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FRONTIERS IN LIFE SCIENCE

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# Pro-GA <Cell-permeable GGCT inhibitor>

Research use only. Not for use in human.

This product has been commercialized with the support of Kyoto Pharmaceutical University.

## Product Background

**γ-G**lutamyl**c**yclo**t**ransferase (GGCT) catalyzes the formation of 5-oxoproline and free amino acids from γ-glutamyl dipeptides for maintaining glutathione homeostasis. Although the enzymatic activity of GGCT was firstly reported in 1956, the gene of GGCT had not been identified for many years. In 2008, a biochemical study revealed C7orf24, a functionally unknown and highly expressed protein in bladder cancer cells, shows GGCT activity (ref.1, 2). Many studies show GGCT is highly expressed in various cancer cells as compared with normal tissues. Overexpression of GGCT promotes cell proliferation in NIH-3T3 with low-level GGCT expression and depletion of GGCT mRNA by RNA interference inhibits cell-growth in several types of cancer cell lines which show high expression of GGCT. A potent inhibitor of GGCT like RNAi methods will be powerful tool to investigate the physiological function of GGCT. *N*-Glutaryl-*L*-alanine (GA) is one of the potent inhibitor for GGCT activity, but GA has little membrane-permeability. Pro-GA is a novel diester-type cell-permeable GGCT inhibitor pro-drug and shows the inhibitory effects for proliferation of multiple malignant cancer cells *in vitro* and tumor growth *in vivo* (Fig.2, ref.3).

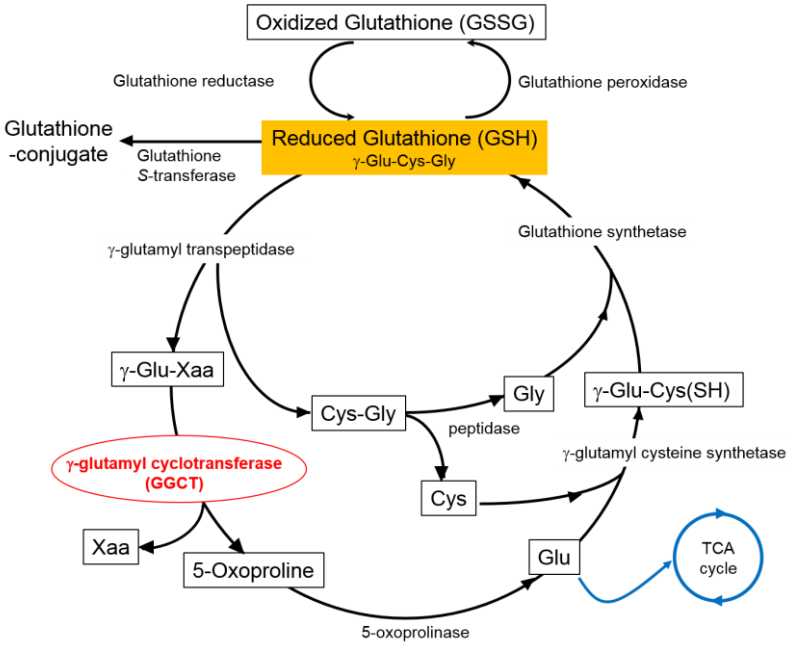



**Figure 1. Overview of glutathione metabolic cycle**

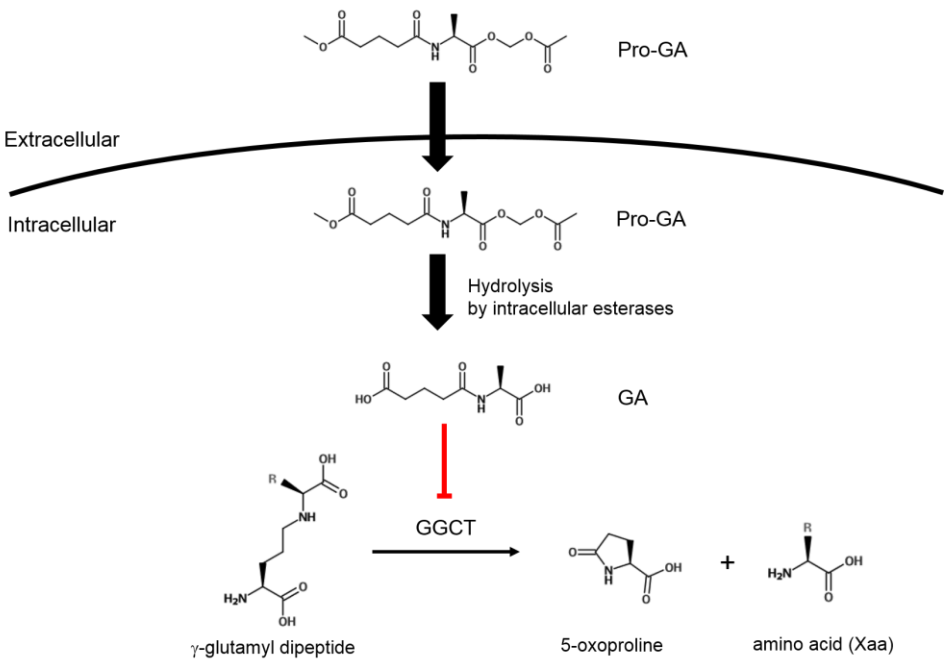



**Figure 2. Principle of pro-drug type inhibitor Pro-GA for GGCT**

## Description

Catalog Number: FDV-0019
Size : 2 mg
Formulation : $C_{12}H_{19}O_7N$
Molecular weight : 289.28 g/mol
Solubility : Soluble in DMSO

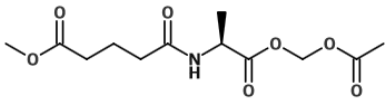


**Figure 3. Chemical structure of Pro-GA**

## Reconstitution and Storage

Reconstitution : 0.2-1 M stock solution in 100% DMSO.
Storage (solution) :
Store powder at -20ºC.
After reconstitution in DMSO, aliquot and store at -20 °C. Avoid repeated freeze-thaw cycles.

## How to use

### General procedure of cell-based inhibition of GGCT by Pro-GA

<Before Use>

pH of assay medium is very important to avoid non-enzymatic hydrolysis of Pro-GA. Because both acidic and basic conditions can induce hydrolysis of Pro-GA, pH should be neutral. As the assay medium, DMEM containing 25 mM HEPES (DMEM/HEPES) is strongly recommended.

1. Prepare 100 μM of Pro-GA in DMEM/HEPES just before use.

Note: Pro-GA is not stable in DMEM/HEPES for long time.
**Please use Pro-GA in DMEM/HEPES <u>within 5 min</u>.**

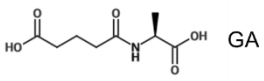
2. Exchange culture medium to the Pro-GA in DMEM/HEPES and culture cells for 1-120 hours.

Note: Pro-GA is immediately penetrated into cells and converted to GA inside the cells by esterases. Regarding detail information, please find “GA-release efficiency of Pro-GA in cells” below.

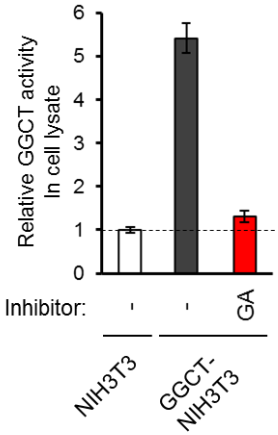
## Application data

### GGCT inhibition activity of parent compound GA *in vitro*

GGCT activities in cell lysates of NIH3T3 or GGCT-overexpressed NIH3T3 were measured by fluorescent substrate LISA-101. 100 μM GA strongly inhibited endogenously expressed GGCT activity.



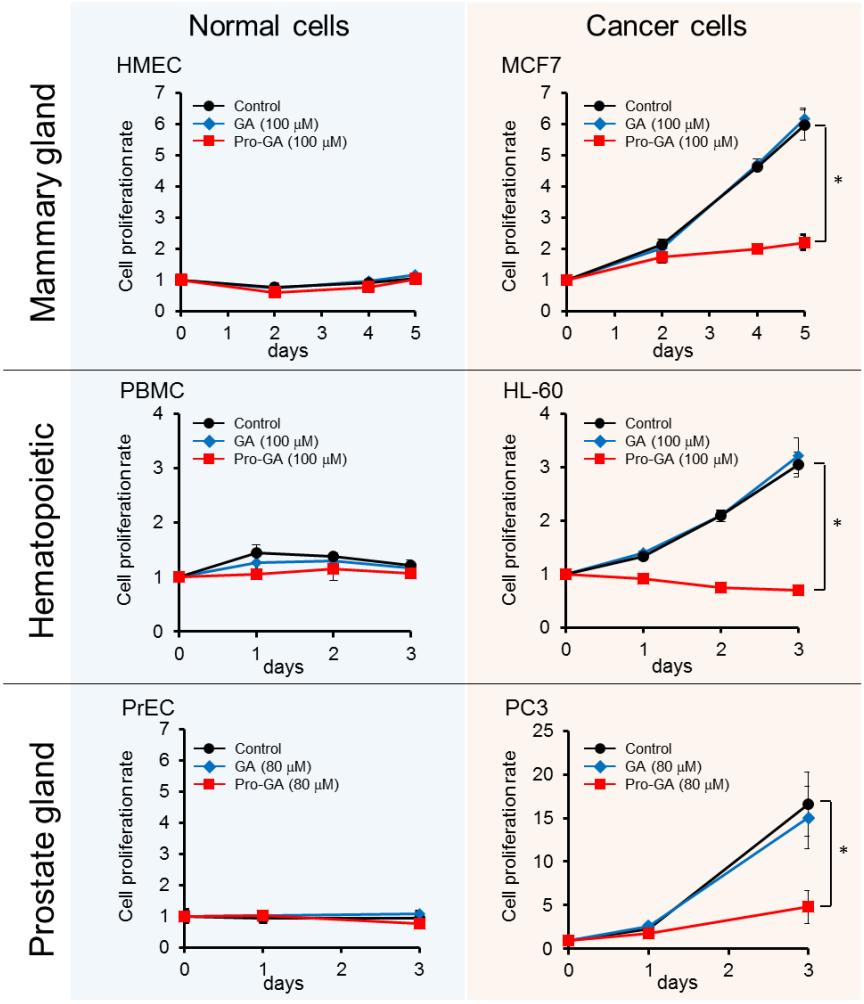

GA




Note: This data is only for reference of an inhibition activity of GA *in vitro*. Pro-GA could not be used *in vitro* assay such as enzymatic activity in the cell lysate.

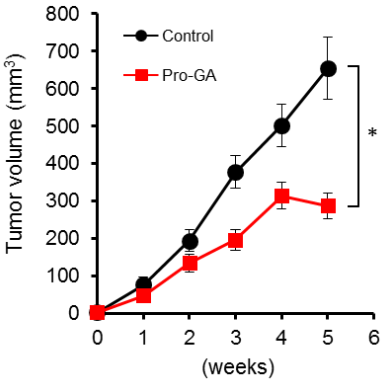
### Pro-GA inhibits the proliferation of cancer cells

Indicated normal cells and cancer cells were cultured in serum starve condition with DMSO (as control), GA and Pro-GA for the indicated number of days. Cell proliferation was calculated by the WST cell viability assay. Pro-GA specifically inhibited proliferation of cancer cells.



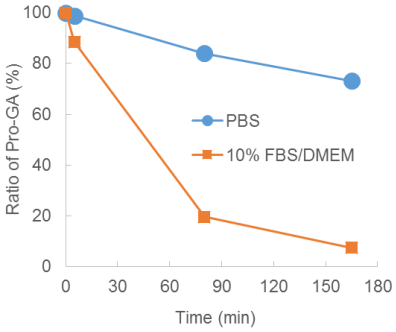
## Pro-GA inhibits tumor growth in xenograft mouse model

PC3 tumor cells were implanted into CB17 SCID mice and DMSO (as control) or Pro-GA was intraperitoneally administrated twice a week (n=5 per group). Administration of Pro-GA significantly inhibited tumor growth *in vivo*.



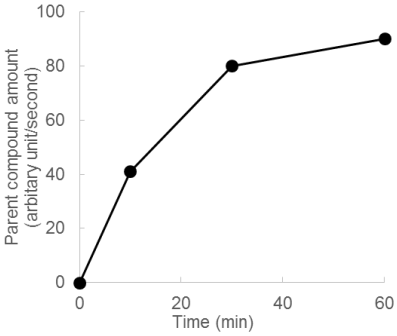

## Stability of Pro-GA in culture medium at room temperature

Stabilities of fluorescent NBD-conjugated Pro-GA in PBS or 10% FBS/DMEM were monitored by HPLC analysis. While conversion of Pro-GA to GA were slowly in PBS ($t_{1/2}$>5 hours), Pro-GA was quickly converted into GA in 10% FBS/DMEM by esterase activity in FBS ($t_{1/2}$~50 min). Please prepare Pro-GA solution in assay medium just before use.




## GA-release efficiency of Pro-GA in cells

MCF7 cells were treated with 100 μM of fluorescent NBD-conjugated Pro-GA for 0-60 min. After wash cells, cell lysates were prepared and analyzed by HPLC to evaluate GA amount released from Pro-GA. NBD-conjugated Pro-GA was immediately incorporated into cells within a few minutes and converted to GA in the cells.




## Reference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2. Kageyama *et al., Proteomics Clin. Appl.*, **1**, 192-199 (2007)
3. Ii *et al., ChemMedChem.*, **13**, 155-163 (2018)



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