Enhancement of Lymphocyte Infiltration in Solid Tumors: A Strategic Approach to Augment Immunotherapeutic Efficacy

Yuchen He[1,2], Naibo Yin[1,2], Zhiwen Zhang[3], Guo-liang Lu[2,4], Jingyuan Wen[1,4]

[1]School of Pharmacy, Faculty of Medical and Health Sciences, the University of Auckland, New Zealand. [2]Auckland Cancer Society Research Centre, Faculty of Medical and Health Sciences, the University of Auckland, New Zealand. [3]School of Pharmacy, Fudan University, China. [4]Maurice Wilkins Centre, the University of Auckland, New Zealand

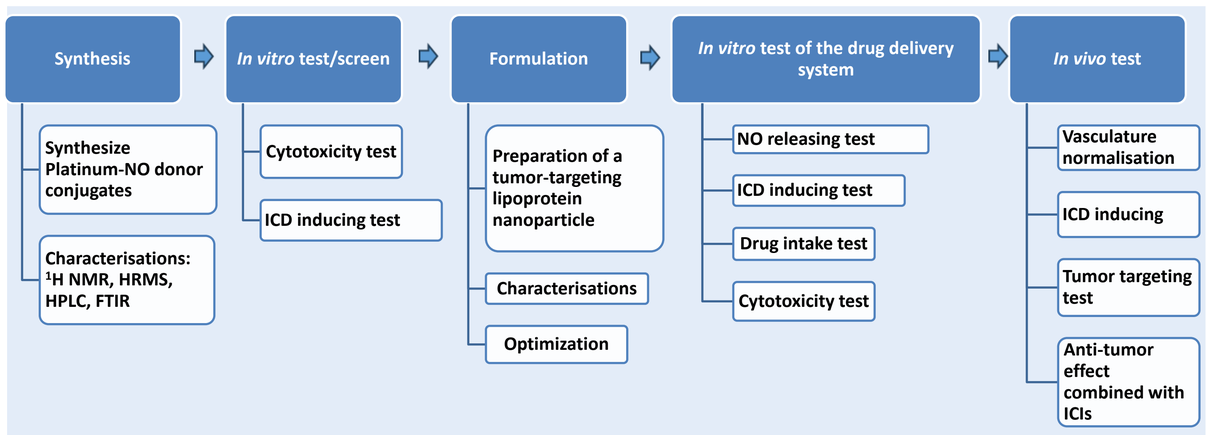
Background

Immune checkpoint inhibitors (ICIs) show limited efficacy (10-40%) in patients with solid tumors due to poor cytotoxic T lymphocyte (CTL) infiltration into tumor sites. Abnormal tumor vasculature creates physical barriers that limit CTL access. Novel strategies to enhance CTL infiltration are crucial for improving ICI therapy outcomes. Nitric oxide (NO), as an endogenous gas molecule, has been reported to promote tumor vessel normalization, thereby improving the effectiveness of anticancer therapies.

Aim & Objectives

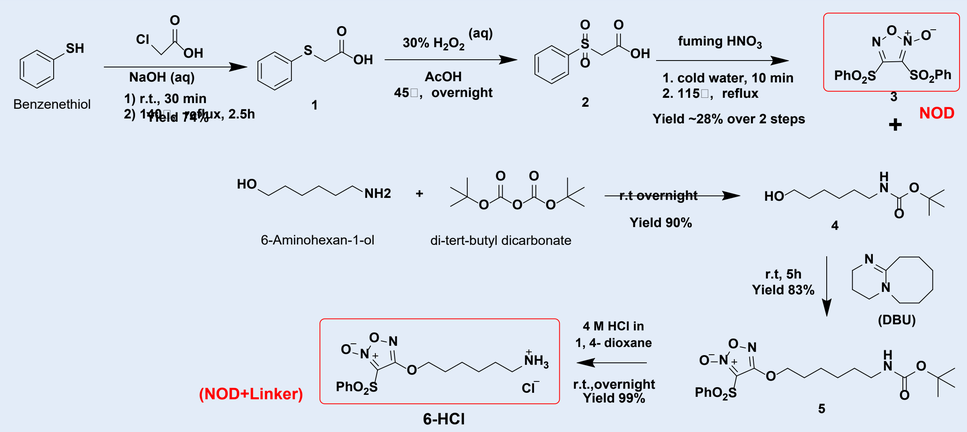
To develop glutathione-sensitive nitric oxide donor (NOD)-platinum conjugates and encapsulate them into a tumor-targeted lipoprotein nano-drug delivery system to enable specific drug release within tumor cells, normalize tumor vasculature, and induce immunogenic cell death (ICD), thereby enhancing CTL infiltration and synergizing with ICIs.

Methodology

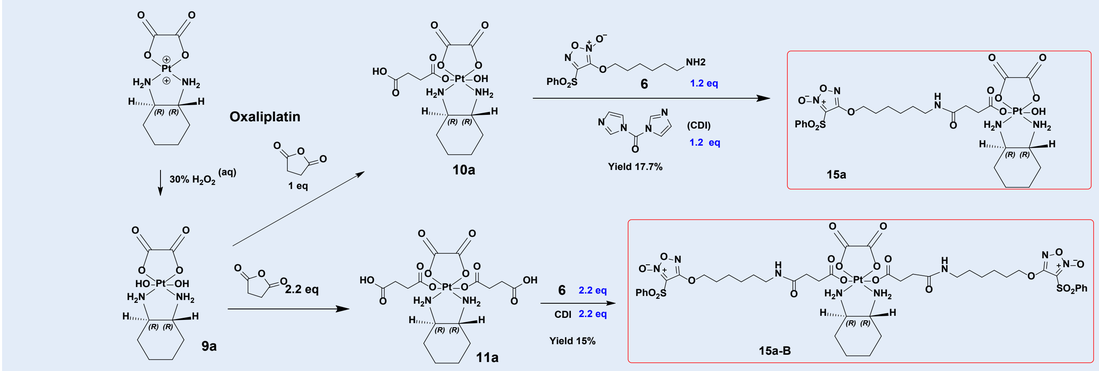


Preliminary Results

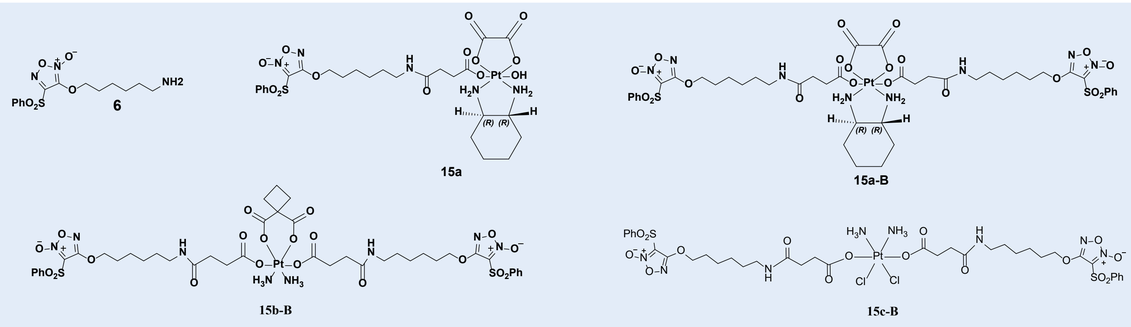
Synthesis route of NOD



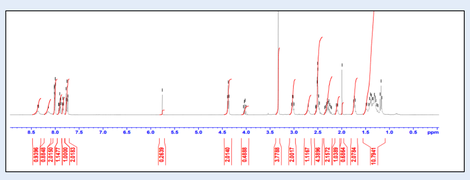
General synthesis route for NOD-Platinum conjugates



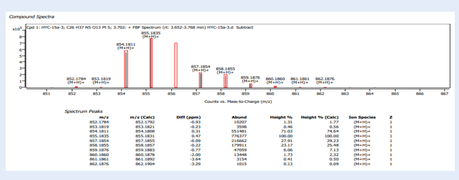
Synthesized NOD-Platinum conjugates



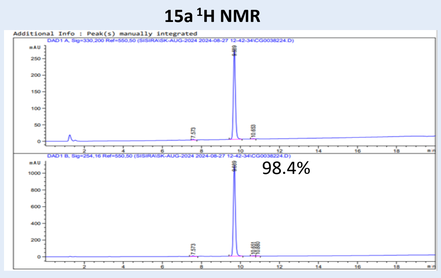
Free drug characterization



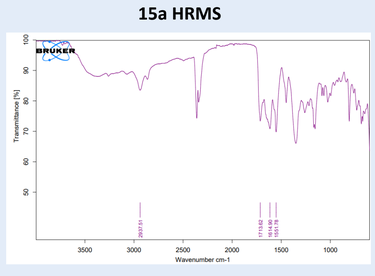
15a ^{1}H NMR



15a HRMS



15a HPLC Purity



15a FTIR

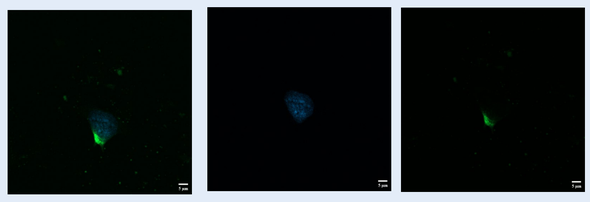
In vitro tests for free drug

- ***Cell Viability Assay (Free drugs)***

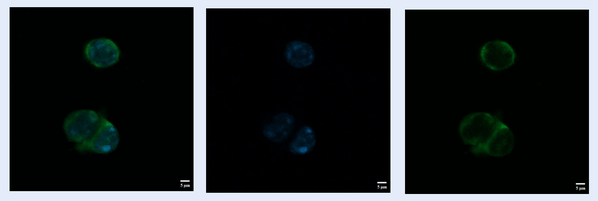
An MTT assay was performed to examine the viability of 4T1 cells after treatment with NOD-platinum conjugates. In brief, 4×10^3 4T1 cells were plated into a 96-well plate. Then, cells were treated with different concentrations of each NOD-Platinum conjugate. After 48 h, 10 µL of MTT (5 mg/mL) was added to each well and incubated for 4 h. The medium was then removed, and 150 µL of DMSO was added to each well and incubated for 15 min. The absorbance was then measured at 570 nm.

- ***Immunogenic Cell Death (ICD) Inducing Ability (Free drug)***

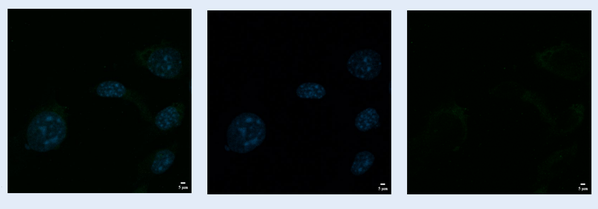
In brief, 4T1 cells were seeded into a chamber slide (1.5×10^4 cells/well) and allowed to attach overnight. The cells were then treated with Oxaliplatin, Carboplatin, Cisplatin, 15a, 15a-B, 15b-B, 15c-B, and NOD separately for 48 h. Then the cells were fixed and sequentially stained with anti-CRT antibody (Abcam, ab2907, 1:250 dilution), Alexa Fluor® 488 Goat Anti-Rabbit (Thermo Fisher, A-11008, 1:300 dilution), and DAPI (Merck, D9542, 1 µg/mL). The fluorescence intensity of surface-exposed CRT across the different treatment groups was used to characterize the ICD-inducing ability of each drug. The cells were imaged on a confocal microscope (Zeiss LSM 800 Airyscan inverted confocal microscope).



Untreated (Negative Control)



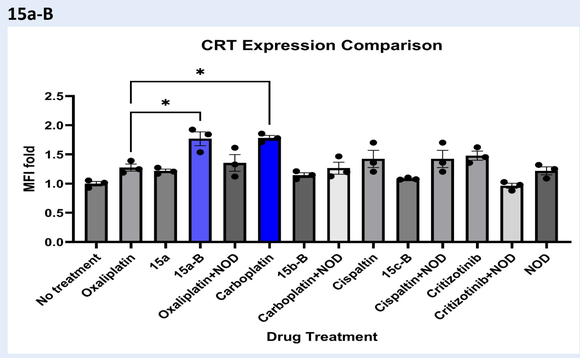
Oxaliplatin (Positive Control)



Secondary Antibody Control

15a-B

The preliminary results (right figure) show that the 15a-B has a significantly improved ICD-inducing ability than Oxaliplatin (Adjusted $P < 0.05$) and also shows better performance compared to other NOD-Platinum conjugates. Therefore, 15a-B was selected for encapsulation into lipoprotein nanoparticles in the next step.



Development of Lipoprotein Nanoparticles

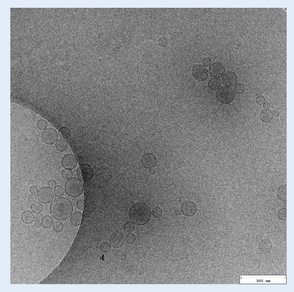
- ***Microfluidics***

A microfluidic device was used for a single-step, continuous, and self-assembly-based synthesis of lipoprotein nanoparticles. Briefly, an organic phase in ethanol and an aqueous phase containing Apolipoprotein A1 (ApoA1) were introduced into the microfluidic device. The solution from the outlet was collected and transferred to a centrifugal membrane filter (Millipore, 100 kDa) for purification (three PBS exchanges).

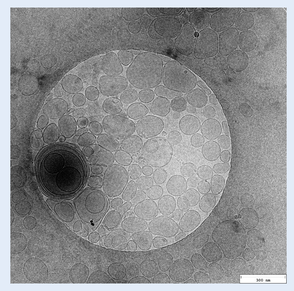
- ***Thin-film hydration***

Materials were dissolved in an organic solvent. The solution was evaporated under vacuum to produce a homogeneous lipid film at 60℃ using a rotary evaporator. The film was dispersed in PBS to form a suspension, which was then rotated at 60°C under normal pressure, followed by sonication in an ice bath. ApoA1 was added to the suspension, which was then sonicated in a cold bath and filtered (0.22 µm, Millipore) to obtain lipoprotein nanoparticles.

**The nanoparticles were imaged under a Cryo-TEM (FEI Tecnai F20).*



Cryo-TEM image of microfluidics method



Cryo-TEM image of the thin-film hydration method

Conclusion and Discussion

A series of NOD–platinum conjugates were successfully synthesized and fully characterized. Preliminary cytotoxicity and immunocytochemistry assays in 4T1 cells identified compound 15a-B as the lead candidate, exhibiting the strongest cytotoxicity and ICD-inducing activity among the conjugates tested. A tumor-targeting peptide-modified lipoprotein nanoparticle is currently being developed using two approaches, with the microfluidics method yielding a smaller and more uniform particle size than the conventional thin-film hydration method. This drug-loaded, tumor-targeted nano-delivery system is expected to offer three synergistic advantages: selective tumor targeting through peptide–receptor-mediated recognition, GSH-triggered intratumoral drug release, and multi-mechanistic antitumor activity via tumor vasculature normalization and ICD induction.

Acknowledgement

This work was supported by the Health Research Council of New Zealand (HRC-22/821) and the Auckland Medical Research Foundation (AMRF-1124013). We thank Adrian Blaser, Sisira Kumara, Sree Sreebhavan, Adrian Turner, and Karin Tan for their expert technical support, and gratefully acknowledge Jingjing Wang, Wilson Sun, Jiney Jose, Kenneth Sue, Amir Ashoorzadeh, Daniel Conole, Yuge Liu, Kaiyun Yang, Marvin Liu, Ziyue Li, Lizhuo Song, and Mahima Bansal for their valuable contributions and support. Special thanks to Sata Tech.

Contact author:
Yuchen He
(Serena)
PhD Candidate
MRes
BSc

yuchen.he@auckland.ac.nz