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# Development of L-γ-Methyleneglutamine-based Compounds for Cancer

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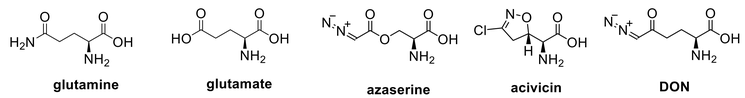
## Cancer metabolism

- In cancer cells, glutaminolysis is the primary source of biosynthetic precursors, fueling the TCA cycle with glutamine-derived α-ketoglutarate.
- α-ketoglutarate provides carbons for the citric acid cycle to produce glutathione, fatty acids, and nucleotides.
- It also contributes nitrogen to produce hexosamines, nucleotides, and many nonessential amino acids.
- Efforts to inhibit glutamine metabolism in cancer using amino acid analogs have been extensive.

## Glutamine analogues

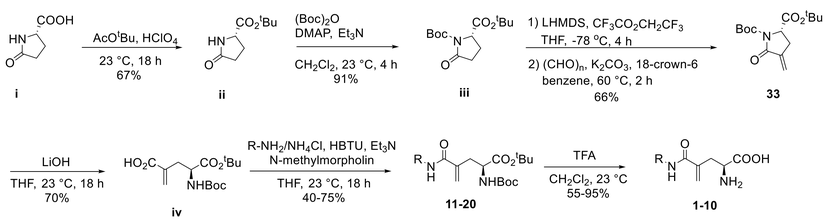
- There are a number of naturally occurring glutamine analogues, such as azaserine, acivicin, and 6-diazo-5-oxo-L-norleucine (DON).
- These inhibit different glutamine- dependent enzymes including glutaminase, NAD synthase, CTP synthetase, FGAR aminotransferase.
- Therefore, they demonstrated variable degrees of gastrointestinal toxicity, myelosuppression, and neurotoxicity, due to their non-selectivity.



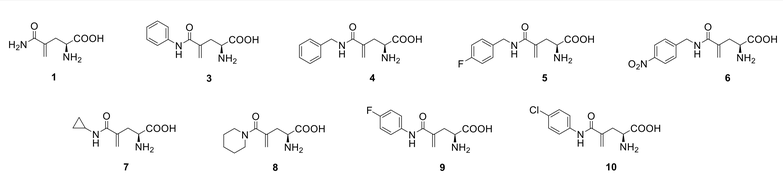

## L-γ-methyleneglutamate

- L-γ-Methyleneglutamine (**1**) were first isolated from groundnut seedling (Arachis hypogaea) in 1952.
- It plays a major role in nitrogen transport in Arachis and Amorpha plants.
- The syntheses of racemic mixture of **1** were reported in 1955. However, no efficient synthetic route was reported before our work.

## General Synthetic scheme

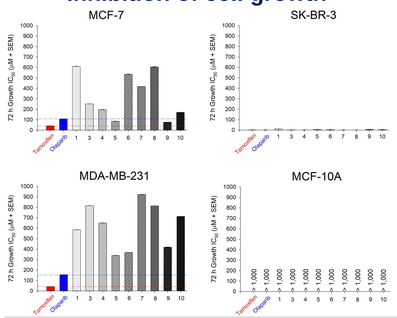



## L-γ-methyleneglutamate analogues

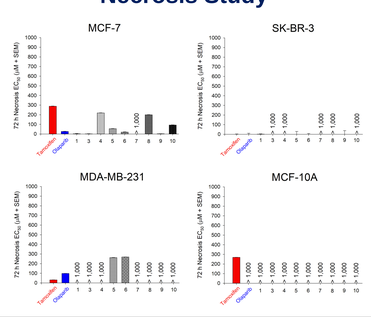



## Results

### Inhibition of cell growth




### Necrosis Study



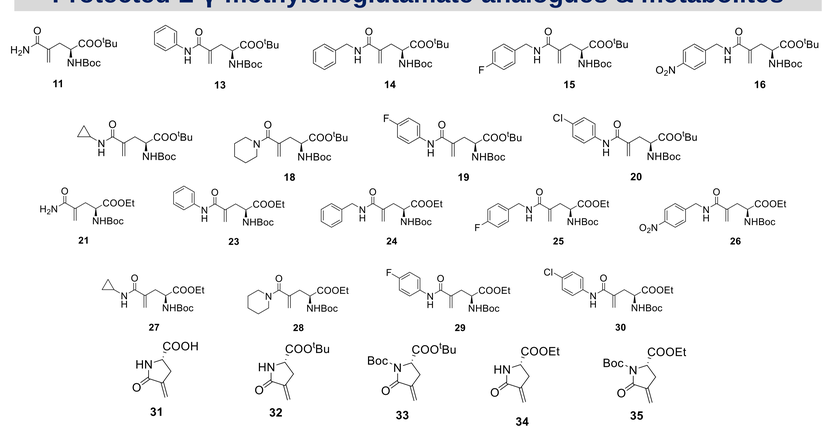

## Discussion

- Amides with primary amine or aromatic amine are more potent in inhibiting the cancer cells growth than with secondary amine or branched alkyl amine.
- Within each subset, the amines with a stronger electron withdrawing group exhibit better potency.
- N-benzyl amides with an electron withdrawing group at the para position were the only compounds to inhibit the growth of triple-negative MDA-MB-231 cells commensurate to olaparib.

## Current work

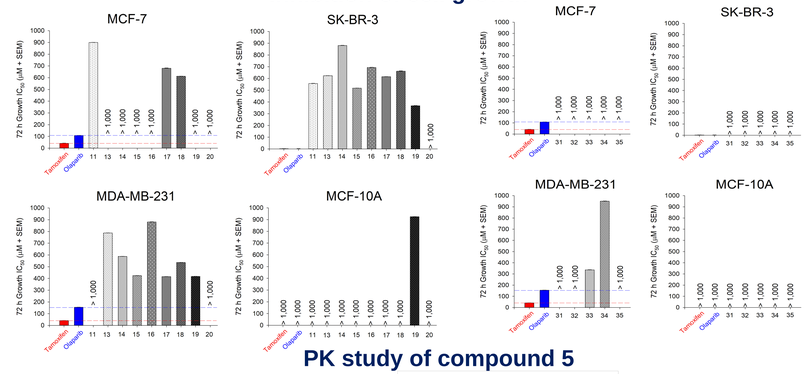
- We will assay the anticancer activity of protected L-γ-Methyleneglutamine analogues against MCF-7, SK-BR-3, MDA-MB-231 cell lines.
- For most potent compound **5**, we will do the PK/PD study.
- We will also investigate the anticancer activity of both metabolite and protected metabolites of L-γ-Methyleneglutamine analogues.
- For selected compounds from **1**-**10**, we will study anticancer activity against brain, and head and neck cancer.

### Protected L-γ-methyleneglutamate analogues & metabolites

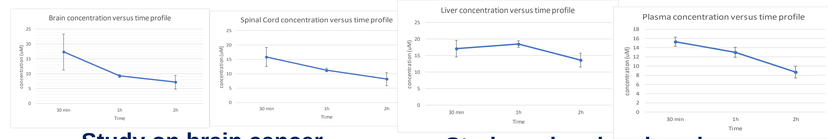



## Results

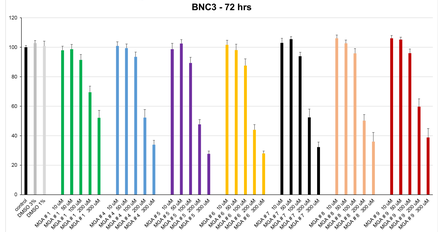
### Inhibition of cell growth



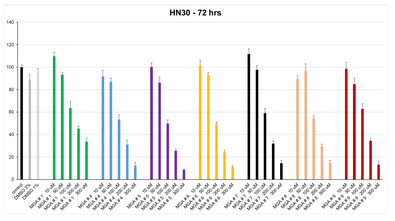

### PK study of compound 5




### Study on brain cancer




### Study on head and neck cancer




## Discussion

- Compare to compound **5** and **6**, protected L-γ-methyleneglutamate analogues, compound **11**-**30** did not show promising activity in MCF-7, SK-BR-3, MDA-MB-231 cell lines.
- Both metabolites and protected metabolites, compound **31**-**35** also did not show promising activity. However, compound **33** showed better activity in MDA-MB-231 than others.
- PK study showed the good distribution of compound **5** in brain after 30 minutes.
- Study of compound **1**,**4**-**9** in brain, and head and neck cancer cell lines showed the concentration dependent inhibition of cell growth of these cancer cells.

## Conclusion & Future Plan

- Compound **5** and **6,** compare to other analougues showed most potent in different cancer cell lines whereas, every analogues is non toxic to MCF-10.
- We will do further study in zebra fish animal model to investigate binding target.
- We will also assay these two compounds in different mitochondrial targets.

References: Hensley et al, J. Clin. Invest. **2013**, 123(9),3678-3684; Shapiro et al, J. Biol. Chem. **1979**,254(8),2835-2838; Wailes et al, Nature, **1954**,174(4420),130-131; Done et al, BioChem. J. **1952**,51(4),451-458; Hossain et al, RSC Adv.,**2021**,11,7115.