PROF. FATMA YURT LAMBRECHT (Orcid ID : 0000-0002-9394-6908)

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Synthesis and investigation of anti-cancer potential of radiolabelled naphthalene monoimide bearing imidazolium salt

Fatma Yurt Lambrecht[1], Kasim Ocakoglu[2,3], Suleyman Gokhan Colak[2], Onur Alp Ersoz[1], Ozge Er[1]

[1]*Ege University, Department of Nuclear Applications, Institute of Nuclear Science, Bornova, 35100, Izmir, Turkey.*

[2]*Mersin University, Advanced Technology Research & Application Center, Ciftlikkoy Campus, TR33343, Yenisehir, Mersin, Turkey.*

[3]*Mersin University, Department of Energy Systems Engineering, Tarsus Faculty of Technology, 33480 Mersin, Turkey.*

Corresponding author email id: ftmyurt@gmail.com

Imidazolium salts and derivatives have anti-tumor efficacy and toxic effects in different microorganisms. In present study, an imidazolium bromide salt (NMI) was synthesized, and its antitumor potential was investigated by *in vitro* studies. Radiolabeling of synthesized NMI was carried out by iodogen method using ^{131}I radionuclide. The yield of radiolabeling was determined as 98.5±0.1%. After that, cytotoxicity and intracellular uptake studies were evaluated in various cell lines. The cytotoxicity of NMI was determined as 35 µM, 20 µM, 10 µM and 1 µM for HEK-293, PC-3, CaCo-2 and MCF-7 cells, respectively. In addition, the intracellular uptake of ^{131}I-NMI was investigated in the cell lines, and the uptake was

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significantly found as 4 h for MCF-7 and 6 h for PC-3. In future studies, antitumor efficacy of ^{131}I-NMI on tumor bearing animal model might be studied in the light of these results.

Keywords: nuclear medicine, ^{131}I, radiolabeling, imidazolium salts, anti-cancer potential.

*Corresponding author Fatma Yurt Lambrecht, Department of Nuclear Applications, Institute of Nuclear Science, Ege University, Bornova, 35100, Izmir, Turkey Tel: +902323113460; fax: +902323113433

Introduction

In recent years, imidazolium salts have attracted increasing interest and been successfully used in a variety of application areas due to their inherent merits such as less toxicity, biocompatibility, low cost, high chemical and thermal stability, non-flammability, relatively low viscosity and vapor pressure, high ionic conductivity, and a wide electrochemical window (1-6). They can also be readily prepared with relatively simple equipment under moderate reaction conditions.

Imidazolium salts (IMSs) which are a part of many biological systems can be found in natural products such as Lepidiline A and Lepidiline B were isolated from the roots of Lepidium meyenii (1). These organic salts can easily be prepared by the alkylation of both nitrogen atoms of imidazole ring with different functional groups which greatly affect the properties and application areas of IMSs. For instance, the hydrophilic cationic segments of IMSs show toxic effects on multivarious microorganisms (7, 8). The main reason of this toxicity is strong interactions between IMSs and biological components such as cell membranes and DNA. The toxicity and bio-activity of the IMSs can be adjusted by changing their amphiphilic structure (9). Imidazole derivatives also show antibacterial activities, and recently several inhibitors containing the imidazole moiety have been studied (10).

In the course of development of new cancer drugs in current chemotherapy studies more effective strategies have been shown for reducing toxicity (11). Nitrogen atoms of the imidazolium cation cause increase in the anti-tumor activity, and these properties can be improved by using different amphiphilic structures (12). Adjusting the toxicity of imidazolium salts for their applications as anti-tumor agents has attracted much attention, and one of these methods is to use metal complexes bearing N-heterocyclic carbene ligands as anti-cancer drugs (13).

In vitro studies results have shown that imidazolium and pyridinium salts can prevent the action of acetylcholinesterase with an enzyme that could be seen in bacteria, fungi, plants and higher organisms except for yeasts (14). The tests with Phosphonium salts indicated that their inhibitory properties were less than the imidazolium and pyridinium salts, and they can show different *in vivo* uptake when compared to imidazolium salts (15).

The studies with indoline-2-carboxylic acid N-(substituted) phenylamide by Kwak et al. revealed the cytotoxic activities against human lung (NCI-H23) and prostate carcinoma cells (PC-3) (16).

The toxicity of IMSs depends on different factors such as lipophilicity and molecular structure (17, 18). However, the toxicity of ionic liquids could cause through various modes of action. For instance, the researchers put forward that there is a very small effect of the anion on toxicity although some studies have revealed a stronger effect of the anion (18).

Radiopharmaceuticals are widely used materials in nuclear medicine nowadays, and they are used in diagnosis or treatment of cancer and diagnosis of infection. Radioactive Iodine-131 (^{131}I) is one of radionuclide that is used routinely in nuclear medicine. ^{131}I has been used for treatment of benign and malignant conditions of the thyroid because of its uptake mechanism since 1940's (19, 20). Iodine-131 is a long half-life radionuclide (8 days) and has beta energy

of 0.192 MeV and gamma energy of 0.364 MeV (21). For these reasons it is commonly used in the studies of Single-photon emission computed tomography (SPECT) which is a nuclear medicine tomographic imaging technique.

This study reports the synthesis of an imidazolium salt derivative (NMI) and investigation of anti-cancer potential of radio iodinated NMI with *in vitro* studies.

Methods

All cell culture mediums were supplied from Biological Industries. Iodogen was purchased from Sigma-Aldrich. The other chemicals and thin-layer chromatography-cellulose sheers (ITLC-F plastic sheets 20x20) were supplied from Merck. The cell culture studies were held in Thermo MSC Advantage 1.2 Laminar Air Flow. Olympus Japan Inverted and light microscope were used to count cells. IC_{50} values of NMI were determined by using Thermo Multimode microplate reader.

1-(3-Aminopropyl) imidazole, 1,8-naphthalic anhydride, 1-bromooctane were purchased from Aldrich and used without any treatment. ^{1}H-NMR spectra was measured on a Bruker 400 MHz spectrometer.

Synthesis of N-(3-propylimidazole)-1,8-naphthalene monoimide (1)

N-(3-propylimidazole)-1,8-naphthalene monoimide (1) was synthesized according to the previously reported method (23). ^{1}H NMR (δ_H, ppm, 400 MHz, $CDCl_3$): 8.49 (d, 2H, *J*= 7.2 Hz Ar); 8.14 (d, 2H, *J*= 7.6 Hz, Ar); 7.68 (t, 2H, *J*= 7.6 Hz, Ar); 7.52 (s, 1H, Ar); 6.97 (s, 2H, Ar); 4.17-4.13 (t, 2H, *J*= 6.8 Hz); 4.05- 4.01 (t, 2H, *J*= 7.6 Hz); 2.23-2.16 (m, 2H, *J*= 7.2 Hz).

^{13}C NMR (δ_c, ppm, 400 MHz, DMSO): 163.6, 136.0, 134.4, 131.2, 130.7, 127.4, 127.2, 122.4, 122.3, 122.0, 48.8, 46.9, 36.6, 30.7, 29.2, 28.2, 28.2, 25.4.

Synthesis of 1,8-naphthalene monoimide bearing imidazolium salt (NMI)

Imidazolium bromide salt was synthesized through of N-alkylation reaction (24) between N-(3-propylimidazole)-1,8-naphthalene monoimide and corresponding alkyl bromide compound (Figure 1). N-(3-propylimidazole)-1,8-naphthalene monoimide (430 mg, 1.4 mmol) was dissolved in 10 ml $CHCl_3$ and under inert atmosphere. 1-bromooctane (3.3 g, 1.7 mmol) was added dropwise to the solution, and the reaction mixture was heated under reflux for 24 h. The mixture was allowed to cool down to room temperature, and the solid was filtered. A white solid was recrystallized from CH_2Cl_2/diethyl ether mixture. The compound was obtained as a white solid in 15% yield.^{1}H NMR (δ_H, ppm, 400 MHz, $CDCl_3$): 10.48 (s, 1H, Ar); 8.53 (dd, 2H, *J*=1.04 Hz, 7.28 Hz, Ar); 8.20 (dd, 2H, *J*=1.04 Hz, 7.28 Hz, Ar); 7.74-7.70 (m, 3H, Ar); 7.41(t, 1H, *J*= 1.78 Hz, Ar); 4.49 (t, 2H, *J*= 6.6 Hz); 4.38 (t, 2H,*J*= 7.46 Hz); 4.20 (t, 2H, *J*=6.5 Hz); 2.42 (q, 2H, *J*= 13.2 Hz); 1.97-1.89 (m, 4H); 1.27 (m, 8H,); 0.83 (t, 3H, *J*= 6.9 Hz). ^{13}C NMR (δ_c, ppm, 400 MHz, $CDCl_3$): 164.2, 137.1, 134.3, 131.3, 127.8, 126.9, 122.4, 121.9, 50.1, 47.7, 36.7, 31.5, 30.2, 28.9, 28.8, 26.1, 22.4, 13.9.

Radiolabeling of NMI

NMI (10 mg) was dissolved in 10 mL distilled water with stirring. Iodogen coated tube was prepared by dissolving 1 mg Iodogen in 1 mL dichloromethane, and then the solvent was evaporated. 25 µL of prepared solution was diluted with 500 µL distilled water. The NMI

solution was transferred into the iodogen coated tube. Then 10-100 MBq ^{131}I was added the tube and the tube was incubated at room temperature for 30min.

The radiochemical purity of radiolabeled NMI was determined by Thin Layer Radio Chromatography (TLRC) method. In this study, the sample from the tube was dropped on cellulose-coated plastic (ITLC-cellulose) sheets (1×10 cm, Merck). The sheets were put in mobile phases [mobile phase-1:n-butanol-water-acetic acid (4-2-1) and mobile phase-2: isopropyl alcohol-n-butanol-0.2 M $NH_4(OH)_2$ (2-1-1)]. After developing, the ITLC-cellulose sheets were removed from the mobile phases, dried and scanned on a TLC scanner (BioScan AR-2000 Washington DC, USA).

In vitro stability

For defining shelf life of radiolabeled compound, the stability was studied to determine the stability of ^{131}I-NMI after labeling on optimum conditions (room temperature, 1 mg iodogen and reaction time 30 min). The yield was checked at different time intervals (30, 60, 120, 240 and 1440 min).

Lipophilicity

Lipophilicity test was performed to determinate ^{131}I-NMI. 150 µL of radiolabeled NMI was added to 3 mL of n-octanol, 3 mL of distilled water. The solution was centrifuged (2500 rpm, 5 min.) for the separation of the phases after mixing the solution with a vortex for 1 hour. Subsequently, 500 µl of samples were taken from each phase and counted by Cd (Te) RAD-501 single channel analyzer.

Cell culture

Cell culture studies were performed by using CaCo-2 [ATCC HTB-37™, tissue: human colon, colorectal adenocarcinoma], PC-3 [ATCC CRL-1435™, tissue: human prostate, adenocarcinoma], MCF-7 [ATCC HTB-22™, tissue: human epithelial breast adenocarcinoma] and HEK-293 [ATCC CRL-1573™, human epithelial embryonickidney] cell lines. These cells were cultured in MEM consist of Non-Essential Amino Acid Solution containing 100 IU/mL penicillin G, 100 mg/mL streptomycin and 10 % heat-inactivated Fetal Bovine Serum (FBS) and maintained at 37°C in incubator containing 5 % CO_2.

Cytotoxicity assay of NMI

MTT method was used for determining IC_{50} values of NMI in HEK-293, CaCo-2, MCF-7 and PC-3 cell lines. The cells was formed in a 96-well culture plate (1×10^5 cells in each well) and various concentration of NMI prepared with MEM solution (w/o FBS) was added in each well after 1 day incubation. The medium on cells was removed and MTT solution was added in each well after twenty four hours. After 4 h incubation, DMSO solution was accrued for solving MTT and absorbance of wells were measured in a micro plate reader (Varioskan Flash Multimode Reader Thermo, Finland) at 560 nm. Afterwards, the percentage of cytotoxicity was calculated.

Intracellular uptakes of ^{131}I-NMI

The in vitro cellular uptake of ^{131}I labeled NMI was performed in CaCo-2, MCF-7, and PC-3 cancer cell lines and HEK-293 normal cell line was used as a control group. The cells were placed in 24-well culture plates (1×10^5 cells in each well) for 2 days in 37 °C incubator. After

two days, the medium on the cells was removed and the wells were washed 2 times with 0.9 % NaCl solution. The radiolabeled NMI (25 µM, activity: 0.9 Bq) diluted with MEM (without FBS) was added on the cells. However, the intracellular uptake of $Na^{131}I$ was assayed at the same conditions. At this step, it was checked if the uptake was caused by free iodine or radioiodinated compound. After determining of time period incubation (1, 2, 4, 6 and 24 h), the wells were counted by Cd(Te) RAD501 signal channel analyzer. Then, the radioactive medium on cells was removed, washed and 0.9 % NaCl solution was added in each well. The wells had been counted again. The data of intracellular uptake study were analyzed, and the percentage of uptake was calculated. Statistical analysis of the intracellular uptake results were performed with Minitab 16. Two sample T-Test were carried out for the uptake of ^{131}I labeled NMI in MCF-7 and HEK-293; PC-3 and HEK-293; CaCo-2 and HEK-293 cell lines. At the same time, two sample T-Test was performed for the uptake of $Na^{131}I$ and ^{131}I labeled NMI in MCF-7, PC-3 and CaCo-2 cells.

Results and Discussion

Radiolabeling of NMI

TLRC analysis results of radiolabeled NMI showed that the relative front (R_f) values of $Na^{131}I$ and ^{131}I-NMI were 0.5, 0.2 (mobile phase-1) and 0.8, 0.3 (mobile phase-2), respectively. Radiolabeling yield of ^{131}I-NMI was determined as 98.5±0.1%.

Radiolabeling efficiency of ^{131}I-NMI was found as quite high. Oxidized iodine (I^+) has high affinity towards an aromatic ring in the molecule. It can easily bind to the aromatic ring via replacing with H^+ atom (25-29).

In the stability experiment, a little bit change in the stability of ^{131}I-NMI until 2 h is observed (Figure 2). This result showed the stability time of radiolabeled NMI has enough shelf life for using on nuclear imaging.

The results of lipophilicity measurements showed that lipophilicity of ^{131}I-NMI is 0.04, and thus the radiolabeled compound has low lipophilic property. The lipophilicity is important for intracellular uptake mechanism of ^{131}I-NMI. Since, the lipophilic compounds show higher uptake by tumor cells than normal cells (30-32).

Cytotoxicity of NMI

According to MTT cytotoxicity test results, IC_{50} values were determined as to be 35 µM, 10 µM, 20 µM and 1 µM for HEK-293, CaCo-2, PC-3 and MCF-7, respectively. It is seen that IC50 values are quite low, and for this reason, NMI can be considered to have a potential as chemotherapy agent (Figure 3-6).

Intracellular uptake

The intracellular uptakes of ^{131}I-NMI in CaCo-2, MCF-7, PC-3 and HEK-293 cell lines were determined (n=3). The uptake of ^{131}I-NMI was evaluated in CaCo-2, MCF-7, PC-3 cell lines and compared with HEK-293 cell line (Figure 7). The uptake of ^{131}I in all cell lines was also shown in Figure 8. The highest uptake of labeled compound was seen in PC-3 cell lines at sixth hour (1.15±0.12%), and another high uptake of ^{131}I-NMI was determined in MCF-7 cell lines at 4 h (1.01±0.18%). On the other hand, maximum uptake of ^{131}I-NMI in CaCo-2 cell line was found as 0.58±0.10%.

The maximum uptake of ^{131}I-NMI in MCF-7 was calculated as seven times higher and six times higher in PC-3 than its uptake in HEK-293. It is reported in the literature that imidazolium salts derivatives showed remarkable uptake in MCF-7 (33, 34) and PC-3 (35) cell lines. Tumor to non-tumor (T/NT) ratio of a compound must be two fold or more for imaging of tumor (36). The tumor (MCF-7 and PC-3) to non-tumor (HEK-293) ratio of NMI is suitable for nuclear imaging. Moreover, the intracellular uptake of ^{131}I was much lower than that of uptake obtained for ^{131}I–NMI in all cell lines, and these results show that the uptake in the cell lines is not based on iodine.

At the same time the intracellular uptake results were found significant by statistical analysis. P values for the uptke of ^{131}I–NMI in HEK-293 vs MCF-7, HEK-293 vs PC-3 and HEK-293 vs CaCo-2 were determined respectively as 0.033, 0.027 and 0.006. On the other hand, P values for Na^{131}I vs ^{131}I–NMI in MCF-7, PC-3 and CaCo-2 were found respectively as 0.013, 0.107 and 0.002.

The synthesis and radiolabeling of 2-Nitroimidazole derivatives of cyclam has been studied by Engelhard et al. In this study, the compounds were labeled with ^{99m}Tc, ^{67}Cu and ^{64}Cu radionuclides. Their uptake mechanisms into tumor cells were characterized and the biodistribution and clearance kinetics of the radiolabeled compounds were determined. According to the results, ^{99m}Tc labeled compound has almost 10 times lower tumor uptake than that of radiolabeled compounds with copper isotopes (37).

In the study reported by Kurtdede et al., ^{131}I labeled 4-benzoyl-1-(4-carboxyphenyl)-5-phenyl-1H- pyrazole-3-carboxylic acid (P3CA) was studied, and *in vivo* studies were performed on Albino Wistar rats. According to the results, the maximum uptake of ^{131}I-P3CA was seen in lungs, stomach and spleen at 15 min (38). Haque et al. has been studied with ortho/paraxylyl linked bis-imidazolium salts and Ag (I) N-heterocyclic carbine (NHC) complexes, and

reported cytotoxicity tests results of all compounds against human colorectal cancer (HCT 116) and breast cancer cell lines (MCF-7). The IC_{50} values were found as 5.6- 20.3 μM for HCT 116 and 1.12-6.38 μM for MCF-7 (39). An investigation of 2-substituted indoline imidazolium salt derivatives against a panel of human tumor cell lines was reported by Xu et al. According to their results, the range of IC_{50} values of the compound 25 were 0.24– 1.18 μM against MCF-7, SW480, SMMC-7721 and HL-60 cell lines. On the other hand, it is showed that compound 26 inhibited powerfully SMMC-7721 and A549 cell lines (40).

Conclusions

In this study, the antitumor potential of ^{131}I-NMI in HEK-293, CaCo-2, MCF-7 and PC-3 cell lines were evaluated with the intracellular uptake studies. ^{131}I labeled NMI showed significant uptake efficiency in MCF-7 and PC-3 cell lines. Further investigation is also necessary in tumor bearing animals to clarify the potential of radiolabeled NMI for breast and prostate tumor imaging.

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Conflict of Interest

There is no conflict of interest.

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Figures Legends

Figure 1. Synthetic route for the imidazolium bromide salt.

Figure 2. In-vitro stability of ^{131}I-NMI.

Figure 3. IC_{50} values for HEK-293 cell line.

Figure 4. IC_{50} values for CaCo-2 cell line.

Figure 5. IC_{50} values for MCF-7 cell line.

Figure 6. IC_{50} values for PC-3 cell line.

Figure 7. Intracellular uptake of ^{131}I labeled NMI in HEK-293, MCF-7, PC-3 and CaCo-2 cells through 24 h.

Figure 8. Intracellular uptake of ^{131}I in HEK-293, MCF-7, PC-3 and CaCo-2 cells through 24 h.