Bioorganic & Medicinal Chemistry Letters 21 (2011) 3222–3226

Contents lists available at ScienceDirect

Bioorganic & Medicinal Chemistry Letters

journal homepage: www.elsevier.com/locate/bmcl

Radiosynthesis of [^{11}C]Vandetanib and [^{11}C]chloro-Vandetanib as new potential PET agents for imaging of VEGFR in cancer

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ARTICLE INFO

Article history:
Received 8 March 2011
Revised 8 April 2011
Accepted 12 April 2011
Available online 20 April 2011

Keywords:
[^{11}C]Vandetanib
[^{11}C]Chloro-Vandetanib
Vascular endothelial growth factor receptors (VEGFR)
Radiosynthesis
Positron emission tomography (PET)
Cancer

ABSTRACT

Vandetanib (ZD6474) and its chlorine analogue chloro-Vandetanib are potent and selective vascular endothelial growth factor receptor (VEGFR) tyrosine kinase inhibitors with low nanomolar IC_{50} values. [^{11}C]Vandetanib and [^{11}C]chloro-Vandetanib, new potential PET agents for imaging of VEGFR in cancer, were first designed, synthesized and labeled at nitrogen and oxygen positions from their corresponding N- and O-des-methylated precursors, in 40–50% decay corrected radiochemical yield and 370–555 GBq/μmol specific activity at end of bombardment (EOB).

Vascular endothelial growth factors (VEGF) are the most common cancer causing angiogenic factors, and their receptors (VEGFR) are overexpressed in tumor-associated endothelial cells.[1] Angiogenesis contributes in particular to tumor growth.[2] Vandetanib [*N*-(4-bromo-2-fluorophenyl)-6-methoxy-7-((1-methylpiperidin-4-yl)methoxy)quinazolin-4-amine] (ZD6474), is an orally bioavailable antitumor drug developed by AstraZeneca. It acts as an antagonist for both VEGFR and epidermal growth factor receptor (EGFR), and also inhibits tyrosine kinase (tk) activity.[3] Vandetanib selectively inhibits the tyrosine kinase activity of VEGFR-2 and VEGFR-3, thereby blocking VEGF-stimulated endothelial cell proliferation and migration, thus reducing tumor vessel permeability. This agent also blocks the activity of EGFR, a receptor tyrosine kinase that mediates tumor cell proliferation and migration and angiogenesis.[4] IC_{50} values of VEGFR-2, VEGFR-3, and EGFR were determined to be 40, 110, and 500 nM, respectively.[5] A chlorine analogue of Vandetanib [chloro-Vandetanib, *N*-(4-chloro-2-fluorophenyl)-6-methoxy-7-((1-methylpiperidin-4-yl)methoxy)quinazolin-4-amine] displays similar or superior biological activities over Vandetanib.[3] Carbon-11-labeled Vandetanib and its chlorine analogue may serve as new probes for monitoring and imaging VEGFR in cancer by biomedical imaging technique positron emission tomography (PET).[6] In our previous work, we developed a PET agent [^{11}C]Gefitinib ([^{11}C]Iressa) for imaging EGFR-tk, as indicated in Figure 1.[7] The goal of this study is to radiolabel therapeutic agents as diagnostic probes to image VEGFR and monitoring its therapeutic efficacy as VEGFR-tk inhibitors, we have first radiosynthesized [^{11}C]Vandetanib [*N*-(4-bromo-2-fluorophenyl)-6-methoxy-7-((1-[^{11}C]methylpiperidin-4-yl)methoxy)quinazolin-4-amine, or *N*-(4-bromo-2-fluorophenyl)-6-[^{11}C]methoxy-7-((1-methylpiperidin-4-yl)methoxy)quinazolin-4-amine] and [^{11}C]chloro-Vandetanib [*N*-(4-chloro-2-fluorophenyl)-6-methoxy-7-((1-[^{11}C]methylpiperidin-4-yl)methoxy)quinazolin-4-amine, or *N*-(4-chloro-2-fluorophenyl)-6-[^{11}C]methoxy-7-((1-methylpiperidin-4-yl)methoxy)quinazolin-4-amine] as new potential PET agents.

As illustrated in Scheme 1, Vandetanib (**6b**) and chloro-Vandetanib (**6a**) as well as their corresponding N-des-methylated precursors **5b** and **5a** were synthesized following the published synthetic protocols.[3,8,9] 7-Benzyloxy-4-chloro-6-methoxyquinazoline (**1**) was reacted with anilines under acid catalysis in a protic solvent *i*-propanol to give corresponding C-7-benzyloxyanilinoquinazolines **2a**, **2b** in 94% and 93% yield, respectively. Deprotection of the C-7-benzyl moiety was subsequently achieved using trifluoroacetyl acid (TFA) and led to the key intermediate 7-hydroxy-4-anilinoquinazolines **3a**, **3b** in 91% and 93% yield, respectively. Coupling reaction of **3a**, **3b** with *tert*-butyl 4-({[(4-methylphenyl)sulfonyl]oxy}methyl)piperidine-1-carbonate under alkylation conditions (K_2CO_3/DMF) provided compounds **4a**, **4b** in 70% and 68% yield, respectively. The *tert*-BOC protecting group of **4a**,

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doi:10.1016/j.bmcl.2011.04.049

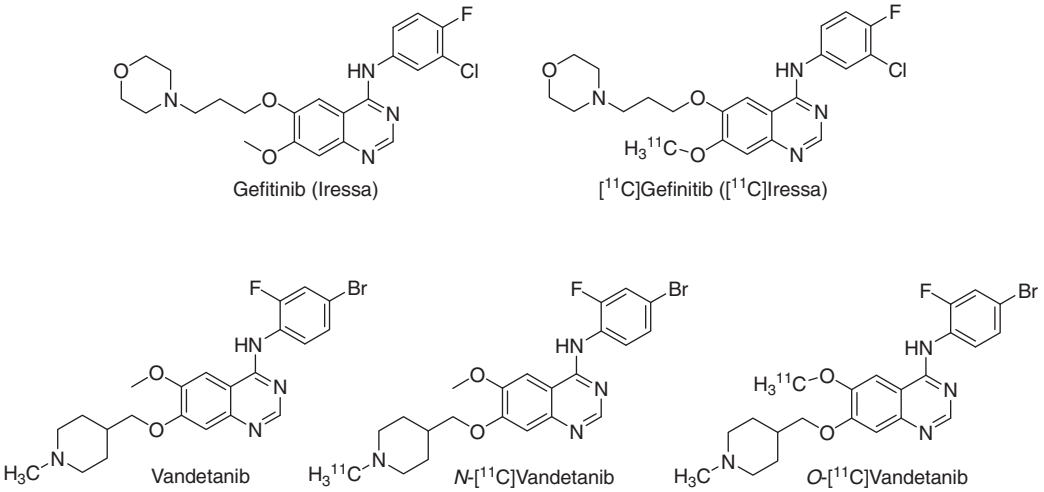
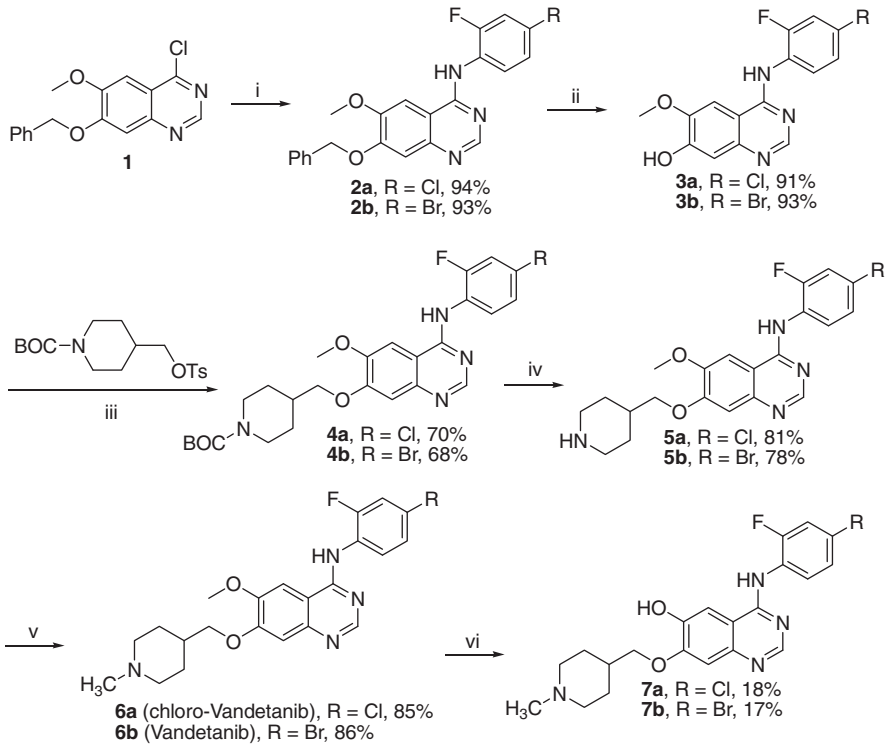


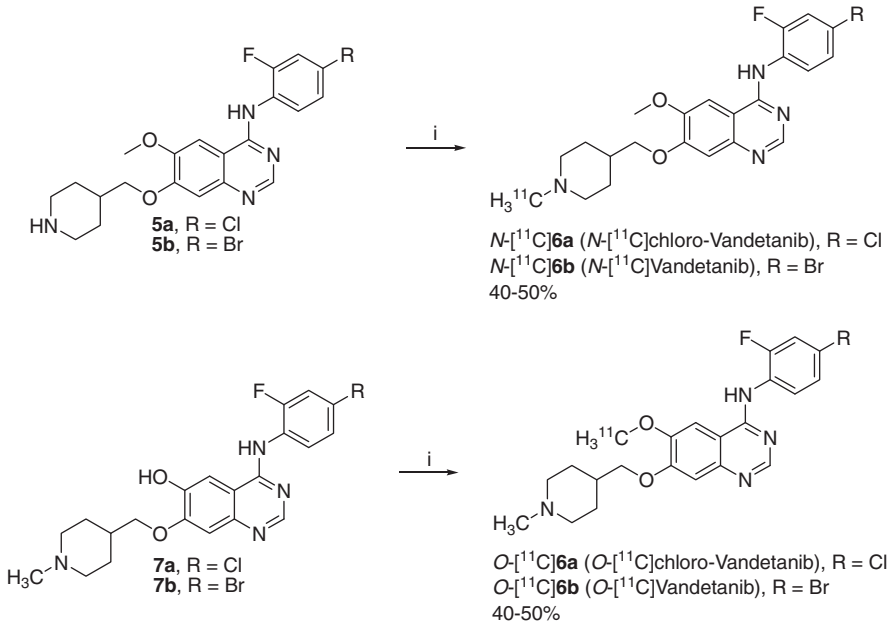
Figure 1. Chemical structure of [^{11}C]Gefitinib and [^{11}C]Vandetanib.



Scheme 1. Synthesis of Vandetanib, chloro-Vandetanib and their corresponding N- and O-des-methylated precursors. Reagents and conditions: (i) R-Ar-NH_2/*i*-PrOH/HCl/reflux; (ii) TFA/reflux; (iii) K_2CO_3/DMF/100 °C; (iv) TFA/CH_2Cl_2/rt; (v) HCHO/CH_3COOH/$NaBH(OAc)_3$/CH_2Cl_2/MeOH/rt; (vi) Py·HCl, 190 °C.

4b was subsequently removed by treating TFA to release the basic piperidine nitrogen yielding N-des-methylated precursors **5a**, **5b** in 81% and 78% yield, respectively. N-Methylation of the piperidine nitrogen of **5a**, **5b** using formaldehyde under reducing condition with sodium triacetoxyborohydride ($NaBH(OAc)_3$) gave the target compounds chloro-Vandetanib and Vandetanib **6a**, **6b** in 85% and 86% yield, respectively. O-Desmethylation of **6a**, **6b** to produce O-des-methylated precursors **7a**, **7b** proved to be difficult. A variety of protocols were screened for this purpose including Lewis acids (LiCl, LiBr), base (EtSNa), protic acid (HBr) and organic salt (pyridine hydrochloride).[10] Pyridine hydrochloride was identified as a suitable O-desmethylation agent to produce **7a**, **7b** in 18% and 17% yield, respectively.

Radiosynthesis of the target radiotracer [^{11}C]Vandetanib ([^{11}C]**6b**) and [^{11}C]chloro-Vandetanib ([^{11}C]**6a**) is indicated in Scheme 2. N-des-Methylated precursor **5a** or **5b** was labeled by [^{11}C]methyl triflate ([^{11}C]CH_3OTf) prepared from [^{11}C]CO_2,[11,12] in the presence of 2 N NaOH in acetonitrile through the N-[^{11}C]methylation[13,14] to provide N-[^{11}C]-methylated product *N*-[^{11}C]**6a** or *N*-[^{11}C]**6b**. Likewise, O-des-methylated precursor **7a** or **7b** was labeled by [^{11}C]CH_3OTf in the presence of 2 N NaOH in acetonitrile through the O-[^{11}C]methylation[15,16] to provide O-[^{11}C]-methylated product *O*-[^{11}C]**6a** or *O*-[^{11}C]**6b**. The target tracer was purified by semi-preparative high performance liquid chromatography (HPLC). The synthesis was performed in an automated multi-purpose [^{11}C]-radiosynthesis module, allowing measurement of specific



Scheme 2. Radiosynthesis of [^{11}C]Vandetanib and [^{11}C]chloro-Vandetanib. Reagents and conditions: (i) [^{11}C]CH_3OTf, CH_3CN, 2 N NaOH, 80 °C.

activity during synthesis.[17,18] The radiochemical yield for the target tracer was 40–50%, decay corrected to end of bombardment (EOB), based on [^{11}C]CO_2. The specific activity of [^{11}C]**6a**, [^{11}C]**6b** was in a range of 370–555 GBq/μmol at EOB measured by the on-the-fly technique using semi-preparative HPLC during synthesis[18] and 185–278 GBq/μmol at the end of synthesis (EOS) determined by analytical HPLC.[19] Chemical purity and radiochemical purity were determined by analytical HPLC.[19] The chemical purity of the precursors **5a**, **5b**, **7a**, **7b** and reference standard **6a**, **6b** was >95%. The radiochemical purity of the target tracer [^{11}C]**6a**, [^{11}C]**6b** was >99% determined by radio-HPLC through γ-ray (PIN diode) flow detector, and the chemical purity of the target tracers [^{11}C]**6a**, [^{11}C]**6b** was >93% determined by reversed-phase HPLC through UV flow detector.

The experimental details and characterization data for compounds **2a**,**b**–**7a**,**b** and for the tracers [^{11}C]**6a**,**b** are given.[20]

In summary, [^{11}C]Vandetanib and [^{11}C]chloro-Vandetanib were first designed and synthesized as new potential PET agents for imaging of VEGFR in cancer. An automated self-designed multipurpose [^{11}C]-radiosynthesis module for the synthesis of [^{11}C]Vandetanib and [^{11}C]chloro-Vandetanib has been built, featuring the measurement of specific activity by the on-the-fly technique. The radiosynthesis employed either N-[^{11}C]methylation or O-[^{11}C]methylation radiolabeling on nitrogen or oxygen position of the precursor. Radiolabeling procedures incorporated efficiently with the most commonly used [^{11}C]methylating agent, [^{11}C]CH_3OTf, produced by gas-phase production of [^{11}C]methyl bromide ([^{11}C]CH_3Br) from our laboratory. The target tracers were isolated and purified by a semi-preparative HPLC procedure in high radiochemical yields, short overall synthesis time, and high specific activity. These results facilitate the potential preclinical and clinical PET studies of [^{11}C]Vandetanib and [^{11}C]chloro-Vandetanib in animals and humans.

Acknowledgments

This work was partially supported by the Breast Cancer Research Foundation and Indiana Genomics Initiative (INGEN) of Indiana University, which is supported in part by Lilly Endowment Inc. The authors would like to thank Dr. Bruce H. Mock and Barbara E. Glick-Wilson for their assistance in production of [^{11}C]CH_3OTf. ^{1}H NMR spectra were recorded on a Bruker Avance II 500 MHz NMR spectrometer in the Department of Chemistry and Chemical Biology at Indiana University Purdue University Indianapolis (IUPUI), which is supported by a NSF-MRI grant CHE-0619254.

References and notes

1. Wheeler, W. J.; Clodfelter, D. K. *J. Labelled Compd. Radiopharm.* **2008**, *51*, 175.
2. Dempke, W. C.; Zippel, R. *Anticancer Res.* **2010**, *30*, 4477.
3. Hennequin, L. F.; Stokes, E. S.; Thomas, A. P.; Johnstone, C.; Plé, P. A.; Ogilvie, D. J.; Dukes, M.; Wedge, S. R.; Kendrew, J.; Curwen, J. O. *J. Med. Chem.* **2002**, *45*, 1300.
4. NCI Drug Dictionary, **2011**. <http://cme.nci.nih.gov/drugdictionary/?CdrID=269177>.
5. Morabito, A.; Piccirillo, M. C.; Falasconi, F.; De Feo, G.; Del Giudice, A.; Bryce, J.; Di Maio, M.; De Maio, E.; Normanno, N.; Perrone, F. *Oncologist* **2009**, *14*, 378.
6. Samén, E.; Thorell, J. O.; Lu, L.; Tegnebratt, T.; Holmgren, L.; Stone-Elander, S. *Eur. J. Nucl. Med. Mol. Imaging* **2009**, *36*, 1283.
7. Wang, J.-Q.; Gao, M.; Miller, K. D.; Sledge, G. W.; Zheng, Q.-H. *Bioorg. Med. Chem. Lett.* **2006**, *16*, 4102.
8. Hennequin, L. F.; Thomas, A. P.; Johnstone, C.; Stokes, E. S.; Plé, P. A.; Lohmann, J. J.; Ogilvie, D. J.; Dukes, M.; Wedge, S. R.; Curwen, J. O.; Kendrew, J.; Lambert-van der Brempt, C. *J. Med. Chem.* **1999**, *42*, 5369.
9. Gangjee, A.; Zaware, N.; Raghavan, S.; Ihnat, M.; Shenoy, S.; Kisliuk, R. L. *J. Med. Chem.* **2010**, *53*, 1563.
10. Wang, M.; Gao, M.; Miller, K. D.; Sledge, G. W.; Hutchins, G. D.; Zheng, Q.-H. *J. Labelled Compd. Radiopharm.* **2008**, *51*, 6.
11. Jewett, D. M. *Int. J. Radiat. Appl. Instrum. A* **1992**, *43*, 1383.
12. Mock, B. H.; Mulholland, G. K.; Vavrek, M. J. *Nucl. Med. Biol.* **1999**, *26*, 467.
13. Zheng, Q.-H.; Mulholland, G. K. *Nucl. Med. Biol.* **1996**, *23*, 981.
14. Gao, M.; Mock, B. H.; Hutchins, G. D.; Zheng, Q.-H. *Nucl. Med. Biol.* **2005**, *32*, 543.
15. Gao, M.; Wang, M.; Hutchins, G. D.; Zheng, Q.-H. *Appl. Radiat. Isot.* **2008**, *66*, 1891.
16. Yoder, K. K.; Hutchins, G. D.; Mock, B. H.; Fei, X.; Winkle, W. L.; Gitter, B. D.; Territo, P. R.; Zheng, Q.-H. *Nucl. Med. Biol.* **2009**, *36*, 11.
17. Mock, B. H.; Zheng, Q.-H.; DeGrado, T. R. *J. Labelled Compd. Radiopharm.* **2005**, *48*, S225.
18. Mock, B. H.; Glick-Wilson, B. E.; Zheng, Q.-H.; DeGrado, T. R. *J. Labelled Compd. Radiopharm.* **2005**, *48*, S224.
19. Zheng, Q.-H.; Mock, B. H. *Biomed. Chromatogr.* **2005**, *19*, 671.
20. (a) *General:* All commercial reagents and solvents were purchased from Sigma-Aldrich and Fisher Scientific, and they were used without further purification. [^{11}C]CH_3OTf was prepared according to a literature procedure.[12] Melting points were determined on a MEL-TEMP II capillary tube apparatus and were uncorrected. ^{1}H NMR spectra were recorded on Bruker Avance II 500 MHz NMR spectrometers using tetramethylsilane (TMS) as an internal standard. Chemical shift data for the proton resonances were reported in parts per million (ppm, δ scale) relative to internal standard TMS (δ 0.0), and coupling constants (J) were reported in hertz (Hz). Liquid chromatography-mass spectra (LC–MS) analysis was performed on an Agilent system, consisting of an 1100

series HPLC connected to a diode array detector and a 1946D mass spectrometer configured for positive-ion/negative-ion electrospray ionization. The high resolution mass spectra (HRMS) were obtained using a Waters/Micromass LCT Classic spectrometer. Chromatographic solvent proportions are indicated as volume: volume ratio. Thin-layer chromatography (TLC) was run using Analtech silica gel GF uniplates (5×10 cm^2). Plates were visualized under UV light. Preparative TLC was run using Analtech silica gel UV 254 plates (20×20 cm^2). Normal phase flash column chromatography was carried out on EM Science silica gel 60 (230–400 mesh) with a forced flow of the indicated solvent system in the proportions described below. All moisture- and air-sensitive reactions were performed under a positive pressure of nitrogen maintained by a direct line from a nitrogen source. Analytical HPLC was performed using a Prodigy (Phenomenex) 5 μm C-18 column, 4.6×250 mm; mobile phase 3:1:1 CH_3CN/MeOH/20 mm, pH 6.7 phosphate (buffer solution); flow rate 1.5 mL/min; and UV (254 nM) and γ-ray (PIN diode) flow detectors. Semi-preparative HPLC was performed using a YMC-Pack ODS-A, S-5 μm, 12 nM, 10×250 mm C-18 column; 3:1:1 CH_3CN/MeOH/20 mm, pH 6.7 phosphate (buffer solution) mobile phase; 5.0 mL/min flow rate; UV (254 nM) and γ-ray (PIN diode) flow detectors. Sterile Millex-GS 0.22 μm vented filter unit was obtained from Millipore Corporation, Bedford, MA.

(b) 7-(Benzyloxy)-*N*-(4-chloro-2-fluorophenyl)-6-methoxyquinazolin-4-amine hydrochloride (**2a**). Hydrogen chloride (6.5 M, 2.54 mL) was added to a mixture of compound **1** (4.51 g, 15.0 mmol) and 4-chloro-2-fluoroaniline (2.40 g, 16.5 mmol) in 2-propanol (160 mL), then the mixture was heated at reflux for 2 h. The mixture was cooled and solid was filtered. The solid was then washed with 2-propanol, followed by Et_2O, and dried under vacuum overnight to give **2a** (7.9 g, 94%) as a white solid: mp 243–245 °C; ^{1}H NMR (DMSO-d_6) δ 4.00 (s, 3H, CH_3O), 5.35 (s, 2H, CH_2O), 7.39–7.55 (m, 7H, Ar–H), 7.60 (t, J = 8.5 Hz, Ar–H), 7.67 (dd, J = 2.0, 10.0 Hz, 1H, Ar–H), 8.37 (s, 1H, H5), 8.80 (s, 1H, H2), 11.71 (s, 1H); MS (ESI, m/z): 410 ([M+H]$^+$, 100%).

(c) 7-(Benzyloxy)-*N*-(4-bromo-2-fluorophenyl)-6-methoxyquinazolin-4-amine hydrochloride (**2b**). A similar procedure for **2a** was used to prepare **2b** (93%) as a white solid: mp 244–246 °C. ^{1}H NMR (DMSO-d_6) δ 4.01 (s, 3H, CH_3O), 5.35 (s, 2H, CH_2O), 7.43–7.58 (m, 8H, Ar–H), 7.78 (dd, J = 2.0, 10.0 Hz, 1H, Ar–H), 8.35 (s, 1H, H5), 8.80 (s, 1H, H2), 11.66 (s, 1H); MS (ESI, m/z): 456 ([M+H]$^+$, 100%).

(d) 4-((4-Chloro-2-fluorophenyl)amino)-6-methoxyquinazolin-7-ol (**3a**). A solution of **2a** (4.46 g, 10.0 mmol) in TFA (30 mL) was refluxed for 1 h. After the reaction mixture was evaporated, the mixture was added cold aqueous $NaHCO_3$ and concentrated $NH_3.H_2O$, and pH of solution was then adjusted to 10. The resulted precipitate was filtered, washed with water and Et_2O, and dried under vacuum to give **3a** (2.91 g, 91%) as a white solid: R_f = 0.20 (1:1 EtOAc/hexanes); mp 267–269 °C; ^{1}H NMR (DMSO-d_6) δ 3.93 (s, 3H, CH_3O), 7.05 (s, 1H, H8), 7.32 (dd, J = 2.0, 8.5 Hz, 1H, H6′), 7.52 (dd, J = 2.0, 10.0 Hz, 1H, H5′), 7.58 (t, J = 8.5 Hz, 1H, H3′), 7.74 (s, 1H, H5), 8.26 (s, 1H, H2), 9.30 (s, 1H, OH); MS (ESI, m/z): 320 ([M+H]$^+$, 100%).

(e) 4-((4-Bromo-2-fluorophenyl)amino)-6-methoxyquinazolin-7-ol (**3b**). A similar procedure for **3a** was used to prepare **3b** (93%) as a white solid: R_f = 0.20 (1:1 EtOAc/hexanes); mp 268–270 °C; ^{1}H NMR (DMSO-d_6) δ 3.93 (s, 3H, CH_3O), 7.02 (s, 1H, H8), 7.44 (dd, J = 2.0, 8.5 Hz, 1H, H6′), 7.52 (t, J = 8.5 Hz, 1H, H3′),7.63 (dd, J = 2.0, 10.0 Hz, 1H, H5′), 7.75 (s, 1H, H5), 8.26 (s, 1H, H2), 9.30 (s, 1H, OH); MS (ESI, m/z): 366 ([M+H]$^+$, 100%).

(f) *tert*-Butyl 4-(((4-((4-chloro-2-fluorophenyl)amino)-6-methoxyquinazolin-7-yl)oxy)methyl)piperidine-1-carboxylate (**4a**). K_2CO_3 (1.38 g, 10.0 mmol) was added to a suspension of compound **3a** (1.60 g, 5.0 mmol) and *tert*-butyl 4-({[(4-methylphenyl)sulfonyl]oxy}methyl)piperidine-1-carbonate (2.07 g, 5.6 mmol) in DMF (45 mL), and stirred at room temperature (rt) for 1 h and heated at 90 °C for 3 h. After the mixture was cooled, it was poured into cold water, and extracted with EtOAc (150 mL × 3). The organic layers were washed brine, dried over Na_2SO_4, filtered, and evaporated to give a residue, which was purified by column chromatography with eluent (2% MeOH/CH_2Cl_2) on silica gel to afford **4a** (1.80 g, 70%) as a white solid: R_f = 0.50 (5% MeOH/CH_2Cl_2); mp 222–224 °C; ^{1}H NMR (DMSO-d_6) δ 1.20–1.22 (m, 2H, piperidine-H), 1.40 (s, 9H, CH_3) 1.77 (d, J = 11.0 Hz, 2H, piperidine-H), 1.98–2.07 (m, 1H, piperidine-H), 2.70–2.85 (m, 2H, piperidine-H), 3.94 (s, 3H, CH_3O), 3.98 (br s, 2H, piperidine-H), 4.02 (d, J = 6.5 Hz, 2H, OCH_2), 7.18 (s, 1H, H8), 7.33 (ddd, J = 1.0, 2.0, 8.5 Hz, 1H, H6′), 7.54 (dd, J = 2.0, 10.0 Hz, 1H, H5′), 7.58 (t, J = 8.5 Hz, 1H, H3′), 7.79 (s, 1H, H5), 8.35 (s, 1H, H2), 9.54 (s, 1H, NH); MS (ESI, m/z): 517 ([M+H]$^+$, 100%).

(g) *tert*-Butyl 4-(((4-((4-bromo-2-fluorophenyl)amino)-6-methoxyquinazolin-7-yl)oxy)methyl)piperidine-1-carboxylate (**4b**). A similar procedure for **4a** was used to prepare **4b** (68%) as a white solid: R_f = 0.50 (5% MeOH/CH_2Cl_2); mp 223–225 °C. ^{1}H NMR (DMSO-d_6) δ 1.20–1.30 (m, 2H, piperidine-H), 1.40 (s, 9H, CH_3) 1.77 (d, J = 11.0 Hz, 2H, piperidine-H), 1.98–2.07 (m, 1H, piperidine-H), 2.70–2.85 (m, 2H, piperidine-H), 3.94 (s, 3H, CH_3O), 3.98–4.01 (m, 2H, piperidine-H), 4.02 (d, J = 6.5 Hz, 2H, OCH_2), 7.18 (s, 1H, H8), 7.45 (dd, J = 2.0, 8.5 Hz, 1H, H6′), 7.53 (t, J = 8.5 Hz, 1H, H3′), 7.64 (dd, J = 2.0, 10.0 Hz, 1H, H5′), 7.79 (s, 1H, H5), 8.35 (s, 1H, H2), 9.53 (s, 1H, NH); MS (ESI, m/z): 563 ([M+H]$^+$, 100%).

(h) *N*-(4-Chloro-2-fluorophenyl)-6-methoxy-7-(piperidin-4-ylmethoxy)quinazolin-4-amine (**5a**). TFA (5 mL) was added to a suspension of compound **4a** (1.03 g, 2.0 mmol) in CH_2Cl_2 (20 mL), and stirred at rt for 2 h, and the volatiles were removed under vacuum. The reaction mixture was quenched with water and extracted with Et_2O. The organic layer was separated, and the aqueous layer was adjusted to pH 10 with 3 N NaOH, and then extracted with CH_2Cl_2. The combined organic layers were dried over $MgSO_4$, and the solvent was removed under vacuum. The crude product was purified by column chromatography with eluent (20–50% MeOH/CH_2Cl_2) on silica gel to afford **5a** (0.67 g, 81%) as a white solid: R_f = 0.15 (50:50:1 MeOH/CH_2Cl_2/$NH_3.H_2O$); mp 220–222 °C.; ^{1}H NMR ($CDCl_3$) δ 1.30 (ddd, J = 4.0, 12.5, 25.0 Hz, 2H, piperidine-H), 1.87 (d, J = 12.5 Hz, 2H, piperidine-H), 2.09–2.12 (m, 1H, piperidine-H), 2.65 (dt, J = 2.5, 12.0 Hz, 2H, piperidine-H), 3.13 (d, J = 12.0 Hz, 2H, piperidine-H), 4.00 (d, J = 6.5 Hz, 2H, CH_2O), 4.02 (s, 3H, CH_3O), 7.01 (s, 1H, Ar–H), 7.20 (s, 1H, Ar–H), 7.22 (s, 1H, Ar–H), 7.24 (s, 1H, Ar–H), 7.28 (s, 1H, Ar–H), 8.52 (t, J = 8.5 Hz, 1H, Ar–H), 8.67 (s, 1H, Ar-NH); MS (ESI, m/z): 417 ([M+H]$^+$, 100%).

(i) *N*-(4-Bromo-2-fluorophenyl)-6-methoxy-7-(piperidin-4-ylmethoxy)quinazolin-4-amine (**5b**). A similar procedure for **5a** was used to prepare **5b** (78%) as a white solid: R_f = 0.15 (50:50:1 MeOH/CH_2Cl_2/$NH_3.H_2O$); mp 221–223 °C; ^{1}H NMR ($CDCl_3$) δ 1.30 (ddd, J = 4.0, 12.0, 25.0 Hz, 2H, piperidine-H), 1.86 (d, J = 12.5 Hz, 2H, piperidine-H), 2.08–2.11 (m, 1H, piperidine-H), 2.66 (dt, J = 2.5, 12.0 Hz, 2H, piperidine-H), 3.13 (d, J = 12.0 Hz, 2H, piperidine-H), 3.98 (d, J = 6.5 Hz, 2H, CH_2O), 4.00 (s, 3H, CH_3O), 7.01 (s, 1H, Ar–H), 7.23 (s, 1H, Ar–H), 7.33 (s, 1H, Ar–H), 7.35 (s, 1H, Ar–H), 7.36 (s, 1H, Ar–H), 8.46 (t, J = 8.5 Hz, 1H, Ar–H), 8.67 (s, 1H, Ar-NH); MS (ESI, m/z): 463 ([M+H]$^+$, 100%).

(j) *N*-(4-Chloro-2-fluorophenyl)-6-methoxy-7-((1-methylpiperidin-4-yl) methoxy)quinazolin-4-amine (**6a**, chloro-Vandetanib). 37% aqueous solution of formaldehyde (40 mg, 0.52 mmol) followed by $NaBH(OAc)_3$ (120 mg, 0.56 mmol) were added in portions to the solution of **5a** (167 mg, 0.4 mmol) and acetic acid (28 mg, 0.48 mmol) in CH_2Cl_2 (10 mL) and methanol (20 mL). After the reaction mixture was stirred at rt for 2 h, and the solvents were removed under vacuum. The resulting residue was added aqueous $NaHCO_3$, the precipitate was filtered, washed with water and brine, and dried to obtain white solid; the aqueous layer was extracted with CH_2Cl_2, dried over $MgSO_4$, filtered and evaporated to provide a residue, which was washed with Et_2O to obtain white solid. The combined white solid gave **6a** (146 mg, 85%): R_f = 0.36 (50:50:1 MeOH/CH_2Cl_2/$NH_3.H_2O$); mp 226–228 °C; ^{1}H NMR ($CDCl_3$) δ 1.45 (ddd, J = 4.0, 12.5, 25.0 Hz, 2H, piperidine-H), 1.88 (d, J = 12.5 Hz, 2H, piperidine-H), 1.94–1.96 (m, 3H, piperidine-H), 2.29 (s, 3H, NCH_3), 2.90 (d, J = 12.0 Hz, 2H, piperidine-H), 4.02 (s, 3H, CH_3O), 4.03 (d, J = 5.5 Hz, 2H, CH_2O), 7.00 (s, 1H, Ar–H), 7.20 (s, 1H, Ar–H), 7.22 (s, 1H, Ar–H), 7.23 (s, 1H, Ar–H), 7.24 (s, 1H, Ar–H), 8.53 (t, J = 8.5 Hz, 1H, Ar–H), 8.68 (s, 1H, Ar-NH); MS (ESI, m/z): 431 ([M+H]$^+$, 100%).

(k) *N*-(4-Bromo-2-fluorophenyl)-6-methoxy-7-((1-methylpiperidin-4-yl) methoxy)quinazolin-4-amine (**6b**, Vandetanib). A similar procedure for **6a** was used to prepare **6b** (86%) as a white solid: R_f = 0.36 (50:50:1 MeOH/CH_2Cl_2/$NH_3.H_2O$); mp 227–229 °C; ^{1}H NMR ($CDCl_3$) δ 1.45 (ddd, J = 4.0, 12.5, 25.0 Hz, 2H, piperidine-H), 1.88 (d, J = 12.5 Hz, 2H, piperidine-H), 1.94–2.00 (m, 3H, piperidine-H), 2.29 (s, 3H, NCH_3), 2.90 (d, J = 12.0 Hz, 2H, piperidine-H), 4.02 (s, 3H, CH_3O), 4.03 (d, J = 5.5 Hz, 2H, CH_2O), 6.99 (s, 1H, Ar–H), 7.24 (s, 1H, Ar–H), 7.25 (s, 1H, Ar–H), 7.34 (d, J = 1.0 Hz, 1H, Ar–H), 7.36 (d, J = 1.0 Hz, 1H, Ar–H), 8.51 (t, J = 8.5 Hz, 1H, Ar–H), 8.68 (s, 1H, Ar-NH); MS (ESI, m/z): 475 ([M+H]$^+$, 100%).

(l) 4-((4-Chloro-2-fluorophenyl)amino)-7-((1-methylpiperidin-4-yl) methoxy) quinazolin-6-ol (**7a**). A mixture of compound **6a** (151 mg, 0.35 mmol) and pyridine hydrochloride (3.2 g, 30 mmol) was heated at 190–200 °C for 80 min and then cooled to rt. Aqueous $NaHCO_3$ was added to reaction mixture to adjust pH of solution to 9, and the solution was extracted with EtOAc (80 mL × 3). The organic layers were washed with water and brine, dried over Na_2SO_4, and evaporated. The residue was purified by column chromatography with eluent (20–50% MeOH/CH_2Cl_2) on silica gel to give **7a** (26 mg, 18%) as a white solid: R_f = 0.30 (50:50:1 MeOH/CH_2Cl_2/$NH_3.H_2O$); mp 219–221 °C; ^{1}H NMR (MeOH-d_4) δ 1.49–1.53 (m, 2H, piperidine-H), 1.96–1.99 (m, 3H, piperidine-H), 2.12 (t, J = 11.5 Hz, 2H, piperidine-H), 2.31 (s, 3H, NCH_3), 2.95 (d, J = 11.0 Hz, 2H, piperidine-H), 4.04 (d, J = 5.5 Hz, 2H, CH_2O), 7.12 (s, 1H, Ar–H), 7.25 (d, J = 8.0 Hz, 1H, Ar–H), 7.30 (dd, J = 2.0, 10.0 Hz, 1H, Ar–H), 7.50 (s, 1H, Ar–H), 7.66 (t, J = 8.0 Hz, 1H, Ar–H), 8.26 (s, 1H, Ar–H); MS (ESI, m/z): 417 ([M+H]$^+$, 100%); HRMS (ESI, m/z): Calcd for $C_{21}H_{23}N_4O_2FCl$ 417.1494 ([M+H]$^+$), found 417.1477.

(m) 4-((4-Bromo-2-fluorophenyl)amino)-7-((1-methylpiperidin-4-yl) methoxy) quinazolin-6-ol (**7b**). A similar procedure for **7a** was used to prepare **7b** (17%) as a white solid: R_f = 0.30 (50:50:1 MeOH/CH_2Cl_2/$NH_3.H_2O$); mp 223–225 °C; ^{1}H NMR (MeOH-d_4) δ 1.50–1.54 (m, 2H, piperidine-H), 1.96–1.99 (m, 3H, piperidine-H), 2.13 (t, J = 11.0 Hz, 2H, piperidine-H), 2.32 (s, 3H, NCH_3), 2.95 (d, J = 11.0 Hz, 2H, piperidine-H), 4.05 (d, J = 5.5 Hz, 2H, CH_2O), 7.13 (s, 1H, Ar–H), 7.40 (d, J = 8.5 Hz, 1H, Ar–H), 7.44 (dd, J = 2.0, 10.0 Hz, 1H, Ar–H), 7.53 (s, 1H, Ar–H), 7.62 (t, J = 8.0 Hz, 1H, Ar–H), 8.26 (s, 1H, Ar–H); MS (ESI, m/z): 461 ([M+H]$^+$, 100%); HRMS (ESI, m/z): Calcd for $C_{21}H_{23}N_4O_2FBr$ 461.0988 ([M+H]$^+$), found 461.0980; and 463.0971 ([M+H]$^+$), found 463.0956.

(n) *N*-(4-Chloro-2-fluorophenyl)-6-methoxy-7-((1-[^{11}C]methylpiperidin-4-yl) methoxy)quinazolin-4-amine (*N*-[^{11}C]chloro-Vandetanib, *N*-[^{11}C]**6a**), *N*-(4-bromo-2-fluorophenyl)-6-methoxy-7-((1-[^{11}C]methylpiperidin-4-yl)methoxy) quinazolin-4-amine (*N*-[^{11}C]Vandetanib, *N*-[^{11}C]**6b**), *N*-(4-chloro-2-fluorophenyl)-6-[^{11}C]methoxy-7-((1-methylpiperidin-4-yl)methoxy)quinazolin-4-amine (*O*-[^{11}C]chloro-Vandetanib, *O*-[^{11}C]**6a**), and *N*-(4-bromo-2-fluorophenyl)-6-[^{11}C]methoxy-7-((1-methylpiperidin-4-yl)methoxy)quinazolin-4-amine (*O*-[^{11}C]Vandetanib, *O*-[^{11}C]**6b**). [^{11}C]CO_2 was produced by the ^{14}N(p,α)^{11}C nuclear reaction on ultra high purity nitrogen (+1% O_2) in the small volume (9.5 cm^3) aluminum gas target of the Siemens Eclipse RDS-111 cyclotron. Precursor **5a**, **5b**, **7a**, or **7b** (0.1 mg) was dissolved in CH_3CN (500 μL) and added to the 5 mL reaction vial of the methylation module, along with NaOH (2 N, 2 μL). Carrier-free (high specific activity) [^{11}C]CH_3OTf produced by the gas-phase production method[12] from [^{11}C]CO_2 through [^{11}C]CH_4 and

[^{11}C]CH_3Br with silver triflate (AgOTf) column was passed into the reaction vial at rt until radioactivity reached a maximum, and then the reaction vial was isolated and heated at 80 °C for 3 min. The reaction mixture was cooled to ~50 °C, diluted with $NaHCO_3$ (0.1 M, 1 mL) and injected onto the semi-preparative HPLC column through a 3 mL injection loop for purification. The product fraction was collected, the solvent was removed by rotatory evaporation under vacuum, and the final product *N*-[^{11}C]**6a**, *N*-[^{11}C]**6b**, *O*-[^{11}C]**6a**, or *O*-[^{11}C]**6b** was formulated in saline, sterile-filtered through a sterile vented Millex-GS 0.22 μm cellulose acetate membrane, and collected into a sterile vial. Total radioactivity was assayed and total volume was noted for tracer dose dispensing. Retention times in the semi-preparative HPLC system were: t_R **5a** = 6.12 min, t_R **6a** = 8.92 min, t_R *N*-[^{11}C]**6a** = 8.92 min; t_R **5b** = 6.67 min, t_R **6b** = 9.13 min, t_R *N*-[^{11}C]**6b** = 9.13 min; t_R **7a** = 6.35 min, t_R **6a** = 8.92 min, t_R *O*-[^{11}C]**6a** = 8.92 min; t_R **7b** = 6.56 min, t_R **6b** = 9.13 min, t_R *O*-[^{11}C]**6b** = 9.13 min. Retention times in the analytical HPLC system were: t_R **5a** = 2.76 min, t_R **6a** = 4.72 min, t_R *N*-[^{11}C]**6a** = 4.72 min; t_R **5b** = 2.73 min, t_R **6b** = 4.98 min, t_R *N*-[^{11}C]**6b** = 4.98 min; t_R **7a** = 2.63 min, t_R **6a** = 4.72 min, t_R *O*-[^{11}C]**6a** = 4.72 min; t_R **7b** = 2.75 min, t_R **6b** = 4.98 min, t_R *O*-[^{11}C]**6b** = 4.98 min. The radiochemical yields were 40–50% decay corrected to EOB, based on [^{11}C]CO_2.