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Preliminary test of the radiopharmaceutical potential of PDI-Pyr radiolabeled with ^{131}I

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Abstract

Objective: Cancer is a fatal disease that arises as a result of unregulated growth and spread of malignant cells. According to the reports of World Health Organization (WHO), cancer related deaths are projected to increase in the future with the value of about 13.1 million deaths by the year 2030. In order to control this danger and growing burden, new technologies and therapeutic methods for improving the life quality of cancer patients are being developed. The overall aim of this work was to design of a new anti-cancer drug.

Methods: Radiolabeling and quality control studies of PDI-Pyr were carried out by using thin layer radiochromatography. Scintigrams were obtained using a gamma camera (Infinia, GE), which was adjusted to detect γ radiations of ^{131}I.

Results: Radiolabeling yield of ^{131}I-PDI-Pyr was obtained to be about 97%. Also, the highest uptakes of ^{131}I-PDI-Pyr were observed in the stomach, the liver, the lung and the bladder.

Conclusion: The novel PDI-Pyr compound was successfully radiolabeled with ^{131}I using iodogen method for the first time. The preliminary results obtained in this study have indicated that in the case of verification of selective accumulation in some stomach liver, lung and bladder cancer cells, perylene chromophore derivatives promise to be used as new anticancer agents.

Keywords: Radiolabelling, Thin Layer Radiochromatography, Scintigraphy, PDI Dyes

Introduction

Cancer remains the second leading cause of death all over the world [1]. Cancer chemotherapy is generally accompanied by side effects. If an anticancer drug could be delivered only to the right site in the right concentration at the right time, cancer could be cured without any side effects [2-3]. Thus, anticancer and antitumor properties of products have great importance.

Perylene diimide derivatives have been widely studied as G-quadruplex interactive compounds and telomerase inhibitors.[4-13]. G-quadruplexes are unusual DNA secondary structures based on planes of four guanines (G-tetrads) stabilized by Hoogsteen G-G pairings and monovalent cations. The central aromatic core of the perylene diimides is suitable for π-π stacking interactions with the terminal G-tetrad of DNA Gquadruplex, while the hydrophilic side chains interact with the DNA grooves. By means of these two kinds of interactions, these molecules are able to induce and stabilize G-quadruplex structures in G-rich singlestranded Oligonucleotides.

This is of great pharmaceutical interest, since the terminal ends of eukaryotic chromosomes (telomeres) are characterized by the presence of a single-stranded G-rich overhang that represents the substrate of a reverse transcriptase enzyme, the ribonucleoprotein telomerase, which is involved in the maintenance of telomere length. This enzyme is not active in most somatic cells but is active in most human tumors, and is, therefore, considered as being of high potential as a selective target for different anti-tumor strategies [4]. Several investigators have experimentally observed that if an ^{125}I radionuclide is incorporated into the nucleus of a cell, it results in a high radiotoxic effectiveness when its decay occurs [14-15]. The same authors have also demonstrated that this radiotoxicity of ^{125}I becomes more effective when ^{125}I decay occurs in the structure of DNA or very close to it. This extreme radiotoxicity is at least 10 times greater than that of other radionuclides such as ^{131}I, ^{14}C, or ^{3}H [16]. In the case of the combination of radiolabeled perylene diimide with the high radiotoxicity of an appropriate radionuclide such as ^{125}I, Auger and/or α-emitting radionuclides, it is expected that a very effective radiolabeled anti-cancer drug can be designed, which will have large potential applications in cancer therapy. In addition, in the case of labeling this drug with an appropriate radionuclide, this may similarly be used for early diagnosis of some kinds of tumors.

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One of the more novel and beneficial application of singlet oxygen in medicine is the cure of tumors. Today, photodynamic therapy (PDT) has become a potential candidate for the major utilization in the modern operation of cancer. Targeted photodynamic therapy is a new strategy that aims to direct the photosensitizers specifically towards the tumor tissues to enhance the efficiency and specificity of PDT [17].

Starting from this consideration, PDI-Pyr (N-(2,6-diisopropylphenyl)-N′-(3-carboxy-2-pyridyl)-1,7-bis{4-[(4-pyrene-1-ylbutanoyl)oxy]phenoxy}perylene-3,4,9,10-tetracarboxylicdiimide) which was considered as an anti-cancer drug was synthesized and subjected to radioiodination, and its biological activities in the metabolism of rats were examined for preliminary testing of the anti-cancer potential of PDI-Pyr (Fig. 1).

As a result, it is hoped that PDI-Pyr will be able to be used as an appropriate carrier for selective incorporation of radioactive iodine atoms into the nucleus of tumor cells, and in addition, the radiotoxicity, for example, of ^{125}I can be realized on the same agent, which will be used as an effective anti-cancer drug for cancer types. Thus, the overall aim of this work was to design of a new anti-cancer drug.

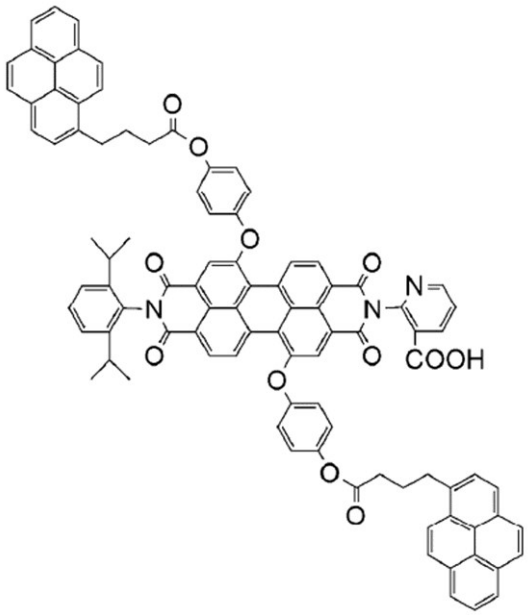


Figure 1. Chemical structure of PDI-Pyr

Material and Methods

Preparing the iodogen coated tubes

1 mg amount of iodogen was dissolved in CH_2CI_2 and transferred to closed tubes. CH_2CI_2 was evaporated by air flow and iodogen was deposited on the walls of glass tubes as a thin film. These tubes were stored at +4 °C until use.

Radioiodination procedure

PDI-Pyr was radioiodinated with ^{131}I using the iodogen method under the same conditions as earlier described by Avcıbaşı et al [18-21]. In order to label PDI-Pyr with ^{131}I, 100 µg of PDI-Pyr was added into the iodogen coated tube and then 1mCi (37 MBq) of $Na^{131}I$ was added. This reaction mixture was kept at room temperature without stirring for 15 min. At the end of this time, the mixture was transferred to another tube by a syringe, and then quality control was performed.

TLRC Studies

For TLRC studies, TLC Aluminum sheets (Merck, 20×20 cm code: 5552) were used, and citric acid monohydrate (100%, pH:6) was used as the mobile phase. The TLRC technique was used to determine the R_f values of the radioiodinated products. Each TLRC sheet was covered by an adhesive band after its development and was cut into 0.5 cm width. Those pieces of TLRC were then counted by using a Cd(Te) detector equipped with a RAD 501 single-channel analyzer. The R_f values were determined and given in Tables 1.

Scintigraphic Studies

The imaging studies were performed on healthy male Albino Wistar rats using a gamma camera (Infinia, GE, Tirat Hacermel, Israel) at Department of Nuclear Medicine of Celal Bayar University. ^{131}I- PDI-Pyr and $Na^{131}I$ were intravenously injected into male Albino Wistar rats via the tail vein after anesthetizing by the mixture of ksilazin and ketamin to determine the dynamic and static situations in the metabolism. Dynamic and static scintigrams were obtained using a gamma camera (Infinia, GE), which was adjusted to detect γ radiations of ^{131}I. Dynamic scintigrams were obtained over the first half hour with frames of 1 minute following the administration of the labelled compound. Static images were obtained from posterior projection after different time intervals up to about 2 hours following the administration of the ^{131}I-PDI-Pyr and $Na^{131}I$. Dynamic and static scintigrams of ^{131}I-PDI-Pyr and $Na^{131}I$ were given in Fig. 3,5 and Fig. 4,6 respectively.

Table 1. R_f values for radiolabeled compound and $Na^{131}I$.

Comounds	Rf
^{131}I-PDI-Pyr	0.88
$Na^{131}I$	0.81

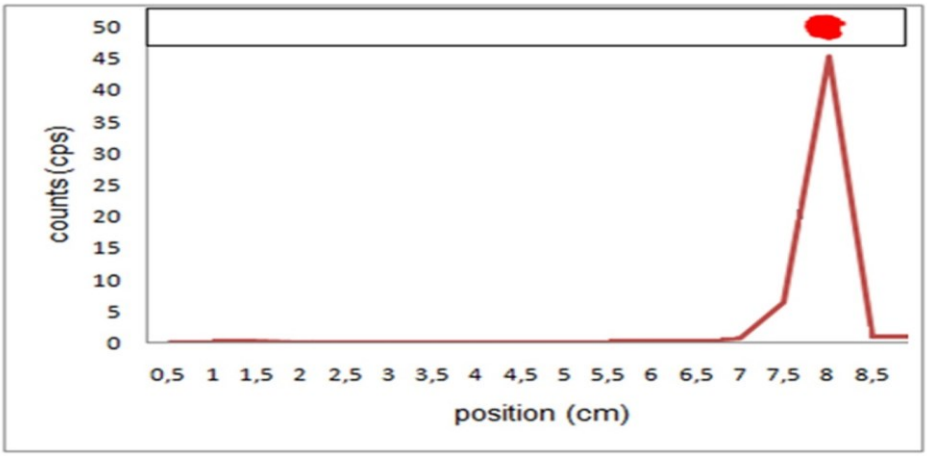


Figure 2. The radiochromatogram of ^{131}I-PDI-Pyr developed with citrate buffer (citric acid mono hydrate) 100% (10 mM) pH:6

Results

Results of TLRC Studies

The results of TLRC studies showed that citric acid mono hydrate, 100%, was the most suitable developing solvent to establish their R_f values given in Table 1. The best labeling yield was obtained at about 97% when the pH value was set to 6. Radiochromatograms of ^{131}I-PDI-Pyr was given in Fig. 2.

Results of Scintigraphy Studies

After administration of ^{131}I-PDI-Pyr and Na^{131}I to rats, static and dynamic scintigrams were obtained. Fig. 3 and 5 show the dynamic images of ^{131}I-PDI-Pyr and Na^{131}I, respectively. Fig. 4 and 6 show the static images corresponding to 30, 60 and 120 min after the administrations of these compounds. Table 1 and 2 also showed organ/background (BG) ratios of ^{131}I-PDI-Pyr and Na^{131}I.

As seen dynamic scintigrams of ^{131}I-PDI-Pyr, it was clearly observed that there were an important accumulation in the abdominal and the chest zone. ^{131}I-PDI-Pyr was significantly localized in the stomach within 30 min. Another result of dynamic images of related compound was the presence of high uptake in the heart, the lung and the liver. As seen dynamic scintigrams of Na^{131}I, it was clearly seen that metabolism of Na^{131}I was completely different from that of ^{131}I-PDI-Pyr. Na^{131}I was highly accumulated in the stomach and the bladder after the administration within 1-2 min. There was a high accumulation in the bladder in the 30 min.

In the static images of the ^{131}I-PDI-Pyr, activities mentioned above were seemed to be increased at 120 min. Accumulation in these zones could probably be caused by the increased specificities of the drug into the liver, the lung, the stomach and the bladder.

Similar results were obtained from a pyridine substituted PDI derivative which was radioiodinated with ^{131}I, and this product was accumulated in the stomach of a male Albino Wistar rats over about 30 minutes [22]. Organ/background (BG) ratios of ^{131}I-PDI-Pyr and Na^{131}I were given in Table 2 and 3, respectively. If the regions of interest values of radiolabeled ^{131}I-PDI-Pyr are evaluated with time, the highest uptake of radioiodinated PDI-Pyr is evidently seen in the stomach within 120 min.

Conclusion

^{131}I-PDI-Pyr which has diagnostic and therapeutic applications potential in nuclear medicine was synthesized and firstly radioiodinated in this study. Radiolabeling of PDI-Pyr with ^{131}I means that it can also be radioiodinated with other radioiodine isotopes such as ^{123}I, ^{124}I, ^{125}I under the similar conditions. The principal aim of this study was to progress the synthesis of an effective radioiodinated anticancer drug. The preliminary results obtained in this study have indicated that in the case of verification of selective accumulation in some cancer cells, perylene chromophore derivatives promise to be used as new anti-cancer agents.

At the next step of this study, the selective incorporation of ^{125}I- PDI-Pyr into some cancer cells should be examined using tumor bearing laboratory animals or cultured cancer cell lines.

Table 2. Organ/BG (background) ratios of ^{131}I-PDI-Pyr and Na^{131}I in 30, 60 and 120 min

Organs	^{131}I-PDI-Pyr			Na^{131}I		
	30 min.	60 min.	120 min.	30 min.	60 min.	120 min.
Head	2.80	2.40	2.03	4.78	2.61	2.59
Thyroid	2.42	2.30	2.90	3.43	4.05	4.63
Right Lung	2.75	2.65	3.20	2.85	3.06	3.19
Left Lung	2.69	2.50	3.20	2.93	2.83	3.08
Heart	3.64	4.00	4.00	3.65	3.40	3.52
Liver	4.39	3.90	4.00	3.14	3.60	3.73
Stomach	4.73	4.70	5.04	4.32	4.40	4.48

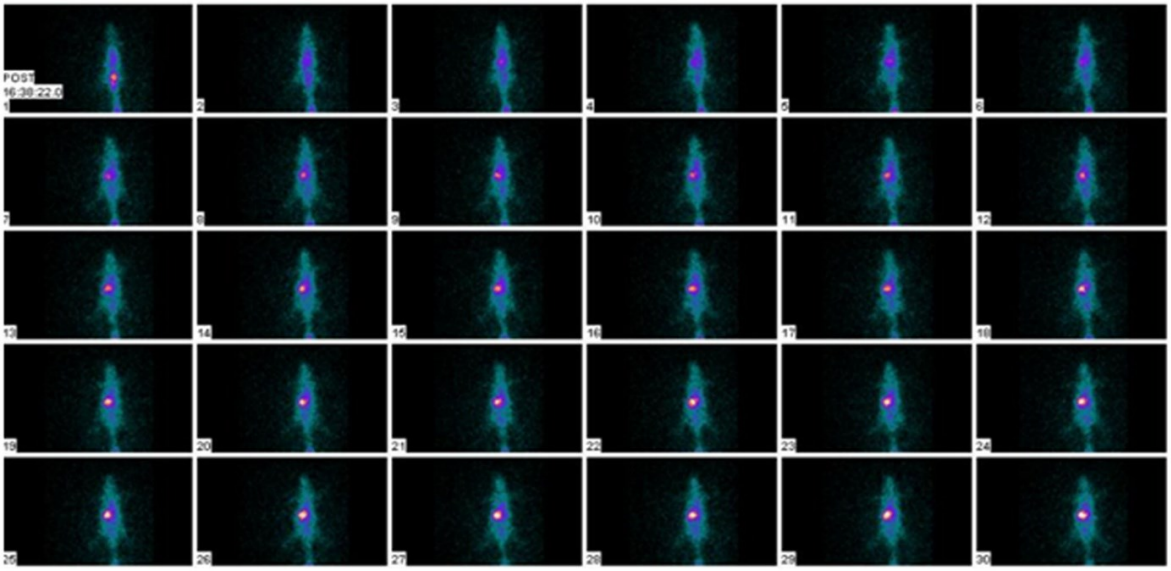


Figure 3. Dynamic scintigrams of ^{131}I-PDI-Pyr which was administered to a male Albino Wistar rats via the tail vein in 30 min. (The mixture of ksilazin and ketamin anesthesia was used in the scintigraphy studies. Dynamic scintigrams were obtained over the first half hour with frames of 1 min following the administration of the labeled compound)

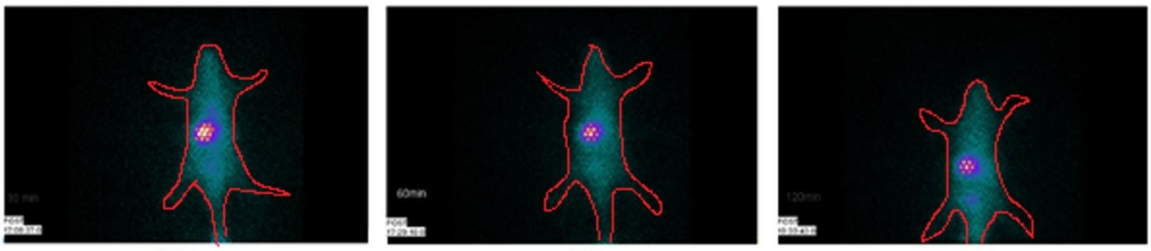


Figure 4. Static scintigrams of ^{131}I-PDI-Pyr which was administered to a male Albino Wistar rats via the tail vein in 30 min (a), 60 min (b), and 120 min (c)

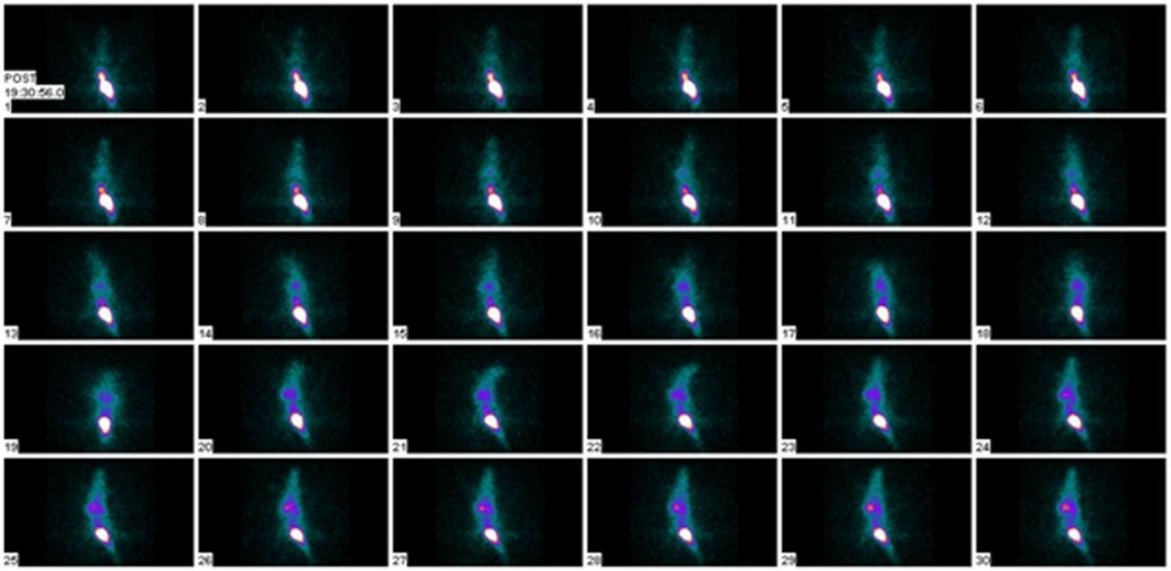
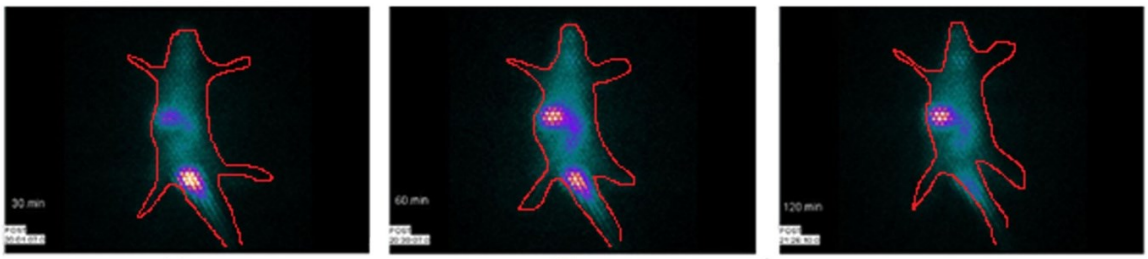


Figure 5. Dynamic scintigrams of $Na^{131}I$ which was administered to a male Albino Wistar rats via the tail vein in 30 min. (The mixture of ksilazin and ketamin anesthesia was used in the scintigraphy studies. Dynamic scintigrams were obtained over the first half hour with frames of 1 min following the administration of the labeled compound)



(a) (b) (c)

Figure 6. Static scintigrams of $Na^{131}I$ which was administered to a male Albino Wistar rats via the tail vein in 30 min (a), 60 min (b), and 120 min (c)

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Conflict of Interest: The authors declared that they had no conflicts of interest.

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