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Preclinical paper

Biodistribution and scintigraphy of [^{111}In]DTPA–adriamycin in mammary tumor-bearing rats

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The aim of this study was to develop an ^{111}In-labeled diethylenetriamine pentaacetic acid–adriamycin (DTPA–ADR) conjugate to image breast cancer. DTPA–ADR was synthesized by reacting adriamycin with DTPA anhydride in the presence of carbonyldiimidazole. After dialysis (MW cut off was 500), the product was freeze-dried (yield 40–50%). An *in vitro* cell culture study was performed using cells from the 13762 Fischer rat mammary tumor line. Drug concentrations tested were 0.1–100 μM. Biodistribution studies were conducted at 0.5, 2, 24 and 48 h in mammary tumor-bearing rats (n = 3/time interval, 10 μCi/ rat, i.v.) with 13762 cells (10^6 cells/ rat, s.c.). Planar imaging and autoradiograms were obtained at the same intervals. *In vitro* cell culture assays showed an IC_{50} of 0.1 ± 0.01 μM for ADR and 7.2 ± 0.29 μM for DTPA–ADR, respectively. In biodistribution studies, tumor/blood uptake ratios of [^{111}In]DTPA–ADR at 0.5, 2, 24 and 48 h were 0.55 ± 0.17, 0.94 ± 0.17, 3.06 ± 0.53 and 3.66 ± 0.35, respectively, whereas those for [^{111}In]DTPA (control) were 1.19 ± 0.69, 0.84 ± 0.07, 0.56 ± 0.10 and 0.60 ± 0.03, respectively. The tumor uptake value (%ID/g) of [^{111}In]DTPA–ADR at 0.5 h was 0.20 ± 0.06. Planar images and autoradiograms showed good visability of tumors. Biodistribution, autoradiography and radionuclide imaging of [^{111}In]DTPA–ADR in breast tumor-bearing rats showed that tumor-to-blood ratios increased steadily between 30 min and 48 h. These results indicate that DTPA–ADR, a new cancer imaging agent, might be useful in the diagnosis of breast cancer and may predict a therapeutic effect prior to treatment. [© 1999 Lippincott Williams & Wilkins.]

Key words: Biodistribution, breast cancer, DTPA–adriamycin, scintigraphy.

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Introduction

Adriamycin (ADR; doxurubicin, rubex), a potent topoisomerase II inhibitor, has been widely used to treat various cancer types including breast cancer. The standard dose of ADR in breast cancer therapy is 60-75 mg/m^2 every 21 days as a cycle with a major side effect being cardiotoxicity.[1] Usually, a patient with breast cancer undergoes four to six cycles of therapy with ADR and treatment is expensive. If the binding of ADR to breast tumors can be demonstrated with scintigraphy, then such a labeled ADR may predict the response of ADR therapy for breast cancer. Additionally, such a radiotracer may provide early diagnosis of cardiotoxicity induced by ADR.

Several reports have shown the possibility of using a labeled drug to select the patients who may benefit from such a drug therapy. For instances, positron emission tomography (PET) imaging using [^{18}F]fluorotamoxifen as the radiotracer provides useful information in predicting the effect of taxoxifen therapy in patients with recurrent or metastatic estrogen receptor-positive breast cancer. Those tumors that showed good uptake of the radiolabeled tamoxifen had positive responses to tamoxifen therapy.[2-4] The use of tumor hypoxia marker has shown it is possible to predict the development of radiation resistance in individual tumors.[5] In this report, synthesis and tumor imaging potential of a new diethylenetriamine pentaacetic acid (DTPA)-ADR conjugate were evaluated.

Experimental

Synthesis of DTPA–ADR conjugate

The synthetic scheme of DTPA-ADR is shown in Figure 1. DTPA-ADR was synthesized by reacting

ADR hydrochloride (300 mg, 0.52 mmol) with DPTA anhydride (923 mg, 2.58 mmol) (Sigma, St Louis, MO) in water (10 ml) containing NaOH (2 N, 5 ml). After dialysis (MW cut off at 500), the product was lyophilized and yielded 247 mg (52%). m.p. 200-201°C (dec, product), 219-220°C (dec, DTPA), 216°C (ADR). ^{1}H-NMR (D_2O) δ 1.18 (s, 3H, sugar-CH_3), 1.80-2.67 (m, OH, cyclohexane-CH_2-sugar) and 2.78-2.79 (m, 8H, DTPA N-CH_2-CH_2-N), 3.20-3.26 (m, 10H, DTPA N-CH_2-COOH), 3.60-3.66 (s, 3H, aromatic-OCH_3), 4.08-4.09 (m, 1H, cyclohexane-H-O-sugar, 5.29 (s, 2H, -$COCH_2OH$), 6.98-6.99 (d, 1H, aromatic), 7.07-7.08 (d, 1H, aromatic), FAB MS *m/z* calculated $C_{41}H_{48}O_{20}N_4Na_2(M^+)$ 962.43, 918.45 (free), found 918.3 (salt free), 632, 580.2 (ADR). Radiosynthesis of [^{111}In]DTPA-ADR was achieved using a previously published procedure.[6] Briefly, DTPA-ADR conjugate (5 mg) was dissolved in 1 ml of water and was treated with $^{111}InCl_3$ (0.7 mCi, in 20 μl, 0.04 N HCl; NEN Dupont, Boston, MA) in a buffer solution of sodium acetate (0.6 N, 20 μl) and sodium citrate (0.06 N, 20 μl). The mixture stood for 30 min. [^{111}In]DTPA-ADR was reconstituted with saline and was given to rats. Radiochemical purity was determined to be greater than 99% [using 1 M ammonium acetate:methanol; 4:1, R_f=0.73 (ITLC SG, Gelman Sciences, Ann Arbor, MI; Bioscan, Washington, DC] demonstrated in Figure 2.

In vitro cell culture study of ADR and DTPA–ADR

In vitro cell culture study was performed with cells from the 13762 Fischer rat mammary tumor line. Drug concentrations tested ranged from 0.1 to 100 μM. In each well, 100 μl of drug solution was added to 500 ml of cell media. Cell medium was incubated in 96-well plates. Each well had 10^6 cells. The drug concentration required to inhibit 50% of cancer cell growth (IC_{50}) was determined.

Stability assay of [^{111}In]DTPA–ADR

Stability of [^{111}In]DTPA-ADR was tested in dog serum samples. Briefly, 20 μCi of 1 mg [^{111}In]DTPA-ADR was incubated in serum (200 μl) at 37°C. At each time point, the serum sample was diluted with 50% methanol in water. Radio-TLC was performed using 1 M ammonium acetate:methanol (4:1) as an eluant.

Biodistribution of [^{111}In]DTPA–ADR in mammary tumor-bearing rats

The animal experiments were carried out in compliance with the relevant national laws relating to the conduct of animal experimentation. The animal

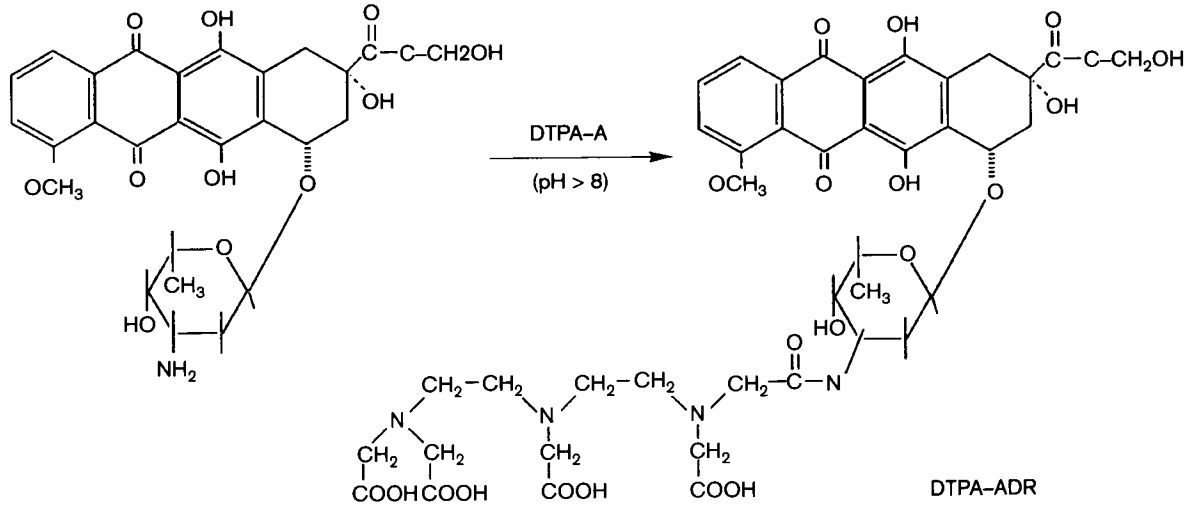

Figure 1. Synthesis of the DTPA–ADR conjugate.

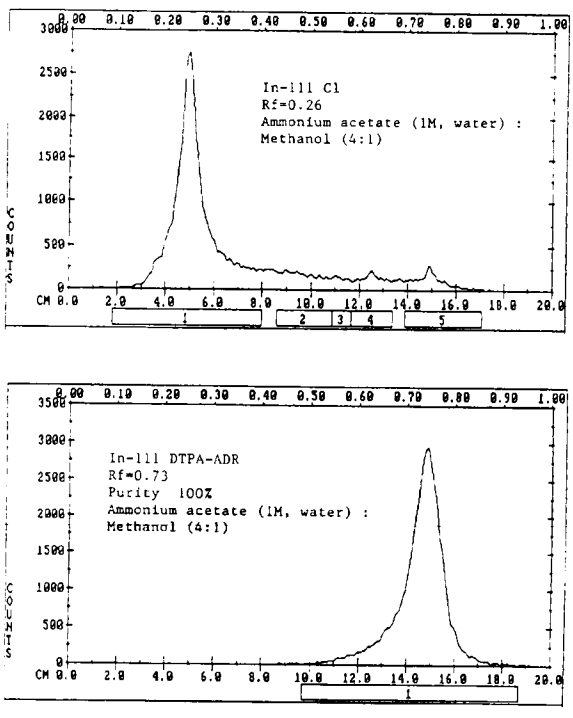

Figure 2. Radio thin-layer chromatographic analysis of [^{111}In]DTPA–ADR.

protocol was approved by the University of Texas MD Anderson Institutional Animal Care and Use Committee (IACU). Female Fischer 344 rats (250–275 g each) (Harlan, Indianapolis, IN) were inoculated with mammary tumor cells from the 13762 tumor cell line (s.c. 10^6 cells/rat, a tumor cell line specific to Fischer rats). After 14 days, tumors of 1–2 cm in size were observed. In tissue distribution studies, five groups of rats (n=3/group) were anesthetized with ketamine (10–15 rat). The [^{111}In]DTPA-ADR reconstituted in saline was given to the five groups of rats (10 μCi/rat, i.v.), and tissue distribution was studied at 0.5, 2, 24 and 48 h. The mass of [^{111}In]DTPA-ADR injected was 10 mg/rat. The tissues were excised, weighed and counted for radioactivity by a γ counter (Packard Instruments, Downers Grove, IL). The percent of injected dose per gram of tissue weight was determined.

Autoradiographic studies of [^{111}In]DTPA–ADR in mammary tumor-bearing rats

Female mammary tumor-bearing rats (three rats per group) were killed at 0.5, 2, 24 and 48 h after receiving [^{111}In]DTPA-ADR (300 μCi/rat, i.v.). Each rat body was imbeded in carboxymethyl cellulose (4%). The frozen body was mounted onto a cryostat (LKB 2250 cryomicrotome) and cut into 40 μm coronal sections. Each section was thawed and mounted on a slide. The slide was then placed in contact with X-ray film (X-Omat AR; Kodak, Rochester, NY) and exposed for 48 h.

Scintigraphic imaging

Scintigraphic images were obtained with a γ camera (Siemens Medical System, Hoffman Estades, IL) equipped with a high-resolution, medium-energy, parallel-hole collimator. Five mammary-tumor-bearing rats were anesthetized by ketamine (10–15 mg/rat; i.p.) and then received 300 μCi of [^{111}In]DTPA-ADR, and whole-body planar images were obtained at 0.5, 2, 24 and 48 h; 300 000 counts were acquired in 256 × 256 matrix.

Results

In vitro cell culture study of ADR and DTPA–ADR

In vitro cell culture assay showed IC_{50} of 0.1 ± 0.01 μM for ADR and 7.2 ± 0.29 μM for DTPA-ADR, respectively.

Stability of [^{111}In]DTPA–ADR

[^{111}In]DTPA-ADR was stable at 0.5, 2, 24 and 48 h in serum samples. There was no degradation of the product observed.

In vivo biodistribution

Tissue distribution of [^{111}In]DTPA-ADR and [^{111}In]-DTPA in mammary-tumor-bearing rats are shown in Tables 1 and 2. Tumor uptake value (%ID/g) of [^{111}In]DTPA-ADR at 0.5, 2, 24 and 48 h was 0.204 ± 0.068, 0.115 ± 0.031, 0.051 ± 0.009 and 0.035 ± 0.003, respectively (Table 1), whereas those for [^{111}In]DTPA were 0.297 ± 0.159, 0.020 ± 0.001, 0.020 ± 0.003 and 0.017 ± 0.002, respectively (Table 2). Although the average tumor uptake value at 0.5 h was higher in the [^{111}In]DTPA group compared to the [^{111}In]DTPA-ADR group, it was not significantly different. At 2, 24 and 48 h post-administration of [^{111}In]DTPA-ADR, tumor uptake values were significantly higher than those with

Table 1. Biodistribution of [^{111}In]DTPA–ADR in mammary-tumor-bearing rats

	0.5 h	2 h	24 h	48 h
Blood	0.37±0.072	0.12±0.014	0.02±0.003	0.01±0.001
Lung	0.49±0.125	0.15±0.034	0.04±0.007	0.03±0.008
Liver	0.28±0.031	0.20±0.015	0.21±0.051	0.16±0.028
Kidney	1.07±0.120	0.53±0.231	0.24±0.057	0.23±0.057
Uterus	0.42±0.177	0.17±0.102	0.02±0.008	0.03±0.007
Muscle	0.06±0.014	0.02±0.003	0.01±0.001	0.01±0.001
Bone	0.06±0.010	0.10±0.111	0.02±0.004	0.02±0.003
Tumor	0.20±0.067	0.11±0.030	0.05±0.009	0.04±0.003

Each rat received [^{111}In]DTPA–adriamycin (10 μCi, i.v.). Each value is percent of injected dose per gram weight (n=3)/time interval. Each data represents mean of three measurements with standard deviation.

Table 2. Biodistribution og [^{111}In]DTPA in mammary-tumor-bearing rats

	0.5 h	2 h	24 h	48 h
Blood	0.26±0.043	0.02±0.003	0.04±0.001	0.03±0.004
Lung	0.17±0.025	0.01±0.001	0.01±0.002	0.01±0.001
Liver	0.15±0.019	0.16±0.057	0.16±0.074	0.11±0.020
Kidney	0.83±0.015	0.19±0.016	0.46±0.351	0.32±0.023
Uterus	0.13±0.018	0.01±0.005	0.01±0.003	0.01±0.002
Muscle	0.09±0.041	0.01±0.001	0.01±0.001	0.01±0.001
Bone	0.15±0.075	0.01±0.002	0.01±0.001	0.01±0.001
Tumor	0.30±0.159	0.02±0.001	0.02±0.003	0.02±0.002

Each rat received [^{111}In]DTPA (10 μCi, i.v.). Each value is percent of injected dose per gram weight (n=3)/time interval. Each data represents mean of three measurements with standard deviation.

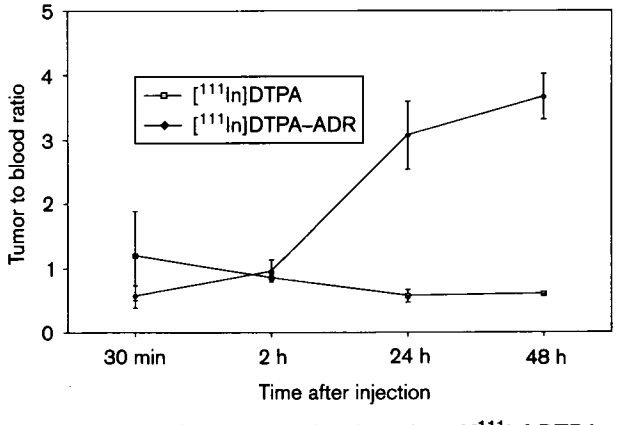

Figure 3. Tumor/blood count density ratios of [^{111}In]-DTPA–ADR in mammary-tumor-bearing rats as a function of time. Data are expressed as the mean ± SE for n=3 rats per group.

[^{111}In]DTPA. Tumor/blood count density ratios of [^{111}In]DTPA-ADR at 0.5, 2, 24 and 48 h were 0.55±0.17, 0.94±0.17, 3.06±0.53 and 3.66±0.35, respectively, whereas those for [^{111}In]DTPA were 1.19±0.69, 0.84±0.07, 0.56±0.10 and 0.60±0.03, respectively (Figure 3). From these data, it is evident

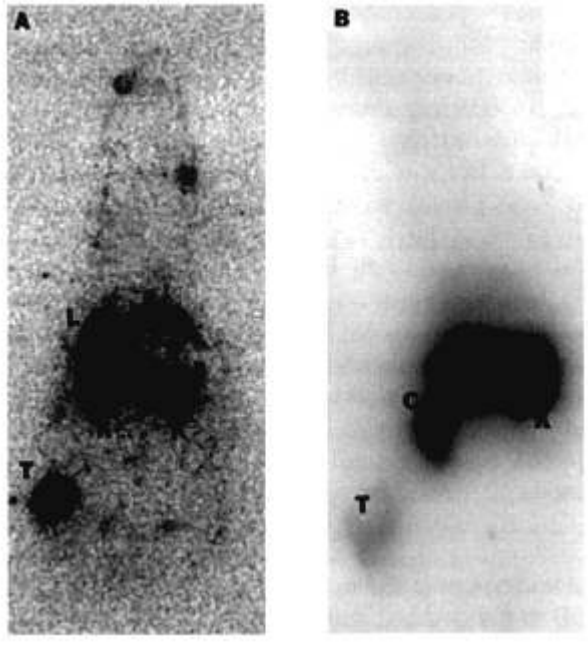

Figure 4. Whole-body autoradiograph (coronal section) of mammary-tumor-bearing rat showed tumor (T), kidney (K), liver (L), spleen (S) and colon (C) uptakes at 0.5 (A) and 24 (B) h after intravenous injection of [^{111}In]DTPA–ADR (300 μCi).

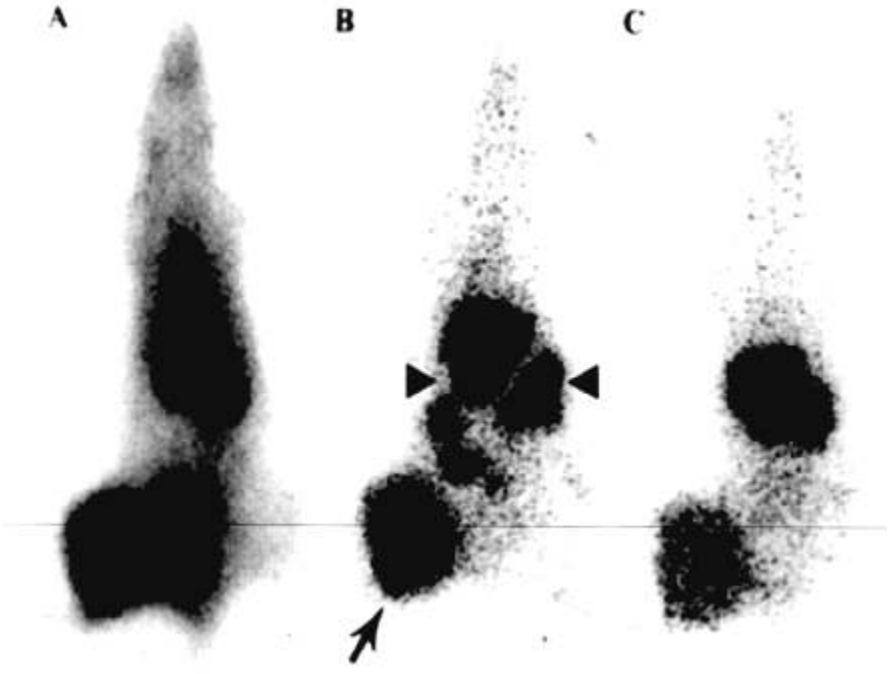

Figure 5. Gamma scintigraphy images (ventral view) of mammary-tumor-bearing rats showed tumor (arrow) and kidney (arrow-heads) uptakes at 0.5 (A), 24 (B) and 48 (C) h after i.v. injection of [^{111}In]DTPA–ADR (300 μCi).

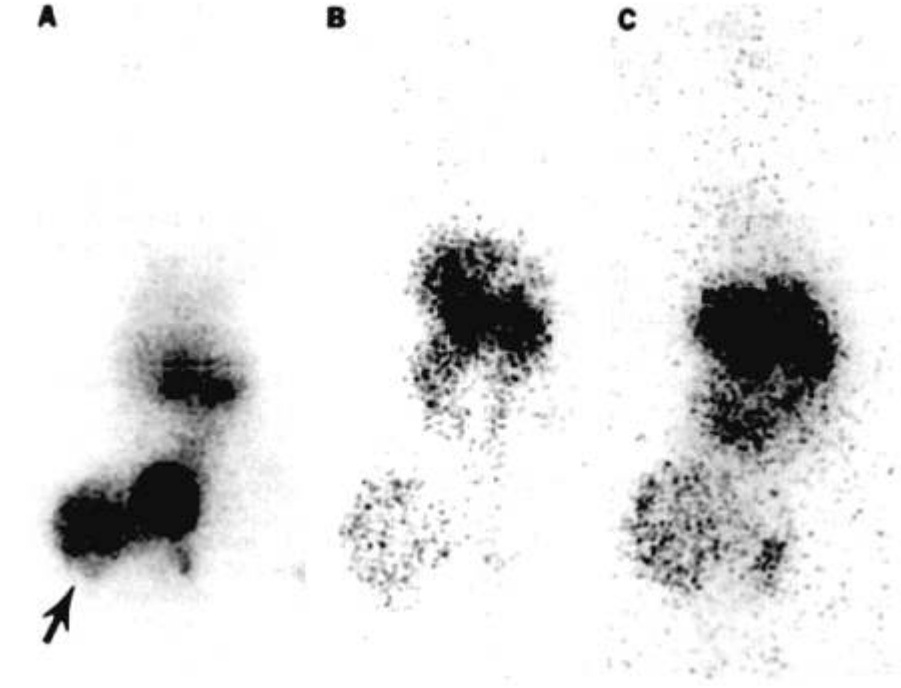

Figure 6. Anterior images (ventral view) of control [^{111}In]DTPA (300 μCi) injected rats at various time points (0.5–48 h). Early image at 0.5 h (A) showed tumor uptake (arrow) reflecting blood perfusion; however, delayed images at 24 (B) and 48 (C) h showed no tumor uptake.

that [^{111}In]DTPA-ADR showed a significant retention in the tumor.

Autoradiogrphic studies and scintigraphic imaging of [^{111}In]DTPA–ADR

In vivo autoradiographic and planar imaging studies in mammary-tumor-bearing rats indicated that the tumor could be visualized well at the time intervals studied (Figures 4 and 5).

Discussion

Numerous studies have modified ADR to treat various tumors.[6-8] Since it is the drug of choice for treating various tumors, it would be ideal to label ADR to diagnose tumors and also predict drug response. The DTPA chelate has been widely used in labeling monoclonal antibodies,[9,10] proteins,[11,12] peptides,[13,14] and small molecules, such as bleomycin,[15-19] paclitaxel[20] and tamoxifen.[21] It has been also widely used as a renal imaging agent as well as in magnetic resonance imaging contrast media. To our knowledge, there have been no reports of studies using radioactively labeled ADR. Using a simple dialysis technique, DTPA-ADR could be synthesized efficiently and chelated with ^{111}In with greater than 96% efficiency.

In vitro assay indicated that ADR is more potent than DTPA-ADR. This may be due to the change of structure identity of ADR. Thus, it is important to determine the specificity of DTPA-ADR. Although *in vitro* cell culture studies demonstrated the stability of this ligand, the harsh chemical environment of the serum *in vivo* may dissociate metallic radionuclide from DTPA attached to proteins. The quality of planar images and autoradiograms of DTPA-ADR which reflects tumor to background ratio is superior to that of DTPA, suggesting the stability and usefulness of this ligand for the detection of tumors *in vivo*.

In summary, DTPA-ADR could be prepared in high yield and radiolabeled for the visualization of tumors. Based upon these findings, research on diagnostic accuracy and prediction of ADR response are warranted.

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