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Research Article

Synthesis of [^{11}C]gefitinib for imaging epidermal growth factor receptor tyrosine kinase with positron emission tomography

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Summary

We have synthesized N-(3-chloro-4-fluorophenyl)-7-[^{11}C]methoxy-6-[3-(morpholin-4-yl)propoxy]quinazolin-4-amine, [^{11}C]gefitinib ([^{11}C]Iressa), a high affinity (IC_{50} = 2 n*M*) inhibitor of the epidermal growth factor receptor tyrosine kinase (EGFR-TK), in solution and in a semi-automated stainless loop methylation system using [^{11}C]methyl triflate. The trapping efficiency for [^{11}C]methyl triflate in solution was higher than in the solvent film generated in the loop system, thus the overall radiochemical yield was considerably higher for the synthesis in solution. The average radiochemical yield for the solution chemistry was 15% with an average specific radioactivity of approximately 9000 mCi/μmole at EOS in one step from its corresponding desmethyl phenol precursor. Copyright © 2006 John Wiley & Sons, Ltd.

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Introduction

A major goal in the development of new cancer chemotherapeutics is to use these promising new agents in the appropriate populations, i.e. in patients whose tumors actually harbor the target being sought. To that end, we and

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others have undertaken a program to use positron emission tomography (PET) in conjunction with radiolabeled chemotherapeutic agents to assess the pharmacokinetics of those agents *in vivo*[1–5]. The ultimate goal of such work is to predict which tumors will respond to the intended chemotherapeutic agent by determining if the tumors can be visualized by the corresponding imaging agent so patients may be separated into appropriate treatment groups.

The epidermal growth factor tyrosine kinese (EGFR-TK) is a promising target for the treatment of a subset of patients with non-small cell lung cancer (NSCLC) as well as other malignancies.[6] Low molecular weight inhibitors of EGFR-TK have entered clinical trials with mixed results. Specifically, gefitinib (Iressa^{TM}) has been successful in a phase II clinical trial in a very select sub-population of patients with NSCLC, who possess a somatic mutation that confers susceptibility to this agent.[7,8]

Recently, DeJesus *et al.* [9] have synthesized [^{18}F]gefitinib. Ben-David *et al.* have synthesized [^{11}C]ML03, an irreversible EGFR-TK ligand, from [^{11}C]acryloyl chloride.[10] Also, Seimbille *et al.* [11] have developed a general route to fluorine-18-labeled analinoquinazoline derivatives, including [^{18}F] gefitinib, [^{18}F]erlotinib ([^{18}F]Tarceva), and [^{18}F]ZD6474. To our knowledge, no biodistribution studies for these radiolabeled EGFR-TK inhibitors have been reported.

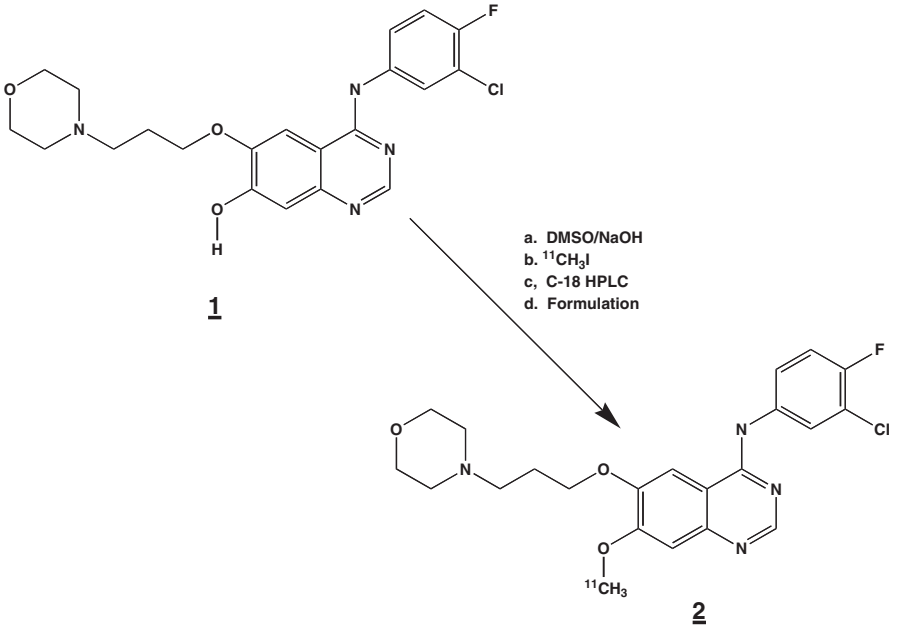

Figure 1.

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These past efforts by others have focused on radiolabeling with fluorine-18. We report here the synthesis of [^{11}C]gefitinib **2** (Figure 1) for evaluation of EGFR-TK *in vivo*.

Experimental

Dimethylsulfoxide (DMSO) was distilled under reduced pressure from barium oxide. The HPLC consisted of model 7126 injectors (Rheodyne, Rohnert, CA), model 590 EF pumps (Waters, Milford, MA), a model 486 ultraviolet (UV) absorbance detector (254 nm) (Waters), and a Bioscan FC-3200 NaI detector connected to FC-1000 base unit. Varian Galaxie Chromatography Data System was used to record and analyze HPLC chromatograms. Semi-preparative (7.8 × 300 mm) and analytical (3.9 × 150 mm) reverse-phase HPLC columns (NovaPak C-18, Waters Associates) were used for purification and quality control of the radiotracer, respectively.

Radiochemical synthesis of [^{11}C]Iressa in solution

The gefitinib precursor (0.4–0.5 mg, approximately 1 μmole) was dissolved in 50 μl of DMSO and 5 μL of 1 M NaOH were added. The vial was sealed and the solution was shaken for 1 min. [^{11}C]Methyl iodide was produced using the GE Tracerlab MeI Microlab system starting from cyclotron produced [^{11}C]CO_2. The [^{11}C]methyl iodide was transferred from the GE Microlab system through a silver triflate furnace. [12] The [^{11}C]methyl triflate was bubbled through the precursor solution in a stream of helium (30 ml/min) for approximately 2 min before flow was stopped. The vial was heated at 80°C for 3 minutes after which semi-preparative HPLC purification was initiated

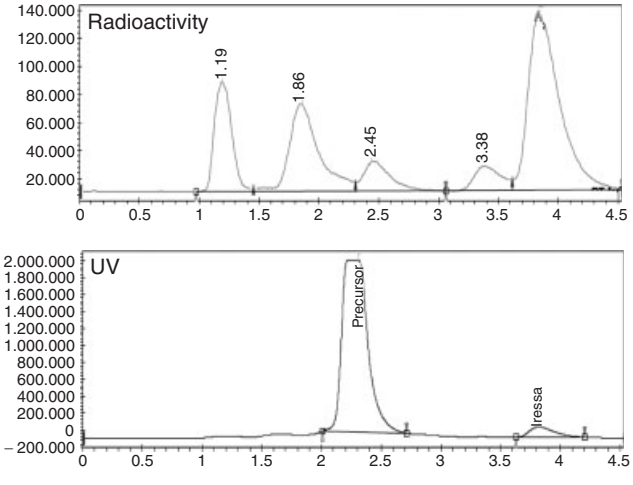

Figure 2. Semi-preparative HPLC Purification of [^{11}C]Iressa

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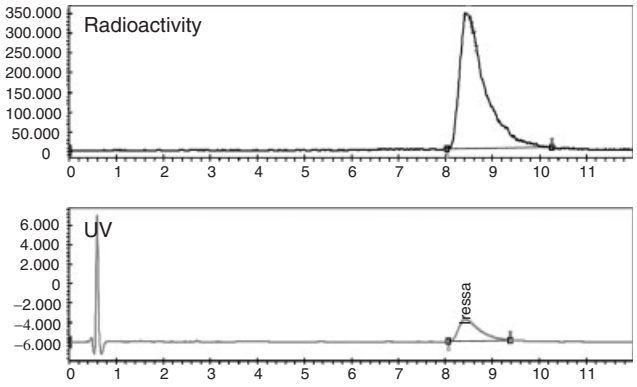

Figure 3. Analytical HPLC of final formulation of [^{11}C]Iressa

using a Waters NovaPak column eluted with 40% acetonitrile/60% 0.1 M ammonium formate at 10 ml/min with the precursor eluting at 2.3 min and the product eluting at 3.8 min (Figure 2). The product was collected in a pressure reservoir where it was diluted with 50 ml of water, then loaded onto a Waters C-18 SepPak Plus. The SepPak containing the purified [^{11}C]gefitinib was washed with 10 ml of water before the product was eluted with 1 ml of absolute ethanol followed by 9 ml of normal saline.

The radiochemical purity and specific activity were determined by analytical radio-HPLC with UV detection at 254 nm eluted with 30% acetonitrile/70% 0.1 M ammonium formate at 2.5 ml/min (Iressa $R_t = 8.5$ min; precursor $R_t = 3.1$ min; Figure 3).

Semi-automated radiochemical synthesis of [^{11}C]Iressa

The gefitinib precursor (0.4–0.5 mg, approximately 1 μmole) was dissolved in 60–200 μL of DMSO and 5 μl of 1 M NaOH were added. The vial was sealed and the solution was vortexed for 1 min. The precursor solution was injected into the Bioscan AutoLoop System and flushed with argon (30 ml/min) for 5 s. [^{11}C]Methyl triflate, produced as previously described, was transferred into the Bioscan Autoloop (Bioscan Inc, Washington, DC) in a stream of helium (30 ml/min). The [^{11}C]methyl triflate was trapped in the AutoLoop for 2.5 min before flow was stopped and the precursor allowed to react for an additional 4 min. After the 4 min reaction, the sample was automatically transferred to the semi-preparative HPLC and worked up as described earlier.

Results and discussion

We have synthesized [^{11}C]gefitinib **2** with an average radiochemical yield of $15 \pm 2\%$ ($n = 3$), determined from [^{11}C]methyl triflate collected in solution,

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and $3 \pm 2\%$ ($n = 6$) in the loop methylation device; neither have been corrected for decay. The average specific radioactivities at end of synthesis (EOS) were 9728 ± 1307 mCi/μmole from the synthesis in solution, and 4466 ± 1723 mCi/μmole from the loop methylation. The radiosynthesis, HPLC purification and formulation were completed in an average time of 25 min ($n = 9$) for both methods. The final formulation was chemically (95%) and radiochemically pure (>99%) as determined by analytical HPLC for both methods (see Figure 3).

The [^{11}C]methyl triflate trapping efficiency in the loop was approximately 25%, trapping an estimated average of 146 mCi ($n = 6$). For comparable productions of [^{11}C]carbon dioxide (i.e. same beam current and length of bombardment), the trapping efficiency for the solution chemistry was >95%, trapping an average of 580 mCi ($n = 3$). It is not clear at this time why there was a difference in trapping efficiency of [^{11}C]methyl triflate between the two methods. The crude product yield based on integration of the radioactivity chromatogram from the semi-preparative HPLC and independent of the amount of radioactivity trapped showed a yield of $15 \pm 6\%$ from the loop methylation and $39 \pm 9\%$ for the solution chemistry.

Conclusion

[^{11}C]Gefitinib **2** was synthesized in high yield and at high specific radioactivity in one step from the phenol precursor and [^{11}C]methyl triflate in solution. The radiotracer was also synthesized, albeit at a lower radiochemical yield, in a semi-automated loop methylation device. The yields and specific radioactivities achieved are sufficient for in vivo animal biodistribution studies. Studies in appropriate experimental animal models are currently under way.

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