Identification of Novel Inhibitors of Vascular Calcification

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Objective

1. **Screening a library of IP6 analogues for novel inhibitors of vascular calcification.**
2. **Investigating the hit series for markers of metabolic stability and toxicity in order to identify lead compounds**.

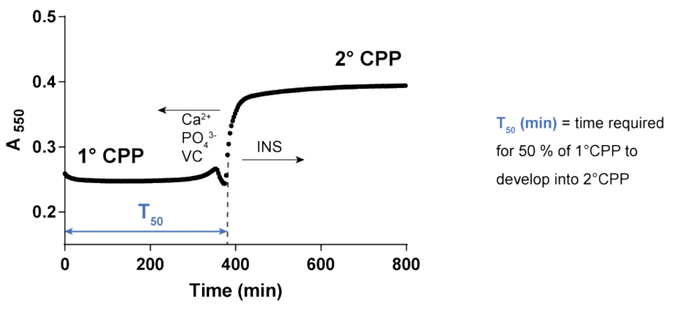
Introduction

Calcification is a multifactorial, dynamic process that is tightly regulated in the human body. Loss of homeostasis may lead to **vascular calcification (VC)**, which is highly prevalent in aged, diabetic or chronic kidney disease patients and associated with **major adverse cardiovascular events**. Currently, there are no pharmacological therapies approved for the **treatment or prevention** of VC.

Circulating protein-mineral nanocrystals, termed **calciprotein particles (CPPs)** are increasingly recognised as markers for mineral stress.

A novel clinical CPP-based test for measuring the propensity for calcification of serum, called **T_{50} assay**, strongly correlates T_{50} with patient survival (Fig.1).[1]

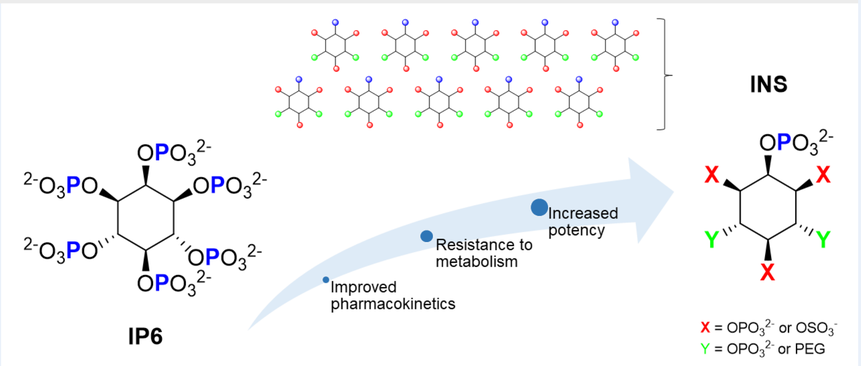
Fig. 1: The T_{50} screening assay



***Myo*-inositol hexaphosphate (IP6)** is an ubiquitous, anionic natural molecule and in **clinical development** for the treatment of VC in end stage renal disease patients.

The screening library consists of ten IP6 analogues with phosphates substituted for poly(ethylene glycol) (PEG) chains or sulphate groups (INS).

Fig. 2: The screening library



Results and Discussion

1. Screening assay & hit identification

Fig. 3: Compound library screening

The INS compounds' activity was defined as the concentration necessary to delay T_{50} to 350 min (c350).

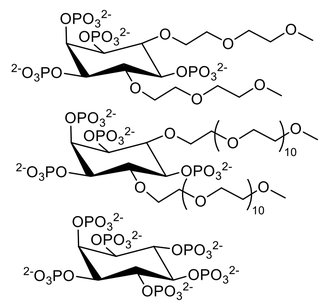
INS-3041 displayed the highest activity, with **c350 = 3.8 ± 0.3 µM**, being ten times as potent as IP6.

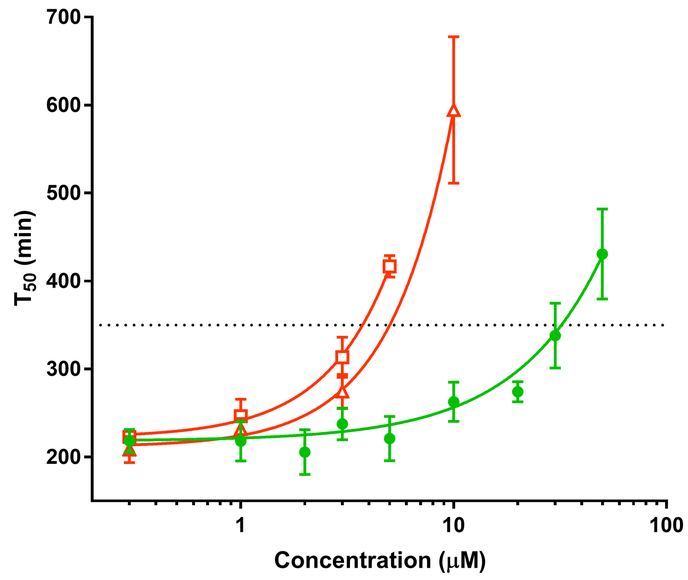
Legend:

INS-3001

INS-3041

IP6





2. Characterising hit series: Stability and toxicity markers

Fig. 4: Enzyme metabolism

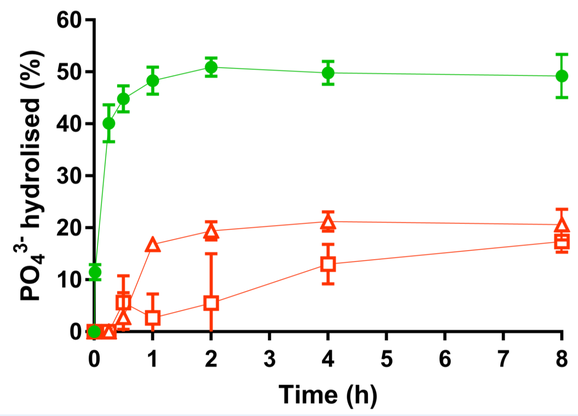


Fig. 5: Stability in human serum

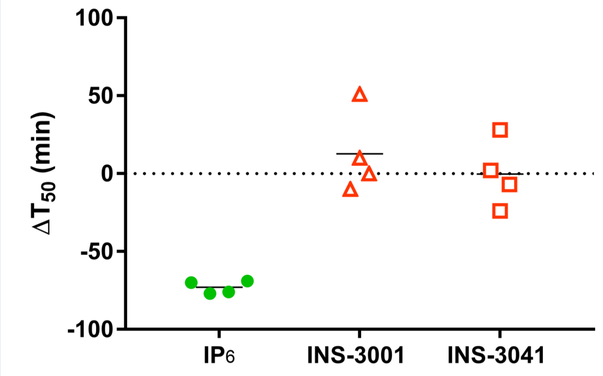


Fig. 6: Free serum Ca^{2+}

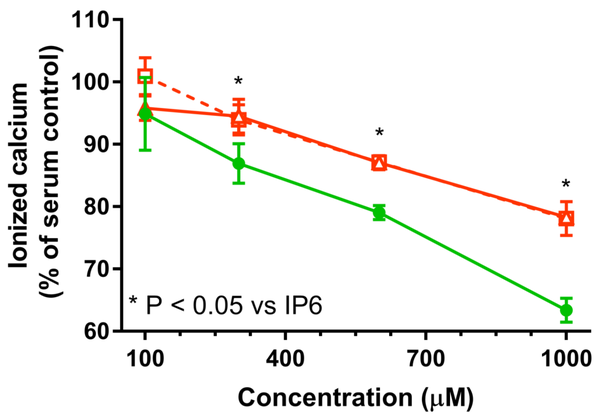
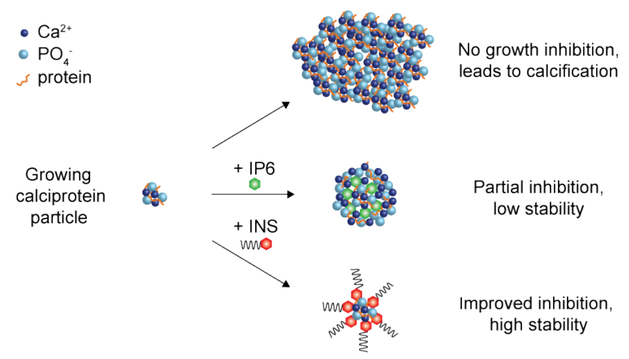


Fig. 7: Proposed mechanism of action



Materials and Methods

T_{50} assay Human serum was spiked with Ca^{2+} and PO_4^{3-} to induce CPP formation. Increasing concentrations of INS were added, and the time required for primary CPPs to develop into larger, crystalline secondary CPPs was detected by time-resolved changes of light scattering.

Enzyme assay INS were incubated with 3-phytase at pH 7.4 and 37 °C. PO_4^{3-} hydrolysis was monitored via formation of a phosphomolybdate malachite green complex.

Serum incubation assay INS were incubated for 4 h in fresh human serum and thereafter tested in the T_{50} assay.

Free serum Ca^{2+} assay Human serum was spiked with increasing concentrations of INS, and free ionic Ca^{2+} was measured with the *o*-cresolphthalein method.

Conclusion

PEGylated IP6 analogues were identified as novel potential **inhibitors of VC**.

INS-3041 & INS-3001 displayed highest efficacy and **resistance to metabolism**.

INS displays **low reduction in free serum Ca^{2+}**, which is related to potential toxic effects.

Outlook

Study the **inhibitory effect of INS on *in vitro* calcification** of human vascular smooth muscle cells (VSMCs).

Perform ***in vivo* studies** (Pharmacokinetics, toxicity, efficacy).

References and Acknowledgements

[1]Pasch, Andreas et al. 2012. "Nanoparticle-Based Test Measures Overall Propensity for Calcification in Serum." Journal of the American Society of Nephrology 23(10): 1744–52.

This work was financially supported by the Commission for Technology and Innovation (25174.1 PFLS-LS).

Abbreviations

VC vascular calcification, CPP calciprotein particle, IP6 *myo*-inositol hexaphosphate, PEG poly(ethylene glycol), INS PEGylated *myo*-inositol hexaphosphate analogues, VSMCs vascular smooth muscle cells

Drug Formulation & Delivery