

Nature Products Inspiration for a Medicinal Chemist to Develop anti-Cancer Agents

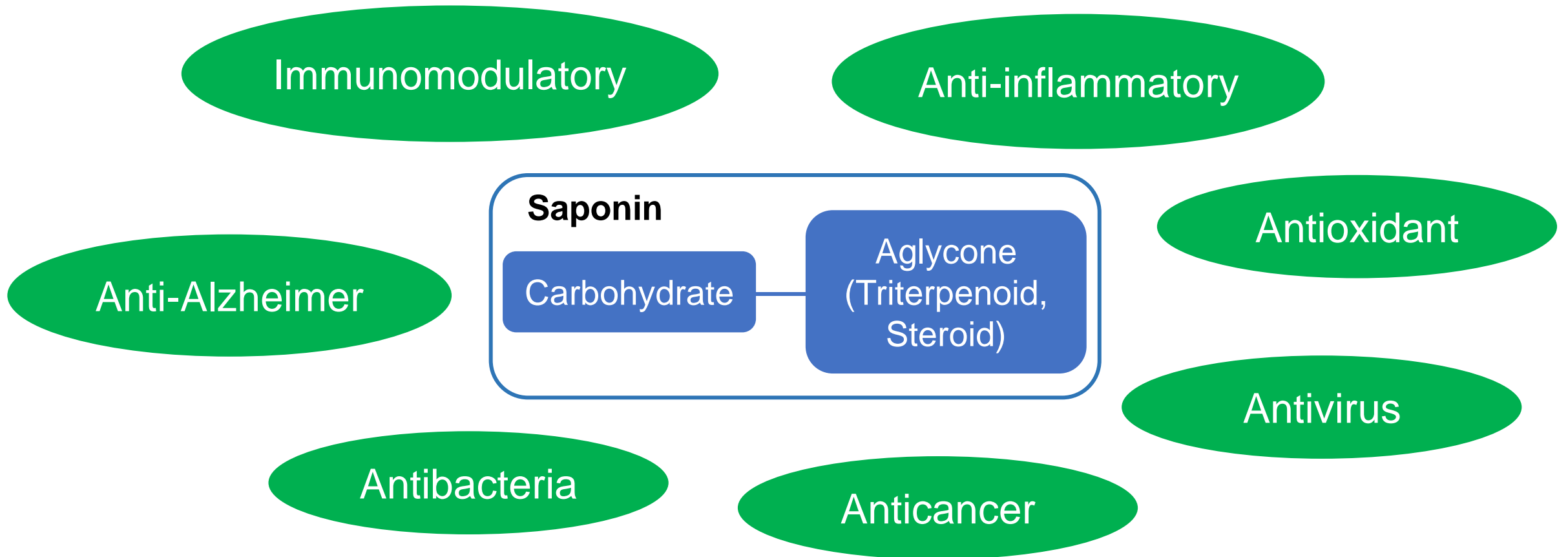
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National Taiwan University



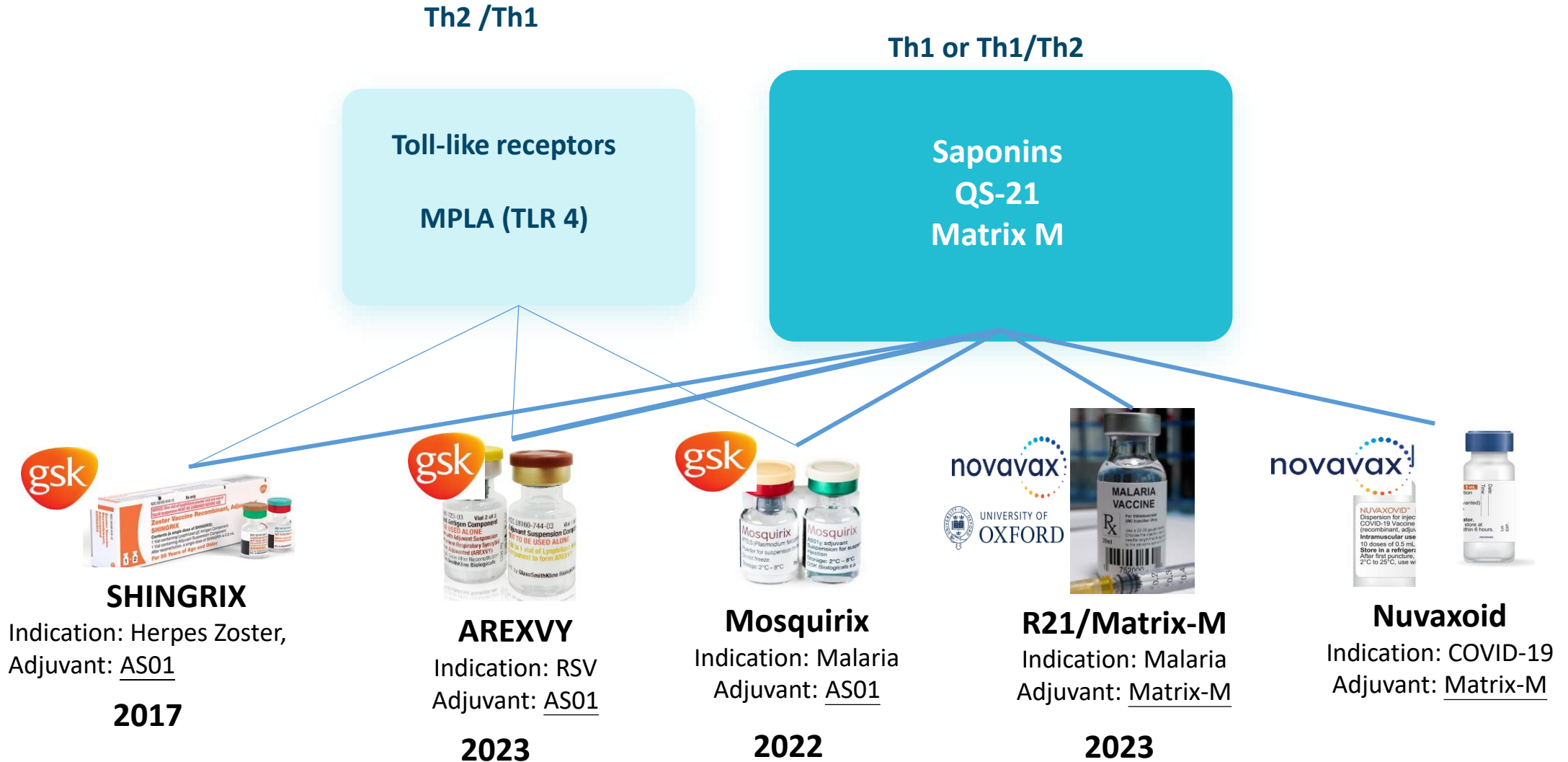
Outline

- ✓ Saponin--- Quillaic Saponin and Oleanolic Saponin Studies
- ✓ Phoretin as a ligand for Small Molecule Drug Conjugation
- ✓ Jugalone as a lead Targeting Protein Disulfide Isomerase Inhibition

Multifunctional Role of Saponins

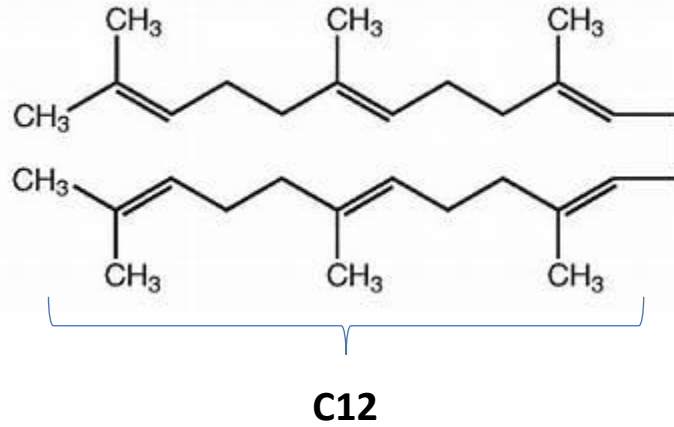


Current trend of adjuvants: Combinations of multiple mechanisms

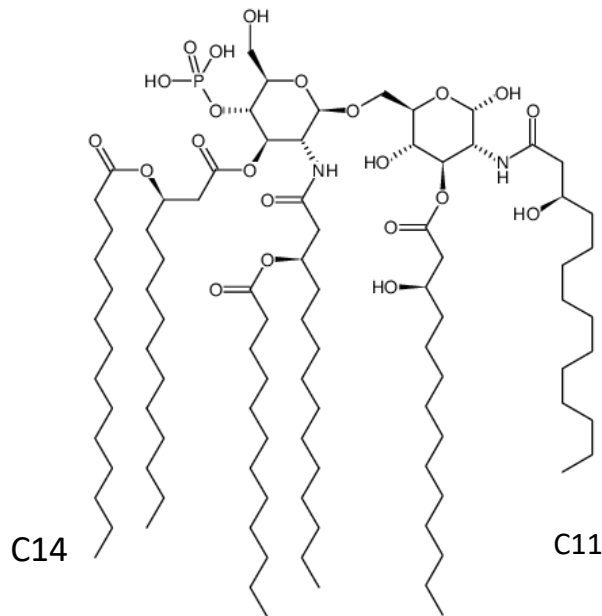


Structures that used in Adjuvant Formulation

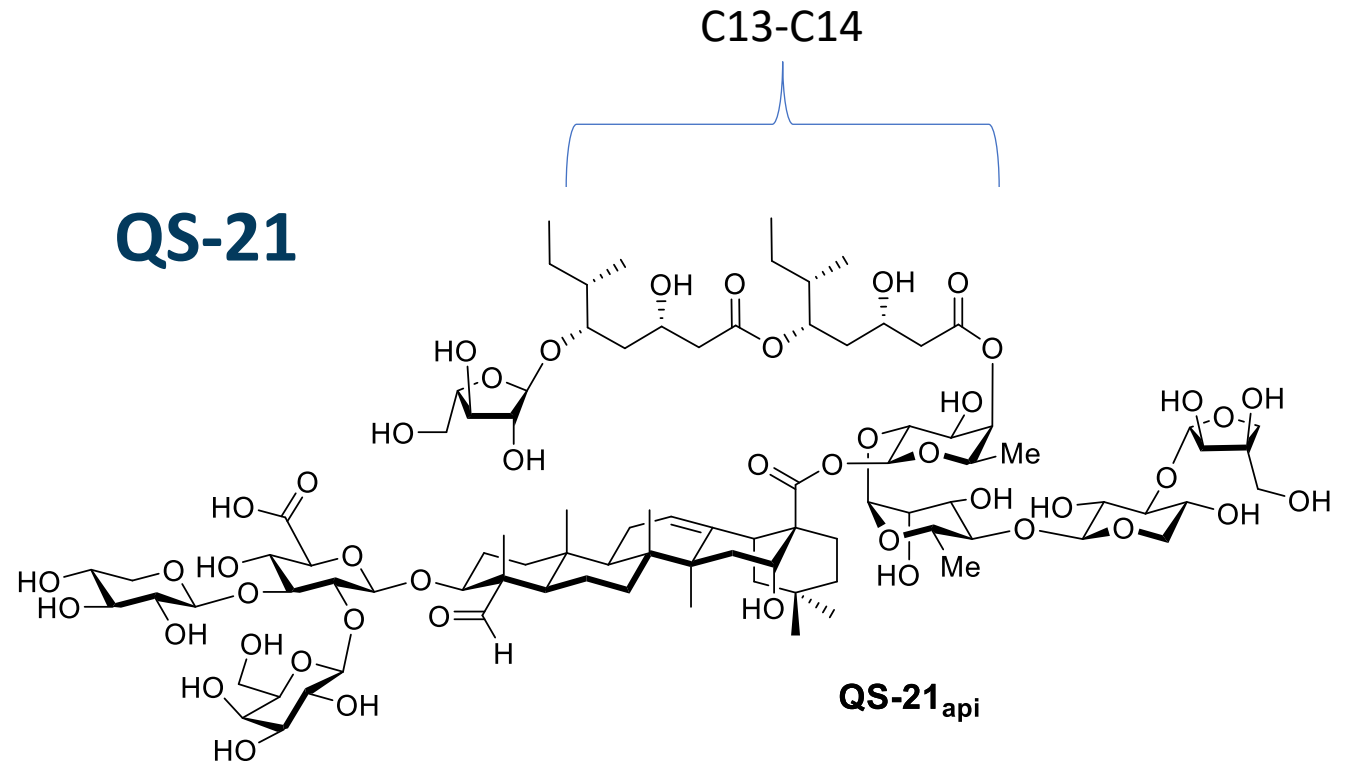
Squalene



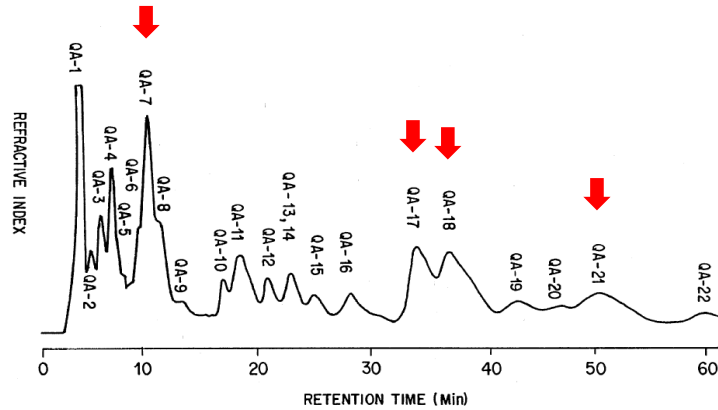
Monophosphoryl lipid A (MPL)



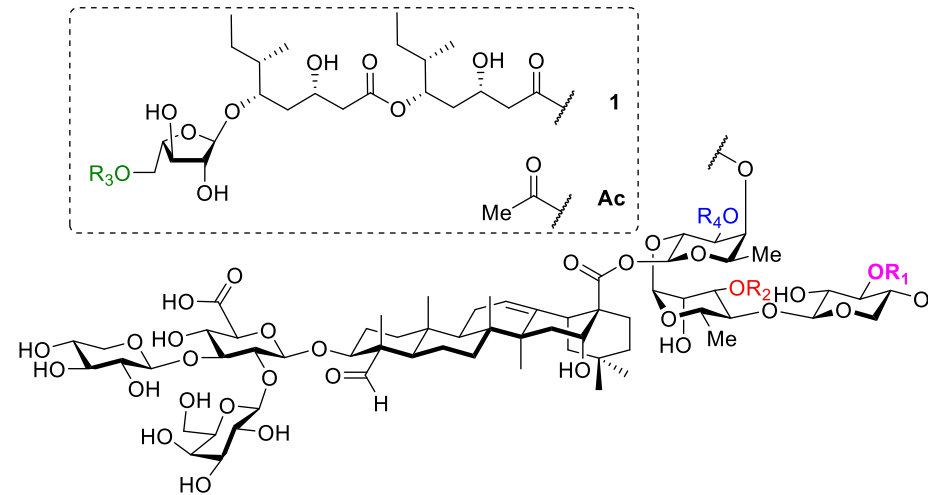
QS-21



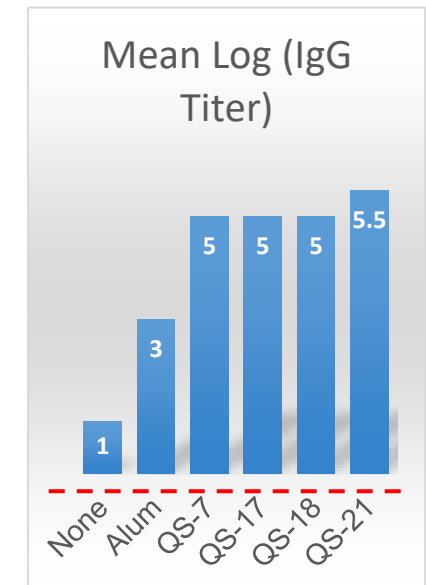
Structures of Saponins isolated from *Quillaja Saponaria*



US 5,583,112, Dec. 10, 1996.



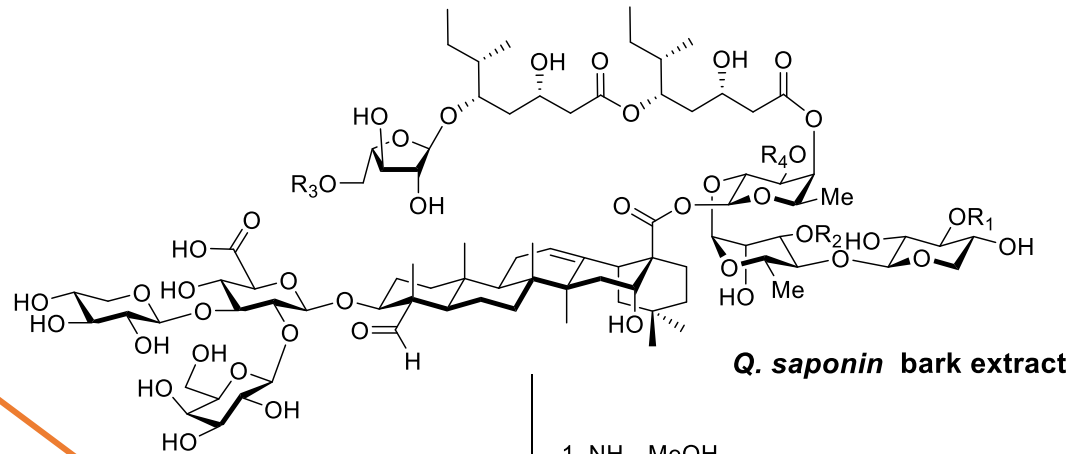
| Compound | Mol wt | Acyl chain | R ₁ | R ₂ | R ₃ | R ₄ | |
|--|--------|------------|------------------------------------|-----------------|-------------------|-------------------|------------------|
| QS-7 | 1861 | Ac | β -D-Apif | β -D-Glcp | N/A | α -L-Rhamp | Less amount |
| QS-17 | 2330 | 1 | β -D-Apif | β -D-Glcp | α -L-Rhamp | H | Highest toxicity |
| QS-18 | 2167 | 1 | β -D-Apif | β -D-Glcp | H | H | |
| QS-21_{api} QS-21_{xyl} | 1988 | 1 | β -D-Apif β -D-Xylp | H | H | H | Potent adjuvant |



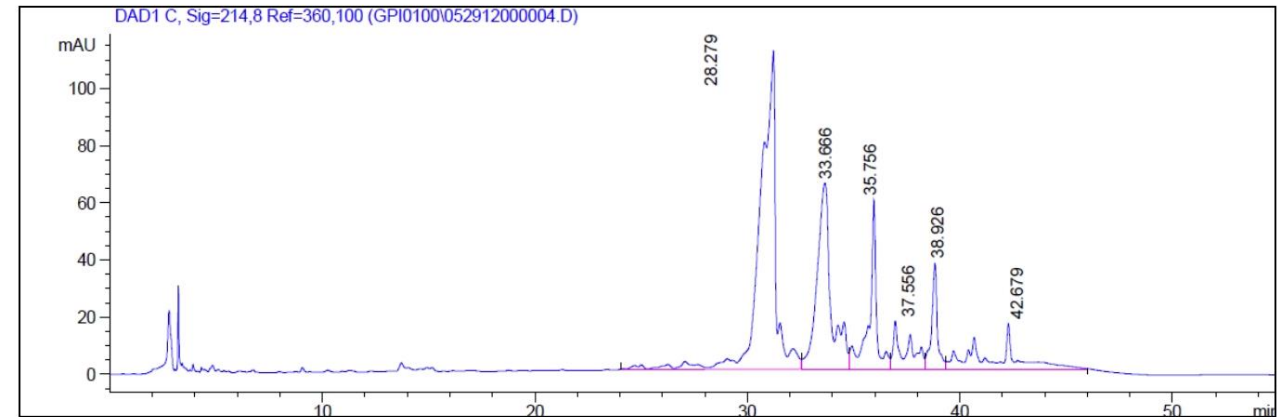
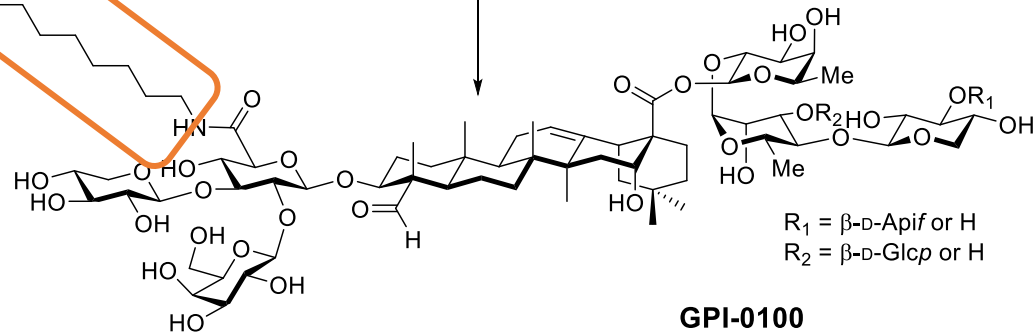
J. Immunol. **1991**, 146, 431-437.

Semisynthesis of *Quillaja saponin* to develop new-generation of saponins-based adjuvants

- Improve adjuvant efficacy (cellular immunity), and reduce toxicity.



1. NH₃, MeOH
2. Dodecylamine, DCC, NHS, py

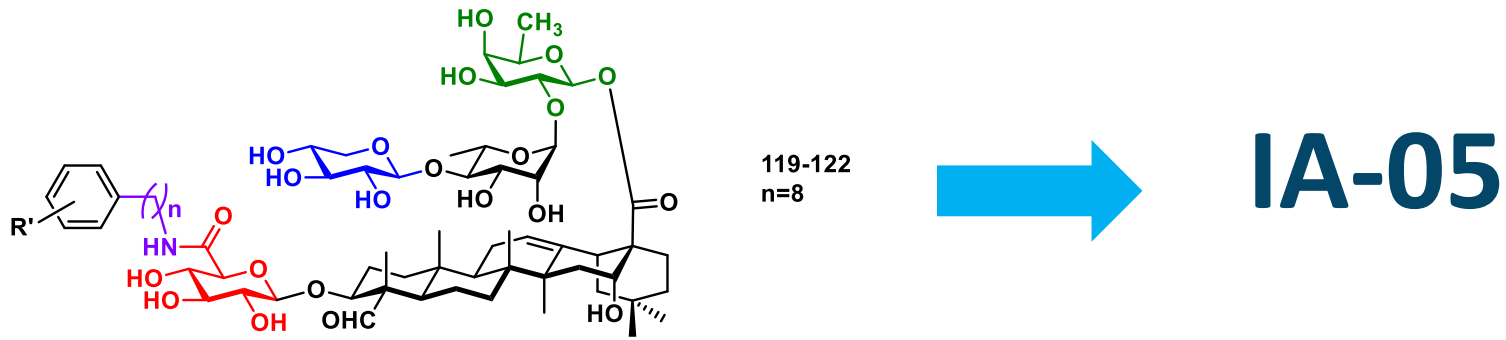


| Lot | Major peaks |
|-----------|---|
| 3849 | 1841, <u>1856</u> , 1709, 1679, <u>1694</u> |
| 3850 | <u>1841</u> , <u>1856</u> |
| 3873 | <u>1841</u> , <u>1856</u> , <u>1709</u> , <u>1679</u> |
| Bu | <u>1841</u> , <u>1856</u> , <u>1709</u> , <u>1679</u> |
| EA | 1841, <u>1856</u> , <u>1709</u> , <u>1694</u> |
| Lot1 | <u>1856</u> , <u>1724</u> , <u>1694</u> |
| GPI QS-17 | <u>1841</u> |
| GPI QS-18 | <u>1841</u> , 1709, 1577, <u>1679</u> |
| GPI QS-21 | 1754, 1841, <u>1679</u> |

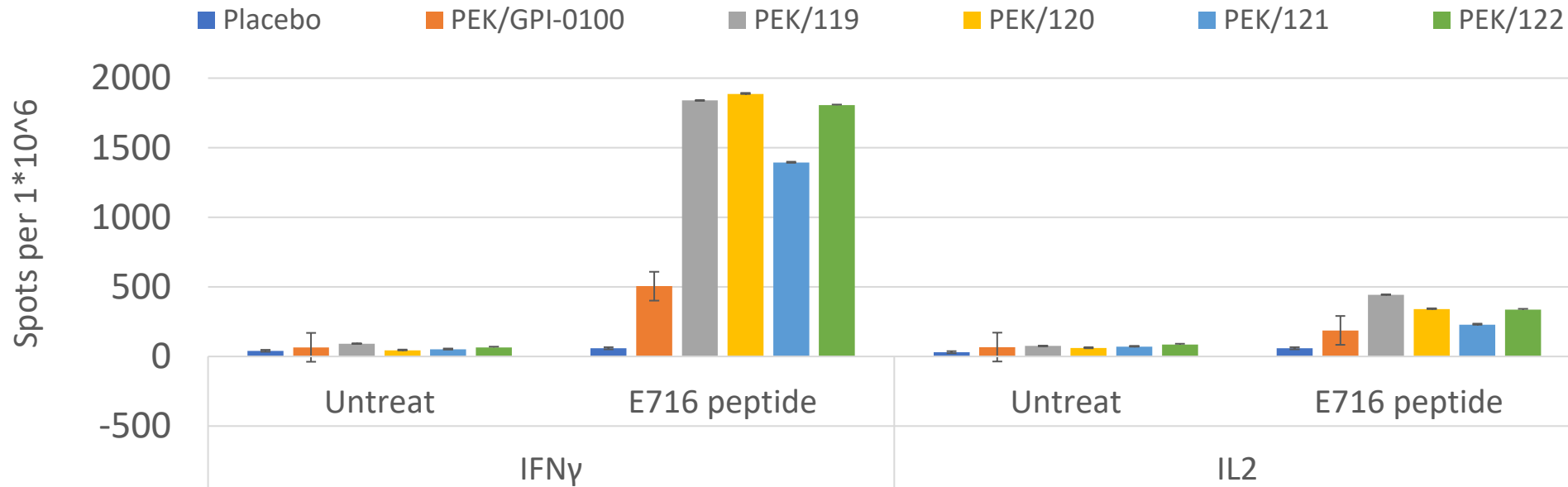
- NOAEL > 2000 ug

Marciani et al. Vaccine 2000, 18, 3141-51.

ELISpot Analysis for truncated compounds and invention of IA-05

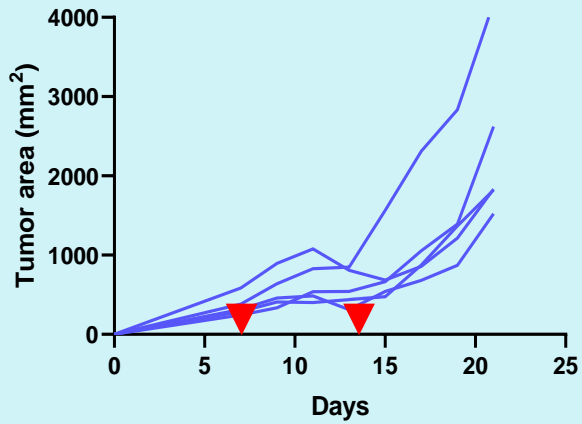


HPV



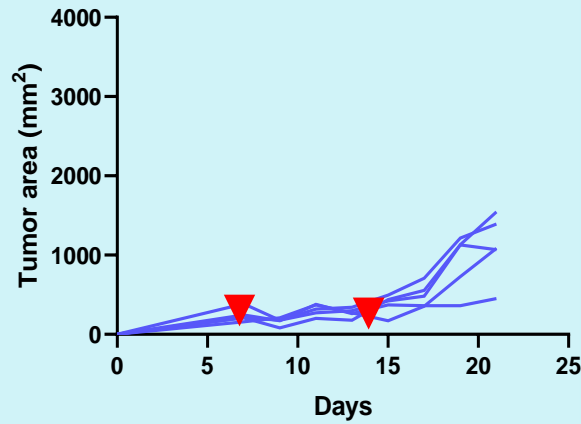
TVGV-1 incorporating IA-05 significantly reduced tumor volume in mice—antitumor model

TVGV-1 (50 ug)



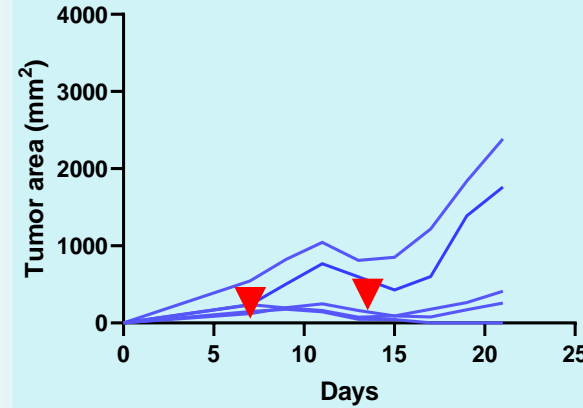
TC-1

TVGV-1 (50 ug) + QS-21 (50 ug)



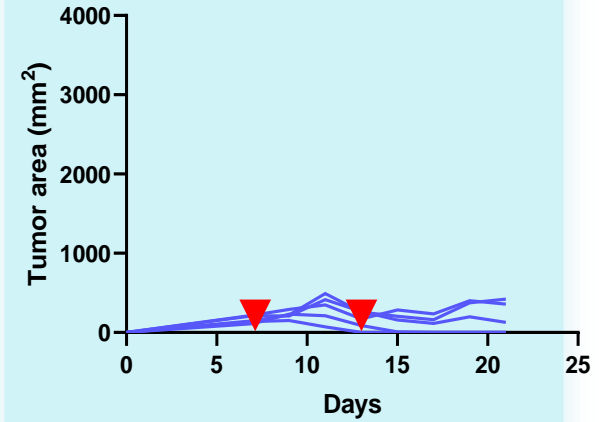
100 mcg of QS-21 was not tolerated

TVGV-1 (50 ug) + IA-05 (50 ug)

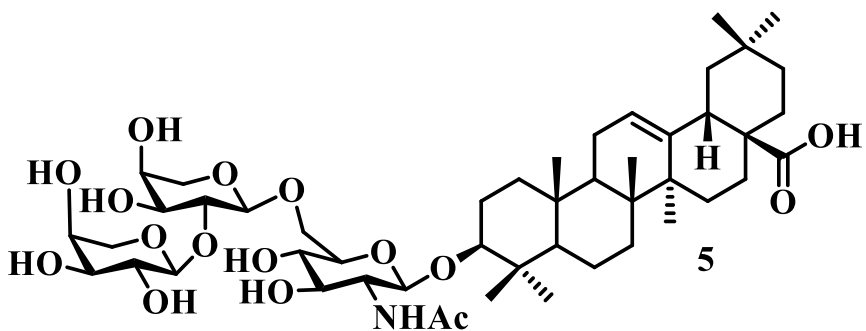


▼ : vaccination

TVGV-1 (50 ug) plus IA-05 (100 ug)

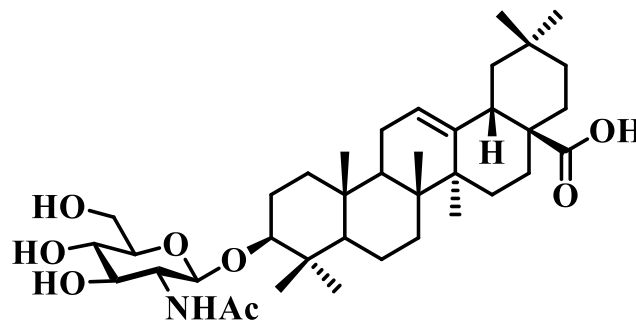


Development of Glucosamine-Bearing OA Saponins



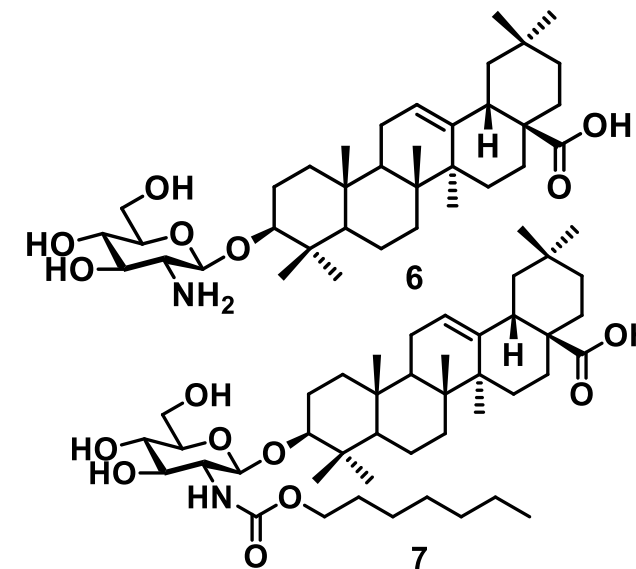
- Isolated from MeOH extract of stems and infructescence of *Albizia subdimidiata*
- $IC_{50} = 0.8 \mu M$ against A2780 ovarian cancer cells

Abdel-Kader, M. et al. *J. Nat. Prod.* **2001**, 64, 536



- Synthesized N-acetylglucosamine oleanolic saponin
- Exhibit 82.6% inhibition against HL-60 leukemia cells at $10 \mu M$

Wang, P. et al. *Carbohydr. Res.* **2010**, 345, 607



- The cytotoxicity of **7** against HL-60 was improved to $IC_{50} = 0.76 \mu M$

Zeng, Y. B. et al. *Mol. Divers.* **2014**, 18, 13
Lin, Y. Y. et al. *Eur. J. Med. Chem.* **2018**, 143, 1942

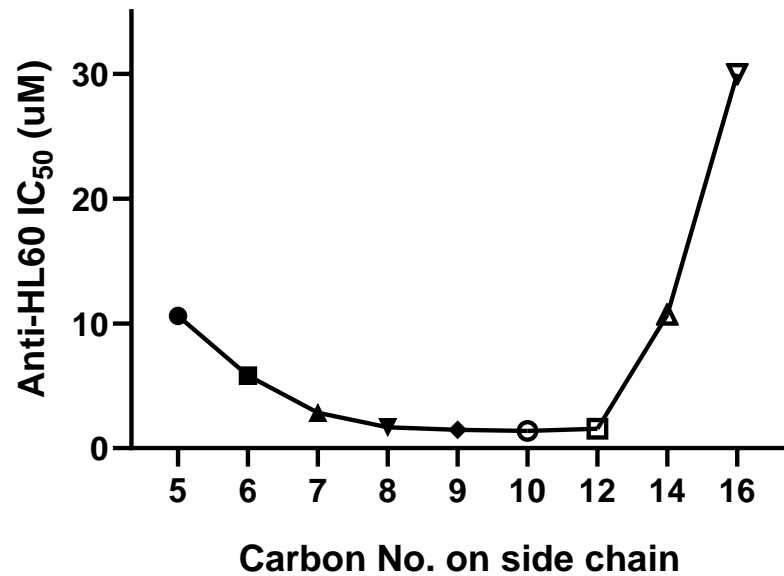
Literature reports

Previous work in our lab

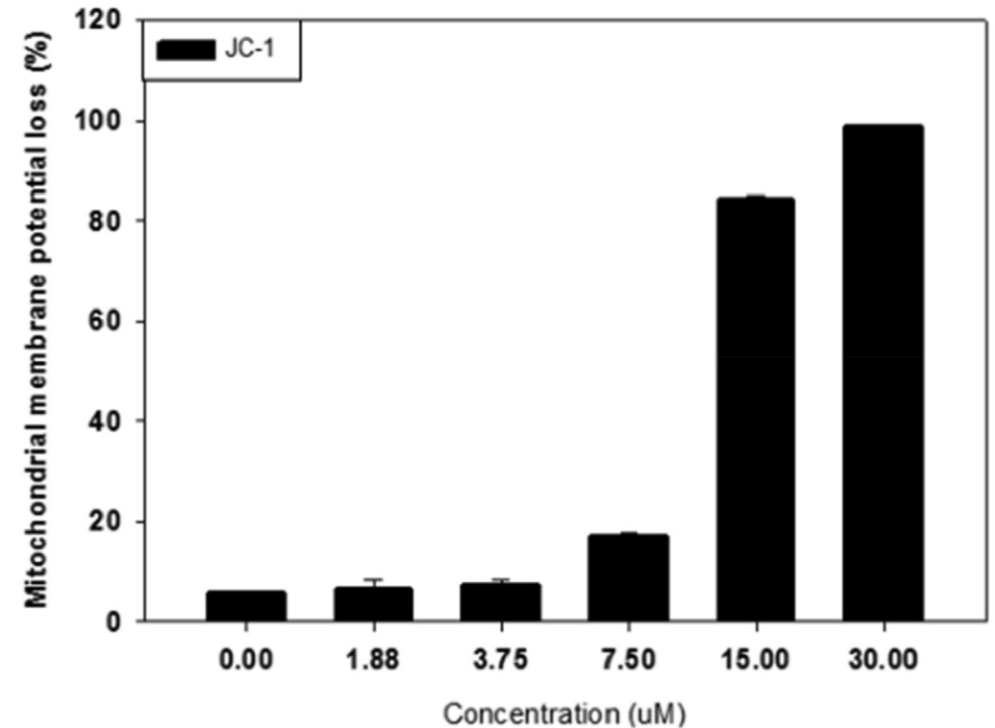
Motivation

Elucidation of bell-shaped correlation

Correlation between Chain Length and Activity

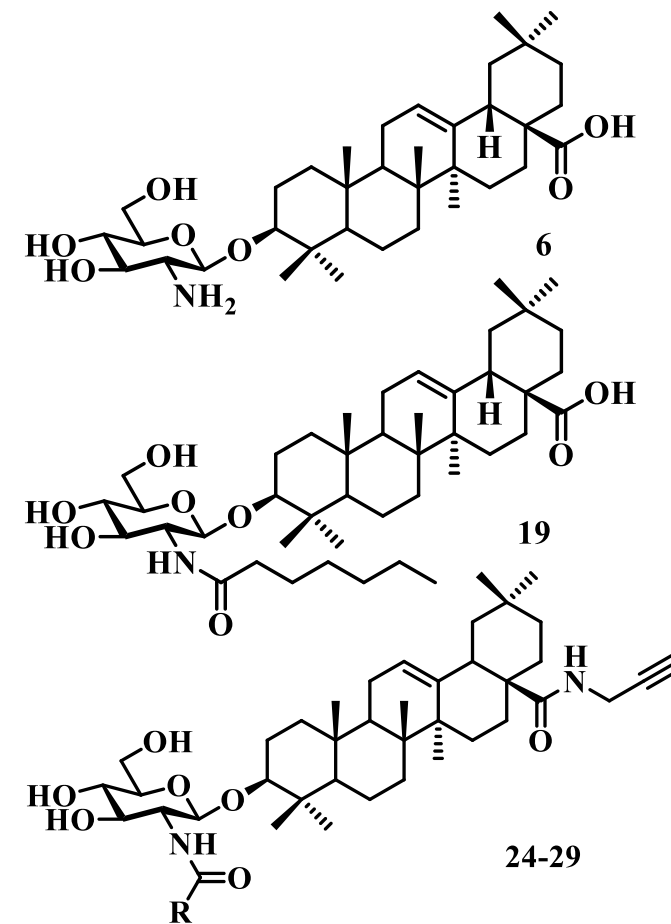


Mechanisms of anti-leukemic efficacy



Cytotoxicity Assay of Alkyne-Modified Saponins

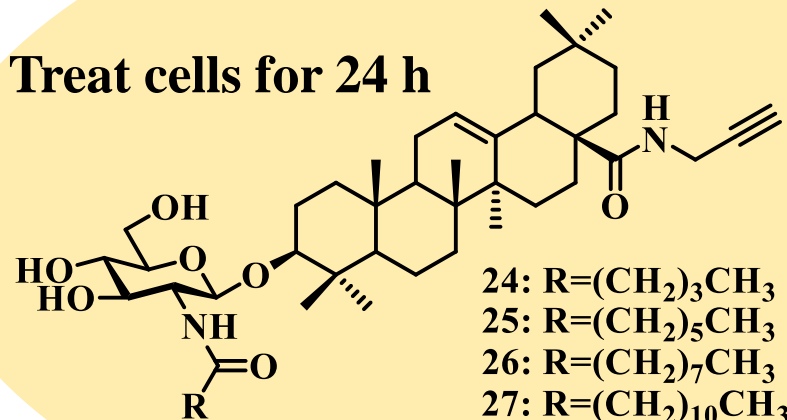
| | IC ₅₀ (μM) ^a | | | |
|-----------------|------------------------------------|-------------|-------------|-------------|
| | HL-60 | PC-3 | HCT116 | CT26 |
| 6 | 2.65 ± 0.01 | 25.0 ± 1.59 | 1.21 ± 0.21 | 9.91 ± 0.35 |
| 19 | 2.85 ± 0.01 | >30 | 8.79 ± 0.97 | 18.2 ± 6.40 |
| 24 (5C) | 11.6 ± 0.24 | 10.7 ± 0.93 | 7.30 ± 1.07 | 6.87 ± 0.46 |
| 25 (7C) | 12.5 ± 0.06 | 10.5 ± 2.12 | 2.68 ± 0.85 | 6.33 ± 0.34 |
| 26 (9C) | 5.74 ± 1.15 | 11.2 ± 2.34 | 2.78 ± 0.36 | 7.63 ± 0.86 |
| 27 (12C) | >30 | >30 | 6.74 ± 0.54 | 17.8 ± 1.15 |
| 28 (14C) | >30 | >30 | >30 | >30 |
| 29 (18C) | >30 | >30 | >30 | >30 |



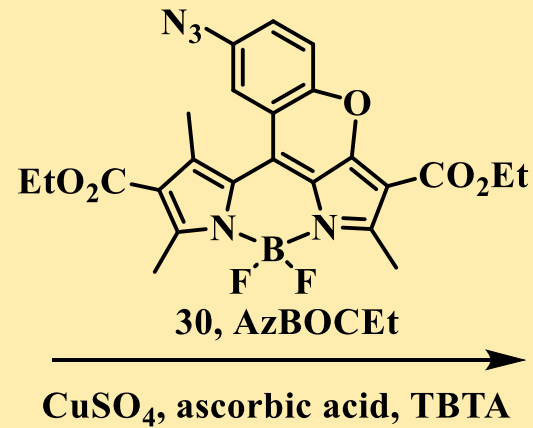
In Situ Generation of Fluorogenic Saponin Probes

HL-60 Cell

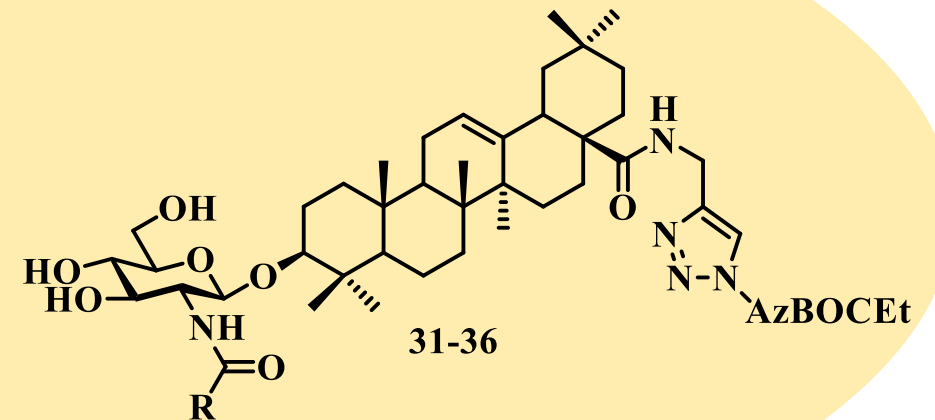
Treat cells for 24 h



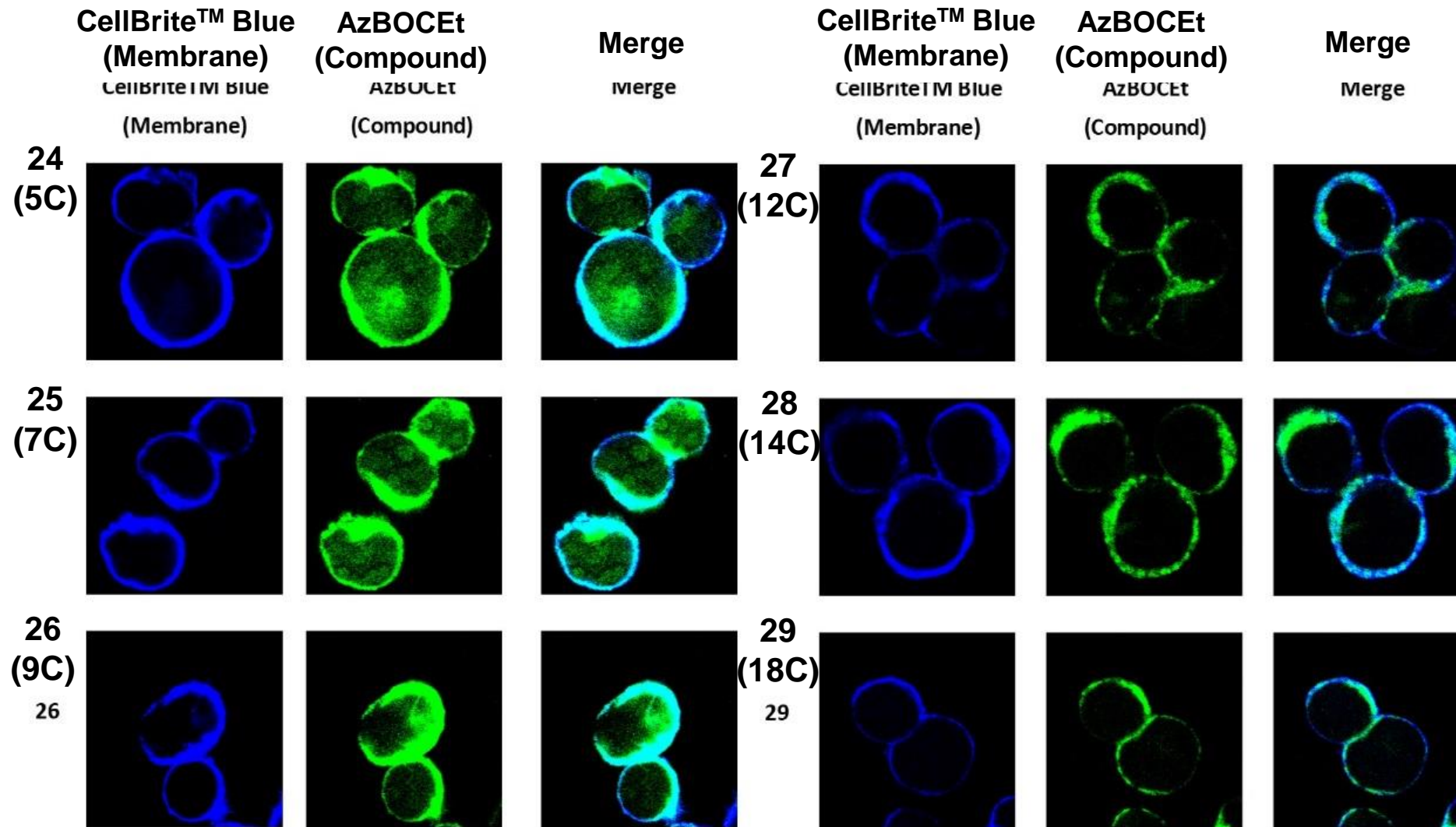
- 24: R=(CH₂)₃CH₃
- 25: R=(CH₂)₅CH₃
- 26: R=(CH₂)₇CH₃
- 27: R=(CH₂)₁₀CH₃
- 28: R=(CH₂)₁₂CH₃
- 29: R=(CH₂)₁₆CH₃



CuSO₄, ascorbic acid, TBTA



Distribution Analysis using Confocal Microscopy



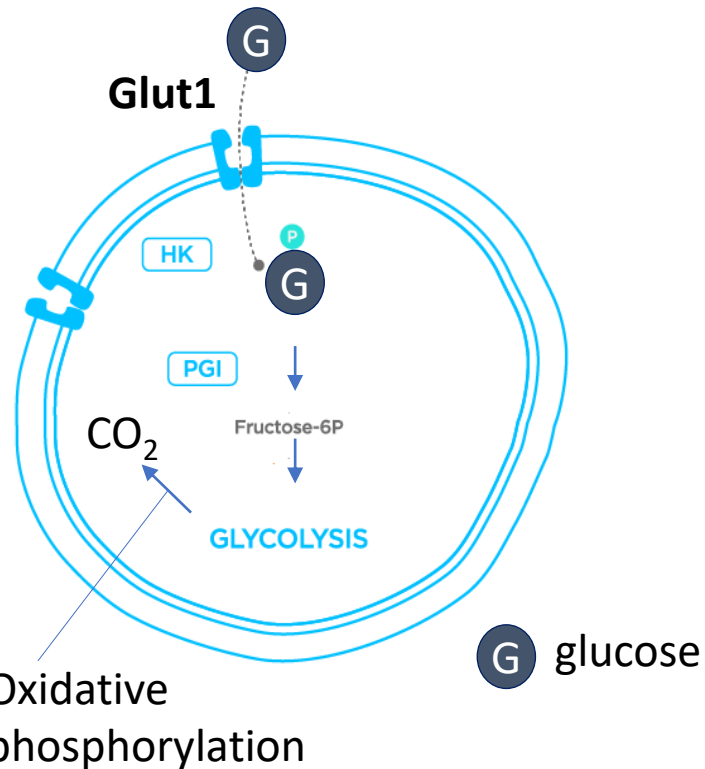
Phoretin as a ligand for Small Molecule Drug Conjugation

Chiu, Pei-F.; Chang, C.-K.; Huang, P.-S.; Lin, Y.-Y.; Lin, C.-S.; Yang, H.-Y. ; Hsu, L.-C.; Yu, L. C.-H. ; Liang, P.-H.* J. Med. Chem. 2023, 66, 14, 9684–9696.

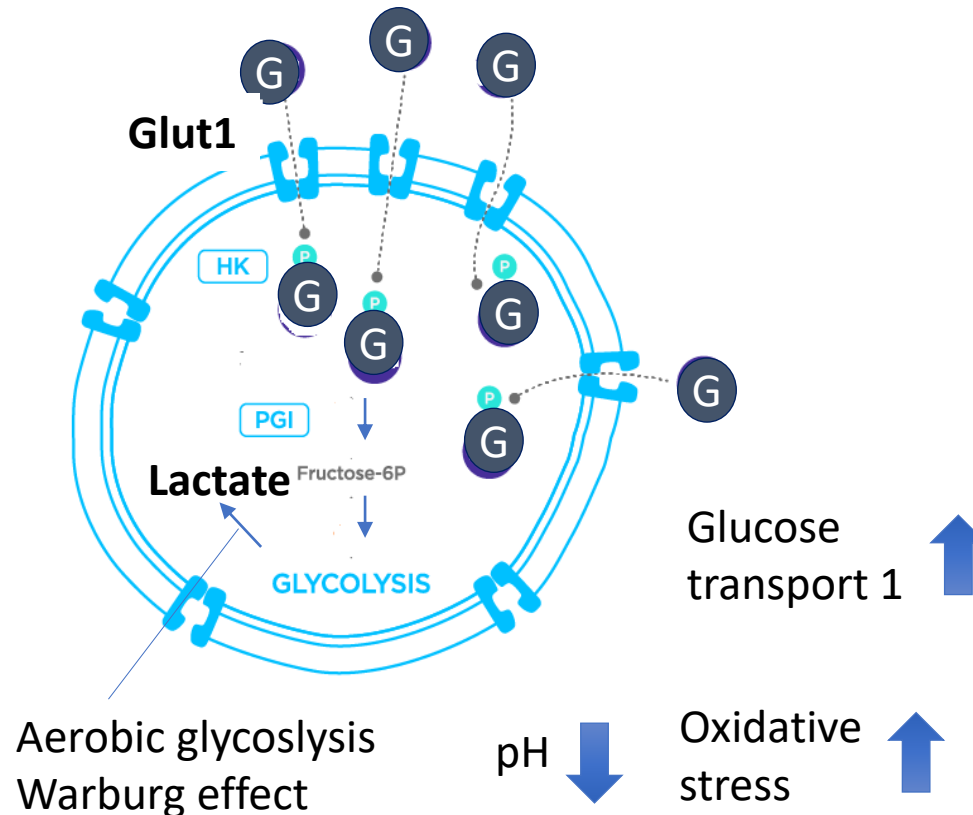
Chang, C.-K.; Chiu, P.-F.; Yang, H.-Y.; Juang, Y.-P.; Lai, Y.-H.; Lin, T.-S.; Hsu, L.-C.; Yu, L. C.-H.; Liang, P.-H.* J. Med. Chem. 2021, 64, 8, 4450–4461.

Cancer cells rely on aerobic glycolysis and overexpress glucose transporters to meet their energy demands, therefore cancer microenvironment is usually pH value lower than normal cells and has higher oxidative stress

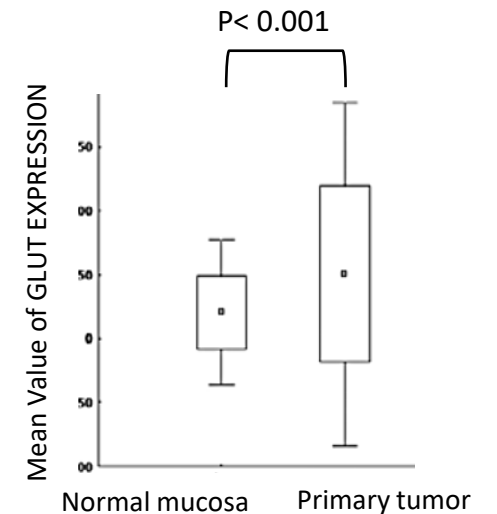
Normal Cells



Cancer cells



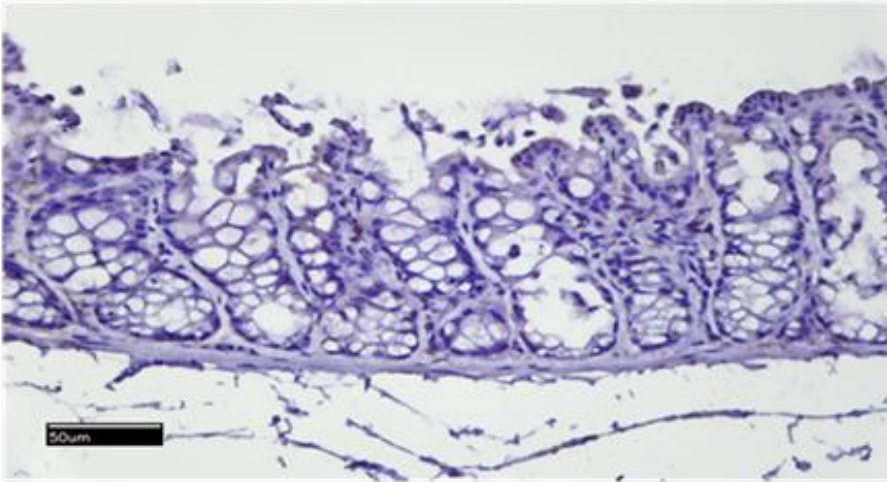
CRC



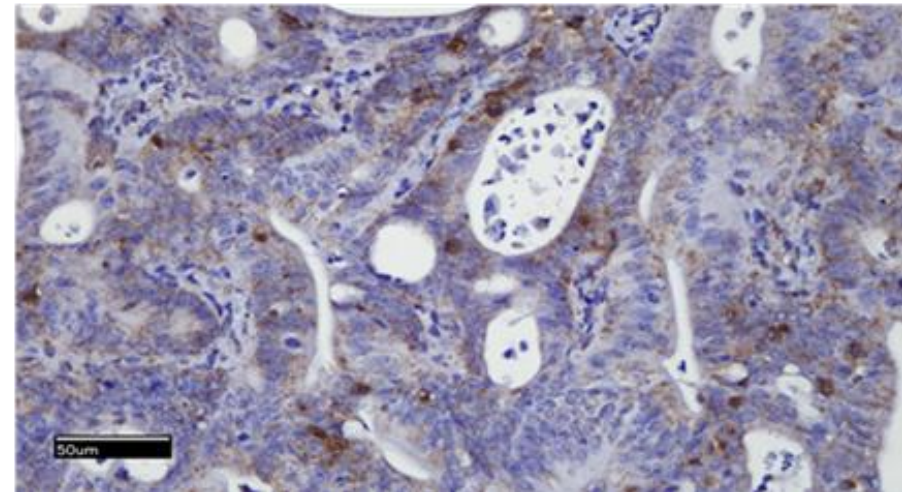
Int J Biol Markers 2011; 26,166-172.

Tumor site has higher expression level of enzyme B compared to the normal colon

A. Healthy mouse

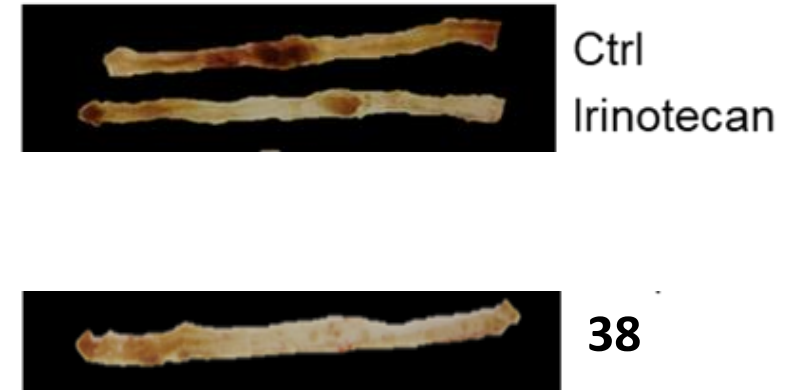
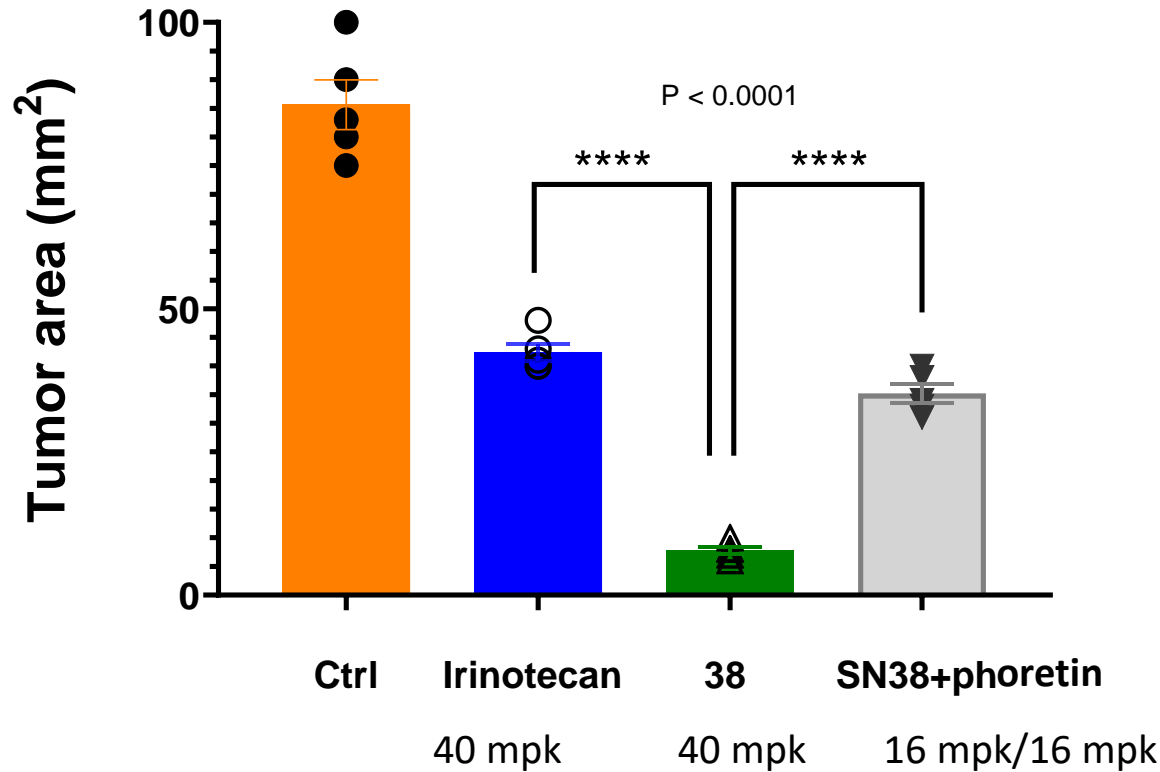
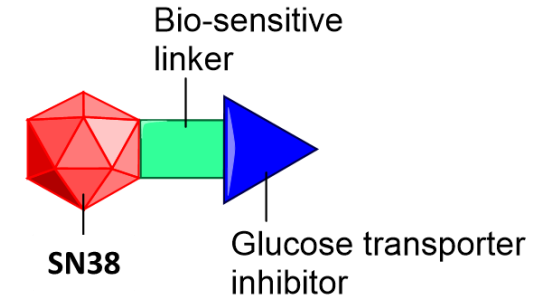
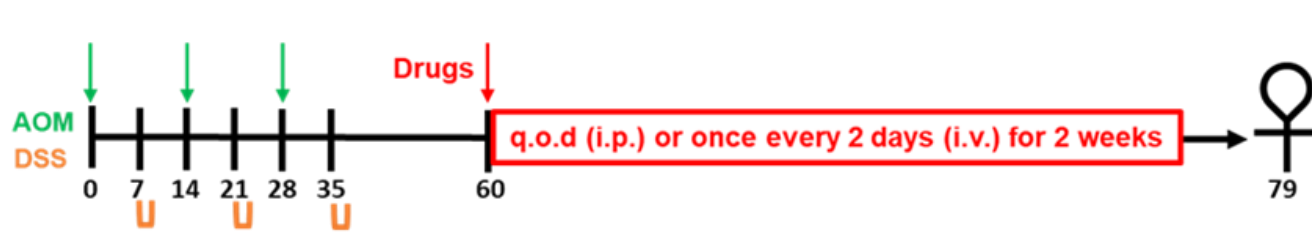


B. CRC mouse

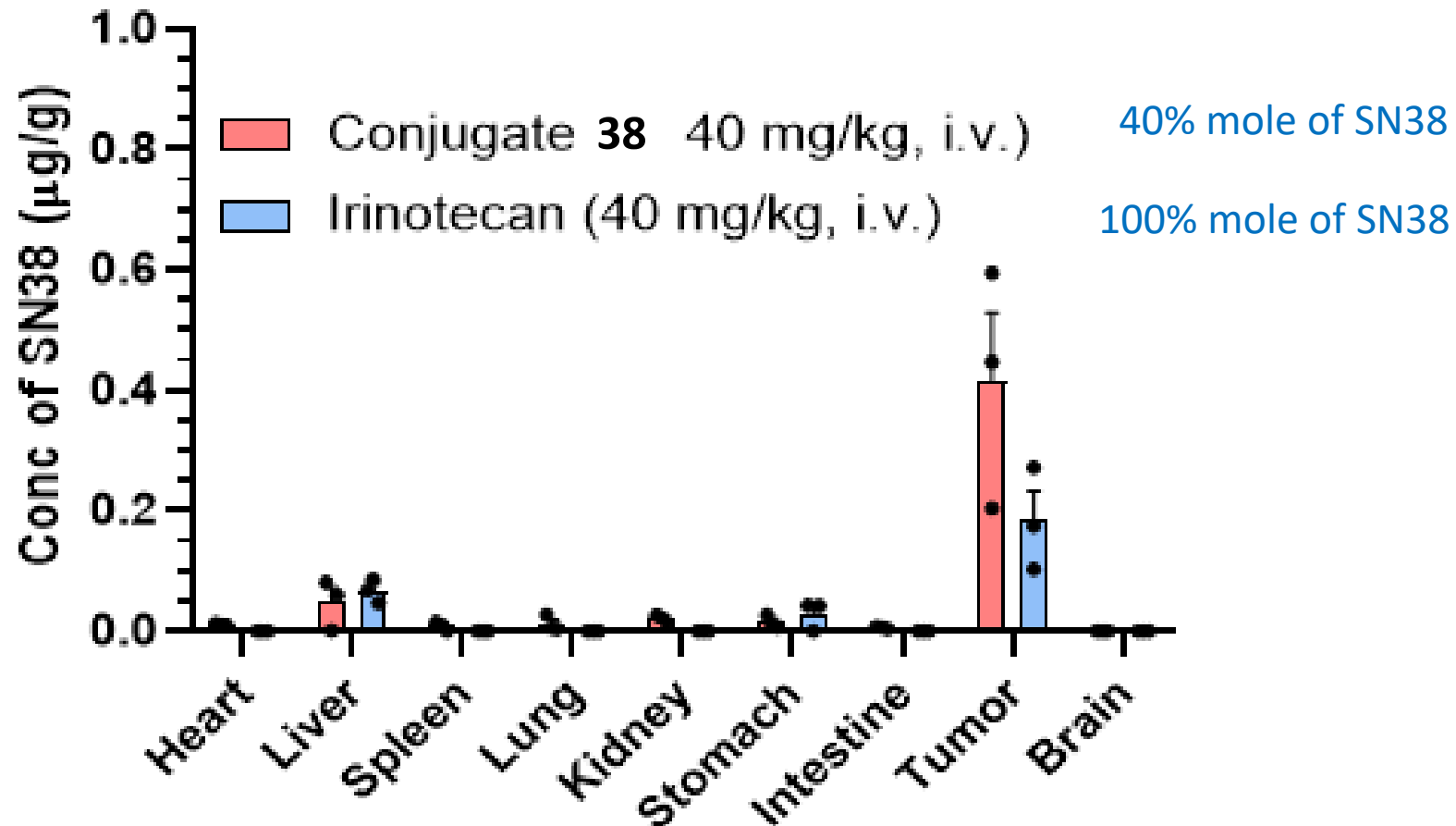


Immunohistochemical (IHC) staining of enzyme B in paraffin-embedded colon sections of (A) healthy mouse, scale bar = 50 μ m, x 100 magnification; (B) CRC mouse, scale bar = 50 μ m, x 100 magnification. Immunohistochemical labeling of enzyme was detected using 3,3'-diaminobenzidine (DAB) substrate (brown color). Cell nuclei were counterstained using haematoxylin (blue/purple).

Conjugate **38** contains less SN38 (40% compared to irinotecan), but it has better therapeutic effect (90% reduction) *in* orthotropic CRC mice model



Conjugate **38** has higher concentration of SN38 in the tumor site with only 40% of SN38 loading, indicating its superior targeting effect



Design, Synthesis and Antiplatelet Evaluation of PDI Inhibitors based on Jugalone

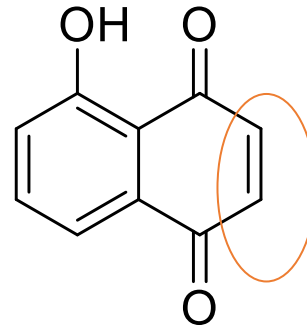
- Juang, Y.-P.; Tsai, J.-Y.; Gu, W.-L.; Hsu, H.-C.; Lin, C.-C.; Wu, C.-C.;* Liang, P.-H.* J. Med. Chem. 2024, 67(5):3626-3642.

Introduction

Juglone



- **Juglandaceae family (胡桃科)**



Juglone (**11**)

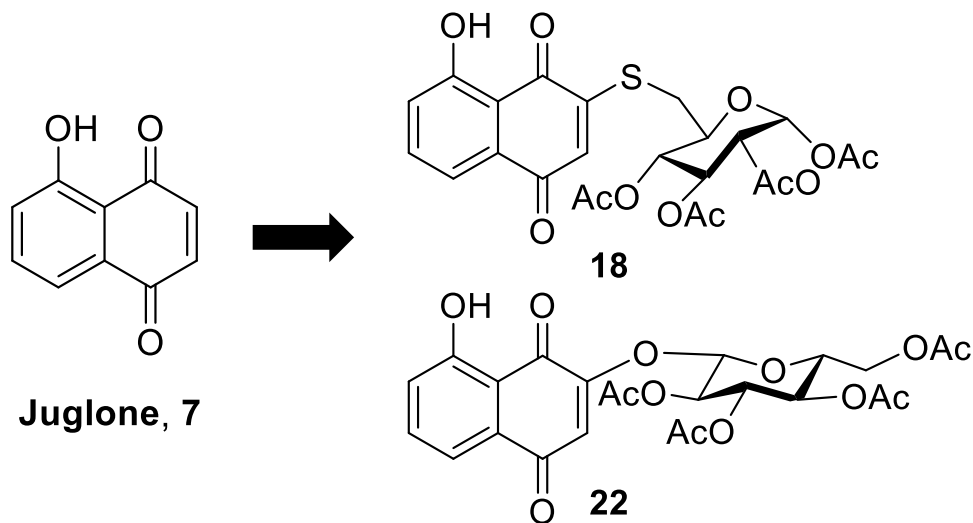
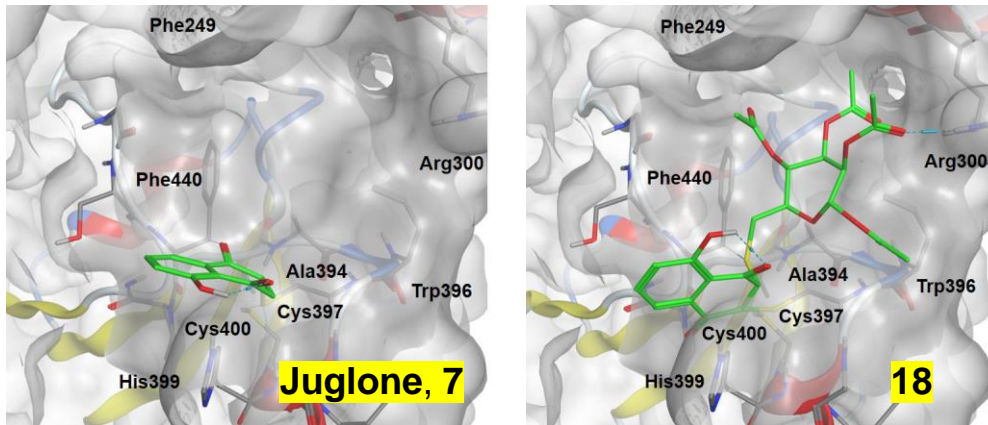
IC₅₀ of PDI = 0.54 μM

IC₅₀ of Platelet inhibition = 1.18 μM

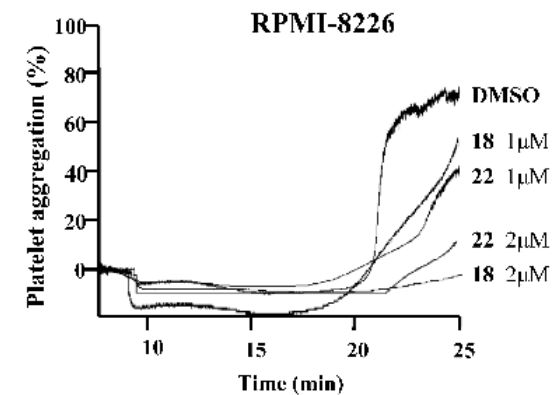
IC₅₀ of cell toxicity = 11 μM

Wu, C.-C. et al. 2021, Phytomedicine, 82, 153449.

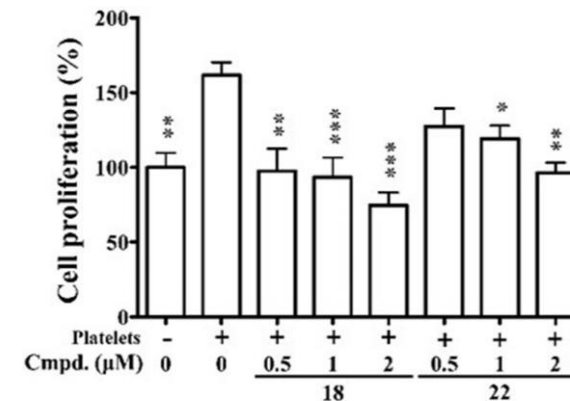
18 and **22** showed the IC_{50} values reached 61 and 48 nM, in cell viability test. In addition, **18** and **22** were able to prevent tumor cell-induced platelet aggregation as well as platelet-enhanced tumor cell proliferation, which was associated with a reduction of platelet secretion of PDGF.



Tumor Cell-Induced PLT Aggregation



PLT-Induced Tumor Cell Proliferation



Summary

Nature products provide a universal platform for new drug developments .

- ✓ Saponin--- Quillaic Saponin ----development of vaccine adjuvant in anti-cancer vaccine
- ✓ Phoretin as a ligand for Small Molecule Drug Conjugation
- ✓ Jugalone derivatives for as a lead Targeting Protein Disulfide Isomerase Inhibition for Tumor Cell-induced Platelet Aggregation

Acknowledgement

Collaborators

Prof. Jih-Hwa Guh (School of Pharmacy, NTU)

Prof. Li-Ching Hsu (School of Pharmacy, NTU)

Prof. Linda Chia-Hui Yu (Graduate Institute of Physiology, NTU)

Prof. Hong-Chi Yang (Graduate Institute of Microbiology, NTU)

Prof. Yung-Ling Leo Lee (IBMS, Academia Sinica, AS)

Prof. Chin-chung Wu, Kaohsiung Medical University

Team members of the Lab

