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A novel radiolabeled indole derivative as solid tumor imaging agent: in silico and preclinical pharmacological study

H. A. Shamsel-Din[1] · A. B. Ibrahim[1]

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Abstract Biologically active indole derivative was synthesized and screened for its cytotoxicity against breast cancer cell line (MCF-7) with the IC_{50} equals 17.5 μg/mL. Docking study showed its high binding affinity to the COX-2 enzyme. It's radiolabeling with ^{99m}Tc and showed high radiochemical yield (92.10 ± 0.57%) and in vitro stability in serum up to 10 h. Biodistribution studies of a novel ^{99m}Tc-NSIC complex in tumor-bearing mice showed high T/NT ratio (6.17 ± 0.08 at 30 min. post-injection), suggesting this complex could be used as a solid tumor imaging agent.

Keywords Indole · ^{99m}Tc · Tumor imaging · Docking · Cox2 · Cytotoxicity

Introduction

The diagnosis of a tumor early and accurate will intensively increase the patient survival [1, 2]. The diagnostic techniques have introduced in nuclear medicine field that is capable of providing informative images of deep solid tumors as example single photon emission computed tomography (SPECT) and positron emission tomography (PET). That important field is interested in the preparation of new radiolabeled imaging agents for diagnosis [3] and the need for selective compounds has a high binding affinity against tumor cell is required. The COX-2 inhibitors have been shown to markedly inhibit tumor growth and metastasis in several animal models of colon, [4–6] skin, [7, 8] lung, [9] bladder, [10] and breast cancers. [11, 12] As a result, COX-2 inhibitors are very selective agents that act to bind with the tumor cells. In recent studies, novel series of indomethacin analogs [13–15] were synthesized which were approved as good COX-2 selective inhibitors. So, these results encouraged us to continue the research on such type of compounds.

Molecular docking is one of the effective approaches for selection of the most suitable compound for each target, through the calculation of the binding affinity and free energy of ligands within a specified active site. Recently, several selective imaging agents for solid tumor such as ^{99m}Tc-DETA, ^{99m}Tc-TEPA, ^{99m}Tc-nitride-pyrazolo[1,5 a] pyrimidine and ^{99m}Tc-2-methoxyisobutyl-isonitrile [16–20], however, they showed some defects like lower tumor uptakes and slow blood clearance. The main target of that research was to discover a new selective imaging agent for solid tumor. This was proposed by the selection of a COX-2 inhibitor with high computational binding affinity to a COX-2 enzyme. Then, it's radiolabeling with ^{99m}Tc and biological evaluation of its selective imaging ability of tumor cells.

Experimental

Materials and methods

All chemicals were of reagent grade and were obtained from Merck Company. ^{99m}Tc was obtained as $^{99m}TcO_4^-$ through elution from a ^{99}Mo/^{99m}Tc generator, Turkey. Whatman No.1 paper chromatography (PC) was purchased from Whatman International Ltd, Merck Company, Germany. The development of the reactions was monitored by

✉ H. A. Shamsel-Din
hisham86@yahoo.com

[1] Labeled Compounds Department, Hot Labs Center, Atomic Energy Authority, P.O. Box 13759, Cairo, Egypt

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thin layer chromatography using aluminum silica gel plates 60 F 245. A NaI (Tl) γ-ray scintillation counter (Scaler Ratemeter SR7 model, England) had used for the measurement of γ-ray radioactivity. High performance liquid chromatography, (Shimadzu HPLC) which consists of UV spectrophotometer detector SpD-6A, Reversed phase Waters Symmetry C18 (RP-18) column (4 mm × 250 mm; 5 μm), Lischrosorb, Merck, pump LC-9A and fraction Collector-LKB, Bromes was used for determining the radiochemical yield. The ^{1}H-NMR spectra were detected on a Varian Gemini 200 NMR Spectrometer at 300 MHz with TMS as a standard, IR spectra were measured on a Perking Elmer 1430 ratio recording infrared spectrophotometer with CDS data station using KBr Wafer technique, and Elemental Analysis at the Micro Analytical Center, Cairo University, Egypt.

Molecular docking studies

The crystal structure of cyclooxygenase (COX)-2 enzyme was downloaded from Protein Data Bank (PDB ID: 3LN1) [21]. The protein was loaded into the Molegro virtual docker (MVD) [22] and all cofactors had removed. The structure of indole derivative (NSIC) was designed using Marvin sketch. Minimization of energy was done to identify the bioactive conformer. Each binding site of oligomeric structure was determined, and the lowest energy pose (based on the Mol Dock Score) of ligand across enzyme COX-2 structure [23].

Synthesis procedure

1-(2-nitrophenylsulfonyl)-1H-indole-3-carbaldehyde (NSIC)

A solution of indole-3-carboxaldehyde (0.145 g, 1 mmol) was added to a solution of sodium hydride (0.036 g, 1.5 mmol) in dry THF (10 mL) and the reaction mixture was stirred for 5 min at 0 °C. Then, 2-nitrobenzenesulfonyl chloride (0.221 g, 1 mmol) was added and the mixture was stirred at 0 °C for 24 h. The reaction mixture was poured onto crushed ice. The product formed was filtered, washed with cold water, purified by silica gel column chromatography using chloroform as eluent, and then the solvent was evaporated and recrystallized from ethanol with yield about 66%. Its physical properties are a brown powder and M.p. 148–152 °C.

IR [KBr, ν_{max} (cm^{-1})]: 1552 (C=C), 1677 (C=O), 2806, 2838 (CH), 3090 (aromatic-CH); ^{1}H-NMR (300 MHz, *DMSO*-d_6, δ (ppm)): 7.44–8.32 (m, 8H, Ar–H), 8.80 (s, 1H, indole H-2), 10.17 (s,1H,CHO); Elemental analysis: Calc. for $C_{15}H_{10}N_2O_5S$: C, 54.54; H, 3.05; N, 8.48; found: C, 54.82; H, 2.99; N, 8.23%.

Evaluation of cytotoxic activity of indole derivative (NSIC)

Evaluation of cytotoxicity activity using the breast carcinoma cell line (MCF7) was performed in the National Cancer Institute, Cairo University, Cairo, Egypt, using the method of [24]. Before treatment with the compound, cancer cells were plated in 96-multiwell plate for 24 h. (10 cells/well) to allow attachment of the cell to the plate wall. The 100 μg/mL of the target compound was added to the cell monolayer triplicate wells and was incubated at 37 °C with compound for 48 h. and in an atmosphere of 5% CO_2. Cells were fixed, washed and stained with Sulfo-Rhodamine-B stain after 48 h. Excess stain was washed with acetic acid and Tris EDTA buffer was used for recovered attached stain. Color intensity was determined by an ELISA reader. The relation between drug concentration and surviving fraction as plotted to show the survival curve of tumor cell line after the target compound was added. IC_{50} value of the NSIC compound was calculated by Graph pad prism-5 software.

Preparation of ^{99m}Tc-NSIC complex

^{99m}Tc-NSIC complex was prepared by the direct radiolabeling technique using sodium borohydride to reduce ^{99m}Tc (VII) into suitable oxidation state for the complexation with NSIC. Radiolabelling by using sodium borohydride as reducing agent was found to be rapid. It has been reported in the literature that stannous salts lead to the formation of radiocolloids [25, 26]. Another advantage of using sodium borohydride is that we can use physiological pH (i.e. 7.4, alkaline pH) in labeling procedure (in case of stannous reduction method, we need to use acidic pH). [27] The sodium borohydride amount, NSIC amount, pH of the reaction and reaction time were optimized to maximize the radiochemical yield as follow:

In an evacuated vial, 100 μL of $^{99m}TcO_4^-$ (400 MBq) in saline and required amounts of solid $NaBH_4$ (0.1–10 mg) were added with continuous stirring followed by 400 μL of NSIC solution in DMSO containing 10–250 μg of NSIC. Various volumes of 0.1 M HCl and/or 0.1 M NaOH solutions have been used for adjusting pH of reaction mixture in the range of 3–11. The reaction mixture was left at different reaction time intervals before investigating the radiochemical yield. Then the in vitro stability was investigated at the optimum condition. In vitro stability was studied by incubating 1 mL of the human serum and 0.1 mL of ^{99m}Tc-NSIC at 37 °C for 12 h. 0.2 mL was withdrawn during the incubation at various time intervals for up to 12 h and the percentage of the ^{99m}Tc-NSIC complex was analyzed using paper chromatography.

Radiochemical yield assay

The radiochemical yields and in vitro stability of ^{99m}Tc-NSIC complex was determined using ascending paper chromatography (PC) and high performance liquid chromatography (HPLC).

Ascending paper chromatographic (PC) analysis

The percent of ^{99m}Tc–NSIC, free pertechnetate ion and reduced hydrolyzed-$^{99m}TcO_2$ colloid were evaluated by paper chromatography using two different mobile phases as follows: The reaction product was spotted on line (origin) of paper chromatography (13 cm long and 1 cm wide) at the distance of 2 cm from the bottom. Acetone, as a mobile phase, was used for determination of free pertechnetate ion which moved with the solvent front ($R_f = 1$), while ^{99m}Tc-NSIC and reduced hydrolyzed-$^{99m}TcO_2$ colloid remained at the origin ($R_f = 0$). Saline, as anther developing solvent, for development another paper strip where colloid remained at the origin ($R_f = 0$) while free pertechnetate ion and ^{99m}Tc-NSIC species developed with the solvent front ($R_f = 1$). The radiolabeling yield percent of the ^{99m}Tc-NSIC complex was calculated as follows:

% Radiochemical yield = 100—(% Free pertechnetate ion + % colloid).

High performance liquid chromatography (HPLC)

The radiochemical yield of the ^{99m}Tc-NSIC was confirmed by the injection of 50 µL of the reaction mixture into RP-18 column of the HPLC system. Using a mixture of acetonitrile: H_2O (65:34 by volume) as a mobile phase and at flow rate of 1 mL/min with wave length of 254 nm. Then the eluted fractions were collected and counted using a well type γ-counter. The retention times of ^{99m}Tc-NSIC and free $^{99m}TcO_4^-$ were 7.65 and 4.3 min, respectively, as shown in Fig. 1.

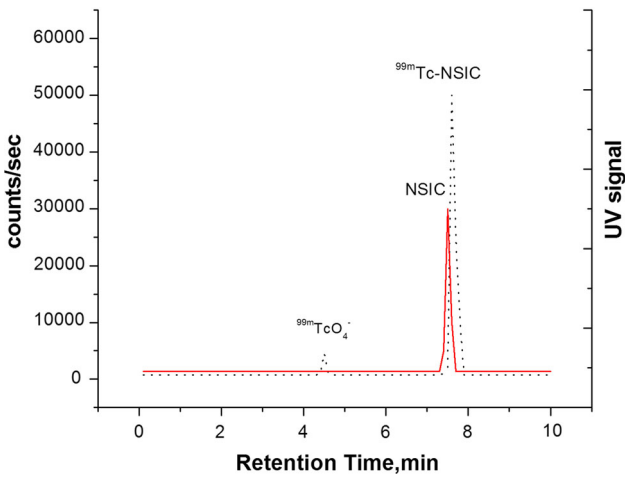


Fig. 1 HPLC radio-chromatogram of ^{99m}Tc-NSIC complex

Biodistribution studies in solid tumor-bearing mice

The study was approved by the animal ethics committee and was in accordance with the guidelines set out by the Egyptian Atomic Energy Authority. Ehrlich Ascites Carcinoma (EAC) The parent tumor line (Ehrlich Ascites Carcinoma), is one of the experimental breast tumor derived from a murine mammary carcinoma [28, 29], had been derived from 7 days old donor female Swiss Albino mice and diluted with sterile physiological saline solution. Exactly 0.2 mL solution was then injected intramuscularly in the right thigh to produce a solid tumor evaluated in female Albino for 4–6 days [30–32].

The biodistribution study of ^{99m}Tc–NSIC complex was evaluated in solid tumor-bearing albino mice of body mass 15–22 g ($n = 5$ mice/time point). A volume of 0.2 mL of a solution containing on ^{99m}Tc–NSIC complex (200–2000 KBq) was injected into the tail vein of mice intravenously. The mice were anesthetized by chloroform at the different time point and their organs and fluids were collected, weighted and their radioactivity was assayed using a NaI(Tl) γ-ray counter. Biological evaluation of ^{99m}Tc–NSIC complex was studied at different time points 5, 15, 30, 60, 120 and 240 min post-injection (p.i.) in mice. The percentages of the injected dose/g organ or fluids (% ID/g) were determined. Blood, bone, and muscles were calculated to be 7, 10 and 40% by the weight of the total body, respectively [33]. Solid tumor to normal muscle (T/NT) was determined from % ID/g for solid tumor and normal muscle.

Statistical analysis

One way ANOVA test was used to evaluate differences in the data. Each result was repeated three times. All results were given as mean ± SEM. The significance level was set at $P < 0.05$.

Results and discussion

Molecular docking study

Molecular docking study was done to evaluate the binding affinity and interaction modes between our compound and the putative target enzyme using the Molegro Virtual Docker (MVD). According to the molecular docking study, the docking interactions analysis for indole derivative (NSIC) indicated different modes of binding in the putative active site of the COX-2 enzyme. As showed in Fig. 2, the

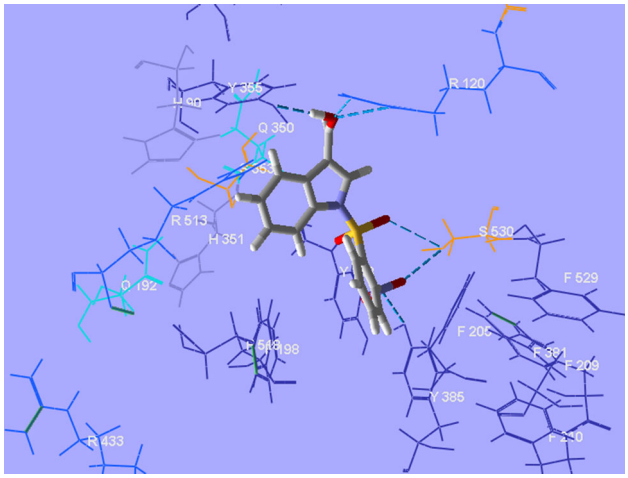


Fig. 2 The Interaction between the NSIC with the active site of COX-2 enzyme

CHO group of NSIC was able to form two hydrogen bonds with NH_2 of Arg A120 and with OH of Tyr A385. One hydrogen bond was observed for SO_2 group with OH of Ser A530, N of NO_2 group with OH of Tyr A385 and O of NO_2 with OH 0f Ser A530.

The docking score of NSIC was displayed the lower binding free energy ($-$ 131.09 kcal mol^{-1}) which indicates the excellent binding affinity with the COX-2 enzyme. From the docking process, we can conclude that NSIC compound predicted to bind selectively to the tumor cells via binding to COX-2. As a result, we selected NSIC to be synthesized and tested its cytotoxicity activity. In addition to, it's radiolabeling with ^{99m}Tc and in vivo evaluation of its selective imaging ability of cancer cells.

Synthesis of NSIC

Synthesis of the NSIC was accomplished according to the sequence indicated in Scheme 1. The synthesized compound was purified by column chromatography on silica gel and confirmed on the basis of elemental and spectral (IR and ^{1}H-NMR) studies. In IR spectrum of NSIC, the carbonyl group showed band at 1677 cm^{-1}, the absorption bands appeared at 2806 and 2838 cm^{-1} for aldehydic CH group and the band near 1200 cm^{-1} for the (S=O) bond. Disappearance band for indole NH group which confirm that the indole derivative was successfully prepared.

The ^{1}H-NMR spectrum showed the chemical shifts at 7.44–8.32 δ as multiplet for aromatic protons. The singlet signals observed at 8.80 and 10.17 δ for indole H-2 and aldehydic proton, respectively. Its elemental analysis data were also in line with theoretical data. All the above data confirm that the indole derivative (NSIC) was successfully prepared.

Preparation of ^{99m}Tc-NSIC complex

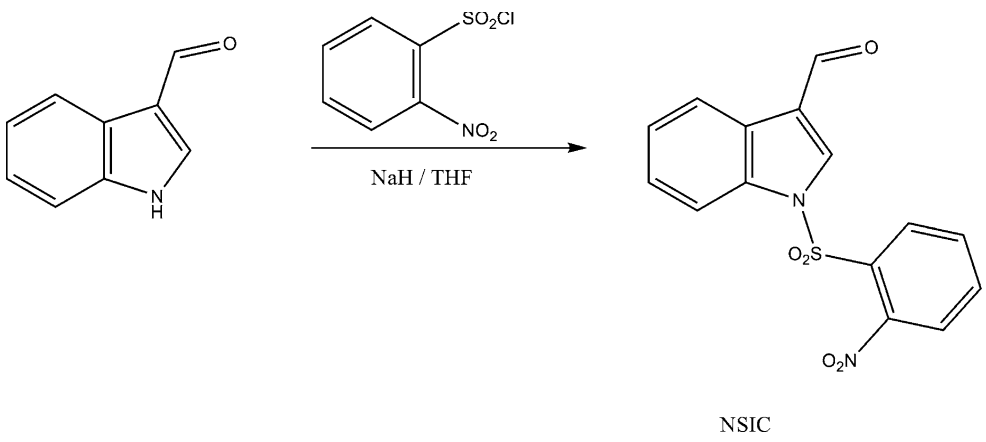
Effect of NSIC amount

The optimum ligand amount was at 150 µg due to that amount was sufficient to chelate all the reduced ^{99m}Tc and the maximum radiochemical yield of 92 ± 0.41% was obtained. Increasing NSIC amount above 150 µg showed no significant change in radiochemical yield as shown in Fig. 3.

Effect of reducing agent

The influence of sodium borohydride amount on the radiochemical yield of ^{99m}Tc–NSIC is shown in Fig. 4. NSIC was radiolabeled with ^{99m}Tc using $NaBH_4$ reducing agent for reduction of ^{99m}Tc from (VII) to lower oxidation state in order to facilitate its chelation with various organic molecules [30]. At low reducing agent amounts, the yield of ^{99m}Tc–NSIC was small because using little $NaBH_4$ amount may lead to an incomplete reduction of $^{99m}TcO_4^-$ and hence, it may lead to the unreliable yield of the ^{99m}Tc-ligand complex due to the presence of free $^{99m}TcO_4^-$. The

Scheme 1 Synthesis of indole derivative (NSIC)



NSIC

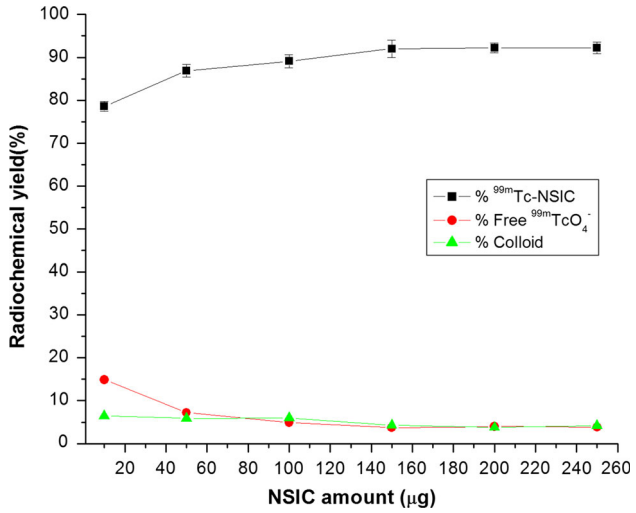


Fig. 3 Effect of NSIC amount on the percent radiochemical yield of ^{99m}Tc–NSIC

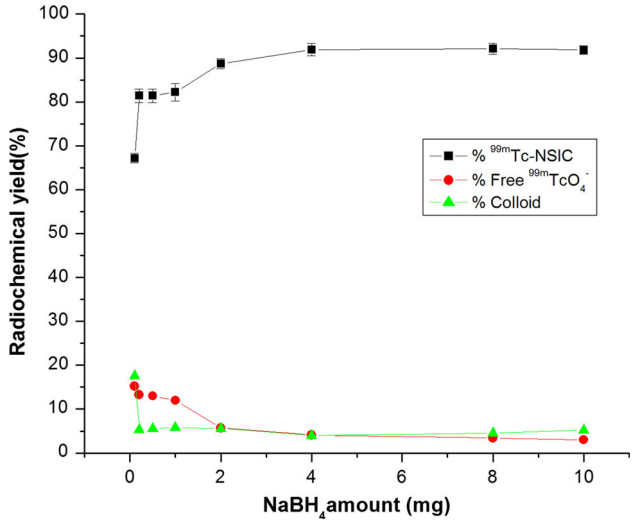


Fig. 4 Effect of $NaBH_4$ amount on the percent radiochemical yield of ^{99m}Tc–NSIC

maximum yield of 91.90 ± 0.81% was achieved by increasing the sodium borohydride amount to 4 mg.

Effect of pH

The effect of pH of the reaction mixture on the radiochemical yield of ^{99m}Tc–NSIC is shown in Fig. 5 in the range of 3–11 by using different volumes of 0.1 M HCl and 0.1 M NaOH solutions. The maximum yield of ^{99m}Tc–NSIC (92.1 ± 0.04%) was obtained at pH 9. At acidic pH values, the radiochemical yield was significantly decreased (60.7 ± 0.1% at pH 3).

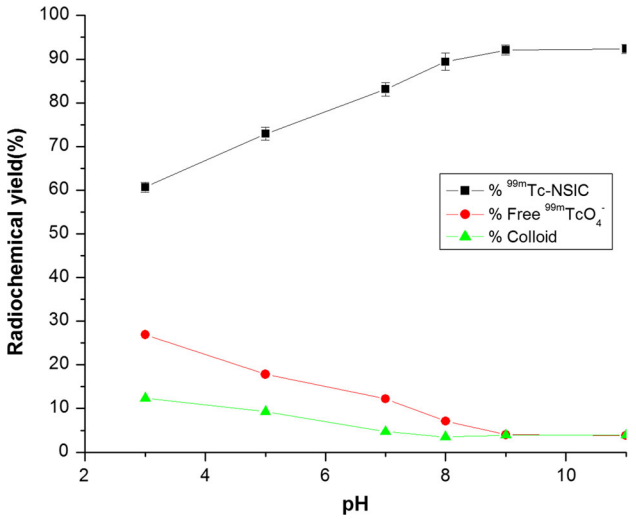


Fig. 5 Effect of pH on the percent radiochemical yield of ^{99m}Tc–NSIC

Effect of reaction time and in vitro stability study

The effect of time, on the radiochemical yield and the in vitro stability of the ^{99m}Tc complexes, was investigated for determining the most suitable range of time during which the complex can be used. Oxidation of the ^{99m}Tc-complex may take place during storage after being radiolabeled with ^{99m}Tc in addition to the radiolysis. As indicated from Fig. 6 that the radiochemical yield of ^{99m}Tc–NSIC complex was increased when the times of reaction had increased from 0 min up to 45 min and the maximum yield of 92.1 ± 0.04% was obtained and showed stability for up to 8 h. Also, the stability of ^{99m}Tc–NSIC in serum was determined by Paper chromatography at various time

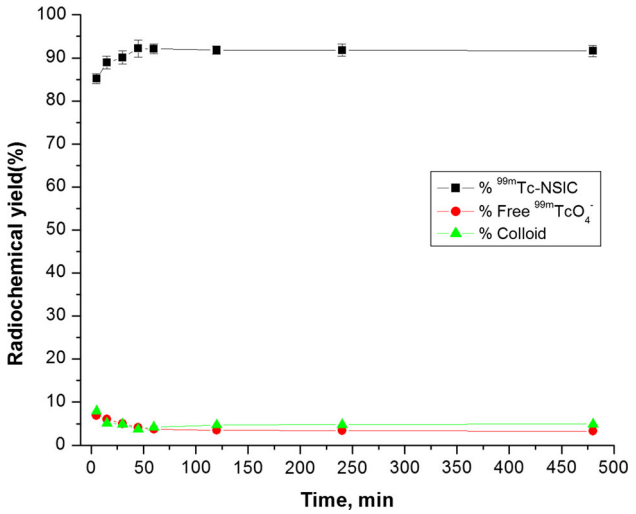


Fig. 6 Effect of reaction time

DRUG CYTOTOXICITY

CONC: ug/ml	MCF7-9D
0.000	1.000
5.000	0.910
12.500	0.584
25.000	0.362
50.000	0.258

MCF7

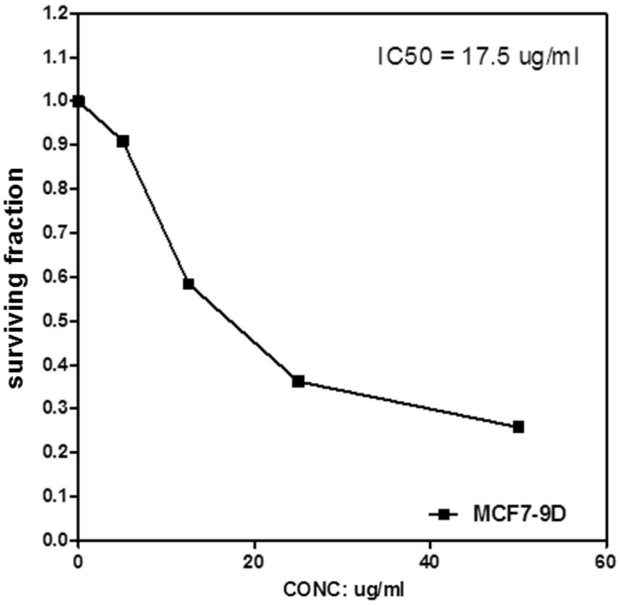


Fig. 7 The IC_{50} value of the NSIC compound

intervals. The results showed that ^{99m}Tc–NSIC was stable in serum showing maximum labeling yield of 91.72 ± 0.32% with no significant decrease up to 10 h.

Biological evaluation

Evaluation of cytotoxic activity of indole derivative (NSIC)

The synthesized target compound was evaluated in vitro for its cytotoxic activity against MCF-7 cell line and compared with doxorubicin as standard drug. From the results of single-dose experiment, synthesized compound (NSIC) exhibited the high percent of inhibition 73.5% at the dose 100 μg/mL. The IC_{50} value of the NSIC compound was found to be 17.5 μg/mL as shown in Fig. 7. Thus, those results had confirmed its high cytotoxicity against breast cancer cell.

Biodistribution of ^{99m}Tc-NSIC in solid tumor-bearing mice

Table 1 shows the biological distribution of ^{99m}Tc-NSIC at 5, 15, 30, 60, 120 and 240 min post injection (p.i) in solid tumor-bearing mice (% ID/g). As shown, ^{99m}Tc-NSIC was primarily excreted via the urinary pathway.

The selectivity and sensitivity of ^{99m}Tc-NSIC as a solid tumor imaging agent was evaluated through the target/non-target (T/NT) ratio is between the tumor tissue (mouse right leg muscle) and normal tissue (mouse left leg muscle). As indicated from Fig. 8 that ^{99m}Tc-NSIC is highly selective to the solid tumor cells with an accumulation ratio 6.17 ± 0.08 at 30 min p.i. Low uptake of ^{99m}Tc-NSIC in stomach at different time intervals post-injection indicates the good in vivo stability of the complex [34, 35]. The target/non-target ratio of ^{99m}Tc-NSIC is higher than that of many other radiopharmaceuticals such as ^{99m}Tc-DETA, ^{99m}Tc-TEPA, ^{99m}Tc-nitride-pyrazolo[1,5 a]pyrimidine and ^{99m}Tc-2-methoxyisobutyl- isonitrile [16–20]. Thus, that preclinical study suggests that ^{99m}Tc-NSIC complex is a

Table 1 Biological distribution of ^{99m}Tc-NSIC in solid tumor bearing mice at different time intervals post-injection (% ID/gram ± S.E, $n = 5$)

Organs and body fluids	% Injected dose/gram at different time intervals (min)					
	5	15	30	60	120	240
Tumor/muscle (T/NT)ratio	2.71 ± 0.02	4.09 ± 0.02	6.17 ± 0.08	5.83 ± 0.07	4.25 ± 0.09	3.66 ± 0.07
Tumor	6.31 ± 0.01	7.15 ± 0.02	9.07 ± 0.07	7.69 ± 0.05	3,78 ± 0.08	2.48 ± 0.06
Muscle	2.33 ± 0.04	1.75 ± 0.03	1.47 ± 0.01	1.32 ± 0.07	0.89 ± 0.02	0.68 ± 0.02
Blood	15.97 ± 0.12	15.15 ± 0.07	8.95 ± 0.18	7.35 ± 0.16	4.67 ± 0.12	2.23 ± 0.11
Kidneys	18.45 ± 0.10	22.67 ± 0.12	30.18 ± 0.12	38.81 ± 0.09	42.72 ± 0.10	47.62 ± 0.18
Liver	3.62 ± 0.04	3.78 ± 0.09	5.16 ± 0.10	6.92 ± 0.03	8.23 ± 0.08	10.02 ± 0.06
Stomach	4.92 ± 0.04	5.02 ± 0.12	3.65 ± 0.12	5.39 ± 0.16	6.39 ± 0.14	6.48 ± 0.32
Spleen	4.25 ± 0.02	3.81 ± 0.03	3.25 ± 0.06	2.14 ± 0.09	1.80 ± 0.09	1.66 ± 0.02
Intestine	0.95 ± 0.02	4.74 ± 0.08	5.34 ± 0.01	5.13 ± 0.02	4.23 ± 0.03	3.99 ± 0.08
Lungs	0.73 ± 0.07	0.81 ± 0.01	3.87 ± 0.05	10.06 ± 0.1	6.96 ± 0.09	3.56 ± 0.05
Heart	6.82 ± 0.06	3.49 ± 0.09	3.04 ± 0.04	2.02 ± 0.05	0.37 ± 0.05	0.23 ± 0.02
Bone	2.95 ± 0.1	8.90 ± 0.04	8.92 ± 0.09	6.32 ± 0.06	3.08 ± 0.04	0.88 ± 0.09

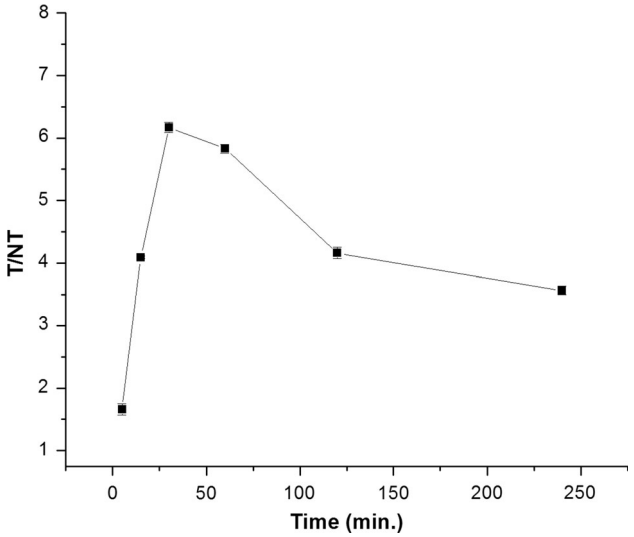


Fig. 8 the variation of T/NT of ^{99m}Tc-NSIC at different time intervals post injection

selective agent for solid tumor imaging and can be used as a hopeful solid tumor imaging radiotracer.

Conclusions

The docking study displayed that the synthesized compound (NSIC) showed promising affinity to inhibit COX-2. NSIC showed a high binding affinity of $-$ 131.09 kcal mol^{-1} and the cytotoxicity study indicated its high inhibition activity (73.5% at 100 μg/mL) against breast cancer cell (MCF-7). Then, NSIC was radiolabeled using ^{99m}Tc and gave a high radiochemical yield of $\sim$ 92.10% with high in vitro stability in serum up to 10 h. Furthermore, the preclinical biodistribution studies revealed that ^{99m}Tc-NSIC complex has a high T/NT ratio of $\sim$ 6.17 at 30 min. post injection in solid tumor-bearing mice and is rapidly cleared out of body organs and excreted via the urinary pathway. So, in conclusion, this research article could introduce ^{99m}Tc-NSIC complex as a selective potential radiopharmaceutical for solid tumor imaging.

References

1. Boonstra MC, de Geus SWL, Prevoo HAJM, Hawinkels LJAC, van de Velde CJH, Kuppen PJK, Sier CFM (2016) Biomark Cancer 8:119–133
2. Kapetas P, Pinker K, Helbich T (2017) Hell J Radiol 2(3):5
3. Jurisson S, Beming D, Jia W, Ma D (1993) Chem Rev 93(3):1137–1156
4. Yang X, Xu Y, Wang T, Shu D, Guo P, Miskimins K, Qian SY (2017) Redox Biol 11:653–662
5. Zeng Z, Wei Z, Ma L, Xu Y, Xing Z, Niu H, Huang W (2017) J Mater Chem B 5(33):6860–6868
6. Yoysungnoen B, Bhattarakosol P, Changtam C, Patumraj S (2016) Biomed Res Int 2016:1781208
7. Jacoby RF, Seibert K, Cole CE (2000) Cancer Res 60:5040–5044
8. Wilgus TA, Ross MS, Parrett ML (2000) Postaglandins Other Lipid Mediat 62:367–384
9. Lu J-J, Fu L, Tang Z, Zhang C, Qin L, Wang J, Deng W (2016) Oncotarget 7(3):2985–3001
10. Yang X, Shi L, Yi C, Yang Y, Chang L, Song D (2017) Am J Cancer Res 7(8):1738–1753
11. Harris RE, Alshafie GA, Abou-Issa H (2000) Cancer Res 60:2101–2103
12. Yun E-J, Song K-S, Shin S, Kim S, Heo J-Y, Kweon G-R, Lim K (2016) Oncotarget 7(31):49961–49971
13. Khaled RA, Elsaady MT, Amin NH, Hefny AA (2017) Der Pharma Chem 9(14):94–99
14. Abdellatif KR, Lamie PF, Omar HA (2016) J Enzyme Inhib Med Chem 31(2):318–324
15. Lamie PF, Ali WA, Bazgier V, Rárová L (2016) Eur J Med Chem 123:803–813
16. Wang J, Yang J, Yan Z, Duan X, Tan C, Shen Y, Wu W (2011) J Radioanal Nucl Chem 287(2):465–469
17. Ding R, He Y, Xu J, Liu H, Wang X, Feng M, Qi C, Zhang J, Peng C (2012) Med Chem Res 21(4):523–530
18. Machac J, Krynyckyi B, Kim C (2002) Semin Nucl Med 32(4):276–292
19. Hruska CB, Conners AL, Jones KN, Weinmann AL, Lingineni RK, Carter RE, Rhodes DJ, O'Connor MK (2014) EJNMMI Res 4(1):5–18
20. Hsia CC, Huang FL, Hung LH, Shen LH, Chen CL, Wang HE (2011) Appl Radiat Isot 69(4):649–655
21. Wang JL, Limburg D, Graneto MJ, Springer J, Hamper JR, Liao S, Pawlitz JL, Kurumbail RG, Maziasz T, Talley JJ, Kiefer JR (2010) Carter. J Bioorg Med Chem Lett 20:7159–7163
22. Thomsen R, Christensen M (2009) Molegro ApS: Aarhus, Denmark, 125-133
23. Akaho E, Fujikawa C, Runion H, Hill C, Nakaho H (1999) J Chem Softw 4:147–162
24. Skehan P, Storeng R, Scudiero D, Monks A, McMahon J, Vistica D, Warren JT, Bokesch H, Kenney S, Boyd MR (1990) J Natl Cancer Inst 82(13):1107–1112
25. Banerjee T, Singh AK, Sharma RK, Maitra AN (2005) Int J Pharm 289:189–195
26. Banerjee I, De K, Chattopadhyay S, Bandyopadhyay AK, Misra M (2014) J Radioanal Nucl Chem 302:837–843
27. Jain V, Swarnakar NK, Mishra PR, Verma A, Kaul A, Mishra AK, Jain NK (2012) Biomaterials 33:7206–7220
28. Abu-Zeid M, Hori H, Nagasawa H, Uto Y, Inayama S (2000) Biol Pharm Bull 23(2):195–198
29. Rhodes BA (1974) Semin Nucl Med 4(3):281–293
30. Motaleb MA, Abdel-Ghaney IY, Abdel-Bary HM, Shamsel-Din HA (2016) J Radioanal Nucl Chem 307:363–372
31. Sakr TM, Essa BM, El-Essawy FA, El-Mohty AA (2014) Radiochemistry 56(1):76–80
32. Wan W, Yang M, Pan S, Yu C, Wu N (2008) Drug Dev Res 69(8):520–525
33. Abdel-Ghaney I, Moustafa K, Abdel-Bary HM, Shamsel-Din H (2013) J Radioanal Nucl Chem 295(2):1273–1281
34. Harden RM, Alexander WD, Kennedy I (1967) Lancet 289:1305–1307
35. Rashed HM, Marzook FA, Farag H (2016) Radiol Med 121:935–943