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Short communication

Structural modification of FAPI-04: Substitution of DOTA with HYNIC for 99mTechnetium radiolabeling

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Introduction

Fibroblast activation protein inhibitor (FAPI) ligands represent an important class of radiopharmaceuticals for imaging and potential therapy of cancer-associated fibroblasts. While FAPI-04 is commonly labeled with radionuclide Lutetium-177(^{177}Lu) via the DOTA chelator for therapeutic applications. Its adaptation for diagnostic imaging with technetium-99m (^{99m}Tc) requires structural modification of the chelator system. In this context, substitution of DOTA with hydrazinonicotinamide (HYNIC) enables efficient complexation with ^{99m}Tc and supports ready to use kit-formulation suitable for routine clinical use (Jiang et al., 2023; Yang et al., 2023).

The aim of this study is to develop a kit-based formulation enabling dual radiolabeling of a single FAPI-04 molecular scaffold for both diagnostic (^{99m}Tc) and therapeutic (^{177}Lu) applications.

Materials and methods

Structural modification and synthesis of HYNIC-FAPI-04

The original FAPI-04 structure, commercially available containing DOTA for ^{177}Lu radiolabeling, was chemically modified by replacing the macrocyclic DOTA chelator with hydrazinonicotinamide (HYNIC). This modification was designed to preserve the quinoline-based pharmacophore responsible for FAP targeting while introducing a bifunctional chelator suitable for ^{99m}Tc coordination. Conjugation was performed via standard peptide coupling chemistry, followed by purification and structural confirmation of the modified ligand (Jiang et al., 2023).

Radiolabeling with ^{99m}Tc and quality control

Radiolabeling of HYNIC-FAPI was performed using a stannous-reduced ^{99m}Tc system in the presence of appropriate co-ligands as EDDA and Tricine, enabling formation of a stable ^{99m}Tc–HYNIC complex. Labeling efficiency was evaluated

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under optimized reaction conditions. Quality control included radiochemical purity assessment by chromatographic methods and in vitro stability testing of radiolabelled product. The labeling results was conceptually compared with the ^{177}Lu-DOTA-FAPI product, highlighting key differences in coordination chemistry, stability, and intended diagnostic versus therapeutic application (Yang et al., 2023).

Results and discussion

Radiolabeling of DOTA-FAPI-04 with ^{177}Lu demonstrated consistently high efficiency, with radiochemical yields exceeding 98% across all evaluated ITLC systems. This confirms the strong stability and favorable coordination chemistry of the DOTA macrocyclic chelator with lutetium(III), where complex formation is governed by thermodynamically stable and kinetically inert metal–ligand interactions. The robustness of this system resulted in excellent reproducibility and minimal sensitivity to minor variations in reaction conditions, supporting its suitability for therapeutic radiopharmaceutical applications (Yang et al., 2023).

In contrast, radiolabeling of HYNIC-modified FAPI-04 with ^{99m}Tc showed more variable radiochemical yields, strongly dependent on reaction conditions and coordination environment. This variability reflects the fundamentally different coordination chemistry of technetium compared to lanthanides, requiring a finely balanced interaction between the HYNIC moiety, reducing conditions, and co-ligand system.

The use of co-ligands such as EDDA, tricine and deep vacuum conditions to avoid oxydation of obtained complex was essential for stabilization of the ^{99m}Tc core and completion of the coordination sphere. The ratio between HYNIC and co-ligands emerged as a critical parameter influencing labeling efficiency, directly affecting the formation of the desired ^{99m}Tc–HYNIC complex versus undesired side species. Additionally, reduction efficiency mediated by $SnCl_2$ played a crucial role in controlling the oxidation state of technetium and subsequent complex formation.

Further analysis showed that parameters such as pH, ligand concentration ratios, and the stability of freshly prepared $SnCl_2$ significantly affected reproducibility. Even minor deviations in these conditions resulted in noticeable fluctuations in radiochemical yield, emphasizing the higher sensitivity of the ^{99m}Tc–HYNIC system compared to the more robust ^{177}Lu–DOTA chemistry.

These findings are consistent with previously reported studies on HYNIC-based technetium labeling systems, where co-ligand-dependent stabilization is essential for achieving high radiochemical purity and in vivo stability (Jiang et al., 2023; Yang et al., 2023). Additionally, the importance of process optimization in radiopharmaceutical formulation and lyophilization is well documented in the literature, particularly for protein-based systems (Rustemi-Ahmeti et al., 2025). Overall, the comparison clearly highlights the superior coordination stability of ^{177}Lu–DOTA-FAPI-04, while demonstrating that optimized HYNIC-based systems can achieve high radiolabeling performance under strictly controlled conditions.

Conclusion

Replacement of DOTA with HYNIC enables successful adaptation of FAPI-04 for ^{99m}Tc radiolabeling, providing a feasible approach toward diagnostic imaging of cancer-associated fibroblasts. This structural modification supports efficient kit-type preparation and expands the theranostic potential of the FAPI platform through complementary use of ^{99m}Tc for imaging and ^{177}Lu for therapy (Jiang et al., 2023; Yang et al., 2023; Rustemi-Ahmeti et al., 2025).

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