

Review article

Synthetic Approach to ^{99m}Tc -labeled SPECT Radiotracers with Multi-nitroimidazoles for Hypoxia

Anh Thu Nguyen^{1,2}, Hee-Kwon Kim^{1,2}

¹Department of Nuclear Medicine, Jeonbuk National University, Jeonju, Korea

²Department of Medical Science, Jeonbuk National University, Jeonju, Korea

*Corresponding Author : Hee-Kwon Kim, Department of Nuclear Medicine, Jeonbuk National University, 20, Geonji-ro, Deokjin-gu, Jeonju-si, Jeollabuk-do, 54907, Rep. of Korea, Tel : +82-63-250-2768, E-mail : hkim717@jbnu.ac.kr

ABSTRACT

Hypoxia, defined as the deficiency of oxygen, is a significant hallmark of cancers presenting in the majority of solid tumors. Detection of tumor hypoxia is essential in cancer diagnosis to prevent cancer progression, metastasis, and resistance to cancer therapies in clinical practices. Single-photon emission computed tomography (SPECT) is one of the methods studied and applied for hypoxia detection with the use of radiolabeled imaging agents in which ^{99m}Tc is the common radioisotope used for radiolabeling. Nitroimidazoles are the hypoxia-targeting moieties presenting in numerous ^{99m}Tc -radiolabeled imaging agents due to their bio-reducible ability in hypoxic environments. Recently, in addition to ^{99m}Tc -labeled radiopharmaceuticals containing one nitroimidazole unit, there has been considerable attention given to ^{99m}Tc -radiopharmaceuticals bearing two or more nitroimidazole units. This review summarizes the synthesis of hypoxia-targeting chelators and radiolabeling processes to produce these ^{99m}Tc -radiopharmaceuticals for SPECT imaging.

Key words Radiopharmaceuticals, SPECT, technetium-99m, hypoxia, tumor

Introduction

Molecular imaging is a non-invasive imaging technique that can monitor biological activities at the cellular and sub-cellular levels [1,2]. Positron emission tomography (PET) and single photon emission computed tomography (SPECT) are the common molecular imaging techniques that have been used in the detection, characterization, and quantification of a variety of diseases such as cancer, heart diseases, and neurodegenerative disorders [3-8]. In SPECT, gamma-emitting radioisotopes are used as the radiotracers to generate radiation that can be detected by gamma cameras from different angles, thus creating three-dimensional images of their accumulation in cells and organs, indicating biological events happening in the body [9,10]. Several gamma-emitting radioisotopes are commonly employed in SPECT imaging, namely, ^{99m}Tc , ^{123}I , ^{133}Xe , ^{201}Tl , and ^{111}In . Among these radioisotopes, ^{99m}Tc is preferable for SPECT imaging because ^{99m}Tc only emits gamma radiation, has a photon energy of 140.5 keV which is ideal for gamma cameras, and the preparation of ^{99m}Tc labeled radiopharmaceuticals is convenient with the use of cold labeling kits [11].

Hypoxia is a phenomenon in which there is an inadequacy of oxygen levels in tissues and cells due to the imbalance of oxygen intake and consumption [12]. In cancer research, hypoxia is recognized as one of the important hallmarks of solid cancer and plays many roles in cancer progression [9,10,13]. In addition, hypoxia is also related to the resistance of cancer cells to therapies and treatment failure [14,15]. Therefore, targeting hypoxia plays an important role in cancer treatment and diagnosis [14]. Because nitroimidazole moieties selectively retain in low-oxygen cells and tissues, they have been widely employed in the development of hypoxia-detection radiopharmaceuticals [16]. Moreover, ^{99m}Tc can create

various complexes with a diverse array of chelates [17].

Besides the common ^{99m}Tc -labeled radiopharmaceuticals containing one moiety of nitroimidazole in the structures, there has been significant interest in the past decades in the development of hypoxia targeting agents that feature ^{99m}Tc -radiopharmaceuticals containing two or more nitroimidazole moieties. This review provides a summary of developments since 2013 in the production of hypoxia-targeting agents labeled with ^{99m}Tc that contain several nitroimidazole units in their structures.

1. Development of ^{99m}Tc -labeled radiotracers with two nitroimidazoles

1. 1. Synthesis of $^{99m}\text{TcN-N4IPDTC}$, $^{99m}\text{TcO-N4IPDTC}$ and $^{99m}\text{Tc}(\text{CO})_3\text{-N4IPDTC}$

In 2015, Zhang and co-workers synthesized the ligand 3-(4-nitro-1H-imidazolyl)propyl dithiocarbamate (N4IPDTC) and labeled it with ^{99m}TcN , ^{99m}TcO , and $^{99m}\text{Tc}(\text{CO})_3$ [18]. The substitution reaction of 4-nitroimidazole **1** with N-(3-bromopropyl)phthalimide **2** in the presence of K_2CO_3 generated compound **3**. The addition of hydrazine to **3**, followed by addition of HCl provided amine hydrochloride **4** bearing 4-nitroimidazole. Ligand N4IPDTC was synthesized via the reaction of **4** with carbon disulfide in the presence of NaOH (Fig. 1). Precursor $[\text{}^{99m}\text{TcN}]^{2+}$ was synthesized from $[\text{}^{99m}\text{TcO}_4]^-$ and succinic dihydrazide (SDH). Substitution reaction of ligand N4IPDTC and $[\text{}^{99m}\text{TcN}]^{2+}$ generated $^{99m}\text{TcN-N4IPDTC}$. $^{99m}\text{TcO-N4IPDTC}$ was afforded via the substitution reaction of N4IPDTC with $^{99m}\text{Tc-GH}$, which was produced from

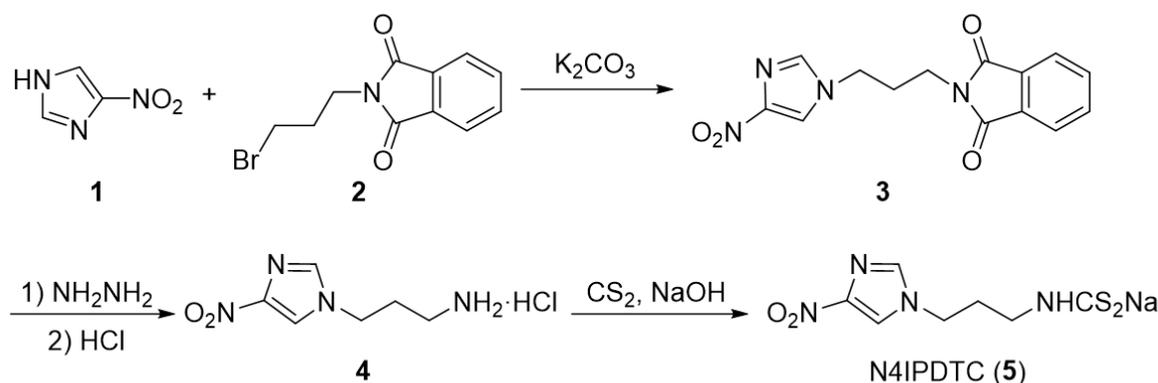


Fig. 1. Synthesis of the N4IPDTC ligand.

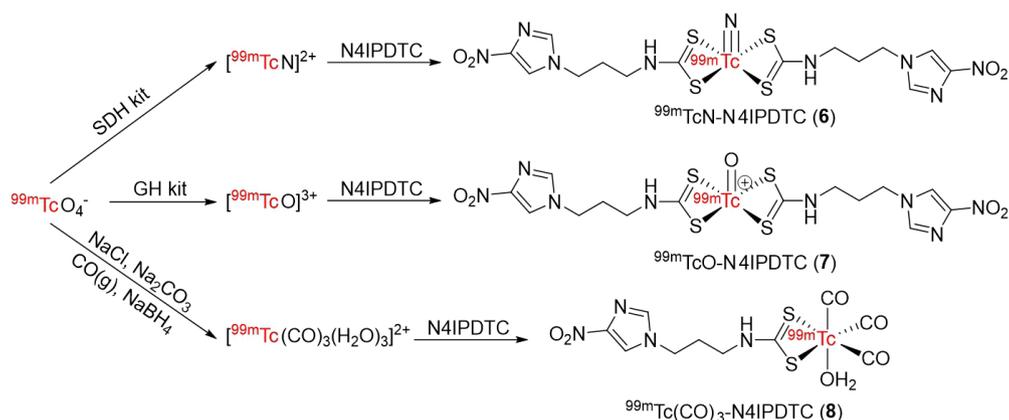


Fig. 2. Radiosynthesis of $^{99m}\text{TcN-N4IPDTC}$, $^{99m}\text{TcO-N4IPDTC}$ and $^{99m}\text{Tc}(\text{CO})_3\text{-N4IPDTC}$.

$[\text{}^{99m}\text{TcO}_4]^-$ by using a glucoheptonate (GH) kit (Fig. 2). The $\text{fac-}[\text{}^{99m}\text{Tc}(\text{CO})_3(\text{H}_2\text{O})_3]^+$ precursor was prepared via the reaction with $[\text{}^{99m}\text{TcO}_4]^-$, Na_2CO_3 , and CO in saline. Substitution of H_2O in a complex with N4IPDTC produced the desired $^{99m}\text{Tc}(\text{CO})_3\text{-N4IPDTC}$. Three ^{99m}Tc -labeled radiotracers were obtained in high yield and with $>95\%$ radiochemical purity.

1.2. Synthesis of $^{99m}\text{TcN-SNXT}$ and $^{99m}\text{TcO-SNXT}$ complexes

In 2018, a xanthate derivative of secnidazole (SNXT) was synthesized by Zhang and co-workers serving as a bidentate ligand. They then proceeded to label this ligand using $[\text{}^{99m}\text{TcN}]^{2+}$ and $[\text{}^{99m}\text{TcO}]^{3+}$ precursors [19]. The SNXT ligand (**10**) was prepared via a reaction between secnidazole, carbon disulfide, and NaOH in water (Fig. 3). The precursors $[\text{}^{99m}\text{TcN}]^{2+}$ and $[\text{}^{99m}\text{TcO}]^{3+}$ were obtained from $^{99m}\text{TcO}_4^-$ by using a succinic dihydrazide (SDH) kit and GH kit, respectively. Reactions of the SNXT ligand with $[\text{}^{99m}\text{TcN}]^{2+}$ and $[\text{}^{99m}\text{TcO}]^{3+}$ cores were carried out in the presence of Na_2CO_3 and CO in saline (Fig. 4) to produce two ^{99m}Tc -labeled complexes ($^{99m}\text{TcN-SNXT}$ and $^{99m}\text{TcO-SNXT}$) in high radiochemical purity.

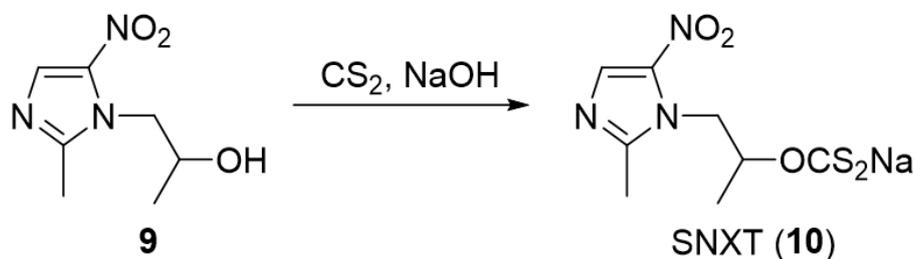


Fig. 3. Synthesis of SNXT ligand.

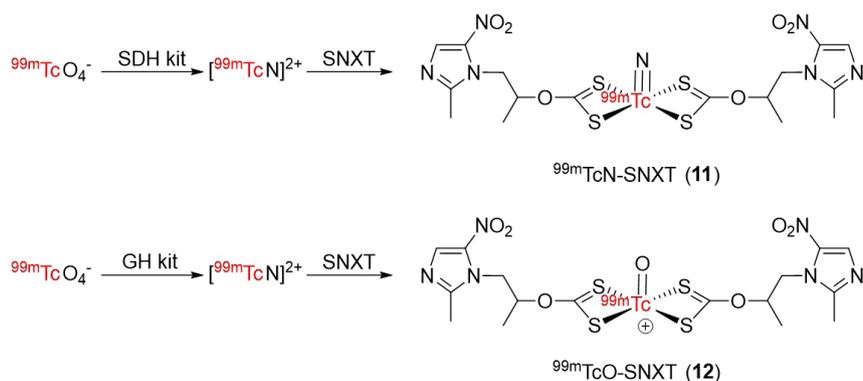


Fig. 4. Radiolabeling reactions of SNXT ligand for the synthesis of $^{99m}\text{TcN-SNXT}$ and $^{99m}\text{TcO-SNXT}$ complexes.

1.3. Synthesis of $^{99m}\text{TcN-NMXT}$ and $^{99m}\text{TcO-NMXT}$

In 2020, two complexes were synthesized and labeled with ^{99m}Tc by Zhang and co-workers, namely $^{99m}\text{TcN-NMXT}$ and $^{99m}\text{TcO-NMXT}$, both bearing 4-nitroimidazole xanthate ligands (NMXT) [20]. The reaction of 2-methyl-4-nitroimidazole **13** with 4-bromobutan-1-ol **14** in the presence of K_2CO_3 in acetonitrile provided compound **15** (NMOH), which was then reacted with carbon disulfide and NaOH in water to generate the sodium salt of the desired ligand NMXT (**16**) (Fig. 5). The $[\text{}^{99m}\text{TcN}]^{2+}$ core was prepared from $^{99m}\text{TcO}_4^-$ by using an SDH kit. Radiolabeling NMXT ligand with $[\text{}^{99m}\text{TcN}]^{2+}$ core provided $^{99m}\text{TcN-NMXT}$ with $>95\%$ radiochemical purity. The $[\text{}^{99m}\text{TcO}]^{3+}$ core was

synthesized from $^{99m}\text{TcO}_4^-$ by using a GH kit. $^{99m}\text{TcO-NMXT}$ was obtained in high radiochemical purity (>95%) via a radiolabeling reaction of the $[\text{}^{99m}\text{TcO}]^{3+}$ core with the NMXT ligand (Fig. 6).

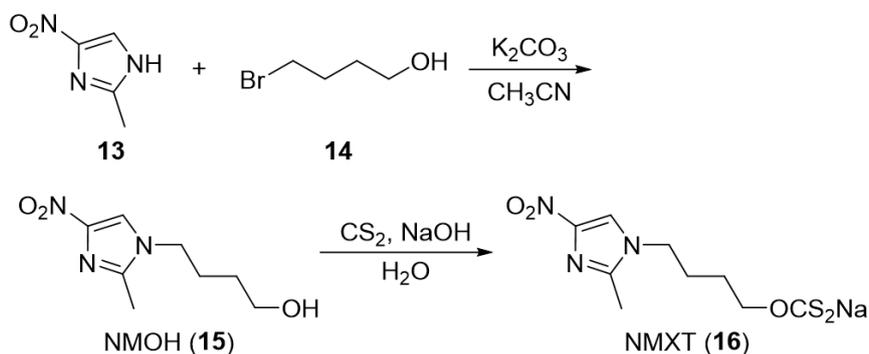


Fig. 5. Synthetic pathway to the NMXT ligand.

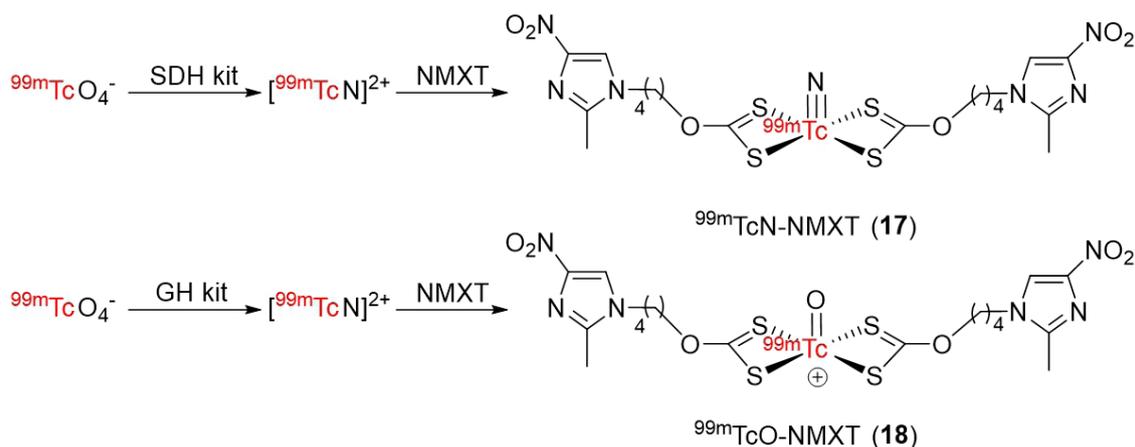


Fig. 6. Synthesis of ^{99m}Tc -labeled complexes $^{99m}\text{TcN-NMXT}$ and $^{99m}\text{TcO-NMXT}$.

1.4. Synthesis of MetroNC- $[\text{}^{99m}\text{TcN(PNP)}]$ complex

In 2016, Banerjee and co-workers introduced a $^{99m}\text{TcN(PNP)}$ -complex featuring a metronidazole isocyanide (MetroNC) ligand [21]. Compound 2-(2-methyl-5-nitro-1H-imidazol-1-yl)ethanamine hydrochloride **19** was transformed into a formamide derivative of 5-nitroimidazole **20** via a reaction with ethylformate and triethylamine. The reaction of **20** with p-TsCl and pyridine in DCM changed the formamide group of **20** to the isocyanide of compound **21** (Fig. 7). The $[\text{}^{99m}\text{TcN}]^{2+}$ intermediate was prepared via a reaction of $\text{Na}^{99m}\text{TcO}_4$ with succinic dihydrazide (SDH) in the presence of SnCl_2 in ethanol, followed by a reaction with PNP2 ligand to give precursor $[\text{}^{99m}\text{TcN(PNP)}]^{2+}$. [MetroNC] (**21**) was added to precursor $[\text{}^{99m}\text{TcN(PNP)}]^{2+}$ to generate MetroNC- $[\text{}^{99m}\text{TcN(PNP)}]$ (**22**) in high radiochemical purity with two proposed structures (Fig. 8).

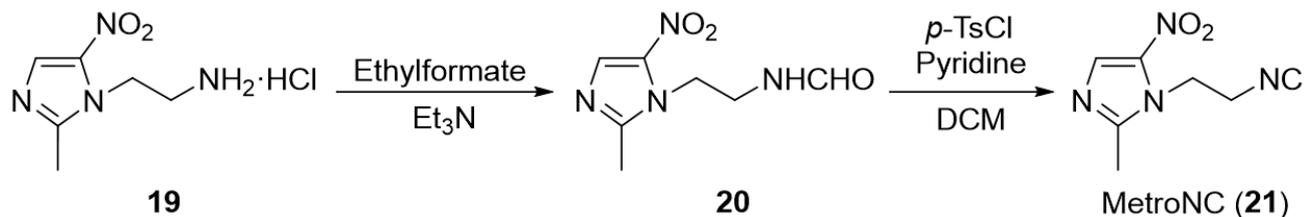


Fig. 7. Synthetic pathway to metronidazole isocyanide (MetroNC) ligand.

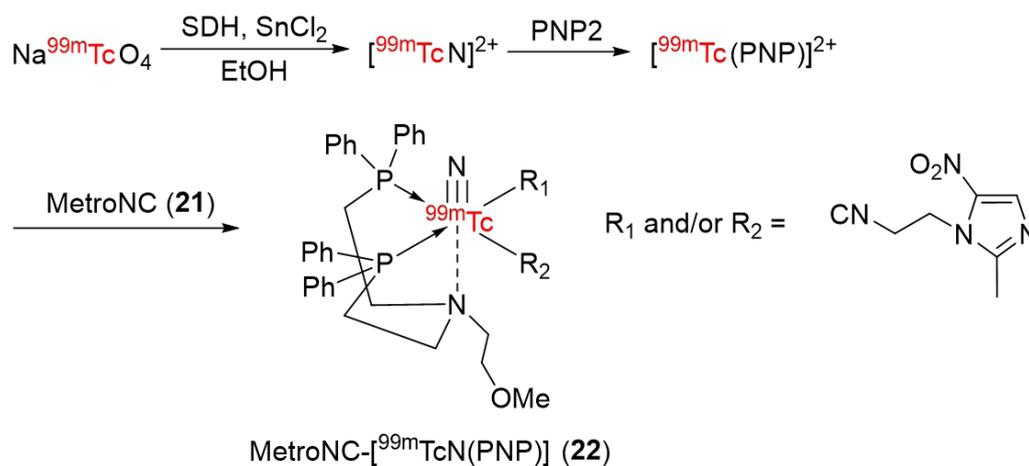


Fig. 8. Radiolabeling reaction of MetroNC ligand and the proposed structures of MetroNC- $[\text{}^{99m}\text{TcN}(\text{PNP})]$ complex.

2. Development of ^{99m}Tc -labeled radiotracers with multi-nitroimidazoles

2. 1. Synthesis of $[\text{}^{99m}\text{Tc}(\text{CO})_3(\text{MetroNC})_3]^+$ complex

Mallia and co-workers synthesized a hypoxia-targeting complex in 2018, incorporating three metronidazole isocyanide moieties (MetroNC) with $^{99m}\text{Tc}(\text{CO})_3$ [22]. The MetroNC ligand was prepared via the reaction of metronidazole **23** with ethylformate and triethylamine, followed by a reaction with p-TsCl and pyridine in DCM (Fig. 9), which was similar to the synthesis of the MetroNC ligand for the preparation of MetroNC- $[\text{}^{99m}\text{TcN}(\text{PNP})]$ [21]. The $[\text{}^{99m}\text{Tc}(\text{CO})_3(\text{H}_2\text{O})_3]^+$ precursor was prepared from $\text{Na}^{99m}\text{TcO}_4$ by using an Isolink® kit vial. MetroNC ligand was radiolabeled with freshly prepared $[\text{}^{99m}\text{Tc}(\text{CO})_3(\text{H}_2\text{O})_3]^+$ in ethanol to afford the $[\text{}^{99m}\text{Tc}(\text{CO})_3(\text{MetroNC})_3]^+$ complex (Fig. 10) with high radiochemical purity (>95%).

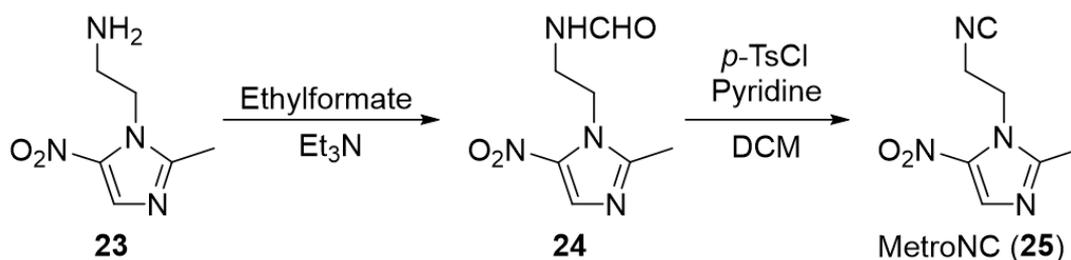


Fig. 9. Synthetic pathway to MetroNC ligand.

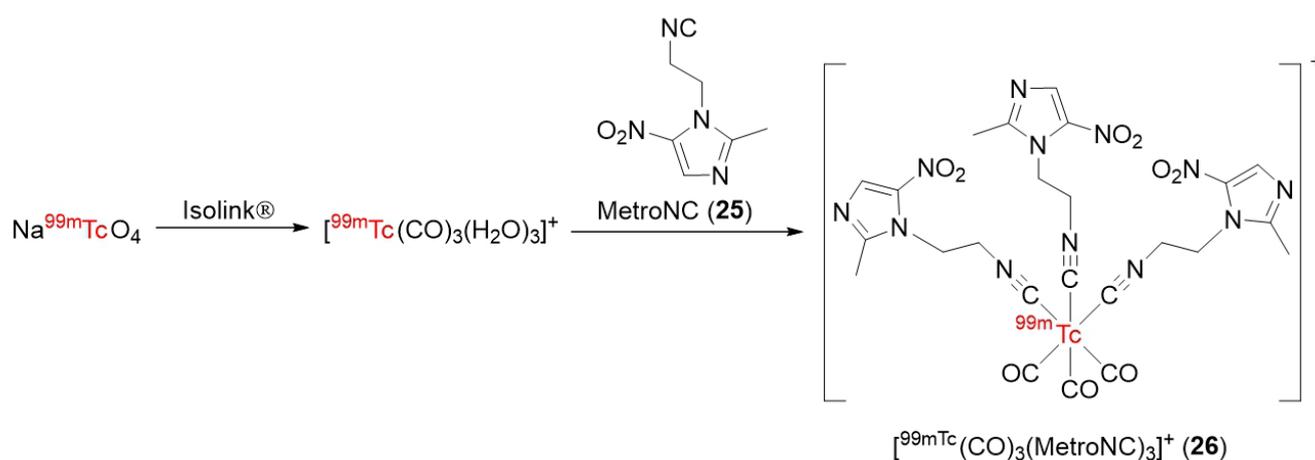


Fig. 10. Radiosynthesis of the $[\text{}^{99m}\text{Tc}(\text{CO})_3(\text{MetroNC})_3]^+$ complex.

2.2. Synthesis of $[\text{}^{99m}\text{Tc}]$ 34 and $[\text{}^{99m}\text{Tc}]$ 35

In 2020, two $^{99m}\text{Tc}(\text{CO})_3$ complexes bearing isocyanide derivatives of 4-nitroimidazole (**32**) were developed by Zhang and co-workers [23]. The substitution reaction of 4-nitroimidazole **27** and *N*-(2-bromoethyl)phthalimide **28** in the presence of K_2CO_3 in DMF at reflux generated compound **29**. Compound **30** bearing primary amine was formed via the reaction of **29** with hydrazine hydrate in ethanol at reflux. The amine group of **30** was then transformed into the isocyanide group of **32** via the reaction of **30** and **31** in the presence of triethylamine in methanol (Fig. 11). The $[\text{}^{99m}\text{Tc}(\text{CO})_3(\text{H}_2\text{O})_3]^+$ precursor was synthesized by adding $\text{Na}^{99m}\text{TcO}_4$ to a solution of Na_2CO_3 , NaBH_4 , and potassium sodium tartrate after purging the solution with CO. Interestingly, radiolabeling ligand **32** with the $[\text{}^{99m}\text{Tc}(\text{CO})_3(\text{H}_2\text{O})_3]^+$ precursor at 100 °C and 25 °C gave the corresponding products, $^{99m}\text{Tc}(\text{CO})_3$ -**34** containing three ligands **32** and $^{99m}\text{Tc}(\text{CO})_3$ -**35** containing two ligands **32**, respectively, with high RCY and >95% radiochemical purity (Fig. 12).

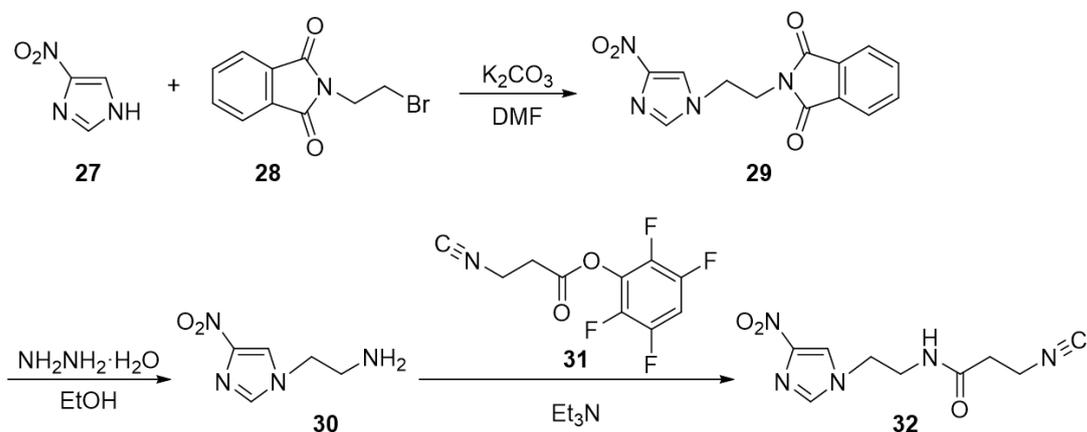


Fig. 11. Synthetic pathway to isocyanide derivative of 4-nitroimidazole.

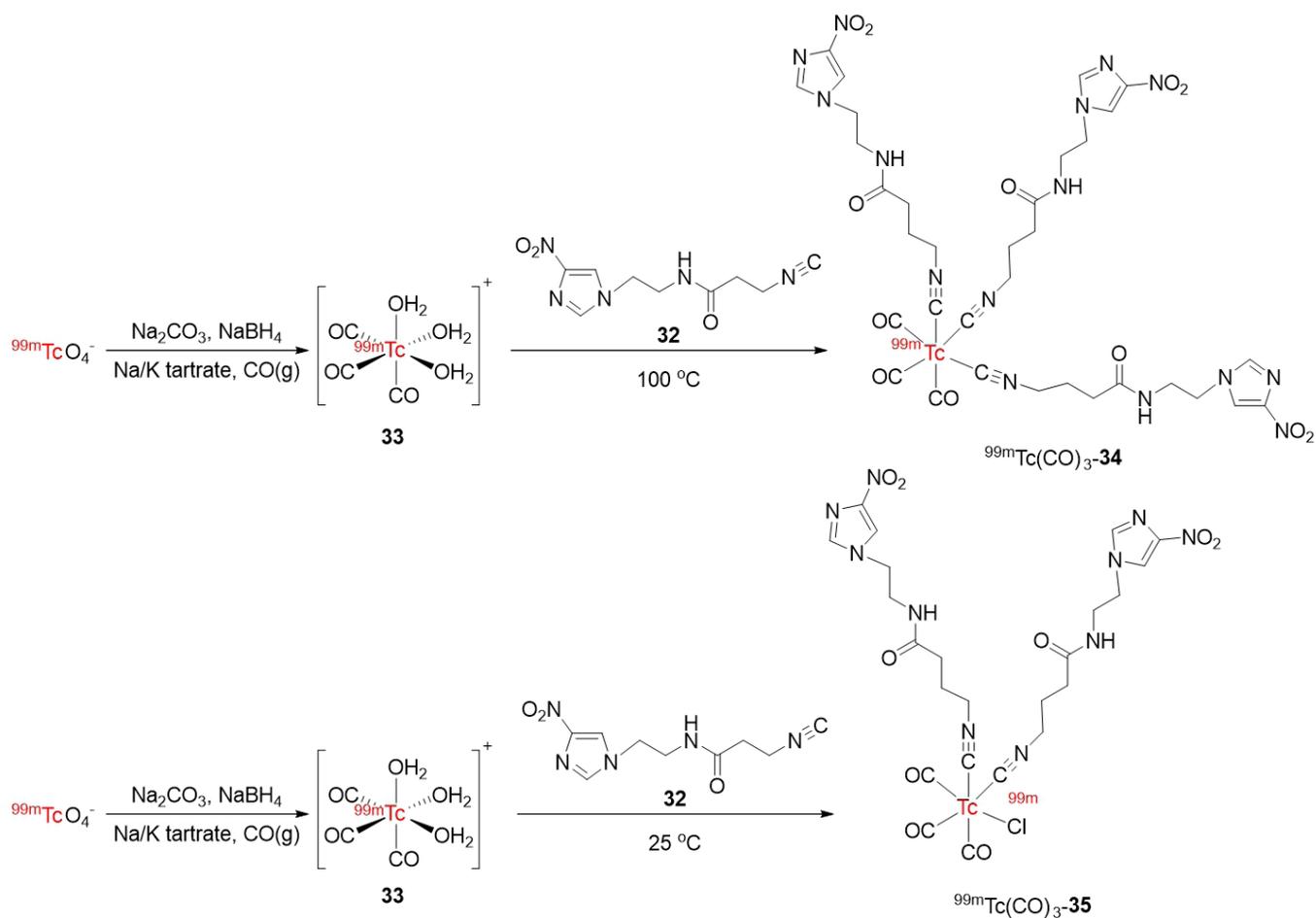


Fig. 12. Radiosynthesis of $^{99m}\text{Tc}(\text{CO})_3\text{-34}$ and $^{99m}\text{Tc}(\text{CO})_3\text{-35}$ complexes.

2.3. Synthesis of $^{99m}\text{Tc}(\text{M})_6^+$ and $^{99m}\text{Tc}(\text{CO})_3(\text{M})_3^+$

In 2020, Zhang and co-workers prepared the isocyanide derivative of 4-nitroimidazole, and proceeded to radiolabel it with $^{99m}\text{Tc}(\text{I})^+$ and $^{99m}\text{Tc}(\text{I})(\text{CO})_3^+$ [24]. Nucleophilic substitution of 4-nitroimidazole **36** and N-(2-bromoethyl)-phthalimide **37** in the presence of K_2CO_3 in DMF generating compound **38** was subsequently converted to compound **39** bearing primary amine via reaction with hydrazine hydrate in ethanol. Ligand **M** was obtained via the amidation of **39** and 2,3,5,6-tetrafluorophenyl 2-isocyanoacetate **40** in the presence of triethylamine in methanol (Fig. 13). Reduction of ^{99m}Tc -pertechnetate with SnCl_2 and ligand **M** in the presence of sodium citrate at room temperature gave the six-coordinated complex $^{99m}\text{Tc}(\text{M})_6^+$. $\text{fac-}^{99m}\text{Tc}(\text{CO})_3(\text{H}_2\text{O})_3^+$ precursor **43** was produced by adding $^{99m}\text{TcO}_4^-$ to the solution of Na_2CO_3 , NaBH_4 , and potassium sodium tartrate and flushed with CO at 80 °C. $^{99m}\text{Tc}(\text{CO})_3(\text{M})_3^+$ was synthesized via the reaction of **M** ligand with prepared $\text{fac-}^{99m}\text{Tc}(\text{CO})_3(\text{H}_2\text{O})_3^+$ precursor at pH 5-6. Two ^{99m}Tc -labeled complexes were both obtained with high RCYs and >95% radiochemical purities (Fig. 14).

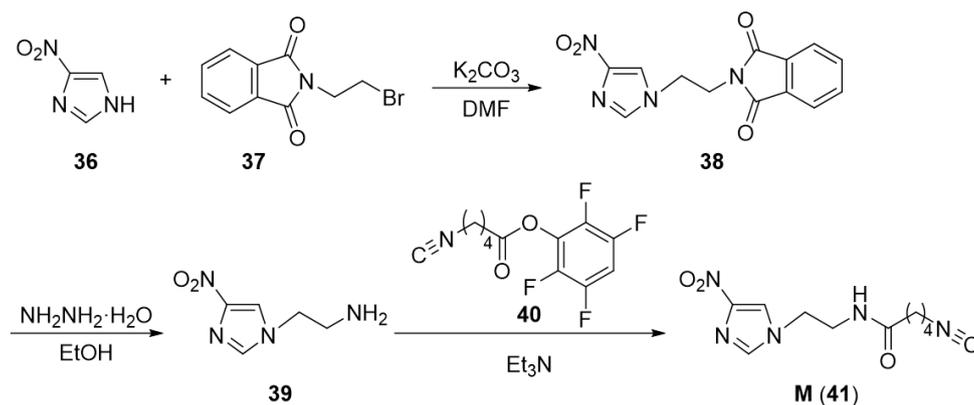


Fig. 13. Synthetic route to 4-nitroimidazole isocyanide derivative **M**.

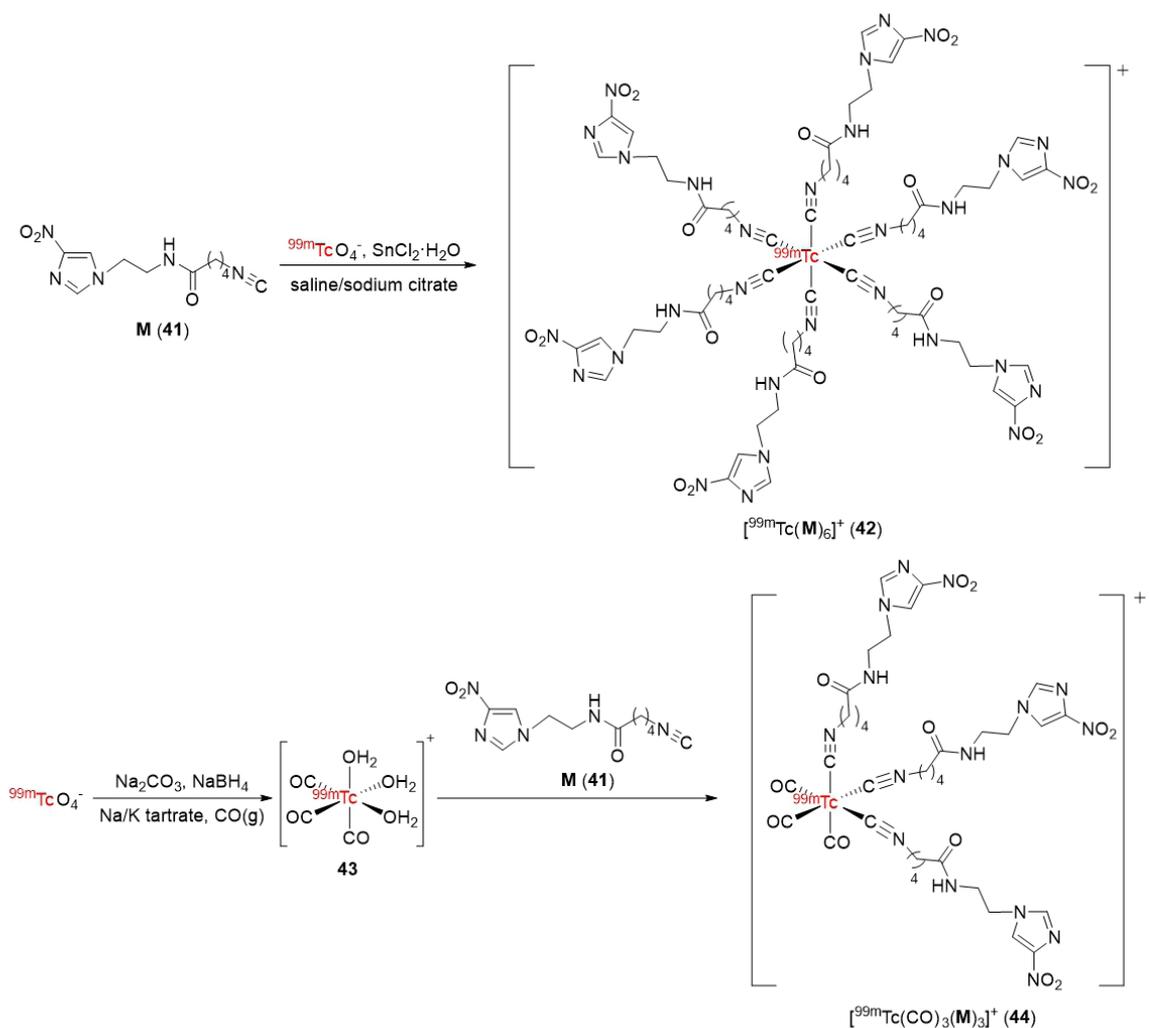


Fig. 14. Radiolabeling reactions for the synthesis of $[\text{}^{99m}\text{Tc}(\text{M})_6]^+$ and $[\text{}^{99m}\text{Tc}(\text{CO})_3(\text{M})_3]^+$.

2.4. Synthesis of $[\text{}^{99m}\text{Tc}]$ 57-60

In 2018, four chelates containing derivatives of 2-nitroimidazole isocyanide were synthesized and radiolabeled with ^{99m}Tc by Zhang and co-workers [25]. The substitution reaction of 2-nitroimidazole **45** with N-(2-bromoethyl)phthalimide **46** in the presence of K_2CO_3 in DMF produced compound **47**, which was then reacted with hydrazine in ethanol to give intermediate **48**. Reactions of **48** with four active esters bearing isocyanide (**49-52**) in the presence of triethylamine in methanol provided the corresponding ligands **174a-d** (Fig. 15). ^{99m}Tc -complexes **57-60** were synthesized in high radiochemical purity (>95%) via direct radiolabeling reactions of ligands **49-52** with $\text{Na}^{99m}\text{TcO}_4$ and reducing agent SnCl_2 in sodium citrate buffer (Fig. 16).

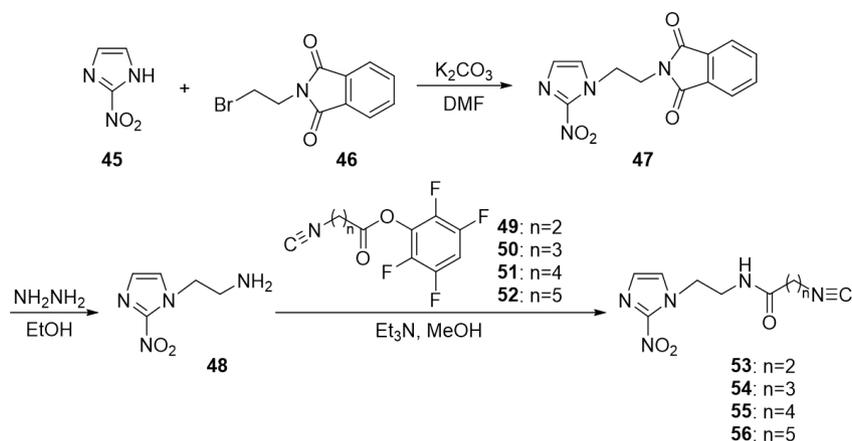


Fig. 15. Synthesis of 2-nitroimidazole isocyanide ligands.

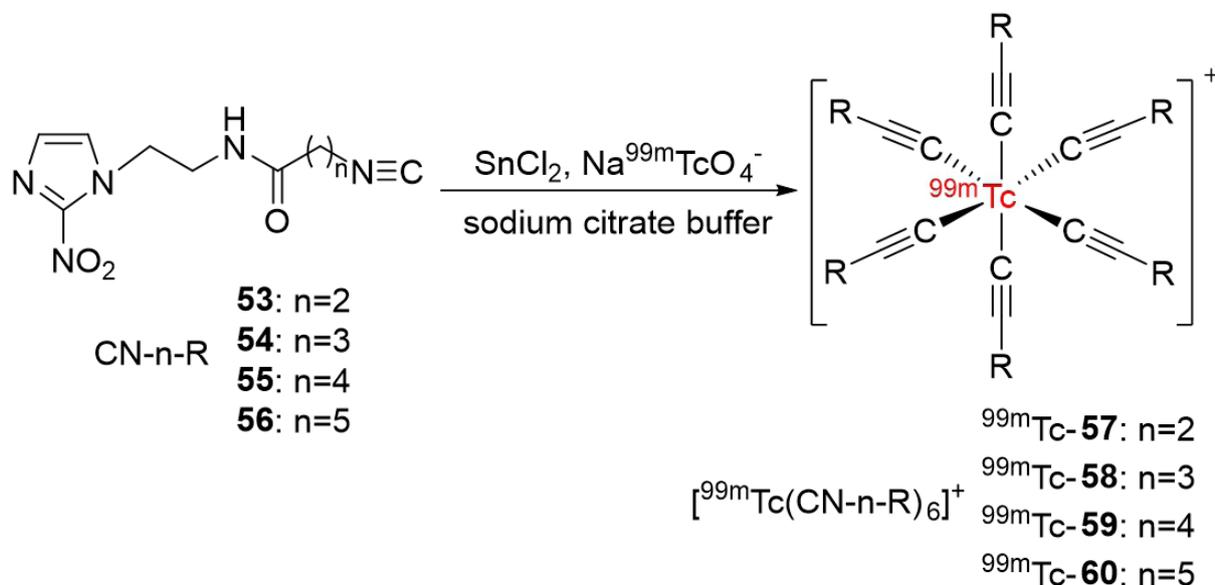


Fig. 16. Radiolabeling reactions for the synthesis of complexes ^{99m}Tc -57-60.

Conclusion

Detection of hypoxia in solid tumors is crucial in the early diagnosis of cancers and prevention of cancer progression and metastasis. Many approaches have been made to detect tumor hypoxia in which SPECT imaging is a promising non-invasive approach to identify and quantify hypoxic regions in vivo. In recent years, numerous ^{99m}Tc -labeled radiopharmaceuticals bearing nitroimidazoles as hypoxia-targeting moieties have been developed due to the versatility of ^{99m}Tc in forming different types of complexes with a wide range of chelators. ^{99m}Tc -labeled radiopharmaceuticals bearing two or more nitroimidazoles in their structures have been successfully prepared, providing a potential approach to enhance the hypoxia selectivity and effectiveness of the hypoxia-targeting agents for SPECT imaging.

REFERENCES

1. Massoud TF, Gambhir SS. Integrating noninvasive molecular imaging into molecular medicine: an evolving paradigm. *Trends Mol Med.* 2007;13(5):183-91.
2. Pysz MA, Gambhir SS, Willmann JK. Molecular imaging: current status and emerging strategies. *Clin Radiol.* 2010;65(7):500-16.
3. Son H, Jang K, Lee H, Kim SE, Kang KW, Lee H. Use of molecular imaging in clinical drug development: a systematic review. *Nucl Med Mol Imaging.* 2019;53(3):208-15.
4. Galbán CJ, Galbán S, Van Dort ME, Luker GD, Bhojani MS, Rehemtulla A, Ross BD. Applications of molecular imaging. *Prog Mol Biol Transl Sci.* 2010;95:237-98.
5. Willmann JK, Van Bruggen N, Dinkelborg LM, Gambhir SS. Molecular imaging in drug development. *Nat Rev Drug Discov.* 2008;7(7):591-607.
6. Saraste A, Nekolla SG, Schwaiger M. Cardiovascular molecular imaging: an overview. *Cardiovasc Res.* 2009;83(4):643-52.
7. Strafella AP, Bohnen NI, Perlmutter JS, Eidelberg D, Pavese N, Van Eimeren T, et al. Molecular imaging to track Parkinson's disease and atypical parkinsonisms: new imaging frontiers. *Mov Disord.* 2017;32(2):181-92.
8. Weissleder R. Molecular imaging in cancer. *Science.* 2006;312(5777):1168-71.
9. Emami Nejad A, Najafgholian S, Rostami A, Sistani A, Shojaeifar S, Esparvarinha M, et al. The role of hypoxia in the tumor microenvironment and development of cancer stem cell: a novel approach to developing treatment. *Cancer Cell Int.* 2021;21(1):1-26.
10. Hayashi Y, Yokota A, Harada H, Huang G. Hypoxia/pseudohypoxia - mediated activation of hypoxia - inducible factor - 1 α in cancer. *Cancer sci.* 2019;110(5):1510-7.
11. Crişan G, Moldovean-Cioroianu NS, Timaru DG, Andrieş G, Căinap C, Chiş V. Radiopharmaceuticals for PET and SPECT imaging: A literature review over the last decade. *Int J Mol Sci.* 2022;23(9):5023.
12. Li Y, Zhao L, Li XF. Hypoxia and the Tumor Microenvironment. *Technol Cancer Res Treat.* 2021;20:15330338211036304.
13. Farina AR, Cappabianca L, Sebastiano M, Zelli V, Guadagni S, Mackay AR. Hypoxia-induced alternative splicing: the 11th Hallmark of Cancer. *J Exp Clin Cancer Res.* 2020;39:1-30.
14. Godet I, Doctorman S, Wu F, Gilkes DM. Detection of hypoxia in cancer models: significance, challenges, and advances. *Cells.* 2022;11(4):686.
15. Walsh JC, Lebedev A, Aten E, Madsen K, Marciano L, Kolb HC. The clinical importance of assessing tumor hypoxia: relationship of tumor hypoxia to prognosis and therapeutic opportunities. *Antioxid Redox Signal.* 2014;21(10):1516-54.
16. Mittal S, Mallia MB. Molecular imaging of tumor hypoxia: evolution of nitroimidazole radiopharmaceuticals and insights for future development. *Bioorg Chem.* 2023;20:106687.
17. Papagiannopoulou D. Technetium - 99m radiochemistry for pharmaceutical applications. *J Label Compd Radiopharm.* 2017;60(11):502-20.
18. Li Z, Zhang J, Jin Z, Zhang W, Zhang Y. Synthesis and biodistribution of novel ^{99m}Tc labeled 4-nitroimidazole dithiocarbamate complexes as potential agents to target tumor hypoxia. *MedChemComm.* 2015;6(6):1143-8.
19. Lin X, Ruan Q, Zhang X, Duan X, Teng Y, Zhang J. ^{99m}Tc labelled complexes with secnidazole xanthate: Synthesis and evaluation as potential radiotracers to target tumor hypoxia. *Appl Radiat Isot.* 2018;140:289-93.
20. Ruan Q, Zhang X, Gan Q, Zhang J. Synthesis and evaluation of [^{99m}TcN]²⁺ core and [^{99m}TcO]³⁺ core labeled complexes with 4-nitroimidazole xanthate derivative for tumor hypoxia imaging. *Bioorg Med Chem Lett.* 2020;30(22):127582.
21. Vats K, Mallia MB, Mathur A, Sarma HD, Banerjee S. Synthesis and evaluation of a novel ^{99m}TcN(PNP)-complex with metronidazole isocyanide ligand as a marker for tumor hypoxia. *J Radioanal Nucl Chem.* 2016;308:363-9.
22. Mallia MB, Mathur A, Sharma R, Kumar C, Sarma HD, Banerjee S, Dash A. Preparation and preliminary evaluation of a tris-metronidazole-^{99m}Tc(CO)₃ complex for targeting tumor hypoxia. *J Radioanal Nucl Chem.* 2018;317:1203-10.
23. Ruan Q, Gan Q, Zhang X, Fang SA, Zhang J. Preparation and Bioevaluation of Novel ^{99m}Tc-Labeled Complexes with a 2-Nitroimidazole HYNIC Derivative for Imaging Tumor Hypoxia. *Pharmaceuticals.* 2021;14(2):158.
24. Ruan Q, Zhang X, Zhang J. Radiosynthesis and evaluation of novel [^{99m}Tc(I)]⁺ and [^{99m}Tc(I)(CO)₃]⁺ complexes with a 4 - nitroimidazole isocyanide for imaging tumor hypoxia. *Appl Organomet Chem.* 2020;34(9):e5798.
25. Ruan Q, Zhang X, Lin X, Duan X, Zhang J. Novel ^{99m}Tc labelled complexes with 2-nitroimidazole isocyanide: design, synthesis and evaluation as potential tumor hypoxia imaging agents. *MedChemComm.* 2018;9(6):988-94.