inhibitors on breast cancer cells.



2-(2-(5-Amino-***1H***-tetrazol-1-il)asetil)-N-(p-tolil)hidrazin-1-karboksamit

tetrazole



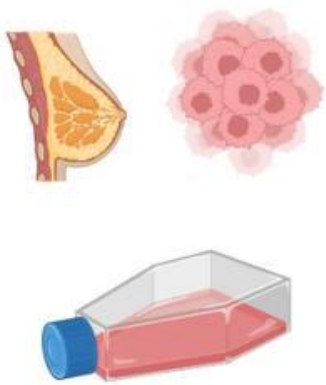
3-(5-Amino-1H-tetrazol-1-il)-N-(p-tolil)-1,2,4-oksadiazol-5-karboksamit

tetrazole + oxadiazole

- Molecules were synthesized by Assoc. Prof. Dr. Hamdi Özkan at Gazi University, Faculty of Science, Department of Chemistry.

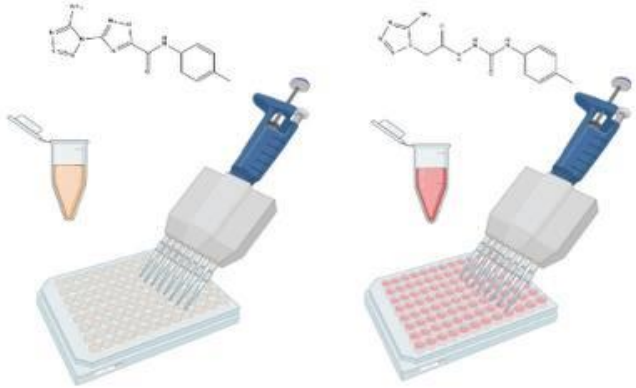
METHOD

Cell culture

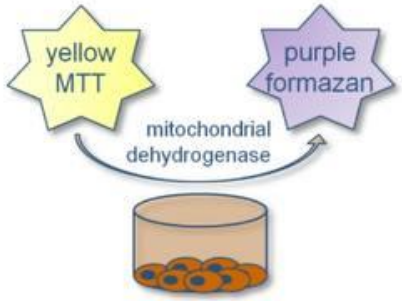


MCF-7 ER+ Breast cancer cell line

Cytotoxicity

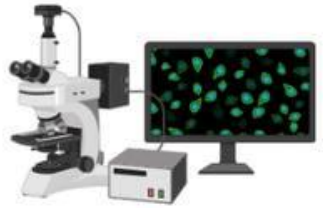


IC50 value and the most effective time determination



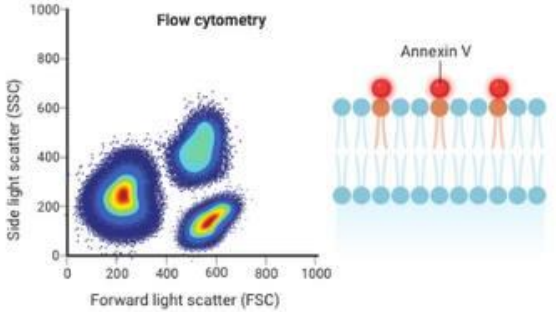
MTT cell viability asssay

Apoptosis



AO/EB double staining

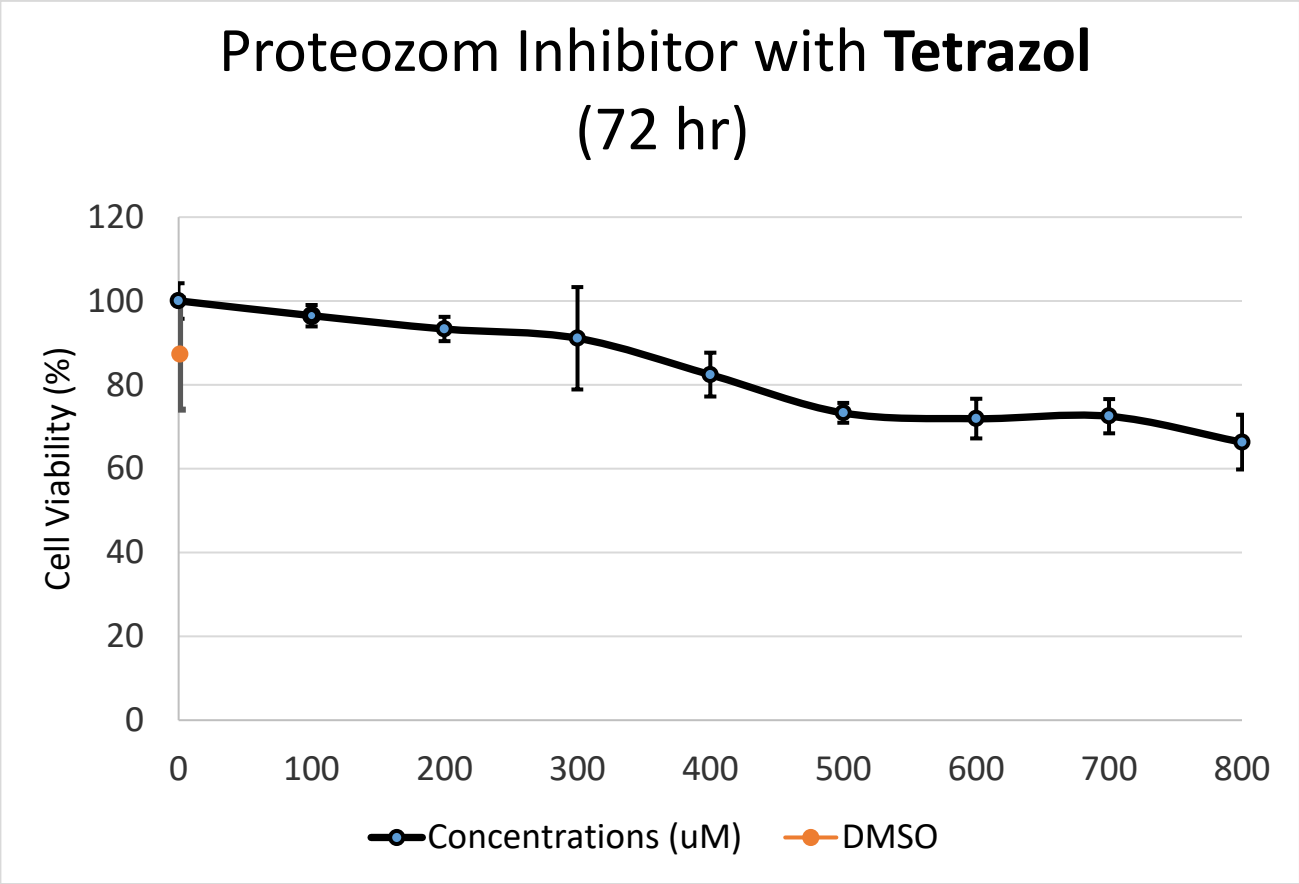
Immunofluorescence microscopy

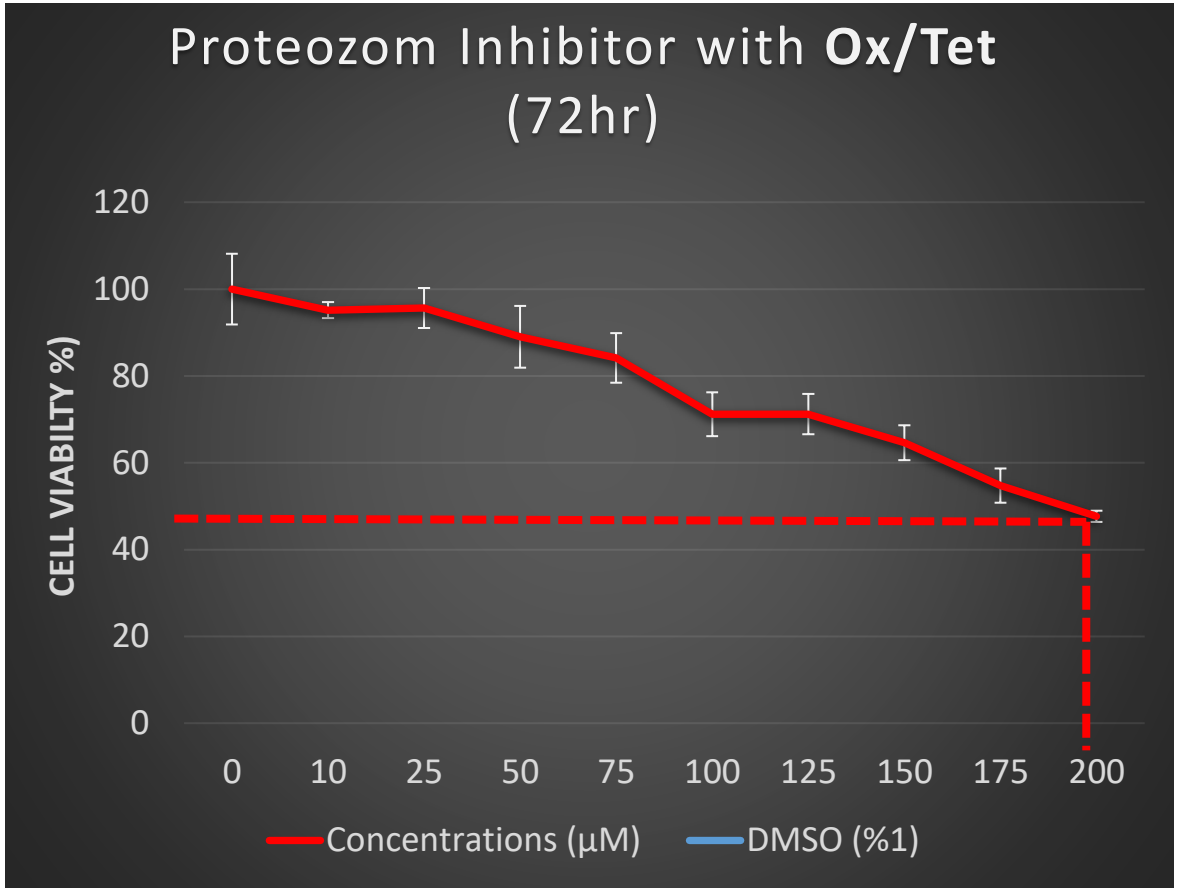


Annexin V/PI staining

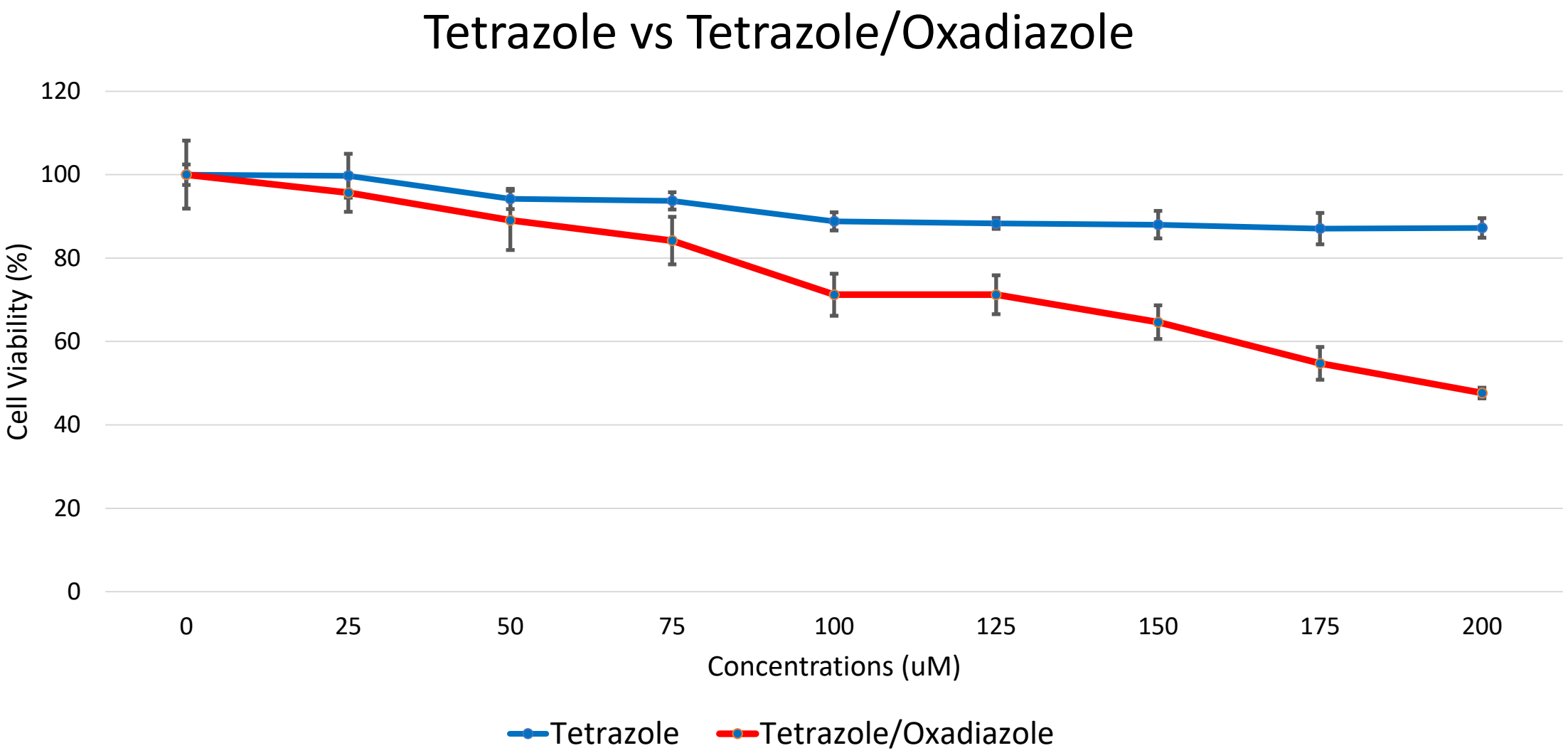
Flow Cytometry

RESULTS

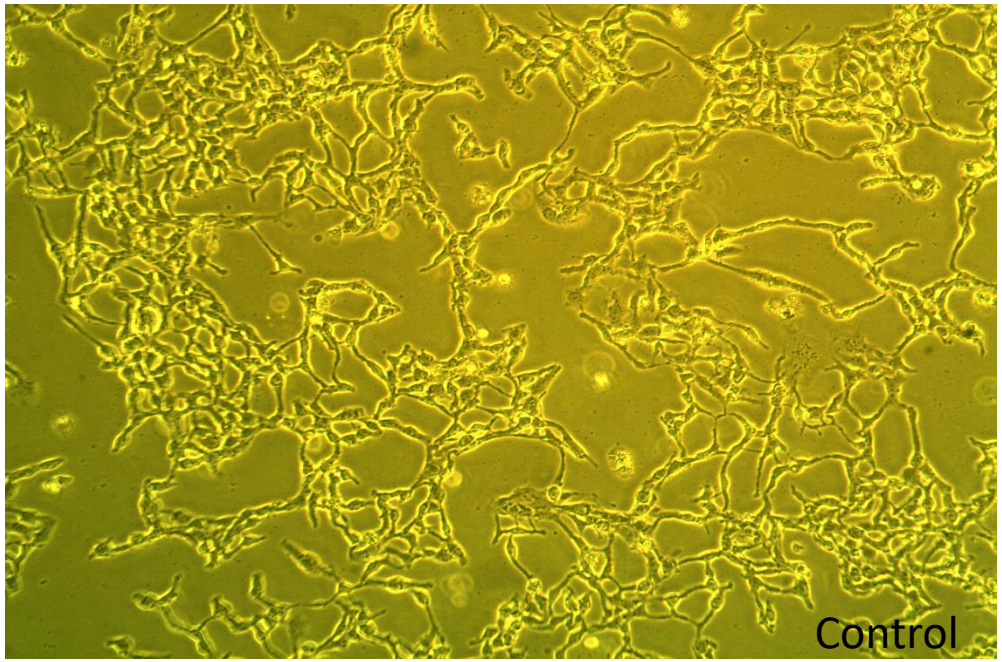


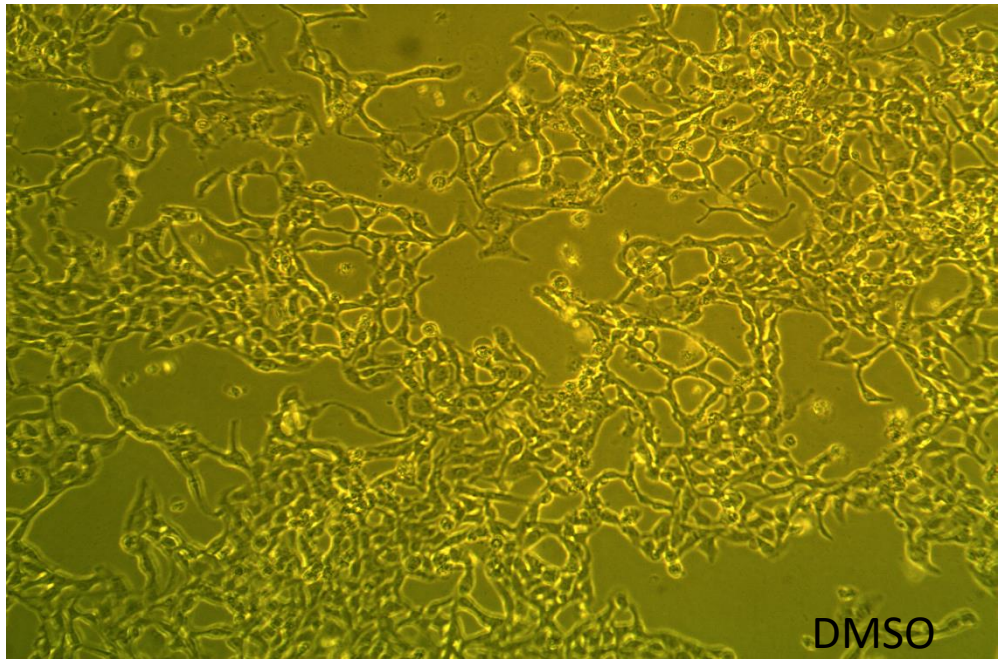


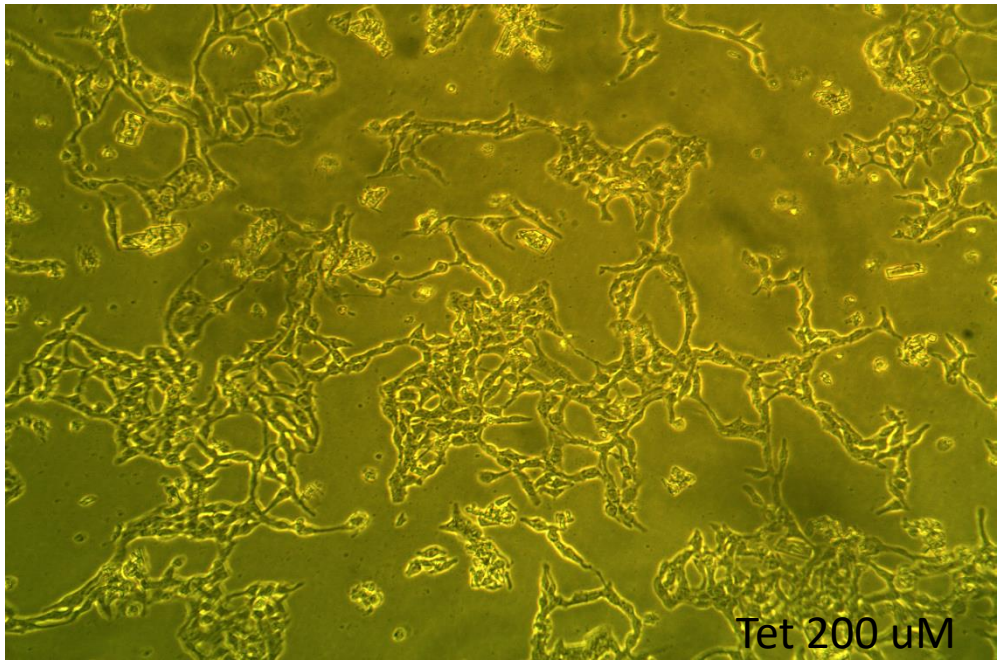
- IC50 value of the molecule containing tetrazole and oxadiazole together as 200µM.
- could not calculate the IC50 value of the molecule containing only tetrazole even at the max concentration of 800 µM, cell viability was 60%.

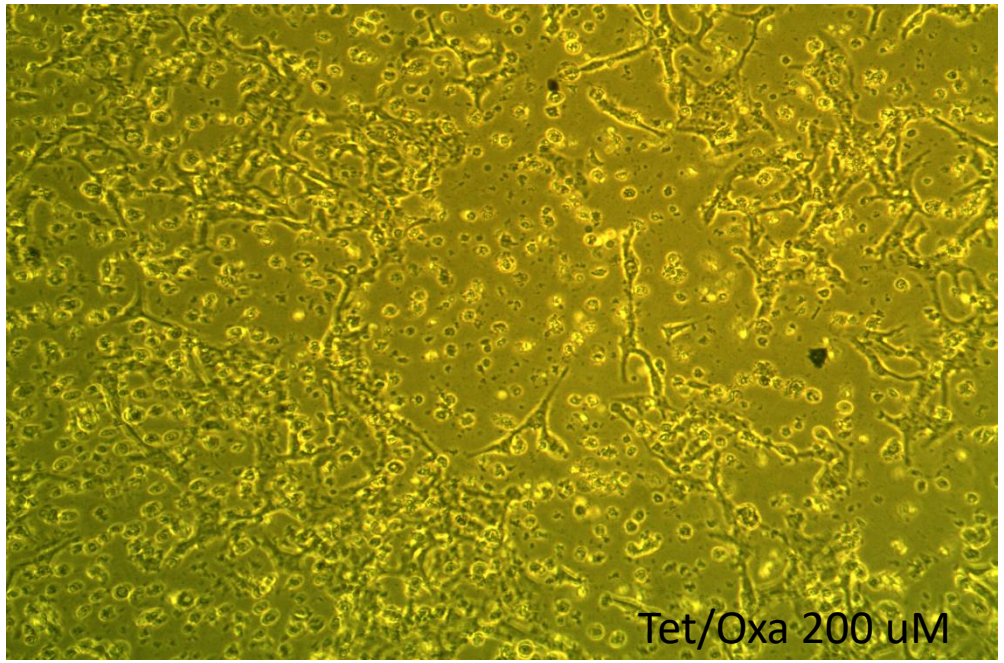


While the cell viability rate of the tetrazole/oxadiazole combination molecule was 50% at 200 µM resolution, the molecule that only contains tetrosal was at 15%.

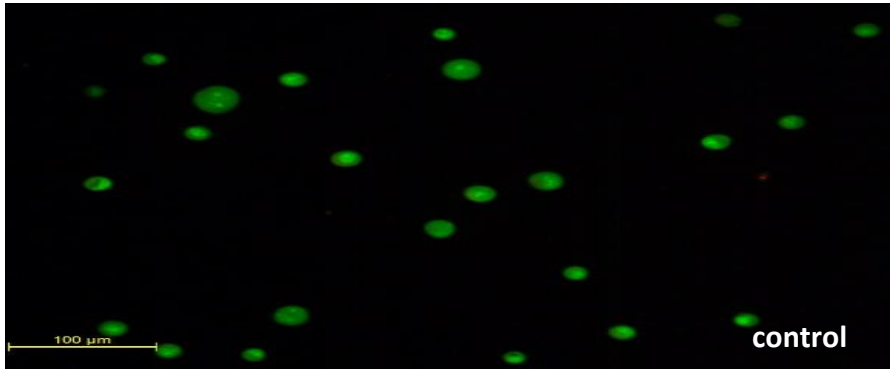

Control

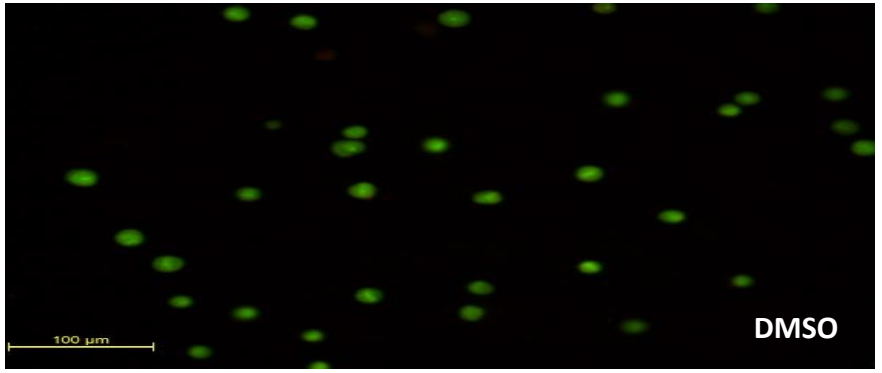

DMSO

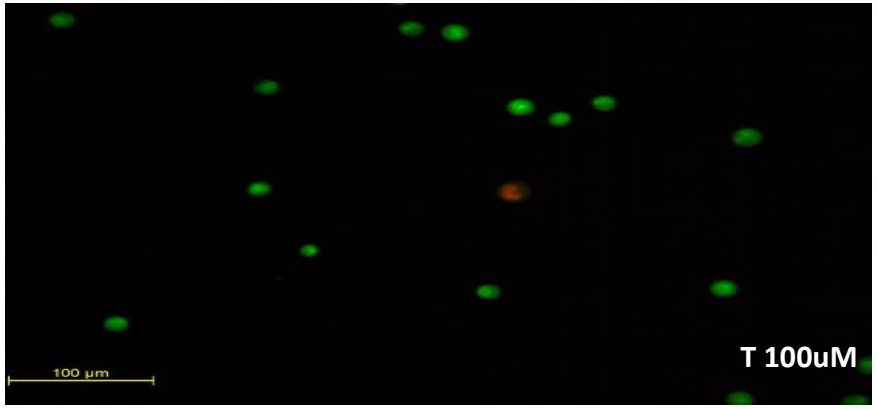

Tet 200 uM

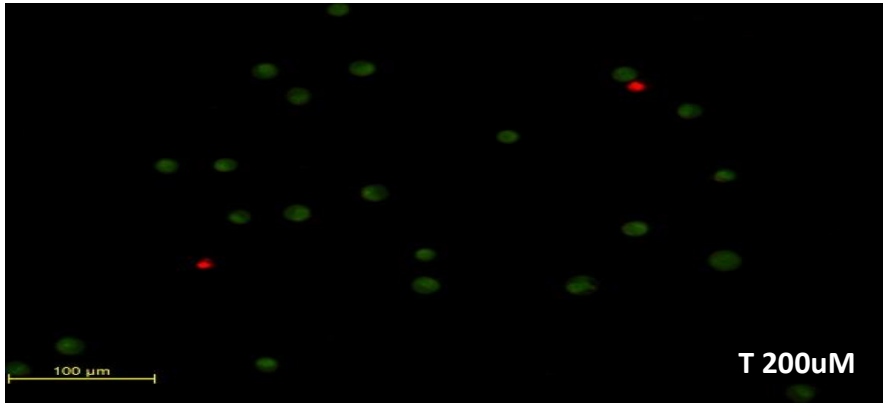

Tet/Oxa 200 uM

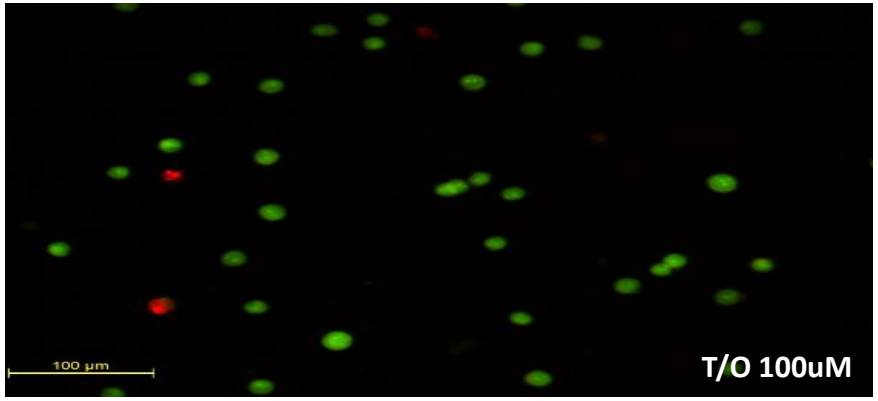
Acridine Orange / Etidium Bromide Double Staining

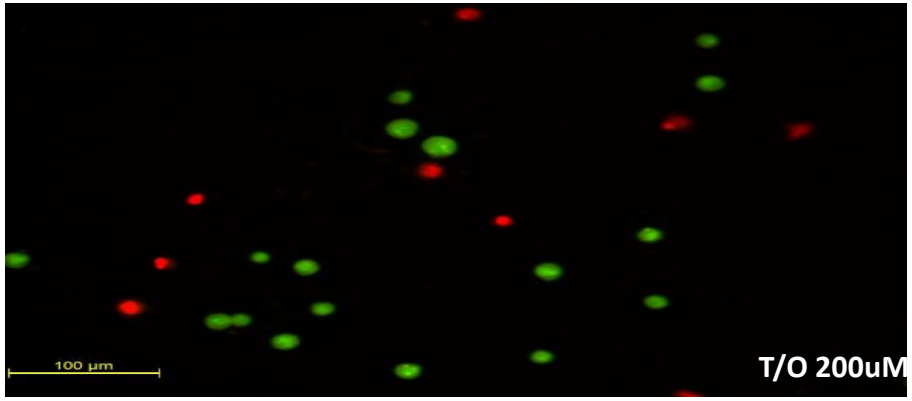


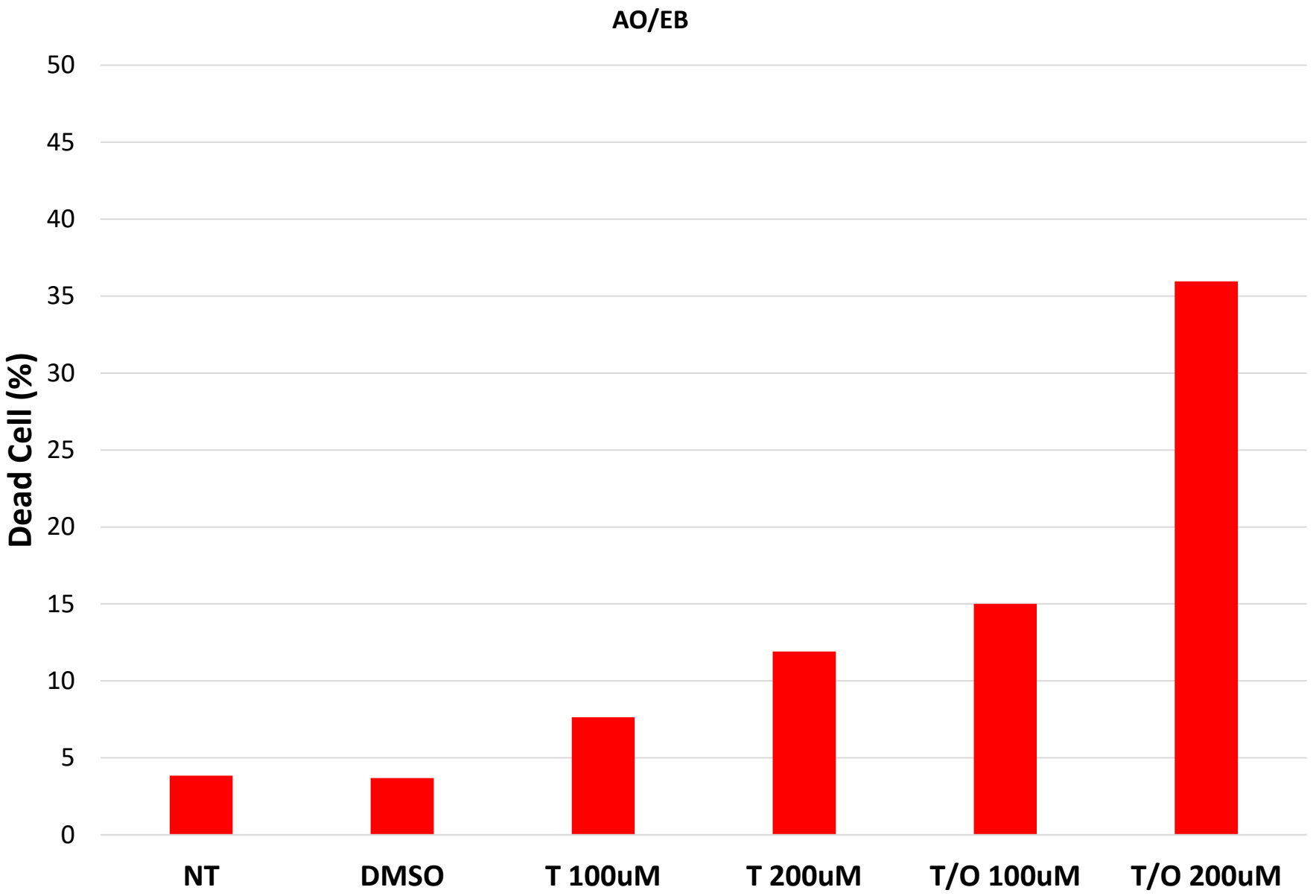








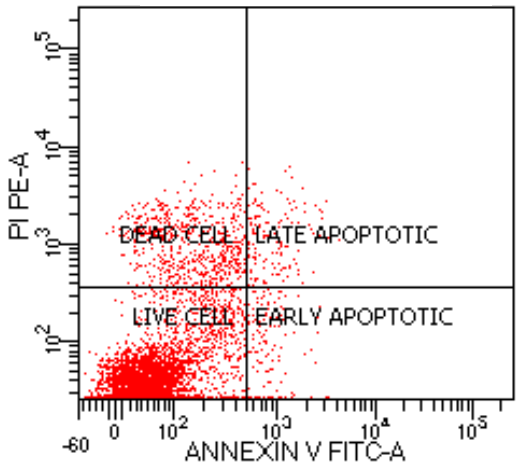



AO/EB
Dead Cell (%)
50
45
40
35
30
25
20
15
10
5
0
NT
DMSO
T 100uM
T 200uM
T/O 100uM
T/O 200uM

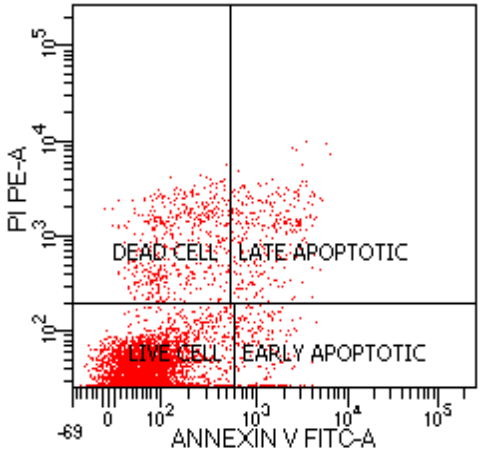
Annexin V/PI Staining

- detects necrotic or late apoptotic cells by the loss of the integrity of the plasma, and nuclear

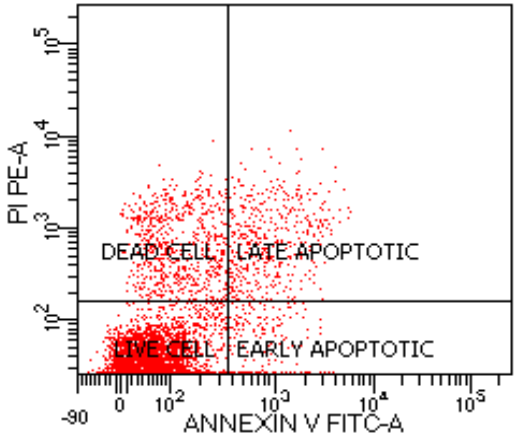
Control



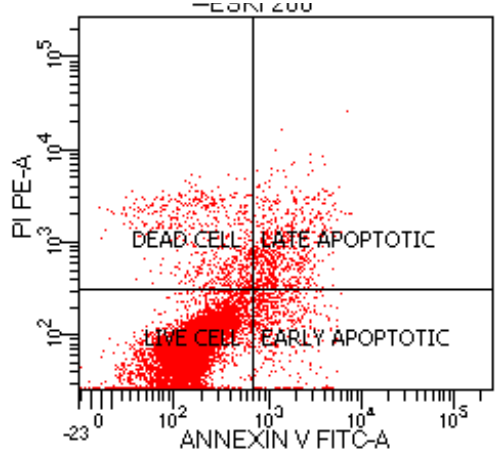
DMSO



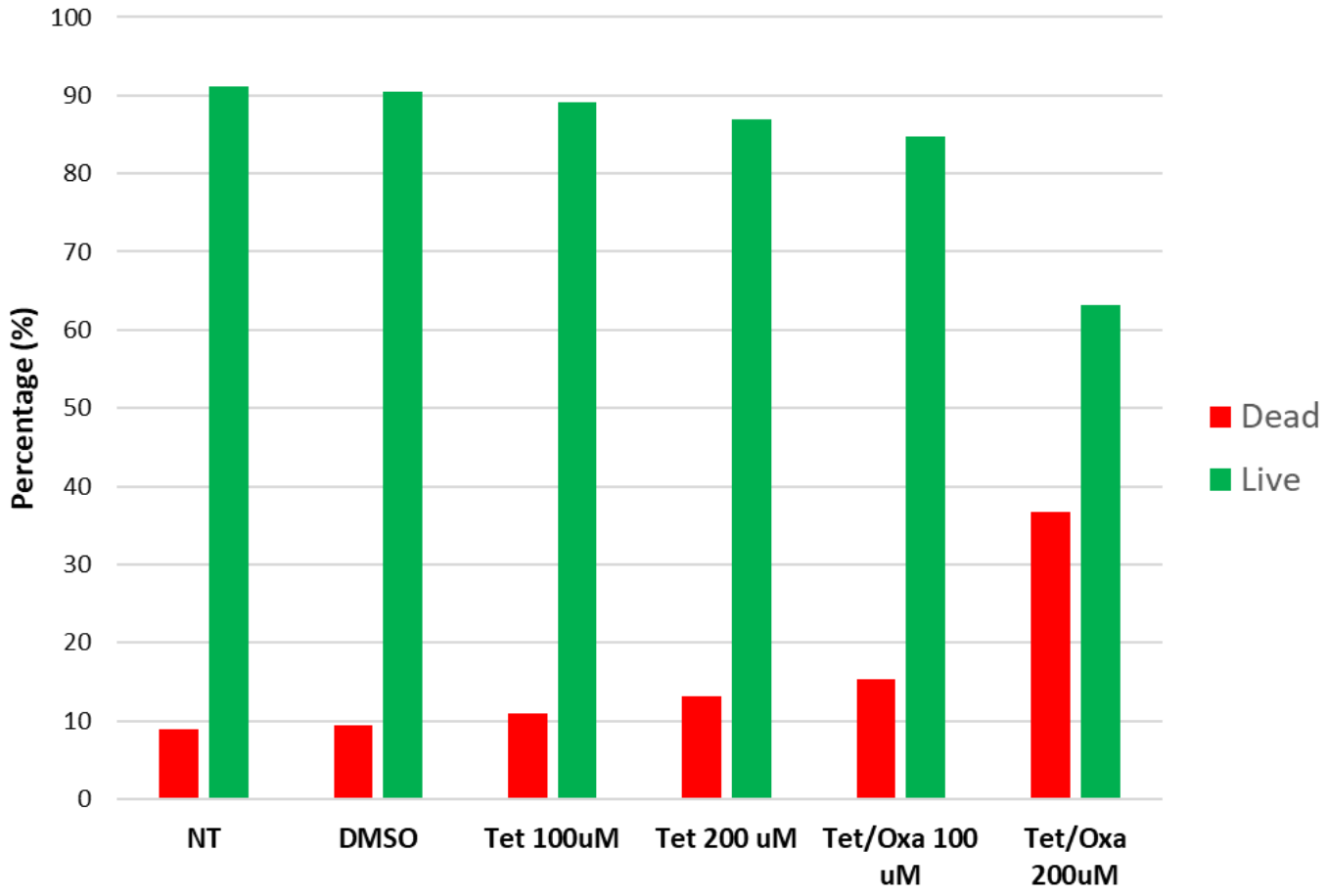
Tetrazole



Tetrazole+oxadiazole



Annexin V/PI Staining



Conclusion

- The results of this study showed that proteasome inhibitor containing a tetrazole ring have some anti-cancer effect, but the addition of an oxadiazole ring to the molecule leads to an increase in cell death caused by the inhibitor.

Thank you for listening,

Do you have any questions?