Atlihan-Gundogdu Evren (Orcid ID: 0000-0002-4095-2714)

Radiolabeling and in vitro evaluation of a new 5-Fluorouracil derivative with cell culture studies

[a] Derya Ilem-Ozdemir, [a] Evren Atlihan-Gundogdu, [a] Meliha Ekinci, [b] Erkan Halay, [c] Kadir Ay, [d] Tamer Karayildirim, [a] Makbule Asikoglu

a. Department of Radiopharmacy, Faculty of Pharmacy, Ege University, Bornova, Izmir, Turkey.

b. Scientific Analysis and Technological Application and Research Center, Usak University, Usak, Turkey.

c. Department of Chemistry, Faculty of Art and Sciences, Manisa Celal Bayar University, Yunusemre, Manisa, Turkey.

d. Department of Chemistry, Faculty of Sciences, Ege University, Bornova, Izmir, Turkey.

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ABSTRACT

The clinical impact and accessibility of ^{99m}Tc tracers for cancer diagnosis would be greatly enhanced by the availability of a new, simple and easy labeling process and radiopharmaceuticals.

5-Fluorouracil (5-FU) is an anti-tumor drug which has played an important role for the treatment of breast carcinoma. In the present study, a new derivative of 5-FU was synthesized as (1-[{1′-(1″-deoxy-2″,3″:4″,5″-di-O-isopropylidene-β-D-fructopyranose-1″-yl)-1′H-1′,2′,3′-triazol-4′-yl}methyl]-5-fluorouracil) (**E**), and radiolabeled with ^{99m}Tc. It was analyzed by radio thin layer chromatography (RTLC) for quality control and stability. The radiolabeled complex was subjected to *in vitro* cell binding studies to determine healthy and cancer cell affinity using HaCaT and MCF-7 cells, respectively. In addition, *in vitro* cytotoxicity studies of compound E were performed with HaCaT and MCF-5 cells.

The radiochemical purity of the [^{99m}Tc]Tc**E** was found to be higher than 90 % at room temperature up to 6 h. The radiolabeled complex showed higher specific binding to MCF-7 cells than HaCaT cells. IC_{50} values of E were found 31.5±3.4 and 20.7±2.77 µM for MCF-7 and HaCaT cells, respectively.

The results demonstrated the potential of a new radiolabeled E with ^{99m}Tc has selective for breast cancer cells.

Key words: 5-fluorouracil derivative, Radiolabeled compound, Technetium-99m, Breast cancer, Cell binding.

INTRODUCTION

Breast cancer is the most common cancer among women worldwide [1]. Prognosis is very good if the disease is detected in early stages, however, in the presence of metastatic disease, 5-year survival drops dramatically. Since early diagnosis is critical for patient survival, there is an acute need for developing novel sensitive breast cancer imaging agents and techniques [2, 3]. Nuclear medicine imaging provides noninvasive functional information at molecular and cellular level that contributes to the determination of health status by measuring the uptake and turnover of target-specific radiotracers in the tissue. By using nuclear imaging procedures, abnormalities are often identified in very early stages. The early detection allows more successful prognosis possibility for the treatment of disease [1].

Cancer imaging studies by using radiopharmaceuticals targeted to specific receptors have been employed successfully up to now [4]. Radiopharmaceuticals consist of two components, a biomolecule and a radionuclide with a specific radiation [4, 5]. For diagnostic purposes, gamma emitter radionuclides are preferred because of their low linear energy transfer which results in low tissue damage in the targeted organ [5]. The ideal nuclear properties of ^{99m}Tc likewise; 140 keV pure gamma photon emission, 6 h physical half-life, low cost, readily availability are optimum for preparing radiopharmaceuticals for diagnostic purposes [6-8]. The biomolecules of the radiopharmaceuticals are responsible for the accumulation of the radionuclide in the targeted tissue. High accumulation in the target and low accumulation in non-target tissue is preferred to increase the resolution and sensitivity of the images and to reduce radiation damage in the rest of the body. The better target/non target ratios are achieved with radiolabeled receptor-specific molecules for cancer imaging and therapy.

5-Fluorouracil (5-FU) is an effective anti-tumor drug, which has been used either as a single agent or in combination with other chemotherapeutic agents for the treatment of tumors such as breast and colorectal carcinoma [9-12]. 5-FU is an antimetabolite drug and works by inhibiting essential biosynthetic processes, or by being incorporated into macromolecules, such as DNA and RNA. Also, 5-FU is an analogue of uracil with a fluorine atom at the C-5 position in place of hydrogen. It rapidly enters the cell using the same facilitated transport mechanism as uracil and is converted intracellularly to several active metabolites: Fluorodeoxyuridine monophosphate (FdUMP), fluorodeoxyuridine triphosphate (FdUTP) and fluorouridine triphosphate (FUTP)— which disrupt RNA synthesis. There are some studies showing a higher selective potential for cancer cells than normal cells. The mechanism of

cytotoxicity of 5-FU on cancer cells has been ascribed to the misincorporation of fluoronucleotides into RNA and DNA and to the inhibition of the nucleotide synthetic enzyme thymidylate synthase [13, 14]. Since 5-FU is poorly tumor selective, high incidences of bone marrow, gastrointestinal tract and central nervous system toxicity are common in its therapeutic use. To tackle these problems, numerous structural modifications of the 5-FU have been performed. Thus, a series of 5-FU prodrugs in which 5-FU is attached to amino acids, peptides, phospholipids and polymers have been reported [15-23]. These N-1 and/or N-3 substituted derivatives have exhibited improved pharmacological and pharmacokinetic properties, including increased bioactivity, selectivity, metabolic stability, absorption and lower toxicity [20]. According to this strategy, in this study a new derivative of 5-FU that contains nucleobase and sugar molecules, 1-[{1′-(1″-*deoxy*-2″,3″:4″,5″-di-*O*-isopropylidene-*β*-D-fructopyranose-1″-yl)-1′*H*-1′,2′,3′-triazol-4′-yl}methyl]-5-florouracil (Compound **E**), was reported [20] and synthesized for higher specificity to breast cancer.

The synthesis of new derivatives of 5-FU could be beneficial for receptor mediated uptake of 5-FU by utilizing these elevated receptors on cancer cells. In this study, we propose that radiolabeled compound of 5-FU derivative bearing pyranose sugar and nucleobase groups could be beneficial for increasing 'MFC-7/HaCaT cellular uptake ratios' and aim to radiolabel compound E with ^{99m}Tc and show cellular binding capacity for different types of cells by using in vitro cell culture studies. For this purpose, compound E was radiolabeled with ^{99m}Tc under varying conditions. Radiochemical purity was determined with radioactive thin layer chromatography (RTLC). After determining the optimum labeling conditions for maximum labeling efficiency and stability, the *in vitro* cell incorporation affinity of developed formulation to breast carcinoma (MCF-7) and the human keratinocyte (HaCaT) cell lines was investigated.

MATERIALS AND METHODS

Materials

Stannous chloride dehydrate was purchased from Sigma-Aldrich (USA). [^{99m}Tc]TcO_4^- was eluted from the Molybdenum-99 (^{99}Mo)/^{99m}Tc generator. All solvents were obtained from Merck (Germany). Cell culture reagents and supplies were obtained from American Type Culture Collection (ATCC). Radioactive samples counted in a counting unit (Atomlab 100 Dose Calibrator Biodex Medical Systems). All other materials were of analytical grade.

Experimental

Synthesis of (1-[{1′-(1′′-*deoxy*-2′′,3′′:4′′,5′′-di-*O*-isopropylidene-*β*-D-fructopyranose-1′′-yl)-1′*H*-1′,2′,3′-triazol-4′-yl}methyl]-5-fluorouracil) (E)

The azido-fructopyranose (**C**) and alkynated-5-fluorouracil (**D**) derivatives obtained in the first two steps and were reacted in THF:*t*-BuOH:H_2O solvent system in the presence of suitable catalysts to give the desired target compound **E** in high yield via copper-catalyzed azide-alkyne cycloadditon (CuAAC), namely *click* reaction as previously reported (Figure 1) [24].

Radiolabeling Studies

Radiolabeling was obtained with [^{99m}Tc]TcO_4^- (37 MBq) in saline (0.1 mL). The compound **E** (1 mg) was dissolved in 1 mL saline. Stannous chloride was dissolved in 1 mL of 0.02 N HCl and kept under an atmosphere of nitrogen. Reduction of [^{99m}Tc]TcO_4^- was performed by adding different amount of 1 mL stannous chloride solution (10, 50, 250 and 500 µg/mL) to 1 mg/mL **E** solution. and the solution was left to stand at room temperature for 15 min prior to radiochemical analyses. The final volume of reaction medium was 2.1 mL.

To determine and optimize the required concentration of reducing agent and pH value for efficient labeling, radiolabeling process was performed with different concentrations of stannous chloride as reducing agent at different pH values (pH 5 and 7.4). In each optimization, radiochemical purity was assessed by RTLC up to 6 h.

Effect of Reducing Agent on Radiolabeling

Reduction of [^{99m}Tc]TcO_4^- was performed with different amount of stannous chloride in 0.02 N HCl (10, 50, 250 and 500 µg.mL^{-1}). Compound **E** was dissolved in 1 mL saline. Radiolabeling was performed with [^{99m}Tc]TcO_4^- (37 MBq) in saline (0.1 mL) and solution was allowed to stand at room temperature for 15 min prior to radiochemical analyses.

Effect of pH on Radiolabeling

The radiochemical purity of ^{99m}Tc radiopharmaceutical is highly dependent on the pH [6]. The effect of pH on the radiochemical purity of [^{99m}Tc]Tc**E** was examined for pH 5.0 and 7.4. The potassium phosphate monobasic dibasic solution was used to adjust the pH 7.4. The sodium acetate buffer was used to adjust the pH 5.

Stability of [^{99m}Tc]Tc**E** in Different Media

The saline, human serum and cell medium were used to evaluate the stability of radiolabeled complex at different media. [^{99m}Tc]Tc**E** reaction medium (37 MBq-100 µL) was

added to 900 µL of each media. The mixtures were incubated at 37 °C at different time intervals up for 6 h and 24 h. Aliquots were taken out and spotted on chromatographic strips and analyzed by RTLC [25].

For cell medium stability assay, 37 MBq of radiolabeled compound was added into cell medium. The mixture was incubated at 37°C and samples were taken and spotted on chromatographic papers at 15, 60 and 120 min after incubation. The radiochemical purity of samples was analyzed with RTLC.

RTLC Procedure

RTLC was performed using Whatman No:3 paper as stationary phase. Free ^{99m}Tc was determined by using acetone as the mobile phase. Reduced/Hydrolyzed (R/H) ^{99m}Tc was determined by Whatman No:3 paper which developed in saline. The radioactivity on chromatographic papers was measured using a TLC scanner (Bioscan AR 2000), and % radiochemical purity (RP) of [^{99m}Tc]Tc**E** was calculated from the following equation (**Equation 1**) by subtracting from 100 the sum of measured impurities percentages.

RP (%) = 100 – [Free ^{99m}Tc (%) + R/H ^{99m}Tc (%)] **Equation 1**

Cell Culture Studies

MCF-7 (breast carcinoma) and HaCaT (human keratinocyte) cells were in McCoy's 5A supplemented with 10% fetal bovine serum. Cell culture was maintained at 37°C under 90% humidity and 5% CO_2. Subculturing was performed employing a 0.25% Trypsin–0.1% EDTA solution. Cell monolayers were prepared by seeding cells on six wells.

After 24 h seeding, the integrity of each cell monolayer was checked by measuring its transepithelial electrical resistance (TEER) with an epithelial voltammeter (EVOM, World Precision Instrument, Sarasota, FL, USA). The TEER value was calculated from the following equation (**Equation 2**):

$$TEER = \left(R_{monolayer} - R_{blank}\right) \times A \quad \textbf{Equation 2}$$

$R_{monolayer}$ is the resistance of the cell monolayer along with the filter membrane, R_{blank} is the resistance of the filter membrane and A is the surface area of the membrane (4.7 cm^2 in six well plates) [25, 26].

Cell Binding Studies

In vitro cell binding experiments were performed in triplicate. The cells (5×10^5 cells/well) were distributed in 6-well plates and incubated at 37 °C in cell culture medium. The medium was removed, and cells were washed with phosphate-buffered saline (PBS; 1 mL) and incubated at 37 °C in 0.5 mL of McCoy's 5A supplemented with 10% fetal bovine serum. After 2 h, 0.5 mL of 37 MBq [^{99m}Tc]Tc**E** was added and incubation was continued. The incubation medium was removed and cells were washed and harvested at 30, 60, 90 and 120 min. After removal of the radiolabel-containing medium, the isolated cells were consecutively washed with 1 mL of cell medium and 1 mL of PBS to remove free radiolabeled complex. The activities, which in the tubes containing sediment cells and in the tubes containing culture medium were both counted by a gamma counter (Sesa Uniscaller). The cellular uptake was calculated as the percentage of the activity counted in the cells relative to the total activity counted. The percentage radioactivity of cells was calculated from the following equation by dividing the radioactivity of cells to the total radioactivity (radioactivity of cells plus radioactivity of cell medium) (**Equation 3**).

Radioactivity of Cells = (Radioactivity of Cells / Total radioactivity) x 100

Equation 3

In vitro cytotoxicity studies

The cytotoxicity of E was determined by the 3-(4,5-dimethyl-2-thiazolyl)-2,5-diphenyl--tetrazolium bromide (MTT) assay. MCF-7 and HaCaT cells (1×10^5/well) were plated in 1mL of medium/well in 24-well plates (Costar Corning, Rochester,NY). After the cell reaches the confluence, they were incubated in the presence of various concentrations (10, 50, 100, 500, 1000 and 2000 µg/mL) of E in 0.1% DMSO for 48 h at 37 ° C. The sample solutions were removed and 1 mg/mL MTT in phosphate- buffered saline solution was added. Viable cells were determined by the absorbance at 570 nm. Measurements were performed and the concentration required for a 50 % inhibition of viability (IC_{50}) was determined with GraphPad Prism and fitted using a 'one-site total binding' algorithm program. In this program, the concentration of E was varied and IC_{50} was calculated. In this program, XY data table was created. The E concentration is entered as X values, and cellular uptake was entered as Y values. Nonlinear regression was chosen and IC_{50} values were calculated and results viewed in the scene.

Statistical Analysis

The calculation of means and standard deviations were made on Microsoft Excel. Oneway Anova was used to determine statistical significance. Differences at the 95% confidence level ($p<0.05$) were considered significant. Experiments were performed in triplicate unless stated otherwise. Results are reported as mean ± standard error.

RESULTS

Synthesis of (1-[{1′-(1′′-*deoxy*-2′′,3′′:4′′,5′′-di-*O*-isopropylidene-*β*-D-fructopyranose-1′′-yl)-1′*H*-1′,2′,3′-triazol-4′-yl}methyl]-5-fluorouracil) (E)

The structural elucidation of compound **E** was performed by spectroscopic methods and all obtained data are compatible with our previous study [24].

Radiolabeling Studies

A novel, simple, rapid and efficient direct method for labeling of compound **E** with ^{99m}Tc was developed and conducted at room temperature. Labeling efficiency of the [^{99m}Tc]Tc**E** was assessed by RTLC studies. Two solvents were used to distinguish and quantify the amounts of radioactive impurities.

According to RTLC results, The R_f values of [^{99m}Tc]Tc**E** mobile phases were presented in Table 1. The RTLC chromatogram of [^{99m}Tc]Tc**E** was presented in Figure 2. In RTLC, using acetone as the solvent, free ^{99m}Tc migrated with the solvent front, while [^{99m}Tc]Tc**E** and R/H ^{99m}Tc remained at the spotting point. R/H ^{99m}Tc was determined by using saline as the mobile phase where the R/H ^{99m}Tc remained at the point of spotting while free ^{99m}Tc and [^{99m}Tc]Tc**E** moved with the solvent front. Under optimized conditions, [^{99m}Tc]Tc**E** was prepared and at a radioactivity around 37 MBq and radiochemical purity over 90%.

Effect of Reducing Agent on Radiolabeling

Radiolabeling studies were performed with different concentrations of reducing agent to determine the optimum conditions. The effect of reducing agent concentration on the radiochemical purity was evaluated and optimum reducing agent amount was found to be 50 µg. Under these conditions labeling efficiency was around 90 % and did not change significantly during 6 h at room temperature ($p>0.05$) (Table 2).

Effect of pH on Radiolabeling

The effect of pH on radiochemical purity was examined at two different pH values (pH 5.0 and 7.4). The radiochemical purity of ^{99m}Tc radiolabeled complex was found to be greater

than 96 % and 95 % at pH 5 and 7.4, respectively. Our results demonstrate that while keeping other reaction conditions constant and varying the pH of the reaction from 5.0 and 7.4, radiochemical purity was not changed significantly ($p>0.05$) (Figure 3) and the radiolabeled complex was not affected by pH alteration.

Stability of [^{99m}Tc]TcE** in Different Media**

The stability of [^{99m}Tc]Tc**E** was evaluated in different media (saline, human serum and cell medium). The radiochemical purity of the [^{99m}Tc]Tc**E** was found to be higher than 95 %, 90 % and 80 % for 6 h, 24 h and 2 h in saline, human serum and cell medium, respectively at 37 ° C ($p>0.05$) (Figure 4).

Cell Binding Studies

Radiolabeled samples were tested for their cell binding capacity. Due to the available half-life of ^{99m}Tc, radiolabeled complexes were assessed at 2 h. The cell binding results of [^{99m}Tc]Tc**E** for MCF-7 and HaCaT cell lines were illustrated in Fig. 5. Apparently, high cell binding capacity of % [^{99m}Tc]Tc**E** was obtained on MCF-7 cell line. While the cell binding % [^{99m}Tc]Tc**E** ranged from 0.18±0.01 to 0.96±0.06 for MCF-7, the cell binding % [^{99m}Tc]Tc**E** ranged from 0.052±0.03 to 0.35±0.22 for HaCaT cells (Table 3). The radioactivity levels of [^{99m}Tc]Tc**E** in MCF-7 was approximately four times higher than HaCaT cell lines and statistically significant differences were observed between the two cell lines at 30 and 120 min.

Transepithelial electrical resistance measurements (TEER)

The TEER values of cells were found to be between 1028±168.65 and 1302±49.52 ohms.cm^{-1} at the beginning of seeding period (Figure 6). After 24 h, the TEER values were measured and found to be between 1112±14.75 and 1537±43.72 ohms.cm^{-1} (Figure 7).

In vitro cytotoxicity studies

Six different concentrations were used for cytotoxicity studies. While 2000 µg/mL of E produces 75.5 % cell viability, 1000 µg/mL produces 89.9 % cell viability, 500 µg/mL produces 93.5 % cell viability, 100 µg/mL produces 96.3 % cell viability, 50 µg/mL produces 97.8 % cell viability, 10 µg/mL produces 99 % cell viability for MCF-7 cells, 2000 µg/mL of E produces 73.5 % cell viability, 1000 µg/mL produces 89 % cell viability, 500 µg/mL produces 94.5 % cell viability, 100 µg/mL produces 97.5 % cell viability, 50 µg/mL produces 98 % cell viability and 10 µg/mL produces 99.5 % cell viability for HaCaT cells. The IC_{50} values of E for MCF-7 and HaCaT cells were found to be 31.5±3.4 and 20.7±2.77 µM, respectively.

DISCUSSION

Selection of radionuclide and drug/biomolecule is important in the development of new radiopharmaceuticals. While selecting radionuclide as well as biomolecule for the development of a new radiopharmaceutical, compatibility, half-life of the radioisotope, energy emission, stability, stoichiometry and molecular size should always be kept in mind. In this study, 5-FU derivate- compound **E** was used as a pharmaceutical part and ^{99m}Tc was used as radionuclide part of developed radiolabeled compound. Compound **E** contains pyranose sugar and nucleobase groups (Figure 1). These two groups are important role in terms of higher affinity to cancer cells [24]. Because of this, we thought that modification of 5-FU with these groups can affect the cell binding capacity of new radiolabeled compound.

The reducing agent is very crucial for ^{99m}Tc radiopharmaceuticals. The colloid structure occurs in the radiolabeled compound and radiochemical purity ratio begins to decrease by adding reducing agent in high concentrations. With lower concentrations of reducing agent, free technetium ratio decreases. In both cases, radiochemical purity of radiolabeled compound is adversely affected. Mostly stannous salts are preferred to use as reducing agents in ^{99m}Tc radiopharmaceuticals. Technetium normally exists as stable pertechnatate in the +7 oxidative state. While pertechnetate is the most stable state in aqueous solution, technetium compounds have been prepared with oxidation states from 1- to 7+. To promote binding of ^{99m}Tc with the synthesized molecule, reducing agents were used to reduce ^{99m}Tc from the +7 oxidation state to more reactive +5 oxidation state [27,28]. In this study, [^{99m}Tc]Tc**E** was prepared by using stannous chloride. The effect of reducing agent concentration on the labeling efficiency was evaluated, and optimum reducing agent amount was found to be 50 μg (Table 2).

pH can influence the radiolabeling efficiency [29-31]. Generally, ideal pH of a radiopharmaceutical is 7.4 or can vary between 2 and 9. According to the Young *et al*. study, the pH of the reaction medium was found to play unimportant role in the labeling process of 5-FU [9]. While reaction conditions are keeping constant and the reaction pH is varied from 5 to 7.4, no significant differences were observed on labeling efficiency (Figure 3). According to result, the pH of the reaction medium was found to play insignificant role in this study too.

Jung *et al*. was radiolabeled 5-FU by using the stannous chloride reduction method with 555 MBq of ^{99m}Tc. Radiochemical purity of the complex was determined by chromatographic techniques. The overall labeling yield of the^{99m}Tc-5-FU complex was calculated to be more than 98.1±1.2% [10]. Furthermore, Young *et al*. used simple basic radiochemical reactions for

the radiolabeling of the 5-FU. The authors have observed >95% radiochemical purity of this radiolabeled complex [9]. Herein, although the chemical modifications on the main molecule (5-FU), the radiochemical purity of [^{99m}Tc]Tc**E** was found to be greater than 90%. The various complexes of ^{99m}Tc may be formed by interactions between electron donor atoms and reduced technetium. In order to form bonds with technetium, the structure must contain electron donors such as oxygen, nitrogen and sulfur. Although the exact complex structure is not known, results showed that molecule **E** is coordinated with ^{99m}Tc because of its electron donor atoms such as oxygen in its structure [32].

The stability of developed radiopharmaceutical is also a very significant factor as the instability will result in an in vivo dispersion and poor target/non target ratio. In this study, [^{99m}Tc]Tc**E** was found to be stable in saline, human serum and cell culture media (Figure 4).

The retention of radioactivity in tumor cells is following binding of a radiolabeled molecule with factors including the nature of the pharmaceutical part, the chemical structure of radiolabeled group and the properties of the radionuclide [33]. Dar *et al*. [17] evaluated the potential of 5-FU as a diagnostic radiolabeled compound in advanced breast cancer. Their study showed that [^{99m}Tc]Tc-5-FU was a promising agent for diagnosing advanced breast cancer with optimum visualization at 1h. In this study, we used cell culture models to quantitatively evaluate the binding capacity of [^{99m}Tc]Tc**E** at the breast cancer and healthy cells and we found that [^{99m}Tc]Tc**E** is more bound to MCF-7 cell than HaCaT cell (Figure 5) which offer advantages. While optimum visualization of breast cancer with ^{99m}Tc-5-FU was obtained at 1h in Dar et. al. studies, high binding capacity with [^{99m}Tc]Tc**E** was achieved for breast cancer cells in the first 30 minutes. The obtained TEER data supports this binding without damaging healthy cells in this study.

Johnston *et al.* [34] investigated the cellular interaction of 5-fluorouracil and cisplatin in a human colon carcinoma cell line and found that exposure to 5-FU is resulted in a IC_{50} value of 24.2 ±4.5 µM. Another study, Ando *et al.* [35] examined 5-fluorouracil sensitivity in esophageal carcinoma cell lines and found that IC50 value for 5-FU was 39.81 µM. In this study, IC_{50} values of E were found to be 31.5±3.4 and 20.7±2.77 µM for MCF-7 and HaCaT cells, respectively. There is a small change for IC50 values. This finding suggests that the biological inhibition mechanism of 5-FU and molecule E can be similar and molecule E may act via thymidylate synthase (TS) inhibition through its active anabolites such as fluorodeoxyuridine monophosphate (FdUMP) [36].

A high target to non-target ratio is always preferred as it shall result in superior quality imaging of the target organ because there would be little interference from the non-target organs. So, the radiopharmaceutical should have high specificity for the target organ, only then it will result in high target to non-target ratio. If this ratio is not high enough, drug uptake in non-target areas can damage the healthy tissues [4, 12, 32, 33, 37, 38]. Although the exact reason is not explained, the changes have been observed in obtained cell culture results at some time intervals. These changes may be due to the effect of molecule E on the cell. Therefore, the evaluation of cellular uptake ratio will be more accurate for cell culture studies. According to cell culture study results, the highest MCF-7/HaCaT cellular uptake ratio was observed with [^{99m}Tc]Tc**E** at 30 min (15.123±9.151%) and gradually decreased at other times (Table 3).

The use of tracer compounds (drug molecules, radiopharmaceuticals) can interfere with the binding/transport process under study and can also affect the cell integrity. Also, the use of chemical dyes renders the tested cells unusable for further experiments. Therefore, non-invasive techniques are best suited to continuously monitor the cell integrity. TEER is the measurement of electrical resistance across a cellular monolayer and is a very sensitive and reliable method to confirm the integrity and permeability of the monolayer. In this study, the resistance of the cells at the beginning of seeding and after 24 h was measured by TEER. This method addresses different aspects of cell functionally and is widely used as a general criterion of cell viability and integrity [39]. No significant decrease in the TEER values was observed to start binding studies (Figure 6 and 7). The TEER variations were not higher than 40%. If the TEER variation was never higher than 40% the cells were not damaged and the cells were viable and ready for experiments [40].

CONCLUSION

The presented study aimed to develop a new radiolabeled compound which is able to detect the breast cancer. To improve the accumulation percentage of the pharmacocophore in the desired tissue, a new derivative of 5-FU, that contains nucleobase and sugar molecule, was synthesized as (1-[{1′-(1″-deoxy-2″,3″:4″,5″-di-O-isopropylidene-β-D-fructopyranose-1″-yl)-1′H-1′,2′,3′-triazol-4′-yl}methyl]-5-fluorouracil) and optimized.

An easy method of radiolabeling method for novel 5-FU derivative was obtained with high labeling yields at pH 5 and 7.4. To estimate the cancer cell imaging capability, *in vitro* cell binding studies were performed and the incorporation ratios of the labeled compound to

normal and cancer cells were evaluated. 30 min after incubation the affinity of labeled compound to cancer cell lines was found higher than normal cells.

In conclusion, radiolabeled compound has promising properties for detection of breast cancer. Further, investigations are needed for clinical validation *via* animal experiments as well as clinical studies.

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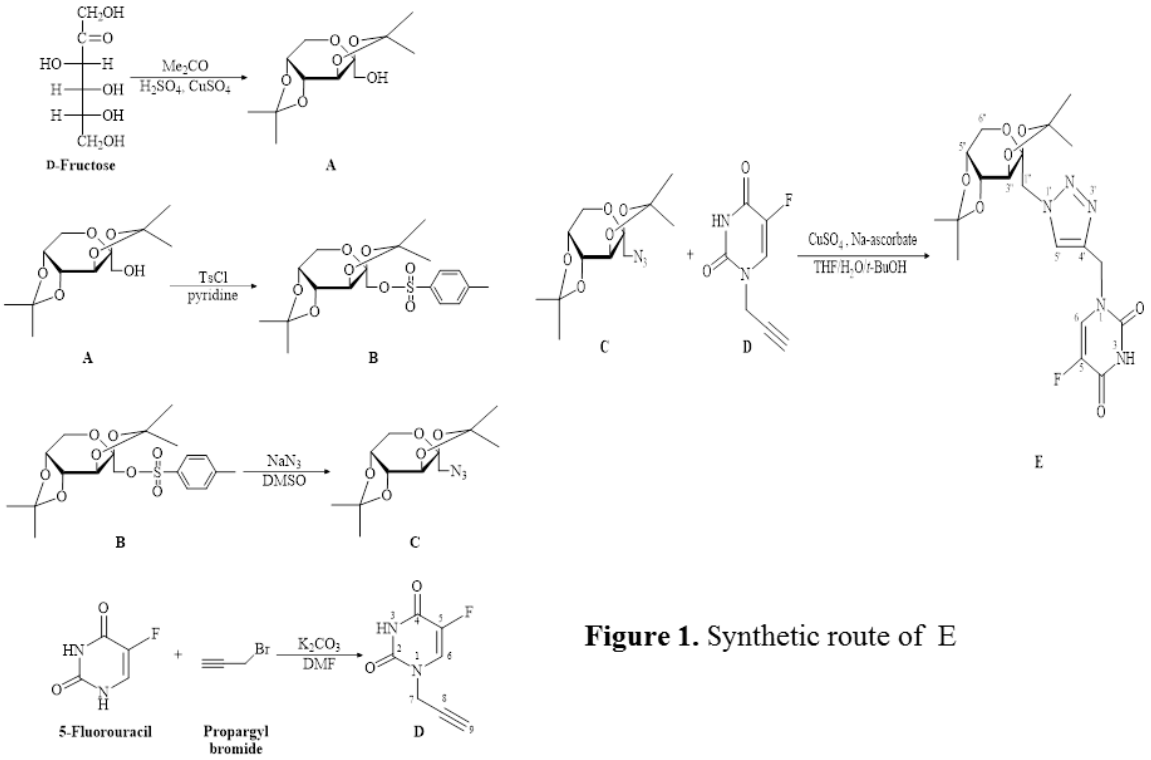

Figure 1. Synthetic route of E

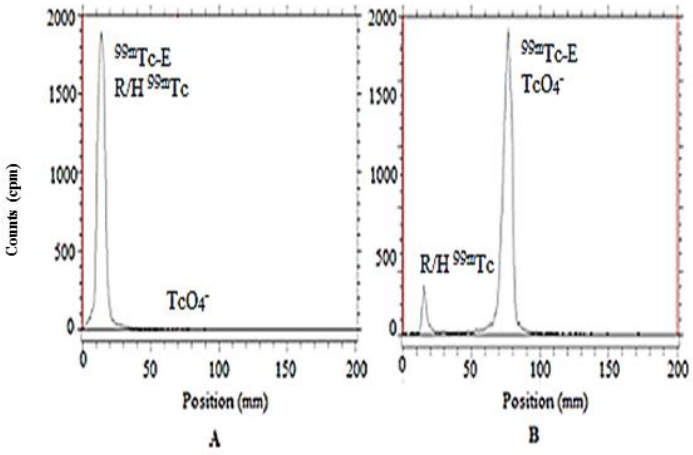

Figure 2. RTLC chromatogram of ^{99m}Tc-E in different mobile phases: A: Acetone, B: Saline.

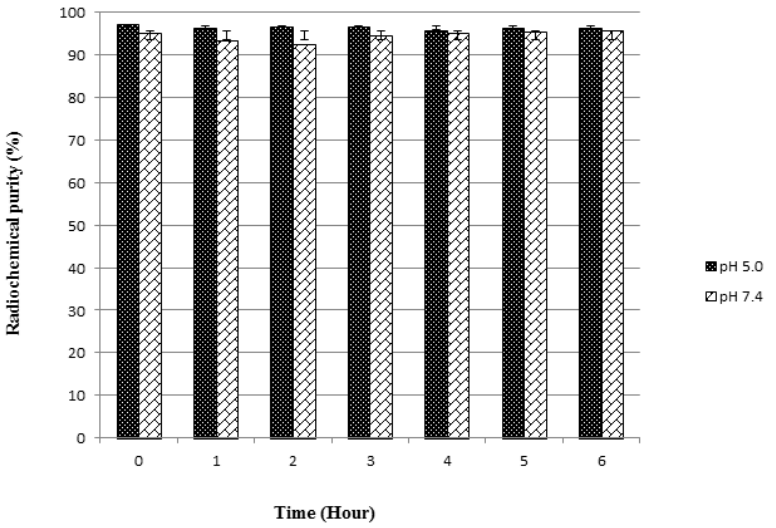

Figure 3. Effect of the pH on radiochemical purity of ^{99m}Tc-**E**

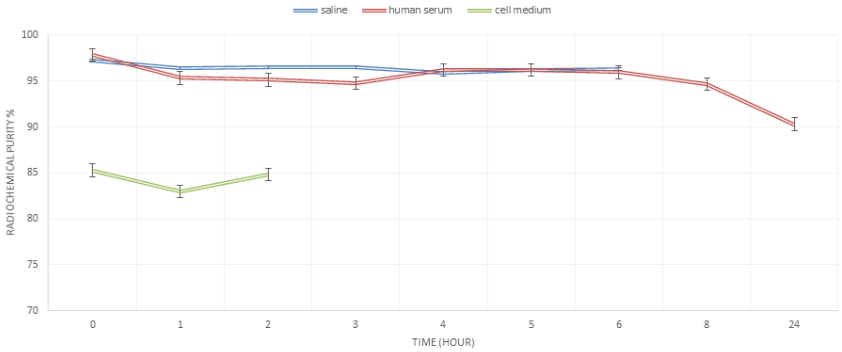

Figure 4. The stability of ^{99m}Tc-**E** in saline, human serum and cell medium

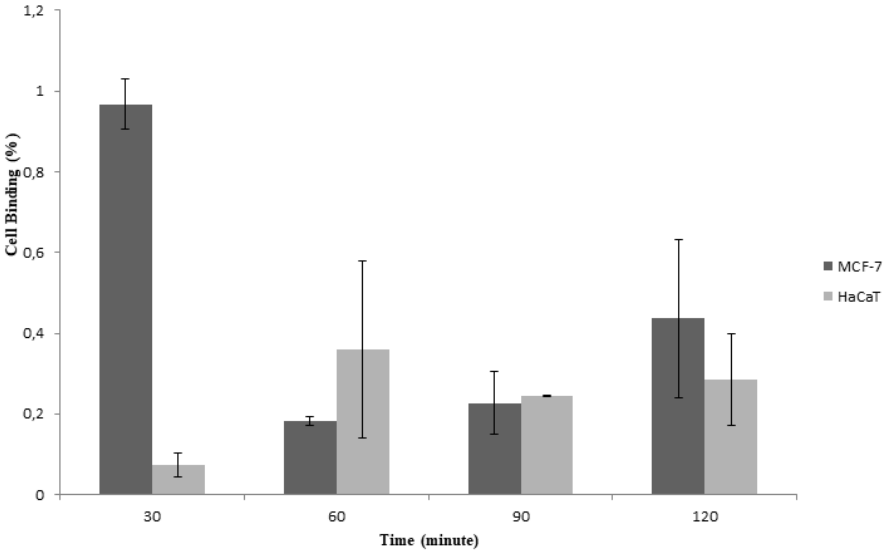

Figure 5. Cell binding percentage of ^{99m}Tc-**E** to MCF-7 and HaCaT cell lines

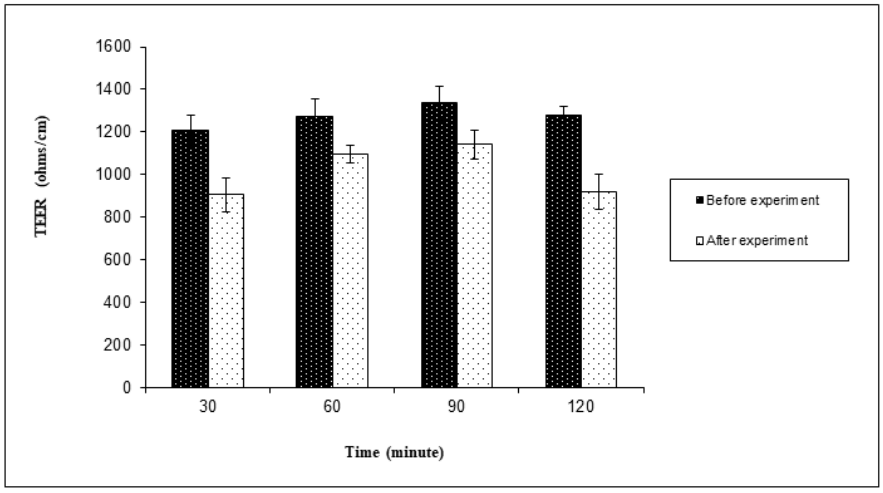

Figure 6. TEER values of MCF-7 cell

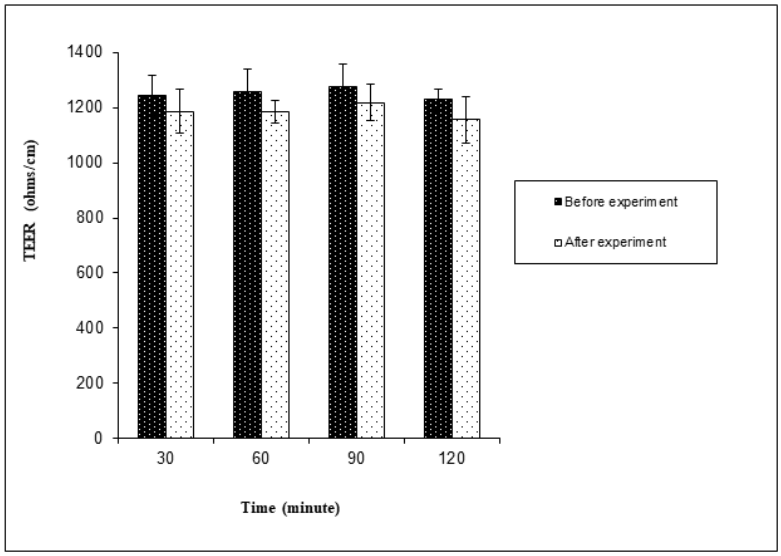

Figure 7. TEER values of HaCaT cell

Table 1. Rf values of ^{99m}Tc-**E** in mobile phases

	Whatman 3MM	
	Acetone	**Saline**
Free ^{99m}Tc	0.8-1.0	0.8-1.0
R/H ^{99m}Tc	0.0	0.0
^{99m}Tc–E	0.0	0.8-1.0

Table 2. Radiochemical purity of ^{99m}Tc-**E** which prepared with different amounts of stannous chloride.

	^{99m}Tc-E (%)			
	Stannous Chloride (µg)			
Time (Hour)	**10**	**50**	**250**	**500**
0	93.47 ± 1.10	97.23 ± 0.16	94.11 ± 1.33	95.91 ± 0.47
1	92.94 ± 1.26	96.43 ± 0.65	93.78 ± 0.72	96.12 ± 0.74
2	90.51 ± 1.75	96.50 ± 0.54	93.97 ± 1.84	94.64 ± 2.08
3	90.59 ± 1.46	96.48 ± 0.30	93.75 ± 1.26	95.53 ± 1.64
4	87.46 ± 5.60	95.87 ± 0.68	92.85 ± 0.51	95.69 ± 0.91
5	82.77 ± 11.49	96.17 ± 0.67	94.25 ± 0.73	94.75 ± 1.63
6	82.47 ± 7.01	96.28 ± 1.08	94.89 ± 0.91	95.29 ± 0.63

Table 3. Cell binding ratios of ^{99m}Tc-**E** to MCF-7 and HaCaT cell lines

Time (Min)	MCF-7/HaCaT ratio
30	15.123 ± 9.151
60	1.638 ± 1.907
90	1.521 ± 0.386
120	1.479 ± 0.709