JOURNAL OF LABELLED COMPOUNDS AND RADIOPHARMACEUTICALS
J Label Compd Radiopharm 2005; **48**: 829–843.
Published online in Wiley InterScience (www.interscience.wiley.com). DOI: 10.1002/jlcr.998

Research Article

Fluorine-18 labeling of 6,7-disubstituted anilinoquinazoline derivatives for positron emission tomography (PET) imaging of tyrosine kinase receptors: synthesis of ^{18}F-Iressa and related molecular probes

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Summary

Inhibitors of tyrosine kinase enzymatic activity represent a promising new class of antineoplastic agents. Although clinical studies performed over the last decade give more insight on the potential therapeutic applications of such drugs, identification of the individual patients who might benefit from them remains a major challenge. We have developed a synthetic strategy for the production of a wide variety of radiolabeled 6,7-disubstituted 4-anilinoquinazolines suitable for noninvasive imaging of tyrosine kinase receptors to predict therapy effectiveness. Three new F-18 labeled radiopharmaceuticals based on the therapeutic agents Tarceva, Iressa, and ZD6474 were synthesized. Decay-corrected yields varied between 25 and 40% for a total synthesis time of 120 min, thus providing F-18 labeled tyrosine kinase inhibitors in quantities and times practical for use as PET radiopharmaceuticals.

Key Words: EGFR; tyrosine kinase inhibitors; fluorine-18; positron emission tomography

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Contract/grant sponsor: California Breast Cancer Research Program; contract/grant numbers: 7KB-0155, DOE DE-FC03-87ER60615

Received 23 March 2005
Revised 5 May 2005
Accepted 6 July 2005

Introduction

The epidermal growth factor receptor (EGFR) signaling pathway contributes to a number of processes important to cancer development and progression.[1,2] Over the last decade, selective small-molecule inhibitors of EGFR tyrosine kinase enzymatic activity have been focused upon as potential anti-cancer agents and as experimental tools for understanding the physiological roles of EGFR signaling.[3] Several 6,7-disubstituted 4-anilinoquinazolines have demonstrated promising antitumor activity *in vitro* and *in vivo* and are currently in clinical trials (Figure 1).[4,5] One of them, Iressa has recently been approved for treatment of advanced lung cancer.[6] Introduction of C-7 basic side chains to 4-anilinoquinazolines induces additional activity against vascular endothelial growth factor receptor (VEGFR), known to play a key role in tumor angiogenesis.[7] However, the clinical expectations have not been fulfilled yet, and one needs to better identify the subset of patients that will benefit from EGFR-inhibitor mediated therapy. Clinical deployment of these compounds would also be greatly facilitated by non-invasive imaging techniques with which to measure drug concentrations in the tumor tissue and monitor inhibition of EGFR and VEGFR kinase activity.[2,6]

A substantial number of ATP competitive inhibitors of the EGFR kinase have already been radiolabeled and evaluated as potential imaging agents.[8–16] However, so far significant EGFR specific tumor uptake has not been convincingly demonstrated *in vivo*. Furthermore, those approaches used EGFR kinase inhibitors not studied in clinical trials, and it is not clear whether those imaging agents can actually be used to assess the biodistribution of clinically used EGFR inhibitors. Our strategy was therefore to develop radiopharmaceuticals with structures identical or very closely related to therapeutic agents that have proven to be clinically successful.

In the present paper, we report a versatile and parallel synthetic route to prepare the different key intermediate 4-chloroquinazolines **8** (Scheme 1). Specific substituents were introduced at the 6-, or 7-, or both positions; well-known for their interactions with key amino acids of EGFR and/or VEGFR.

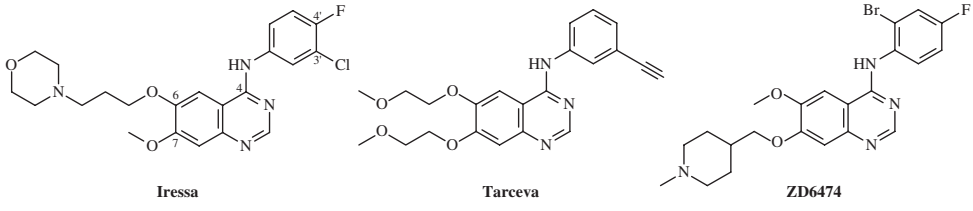
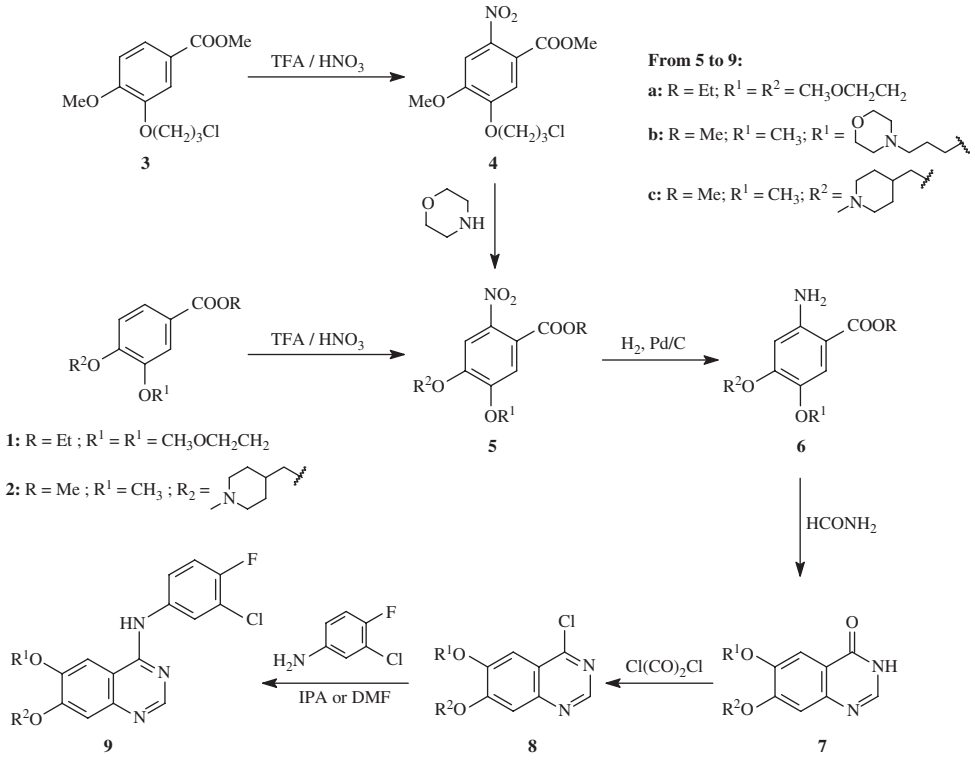


Figure 1. Promising inhibitors of EGFR and VEGFR activity



Scheme 1. Synthesis of 6,7-disubstituted anilinoquinazoline derivatives

The synthetic strategy proposed here can be used to produce a library of 6,7-disubstituted 4-anilinoquinazolines, and subsequently radiolabeled analogs for imaging purposes. Three non-radioactive tyrosine kinase inhibitors (TKIs) were synthesized by condensation of 3-chloro-4-fluoroaniline with the 4-chloroquinazoline intermediates with over 50% yield. We have also investigated the fluorine-18 labeling of those TKIs by coupling of the 4-chloroquinazolines **8** with 3-chloro-4-[^{18}F]fluoroaniline (**12**).

Results and discussion

Chemistry

Anilinoquinazolines **9** were prepared by one main route consisting of the following steps: (1) introduction of the substituents, different from the methoxy group, at the C-6 and/or C-7 positions of the quinazoline nucleus; (2) nitration and subsequent reduction of the nitro group to provide 2-aminobenzoate derivatives; (3) formation of the 4-quinazolones; (4) conver-

sion of the quinazolones into 4-chloroquinazolines; and (5) attachment of the aniline ring (Scheme 1).

Alkylation at both 3- and 4-position by treatment of ethyl 3,4-dihydroxybenzoate with 2-bromoethyl methyl ether in presence of base, followed by nitration at the 2-position of ethyl 3,4-bis(2-methoxyethoxy)benzoate (**1**) with nitric acid in TFA gave the key intermediate **5a** in 60% yield from ethyl 3,4-dihydroxybenzoate.[17] The disubstituted derivative **5a** was subsequently used to obtain a quinazoline structure similar to Tarceva, possessing similar substituents at both 6- and 7-positions.

Introduction of the 3-propoxymorpholino group found in Iressa was realized by treatment of methyl 3-hydroxy-4-methoxybenzoate with 1-bromo-3-chloropropane in presence of potassium carbonate.[18] Aromatic nitration of **3**, and nucleophilic displacement of the aliphatic chlorine of **4** by heating in an excess of morpholine led to the expected nitrobenzoate derivative **5b** in 37% yield from methyl 3-hydroxy-4-methoxybenzoate.

Reaction of methyl vanillate with *N*-BOC-4-piperidinemethanol under Mitsunobu conditions using diethyl azodicarboxylate afforded methyl 3-methoxy-4-(1-*tert*-butyloxycarbonylpiperidin-4-ylmethoxy)benzoate. The *t*-BOC protecting group was subsequently removed by treatment with concentrated HCl in 1,4-dioxane, and the piperidine nitrogen was methylated under Eschweiler–Clarke (HCOOH, HCHO, reflux) to yield **2**, containing the C-7 basic side chain of ZD6474.[19] Treatment of **2** with fuming HNO_3 in TFA provided methyl 5-methoxy-4-(1-methylpiperidin-4-ylmethoxy)-2-nitrobenzoate (**5c**) in 56% yield from methyl vanillate.

Reduction of the nitro group of the intermediates **5** by catalytic hydrogenation and subsequent Niementowski condensation gave the corresponding quinazolones **7**. Treatment of the quinazolones with oxalyl chloride yielded quantitatively the 4-chloroquinazolines **8**. The preparation of the non-radioactive tyrosine kinase inhibitors **9** was achieved by condensation of 3-chloro-4-fluoroaniline with the substituted 4-chloroquinazolines **8** in 2-propanol or DMF with over 50% yield.

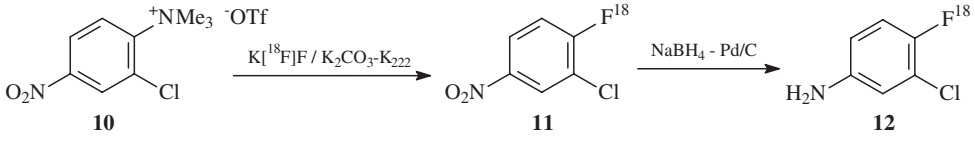
Radiochemistry

The radiolabeled EGFR inhibitors were prepared starting with the standard kryptofix-K_2CO_3-mediated nucleophilic ^{18}F exchange reaction with a trimethylammonium triflate precursor **10** (Scheme 2). Introduction of the fluorine-18 using a no-carrier-added nucleophilic substitution with K[^{18}F]F-K_{222} (K_{222}: Kryptofix [2.2.2]; 4,7,13,16,21,24-hexaoxa-1,10-diazabicyclo[8.8.8]hexacosane) was initially conducted in *N*,*N*-dimethyl sulfoxide (sealed V-vial) at 130°C for 10 min, leading to a rapid degradation of the precursor and low radiochemical yields (Table 1). Therefore, we investigated

whether we could improve the nucleophilic incorporation of [^{18}F]fluoride into the activated trimethylammonium triflate salt by changing the reaction conditions (temperature, time, and solvent). The reaction was then performed in either DMSO or CH_3CN at 110°C, but no significant improvement was observed. Although trimethylammonium triflate salts have been widely used for fluoride ion nucleophilic aromatic substitution reactions, they are known to be thermally unstable. Therefore, we sought to accomplish the first radiochemical step at room temperature, and yields, with respect to initial [^{18}F]fluoride, were approximately 75% (decay corrected) when the reaction occurred in acetonitrile for 25 min vs 20% when the same reaction mixture was heated at 110°C for 15 min.

Then, reduction of the nitro with sodium borohydride in presence of palladium on activated carbon gave 3-chloro-4-[^{18}F]fluoroaniline (**12**) (Table 1). Typically, starting from 3.4 to 6.1 GBq (92–165 mCi) aliquot of a cyclotron-produced [^{18}F]F^- batch, 1.4–2.2 GBq (38–60 mCi) of 3-chloro-4-[^{18}F]fluoroaniline could be obtained in 70–80 min.

Subsequent coupling of 3-chloro-4-[^{18}F]fluoroaniline (**12**) with the 4-chloroquinazoline intermediates **8** in 2-propanol afforded, after HPLC purification, the F-18 labeled 4-anilinoquinazolines in 25–40% decay corrected

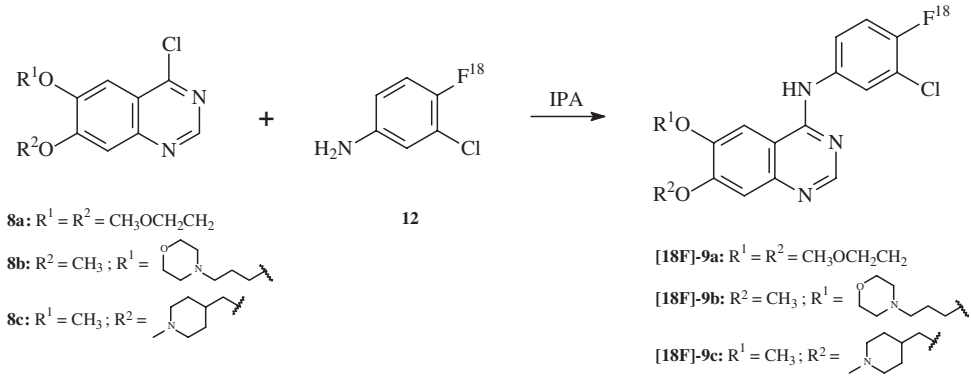


Scheme 2. Preparation of 3-chloro-4-[^{18}F]fluoroaniline (12)

Table 1. Production of 3-chloro-4-[^{18}F]fluoroaniline (12)

	Solvent[a]	Temperature	Time[c]	RCY[d]
Step 1	A	130°C	10	33.3–34%
	A	110°C	15	20.8–44.4%
	A	110°C	20	39.1–46.2%
	B	110°C	15	20%
	B	RT[b]	15	46.1–49.3%
	B	RT	20	52.8–58.9%
	B	RT	25	71.1–75.8%
Step 2	MeOH	RT	5	95.2–97.7%

[a] Solvent A: anhydrous *N*,*N*-dimethyl sulfoxide; solvent B: anhydrous acetonitrile.
[b] RT = room temperature.
[c] Time is expressed in minutes.
[d] RCY = radiochemical yield (decay corrected).



Scheme 3. Synthesis of F-18 labeled anilinoquinazolines ([^{18}F]-9a–c)

radiochemical yields (Scheme 3). The average total synthesis time was about 2 h. Identities of the new radiopharmaceuticals were confirmed by comparing their HPLC mobility with those of the non-radioactive analogues. Specific activity, determined by using on-line measurements of radioactivity and UV absorption, was > 222 GBq/μmol (> 6000 Ci/mmol).

Experimental

General

All reagents, including anhydrous solvents, were purchased from Aldrich, Sigma and Acros, and were used without further purification. Analytical thin layer chromatography (TLC) was performed on Sigma-Aldrich silica gel pre-coated plastic sheets with fluorescent indicator (UV 254). Visualization was achieved with short-wave ultraviolet light. Column chromatography was performed using silica gel (60–200 mesh). High performance liquid chromatography (HPLC) was carried out on an Agilent 1100 Series system. HPLC eluates were monitored for their UV absorbency at 254 nm, and their radioactivity content by connecting the outlet of the UV-photometer to a NaI detector. The recorded data were processed by an Agilent ChemStation software system. ^{18}F-activity was also measured with an ion chamber Capintec CRC11. The HPLC column used for the characterization of the product against standards and for the purification of radioactive compounds was a Phenomenex Luna-C18 (5 μm, 250 mm × 10 mm) column, operated at a flow rate of 2 ml/min. ^{1}H NMR spectrum was taken in deuterated solvents on a Bruker AM360/Wb spectrometer (at 360 MHz) using Me_4Si as an internal standard, and selected proton resonances are reported. Chemical shifts are expressed in ppm (δ) relative to the standard and coupling constants (*J*) in Hz. Mass spectrum was obtained on MALDI-TOF instrument (ABI DE STR) and EI instrument (Micromass, Waters Autospec).

Chemistry

Synthesis of ethyl 3,4-bis(2-methoxyethoxy)benzoate (**1**). To ethyl 3,4-dihydroxybenzoate (2.6 g, 14.3 mmol), potassium carbonate (4.35 g, 31.5 mmol) and tetrabutylammonium iodide (54 mg, 0.14 mmol) in degassed acetone (30 ml) was added 2-bromoethyl methyl ether (5 g, 36 mmol). The mixture was stirred under argon at reflux for 72 h. Ether (45 ml) was added to the mixture and after stirring 30 min at room temperature, the precipitate was removed by filtration. The filtrate was concentrated *in vacuo* and the residue was triturated with hexane to afford **1**, as a white solid (2.9 g, 68%). ^{1}H NMR (360 MHz, $CDCl_3$): δ : 7.67 (dd, $J = 2.1$ and 8.6 Hz, 1H), 7.58 (d, $J = 2.1$ Hz, 1H), 6.91 (d, $J = 8.3$ Hz, 1H), 4.34 (q, $J = 7.2$ Hz, 2H), 4.20 (m, 4H), 3.80 (m, 4H), 3.46 (s, 6H), 1.38 (t, $J = 7.2$ Hz, 3H). HRMS (EI) m/z: calculated for $C_{15}H_{22}O_6$, 298.1416; found, 298.1422.

Synthesis of methyl 3-methoxy-4-(1-methylpiperidin-4-ylmethoxy)benzoate (**2**). Triphenylphosphine (3.4 g, 13 mmol) was added under argon to a suspension of methyl vanillate (1.82 g, 10 mmol) in CH_2Cl_2 (40 ml), followed by the addition of *N*-BOC-piperidinemethanol (2.58 g, 12 mmol) and by a solution of diethyl azodicarboxylate (40% in toluene, 13 mmol) in CH_2Cl_2 (10 ml). After the solution was stirred for 1 h at ambient temperature, the reaction mixture was poured onto a column of silica and was eluted with hexane/EtOAc (from 10/0 to 3/1) to afford methyl 3-methoxy-4-(1-*tert*-butyloxycarbonylpiperidin-4-ylmethoxy)benzoate (3.49 g, 92%). ^{1}H NMR (360 MHz, $CDCl_3$): δ : 7.65 (dd, $J = 1.7$ and 8.6 Hz, 1H), 7.55 (s, 1H), 6.85 (d, $J = 8.3$ Hz, 1H), 4.30–4.05 (m, 2H), 3.91 (s, 3H), 3.89 (s, 3H), 2.75 (m, 2H), 2.15–2.00 (m, 2H), 1.87 (d, $J = 12.6$ Hz, 2H), 1.46 (s, 9H), 1.26 (m, 3H). HRMS (EI) m/z: calculated for $C_{20}H_{29}NO_6$, 379.1995; found, 379.2002. Methyl 3-methoxy-4-(1-*tert*-butyloxycarbonylpiperidin-4-ylmethoxy)benzoate (3 g, 7.9 mmol) was dissolved in 1,4-dioxane (5 ml) and treated with concentrated HCl (1 ml) for 1 h at room temperature. The volatiles were evaporated under vacuum, and the crude material was subsequently dissolved in formic acid (3.5 ml). Formaldehyde (12 M, 37% in water, 3.5 ml) was added and the mixture was stirred overnight at 95°C. The solvent was removed under reduced pressure and the residue was dissolved in water (30 ml). The pH was adjusted to 11 with an aqueous solution of sodium hydroxide (2 M). The mixture was diluted with ethyl acetate and the organic layer was separated. The aqueous layer was further extracted with ethyl acetate, and the organic layers were combined. The organic layers were washed with water, brine, dried over Na_2SO_4, and concentrated under reduced pressure to give the title compound (1.85 g, 80%). ^{1}H NMR (360 MHz, DMSO-d_6): δ : 7.56 (dd, $J =$ 2.1 and 8.3 Hz, 1H), 7.44 (d, $J = 2.1$ Hz, 1H), 7.07 (d, $J = 8.3$ Hz, 1H), 3.88 (d, $J = 5.8$ Hz, 2H), 3.81 (s, 3H), 3.80 (s, 3H), 2.76 (d, $J = 11.5$ Hz, 2H), 2.15

(s, 3H), 1.85 (t, $J = 11.5$ Hz, 2H), 1.72 (m, 3H), 1.29 (m, 2H). HRMS (EI) m/z: calculated for $C_{16}H_{23}NO_4$, 293.1627; found, 293.2629.

Synthesis of methyl 3-(3-chloropropoxy)-4-methoxybenzoate (**3**). To methyl 3-hydroxy-4-methoxybenzoate (3.75 g, 20.6 mmol) and potassium carbonate (5 g, 36.2 mmol) in degassed acetone (25 ml) was added 1-bromo-3-chloropropane (8 g, 50.8 mmol). The mixture was stirred under Argon at reflux for 24 h. The precipitate was removed by filtration and washed with ethyl acetate. The volatiles were removed under reduced pressure. The resulting solid was triturated in hexane, filtered, and dried under vacuum to give **3** (4.08 g, 76%). ^{1}H NMR (360 MHz, $CDCl_3$): δ : 7.69 (dd, $J = 2.1$ and 8.3 Hz, 1H), 7.57 (d, $J = 2.1$ Hz, 1H), 6.90 (d, $J = 8.6$ Hz, 1H), 4.22 (t, $J = 6.1$ Hz, 2H), 3.92 (s, 3H), 3.89 (s, 3H), 3.78 (t, $J = 6.1$ Hz, 2H), 2.31 (q, $J = 6.1$ Hz, 2H). HRMS (EI) m/z: calculated for $C_{12}H_{15}O_4Cl$, 258.0659; found, 258.0655.

Synthesis of methyl 5-(3-chloropropoxy)-4-methoxy-2-nitrobenzoate (**4**). A solution of **3** (2 g, 7.7 mmol) in methylene chloride (8 ml) was cooled to 0–5°C. TFA (4 ml) was added followed by addition in portions over 15 min of a solution of fuming 24 M nitric acid (0.8 ml) in methylene chloride (1.6 ml). After completion of the addition, the solution was allowed to warm up and stirred at ambient temperature for 2 h. The volatiles were removed by evaporation, and the residue was purified by column chromatography eluting with CH_2Cl_2/EtOAc (from 10/0 to 9/1). Evaporation of the fractions containing the expected product gave 1.92 g of **4** (82%), as an orange oil. ^{1}H NMR (360 MHz, $CDCl_3$): δ : 7.45 (s, 1H), 7,11 (s, 1H), 4.26 (t, $J = 6.1$ Hz, 2H), 3.95 (s, 3H), 3.91 (s, 3H), 3.77 (t, $J = 6.1$ Hz, 2H), 2.33 (q, $J = 6.1$ Hz, 2H). HRMS (EI) m/z: calculated for $C_{12}H_{14}NO_6Cl$, 303.0510; found, 303.0502.

A similar procedure was used to prepare ethyl 4,5-bis(2-methoxyethoxy)-2-nitrobenzoate (**5a**) and methyl 5-methoxy-4-(1-methylpiperidin-4-ylmethoxy)-2-nitrobenzoate (**5c**). **5a**: ^{1}H NMR (360 MHz, $CDCl_3$): δ : 7.49 (s, 1H), 7.10 (s, 1H), 4.35 (q, $J = 7.2$ Hz, 2H), 4.23 (m, 4H), 3.78 (m, 4H), 3.44 (s, 6H), 1.33 (t, $J = 7.2$ Hz, 3H). HRMS (EI) m/z: calculated for $C_{15}H_{21}NO_8$, 343.1267; found, 343.1270. **5c**: ^{1}H NMR (360 MHz, DMSO-d_6): δ : 7.67 (s, 1H), 7.35 (s, 1H), 4.04 (d, $J = 6.1$ Hz, 2H), 3.93 (s, 3H), 3.83 (s, 3H), 3.47 (m, 2H), 2.97 (m, 2H), 2.77 (s, 3H), 2.10–1.80 (m, 3H), 1.48 (m, 2H). HRMS (EI) m/z: calculated for $C_{16}H_{22}N_2O_6$, 338.1478; found, 338.1475.

Synthesis of methyl 4-methoxy-5-(3-morpholinopropoxy)-2-nitrobenzoate (**5b**). Morpholine (5 ml) was added to a suspension of **4** (1.83 g, 6 mmol) and the solution was heated at 100°C for 1 h. The residue was diluted with ethyl acetate and poured into ice cold water. The organic layer was washed with a 5% aqueous solution of $NaHCO_3$, twice with H_2O, dried over Na_2SO_4,

and concentrated under reduced pressure to give the title compound (1.28 g, 60%). ^{1}H NMR (360 MHz, $CDCl_3$): δ : 7.45 (s, 1H), 7.10 (s, 1H), 4.18 (t, $J = 6.8$ Hz, 2H), 3.94 (s, 3H), 3.90 (s, 3H), 3.70 (m, 4H), 2.51 (t, $J = 6.8$ Hz, 2H), 2.45 (m, 4H), 2.05 (q, $J = 6.8$ Hz, 2H). HRMS (EI) *m/z*: calculated for $C_{16}H_{22}N_2O_7$, 354.1427; found, 354.1417.

Synthesis of ethyl 2-amino-4,5-bis(2-methoxyethoxy)benzoate (**6a**). A suspension of **5a** (2.06 g, 6 mmol) in methanol (15 ml) containing 10% palladium on activated carbon (300 mg) was hydrogenated until uptake of hydrogen ceased. The mixture was filtered and the volatiles were removed by evaporation. The residue was dissolved in ether and 2 M hydrogen chloride in ether (5 ml) was added. The solid was collected by filtration and dried under vacuum to give **6a** (1.76 g, 94%). ^{1}H NMR (360 MHz, $CDCl_3$): δ : 7.42 (s, 1H), 6.12 (s, 1H), 5.61 (brs, 2H), 4.27 (q, $J = 7.2$ Hz, 2H), 4.07 (m, 4H), 3.71 (m, 4H), 3.43 (s, 6H), 1.34 (t, $J = 7.2$ Hz, 3H). HRMS (EI) *m/z*: calculated for $C_{15}H_{23}NO_6$, 313.1525; found, 313.1531.

A similar procedure was used to prepare methyl 2-amino-4-methoxy-5-(3-morpholinopropoxy)benzoate (**6b**) and methyl 2-amino-5-methoxy-4-(1-methylpiperidin-4-ylmethoxy)benzoate (**6c**). **6b**: ^{1}H NMR (360 MHz, $CDCl_3$): δ : 7.34 (s, 1H), 6.14 (s, 1H), 5.61 (brs, 2H), 4.00 (t, $J = 6.5$ Hz, 2H), 3.84 (s, 3H), 3.83 (s, 3H), 3.73 (m, 4H), 2.54 (t, $J = 7.2$ Hz, 2H), 2.48 (m, 4H), 1.99 (q, $J = 6.8$ Hz, 2H). HRMS (EI) *m/z*: calculated for $C_{16}H_{24}N_2O_5$, 324.1685; found, 324.1675. **6c**: ^{1}H NMR (360 MHz, DMSO-d_6): δ : 7.20 (s, 1H), 6.62 (s, 1H), 3.82 (d, $J = 6.5$ Hz, 2H), 3.76 (s, 3H), 3.69 (s, 3H), 3.42 (m, 2H), 2.96 (m, 2H), 2.70 (s, 3H), 2.10–1.90 (m, 3H), 1.61 (m, 2H). HRMS (EI) *m/z*: calculated for $C_{16}H_{24}N_2O_4$, 308.1736; found, 308.1729.

Synthesis of 6,7-bis(2-methoxyethoxy)-3,4-dihydroquinazolin-4-one (**7a**). **6a** (1.3 g, 4.1 mmol) and ammonium formate (260 mg, 4.1 mmol) were dissolved in formamide (2.2 ml) and the stirred mixture was heated to 160°C under an atmosphere of Argon for 3 h. After cooling, water (7 ml) was added and the precipitate was recovered by filtration, washed with cold water and dried. The filtrate was extracted five times with $CHCl_3$, and the pooled organic extracts were washed with brine, dried over Na_2SO_4, and concentrated. The residue and crude quinazolone precipitate were combined, triturated in hot CH_3CN (9 ml) for 30 min, cooled to 20°C, and treated with ether (9 ml). After cooling to 4°C, **7a** was recovered by filtration as a white solid and dried (753 mg, 62%). ^{1}H NMR (360 MHz, CD_3OD): δ : 7.98 (s, 1H), 7.61 (s, 1H), 7.15 (s, 1H), 4.28 (m, 4H), 3.85 (m, 4H), 3.48 (s, 6H). HRMS (EI) *m/z*: calculated for $C_{14}H_{18}N_2O_5$, 294.1216; found, 294.1213.

Synthesis of 7-methoxy-6-(3-morpholinopropoxy)-3,4-dihydroquinazolin-4-one (**7b**). A solution of **6b** (650 mg, 2 mmol) in 2-methoxyethanol (8 ml) containing

formamidine acetate (208 mg, 2 mmol) was heated at 115°C for 2 h. Formamidine acetate (416 mg, 4 mmol) was added in portions every 30 min during 4 h. Heating was prolonged for 30 min after the last addition. After cooling, the volatiles were removed under vacuum and the residue applied to flash chromatography (CH_2Cl_2/MeOH from 10/0 to 9/1) to give **7b** (498 mg, 78%). ^{1}H NMR (360 MHz, DMSO-d_6): δ : 7.99 (s, 1H), 7.43 (s, 1H), 7.12 (s, 1H), 4.10 (t, $J = 6.5$ Hz, 2H), 3.90 (s, 3H), 3.83 (s, 3H), 3.57 (m, 4H), 2.42 (t, $J = 6.8$ Hz, 2H), 2.36 (m, 4H), 1.92 (q, $J = 6.8$ Hz, 2H). HRMS (EI) *m/z*: calculated for $C_{16}H_{21}N_3O_4$, 319.1532; found, 319.1525.

A similar procedure was used to prepare 6-methoxy-7-[(1-methylpiperidin-4-yl)methoxy]-3,4-dihydroquinazolin-4-one (**7c**). ^{1}H NMR (360 MHz, DMSO-d_6): δ : 7.98 (s, 1H), 7.43 (s, 1H), 7.11 (s, 1H), 3.97 (d, $J = 6.1$ Hz, 2H), 3.87 (s, 3H), 2.78 (d, $J = 11.5$ Hz, 2H), 2.15 (s, 3H), 1.86 (t, $J = 10.6$ Hz, 2H), 1.74 (m, 3H), 1.32 (m, 2H). HRMS (EI) *m/z*: calculated for $C_{16}H_{21}N_3O_3$, 303.1583; found, 303.1580.

Synthesis of 4-chloro-6,7-bis-(2-methoxyethoxy)-quinazoline (**8a**). **7a** (0.5 g, 1.7 mmol) in $CHCl_3$ (10 ml) containing one drop of DMF was added oxalylchloride (720 mg, 5.7 mmol) in several portions over 5 min. Once foaming ceased the solution was refluxed 1.5 h. The solvent was removed under vacuum and the residue was dissolved in 1,2-dichloroethane (100 ml), washed with saturated aqueous Na_2CO_3 (2 × 160 ml), dried over Na_2SO_4, and concentrated under reduced pressure. Purification by flash chromatography (hexane/ethyl acetate from 10/0 to 4/6) afforded the solid title product as a white powder (520 mg, 98%). ^{1}H NMR (360 MHz, $CDCl_3$): δ : 8.86 (s, 1H), 7.44 (s, 1H), 7.33 (s, 1H), 4.34 (m, 4H), 3.89 (m, 4H), 3.50 (s, 3H), 3.49 (s, 3H). HRMS (EI) *m/z*: calculated for $C_{14}H_{17}N_2O_4Cl$, 312.0877; found, 312.0874.

A similar procedure was used to prepare 4-chloro-7-methoxy-6-(3-morpholinopropoxy)-quinazoline (**8b**) and 4-chloro-6-methoxy-7-[(1-methylpiperidin-4-yl)methoxy]-quinazoline (**8c**). **8b**: ^{1}H NMR (360 MHz, $CDCl_3$): δ : 8.87 (s, 1H), 7.40 (s, 1H), 7.33 (s, 1H), 4.28 (t, $J = 6.8$ Hz, 2H), 4.05 (s, 3H), 3.74 (m, 4H), 2.60 (t, $J = 6.8$ Hz, 2H), 2.51 (m, 4H), 2.14 (q, $J = 6.8$ Hz, 2H). HRMS (EI) *m/z*: calculated for $C_{16}H_{20}N_3O_3Cl$, 337.1193; found, 337.1192. **8c**: ^{1}H NMR (360 MHz, DMSO-d_6): δ : 8.88 (s, 1H), 7.46 (s, 1H), 7.40 (s, 1H), 4.10 (d, $J = 6.1$ Hz, 2H), 4.01 (s, 3H), 2.88 (d, $J = 11.2$ Hz, 2H), 2.24 (s, 3H), 2.03 (m, 2H), 1.80 (m, 3H), 1.39 (m, 2H). HRMS (EI) *m/z*: calculated for $C_{16}H_{20}N_3O_2Cl$, 321.1244; found, 321.1251.

Synthesis of 4-(3′-chloro-4′-fluoroanilino)-6,7-bis-(2-methoxyethoxy)-quinazoline (**9a**). A mixture of **8a** (90 mg, 0.29 mmol), 3-chloro-4-fluoroaniline (46 mg, 0.32 mmol) in 2-propanol (1.5 ml) containing pyridine (25 µl) was heated at

reflux for 2 h. The volatiles were removed by evaporation. The residue was taken in chloroform/methanol (9/1), washed with an aqueous solution of $NaHCO_3$ (5%), dried over Na_2SO_4, and concentrated under reduced pressure. Purification by flash chromatography (CH_2Cl_2/MeOH from 10/0 to 19/1) gave the solid title compound (65 mg, 53%). ^{1}H NMR (360 MHz, DMSO-d_6): δ : 9.56 (s, 1H), 8.50 (s, 1H), 8.13 (dd, J = 2.7 and 6.8 Hz, 1H), 7.85 (s, 1H), 7.79 (m, 1H), 7.46 (t, J = 9.0 Hz, 1H), 7.24 (s, 1H), 4.28 (m, 4H), 3.75 (m, 4H), 3.37 (s, 3H), 3.35 (s, 3H). HRMS (MALDI-TOF) m/z: $[M + H]^+$ calculated for $C_{20}H_{22}N_3O_4FCl^+$, 422.1277; found, 422.1257.

Synthesis of 4-(3′-chloro-4′-fluoroanilino)-7-methoxy-6-(3-morpholinopropoxy)-quinazoline (**9b**). A mixture of **8b** (80 mg, 0.24 mmol), 3-chloro-4-fluoroaniline (40 mg, 0.28 mmol) and DMF (2.5 ml) was stirred and heated to 145°C for 1 h. The mixture was cooled to ambient temperature and the volatiles were removed. The residue was applied to flash chromatography (CH_2Cl_2/MeOH from 10/0 to 9/1) to give **9b** (75 mg, 70%). ^{1}H NMR (360 MHz, DMSO-d_6): δ : 9.56 (s, 1H), 8.50 (s, 1H), 8.10 (m, 1H), 7.80 (m, 2H), 7.45 (t, J = 9.2 Hz, 1H), 7.21 (s, 1H), 4.19 (t, J = 6.1 Hz, 2H), 3.94 (s, 3H), 3.58 (m, 4H), 2.40 (m, 6H), 2.00 (m, 2H). HRMS (MALDI-TOF) m/z: $[M + H]^+$ calculated for $C_{20}H_{25}N_4O_3FCl^+$, 447.1594; found, 447.1585.

Synthesis of 4-(3′-chloro-4′-fluoroanilino)-6-methoxy-7-[(1-methylpiperidin-4-yl)methoxy]-quinazoline (**9c**). A mixture of **8c** (60 mg, 0.19 mmol) and 3-chloro-4-fluoroaniline (32.5 g, 0.22 mmol) in 2-propanol (3 ml) containing concentrated HCl (17 μl) was heated at reflux for 2 h. The mixture was cooled and the solid was filtered, washed with 2-propanol (2 ml) followed by ether (5 ml) and dried under vacuum to yield **9c** (71 mg, 88%). ^{1}H NMR (360 MHz, DMSO-d_6): δ : 9.80 (s, 1H), 8.49 (s, 1H), 8.19 (dd, J = 2.9 and 6.9 Hz, 1H), 7.94 (s, 1H), 7.87 (m, 1H), 7.43 (t, J = 9.2 Hz, 1H), 7.17 (s, 1H), 4.05–3.95 (m, 5H), 2.78 (d, J = 10.4 Hz, 2H), 2.15 (s, 3H), 1.86 (t, J = 11.2 Hz, 2H), 1.76 (m, 3H), 1.34 (m, 2H). HRMS (EI) m/z: calculated for $C_{22}H_{24}N_4O_2ClF$, 430.1572; found, 430.1577.

Synthesis of 3-chloro-4-trimethylammonium-nitrobenzene trifluoromethanesulfonate (**10**). A solution of 2-chloro-4-nitroaniline (1.40 g, 8.1 mmol) in THF (15 ml) was added under argon to NaH (60% suspension in mineral oil, 0.7 g, 17.5 mmol). The reaction mixture was stirred 5 min at room temperature under argon, followed by the addition of a solution of iodomethane (2.84 g, 20 mmol) in THF (5 ml). After the solution was stirred for 24 h at ambient temperature, the reaction mixture was poured into cold water. The mixture was diluted with ethyl acetate and the organic layer was separated. The aqueous layer was further extracted with ethyl acetate, and the organic layers were combined. The organic layers were washed with water, brine, dried over Na_2SO_4, and

concentrated under reduced pressure. The residue was applied to flash chromatography (Hexanes/$CHCl_3$ from 10/0 to 7/3) to give 3-chloro-4-(dimethylamino)nitrobenzene (1.35 g, 83%). ^{1}H NMR (360 MHz, $CDCl_3$): δ : 8.22 (s, 1H), 8.06 (d, $J = 8.3$ Hz, 1H), 6.97 (d, $J = 8.3$ Hz, 1H), 3.01 (s, 6H). 3-Chloro-4-(dimethylamino)nitrobenzene (0.6 g, 3.0 mmol) was dissolved CH_2Cl_2 (5 ml) and treated with methyl trifluoromethanesulfonate (0.99 g, 6.0 mmol) for 24 h at room temperature under an argon atmosphere. The mixture was diluted with diethyl ether and the solid residue was filtered off. The residue was recrystallized twice from CH_2Cl_2/diethyl ether to give the title compound (0.89 g, 80%) as white needles. ^{1}H NMR (360 MHz, Acetone-d_6): δ : 8.62 (m, 2H), 8.47 (dd, $J = 2.7$ and 9.6 Hz, 1H), 4.20 (s, 9H).

Radiochemistry

Preparation of 3-chloro-4-[^{18}F]fluoroaniline (**12**). A 5 ml V-vial was loaded with 0.5 ml of a CH_3CN/H_2O solution of potassium carbonate (0.5 mg, 3 μmol) and Kryptofix [2.2.2] (K_{222}, 2.25 mg, 6 μmol). Fluorine-18 in water was added and the V-vial was gently heated at 110°C. Water was removed under a stream of argon by azeotropic distillation with acetonitrile (3 × 0.5 ml) to give the no-carrier-added K[^{18}F]F-K_{222} complex as a white semi-solid residue. A solution of 3-chloro-4-trimethylammonium-nitrobenzene triflate (**10**, 2.2 mg, 6 μmol) in anhydrous acetonitrile or dimethyl sulfoxide (0.5 ml) was added into the vial containing dry cryptate (K[^{18}F]F-K_{222}) and stirred under the given conditions described in Table 1. After cooling the mixture was poured into water (8 ml), and the aqueous solution was passed through a C18 Sep-Pak. The Sep-Pak was rinsed with additional water (5 ml) and 3-chloro-4-[^{18}F]fluoro-nitrobenzene (**11**) was eluted through the column with MeOH (1.5 ml) into a tube containing sodium borohydride (7 mg, 0.18 mmol) and palladium on activated carbon (10%, 1 mg). The methanolic solution was stirred at room temperature for 5 min, and the reaction was quenched with concentrated HCl (0.2 ml). The solution was subsequently filtered, and the filter was washed with MeOH (0.5 ml). Evaporation of the volatiles under reduced pressure gave 3-chloro-4-[^{18}F]fluoroaniline (**12**). Typically, 1.4–2.2 GBq (38–60 mCi) of 3-chloro-4-[^{18}F]fluoroaniline could be obtained in 70–80 min starting from 3.4 to 6.1 GBq (92–165 mCi) aliquot of a cyclotron-produced [^{18}F]F^- batch (overall radiochemical yields, based on starting [^{18}F]fluoride: 58–72% decay corrected).

*Radiosynthesis of 4-(3′-chloro-4′-[^{18}F]fluoroanilino)-6,7-bis-(2-methoxyethoxy)-quinazoline ([^{18}F]-***9a***)*. A solution of **8a** (1 mg, 3 μmol) in 2-propanol (2 ml) was added to the dry 3-chloro-4-[^{18}F]fluoroaniline (**12**) and stirred at 85°C for 15 min. The volatiles were removed under pressure, and the residue was reconstituted into a (1/1) mixture of MeOH/phosphate buffer at pH 5.8. [^{18}F]-**9a**

was purified by HPLC (Phenomenex, Luna-C18, 5 μm, 250 mm × 10 mm) with a linear gradient (0–15 min) of 50–100% MeOH in phosphate buffer (2 ml/min) followed by 15 min isochratic elution with 100% MeOH. Retention time of [^{18}F]-**9a** was 22 min vs 15 and 17 min for the starting materials **12** and **8a**, respectively, under the same elution conditions. Typically, 0.4–0.6 GBq (12–16 mCi) of pure [^{18}F]-**9a** could be obtained in 50–55 min starting from 1.4–1.5 GBq (38–41 mCi) of 3-chloro-4-[^{18}F]fluoroaniline (overall radiochemical yields, based on starting 3-chloro-4-[^{18}F]fluoroaniline: 43–55% decay corrected).

*Radiosynthesis of 4-(3′-chloro-4′-[^{18}F]fluoroanilino)-7-methoxy-6-(3-morpholinopropoxy)quinazoline ([^{18}F]-**9b**).* A solution of **8b** (1 mg, 3 μmol) in 2-propanol (2 ml) was added to the dry 3-chloro-4-[^{18}F]fluoroaniline (**12**) and stirred at 120°C for 15 min. The volatiles were removed under pressure, and the residue was reconstituted into a (1/1) mixture of MeOH/phosphate buffer at pH 5.8. [^{18}F]-**9b** was purified by HPLC (Phenomenex, Luna-C18, 5 μm, 250 mm × 10 mm) with a linear gradient (0–15 min) of 50–100% MeOH in phosphate buffer (2 ml/min) followed by 15 min isochratic elution with 100% MeOH. Retention time of [^{18}F]-**9b** was 21 min vs 17 min for the starting material **8b** under the same elution conditions. Typically, 0.7–1.1 GBq (19–30 mCi) of pure [^{18}F]-**9b** could be obtained in 50–55 min starting from 1.4–2.2 GBq (38–60 mCi) of 3-chloro-4-[^{18}F]fluoroaniline (overall radiochemical yields, based on starting 3-chloro-4-[^{18}F]fluoroaniline: 60–70% decay corrected).

*Radiosynthesis of 4-(3′-chloro-4′-[^{18}F]fluoroanilino)-6-methoxy-7-[(1-methylpiperidin-4-yl)methoxy]-quinazoline ([^{18}F]-**9c**).* A solution of **8c** (1 mg, 3 μmol) in 2-propanol (2 ml) was added to the dry 3-chloro-4-[^{18}F]fluoroaniline (**12**) and stirred at 120°C for 15 min. The volatiles were removed under pressure, and the residue was reconstituted into a (1/1) mixture of MeOH/phosphate buffer at pH 4.7. [^{18}F]-**9c** was purified by HPLC (Phenomenex, Luna-C18, 5 μm, 250 mm × 10 mm) with a linear gradient (0–15 min) of 50–100% MeOH in phosphate buffer (2 ml/min) followed by 15 min isochratic elution with 100% MeOH. Retention time of [^{18}F]-**9c** was 18 min vs 15 and 11 min for the starting materials **12** and **8c**, respectively, under the same elution conditions. Typically, 0.6 GBq (15–17 mCi) of pure [^{18}F]-**9c** could be obtained in 50–55 min starting from 1.4 to 1.7 GBq (38–45 mCi) of 3-chloro-4-[^{18}F]fluoroaniline (overall radiochemical yields, based on starting 3-chloro-4-[^{18}F]fluoroaniline: 46–56% decay corrected).

*Formulation of the ^{18}F labeled TKI ([^{18}F]-**9a**−**c**) and quality control.* HPLC fractions (4–5 ml) containing the ^{18}F labeled product were collected and solvents were evaporated under reduced pressure. The tracer was dissolved in ethanol, and the solution was then diluted to the proper dosage with sterile

physiological saline, so that the injection dose contained no more than 10% of alcohol.

As demonstrated by HPLC analysis (Phenomenex Luna C-18 column, 10×250 mm, 5 μm), radiolabeled anilinoquinazoline derivatives ([^{18}F]-**9a–c**) co-eluted with authentic synthesized unlabeled reference **9a–c**. The radiopharmaceuticals were found to be >95% chemically and radiochemically pure.

Conclusion

In conclusion, this report describes a synthetic strategy for the production of a wide variety of radiolabeled 6,7-disubstituted 4-anilinoquinazolines suitable for imaging tyrosine kinase receptors to predict therapy effectiveness. Three new F-18 labeled radiopharmaceuticals based on the therapeutic agents Tarceva, Iressa, and ZD6474 were synthesized. Conditions for radiosynthesis are suitable for preparation of the compounds in quantities and times which are practical for use as PET radiopharmaceuticals. Cell uptake studies and preliminary microPET imaging in disease-free and tumor bearing animals have been performed, and further evaluation are underway to demonstrate the feasibility of using those F-18 labeled tyrosine kinase inhibitors as potential imaging agents to predict efficacy of those antitumor therapeutics *in vivo*.

Acknowledgements

This work was supported in part by California Breast Cancer Research Program Grant 7KB-0155, and DOE DE-FC03-87ER60615. We thank the cyclotron staff at UCLA and the UCLA Pasarow Mass Spectrometry Laboratory for their collaboration.

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