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A first synthesis of ^{18}F-radiolabeled lapatinib: a potential tracer for positron emission tomographic imaging of ErbB1/ErbB2 tyrosine kinase activity

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Fluorine-18-labeled lapatinib has been successfully synthesized for the first time by the reaction of a dimethylformamide solution of *meta*-[^{18}F]fluorobenzylbromide with a Boc-protected lapatinib precursor fragment. The reaction proceeded in the presence of K_2CO_3 at 110 °C for 10 min in a microwave and was followed by Boc-group deprotection with trifluoroacetic acid. The overall radiochemical yield of the reaction starting from the radiofluorination of the iodonium salt was 8–12% (uncorrected, $n = 6$) in a 140-min synthesis time.

Supplementary information may be found in the online version of this article.

Keywords: Radiolabeling; Fluorine-18; meta-[^{18}F]benzylbromide; [^{18}F]Lapatinib

Introduction

Inhibition of epidermal growth factor receptor tyrosine kinases, which are often overexpressed in tumors, is an attractive modern approach to cancer therapeutics[1–5] and is an area of active investigations. Many drugs to inhibit tyrosine kinase activity have been developed or are under development.[3,6–9] Molecular probes and radiolabeled analogs of epidermal growth factor receptor tyrosine kinase inhibitor drugs engender multiple interests including studies of basic mechanisms of action, preclinical targeting studies, and further drug development for new indications in oncology. In addition, radiolabeled analogs may be useful in clinical applications to verify target presence, establish drug localization indices, and monitor the effects of treatment protocols.[10–19]

Lapatinib ditosylate (Tykerb®) is a dual ErbB1/ErbB2 tyrosine kinase inhibitor from GlaxoSmithKline and is clinically approved for use in combination chemotherapy for the treatment of advanced metastatic breast cancer.[20–24] It is also under investigation in other cancer indications.[25,26] Further clinical and preclinical studies of this drug would be greatly facilitated by noninvasive imaging techniques that allow the measurement of drug concentrations in tumor tissue and ErbB1/ErbB2 tyrosine kinase inhibition.

Ideally, [^{18}F]lapatinib can be synthesized by exchanging the ^{19}F (Figure 1) with [^{18}F]F. Although the product from this reaction is of low specific activity, it could still prove useful, given the high doses of lapatinib used in both preclinical[27] and clinical studies.[28] This direct substitution approach is reported infrequently in the radiochemistry literature,[29–32] mainly because of the unreactive nature of [^{18}F]F under most nucleophilic conditions. Attempts to take a similar approach for an [^{18}F]lapatinib analog resulted in failure.[31] Alternative routes could involve direct nucleophilic fluorination of a suitably activated precursor by [^{18}F]fluoride ion or radiofluorination of a benzyl intermediate prior to final assembly of the lapatinib drug.

Direct nucleophilic fluorination of an aromatic ring is known to be difficult without an electron withdrawing group (NO_2, CN, CHO, RCO, COOR) in an *ortho* or *para* position to the leaving group (NO_2, NMe_3^+, Cl, Br, I). The presence of such a group is posited to reduce the electron density at the target carbon and/or act by resonance stabilization of the putative sigma intermediate complex. Because of the absence of any such electron withdrawing group in lapatinib, the total synthesis of an [^{18}F]lapatinib analog becomes much more challenging. In addition, there are very few examples of a direct replacement of a leaving group (Cl, Br, I, NMe_3^+, NO_2) by [^{18}F]fluoride ion in compounds with an electron withdrawing group (CN, NO_2, Br, CHO) present in the nonactivating *meta* ring position.[29–32] Thus, indirect fluorination was our only viable option for the preparation of [^{18}F]lapatinib.

In this indirect approach,[33–35] a first ^{18}F-labeled prosthetic group or synthon is synthesized and then coupled with the remaining fragment of the molecule. Specifically, the synthesis is based on the ether bond formation between 3-[^{18}F]fluorobenzylbromide and a Boc-protected lapatinib fragment, *tert*-butyl (5-(4-(3-chloro-4-hydroxyphenylamino)quinazolin-6-yl)furan-2-yl)methyl

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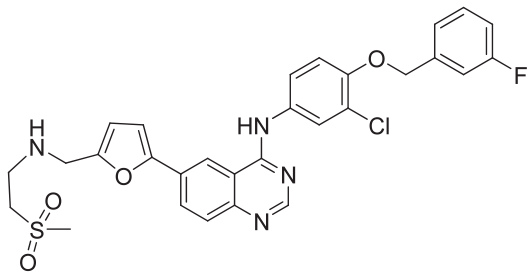


Figure 1. Lapatinib structure.

(2-(methylsulfonyl)ethyl)carbamate, which contains a phenolic group (**2**), followed by deprotection of the *N*-Boc secondary amino protecting group with trifluoroacetic acid (TFA) to give [^{18}F]lapatinib.

Results and discussion

>*tert*-Butyl (5-(4-(4-(benzyloxy)-3-chlorophenylamino)quinazolin-6-yl)furan-2-yl)methyl(2-(methylsulfonyl)ethyl)carbamate (compound **1)** was synthesized according to a modified literature method (see Supplementary Information for detailed procedure and references). Hydrogenolysis of the benzyl ether of **1** afforded an *N*-Boc-protected lapatinib precursor (compound **2**; Scheme 1) with 76% yield. Formation of compound **2** was confirmed by both ^{1}H and ^{13}C NMR and high-resolution mass spectrometry (MS).

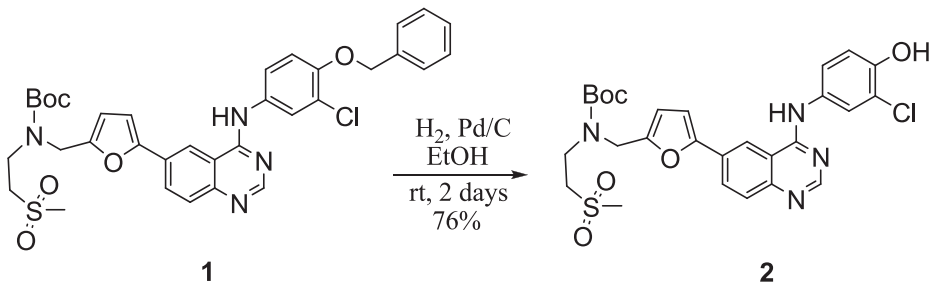
Fluorine-18 labeling of lapatinib involved two steps: first, the preparation of *meta*-[^{18}F]fluorobenzylbromide; second, the coupling of this prosthetic group with the lapatinib precursor compound **2** (Scheme 2).

meta-[^{18}F]Fluorobenzylbromide was synthesized according to the recent procedure[36] reported by our group. *meta*-[^{18}F] Fluorobenzylbromide was purified using a Sep-Pak® plus silica cartridge. The coupling reaction of *meta*-[^{18}F]fluorobenzylbromide with a Boc-protected lapatinib precursor fragment (**2**) was carried out at 110 °C in dimethylformamide in the presence of K_2CO_3 in a microwave. The formation of the product was monitored by HPLC using a semi-preparative column. The complete consumption of *meta*-[^{18}F]fluorobenzylbromide was observed within 10 min. Deprotection of the Boc-protected amine group was achieved with TFA at 100 °C for 10 min. Radio-HPLC analysis showed that the coupling reaction proceeded cleanly with only two minor peaks (Figure 2). The reaction mixture was partially neutralized with 6 N NaOH, and the final product was purified by semi-preparative HPLC. The collected fraction was diluted with 10 mL of water and passed through a Sep-Pak® light C18 cartridge (preconditioned with 5 mL of ethanol, 10 mL of water, 10 mL of air). The trapped [^{18}F] lapatinib was eluted with 1 mL of ethanol. The compound was formulated for further use by evaporating the ethanol at 70 °C under nitrogen, and the [^{18}F]lapatinib was dissolved in 2 mL of 10% ethanol in 1× PBS. The solution was then passed through a 0.25-µm sterile filter (Millipore). The final pH of the product solution was between 6 and 7.

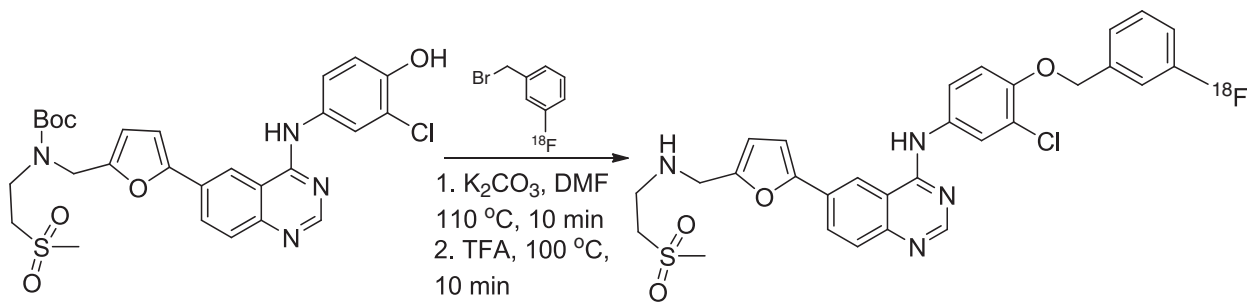
The overall radiochemical yield starting from radiofluorination of the iodonium salt was 8–12% (uncorrected, $n = 6$) in a 140-min synthesis time, with a radiochemical purity of >98% by analytical HPLC (Figure 3a). The specific activity, determined by injection of an aliquot of known activity onto an analytical HPLC and comparison of the integrated sample UV signal area with a calibrated µmol/UV area curve for the nonradioactive lapatinib, was 35–430 Ci/mmol ($n = 6$). The specific activity remained low because of the low amount[37] of starting [^{18}F]fluoride ion (10–50 mCi) used in this manual reduction-to-practice synthesis. The identity of the [^{18}F]lapatinib was further confirmed by comparing its HPLC retention time with that of co-injected authentic nonradioactive lapatinib (Figure 3b), using different analytical HPLC conditions (Supplementary Information).

Materials and methods

4-Chloro-6-bromoquinazoline was purchased from Ark Pharm, Inc. (Libertyville, IL), and used as received. All other commercially available organic precursors and dry solvents were obtained from



Scheme 1. Synthesis of *tert*-butyl (5-(4-(3-chloro-4-hydroxyphenylamino)quinazolin-6-yl)furan-2-yl)methyl(2-(methylsulfonyl)ethyl)carbamate. rt, room temperature.



Scheme 2. Synthesis of [^{18}F]lapatinib.

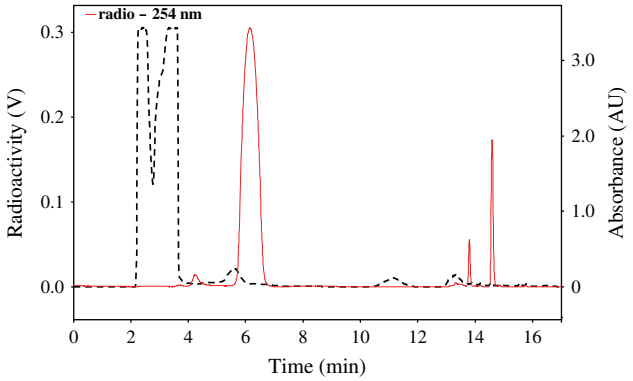


Figure 2. HPLC analysis of the crude reaction mixture of [^{18}F]lapatinib. HPLC conditions: Agilent Eclipse XDB C18 column, 5 µm, 9.4 × 250 mm; eluent, 40% acetonitrile, 60% water with 0.1% TFA; flow rate, 4 mL/min. Solid line, in-line radiodetection; dotted line, UV detection at 254 nm. (This figure is available in color online at http://www.interscience.wiley.com/journal/jlcr).

Sigma-Aldrich (St. Louis, MO) and used as received unless otherwise noted. Compound **1** was synthesized by an improved modification of the literature-described method (see Supplementary Information for detailed procedure and references). *meta*-[^{18}F]Fluorobenzylbromide was prepared using our recently described method.[36] Thin-layer chromatography visualization was achieved using both 254-nm or 360-nm UV detection. Flash column chromatography was performed on an Ana-Logix IntelliFlash 280 system, using Biotage® SNAP Cartridges. Atmospheric pressure chemical ionization MS was performed on a 6130 Quadrupole LC/MS Agilent Technologies instrument equipped with a diode array detector. ^{1}H and ^{13}C NMR spectra were recorded on a Varian spectrometer (400 MHz). Chemical shifts (ppm) are reported relative to the solvent residual peak of dimethyl sulfoxide (δ ^{1}H, 2.50; ^{13}C, 40.45 ppm). High-resolution MS was performed by the Scripps Research Institute Center for Metabolomics and Mass Spectrometry (La Jolla, CA). Fluorine-18 was purchased from PETNET Solutions (North Wales, PA). Radiosynthesis was performed manually. The product was purified by HPLC on a Beckman Coulter System Gold instrument equipped with a multiwavelength detector using an Agilent Eclipse C18 column (5 µm, 9.4 × 250 mm). Analytical HPLC analyses for radiochemical work were performed on an Agilent 1200 Series instrument equipped with multiwavelength detectors using an Agilent Eclipse XDB C18 column (4.6 × 150 mm, 5 µm) with a flow rate of 1.0 mL/min. All the microwave reactions for fluorine-18 labeling were done in a Biotage Initiator 2.5 using Biotage 10-mL V-shaped vials at constant temperature mode. All the Sep-Pak® cartridges used in this synthesis were obtained from Waters (Billerica, MA).

tert-Butyl (5-(4-(3-chloro-4-hydroxyphenylamino) quinazolin-6-yl)furan-2-yl)methyl(2-(methylsulfonyl)ethyl) carbamate (2)

A solution of *tert*-butyl (5-(4-(4-(benzyloxy)-3-chlorophenylamino) quinazolin-6-yl)furan-2-yl)methyl(2-(methylsulfonyl)ethyl)carbamate (**1**; 1.00 g, 1.5 mmol) in EtOH (1.4 L) was hydrogenated for 2 days at room temperature in the presence of 10% Pd/C (0.5 g, 0.5 mmol) and then filtered. The solvent was evaporated to dryness, and the residue was purified by chromatography (30% ethyl acetate in hexane) to afford the title compound *tert*-butyl (5-(4-(3-chloro-4-hydroxyphenylamino)quinazolin-6-yl)furan-2-yl) methyl(2-(methylsulfonyl)ethyl)carbamate (**2**; 0.66 g, 76%) as a

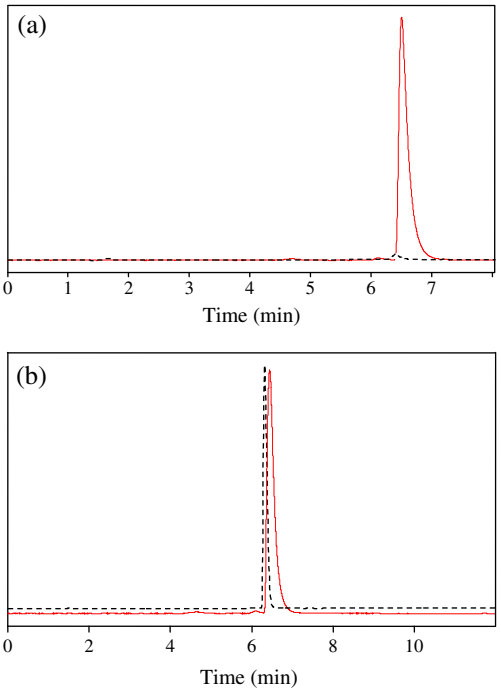


Figure 3. HPLC analysis of (a) [^{18}F]lapatinib after formulation and (b) [^{18}F] lapatinib co-injected with the nonradioactive standard. HPLC conditions: Agilent Eclipse XDB C18 column, 5 µm, 4.6 × 150 mm; 20–90% acetonitrile in water (0.1% TFA) in 10 min; flow rate, 1 ml/min. Solid line, in-line radiodetection; dotted line, UV detection at 254 nm. (This figure is available in color online at http://www.interscience.wiley.com/journal/jlcr).

yellow solid. LC-MS (Method II, positive mode atmospheric pressure chemical ionization): retention time = 4.51 min, *m/z* 573 [M + 1]; high-resolution ESI-TOF MS calculated for $C_{27}H_{30}ClN_4O_6S$, 573.1569, found 573.1557 [M + 1]. ^{1}H NMR (400 MHz, DMSO-d_6): δ 10.07 (s, 1H), 9.77 (s, 1H), 8.73 (d, *J* = 1.3, 1H), 8.53 (s, 1H), 8.10 (dd, *J* = 1.7, 8.8 Hz, 1H), 7.87 (d, *J* = 2.5 Hz, 1H), 7.79 (d, *J* = 8.7 Hz, 1H), 7.57 (dd, *J* = 2.5, 8.8 Hz, 1H), 7.06–7.02 (m, 2H), 6.52 (d, *J* = 3.1 Hz, 1H), 4.55 (s, 2H), 3.70 (br, 2H), 3.40 (br, 2H, mixed with water peak), 2.30 (s, 3H), 1.44 (s, 9H). ^{13}C NMR (100 MHz, DMSO-d_6): δ 157.6, 154.4, 154.2 (br), 152.0, 149.6, 148.8, 131.1, 128.4, 128.2, 127.9, 124.3, 122.8, 118.9, 116.7, 116.1, 115.2, 110.4 (br), 110.1 (br), 107.7, 79.84, 51.75, 44.01, 43.04, 40.59, 27.85.

Radiosynthesis of *meta*-[^{18}F]fluorobenzylbromide

Phenyl(3-formylphenyl)iodonium bromide (1–2 mg), TEMPO (0.5–1 mg), and dried Cs[^{18}F]F (10–50 mCi) were heated at 110 °C in a microwave for 5 min.[36] *meta*-[^{18}F]Fluorobenzaldehyde was then converted to corresponding alcohol by treatment with aqueous $NaBH_4$ and finally converted to *meta*-[^{18}F] fluorobenzylbromide by reaction with triphenylphosphine dibromide. The *meta*-[^{18}F]Fluorobenzylbromide thus obtained was loaded onto a silica plus Sep-Pak® cartridge and eluted with 2 mL of dry dichloromethane.

Radiosynthesis of [^{18}F]lapatinib

A dichloromethane solution of *meta*-[^{18}F]fluorobenzylbromide was evaporated under a gentle flow of nitrogen at 60 °C to obtain dry *meta*-[^{18}F]fluorobenzylbromide. To the residue was added 25 mg of activated (dried overnight at 100 °C in a oven) K_2CO_3 and 2–3 mg of **2** in dimethylformamide (0.5 mL). The

solution was heated in a microwave oven at 110 °C for 10 min. To the solution was added 1 mL of TFA, and the solution was heated in a microwave oven at 100 °C for a further 10 min. The resulting solution was partially neutralized with 6 N NaOH (2 mL) or most of the TFA was evaporated at 80 °C under nitrogen and injected onto a semi-preparative HPLC column (Agilent Eclipse C18, 5 µm, 9.4 × 250 mm; eluent: 40% acetonitrile, 60% water (with 0.1% TFA); flow rate, 4 mL/min) with the product fraction collected at 6.2 min.

Conclusion

In conclusion, we have successfully developed the first reliable and reproducible strategy for the synthesis of [^{18}F]lapatinib using *meta*-[^{18}F]fluorobenzylbromide as the fluorination synthon. The radiosynthesis is suited for the preparation of the compound in quantities and times practicable when considering its use as a positron emission tomography radiopharmaceutical or investigational radiochemical. Further development and work toward an automated radiolabeling protocol are under way.

Supplementary Information

Supplementary information may be found in the online version of this article: full characterization of all compounds and HPLC data of radiolabeling experiments.

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