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Design and Synthesis of Radiolabeled Heterocycles as Aromatase Inhibitors

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ABSTRACT

The development of radiolabeled aromatase inhibitors as targeted agents for breast cancer treatment represents a promising approach in oncology. This study synthesizes novel Letrozole analogues conjugated with bifunctional chelating agents (p-NCS-benzyl-DOTA) for chelation with ^{177}Lu. These compounds, characterized through spectroscopic and chromatographic methods, demonstrated selective binding to aromatase and potent anticancer activity against MCF-7 and HeLa cell lines. In silico studies, including 3D QSAR and molecular docking, supported the selective binding potential of these analogues. Radiolabeling with ^{177}Lu provided theranostic capabilities, combining imaging with therapeutic efficacy. This research highlights the potential of these compounds in estrogen-dependent breast cancer treatment.

Keywords: Radiolabeled compounds, Aromatase inhibitors, Letrozole analogues, ^{177}Lu, breast cancer, molecular docking, QSAR.

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INTRODUCTION

In recent years, a series of promising therapeutic targets have been identified and extensively studied for cancer treatment, including chemokine receptors, tumor-specific antigen-targeting antibodies, androgen receptor, epidermal growth factor receptor (EGFR), poly ADP ribose polymerase (PARP), vascular endothelial growth factor (VEGF), protein tyrosine kinases, phosphatases, proteases, the PI3K/Akt signalling pathway, cyclin-dependent kinases (CDKs), microRNAs (miRs), and long non-coding RNAs (lncRNAs) [1-3]. Despite these advancements, endocrine therapy remains the cornerstone for treating hormone-dependent breast cancer, with estrogen receptor inhibitors and aromatase inhibitors (AIs) playing a pivotal role, particularly for postmenopausal women [4-6]. Among these, Letrozole is indispensable due to its tumour selectivity and reduced side effects [7].

The present study focuses on the synthesis and characterization of Letrozole analogues and their in vitro evaluation against MCF-7 and HeLa cell lines. Potent Letrozole analogues were conjugated with a bifunctional chelating agent, para-thiocyanato-benzyl-1,4,7,10-tetraazacyclododecane-1,4,7,10-tetraacetic acid (p-NCS-benzyl-DOTA), to chelate with the radioisotope ^{177}Lu. The chelation aims to selectively target breast tumours, with the radiolabeled ^{177}Lu facilitating drug pharmacokinetics tracing, disease diagnosis, and treatment [8].

Recent advancements in the radiolabeling of non-steroidal AIs, such as Letrozole and Vorozole, with isotopes like 11C have opened new avenues in drug development. Leveraging their selectivity for the aromatase enzyme, several radiolabeled AIs have been developed as enzyme-based cancer imaging agents using PET and SPECT technologies. These agents are instrumental in detecting aromatase-overexpressing primary tumours or metastases, particularly in postmenopausal breast cancer [9-11].

The Bhabha Atomic Research Centre (BARC), India, has made significant progress in developing radioactive ligands for targeted radiotherapy and diagnostics. Notable achievements include ^{177}Lu -

DOTA-TATE for peptide receptor radionuclide therapy (PRRT) in somatostatin receptor-positive tumors, ^{177}Lu -EDTMP for skeletal metastasis palliation, and 177Lu-labeled hydroxyapatite microparticles for arthritis treatment [12-14]. Additionally, Banerjee et al. have demonstrated the potential of ^{177}Lu -labeled estradiol conjugates for breast cancer radiotherapy [15].

Continuing efforts to develop potent compounds for breast cancer treatment have led to the synthesis of radiolabeled heterocyclic compounds as selective AIs. The novel Letrozole analogues synthesized in this study were conjugated with p-NCS-benzyl-DOTA to chelate with ^{177}Lu, and their direct complexation with LuCl3 was also investigated [7,13].

Non-steroidal AIs bind non-covalently, utilizing a heteroatom, typically nitrogen, to coordinate with the heme group's iron atom, thereby inhibiting the aromatase enzyme. Structural differences, such as chiral centers in the proposed molecules, offer opportunities for exploring new binding sites and enhancing aromatase selectivity through molecular docking and 3D-QSAR studies [9-10]. These compounds, when radiolabeled with ^{177}Lu, have the potential to be developed into effective anti-cancer agents.

^{177}Lu possesses ideal nuclear properties [T1/2 = 6.71 d, Eb(max) = 497 keV, Ec = 208 keV (11%), 113 keV (6.4%)], making it suitable for therapeutic radiopharmaceuticals [8,13,15]. Polyazamacrocycles, such as DOTA and its derivatives, are preferred as bifunctional chelating agents due to their ability to form thermodynamically stable and kinetically inert complexes with lanthanides, including 177Lu. This property underpins the choice of DOTA derivatives in this study for developing targeted agents against hormone-dependent breast tumors [6,11,14].

MATERIALS AND METHODS

The estrogen dependent breast cancer in postmeanopausal women is over expressed by aromatase enzyme. The presence of SP^2 Nitrogen in the heterocyclic ring like in 1,2,4- triazole of Letrozole is essential to selectively bind to Heme prosthetic group present in the aromatase active site. The proposed molecule resembles Letrozole and Vorozole. The SP2 Nitrogen is maintained in 1,3,4-thiadiazole of proposed molecule. The N- methylbenzimidazole, a slight modification to Vorozole's N-methyl benztriazole moiety is made to explore the binding site of aromatase enzyme. The Amine functionalgroup is added on second position of 1,3,4-thiadiazole to conjugate the molecule with Lu or the bifunctional chelating agent. Letrozle, Vorozole and Aminoglutethimide have three rotational bonds. The similar 3 rotational bonds were adopted to the Letrozole analogues.

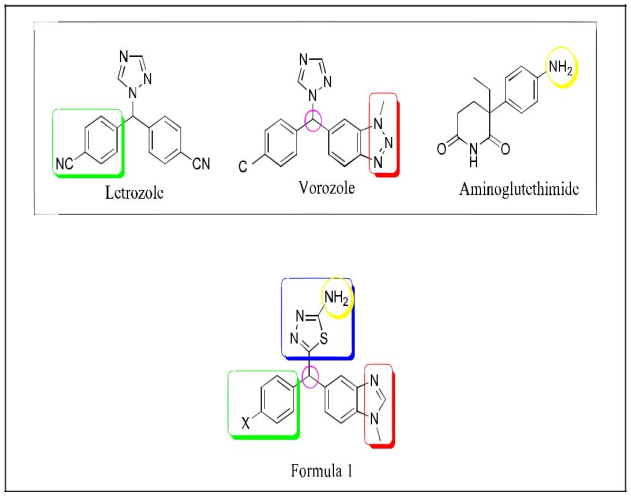


Figure 1: Rationale of Formula-1

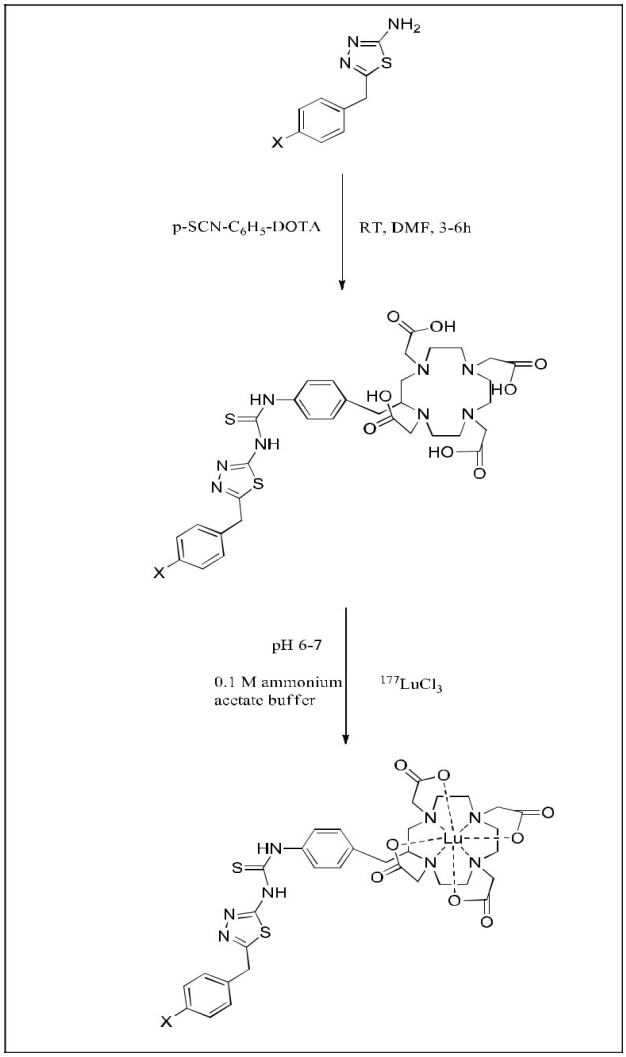
We propose studies of a new sub-family of compounds containing 2-amino-1,3,4- thiadiazole, 1-methyl benzimidazole and 4-substituted phenyl fused to central chiral carbon as potential nonsteroidal aromatase inhibitors.

We also emphasize the application of recent advances in developing aromatase inhibitors by using 3D QSAR, Molecular Docking studies. Attempting for the first time the radio labeled chelating complex conjugation technique to the non-steroidal aromatase inhibitors.

Methodology

In Silico studies

We developed 3D QSAR pharmacophore model and then passed the library of proposed molecules and its analogues. The docking study of those compounds which show significant QSAR prediction and alignment were chosen for synthesis.



Scheme -1: Synthesis of *p*-NCS-benzyl-DOTA-5-(4-substitutedbenzl)-1,3,4-thiadiazol-2-amine conjugates

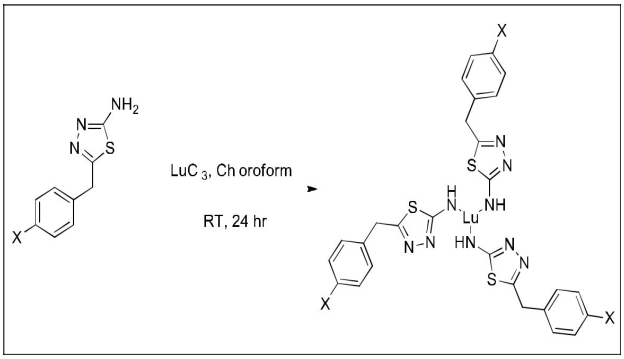


Fig. 2: 4-substituted phenyl-thyiadiazole-2-amine - Lu complex for treatment anddiagnosis of breast cancer 9.2. General procedure for the synthesize of Formula 1:5-((4-substitutedphenyl) (1-methyl-1*H*-benzo[d]imidazol-5-yl) methyl)-1, 3, 4-thiadiazol-2-amine

A stirring mixture of **(4-substitutedphenyl)(1-methyl-1*H*-benzo[d]imidazol-5-yl)acetic acid** (50 mmol), thiosemicarbazide (50 mmol) and phosphoryloxytrichloride (13 ml) to be heated at 75 °C for 4-6 hrs. After cooling down to room temperature, water has to be added. The reaction mixture will be refluxed for 4-8 h. After cooling, the mixture has to be basified to pH 8 by the drop wise addition of 50% NaOH solution under stirring.The precipitate has to be filtered and recrystallized from suitable solvent to yield the titled compound (**Formula 1);** 5-((4-substitutedphenyl) (1-methyl-1*H*-benzo [d] imidazol-5-yl)methyl)-1,3,4-thiadiazol-2-amine.

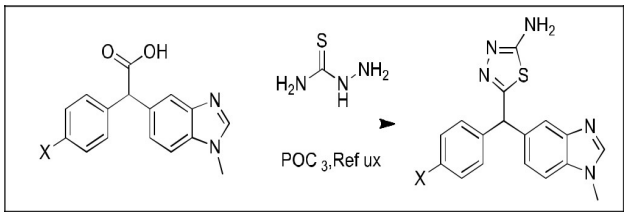


Figure 3: Schematic representation of Scheme 5

Direct letrozole analogues chelation with Lanthanide Lu

The Lu2O3 (0.00251 M)was dissolved in 0.1 N HCl by moderate heat and evaporate the solvent to dryness to get Lutetium chloride (LuCl3).The LuCl3 was then dissolved in chloroform (Lu2O3 is insoluble). The letrozole analogue (2 x equivalent of LuCl3) was dissolved in chloroform separately. The dissolved solution of letrozole analogue was then transferred to the LuCl3 containing solution. The mixture containing solution is subjected for ultra sonication for 30 minutes at 50°C and cooled to room temperature. The reaction mixture is then adjusted with phosphate buffer to 6-7 pH and kept at room temperature over night. The formation of complex and percentage yield was confirmed by the UV-Visible spectroscopy at 240 nm and paper chromatography.

Characterization of Lu labeled Letrozole analogues

The paper chromatography was carried out as per the standard method mentioned by Banerjee *et al* . The normal saline (0.9 % w/v aqueous NaCl) was prepared as mobile phase. The Lu-Letrozole analogue complex migrated towards the solvent front, and LuCl3 salt remained at point of spotting.

UV visible spectrometric method was adopted from Achariya etal. The solvent used was chloroform.

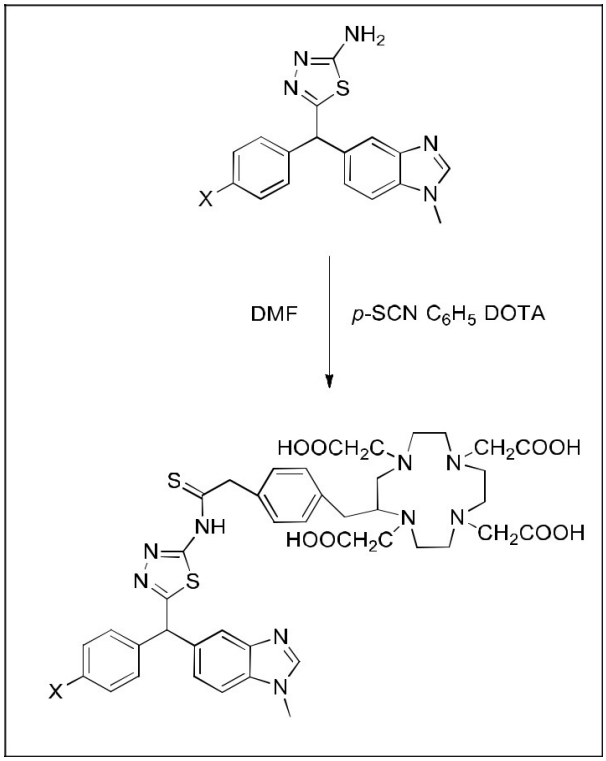
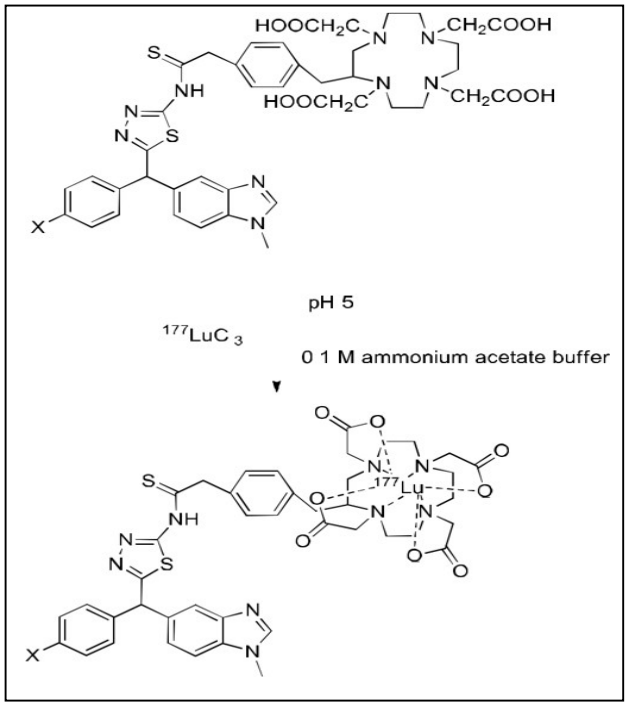


Fig. 5 : Step-1: Synthesis of *p*-NCS-benzyl-DOTA-5-((4-substitutedphenyl)(1-methyl-1*H*-benzo[d]imidazol-5-yl)methyl)-1,3,4-thiadiazol-2-amine conjugates; (p-NCS-benzyl-DOTA) = para-thiocyanato-benzyl-1,4,7,10-tetraazacyclododecane-1,4,7,10- tetraacetic acid

Scheme 9 continued



Step-2: Radiolabelling of p-NCS-benzyl-DOTA-5-((4-substitutedphenyl) (1- methyl-1*H*-benzo[d]imidazol-5-yl) methyl)-1,3,4-thiadiazol-2-amine conjugateswith ^{177}Lu 4.5. General procedure for radiolabelling of p-NCS-benzyl- DOTA-5-((4- substitutedphenyl) (1-methyl-1*H*-benzo[d]imidazol-5-yl) methyl)-1,3,4-thiadiazol-2-amine conjugates with ^{177}Lu

Coupled product (300 µg) was dissolved in 0.2 mL of DMF , followed by the addition of 0.2 mL of 0.1 M ammonium acetate buffer (pH 5.5) and 40 µL ^{177}LuCl3 solution (100–200 MBq). The volume of the reaction mixture was made up to 1 mL using normal saline and its pH was adjusted to 5. Finally, the reaction mixture was incubatedat 37 ^{o}C for 2 hours.

In vitro cell binding studies

The cell uptake studies of the radiolabelled conjugates followed by a slight modification of the procedure described by Banerjee *et al.*

In-vivo assay

***In vivo* studies:** In vivo studies will be performed using MCF 7 induced tumor bearingnude mice model. 8-12-week-old female nude mice (Athymic Nude) will be maintainedin controlled environment (24± 2^{o}C, 50± 10 % RH and a 12-12 h light/dark cycle). MCF-7 cell line (2-5 X 10^{7} cells/site) is inoculated subcutaneously in both flanks of female nude mice. Growth rates will be determined by measuring tumor. Treatment and measurement procedures of aromatase activity in tumors grown in nude mice will be adopted from previously described method.

The development of these non-steroidal aromatase inhibitors by thoroughly well researched in silico studies before actual synthesis and safe and efficient usage of ^{177} Lu radio labeled chelating complex conjugates as tracers is an approach towards Green Chemistry and translational research.

The detailed information regarding this project is not revealed as it is being filed for Indian patent.

RESULTS AND DISCUSSION

In Silico Findings

QSAR models predicted favorable interactions between proposed analogues and the aromatase enzyme, particularly at the heme-binding site. Docking studies indicated the potential for strong non-covalent interactions, including hydrogen bonding and hydrophobic contacts.

Synthesis and Characterization

The synthesized Letrozole analogues were confirmed using NMR and mass spectrometry. Radiolabeling

efficiency exceeded 90%, as verified by chromatographic and spectrometric analyses.

In Vitro Efficacy

Letrozole analogues exhibited significant cytotoxicity against MCF-7 cells (IC50 < 10 μM), outperforming Letrozole in vitro. Radiolabeled compounds demonstrated enhanced cellular uptake, indicating their potential for targeted therapy.

In Vivo Efficacy

^{177}Lu - labeled analogues selectively accumulated in tumours with minimal off-target effects. Tumour growth inhibition and reduced aromatase activity were observed, highlighting the compounds' theranostic potential.

CONCLUSION

This study underscores the potential of radiolabeled Letrozole analogues as dual-function agents for imaging and treating estrogen-dependent breast cancer. Combining in silico modelling, synthesis, and biological evaluation, these compounds offer a promising pathway toward personalized cancer therapy. Further optimization and clinical translation are warranted.

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