Synthetic methodologies toward fibroblast activation protein inhibitors and radiolabelling with ^{68}Ga, ^{64}Cu or ^{89}Zr

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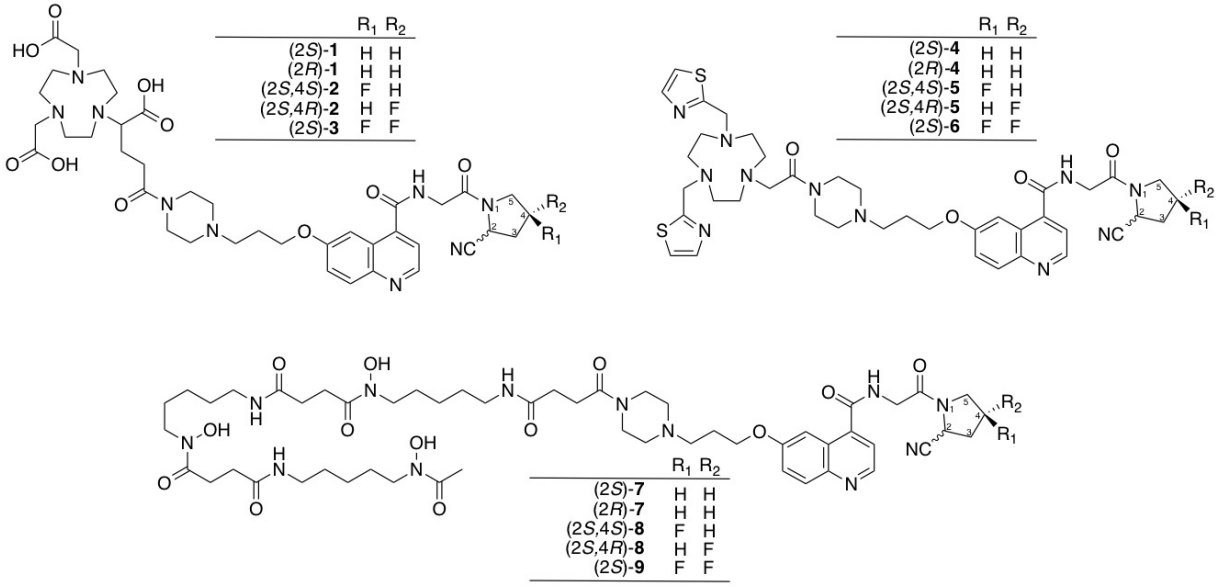
In healthy adult tissues either no or only insignificant levels of fibroblast activation protein (FAP) are detected in uterus, cervix, placenta, breast and skin. Nonetheless, FAP overexpression is significant on reactive stromal fibroblasts in more than 90% of common human epithelial cancers[1] and boosts malignant tumourigenesis. Tumour growth may be diminished by FAP inhibition[2] and several quinoline-based FAP-inhibitors (FAPIs) have been proven to bind specifically to the enzymatic domain of FAP.[3] Radiolabelled quinoline-based FAPI compounds have been reported and show promising results in early-phase clinical trials, but their synthesis remains challenging.[4] Here, we present synthetic routes toward a series of novel FAPI compounds functionalized with different chelates including NODAGA, Hno2th1a and DFO for labelling with ^{68}Ga, ^{64}Cu or ^{89}Zr, respectively.

Five different FAPI units were synthesised by multiple standard organic chemistry reactions combining the key pyrrolidine and quinoline moieties *via* classic amide bond forming reactions. FAPI units were coupled to NODAGA, Hno2th1a and DFO ligands and isolated by reverse-phase HPLC as off-white amorphous solids in 12–18% overall yields. The non-radioactive complexes were prepared by the reaction of the ligands with $Ga(NO_3)_3$, $CuCl_2$ or $ZrCl_4$ and radioactive complexes containing the ^{68}Ga and ^{89}Zr nuclides were also prepared (RCP > 99%; calculated from radio-iTLC), and analysed by radio-HPLC. Single radioactive species were obtained for NODAGA chelate-bearing ligands but DFO bearing-chelate ligands showed potential isomerization which is likely due to the chirality of the isomers formed at the metal coordination complex which results in diastereomers. Additional radiolabelling and biochemical studies are underway to select the most promising radiotracer for PET imaging in animal models.



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