Development and Biological Evaluation of Novel α-Helix Mimetic Prodrugs as Leads for Prostate Cancer Treatment

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1| INTRODUCTION

The androgen receptor (AR) transcription pathway plays a critical role in the proliferation of prostate cancer (PCa) cells.[1] Consequently, traditional PCa treatments focus on androgen suppression and deprivation by the use of AR antagonists and castration. These reduce AR activity however the onset of resistance is inevitable.[2]

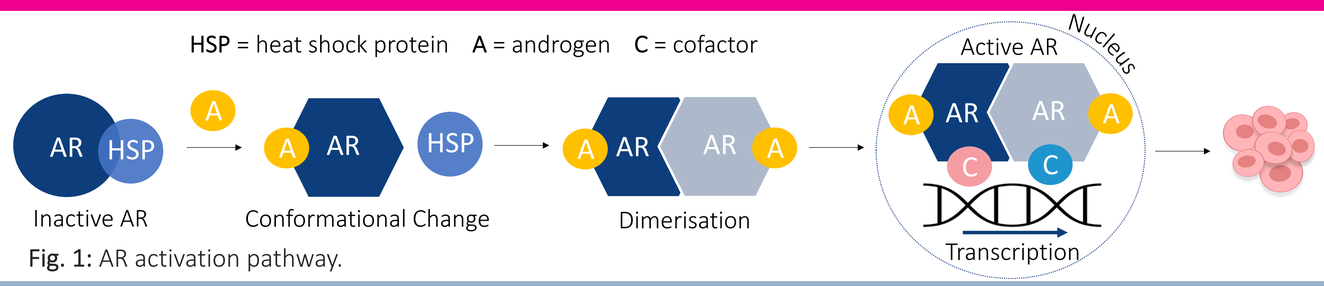

Fig. 1: AR activation pathway.

2| SPIVEY α-HELIX MIMETIC

Our research has focused on the design and development of a novel small drug-like molecule, a coactivator binding inhibitor (CBI), targeting the activating function 2 domain (AF2) within the AR ligand-binding domain.[3]

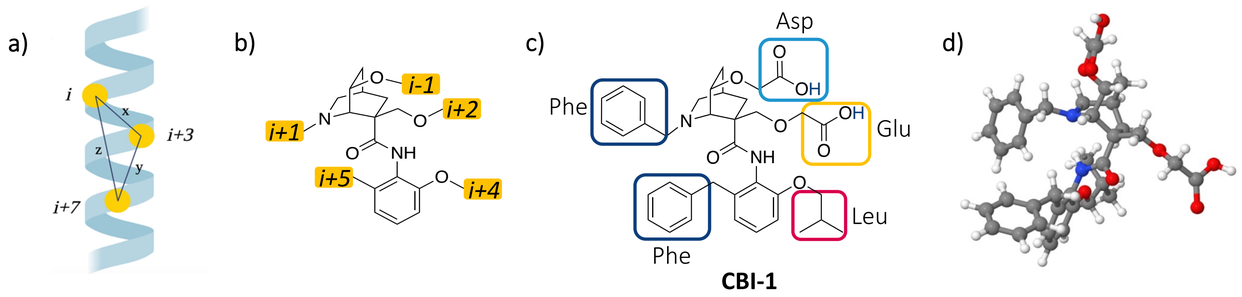

Fig. 2a: Protein α-helix structure. **b**: **CBI** plug and play scaffold. **c**: **CBI-1**, an Asp-Phe-Glu-X-Leu-Phe (FXXLF) Spivey α-helix mimetic structure. **d**: Computationally optimized energy minimized 3D model of **CBI-1**.

CBI-1 contains an α-helix mimetic scaffold imitating the FXXLF epitope of AF2-binding coactivators. With a relatively rigid 3D structure, it is anticipated to be resistant to proteolysis, and to bind to the target with high affinity and at a reduced entropic cost, inhibiting the AR and hence the development of PCa.

3| SYNTHESIS

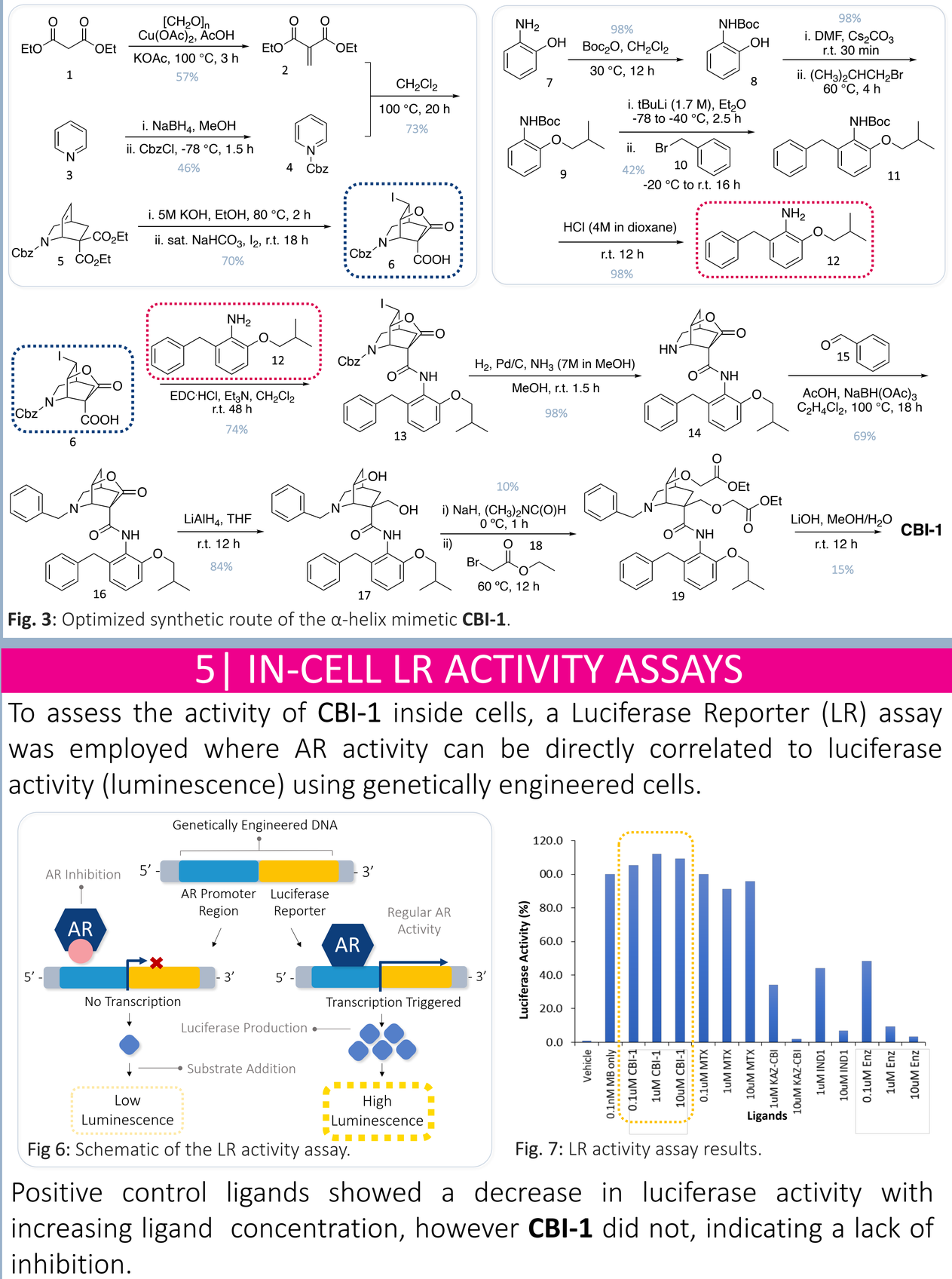

Fig. 3: Optimized synthetic route of the α-helix mimetic **CBI-1**.

4| IN-VITRO FP BINDING ASSAYS

To assess binding of **CBI-1** to the AR, a Fluorescence Polarisation (FP) assay was developed. Exploiting the inverse relationship between molecular size and rotational speed, the extent of light depolarisation by a fluorophore-labelled ligand was monitored and correlated to AR binding.

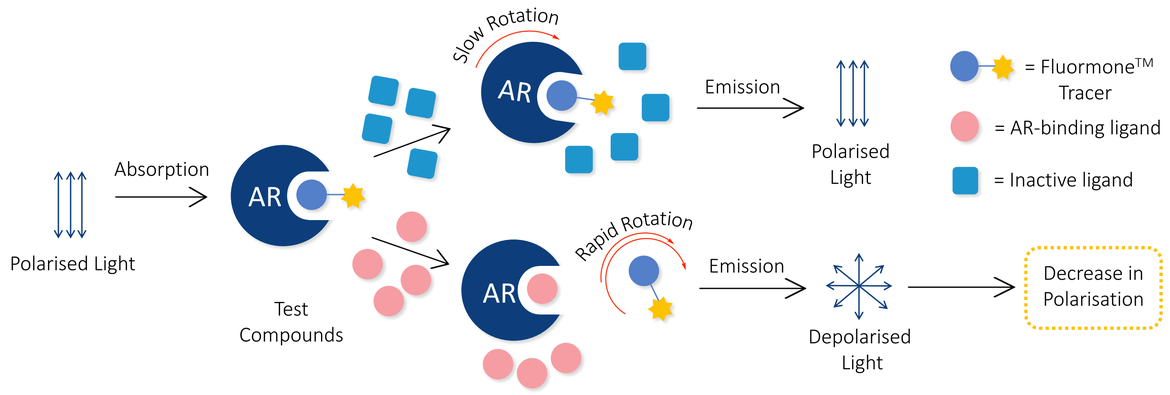

Fig. 4: Schematic of the FP assay where decrease in polarisation indicates binding to the AR.

Alongside **CBI-1,** several positive control ligands known to bind to the AR were screened, all of which resulted in a decrease in polarisation indicating successful binding to the AR.

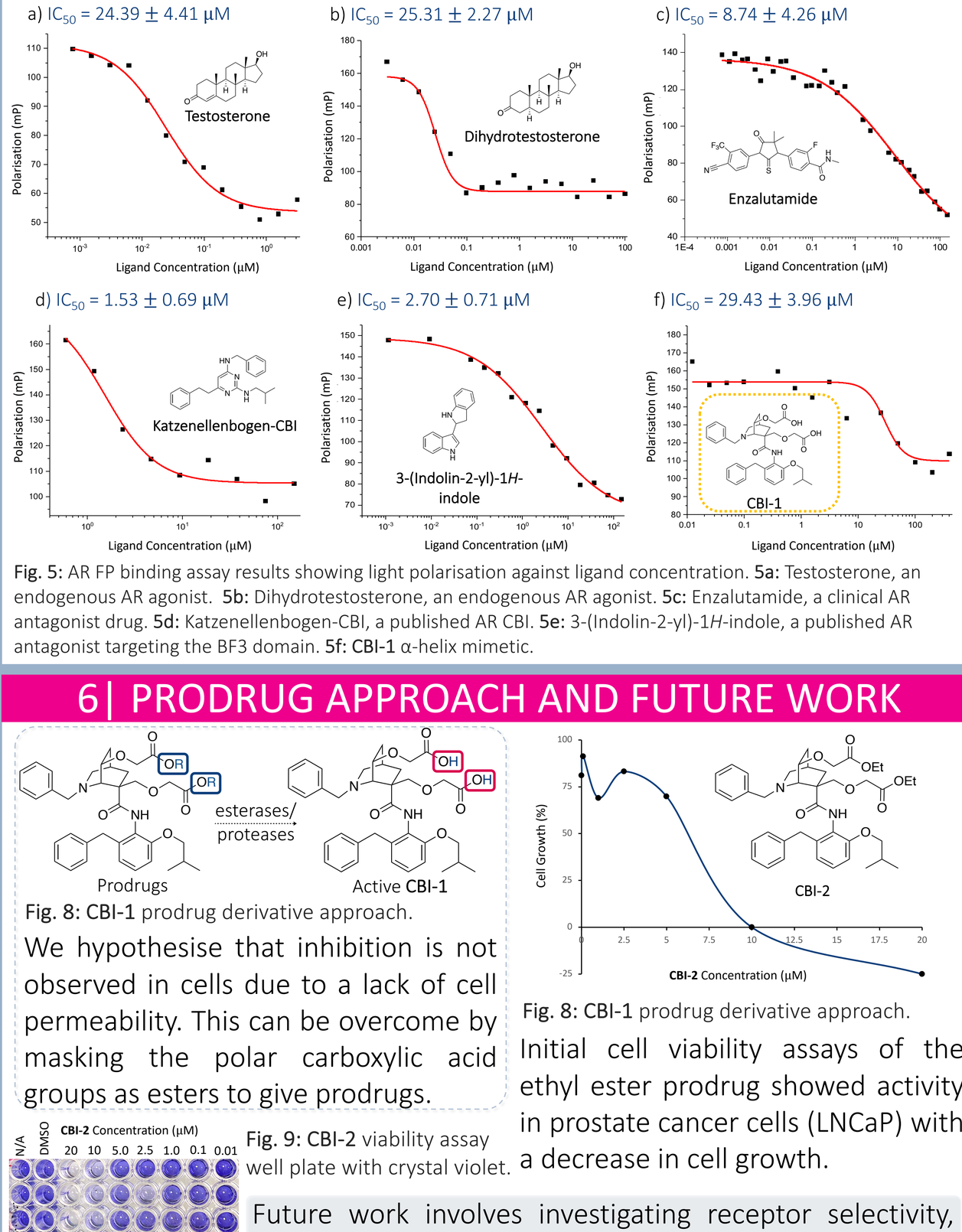

Fig. 5: AR FP binding assay results showing light polarisation against ligand concentration. **5a:** Testosterone, an endogenous AR agonist. **5b:** Dihydrotestosterone, an endogenous AR agonist. **5c:** Enzalutamide, a clinical AR antagonist drug. **5d:** Katzenellenbogen-CBI, a published AR CBI. **5e:** 3-(Indolin-2-yl)-1*H*-indole, a published AR antagonist targeting the BF3 domain. **5f: CBI-1** α-helix mimetic.

5| IN-CELL LR ACTIVITY ASSAYS

To assess the activity of **CBI-1** inside cells, a Luciferase Reporter (LR) assay was employed where AR activity can be directly correlated to luciferase activity (luminescence) using genetically engineered cells.

Fig 6: Schematic of the LR activity assay.

Fig. 7: LR activity assay results.

Positive control ligands showed a decrease in luciferase activity with increasing ligand concentration, however **CBI-1** did not, indicating a lack of inhibition.

6| PRODRUG APPROACH AND FUTURE WORK

Fig. 8: CBI-1 prodrug derivative approach.

We hypothesise that inhibition is not observed in cells due to a lack of cell permeability. This can be overcome by masking the polar carboxylic acid groups as esters to give prodrugs.

Fig. 9: CBI-2 viability assay well plate with crystal violet.

Fig. 8: CBI-1 prodrug derivative approach.

Initial cell viability assays of the ethyl ester prodrug showed activity in prostate cancer cells (LNCaP) with a decrease in cell growth.

Future work involves investigating receptor selectivity, tests on prostate tumour explant models, and X-ray crystallography studies to confirm mode of binding.

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