Investigation into the metabolic stability of ^{18}F-labeled PSMA inhibitor derivatives bearing aryl-fluorosulfates for PET tracer development applications

Guillermo Luque Consuegra[1], Austin Craig[1], Jürgen Kögler[1], Martin Ullrich[1], Cornelius K. Donat[1], Klaus Kopka[1,2], Sven Stadlbauer[1]

[1] Institute of Radiopharmaceutical Cancer Research, Helmholtz-Zentrum Dresden-Rossendorf, Dresden, Germany;

[2] Faculty of Chemistry and Food Chemistry, School of Science, Technische Universität Dresden, Dresden, Germany

Introduction

- Radiolabeled PSMA inhibitors are used in clinics for non-invasive molecular imaging and treatment of prostate cancer [1].
- The sulfur [^{18}F]fluoride exchange ([^{18}F]SuFEx) radiolabeling approach has had promising results to prepare ^{18}F-labeled radiotracers with high RCY and RCP in absence of metal additives and under mild conditions [2,3].
- Recently, the [^{18}F]SuFEx radiolabeling approach was used on FAP inhibitors. Fibroblast Activation Protein alpha (FAPα) inhibition potency was not affected by the addition of aryl-fluorosulfates [4]. However, defluorination in serum (37 ºC) and *in vivo* was observed.
- This work aims to study the influence of aryl-fluorosulfates on PSMA-inhibitors with ([^{18}F]**2**) and subsequently (not this work) without ([^{18}F]**4**) electron withdrawing group.
- Both radioligands will be evaluated with respect to their relative stability to aid future radiotracer development using the [^{18}F]SuFEx radiofluorination approach.

Methods

- Two step radiosynthetic preparation of [^{18}F]**2** *via* the [^{18}F]SuFEx reaction and deprotection. The identity and radiochemical purity (RCP) of [^{18}F]**2** was confirmed by U-HPLC (Fig. 1A).
- Real-time radioligand binding experiments of [^{18}F]**2** on LNCaP cells (Fig. 1B) were performed using LigandTracer Yellow (Ridgeview Instruments AB).
- Stability studies were performed by incubating a sample of [^{18}F]**2** in three different media: PBS buffer (pH 7.4), EtOH and human serum (37 ºC) (Fig. 1C).
- Xenograft tissue binding studies were performed using [^{18}F]**2** and [^{68}Ga]Ga-PSMA-11 as the reference tracer (Fig. 1D).
- PET imaging of LNCaP tumor-bearing mouse using [^{18}F]**2** (8.5 MBq equivalent to 1.7 nmol) (Fig. 1E)
- PET imaging of LNCaP tumor-bearing mouse using [^{18}F]**2** and blocking agent 2-PMPA (6.6 µmol; ~4×10^3-fold molar excess) (Fig. 1F)

Results

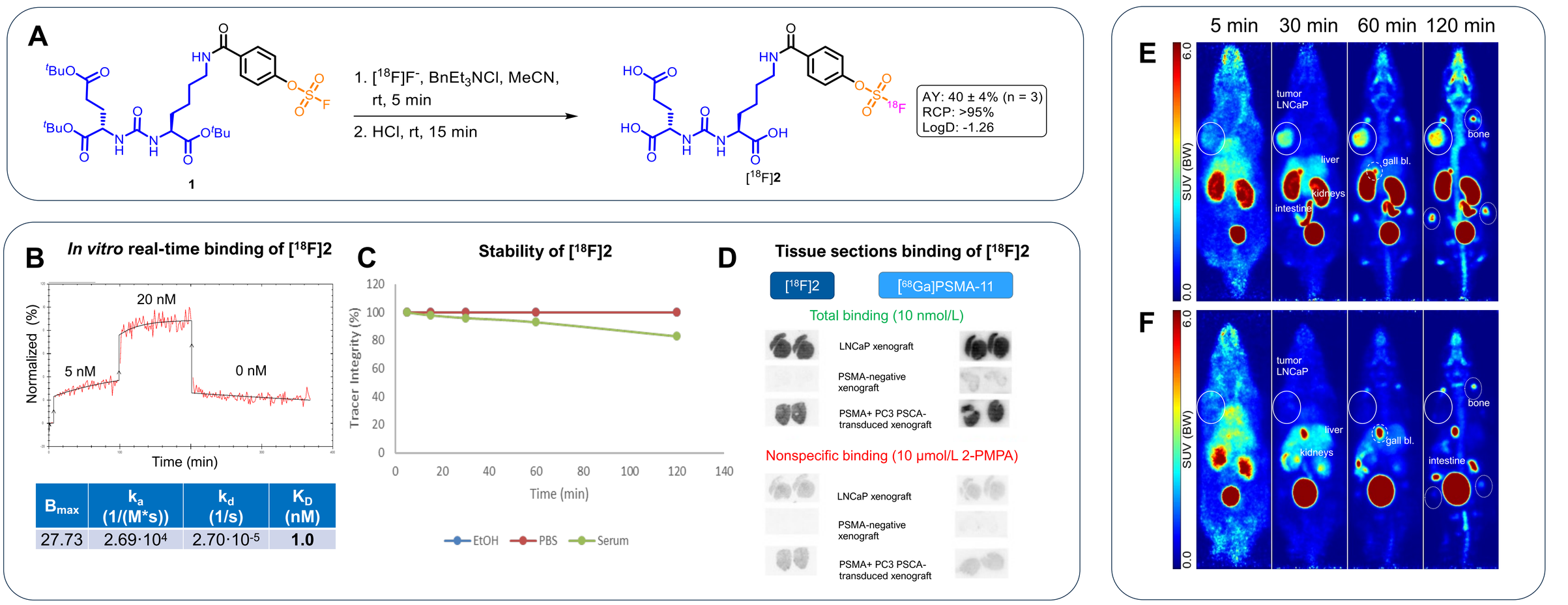

Figure 1. **A)** Radiosynthesis of ^{18}F-labeled PSMA inhibitor [^{18}F]**2**; **B)** *In vitro* real-time binding experiment using [^{18}F]**2** and LNCaP cells (1:1 binding mode accounting for bulk index); **C)** Stability of [^{18}F]**2** in various media; **D)** Autoradiography of [^{68}Ga]Ga-PSMA-11 and [^{18}F]**2** in various xenograft tissue sections; **E)** PET experiments of [^{18}F]**2** (8.5 MBq, equivalent to 1.7 nmol) in an LNCaP tumor bearing mouse (tumor regions indicated); **F)** PET experiments of [^{18}F]**2** in an LNCaP tumor-bearing mouse with blocking agent 2-PMPA (6.6 µmol) (tumor region indicated).

- Radiosynthesis: An ^{18}F-labeled PSMA inhibitor ([^{18}F]**2**) was obtained with activity yield (AY) of 40 ± 3% (n = 3) and >95% RCP in 60 min.
- Binding affinity was found to be 1 nM in real-time radioligand binding experiments. The low dissociation rate constant indicates internalization. Affinity for [^{18}F]**2** was found to be in the nanomolar range as ^{18}F-PSMA-1007 [5].
- Stability experiments show partial (~20% in 120 min) degradation in human serum but no degradation in PBS or EtOH.
- Tissue sections from of LNCaP xenografts show [^{18}F]**2** accumulation in a pattern similar to [^{68}Ga]Ga-PSMA-11.
- *In vivo* PET images show tumor accumulation, but apparent tracer degradation (defluorination) occurs shown by bone accumulation ~60 min post-injection.
- Competitive blocking experiment (Fig. 1F) (2-PMPA; 6.6 µmol) show that tumor accumulation is target-specific.

Conclusions & Further work

- [^{18}F]**2** shows good binding kinetics compared to known radiotracers (1 nM) but ~20% defluorination occurs in human serum after 120 min.
- [^{18}F]**2** binds to LNCaP xenograft sections in a similar pattern as [^{68}Ga]Ga-PSMA-11.
- PET imaging experiments show defluorination but also accumulation in LNCaP tumor xenografts (SUV_{max} = 4.4).
- Further work: Radiosynthesis of [^{18}F]**4** starting from precursor **3** (Fig. 2).

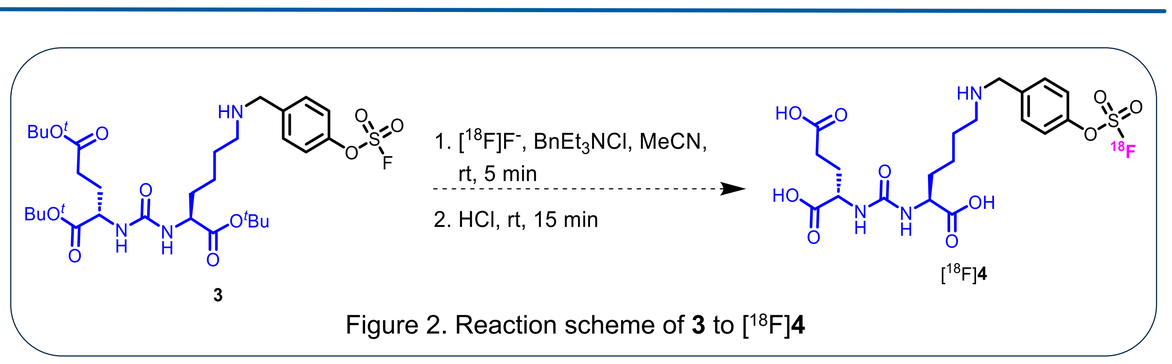

Figure 2. Reaction scheme of **3** to [^{18}F]**4**

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Institute of Radiopharmaceutical Cancer Research
Dr. Guillermo Luque Consuegra · g.luque-consuegra@hzdr.de · www.hzdr.de