Development of *L*-γ-Methyleneglutamine-Based Compounds for Cancer

Md Imdadul H. Khan,[1] Md Imran Hossain,[1] Nicholas S. Akins,[1] Fakhri Mahdi,[1] Patrice Penfornis,[2] Pier Paolo Claudio,[1,2] Jason J. Paris,[1] Hoang V. Le,[1,*]

[1] Department of BioMolecular Sciences and Research Institute of Pharmaceutical Sciences, School of Pharmacy, University of Mississippi, University, MS 38677, U.S.A.

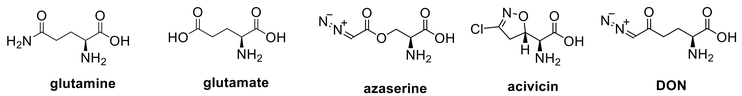
[2] University of Mississippi Medical Center, Jackson, MS 39216 U.S.A.

Cancer Cell 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.
- α-Ketoglutarate also contributes nitrogen to produce hexosamines, nucleotides, and many nonessential amino acids.
- Efforts to inhibit glutamine metabolism in cancer using amino acid analogues have been extensive.

Glutamine Analogues

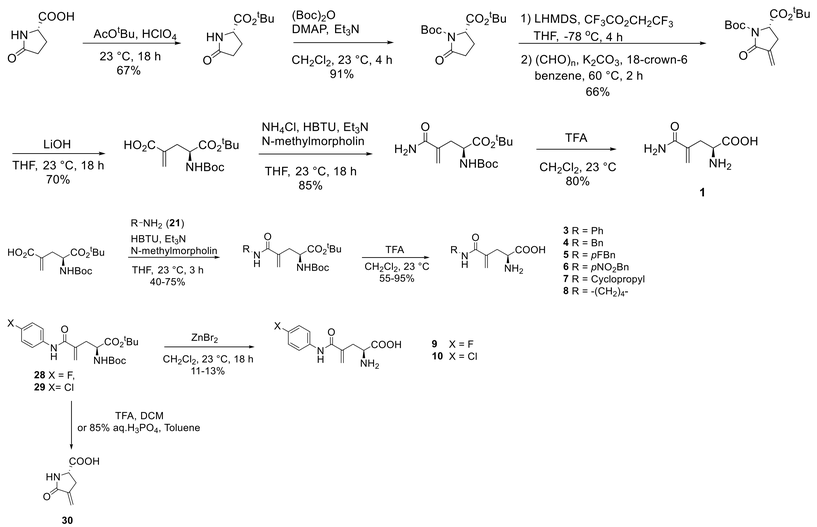
- There are a number of naturally occurring glutamine analogues, such as azaserine, acivicin, and 6-diazo-5-oxo-*L*-norleucine (DON).
- These inhibit different glutamine-dependent enzymes including glutaminase, NAD synthase, CTP synthetase, and FGAR aminotransferase.
- Therefore, they demonstrated variable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



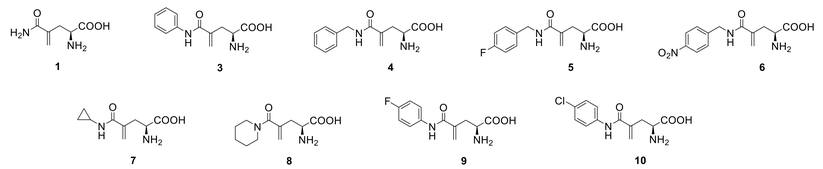
L-γ-Methyleneglutamine

- *L*-γ-Methyleneglutamine (**1**) was first isolated from groundnut seedling (*Arachis hypogaea*) in 1952, also later found in tulip bulbs.
- It plays a major role in nitrogen transport in *Arachis* and *Amorpha* plants.
- The synthesis of racemic mixture of **1** was reported in 1955. However, no synthesis of the biological relevant isomer *L*-γ-methyleneglutamine **1**, nor further research on its biological activity has been reported.

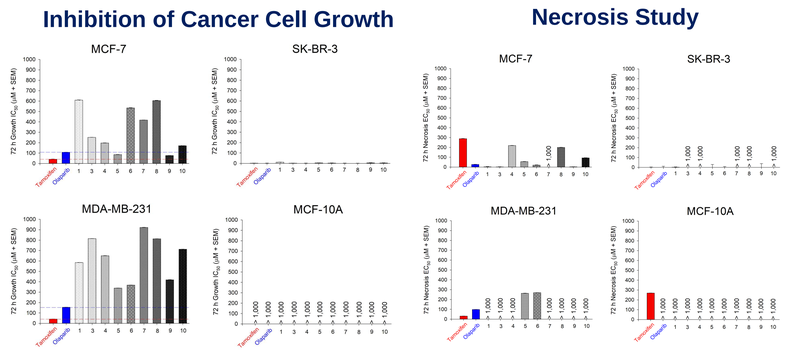
Synthesis of 1 and Its Amide Analogues



L-γ-Methyleneglutamic acid Amides



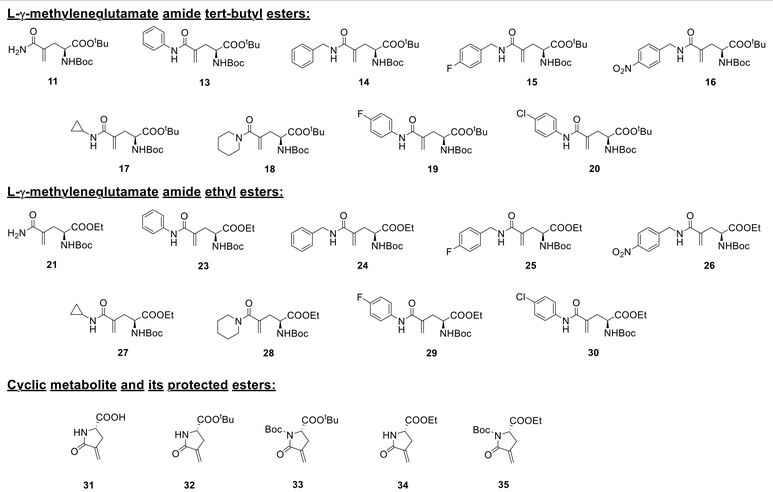
Inhibition of Breast Cancer Cell Growth



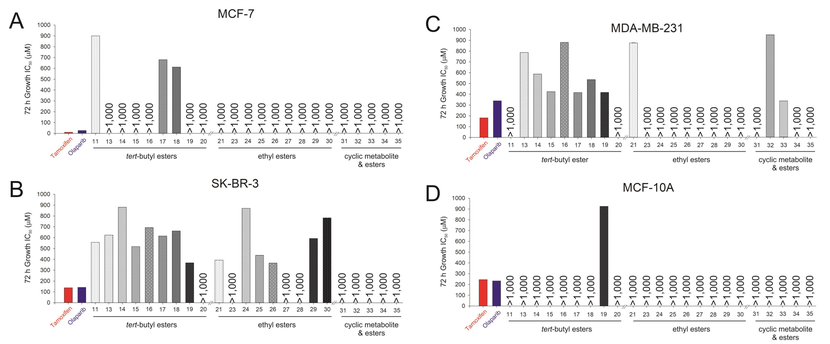
Structure–Activity Relationships

- Amides with primary amine or aromatic amine are more potent in inhibiting cancer cell 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.

Protected *L*-γ-Methyleneglutamic Acid Amides



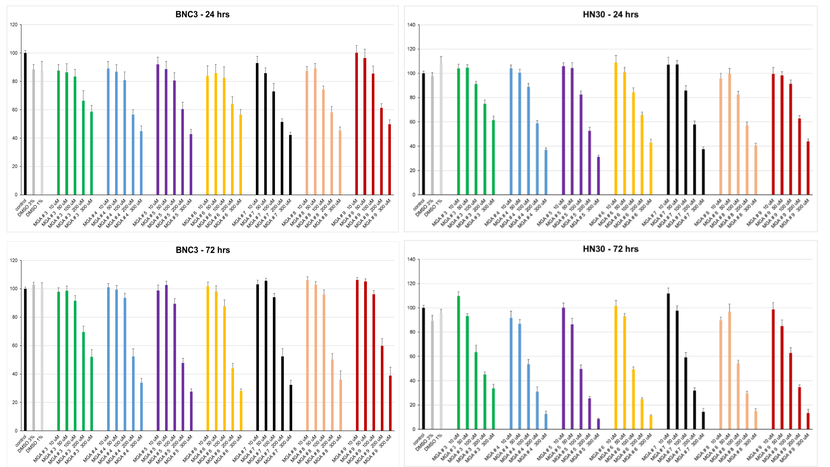
Inhibition of Breast Cancer Cell Growth



Pharmacokinetics Studies of Compound 5

	Routes	$t_{1/2}$ (h)	C_{max} (ng/mL)	T_{max} (h)	$AUC_{0-\alpha}$ (ng/mL*h)
Plasma	Intravenous	0.83	4,830	0.083	1,665
	Intraperitoneal	0.40	1,236	0.083	968
Brain	Intraperitoneal	0.71	31	0.5	44.22
Kidney	Intraperitoneal	0.45	17,681	0.5	21,940
Liver	Intraperitoneal	0.42	7,020	0.5	8,139

Inhibition of Brain Cancer Cell and Head and Neck Cancer Cell Growth



Conclusions

- Amides seem to be the active form, rather than their metabolite.
- Good distribution to the brain with $t_{1/2}$ of compound **5** = 0.71 h
- Exerted concentration-dependent inhibition of growth of brain cancer cells and head & neck cancer cells
- Investigation of the biological targets and studies in animal models are being planned.

References: **1)** Newsholme, P.; Procopio, J.; Lima, M. M. R.; Pithon-Curi, T. C.; Curi, R. *Cell Biochem. Funct.* **2003**, *21* (1), 1–9. **2)** Akins, N. S.; Nielson, T. C.; Le, H. V. *Curr. Top. Med. Chem.* **2018**, *18* (6), 494–504. **3)** Shapiro, R. A.; Clark, V. M.; Curthoys, N. P. *J. Biol. Chem.* **1979**, *254* (8), 2835–2838. **4)** Barclay, R. K.; Phillipps, M. A. *Cancer Res.* **1966**, *26* (2), 282–286. **5)** Wailes, P.; Whitting, M. C.; Fowden, L. *Nature* **1954**, *174* (4420), 130–131. **6)** Done, J.; Fowden, L. *Biochem. J.* **1952**, *51* (4), 451–458. **7)** Hossain, M. I.; Thomas, A. G.; Mahdi, F.; Adam, A. T.; Akins, N. S.; Woodard, M. M.; Paris, J. J.; Slusher, B. S.; Le, H. V. *RSC Adv.* **2021**, *11*, 7115–7128. **8)** Khan, M. I. H.; Mahdi, F.; Penfornis, P.; Akins, N. S.; Hossain, M. I.; Kim, S. J.; Sulochana, S. P.; Adam, A. T.; Tran, T. D.; Tan, C.; Claudio, P. P.; Paris, J. J.; Le, H. V. *Bioorg. Med. Chem.* **2022**, under review. Preprint at *ChemRxiv* **2022**, doi: 10.26434/chemrxiv-2022-rhjcx.