Development of a Smart Dual Acting Drug Delivery System (SDADDS)

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Abstract

The present-day challenge of delivering anti-cancer agents selectively to tumor cells to mitigate systemic toxicity has led to greater focus on drug delivery research using nanoscale carriers. Despite progress in preclinical studies, the therapeutic effects have not lived up to their expectations in the clinical setting. Though promising, these systems typically exploit passive delivery of a single therapeutic to the target tissue, for example, by the encapsulation of drugs in carrier systems followed by drug release under an external trigger. Our project addresses this issue through the design and synthesis of a Smart Dual Acting Drug Delivery System (SDADDS) consisting of monodisperse bifunctional nanocarriers capable of synergistic targeting of multiple drivers of cancer thereby overcoming current limitations to treating cancer.

Triple negative breast cancer (TNBC), accounts for 10-15% of all breast cancers. TNBC is a multidriver disease that grows sporadically due to it having no selective actionable dominant target. This has caused no target therapy to be approved, thus making it an excellent model to explore the efficiency of SDADDS.

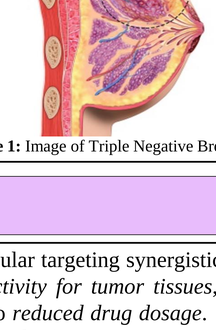


Figure 1: Image of Triple Negative Breast

Objectives

This study aims to utilize two different modes of cellular targeting synergistically that would not only offer superior therapeutic *selectivity for tumor tissues*, but would also decrease chemotherapeutic toxicity due to *reduced drug dosage*. The SDADDS in theory should both target the overexpressed TAM receptors on tumor cells and deliver the therapeutic through nanomaterials to increase the bioavailability and decrease chemotherapeutic toxicity.

Design Mechanism

The SDADDS will comprise of A) extracellular receptor targeting through polyvalent binding to increase selective binding to cancerous cells and B) photocaged linkers that will allow a high local concentration of the anti-neoplastic agent to be released in close proximity to the target tissue through controlled photorelease (**Figure 2**). RU301/302 (**Figure 3**) have been identified as inhibitors if AXL receptors which belong to the TAM family. They will first be modified for dendron attachment appropriately to ensure receptor binding and inhibition functions are maintained (**Figures 4 and 5**).

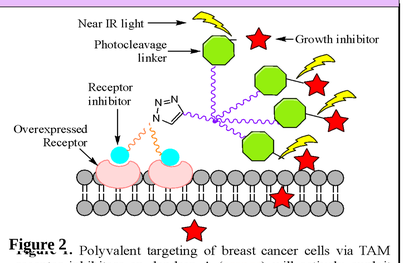


Figure 2. Polyvalent targeting of breast cancer cells via TAM receptor inhibitors on dendron A (orange) will actively exploit the overexpression of the receptor. Photomediated release of the therapeutic on dendron B (purple) will provide the second phase of selective drug delivery to tumor cells.

The second step is to ensure that the photocaged dasatinib complex (dendron B) can be controlled through photorelease. Once uncaged, the drug molecule dasatinib will be released locally targeting the Src intracellular pathway. The final step of the strategy is linking the TAM inhibitor and the coumarin-dasatinib dendrimers together to create the bifunctional targeted drug delivery system (SDADDS), that should effectively inhibit growth of the target cancer cells overexpressing TAM receptors.

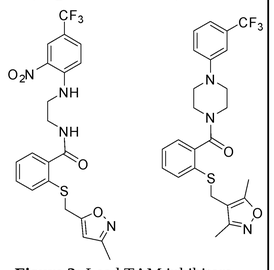


Figure 3: Lead TAM inhibitors RU301 and RU302, repress cellular proliferation

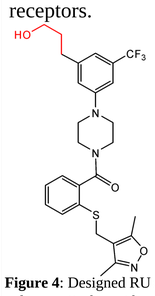


Figure 4: Designed RU analog with polymer-attachment handle position optimized for minimal binding interference

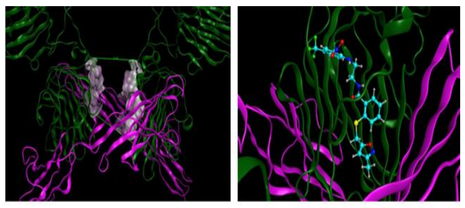


Figure 5 Ribbon models of the dimer AXL receptor-Gas6 complex is shown. (A) The distance between the binding sites identified by Kimani *et al*[6] as a measure of Dendrimer A chain lengths. (B) Binding mode of RU-302 in the binding site that helps in identifying the most suitable position for dendrimer A attachment.

Synthesis of Fluorescent Dendrimer Model

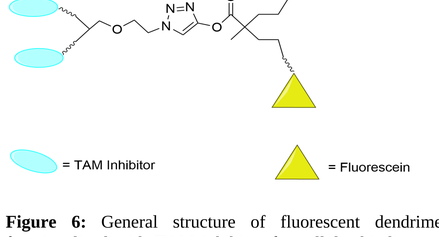
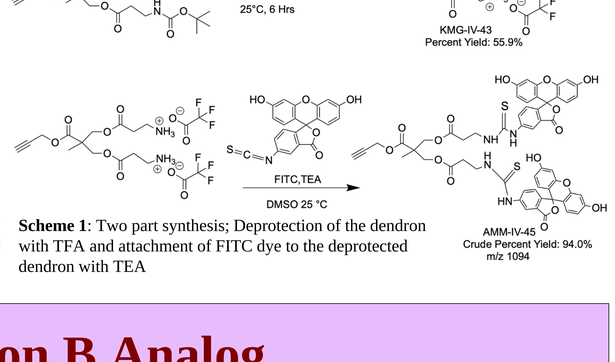


Figure 6: General structure of fluorescent dendrimer functionalized with TAM inhibitor for cellular binding and time lapse studies.



Scheme 1: Two part synthesis; Deprotection of the dendron with TFA and attachment of FITC dye to the deprotected dendron with TEA

Dendron B Analog

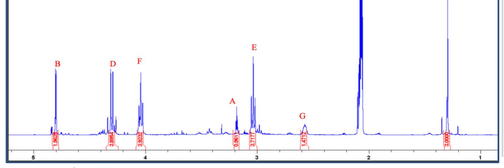


Figure 7: ^{1}H NMR results confirming the deprotection of the dendron. (Scheme 1, step 1)

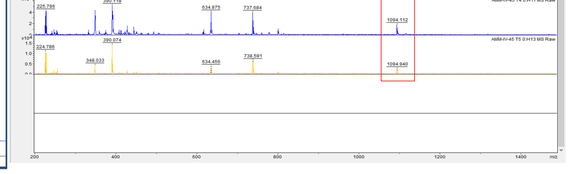
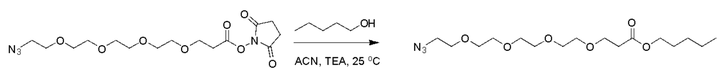


Figure 8: Mass spectrometry results from scheme 1 conjugation (Scheme 1, Step 2)

Dendron A Analog



Scheme 2: Synthesis; Conjugation of Azide-PEG4-NHS and 1-pentanol

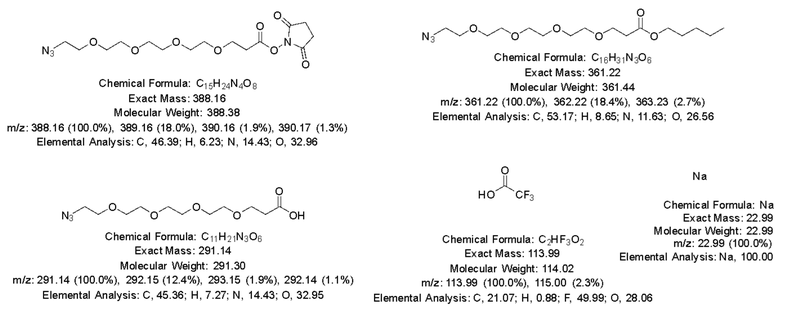
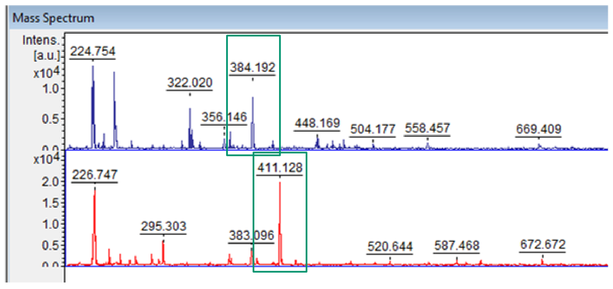
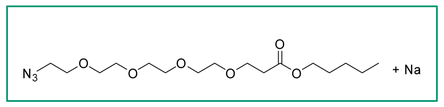


Figure 9: Possible Products of Scheme 2 reaction.





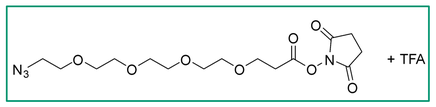


Figure 10: MALDI-ToF Mass spectrometry results from scheme 2

Results

Dendron B: The fluorescent dendron was synthesized via a two-step process. First, the boc-protected terminal amines were deprotected using TFA. The deprotection was confirmed by ^{1}H NMR and MALDI-ToF. The deprotected intermediate product was then conjugated with the fluorescent dye, FITC. The reaction was monitored via MALDI-ToF and showed successful attachment of FITC to both arms of the deprotected dendron. Product purification was attempted using size exclusion via Sephadex LH-20 but was unsuccessful with no product being isolated. No aromatics were detected in ^{1}H or ^{13}C NMRs.

Dendron A: Azide-PEG4-NHS was conjugated to 1-pentanol in order to determine reaction and purification conditions to be used when conjugating the RU analogs to the 2-arm PEG for creation of dendron A. Initial attempts at conjugating 1-pentanol to the NHS-PEG was unsuccessful. The addition of triethylamine (TEA) as a scavenger base resulted in successful attachment as confirmed by MALDI-ToF.

Conclusions/ Next Steps

- The synthesis of the modified TAM inhibitors is underway and will be attached to dendron A once completed using the developed reaction conditions.
- We are in the process of developing purification methods for the dye conjugated fluorescent dendron which will ultimately be attached to dendron A using click chemistry and subsequently used in cellular binding assays.
- Once the fluorescent tag model is complete we will perform the cellular uptake and time-lapse studies to confirm the RU inhibitor binding to TAM(+) cells.
- The long term objective is to make the system customizable so that it can target varying pathways that occur in different cancer type, thus allowing for the creation of personalized treatment for late-stage cancer patients.

References

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