Synthesis and biodistribution of 1-[2-(cyclopentadienyltricarbonyltechnetium-99m)-2-oxo-ethoxy-phenyl]-1,2-di-(p-hydroxyphenyl)but-1-ene for tumor imaging

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A B S T R A C T

The high incidence and mortality of breast cancer and other estrogen receptor related tumors motivates efforts to develop innovative imaging probes to effectively diagnose, evaluate the extent of the tumor, and predict the efficacy of treatments while concurrently and selectively delivering anticancer agents to the cancer tissues. In the present study, 1-[2-(cyclopentadienyltricarbonyltechnetium-99m)-2-oxo-ethoxy-phenyl]-1,2-di-(p-hydroxyphenyl)but-1-ene was prepared and investigated as a potential agent for imaging estrogen receptors (ERs) in associated tumors. The compound was obtained in high radiochemical purity and radiochemical yields. The biodistribution of the radioactive compound in mature female rats showed an ER-mediation in the ovarian target tissues as the uptake was reduced by a blocking dose of estradiol. The nonspecific uptake in muscle was relatively low.

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1. Introduction

Some types of tumors are traditionally classified according to their hormone receptor status: Hormone-dependent tumors (Hormone receptor positive), and hormone-independent tumors (Hormone receptors negative). In the case of breast tumors, this designation comes from the fact that hormone-dependent cancer cells, whose proliferation is induced by estrogen, exhibit an accumulation of a specific intracellular receptor protein: the alpha form of the estrogen receptor (ERα). An adjuvant treatment with antiestrogens or with aromatase inhibitors is used for patients with hormone-dependent breast cancer. These receptors serve as targets for endocrine therapies of these cancers [1–4]. Knowledge of the receptor status can lead to more effective individualized treatment. But the receptors can also be used as targets for diagnostic imaging and radiotherapy. Diagnostic imaging can be achieved by administration of a suitably radiolabeled ligand that accumulates in the receptor-positive tumor cells where it can be detected and quantified by imaging. Such images can sometimes be used to predict whether hormone therapy might be effective or not [5–8]. Therefore, the development of such hormone receptor ligands for diagnostic imaging is a promising area of research.

Nuclear medicine imaging modalities, using specific targeting moieties, play a crucial role in the diagnosis, staging, and follow-up for patients with cancer. Radiotracer-based imaging approaches, such as single-photon emission computed tomography (SPECT) and positron emission tomography (PET) could provide a non-invasive in vivo assessment providing information about tumor localization and progression [9]. Several radionuclides are used in nuclear medicine, including iodine-131, indium-111, thallium-201, fluorine-18, technetium-99m, gallium-67 and others [10]. Technetium—99m (99mTc) is one of the most common radiotracers for imaging. This isotope has suitable physical and chemical characteristics, including a physical half-life of 6.02 h and gamma emission of low energy (140 keV) with the added advantage of abundant availability in nuclear medicine laboratories and low cost [11–13]. An interesting compound containing both 99mTc and Pt, Pt-LQ-Tc
(LQ = mono-[4-(3-[(bis(quinolin-2-ylmethyl)amino)propylcarbamoyl]pyridinium-1-yl], has been designed in order to combine therapeutics and diagnosis in the same molecule [14].

Diagnostic imaging of breast by positron emission tomography (PET) is well established [15] and has been achieved using steroids labeled with fluorine-18 such as 16x-[18F]fluorodeoxyglucose (FDG) [16]. Although a number of steroids labeled with bromine, fluorine and iodine radioisotopes have been prepared and studied [17–25], especially in terms of their potential for selective radiotherapy, their use in imaging studies, particularly in humans, has been more limited. While most of these agents were based on steroidal estrogens, a few investigations were focused on nonsteroidal estrogens or antiestrogens including selective ER modulators (SERMs) [3].

In 1996, Jaouen and coworkers coupled ferrocene to the biovalent hydroxytamoxifen to create a new class of compounds, called ferrocifen [26]. These compounds were initially designed to combine the antiestrogenic properties of tamoxifen with a potential cytotoxic effect of ferrocene to possibly obtain new therapeutic advantages [27,28]. In an effort to develop this class of compounds, 1-(2-ferrocenyl-2-oxoethoxyphenyl)-1,2-bis-(4-hydroxyphenyl)but-1-ene, 1, was synthesized (Chart 1) [29]. This type of ferrocenyl compound is suitable for use as a precursor in the synthesis of the technetium analog, 2 (Chart 1), by performing an organometallic exchange reaction developed for steroids by Wenzel [30] and Katzenellenbogen [31,32].

In 2001, Alberto found an efficient method to generate aqua-ion [99mTc(CO)3(H2O)3]+ [33]. He demonstrated later that the cyclopentadienyltricarbonyltechnetium compound could be conveniently prepared from a reaction between [99mTc(CO)3(H2O)3]+ and cyclopentadienide derivatives [34–36]. 2 was first obtained from the reaction of 1 with [99mTc(CO)3(H2O)3] in DMSO/water at 120 °C [37]. The best yield obtained for 2 was 70%, which is slightly too low to allow the use of this approach for biodistribution study. We have now found good conditions for the double ligand transfer reaction involving 1, [99mTcO4]− and Mn(CO)5Br to produce 2 in 95% yield. For identification of the technetium compound, a manganese or rhenium analog was used for comparison using the HPLC technique [38,39]. Therefore, 1-[4-(2-(cyclopentadienyltricarbonylmanganese)-2-oxoethoxy)phenyl]-1,2-di(p-hydroxyphenyl)-but-1-ene, 3, was also prepared [40]. In the present study, we report the synthesis of 2 and the determination of in vitro radiochemical stability. In addition, blood circulation time was evaluated. A biodistribution study was also performed to demonstrate the feasibility of using 2 as a potential agent for imaging estrogen receptors (ERs) in breast tumor.

2. Experimental

The synthesis of the ferrocenyl complex, 1 [29] and the cyclopentadienyltricarbonylmanganese derivative, 3 [39] have been previously published.

2.1. 1-[2-(cyclopentadienyltricarbonyltechnetium-99)-2-oxoethoxy-phenyl]-1,2-di-(p-hydroxyphenyl)-but-1-ene, 2

1 (1 mg) and Mn(CO)5Br (1 mg) were dissolved in 150 μL of dimethylformamide in a 10 mL glass tube and 150 μL of [99mTcO4]− in saline was added. The vial was sealed and purged with nitrogen for 10 min and heated in an oil bath at 150 °C for 1 h. The mixture was purified by chromatography on a silica gel plate (TLC) (diethyl ether/petroleum ether 8:2) to give 2. Radio-TLC (diethyl ether) gave a peak with an Rf = 0.8 (yield 90%). HPLC retention time ~22.73 min. The solution was purified from [99mTcO2] using a 0.22 mm syringe filter, as previously described by Fernandes et al. [41].

2.2. Biological pH stability study

Purified 2 (100 μL, 60 μCi) was added to potassium phosphate buffer (900 mL, 0.1M) at pH 7.4. The vial was sealed and incubated at 37.0 °C for the duration of study. The solution was examined by radio-HPLC at 30 min. 1 h, 2 h, 3 h and 24 h during incubation to determine the effective stability of the compound.

2.3. In vitro stability study

An in vitro stability study was carried out in isolated heparinized whole rat blood (male, Wistar rats). The whole blood (5 mL) was incubated with 100 μCi of 2 in 100 μL ethanol for 30 min, 1 h, 2 h, 3 h and 24 h at 37.0 °C. A sample of blood (1 mL) was taken from the solution each time. The sample was centrifuged at 5000 rpm for 5 min to separate plasma and cellular fractions and individual fractions were counted in a well counter. The plasma was then treated with CH3CN (1 mL) and centrifuged as before and the supernatant was separated from the precipitated debris. The fractions were again counted with the bulk of activity residing in the plasma supernatant. The radioactive species in the supernatant were analysed by radio-HPLC.

2.4. Kinetic study

The kinetic study was carried out according to the relevant national regulation using mature (5–6 weeks old) female Wistar rats weighing between 180 and 200 g. Purified 2 was dissolved in 15% ethanol/saline buffer and injected via the tail vein. The dose employed was 80 μCi per animal (in 300 mL volume). At the specified time points post-injection of 2, blood samples were collected by heart puncture. The injected dose (ID) was calculated by comparison with dose standards prepared from the injected solution at appropriate counting rates. The data were expressed as percentage of ID per gram of tissue (%ID/g of blood). The biological half-life of compound 2 was determined using the following formula:

\[ T_b = T_x \times T_{eff}/T_p - T_{eff} \]

\[ T_{eff} = 0.693/K_e \]

\[ K_e = log(C_1/C_2)/t_2 - t_1 \]

Ke = elimination constant determined from curve.

2.5. In vivo biodistribution

The biodistribution studies were carried out according to the relevant national regulation using mature (5–6 weeks old) female Wistar rats weighing between 180 and 200 g. Purified 2 was dissolved in 15% ethanol/saline buffer and injected via the tail vein under slight ether anaesthesia. The dose employed was 80 μCi per animal (in 300 mL volume). and one set of animals was co-injected with radiotracer together with a blocking dose (15 mg) of estradiol.

At the specified time points post-injection of 2, groups of three animals each were sacrificed by heart puncture; tissues of interest were removed and weighed; and radioactivity was counted. The injected dose (ID) was calculated by comparison with dose standards prepared from the injected solution at appropriate counting rates. The data were expressed as percentage of ID per gram of tissue (%ID/g of tissue). For each animal group, the data were averaged and standard deviation was calculated. Data were analysed by multifactorial analysis of variance (ANOVA, SPSS 12.0 for Windows) and significant differences among treatments were considered using Student’s t-test at the P < 0.05 level.
3. Results and discussion

3.1. Synthesis

Intensive development of nuclear medicine in the field of cancer diagnosis and therapy is currently taking place. The most important diagnostic and therapeutic radiopharmaceuticals, which selectively distribute within given tissues or organs, are formed by coordination compounds with a diagnostic or therapeutic radiometal firmly attached to a selected biologically active molecule. Among diagnostic radionuclides technetium-99m (T1/2 = 6 h, Eɣ ≈ 140 keV) is most commonly used for SPECT applications due to its favorable physical properties, ready availability from the $^{99m}$Mo-$^{99m}$Tc generator and relatively low cost [42].

The synthesis of the ferrocenyl derivative 1 was published in the literature [29]. The IC$_{50}$ values of 11.3 µM and 7.8 µM were found respectively against hormone-dependent breast cancer cells (MCF-7) and against prostate cancer cells PC3. Its relative binding affinity (RBA) value for the alpha form of the estrogen receptor is 14%. Cyclopentadienyltricarbonylmanganese compound 3 was prepared according to the literature procedure [40].

Scheme 1 shows the synthesis of cyclopentadienyltricarbonyltechnetium compound 2.

Heating the mixture of 1 with [${}^{99m}$TcO$_4^-$] and Mn(CO)$_5$Br in dimethylformamide in a sealed tube at 150 °C for 90 min resulted in formation of technetium compound 2 in 95% yield after purification by TLC chromatography.

The technetium compound, 2, was identified by comparison of its retention time to that of manganese analog 3 (Fig. 1).

The radiolabeling protocol to obtain compound 2 was successfully optimized to achieve high yields. According to the American Pharmacopoeia [43], radiopharmaceutical preparations are suitable for in vivo administration if their radioactive impurities do not exceed 10%. Use of the optimized protocol described in this study resulted in the detection of under 10% impurities, thus indicating that compound 2 can be used in further in vivo assays.

![Scheme 1. Synthesis of cyclopentadienyltricarbonyltechnetium compound 2.](image)

![Fig. 1.](image)

![Fig. 2. In vitro stability of 2 as a function of time in the presence of saline solution (Series 1), at room temperature, or rat plasma (Series 2), at 37 °C.](image)

![Fig. 3. In vivo disappearance of compound 2 in mature female rats.](image)
3.2. Biochemical studies

The in vitro stability study was carried out in isolated whole rat blood following the procedure reported in the literature [39,40,44]. Around 93% of the radioactivity was separated into supernatant from the pellet over the period of 3 h, indicating little binding to cells or cellular proteins in the blood. According to the radio-HPLC analysis of the supernatant, 2 was the only radioactive species observed during 30 min of incubation. Compound 2 was also incubated with a potassium phosphate buffer at 37 °C for 22 h; the same stability results were found (Fig. 2).

Radiolabeling stability is a mandatory characteristic for radio-pharmaceutical platforms. In the event that metal detachment occurs, biodistribution and images will no longer be reliable and may lead to wrong interpretations [36,37]. 2 showed high stability in vitro (over 95%) throughout the experiment (Fig. 2).

The kinetic study was undertaken on female Wistar rats. A solution of purified 2 in 15% ethanol/saline buffer was injected via the tail vein. Blood samples were collected by heart puncture at 30 min, 1 h, 2 h, 3 h et 24 h post injection for concentration determination. Fig. 3 shows that the plasma activity increased significantly during first 30 min before a fast decrease within 5 h. After that time, a slow decrease was observed until 20 h. Using this last curve section for the half-life determination, a value of 14.68 h was found for the half-life of 2.

Comparing to the cyclopentadienyltricarbonyltechnetium-99m 

Tissue biodistribution of compound 2 in mature female rats (%ID/g of tissue).

Table 1

<table>
<thead>
<tr>
<th>Percent injected dose/gram ± S.D. (n = 3)</th>
<th>15 min</th>
<th>1 h</th>
<th>2 h</th>
<th>3 h</th>
<th>6 h</th>
<th>18 h</th>
<th>24 h</th>
</tr>
</thead>
<tbody>
<tr>
<td>Blood</td>
<td>0.46 ± 0.05</td>
<td>0.33 ± 0.11</td>
<td>0.29 ± 0.05</td>
<td>0.31 ± 0.01</td>
<td>0.13 ± 0.00</td>
<td>0.03 ± 0.01</td>
<td>0.02 ± 0.02</td>
</tr>
<tr>
<td>Liver</td>
<td>2.43 ± 0.64</td>
<td>7.78 ± 0.63</td>
<td>9.87 ± 1.68</td>
<td>10.52 ± 0.89</td>
<td>1.89 ± 0.72</td>
<td>0.24 ± 0.04</td>
<td>0.580 ± 0.07</td>
</tr>
<tr>
<td>bone</td>
<td>0.23 ± 0.09</td>
<td>0.12 ± 0.03</td>
<td>0.14 ± 0.031</td>
<td>0.20 ± 0.05</td>
<td>0.06 ± 0.015</td>
<td>0.016 ± 0.0</td>
<td>0.022 ± 0.0</td>
</tr>
<tr>
<td>Muscle</td>
<td>0.09 ± 0.00</td>
<td>0.12 ± 0.02</td>
<td>0.11 ± 0.04</td>
<td>0.17 ± 0.06</td>
<td>0.034 ± 0.003</td>
<td>0.006 ± 0.0</td>
<td>0.007 ± 0.0</td>
</tr>
<tr>
<td>Lung</td>
<td>7.69 ± 0.89</td>
<td>6.62 ± 0.25</td>
<td>21.86 ± 0.70</td>
<td>24.81 ± 0.60</td>
<td>6.20 ± 0.943</td>
<td>1.067 ± 0.30</td>
<td>1.143 ± 0.022</td>
</tr>
<tr>
<td>Uterus</td>
<td>0.19 ± 0.04</td>
<td>0.23 ± 0.14</td>
<td>0.28 ± 0.05</td>
<td>0.17 ± 0.01</td>
<td>0.1 ± 0.01</td>
<td>0.045 ± 0.04</td>
<td>0.033 ± 0.04</td>
</tr>
<tr>
<td>Ovaries</td>
<td>0.19 ± 0.00</td>
<td>0.27 ± 0.06</td>
<td>0.61 ± 0.07</td>
<td>0.56 ± 0.013</td>
<td>0.18 ± 0.01</td>
<td>0.021 ± 0.0</td>
<td>0.036 ± 0.02</td>
</tr>
<tr>
<td>kidney</td>
<td>0.43 ± 0.02</td>
<td>0.47 ± 0.18</td>
<td>1.47 ± 0.023</td>
<td>2.03 ± 0.045</td>
<td>1.13 ± 0.038</td>
<td>0.440 ± 0.016</td>
<td>0.340 ± 0.016</td>
</tr>
<tr>
<td>Spleen</td>
<td>1.32 ± 0.23</td>
<td>6.49 ± 0.43</td>
<td>8.85 ± 0.75</td>
<td>4.84 ± 0.09</td>
<td>0.28 ± 0.09</td>
<td>0.044 ± 0.01</td>
<td>0.081 ± 0.01</td>
</tr>
<tr>
<td>Heart</td>
<td>0.22 ± 0.02</td>
<td>0.21 ± 0.06</td>
<td>0.36 ± 0.08</td>
<td>0.3 ± 0.08</td>
<td>0.15 ± 0.01</td>
<td>0.025 ± 0.0</td>
<td>0.031 ± 0.01</td>
</tr>
</tbody>
</table>

*2 h block indicates co-administration of 15 μg estradiol.

Tissue biodistribution of 2 in mature female rats: blocking study.

Table 2

<table>
<thead>
<tr>
<th>Percent injected dose/gram ± S.D. (n = 3)</th>
<th>2 h</th>
<th>2 h (Block)</th>
</tr>
</thead>
<tbody>
<tr>
<td>blood</td>
<td>0.33</td>
<td>±0.06</td>
</tr>
<tr>
<td>Liver</td>
<td>9.87</td>
<td>±1.68</td>
</tr>
<tr>
<td>bone</td>
<td>0.14</td>
<td>±0.03</td>
</tr>
<tr>
<td>Muscle</td>
<td>0.11</td>
<td>±0.04</td>
</tr>
<tr>
<td>lung</td>
<td>21.86</td>
<td>±0.70</td>
</tr>
<tr>
<td>Uterus</td>
<td>0.28</td>
<td>±0.06</td>
</tr>
<tr>
<td>ovaries</td>
<td>0.61</td>
<td>±0.08</td>
</tr>
<tr>
<td>Kidney</td>
<td>1.47</td>
<td>±0.24</td>
</tr>
<tr>
<td>spleen</td>
<td>8.86</td>
<td>±0.75</td>
</tr>
<tr>
<td>heart</td>
<td>0.37</td>
<td>±0.09</td>
</tr>
<tr>
<td>Uterus/Muscle</td>
<td>3.02</td>
<td>±0.86</td>
</tr>
<tr>
<td>Uterus/blood</td>
<td>0.89</td>
<td>±0.27</td>
</tr>
<tr>
<td>Ovaries/Muscle</td>
<td>6.11</td>
<td>±0.32</td>
</tr>
<tr>
<td>Ovaries/blood</td>
<td>1.94</td>
<td>±0.65</td>
</tr>
</tbody>
</table>

Table 3

<table>
<thead>
<tr>
<th>Compound</th>
<th>Ovaries</th>
<th>Uterus</th>
<th>Ovaries</th>
<th>Uterus</th>
</tr>
</thead>
<tbody>
<tr>
<td>15 min</td>
<td>0.199 ± 0.002</td>
<td>0.197 ± 0.004</td>
<td>1.76 ± 0.10</td>
<td>0.44 ± 0.15</td>
</tr>
<tr>
<td>1 h</td>
<td>0.27 ± 0.060</td>
<td>0.236 ± 0.14</td>
<td>0.42 ± 0.03</td>
<td>0.2 ± 0.005</td>
</tr>
<tr>
<td>2 h</td>
<td>0.61 ± 0.070</td>
<td>0.283 ± 0.05</td>
<td>0.45 ± 0.08</td>
<td>0.2 ± 0.02</td>
</tr>
<tr>
<td>3 h</td>
<td>0.56 ± 0.013</td>
<td>0.17 ± 0.01</td>
<td>0.13 ± 0.01</td>
<td>0.07 ± 0.02</td>
</tr>
<tr>
<td>6 h</td>
<td>0.183 ± 0.01</td>
<td>0.01 ± 0.010</td>
<td>0.12 ± 0.02</td>
<td>0.04 ± 0.01</td>
</tr>
<tr>
<td>18 h</td>
<td>0.021 ± 0.000</td>
<td>0.045 ± 0.04</td>
<td>0.06 ± 0.01</td>
<td>0.04 ± 0.01</td>
</tr>
</tbody>
</table>

* Values from Refs. [39,45].

Fig. 4. Uterus to muscle ratios (a) and ovaries-to-muscle ratios (b) determined by tissue biodistribution at 2 h post injection of compound 2 and 4 [45] respectively.
Long circulation time is an important feature for radiopharmaceuticals since a large number of passages through the targeted areas might favor radiotracer accumulation leading to better target/non-target ratios. For imaging purposes, a stability of compound with a half-life ranging from 4 to 6 h is suitable to achieve a reasonable signal-to-noise ratio [12].

A biodistribution study was thus performed to demonstrate the feasibility of 2 as a radiotracer for breast cancer. Purified 2 was reconstituted in 15% ethanol-saline and injected into mature female Wistar rats (180–200 g) via the tail vein. The dose of 2 employed was 80 μCi/animal. Animals were sacrificed at 15 min, 1 h, 2 h, 3 h, 6 h, 18 h and 24 h post-injection. Table 1 shows the uptake of 2 in selected tissues of rats. The uptake values were calculated as a percent injected dose per gram of tissue (% ID/g).

At 15 min post injection, the uptake by uterus is similar to ovaries and in the same range as for muscle and bone. Interestingly, the ovaries/muscle and uterus/muscle ratios are 2.01 ± 0.01 and 2 ± 0.02, respectively, thus opening the possibility of imaging of these organs.

At 2 h post-injection point, there is an uptake by ovaries with 0.66 ± 0.07% ID/g. Apart from the high uptake by the liver and lung (9.87 ± 1.68 and 21.86 ± 1.70% ID/g, respectively), the uptake of kidney, spleen and heart is higher than that of ovaries. The ovaries/muscle and ovaries/blood ratios are 6.11 and 1.94 respectively. At 6 h post-injection, uptake in the ovaries was reduced to 0.183 ± 0.015% ID/g; while up to 75% of the radioactivity in liver and kidney, spleen and heart is higher than that of ovaries. The ovaries/muscle uptake ratio shows a significant reduction when the re-injection of estradiol is mediated by co-injection of large excess of estradiol means that this uptake is ER mediated. Further studies, involving the metabolic stability of compound 2 in in vivo tumor model experiments, are currently underway.

To determine whether the uptake was mediated by a high affinity, limited-capacity system, a blocking study was carried out by co-injection of 80 μCi of 2 together with 18 μg/g estradiol to fully occupy the ER sites. The results are shown in Table 2. Uptake in ER-rich target tissues (uterus and ovaries) was reduced to 0.11 ± 0.01 and 0.24 ± 0.01, respectively. This result indicates that the uptake of 2 in the ovaries is mediated by binding to ER. No obvious differences in activity levels were observed in blood, liver, lung and spleen between blocked and nonblocked animals.

Table 3 shows the biodistribution of compound 2 and 4 in mature female rats. A compared to 4, 2 attains a maximum uptake at 2 h while the uptake of 4 starts to decrease from 15 min. The uptakes of 2 by ovaries and by uterus are similar, while the uptake of 4 by ovaries is much higher than that by uterus.

Fig. 4 shows uterus to muscle ratios and ovaries to muscle ratios of 2 and 4. 4 has higher uterus to muscle ratios than that of 3. For ovaries, 4 still has better ratio value than 2 at 1 h but lower at 15 min. The difference between the uptake of 2 and 4 may be due to the fact that 4 has good affinity for ER in ER rich tissue (uterus and ovaries) [39].

4. Conclusion

We have developed suitable conditions for the synthesis of 99mTc compound 2. It was obtained in good yields (90%). Tissue distribution showed a significant selective uptake by the ovaries and a low level of activity in muscle. The fact that the ovaries/muscle uptake ratio shows a significant reduction when the receptor is blocked by co-injection of large excess of estradiol means that this uptake is ER mediated. Further studies, involving the metabolic stability of compound 2 in in vivo tumor model experiments, are currently underway.

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References


