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Imaging of prostate cancer: optimizing affinity to prostate specific membrane antigen by spacer modifications in a tumor spheroid model

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ABSTRACT

Early diagnosis of prostate cancer (PCa) is crucial for staging, treatment and management of patients. Prostate specific membrane antigen (PSMA), highly over-expressed on PCa cells, is an excellent target for selective imaging of PCa. In recent years, various scaffolds have been explored as potential carriers to target diagnostic and therapeutic agents to $PSMA^+$ tumour cells. Numerous fluorescent or radioisotope probes linked via a peptide linker have been developed that selectively binds to PCa cells. However, there are very few reports that examine the effects of chemical modifications in the peptide linker of an imaging probe on its affinity to PSMA protein. This report systematically investigates the impact of hydrophobic aromatic moieties in the peptide linker on PSMA affinity and *in vitro* performance. For this, a series of fluorescent bioconjugates **12**–**17** with different aromatic spacers were designed, synthesized, and their interactions within the PSMA pocket were first analysed *in silico*. Cell uptake studies were then performed for **12**–**17** in $PSMA^+$ cell lines and 3D tumour models *in vitro*. Binding affinity values of **12**–**17** were found to be in the range of 36 to 157.9 nM, and **12** with three aromatic groups in the spacer exhibit highest affinity ($K_D = 36$ nM) compared to **17** which is devoid of aromatic groups. These studies suggest that aromatic groups in the spacer region can significantly affect deep tissue imaging of fluorescent bioconjugates. Bioconjugate **12** can be a promising diagnostic tool, and conjugation to near-infrared agents would further its applications in deep-tissue imaging and surgery.

Abbreviations: AMBA: aminomethyl benzoic acid; DCM: dichloro methane; DIPEA: N,N-Diisopropylethylamine; DMF: dimethyl formamide; DUPA: 3-(1,3-dicarboxypropyl)-ureido]pentanedioic acid; DPBS: Dulbecco's phosphate buffer saline; EDTA: ethylenediamine tetra-acetic acid; FACS: fluorescence-activated cell sorting; FDA: fluorescein diacetate; FGS: fluorescent guided surgery; HPLC: high performance liquid chromatography; HRMS: high resolution mass spectrometry; LNCaP: lymph node prostate cancer metastasis; MFI: mean fluorescence intensity; NMR: nuclear magnetic resonance; PI: propidium iodide; 2-PMPA: 2-(phosphonomethyl)pentanedioic acid; PCa: prostate cancer; PSMA: prostate specific membrane antigen; PyBOP: benzotriazol-1-yloxytripyrrolidinophosphonium hexafluorophosphate; SAR: structure activity relationship; SEER: surveillance, epidemiology and end results; SPPS: solid phase peptide synthesis; RP-PFP: reverse phase-pentafluoro phenyl; RPMI 1640: Roswell park memorial institute; 2D: two-dimensional; TFA: trifluoroacetic acid; TIPS: triisopropyl silane; TLC: thin layer chromatography; 3D: three-dimensional

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1. Introduction

Prostate cancer (PCa) continues to be an enormous medical challenge to the large size of the male population, attaining notoriety of being the fifth leading cause of death and the most commonly diagnosed cancer in males worldwide (Siegel et al., 2019). India also has seen a substantial increase in the incidence rate of PCa to 29.8% from 1990 to 2016 (Dhillon et al., 2018). Apart from being an increasing health concern, PCa is also a significant economic burden on health-care systems worldwide. A nationwide study, based on Surveillance, Epidemiology and End Results (SEER)-Medicare program, estimated the costs for treatment and morbidity management within three years of diagnosis to be $14,453 per patient in the USA. The estimated three-years cost to Medicare for detection of PCa amounts to $1.2 billion (Trogdon et al., 2019).

Radical prostatectomy is the gold-standard curative method for localized, locally advanced, and metastatic PCa (Kim & Bullock, 2018). It has demonstrated a significant survival advantage to patients over radiotherapy, based on Munich Cancer Registry and SEER database records (Culp et al., 2014; Gratzke et al., 2014; Hamdy et al., 2016). However, surgical intervention is a hurdle for prostate malignancy,

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