

(19) World Intellectual Property Organization
International Bureau



(43) International Publication Date
9 April 2009 (09.04.2009)

PCT

(10) International Publication Number
WO 2009/045535 A2

(51) International Patent Classification:

A61K 49/00 (2006.01) A61K 31/50 (2006.01)
A61K 31/49 (2006.01)

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(21) International Application Number:

PCT/US2008/011509

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(22) International Filing Date: 6 October 2008 (06.10.2008)

(25) Filing Language:

English

(26) Publication Language:

English

(30) Priority Data:

60/997,783 4 October 2007 (04.10.2007) US

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(81) Designated States (unless otherwise indicated, for every kind of national protection available): AE, AG, AL, AM, AO, AT, AU, AZ, BA, BB, BG, BH, BR, BW, BY, BZ, CA, CH, CN, CO, CR, CU, CZ, DE, DK, DM, DO, DZ, EC, EE, EG, ES, FI, GB, GD, GE, GH, GM, GT, HN, HR, HU, ID, IL, IN, IS, JP, KE, KG, KM, KN, KP, KR, KZ, LA, LC, LK, LR, LS, LT, LU, LY, MA, MD, ME, MG, MK, MN, MW, MX, MY, MZ, NA, NG, NI, NO, NZ, OM, PG, PH, PL, PT, RO, RS, RU, SC, SD, SE, SG, SK, SL, SM, ST, SV, SY, TJ, TM, TN, TR, TT, TZ, UA, UG, US, UZ, VC, VN, ZA, ZM, ZW.

(84) Designated States (unless otherwise indicated, for every kind of regional protection available): ARIPO (BW, GH, GM, KE, LS, MW, MZ, NA, SD, SL, SZ, TZ, UG, ZM, ZW), Eurasian (AM, AZ, BY, KG, KZ, MD, RU, TJ, TM), European (AT, BE, BG, CH, CY, CZ, DE, DK, EE, ES, FI, FR, GB, GR, HR, HU, IE, IS, IT, LT, LU, LV, MC, MT, NL, NO, PL, PT, RO, SE, SI, SK, TR), OAPI (BF, BJ, CF, CG, CI, CM, GA, GN, GQ, GW, ML, MR, NE, SN, TD, TG).

Published:

— without international search report and to be republished upon receipt of that report

(54) Title: FLUORINE-18 DERIVATIVE OF DASATINIB AND USES THEREOF

(57) Abstract: Provided herein are [18F]-labeled Dasatinib derivatives or analogs effective for imaging cells or tissue having an increased tyrosine kinase activity associated with a pathophysiological condition. Also provided are methods for in vivo imaging using the [18F]-labeled Dasatinib derivatives or analogs, particularly methods of imaging utilizing positron emission tomography. These methods are effective for diagnosing a pathophysiological condition susceptible to treatment with Dasatinib or other kinase inhibitor in a subject, or determining whether a cancer in a subject susceptible to being treated with Dasatinib or other kinase inhibitor has developed resistance to the same and for maximizing tumor response to kinase inhibitor with minimal toxicity to the subject.



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FLUORINE-18 DERIVATIVE OF DASATINIB AND USES THEREOF

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BACKGROUND OF THE INVENTION

10 Field of the Invention

The present invention relates to tyrosine kinases and positron emission tomography (PET) visualization of certain cancers *in vivo*. More specifically, the present invention relates to a fluorine-18 analog of Dasatinib and its use in PET to visualize cancers *in vivo*.

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Description of the Related Art

A focus of modern medicine is to develop care that is individualized to each patient. An important facet of this has been kinase inhibitor therapy, and signal transduction modulation in general. Another key aspect of customized care is obtaining a detailed disease
20 profile through non-invasive medical imaging techniques such as PET and using this to assess disease status and determine the optimal course of treatment.

Molecular and functional imaging with radiotracers such as [¹⁸F]-fluoro-2-deoxy-D-glucose (FDG) and [¹⁸F]-3'-deoxy-3'-fluorothymidine (FLT) are particularly useful as
25 surrogate markers in cancer diagnosis and management (1-2). However, more sophisticated tumoral information is necessary to predict response or observe the onset of drug resistance. Radiolabeled small molecule imaging modalities that are matched to a given kinase inhibitor and are capable of querying a specific molecular target are one possible solution.

PET is a non-invasive nuclear medicine imaging technique that produces a
30 virtual three-dimensional computer image that quantifies and localizes a specific biochemical activity or biological target within the tissues and organs of a living subject. The type of biochemical activity, such as enzyme function, or biological target, such as a receptor, that is imaged by PET depends upon the type of radioactive tracer used. A radiotracer is a biological molecule chemically-conjugated to a trace amount of radioactive isotope and that participates
35 in specific biochemical processes or binds to specific biological target(s) of interest. A radiotracer is typically administered to a subject by vein. As the radiotracer distributes throughout the body, it accumulates locally according to the specifically-related biochemical activity, or concentration of the biological target within individual tissues and organs. The PET scanner localizes and quantifies this activity within the body of the subject by detecting the source of photons emitted in the decay of the tracer-radioisotope. Computer analysis of this
40 data generates PET images, which are interpreted by physicians.

PET uses positron-emitting radioisotopes with short half lives (HL) such as fluorine-18 (^{18}F), ^{11}C (HL:~20 min), ^{13}N (HL: ~10 min), ^{15}O (HL:~2 min), and ^{18}F (HL:~110 min). After a positron is emitted, it travels up to a few millimeters until it meets an electron, in which process both particles are annihilated, wherein their masses are converted to a pair of annihilation photons with each departing in opposite directions. These annihilation photons are detected, by PET, when these strike scintillating crystals in the PET scanning device. The energy deposited within a crystal creates a burst of light and this light-signal is, then, amplified by photomultiplier tubes. As the annihilation photons are emitted 180° apart, it is possible to localize their source to a straight-line in space when diametrically-opposite crystals within the ring of a PET scanner are excited simultaneously. Sophisticated computational algorithms analyze and incorporate data from all of these 'lines of response' into generated PET images.

Normal Abl and Src kinases are expressed in a variety of tissues and are tightly regulated and inactive most of the time. Both have many functions and associations *in vivo*, but generally, Src regulates cell adhesion and motility, while Abl is involved in cytoskeletal reorganization (3) and cell death signaling (4). In some leukemias, a reciprocal t(9;22) translocation between the ABL and BCR genes forms the Philadelphia chromosome (Ph), whose mutant gene product, Bcr-Abl, is a constitutively activated tyrosine kinase. Bcr-Abl causes chronic myelogenous leukemia (CML) and some types of acute lymphoblastic leukemia (ALL) (5). Src tyrosine kinase is activated and/or overexpressed in numerous malignancies, mutated in a few examples and is often associated with increased motility, invasiveness or metastasis in cancer (6). The abundance, activation and dysregulation of Bcr-Abl and Src in cancer make these kinases attractive targets for drug development and molecular imaging.

Imatinib, a Bcr-Abl tyrosine kinase inhibitor, is one of the most well known molecularly targeted therapeutics and has revolutionized treatment of CML (7-8). Imatinib is also approved for gastrointestinal stromal tumor (GIST) therapy and acts via inhibition of c-Kit receptor tyrosine kinase (9). While imatinib has been a major breakthrough, resistance to kinase inhibitor therapy arises from a number of mechanisms including kinase-domain point mutations (pre-existing or acquired), upregulation of Bcr-Abl, activation of alternate, compensatory kinase pathways (Src family), and drug transporters (10). The issue of resistance is complicated further by residual quiescent cancer stem cells that are less susceptible to therapy possibly by one or more of the aforementioned mechanisms (11-12). These issues have fueled the development of a number of next-generation Bcr-Abl inhibitors (13-14). Dasatinib (BMS-354825) is a high affinity dual Src/Abl and c-Kit inhibitor recently approved for all categories of imatinib-refractory CML and Ph+ ALL (15-16). Dasatinib is effective in many imatinib resistant Bcr-Abl kinase domain mutants, but the "gatekeeper" mutants like T315I or F317L remain problematic (16).

Dasatinib is a rather toxic anticancer drug. Treatments with dasatinib employ either a fixed dosage (70 mg twice-daily) or the conventional 'maximum tolerated dose' approach, wherein drug dosage starts low and is increased until the patient experiences toxicity. Administered orally, the absorption and pharmacokinetics of dasatinib – ie, the amount of

ingested dasatinib that could actually reach tumor – varies among individuals, influenced by gastric pH & food content, drug interactions, and other factors. A standard starting dose is 70 mg twice-daily, though no linear dose-response relationship is evident, at levels both above and below 70 mg twice-daily. Yet dasatinib-toxicity is clearly dose-related. Severe myelosuppression occurs in >50% of patients, with diarrhea and severe hemorrhage (including CNS) as other major toxicities.

In vitro, dasatinib-sensitive solid tumor cell lines demonstrate a conventional dose-response curve (48). Hence, in cancer patients treated with oral doses of dasatinib, it would be valuable for clinicians to know what amount of a given dose actually reaches tumors, *in vivo*; possessing this knowledge should help clinicians predict tumor response, allowing earlier modification of therapeutic regimens, when satisfactory response is unlikely. Detecting changes in tumor pharmacokinetics may also provide a novel means of identifying the onset of chemoresistance to Dasatinib.

¹⁴C- and ³H-labeled (beta-emitting) radiotracers are produced routinely in drug development, but kinase inhibitors bearing positron-emitting isotopes are much less developed. No kinase inhibitor-based imaging probe exists yet for routine use in humans. Thus far, the majority of effort has been by vanBrocklin, Mishani and others on quinazoline-type small-molecule probes for EGFR tyrosine kinase, which is overexpressed in some cancers (17-22). More recently, Wang, *et al.* reported the synthesis of [¹¹C]-gefitinib (23) and [¹⁸F]-sunitinib (24).

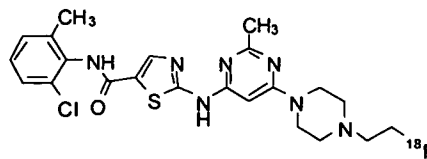
Recently, [¹⁸F]-FLT PET was used to distinguish bone marrow in patients with myeloproliferative disorders from normal (27) [¹¹C]-AG957 was the first example of a Bcr-Abl-targeted radiotracer specifically developed for PET, but this tracer suffers from inherent chemical instability and weak target binding relative to newer inhibitors (28-29). An [¹²⁴I]-pyridopyrimidinone derivative (30) which binds tightly to Bcr-Abl, among other kinases, has been reported but does not possess an ideal logP. LogP is the ratio of concentrations of a compound in the two phases of a mixture of two immiscible solvents at equilibrium, and is a measure of differential solubility of the compound between these two solvents. Recently, Fowler reported [¹¹C]-imatinib imaging in baboon (31). Generally, ¹⁸F is more convenient for PET imaging studies, as it has a 110-minute half-life, unlike ¹¹C (*T*_{1/2}=20 min).

Thus, there is an increasing need in the art for radiofluorinated tracers for PET imaging. Specifically, the prior art is deficient in a better PET tracer for the Abl and c-kit and other kinases in the form of a ¹⁸F derivative of Dasatinib that has both favorable physical properties and strong target binding. The present invention fulfills the longstanding need in the art.

SUMMARY OF THE INVENTION

The present invention is directed to a compound for *in vivo* imaging of cells or tissue having an increased tyrosine kinase activity associated with a pathophysiological

The present invention also is directed to a related [¹⁸F]-labeled compound having the chemical structure



The present invention is directed further still to a method for *in vivo* imaging cells or tissue having an increased tyrosine kinase activity associated with a pathophysiological condition in a subject. The method comprises administering to the subject a sufficient amount of a [¹⁸F]-labeled dasatinib derivative or analog thereof to provide an imageable concentration of the derivative or analog in the cells or tissue. Emissions from the [¹⁸F] label comprising the derivative or analog are detected thereby forming an image of the cells or tissue.

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method comprises administering to the subject an imageable amount of an [18-F]-labeled kinase inhibitor and imaging the subject using positron emission tomography (PET). The imaged tumor uptake of the [18-F]-label with inhibitor is correlated to binding affinity for the tumor. Subsequently, a dose of an unlabeled kinase inhibitor is administered to the subject and shortly after the [18-F]-labeled kinase inhibitor of the present invention is administered to the subject and a PET scan of the subject is obtained. If the PET image indicates a total loss of tumor uptake of the [18-F]-labeled kinase inhibitor, it is determined that the administered dose of the therapeutic inhibitor corresponds to a tumor saturating dose, whereas no loss or partial loss, but not total loss, of [18-F]-labeled kinase inhibitor uptake by the tumor of the subject indicates that the therapeutic dose of the dasatinib or other kinase inhibitor should be increased, thereby maximizing tumor response results, while minimizing side effects thereto.

Other and further aspects, features and advantages of the present invention will be apparent from the following description of the presently preferred embodiments of the invention given for the purpose of disclosure.

BRIEF DESCRIPTION OF THE DRAWINGS

So that the matter in which the above-recited features, advantages and objects of the invention, as well as others which will become clear, are attained and can be understood in detail, more particular descriptions of the invention are briefly summarized. The above may be better understood by reference to certain embodiments thereof which are illustrated in the appended drawings. These drawings form a part of the specification. It is to be noted; however, that the appended drawings illustrate preferred embodiments of the invention and therefore are not to be considered limiting in their scope.

Figures 1A-1B are synthetic schema showing the synthesis of an unlabeled (^{19}F) fluorinated derivative of Dasatinib (**Figure 1A**) and two radiosynthetic routes to an [^{18}F] derivative of Dasatinib (**Figure 1B**).

Figures 2A-2B are cavity-depth (**Figure 2A**) and Connolly (**Figure 2B**) surface renderings of **5** docked into Abl kinase domain.

Figures 3A-3F illustrate inhibition of cellular proliferation of M07e/p210^{bcr-abl} (R10 neg) (**Figure 3A**), M07e (**Figure 3B**), and K562 (**Figure 3C**) cell lines with fluorinated analog **5** versus Dasatinib (**Figures 3D-3F**).

Figure 4 depicts a HPLC chromatogram showing coelution of [^{18}F]-**5** with co-injected non-radioactive reference ^{19}F compound **5**. Phenomenex Luna C₁₈ 4.6_250 mm, 5 μ , isocratic 60% NaOAc / 40% CH₃CN, 1.0 mL/min. (Δ = 0.4min between detectors).

Figure 5 illustrates inhibitory activity of **5** on 21 kinases at 10 nM.

Figure 6 illustrates microPET imaging of a K562 xenograft in a mouse with [^{18}F]-**5** from 60-75 min.

Figures 7A-7D are [^{18}F]-**5** microPET images of a SCID mouse bearing H1975 lung cancer xenograft on its right shoulder (**Figure 7A**) and H1975-DR lung cancer xenograft

on its left shoulder (Figure 7B). Figures 7A-7B are transaxial images showing bilateral tumor uptake (Figure 7A) and competitive inhibition of tracer uptake (Figure 7B) by unlabeled Dasatinib. Figures 7C-7D are coronal images showing bilateral tumor uptake (Figure 7C) and competitive inhibition of tracer uptake in tumor and organs (Figure 7D).

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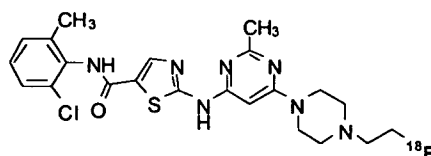
DETAILED DESCRIPTION OF THE INVENTION

As used herein, the term “a” or “an”, when used in conjunction with the term “comprising” in the claims and/or the specification, may refer to “one”, but it is also
10 consistent with the meaning of “one or more”, “at least one”, and “one or more than one”. Some embodiments of the invention may consist of or consist essentially of one or more elements, method steps, and/or methods of the invention. It is contemplated that any device, compound, composition, or method described herein can be implemented with respect to any other device, compound, composition, or method described herein.

15 As used herein, the term “or” in the claims refers to “and/or” unless explicitly indicated to refer to alternatives only or the alternatives are mutually exclusive, although the disclosure supports a definition that refers to only alternatives and “and/or”.

As used herein, the term “subject” is any recipient of compound [¹⁸F]-5 or other [¹⁸F] labeled dasatinib derivative or analog.

20 In one embodiment of the present invention there is provided a compound for *in vivo* imaging of cells or tissue having an increased tyrosine kinase activity associated with a pathophysiological condition, comprising a [¹⁸F] -labeled dasatinib derivative or analog. Further to this embodiment the compound comprises a physiologically acceptable carrier or adjuvant. In both embodiments the [¹⁸F] label may comprise a [¹⁸F]-fluoroethylpiperazinyl moiety. For example, the compound may have the structure:



Also in both embodiments, the physiological condition may be a cancer. Representative examples of a tyrosine kinase are Abl, Ack, Csk, EphA2, EphB4, Kit, PDGFR-alpha, Src or Tec.

30 In a related embodiment the present invention provides a [¹⁸F] labeled compound having the chemical structure as described *supra*. Further provided is a composition comprising the [¹⁸F] labeled compound and the physiologically acceptable carrier or adjuvant as described *supra*.

35 In another embodiment of the present invention there is provided a method for diagnosing a pathophysiological condition susceptible to treatment with dasatinib or other kinase inhibitor in a subject in need of such diagnosis, comprising the steps of administering a sufficient amount of the compound as described *supra* to the subject to provide an imageable

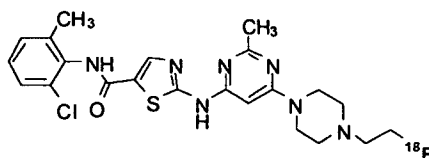
concentration therewithin; imaging the subject using positron emission tomography (PET); and determining whether the intensity of the label in any body area of the subject is increased in comparison with normal background, wherein an increase in intensity of the labeling indicates that the individual has a condition that is susceptible to being treated with dasatinib or another
5 kinase inhibitor.

Further to this embodiment, the method may comprise treating the pathophysiological condition with a pharmacologically effective dose of one or more of dasatinib or other kinase inhibitor. Further still, the method may comprise monitoring the susceptibility of the pathophysiological condition to treatment with dasatinib or other kinase
10 inhibitor(s) to determine whether the subject has developed resistance to such treatment. In this further embodiment, the step of monitoring susceptibility may comprise administering another imageable amount of the compound as described *supra* to the subject; imaging the subject using PET; and comparing the intensity of the label in a body area associated with the pathophysiological condition to a previous label-intensity, wherein a decrease in intensity
15 compared to the previous intensity indicates that the pathophysiological condition is less susceptible to treatment with the dasatinib or other kinase inhibitor. In all embodiments the pathophysiological condition may be as described *supra*.

The present invention is directed further still to a method for maximizing tumor response to a kinase inhibitor with minimal toxicity therefrom in a subject having a cancer.
20 The method comprises administering to the subject an imageable amount of an [18-F]-labeled kinase inhibitor and imaging the subject using positron emission tomography (PET). The imaged tumor uptake of the [18-F]-label with inhibitor is correlated to binding affinity for the tumor. Subsequently, a dose of an unlabeled kinase inhibitor is administered to the subject and shortly after the [18-F]-labeled kinase inhibitor of the present invention is administered to the
25 subject and a PET scan of the subject is obtained. If the PET image indicates a total loss of tumor uptake of the [18-F]-labeled kinase inhibitor, it is determined that the administered dose of the therapeutic inhibitor corresponds to a tumor saturating dose, whereas no loss or partial loss, but not total loss, of [18-F]-labeled kinase inhibitor uptake by the tumor of the subject indicates that the therapeutic dose of the dasatinib or other kinase inhibitor should be
30 increased, thereby maximizing tumor response results, while minimizing side effects thereto.

In yet another embodiment of the present invention there is provided an *in vivo* method for imaging cells or tissue having an increased tyrosine kinase activity associated with a pathophysiological condition in a subject, comprising the steps of administering to the subject a sufficient amount of a [¹⁸F]-labeled dasatinib derivative or analog to provide an imageable
35 concentration of the derivative or analog in the cells or tissue; and detecting emissions from the [¹⁸F] label comprising the derivative or analog, thereby forming an image of the cells or tissue. Further to this embodiment the [¹⁸F]-labeled dasatinib derivative or analog may comprise a physiologically acceptable carrier or adjuvant.

In both embodiments, the [^{18}F] label may comprises a [^{18}F]-fluoroethylpiperazinyl moiety. The [^{18}F]-labeled dasatinib derivative or analog may comprise a [^{18}F] -fluoroethylpiperazinyl moiety and furthermore may have the chemical structure:



- 5 In these embodiments, the detecting step may be by positron emission tomography. Also, the pathophysiological condition may be a cancer and the cells and tissue may comprise a tumor. In addition the tyrosine kinase may be Abl, Ack, Csk, EphA2, EphB4, Kit, PDGFR-alpha, Src or Tec.

- 10 In yet another embodiment of the present invention there is provided a method for maximizing tumor response to a kinase inhibitor with minimal toxicity therefrom in a subject having a cancer, comprising the steps of administering to the subject an imageable amount of an [^{18}F]-labeled kinase inhibitor prior to the subject having been treated with dasatinib or another kinase inhibitor; Administering the subject a therapeutic amount of dasatinib or another kinase inhibitor with similar kinase binding activity; Administering the subject
15 another imageable amount of the compound as described *supra*; imaging the subject using positron emission tomography (PET); correlating the imaged tumor uptake of the [^{18}F]-label in the second PET scan with the first imaged tumor uptake of the [^{18}F]-label PET scan, wherein a disappearance of [^{18}F]-label intensity for any one tumor of the subject compared to the previous intensity indicates that the specific tumor is being treated at a sufficient therapeutic
20 concentration of dasatinib or another kinase inhibitor, while if the intensity of the label remains the same or only diminishes but it doesn't disappear, the specific tumor for which this occurs is not being optimally treated and the therapeutic dose of dasatinib or the other kinase inhibitor should be increased.

- 25 Further to this embodiment the method comprises designing a therapeutic regimen to treat the cancer with minimal toxicity to the subject based on the saturation dose of the kinase inhibitor. In both embodiments [^{18}F]-labeled kinase inhibitor may be [^{18}F]-dasatinib. Also, the kinase may be Abl, Ack, Csk, EphA2, EphB4, Kit, PDGFR-alpha, Src or Tec.

- 30 Provided herein are the radiosynthesis, biological evaluation and *in vivo* micro-PET imaging of a fluorine-18 radiotracer. The [^{18}F]-labeled compounds may be based on a potent, multi-targeted kinase inhibitor, for example, but not limited to, dasatinib, which is approved for the treatment of imatinib-resistant CML and Ph+ ALL. When considering the structure of dasatinib, the hydroxyethylpiperazinyl moiety was ideal for derivatization based on binding orientation. Chemically, the most straightforward approach was at the same site; *N*-alkylation of the unsubstituted piperazine with a simple fluorine-containing group or activated
35 precursor for fluoride displacement (32). Thus, an analog of dasatinib bearing an [^{18}F]

fluoroethyl substituent was constructed. *N*-(2-Fluoroethyl)piperazines are not widely reported, but Katzenellenbogen and Welch described a successful synthesis of activated ethylpiperazines and subsequent displacement with ^{18}F (33). Sterically, hydroxyl-to-fluoro substitution is tolerated well, so long as the hydroxyl is not involved in critical H-bonding.

5 Particularly, Compound 5 has similar target selectivity to dasatinib *in vitro* and retains strong anti-tumor potency. Radiosynthesis of [^{18}F]-5 was accomplished in a two-step approach by radiofluorination of either 2-bromoethyltriflate or ethylene glycol ditosylate and subsequent alkylation of piperazine precursor 4. Production runs of [^{18}F]-5 from 2-bromoethyltriflate had an average specific activity of 2,560 mCi/ μmol ($n=11$) in 125 ± 5 min
10 after end-of-bombardment.

Generally, compound [^{18}F]-5 and all precursors and intermediates are synthesized using known and standard chemical synthetic techniques. Particularly, the synthesis of both ^{18}F radiotracer and ^{19}F reference analogs began with chloropyrimidine 3, an intermediate that was synthesized according to the literature (15). An $\text{S}_{\text{N}}\text{Ar}$ displacement with
15 piperazine gave compound 4 in good yield (78%). The 2-fluoroethyl reference compound 5 was obtained by alkylation of 4 with 1-bromo-2-fluoroethane in the presence of Na_2CO_3 and catalytic KI (Fig. 1A).

A two-step process was used to produce the [^{18}F]-*N*-2-fluoroethyl labeled compound. A two-carbon synthon containing two leaving groups was displaced with F-18 first,
20 then reacted with piperazine 4. A one-step radiosynthesis would be ideal, however the intramolecular cyclization may be a problematic competing reaction in a precursor containing a $\text{X}-\text{CH}_2\text{CH}_2-\text{NR}_2$ system—a piperazine beta to a leaving group that is significantly reactive with fluoride ion (Fig. 1B).

Thus, the present invention provides imaging methods using the [^{18}F]-labeled
25 Dasatinib derivative or analog. These [^{18}F]-labeled dasatinib derivative or analog may be administered in amounts sufficient to produce an imageable concentration in cells or tissues particularly associated with a pathophysiological condition, such as, but not limited to a cancer, e.g., a leukemia. These [^{18}F]-labeled compounds are particularly suited to imaging via positron emission tomography. One of ordinary skill in the art is well-suited to determine
30 amounts of the [^{18}F]-labeled compounds to administer to a subject, the route of administration and the PET imaging conditions necessary to obtain a useable image.

Generally, as the [^{18}F]-labeled compounds are effective to bind to or competitively inhibit a tyrosine kinase, it is contemplated that the [^{18}F]-labeled compounds provided herein are suitable to image and to locate within a body mass a tyrosine kinase
35 associated with a pathophysiological condition. Examples of imageable tyrosine kinases are Abl, Ack, Csk, EphA2, EphB4, Kit, PDGFR- α , Src or Tec. Particularly, as a probe, the [^{18}F]-labeled compound [^{18}F]-5 had significant K562 tumor uptake in mice, and thus can be used as a molecularly-targeted PET imaging probe with *in vivo* models of systemic CML, GIST and other malignancies involving Abl, Src and Kit. Thus, [^{18}F]-5 is effective to visualize tumor
40 characteristics on a molecular level, non-invasively, such as the existence or emergence of

drug-resistant leukemia in bone marrow among others. Established proliferative imaging modalities like [¹⁸F]-FLT or [¹⁸F]-FDG are valuable, but cannot give the same information about the molecular changes occurring during disease progression or the emergence of resistance.

5 One mechanism of tumor resistance to dasatinib therapy involves changes in the tumor receptor-targets that prevent dasatinib-binding. It is an object of the present invention to provide an assay that can detect the inability of dasatinib to bind to its tumor target-receptors which predicts tumor resistance to dasatinib therapy. This spares patients needless toxicity and allows clinicians to make earlier changes in therapeutic regimens.

10 One mechanism of tumor resistance to dasatinib therapy involves increases in the tumor receptor-targets, requiring increased doses of the therapeutic drug. It is an object of the present invention to provide an assay that can detect the inability of dasatinib to completely inhibit its tumor target-receptors which predicts tumor resistance to dasatinib therapy. This allows clinicians to make earlier changes in therapeutic regimens.

15 The lack of a clear linear dose-response relationship, for oral dasatinib therapy, suggests that tumor & systemic pharmacokinetics vary widely, among patients; correlating tumor concentrations of dasatinib or other kinase inhibitor (by PET) to tumor response should allow clinicians a clearer understanding of the dose-response relationship for individual cancer patients. Additionally, [¹⁸F]- dasatinib *PET* allows this tumor dose-response relationship to be studied on a tumor-by-tumor basis within the same patient, in metastatic disease, which no other assay can do. Multiple biopsies of scattered metastases has never been standard clinical practice; visualizing heterogeneous tumor pharmacokinetics, in metastases, may clarify the meaning of changes in tumor size, post-therapy.

20 [¹⁸F]-- dasatinib imaging provides for correlation of tumor response to tumor dosage. Determining how therapeutic dose levels of dasatinib or other kinase inhibitor affect the tumor accumulation of [¹⁸F]--labeled dasatinib, compared to a pre-treatment PET scan, can be effective to determine changes in tumor [¹⁸F]-- dasatinib uptake which then serves as an index of the amount of tumor target therapeutic drug saturation. Determining whether the specific tumor or metastatic tumor is saturated or not by the specific therapeutic dasatinib or other kinase inhibitor levels administered to a patient in need of such treatment, using [¹⁸F]-- dasatinib and PET scan, can be an indicator of whether the dasatinib or other kinase inhibitor dose given the patient should be increased, decreased or unmodified. The ability to visualize the saturation of dasatinib or other kinase inhibitor binding sites, in a tumor, *in vivo*, by PET, allows the relationship between the ingested dose levels of oral dasatinib or other kinase inhibitor therapy and clinical efficacy, as specific tumor shrinkage or lack thereof can be visualized, to be determined.

30 Thus, the present invention also provides an assay which can detect tumor saturation, by dasatinib or other kinase inhibitor, which is useful as a tool for maximizing tumor therapy-response while minimizing drug toxicity. Prescribing doses in excess of the dosage at
40

which tumor saturation occurs increases the risk of chemotoxicity without increasing tumor therapy-response. Yet prescribing a kinase inhibitor dose which fails to saturate tumor target-receptors yields a suboptimal tumor therapy-response. Therefore, an important object of the present invention is that PET imaging with [¹⁸F]--dasatinib changes the dosage goal in the treatment of a cancer patient from maximum tolerated dose to maximum tumor dose or saturation point.

The following example(s) are given for the purpose of illustrating various embodiments of the invention and are not meant to limit the present invention in any fashion.

EXAMPLE 1

Methods and Materials

Chemicals and MS/NMR

All chemicals and solvents were obtained from Sigma-Aldrich (Milwaukee, WI) or Fisher Scientific (Pittsburgh, PA) and used without further purification. ¹H, ¹³C, and ¹⁹F NMR spectra were recorded on a Bruker AMX-400 at 400, 100 and 376 MHz, respectively or a Bruker AVANCE II 500 at 500, 125 or 470 MHz, respectively. Chemical shifts (δ) are determined relative to CDCl₃ (referenced to 7.27 ppm (δ) for ¹H-NMR and 77.0 ppm for ¹³C-NMR) or DMSO-*d*₆ (referenced to 2.49 ppm (δ) for ¹H-NMR and 39.5 ppm for ¹³C-NMR). The internal reference for ¹⁹F-NMR was CFCI₃ (0.0 ppm (δ)). Coupling constants (*J*) are given in Hertz and spectral splitting patterns are designated as singlet (s), doublet (d), triplet (t), quadruplet (q), multiplet or overlapped (m), and broad (br).

Low resolution mass spectra (ionspray, a variation of electrospray) were acquired on a Perkin-Elmer Sciex API 100 spectrometer. HRMS service was obtained from the Mass Spectrometry Lab at UIUC and acquired on a Micromass 70-SE-4F spectrometer using FAB⁺ ionization. HPLC was performed on a Jasco (Easton, MD) system comprised of a PU-2089plus pump, UV-2075plus UV/VIS detector, LCNetII/ADC data acquisition system and Windows PC running EZChrom Elite v3.1.4 software. Flash chromatography was performed using Merck silica gel 60 (mesh size 230-400 ASTM) or using an Isco (Lincoln, NE) CombiFlash Companion or SQ16x flash chromatography system with RediSep columns (normal phase silica gel (mesh size 230-400 ASTM) and Fisher Optima™ grade solvents. Microwave reactions were performed in a CEM Discover microwave reaction system (Matthews, NC). Thin-layer chromatography (TLC) was performed on E. Merck (Darmstadt, Germany) silica gel F-254 aluminum-backed plates with visualization under UV (254 nm) and by staining with potassium permanganate or ceric ammonium molybdate. Molecular modeling was performed using SYBYL 7.1 (Tripos Inc., St. Louis, MO) on an Intel Xeon PC workstation running RedHat Enterprise Linux 3.

Computational Chemistry

Molecular modeling and graphics renderings were performed using the SYBYL 7.1 (Tripos Associates Inc., St. Louis, MO) software package on an Intel Xeon PC workstation

running RedHat Enterprise Linux 3. The Abl kinase:PD166326 kinase inhibitor cocrystal structure was used as the initial model. This file can be obtained from the protein databank (coordinate file 1OPK, www.rcsb.org).

A more appropriate starting structure would be the Abl:Dasatinib cocrystal structure reported by Tokarski, *et al.* (43), but at the time this work was initiated, the coordinate file 2GQG had not been released on the RCSB. The atom types for the inhibitor were corrected, hydrogen atoms were added to the protein and the C and N endgroups were fixed using the SYBYL/BIOPOLYMER module. Protein and inhibitor atomic charges were calculated using MMFF94 force field. The complex was minimized using the SYBYL gradient convergence method with an MMFF94s force field and 0.05 kcal/mol·Å rms gradient as the convergence criterion. All heavy atoms (inherent to the crystal structure) were constrained in an aggregate during minimization.

To create the Abl kinase:Compound 5 model, the inhibitor in the AblK:PD166326 cocrystal structure was replaced with compound 5 in an orientation that preserves the H-bond donor acceptor pair at Met318 and directs the fluoroethylpiperazinyl moiety out into solvent-exposed area (Figs. 2A-2B). The inhibitor atoms were allowed to move freely for minimization. Conformational analysis run on the ligand showed that the fluoroethyl sidechain has considerable freedom of motion. Several lowest energy conformers of the terminal fluoroethyl group were found and minimized, but ultimately showed negligible differences in energy.

Octanol/Water Partition Coefficient Determination

Octanol/water partition coefficients were determined for each radiotracer by shaking 370 KBq (10 µCi) of each radioligand with 10 mL of *n*-octanol and 10 mL of water for 2 hours. Octanol and deionized water were presaturated for at least 24 hours prior to use. The two layers were separated and spun in a centrifuge at 1000 g for 20 minutes. 1 mL samples were recovered with a syringe with a 25 gauge needle from each solvent and counted in a gamma counter. The samples of both layers were also analyzed for impurities by HPLC and the partition coefficient determined.

Protein Binding Assay

Protein binding of the radiotracers were determined adding 37 kBq (1 µCi) of each radioligand to samples of 1 % bovine serum albumin and 1 mL of fresh human serum. The protein was precipitated by adding 1 mL of ice cold 20% trichloroacetic acid and the suspension centrifuged and washing with 1 mL of 20% ice cold trichloroacetic acid. The protein pellets and supernatants were counted in a gamma counter to determine the protein binding of the radioligands.

Tyrosine Kinase Activity Assays

Abl and Src kinase activity was measured according to Trentham (44) with some modifications. For Abl, the reaction was in 25 mM Hepes buffer pH 7.5, 10 mM MgCl₂, 2 mM DTT, 20 mM β-glycerol phosphate, 0.1 mM Na₃VO₄, 120 mM β-NADH, 500 mM phosphoenolpyruvate, and including 3.1 mg/ml L-lactic dehydrogenase, 6.67 mg/ml pyruvate kinase, 0.005% Tween 80, 1% DMSO, 5 nM Abl kinase (Invitrogen), 30 mM peptide substrate EAIYAAPFAKKK (SEQ ID NO: 1) (~1 x K_m), and 200 mM ATP (~10 x K_m). For Src, the same reaction conditions were used except for the following: 10 mM MnCl₂ (instead of MgCl₂), 20 nM Src kinase, 300 mM KVEKIGEGTYGVVYK-OH peptide, SEQ ID NO: 2 (~1 x K_m), 200 mM ATP (~3 x K_m). Kinase was preincubated with inhibitor at 37°C for 10 min prior to starting the reaction upon addition of ATP; Assay was carried out in 384 well clear plates (Corning) and absorbance was measured on SpectraMax plate reader (Molecular Devices). Reaction rates were plotted against inhibitor concentration and fitted using SigmaPlot 9.0 (Systat Software Inc.).

In addition to the rigorous IC₅₀ determinations in Abl and Src kinase above, a set of 21 tyrosine kinases were evaluated using Carina Biosciences' (Kobe, Japan) QuickScout™ service to measure kinase activity inhibition by compound 5 at 10 nM. Staurosporine was used as a control / benchmark inhibitor. Literature K_d values for Dasatinib are included for comparison. An ELISA-based assay was used in which the phosphorylation of an oligopeptide substrate was detected by an HRP-conjugated anti-p-Tyr (PY20) probe. The concentration of ATP was at the approximate K_m of each kinase (0.5 – 100 mM). The data appears in Table 2 in Example 3.

Tumor Cell Culture

The immortalized human hematopoietic Philadelphia chromosome-positive cytokine independent R10(-) M07e^{p210} cell line (46) was maintained in Iscove's modified Dulbecco's medium (Life Technologies, Inc., Grand Island, NY) containing 10% FCS (Hyclone, Logan, UT). The parental M07e megakaryoblastic cell line was a kind gift of Brian Druker and was maintained in the presence of 50 ng/mL kit ligand (SCF) as described (46-47) K562 was obtained from the ATCC. K562 was maintained in suspension in 90% RPMI 1640 medium supplemented with 10% FBS, 2 mM L-glutamine, 10 mM HEPES, 1 mM sodium pyruvate, 1.5 g/L sodium bicarbonate, 4.5 g/L L-glucose, 10% FBS, 100 IU/mL penicillin and 100 μg/ml streptomycin. Tumor cell cultures were maintained in a humidified atmosphere with 5% CO₂ at 37°C (NuAire).

Cellular Proliferation Assay

Figures 3A-3F shows cell growth determined by a [³H]thymidine uptake assay. Cells (10⁴ cells/well) were cultured in 96-well, round-bottomed plates (Fisher Scientific) with diluted DMSO (control) or with varying concentrations of Dasatinib or fluorinated derivative 5 that were resuspended in DMSO for 48 h at 37°C. [³H]Thymidine was added at a concentration of 1 μCi/well, and cells were incubated for an additional 18 h. Cells were harvested with the

Unifilter system, scintillation fluid (25 μ l/well) was added to each well, and [3 H]thymidine incorporation was determined on a Packard Scintillation Counter. Data points for all assays were obtained in triplicate, and background incorporation from cell-free wells was determined and subtracted from all data points. The data was analyzed using a non-linear regression fit to a sigmoidal dose-response curve using Prism 4 (GraphPad Software, Inc).

Toxicity in Mice

Mice were injected i.v. with unlabeled (19 F) reference compound **5** in the tail vein and sacrificed using carbon dioxide at 30, 60 and 120 min post injection. Each group contained three mice. A total of 24 eight-week-old B6D2F1 mice (average initial weight was 22.9 g for male mice and 18.5 g for female mice) were used in the acute toxicity study. There were five males and five females in either control or treatment group. The treatment group received one dose of compound **5** (0.1 mg/kg) intravenously through tail vein injection and the control group received the same amount of vehicle (85% beta-hydroxypropyl cyclodextrine, 5% DMSO, 10% EtOH). All animals were observed for 14 days following treatment. Observation showed no apparent anemia, no weight loss, no agitation, no tachypnea, no GI disturbances and no apparent neurological dysfunction in all mice. In both the control and treatment groups, male mice gained on average 1.8 g in body weight and females gained on average 0.5 g. Mice were sent for pathology, including complete blood cell count, complete chemistry panel and complete necropsy. Two intact male mice and two intact female mice were also sent and used as references. Results showed no significant abnormalities in any control or treated mouse compared with intact mice. There was no evidence of drug-induced or vehicle induced organ toxicities. In conclusion, fluoro derivative **5**, administered at 0.1 mg/Kg intravenously, is safe and nontoxic in B6D2F1 mice.

In vivo metabolism in mice

Mice were injected i.v. with [18 F]-**5** in the tail vein and sacrificed using carbon dioxide at 30, 60 and 120 min post injection. Each group contained three mice. Immediately after sacrifice, about 0.5 ml of blood was collected by cardiac puncture and deposited in a 1.5 ml Eppendorf tube. Disodium EDTA (2.5 mg) was used as anticoagulant. The samples were then maintained at 4°C for subsequent procedures. The total radioactivity in each total blood sample was counted. The samples were then centrifuged at 4°C at 2200 mg. Radioactivity of the serum and pellet measured and about 50% of the total radioactivity was retained in the pellet. The serum was transferred to a 1.5 ml Eppendorf tube containing about 700 μ l of 60% acetonitrile in water and centrifuged again to precipitate any residual proteins. The supernatant was analyzed using HPLC and examined for metabolites. HPLC was carried out on a C-18 Shimadzu 4.6 x 250 mm HPLC column and eluted under gradient conditions 80%A (pH 5.5 50mM NaOAc):20% B (CH_3CN) to 20%A:80%B at 1 ml/min. Radioactivity was detected using Packard Radiomatic FLO-One / beta detector equipped with a PET flow cell containing BGO (bismuthgermanate) windows.

Animal imaging with PET

All animal studies were carried out within the framework of an institutional IACUC approved protocol (No. 86-02-020). Athymic nu/nu mice (National Cancer Institute, Bethesda, Maryland) were inoculated subcutaneously onto the right shoulder with 1×10^7 K562 cells mixed with Matrigel (BD Biosciences, San Jose, CA). K562 is a chronic myelogenous leukemia cell line cultured with IMDM (Iscove's modified Dulbecco's medium; prepared in-house) containing 4 mM L-glutamine, 1.5 g/L sodium bicarbonate, and 10% fetal bovine serum.

Three weeks following tumor inoculation, PET images of mice were obtained using FOCUS 120 microPETTM scanner (Siemens Preclinical Solutions, Knoxville, TN) with [¹⁸F]-**5**. Mice were injected intravenously (via tail vein) with 14 ± 1 MBq (375 ± 25 mCi) of [¹⁸F]-**5** and imaged 60 minutes later under 2% (at 1 L/min) isoflurane anesthesia (Forane, Baxter Healthcare, Deerfield, IL). Image acquisition time was 15 min ($t = 60$ to 75 min) using a 250-750 keV energy window and a 6nsec timing window. List-mode data were sorted into sinograms by Fourier re-binning and reconstructed by filter back-projection without attenuation correction. Count data in the reconstructed images were converted to activity concentration (i.e. % of the injected dose per gram (%ID/gm)) using a system calibration factor determined using a ¹⁸F-filled mouse-sized phantom. Visualization and analyses of microPET images were carried out using AsiPROTM software (Siemens Preclinical Solutions, Knoxville, TN).

EXAMPLE 2

Chemistry

Dasatinib **2** was synthesized according to the procedure of Lombardo, *et al* (15).

N-(2-Chloro-6-methylphenyl)-2-(2-methyl-6-(piperazin-1-yl)pyrimidin-4-ylamino)thiazole-5-carboxamide, **4**

2-(6-Chloro-2-methylpyrimidin-4-ylamino)-*N*-(2-chloro-6-methylphenyl)thiazole-5-carboxamide, **3** (15) (1.00 g, 2.54 mmol), piperazine (2.19 g, 25.4 mmol) and *N,N*-diisopropylethylamine (0.84 mL, 5.07 mmol) were dissolved in 30 mL of dry 1,4-dioxane and refluxed overnight. The solvent was stripped and the residue was triturated several times with DI water / MeOH, MeOH / ether and ether. The white solid was dried under high vacuum to give precursor **4** (0.88 g, 78%). ¹H NMR (DMSO-*d*₆) δ 9.85 (s, 1H), 8.20 (s, 1H), 7.39 (dd, 1H, $J = 7.5, 1.5$ Hz), 7.29 – 7.22 (m, 2H), 6.01 (s, 1H), 3.43 (m, 4H), 2.73 (m, 4H), 2.39 (s, 3H), 2.23 (s, 3H); ¹³C NMR (DMSO-*d*₆) δ 165.1, 162.6, 162.5, 159.9, 156.9, 140.8, 138.8, 133.5, 132.4, 129.0, 128.1, 127.0, 125.6, 82.4, 45.3 (2), 44.8 (2), 25.6, 18.3; FTIR (ATR) $\nu_{\max}$ 3190, 2950, 1619, 1571, 1506, 1410, 1294, 1205, 1185, 769; MS-ESI m/z 445 [M+H]⁺; HRMS (FAB+) calc'd for C₂₀H₂₂ClN₇OS: 443.1295, found: 443.1303; HPLC $t_R = 2.6$

min (Phenomenex Gemini C18 250 x 4.6 mm, 50% 20mM pH 4.1 KH₂PO₄ / 50% CH₃CN, 1 mL/min, λ =254nm).

N-(2-Chloro-6-methylphenyl)-2-(6-(4-(2-fluoroethyl)piperazin-1-yl)-2-methylpyrimidin-4-ylamino)thiazole-5-carboxamide, 5

Piperazine 4 (50 mg, 0.11 mmol), 1-bromo-2-fluoroethane (21 μ L, 0.27 mmol), K₂CO₃ (78 mg, 0.56 mmol), NaI (2 mg, 0.01 mmol) and 5 mL of CH₃CN were added to a 10 mL screw-top tube under argon. The vial was sealed and stirred 2h at 60 °C. Another 21 μ L (0.27 mmol) of 1-bromo-2-fluoroethane (42) was added and the mixture stirred another 2h. The reaction mixture was partitioned between 30 mL of EtOAc and 30 mL water. The organic layer was washed with water and brine, dried over MgSO₄ and concentrated to find a yellow oil. Purification by gradient flash chromatography (SiO₂, 0% to 10% 7N NH₃ in MeOH / CH₂Cl₂) yielded 46 mg (84%) of compound 5, a white powder: ¹H NMR (DMSO-*d*₆) δ 11.44 (s, 1H), 9.85 (s, 1H), 8.20 (s, 1H), 7.39 (d, 1H, *J* = 7.0 Hz), 7.27 – 7.24 (m, 2H), 6.04 (s, 1H), 4.62 – 4.48 (dt, 4H, *J* = 47.8, 4.8 Hz), 3.51 (m, 4H), 2.69 – 2.60 (m, 2H, dt, 4H, *J* = 28.8, 4.8 Hz), 2.68 (m, 4H) 2.39 (s, 3H), 2.22 (s, 3H); ¹³C NMR (DMSO-*d*₆) δ 165.1, 162.5, 162.4, 159.9, 156.9, 140.8, 138.8, 133.5, 132.4, 129.0, 128.1, 127.0, 125.7, 82.6, 81.7 (d, *J* = 164 Hz), 57.5 (d, *J* = 19 Hz), 52.4 (2), 43.5 (2), 25.5, 18.3; ¹⁹F NMR (DMSO-*d*₆) δ -217; FTIR (ATR) $\nu_{\max}$ 3202, 2945, 1622, 1576, 1504, 1413, 1394, 1290, 1188, 768; MS-ESI *m/z* 490 [M+H]⁺; HRMS (FAB+) calc'd for C₂₂H₂₅ClFN₇OS: 489.1514, found: 489.1519; HPLC *t*_R = 5.6 min (Phenomenex Gemini C18 250 x 4.6 mm, 50% 20mM pH 4.1 KH₂PO₄ / 50% CH₃CN, 1 mL/min, λ =254nm).

2-Bromoethyltriflate 6

2-Bromoethyl triflate 6 has been used to install a [¹⁸F]-fluoroethyl moiety on piperazines before and is easily obtained by triflation of 2-bromoethanol and triflic anhydride (33). Compound 6 was produced as in Chi, *et al.*, distilled, aliquated, sealed under argon and stored at -20 °C (33) ¹H NMR (CDCl₃-*d*) δ 4.75 (t, 2H, *J* = 6.4 Hz), 3.61 (t, 2H, *J* = 6.4 Hz); ¹³C NMR (CDCl₃-*d*) δ 118.5 (q, *J* = 320 Hz), 74.2, 26.1; ¹⁹F NMR (CDCl₃-*d*) δ -75.0.

Radiosynthesis

All HPLC solvents were filtered (0.45 μ m, nylon, Alltech) prior to use. Water (ultra-pure, ion-free) was obtained from a Millipore Alpha-Q Ultra-pure water system. Sep-Pak® cartridges were obtained from Waters Corporation (Milford, MA). Radio-TLC was performed on silica gel plates (5x20 cm; 250 μ m thickness; Aldrich, Milwaukee, WI) and analyzed with a BioScan AR-2000 Imaging Scanner (BioScan Inc., Washington D.C.) HPLC was performed using a Shimadzu (Columbia, MD) system composed of a C-18 reversed-phase column (Phenomenex Luna analytical 4.6x250 mm or semi-prep 10x250 mm, 5 μ , 1.0 or 4.0 mL/min, 50mM pH 5.5 NaOAc/CH₃CN), two LC-10AT pumps, an SPD-M10AVP photodiode array detector and a BioScan Flow Count radiodetector using a 25_25 mm NaI(Tl) crystal. Radioactivity was assayed using a Capintec CRC-15R dose calibrator (Ramsey, NJ).

No-carrier-added [^{18}F] fluoride ion was produced by the $^{18}\text{O}(\text{p},\text{n})^{18}\text{F}$ nuclear reaction by bombardment of an enriched [^{18}O] H_2O target with 11 MeV protons using an EBCO-TR19 cyclotron. The ^{18}F fluoride ion was trapped on an AccellTM Plus QMA ion-exchange cartridge (Waters).

5

[^{18}F]-N-(2-Chloro-6-methylphenyl)-2-(6-(4-(2-fluoroethyl)piperazin-1-yl)-2-methylpyrimidin-4-ylamino)thiazole-5-carboxamide, [^{18}F]-5

The radiosynthesis of [^{18}F]-5 was performed via two methods from 2-bromoethyltriflate, **6** (method A) or ethylene glycol ditosylate (method B). Routine production of [^{18}F]-5 currently uses method A.

Method A: The QMA cartridge containing cyclotron-produced [^{18}F] fluoride ion was eluted with a solution containing 420 μL of H_2O and 120 μL of 0.25 M K_2CO_3 into a 10 mL Reacti-vial containing 15 mg of Kryptofix [2.2.2] (4,7,13,16,21,24-hexaoxa-1,10-diazabicyclo[8.8.8]hexacosane) in 1.0 mL CH_3CN . Water was removed azeotropically with CH_3CN (3x1.0 mL) at 100–105 $^\circ\text{C}$. The Reacti-vial was cooled to 0 $^\circ\text{C}$ and to the anhydrous [^{18}F] $\text{KF}/\text{K}_2\text{CO}_3$ complexed with Kryptofix (with [^{18}F]- KF :Kryptofix 2.2.2) was added a solution of 2-bromoethyltriflate, **6**, (0.054 mmole) in *o*-dichlorobenzene (500 μL) and heated to 105 $^\circ\text{C}$ for 10 min. The [^{18}F]-1-bromo-2-fluoroethane ([^{18}F]-7) formed was distilled at 120 $^\circ\text{C}$ by bubbling a stream of argon (100mL/min) into another Reacti-Vial maintained at -25 $^\circ\text{C}$, containing a solution of piperazine precursor **4** (6.5 mg, 14.6 μM), NaI (9.0 mg, 60 μM), and Cs_2CO_3 (5 mg, 15.3 μM) in 500 μL of 1:1 CH_3CN :DMF. The activity in the receiving vial was measured periodically to follow the distillation procedure (5 min).

The Reacti-Vial was fitted with a new, un-pierced septum to minimize loss of [^{18}F]-7 at high temperature. The solution was heated to 120 $^\circ\text{C}$ for 40 min, cooled, diluted with 1.2 mL of 1:4 CH_3CN :50mM pH 5.5 NaOAc and passed through a 13mm syringe filter (0.25 μm). This solution was injected onto a C_{18} semi-preparative HPLC column and eluted under gradient conditions; 80%A (50mM pH 5.5 NaOAc):20%B (CH_3CN) to 20%A:80%B. [^{18}F]-5 eluted at 15.3 min, which was well resolved from precursor **4** ($t_{\text{R}} = 13.4$ min). For intravenous administration, the product-containing fraction was stripped of solvent by rotary evaporation, formulated in 5% BSA in saline to the proper dosage and sterile filtered.

The radiochemical purity of the final formulation was confirmed using analytical HPLC. Co-elution with non-radioactive ^{19}F reference compound **5** confirmed the identity of the radiotracer (Fig. 4). To measure radiochemical and chemical purity (>99%), [^{18}F]-5 was reinjected from the semi-prep HPLC product peak on analytical HPLC (product $t_{\text{R}}=13.2\text{min}$, isocratic 60% 50mM pH 5.5 NaOAc:40% CH_3CN , 1.0 mL/min). Total time of radiosynthesis was 120 ± 5 minutes from EOB. The decay-corrected radiochemical yields ($n=3$) were $25.1\pm 5.8\%$ from [^{18}F]-1-bromo-2-fluoroethane and $6.6\pm 2.3\%$ overall from starting [^{18}F]-fluoride. These conditions were optimized and it was found that [^{18}F]-7 could be distilled rapidly prior to the alkylation of **4**, which improved yields of [^{18}F]-5 somewhat to $9.8\pm 5.0\%$. The specific activity ranged from 108–7350 mCi/ μmol (average 2560 mCi/ μmol , $n=11$).

Method B: The QMA cartridge containing cyclotron-produced [^{18}F] fluoride ion was eluted with a solution containing 420 μL of H_2O and 120 μL of 0.25 M K_2CO_3 (20 μmol) into a 5 mL Reacti-vial containing 10 mg (2.7 μmol) of Kryptofix [2.2.2] in 0.5 mL CH_3CN . Water was removed azeotropically with CH_3CN (3x0.5 mL) at 105–110 $^\circ\text{C}$. To the anhydrous [^{18}F] $\text{KF}/\text{K}_2\text{CO}_3$ complexed with Kryptofix was added a solution of ethylene glycol ditosylate (2.0 mg, 5.4 μmol) in CH_3CN (100 μL) and heated (sealed) in an oil bath at 110 $^\circ\text{C}$ for 10 min (35). The reaction mixture was cooled to room temp. and treated with a solution of 5.5 mg of piperazine precursor 4 in 100 μL of DMSO. The mixture was heated at 160 $^\circ\text{C}$ for 30 min, cooled, and passed through a C-18 Sep-Pak column activated previously with 8 mL of MeOH followed by 10 mL of DI water. The Sep-Pak was washed with water (2x6 mL) and [^{18}F]-5 eluted with CH_3CN (1.2 mL). The Sep-Pak was washed with 0.8 mL of water and the combined [^{18}F] solution purified and formulated as in method A. Total time of radiosynthesis was 120 $\pm$ 10 minutes from EOB. The decay-corrected radiochemical yield was 23 $\pm$ 5% (n=3) over two steps based on starting [^{18}F]-fluoride. The specific activity was 3 – 6 mCi/ μmol (n=3).

[^{18}F]-2-fluoroethyl tosylate 8

Compound 8 is generated in situ in a similar fashion from ethylene glycol ditosylate (35). The decay-corrected radiochemical yield of [^{18}F]-5 from the tosylate, 8, was somewhat better over two steps (23%) but with much lower specific activity of 3–6 mCi/ μmole (n=3). The total time of preparation (radiosynthesis and chromatography and formulation) ranged from 120 to 130 minutes (125 $\pm$ 5 min). Compound 5 has a favorable log $D_{(o/w)}$ of 2.1 $\pm$ 0.6 and is highly protein bound in serum (98.5 $\pm$ 1.0%) and 1% BSA (99.0 $\pm$ 0.3%).

EXAMPLE 3

Compound [^{18}F]-5 kinase inhibition profile

Prior experience with pyridopyrimidinone Src/Abl inhibitors (34) and molecular docking studies into the Abl crystal structure predicted that the Dasatinib pharmacophore would share much of the same binding characteristics in which an arene sits deep within the catalytic pocket and the substituents on N4 of the piperazine would protrude from a solvent accessible hole in the kinase catalytic domain (Figs. 2A-2B). To determine whether compound 5 retains kinase inhibition profile that is similar to Dasatinib, the inhibition of kinase activity was characterized. *In vitro* and cellular assays demonstrated that fluorinated analog 5 has inhibitory activity that closely parallels that of Dasatinib. Compound 5 inhibits Abl and Src kinase activity at roughly half the potency of Dasatinib in our assays (Table 1).

TABLE 1

Compound 5 has kinase and cellular inhibition characteristics similar to Dasatinib.^a

	Dasatinib IC ₅₀ (nM)	Compound 5 IC ₅₀ (nM)
Abl protein	4.2 ± 0.4	9.1 ± 0.8
Src protein	1.5 ± 1.1	3.5 ± 2.2
K562 cells	1.0 ± 0.2	1.1 ± 0.2
R10 neg cells (M07e/p210 ^{bcr-abl})	0.07 ± 0.02	0.10 ± 0.02
M07e cells ^b	1.2 ± 0.8	1.1 ± 0.3

^a Mean of three experiments in each.

^b Grown in 50 ng/mL SCF (Kit ligand)

Compound 5 is equipotent with Dasatinib in inhibiting proliferation of cells dependent on Bcr-Abl for growth. K562 growth was inhibited at an IC₅₀ of 1.1 nM and M07e/p210^{bcr-abl} cells at 0.10 nM. Also, Kit ligand dependent growth of the parental M07e line was inhibited with an IC₅₀ of 1.1 nM. This result correlates well with strong inhibition of Kit kinase as seen in the kinase panel.

Kinase inhibition by 5 at 10 nM was examined in a panel of 21 kinases, which includes many relevant members for malignancies of interest (Fig. 5). As expected, the pattern of kinase binding data for Dasatinib (36) was very similar to the kinase inhibition profile of compound 5 (Table 2). Negative values, particularly for TIE2, are not readily explainable, but should be interpreted as an enhancement of substrate phosphorylation. Abl, Src and Kit are inhibited at >97% at 10nM, which corresponds to IC₅₀'s of <2 nM. Furthermore, 5 potently inhibited Tec kinase and two representative ephrin receptor tyrosine kinases, EphA2 and EphB4. Recently, Tec and Btk kinases were found to be major targets of Dasatinib by chemical proteomics (37). While inhibiting the ephrin receptors may be a double-edged sword for therapeutics due to tumor-suppressor signaling (38), they are upregulated in a variety of cancers (39) and hold promise in molecular imaging (40). Table 3 shows EC50 and IC50 values of [¹⁸F]-5 and Dasatinib for various tyrosine kinases.

TABLE 2
Inhibitory activity of Compound **5** and staurosporine against tyrosine kinases

Kinase	% Inhibition of Kinase Activity		Staurosporine conc. (nM)	Dasatinib ⁴ K _d (nM)
	Compound 5 (10nM)	Staurosporine		
ABL	99.4	87.2	(1000)	0.50
ACK	83.1	96.4	(30)	6.0
TYRO3	23.1	95.3	(1000)	--
CSK	95.2	89.3	(300)	1.0
EGFR	27.2	82.4	(10000)	100
EphA2	98.1	92.6	(10000)	0.80
EphB4	97.0	94.1	(10000)	0.30
FAK	-18.3	81.7	(100)	--
FGFR1	20.4	86.5	(100)	4000
IGF1R	5.9	96.9	(3000)	>10000
JAK3	16.0	95.3	(3)	--
MET	7.2	90.0	(300)	--
FLT3	0.3	96.4	(3)	5000
KIT	98.7	96.7	(10)	0.60
PDGFR_	86.0	88.4	(3)	0.40
SRC	97.5	89.8	(3000)	0.20
SYK	4.5	90.2	(30)	3000
TEC	98.1	92.5	(300)	--
TIE2	-51.7	90.9	(300)	>10000
TRKA	-0.9	94.0	(3)	>10000
KDR	-5.6	91.8	(300)	3000

5

TABLE 3
EC₅₀ and IC₅₀ Values of [¹⁸F]-**5** and Dasatinib against tyrosine kinases

Kinase	F18- 5	Dasatinib
	EC ₅₀ (nM)	IC ₅₀ (nM)
ABL	0.60	< 1
ACK	20	
CSK	5.0	
EGFR	270	180
EphA2	1.9	
EphB4	3.1	
FGFR1	390	880
KIT	1.3	5
PDGFR_	16	
SRC	2.6	0.5
TEC	1.9	

Src/Abl is not selective and interacts with a number of kinases (36). A tumor overexpressing a particular kinase, such as Bcr/Abl or Src, can however selectively uptake a high-affinity probe in the presence of surrounding tissues that have negligible kinase

expression. This selective uptake is possible with a related kinase-targeted radiotracer in Bcr-Abl overexpressing K562 cells (30).

Compound [¹⁸F]-5 efficacy *in vivo*

In *in vivo* studies in mice, 5 has not shown toxicity at a dose of 0.1 mg/kg. HPLC analysis of plasma samples revealed that [¹⁸F]-5 was metabolized significantly over a two-hour time course (see Table 2). Under these conditions three radioactive peaks were observed at 4.4, 15.1 and 16.9 minutes (Table 4). The peak at 16.9 min corresponds to [¹⁸F]-5 whereas the other two are metabolites.

TABLE 4
Metabolic profile of [¹⁸F]-5

Time (min)	Peak (% Radioactivity)		
	1 (4.4 min)	2 (15.1 min)	3 [¹⁸ F]-5 (16.9 min)
30	58 ± 19	10 ± 9	31 ± 11
60	88 ± 4	3 ± 3	9 ± 3
120†	84	7	8

† Only one trial was successful at this timepoint

In vivo results have been obtained in athymic mice bearing subcutaneous K562 (CML) tumor xenografts in the right shoulder. [¹⁸F]-5 (14±1 MBq, 375±25 µCi) was injected through the tail vein and the subjects were imaged in a microPET scanner. Figure 6 shows the microPET scan of one representative mouse 60 minutes after injection; the exposure time was 15 minutes. Tracer activity was evident within the tumor xenograft (white arrow) and was determined to be 1.1% of the injected dose by ROI (region of interest) analysis. The coronal image shows [¹⁸F]-5 activity in the tumor, blood pool activity in the head (H), physiologic excretion into the liver and gastrointestinal (GI) tract as well as into the kidneys and bladder (B). The transaxial image was taken at the level of the known palpable tumor as shown in the coronal section (broken line). The intensity of the radiotracer activity is color-graded as depicted by the colored scale. There was no significant uptake observed in bone, suggesting that [¹⁸F]-5 did not undergo rapid metabolic defluorination. Instead, the compound appears to be cleared via the hepatobiliary route predominantly. This result correlates with the distribution of Dasatinib in mice.

Saturation of available target-binding sites in tumors by Dasatinib

In vivo animal data demonstrates that therapeutic dosages of Dasatinib can completely saturate (or occupy) all available target-binding sites, in tumors. To determine if [¹⁸F]-5 uptake is competitively inhibited *in vivo*, a SCID mouse was injected with H1975 and H1975-DR lung cancer cells on its right and left shoulders, respectively (Figs. 7A-7B).

Transaxial and coronal images (Figs. 7A, 7C) illustrate that tumor uptake is evident bilaterally. Unlabeled Dasatinib was administered (30 mg/kg IP) to the mouse. Transaxial (Fig. 7B) and coronal (Fig. 7D) images illustrate competitive inhibition of tracer uptake in the tumors and organs, e.g., skeleton, thereby confirming specificity of tracer binding.

5

EXAMPLE 4

Determining patient sensitivity to Dasatinib or to other tyrosine kinase inhibitors to which Dasatinib binds

For each PET study, patients receive 10 mCi of [¹⁸F]-Dasatinib radiotracer given intravenously over 1 minute. Patients then undergo an [¹⁸F]-Dasatinib PET study which consists of a dynamic PET scan (approximately 45-minutes in duration) centered on the heart. After the dynamic scan, a PET-CT scan is performed; the PET-CT scan encompasses static images of the head, neck and torso (approximately 55 minutes in duration), to determine biodistribution, tumor localization, & organ dosimetry. The CT component serves the dual purpose of providing transmission data to allow quantification of the emission PET signal and providing anatomic localization for the PET signals. Blood samples are obtained at pre-defined time points during the scanning to determine blood clearance of the radiotracer and Dasatinib.

15

EXAMPLE 5

Determining inhibitor dose requirements

For each PET study, patients receive 10 mCi of [¹⁸F]-Dasatinib radiotracer given intravenously over 1 minute and monitored as in Example 4. The oral Dasatinib dose is provided to a cancer patient in need of such treatment. Three hours later, the patient is placed upon the PET scanner bed. Immediately prior to the radiotracer-injection, blood is obtained for a total Dasatinib level measurement. The [¹⁸F]-Dasatinib injection is then made with a maximal dose of 15mCi of [¹⁸F]-Dasatinib. 30 minutes after injection of the [¹⁸F]-Dasatinib, another blood sample is obtained for a total Dasatinib level measurement. Imaging commences at the start of the [¹⁸F]-Dasatinib injection. A dynamic PET scan (approximately 45 minutes) is performed centered on the heart. Thereafter, a PET-CT scan, including a series of static 2-D PET images of the head, neck and torso (typically 6-7 bed positions) is performed to allow for estimates of whole organ uptake & excretion patterns.

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EXAMPLE 6

Determination of maximum tumor dose or saturation

The cancer patient is injected with [¹⁸F]-Dasatinib, by intravenous bolus; and, after 1-2 hours, the patient lies upon a scanner bed for 20-30 minutes of imaging of the body in the PET camera. If the indication for [¹⁸F]-Dasatinib PET is to demonstrate tumor avidity for Dasatinib, a single pretreatment [¹⁸F]-Dasatinib PET would suffice. This indication is analogous to the use of ¹¹¹In-pentetreotide to predict tumor response to octreotide therapy; radioiodide scintigraphy to predict tumor response to radioiodide therapy; ^{99m}Tc-bisphosphonate

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scintigraphy to predict response to bone-seeking radiopharmaceutical therapy, eg, radiostrontium & radiosamarium; 123I-MIBG to predict response to 131I-MIBG therapy; and so forth.

If the clinician wants to know whether a prescribed dose will saturate tumor target-receptors, patients are imaged twice: first, before the patient ever has been treated with Dasatinib, the patient is administered [¹⁸F]-Dasatinib and analyzed by PET scan to establish tumor Dasatinib-avidity and, second, during Dasatinib therapy, in which decreases in the [¹⁸F]-Dasatinib concentrations found in tumor reflect tumor target-receptor occupancy, by non labeled Dasatinib, and complete loss of tumor [¹⁸F]-Dasatinib uptake indicates tumor saturation with the non radioactive Dasatinib, indicating that higher therapeutic doses are not required to realized full Dastinib therapeutic outcome. If tumor saturation is visualized, the clinician may prescribe a lower dose of oral Dasatinib, as a lower prescribed dose may allow maximal anti-tumor efficacy with less risk of toxicity. If tumor saturation is not visualized, the clinician may prescribe a higher dose of oral Dasatinib, anticipating improved tumor response.

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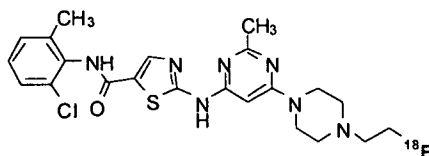
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WHAT IS CLAIMED IS:

1. A compound for *in vivo* imaging of cells or tissue using positron emission tomography having an increased tyrosine kinase activity associated with a pathophysiological condition, comprising:

a [^{18}F] -labeled Dasatinib derivative or analog thereof.

2. The compound of claim 1 having a chemical structure:



3. The compound of claim 1, wherein the pathophysiological condition is a cancer.

4. The compound of claim 1, wherein the tyrosine kinase is Abl, Ack, Csk, EphA2, EphB4, Kit, PDGFR-alpha, Src or Tec.

5. A method for diagnosing a pathophysiological condition susceptible to treatment with Dasatinib or other kinase inhibitor in a subject in need of such diagnosis, comprising the steps of:

administering a sufficient amount of the compound of claim 1 to the subject to provide an imageable concentration therewithin;

imaging the subject using positron emission tomography; and

determining whether the intensity of the label in any body area of the subject is increased in comparison with normal background, wherein an increase in intensity of the labeling indicates that the individual has a condition that is susceptible to being treated with Dasatinib or another kinase inhibitor.

6. The method of claim 5, wherein the pathophysiological condition is a cancer.

7. The method of claim 5, further comprising monitoring the susceptibility of the pathophysiological condition to treatment with Dasatinib or other kinase inhibitors to determine whether resistance has developed.

8. The method of claim 5, wherein monitoring susceptibility comprises the steps of:

administering another imageable amount of the compound of claim 1 to the subject;

5 imaging the subject using PET; and

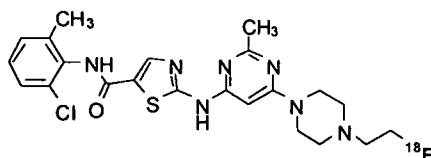
comparing the intensity of the label in a body area associated with the pathophysiological condition to a previous label-intensity, wherein a decrease in intensity compared to the previous intensity indicates that the pathophysiological condition is less susceptible to treatment with the Dasatinib or other kinase inhibitor.

10 9. An *in vivo* method using positron emission tomography for imaging cells or tissue having an increased tyrosine kinase activity associated with a pathophysiological condition in a subject, comprising the steps of:

15 administering to the subject a sufficient amount of a [^{18}F]-labeled Dasatinib derivative or analog thereof to provide an imageable concentration of the derivative or analog in the cells or tissue; and

detecting emissions from the [^{18}F] label comprising the derivative or analog, thereby forming an image of the cells or tissue.

20 10. The method of claim 9, wherein the [^{18}F]-labeled Dasatinib derivative or analog has the structure:



25 11. The method of claim 9, wherein the pathophysiological condition is a cancer.

12. The method of claim 9, wherein the cells or tissue comprise a tumor.

30 13. The method of claim 9, wherein the tyrosine kinase is Abl, Ack, Csk, EphA2, EphB4, Kit, PDGFR-alpha, Src or Tec.

14. A method for maximizing tumor response to a kinase inhibitor with minimal toxicity therefrom in a subject having a cancer, comprising the steps of:

35 administering to the subject an imageable amount of an [^{18}F]- Dasatinib;

imaging the subject using positron emission tomography (PET);

administering to the subject a dose of dasatinib or another unlabeled kinase inhibitor

administering to the subject an imageable amount of an [^{18}F]- Dasatinib;

imaging the subject using positron emission tomography (PET)

5 comparing the imaged tumor uptake of the [^{18}F]-label of the first PET image with the imaged tumor uptake of the [^{18}F]-label of the second PET image

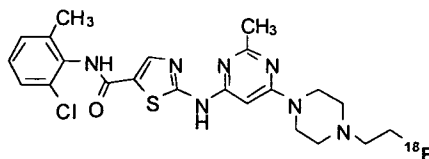
determining inhibitor binding affinity for the tumor; and

determining whether the dose of the unlabeled dasatinib or kinase inhibitor administered to the subject creates a complete loss of tumor uptake of the [^{18}F]-labeled kinase inhibitor in the second PET image

10 determining whether the dose of dasatinib or another unlabeled kinase inhibitor administered to the patient in need of such treatment should be increased or decrease or left the same

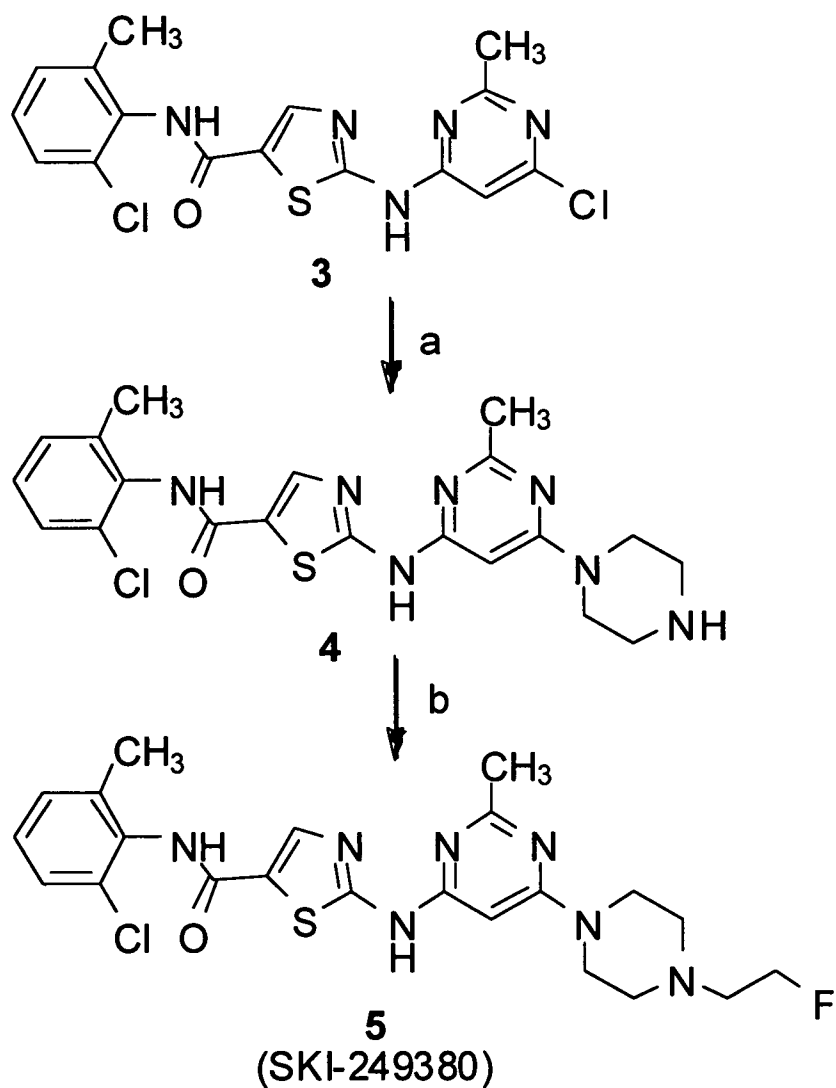
15 15. The method of claim 9, wherein the kinase is Abl, Ack, Csk, EphA2, EphB4, Kit, PDGFR-alpha, Src or Tec.

16. A [^{18}F] labeled compound having the chemical structure:



20 17. A composition comprising the [^{18}F] labeled compound of claim 25 and a physiologically acceptable carrier or adjuvant.

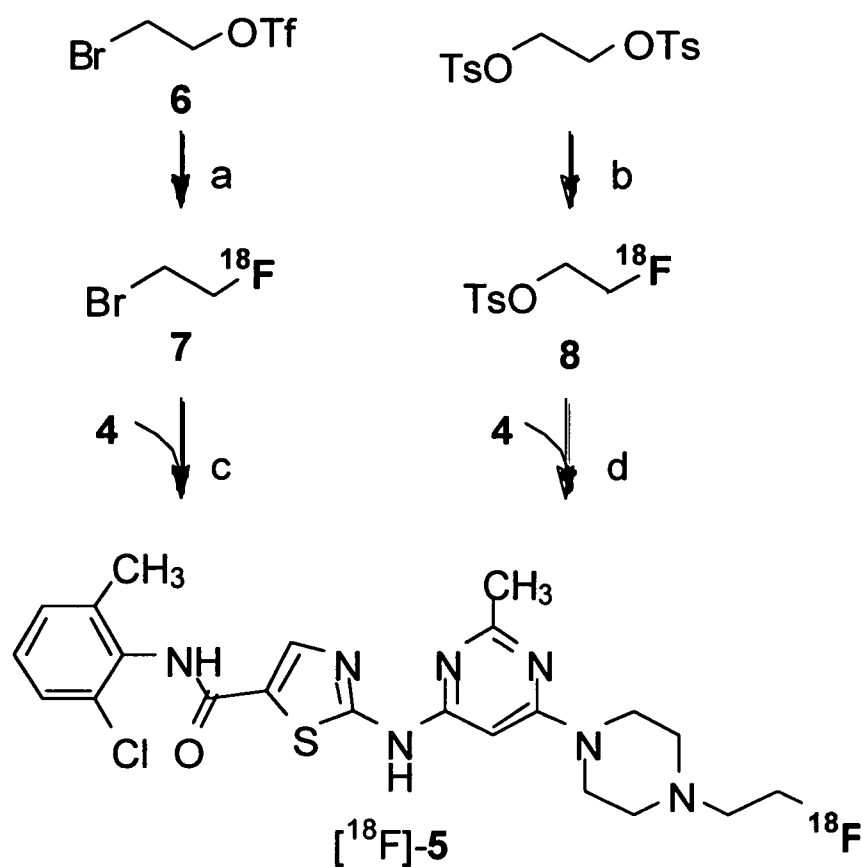
Scheme 1



(a) piperazine, diisopropyl-ethylamine, dioxane, reflux, 12 h, 78%;
(b) 1-bromo-2-fluoroethane, Na₂CO₃, KI, CH₃CN, 60 °C, 4 h, 84%

Fig. 1B

Scheme 2



Reagents and conditions: (a) [^{18}F]-KF:Kryptofix 2.2.2, K_2CO_3 , o-dichlorobenzene, 105 °C, 10 min; (b) [^{18}F]-KF:Kryptofix 2.2.2, K_2CO_3 , CH_3CN , 110 °C, 10 min; (c) NaI, CsCO_3 , 1:1 DMF: CH_3CN , 140 °C, 40 min; (d) DMSO, 160 °C, 30 min.

Fig. 1B

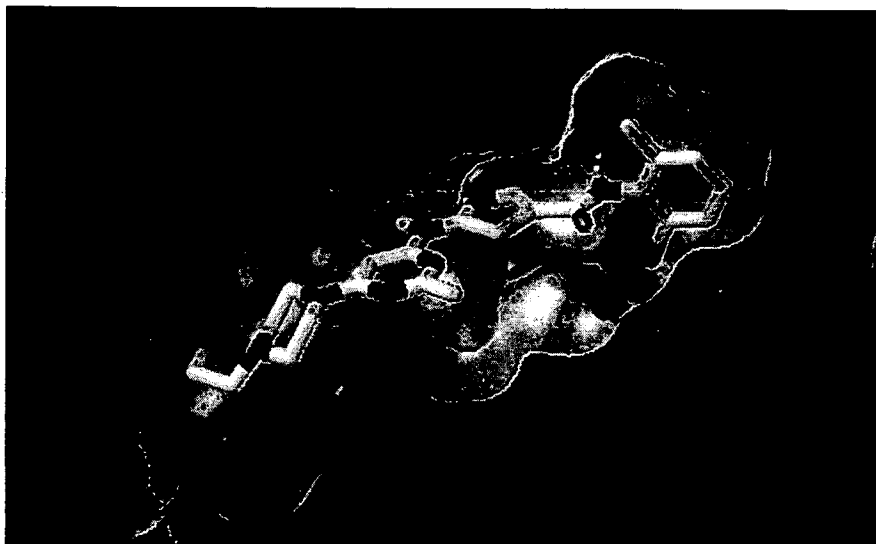


Fig. 2A



Fig. 2B

Fig. 3A

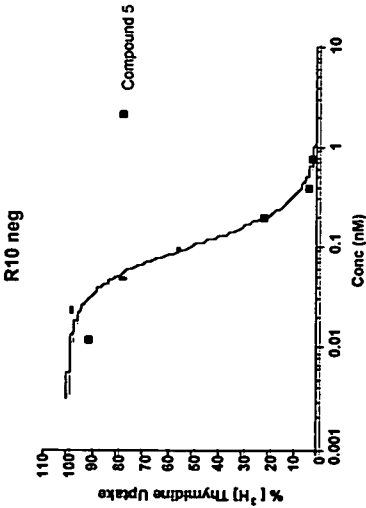


Fig. 3B

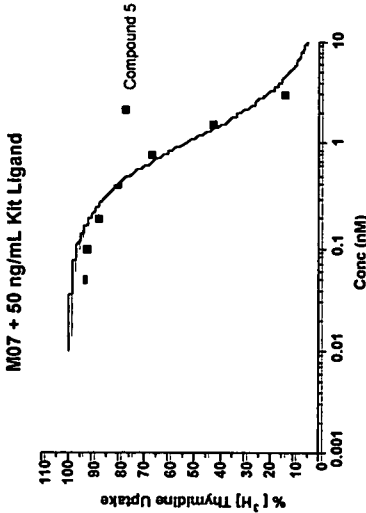


Fig. 3C

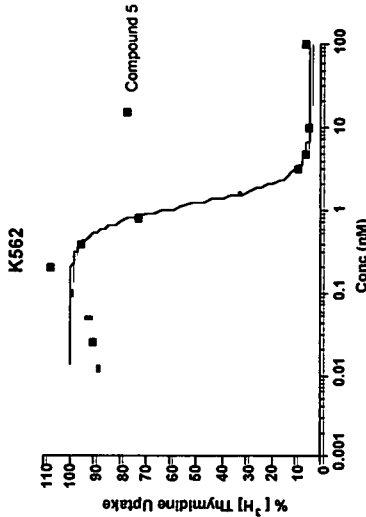


Fig. 3D

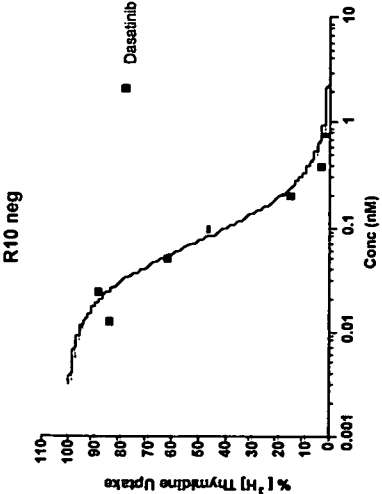


Fig. 3E

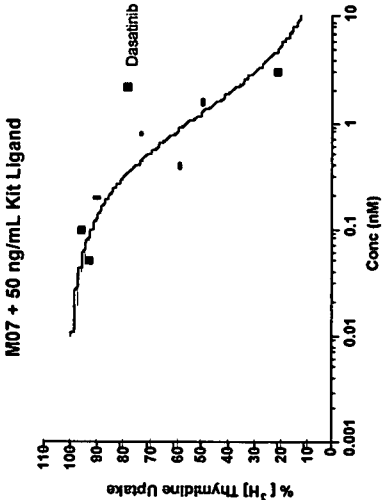
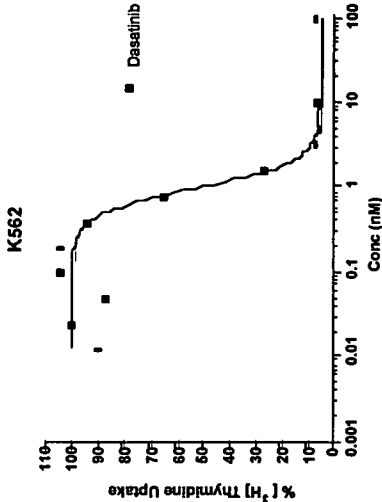


Fig. 3F



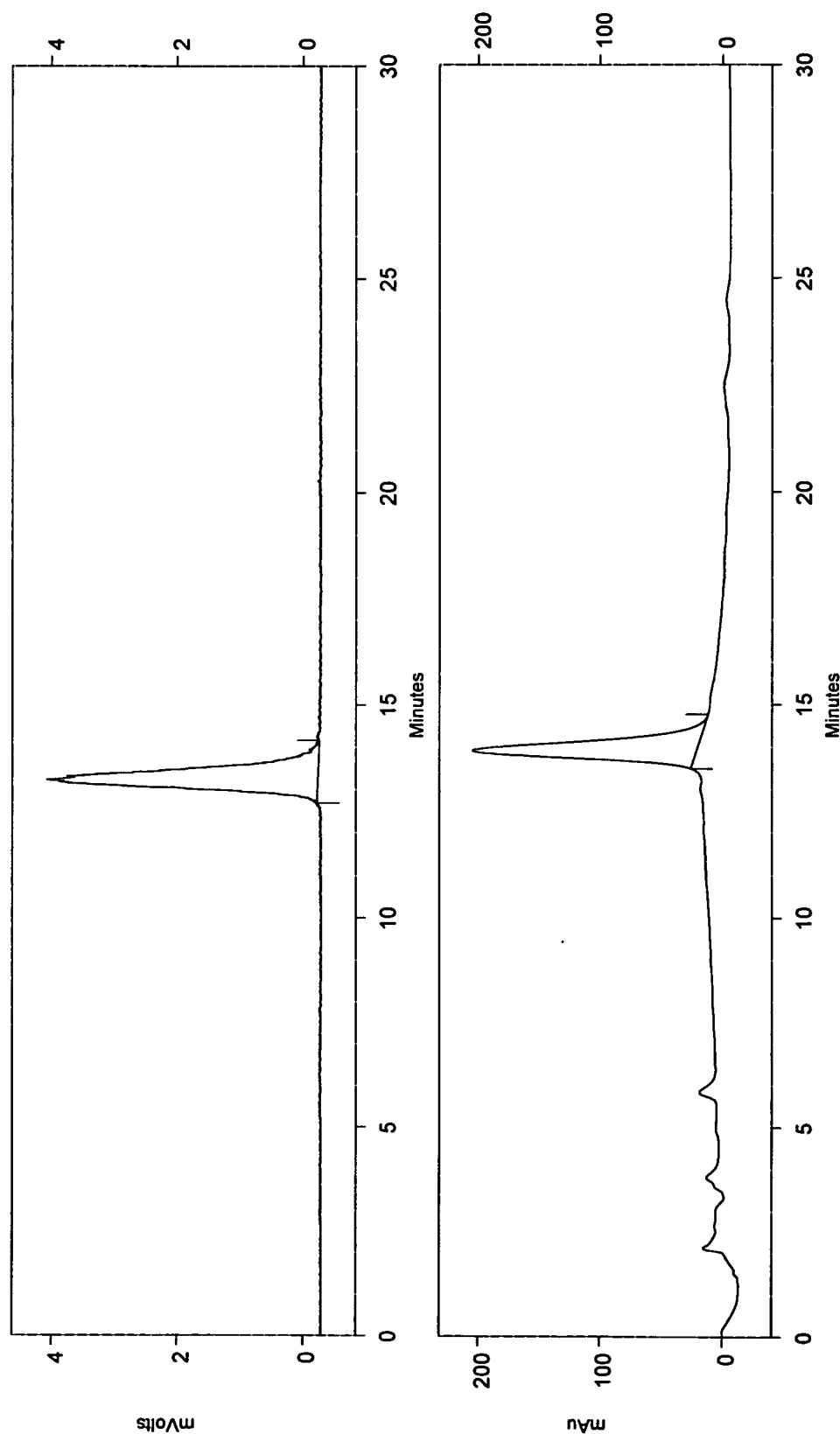


Fig. 4

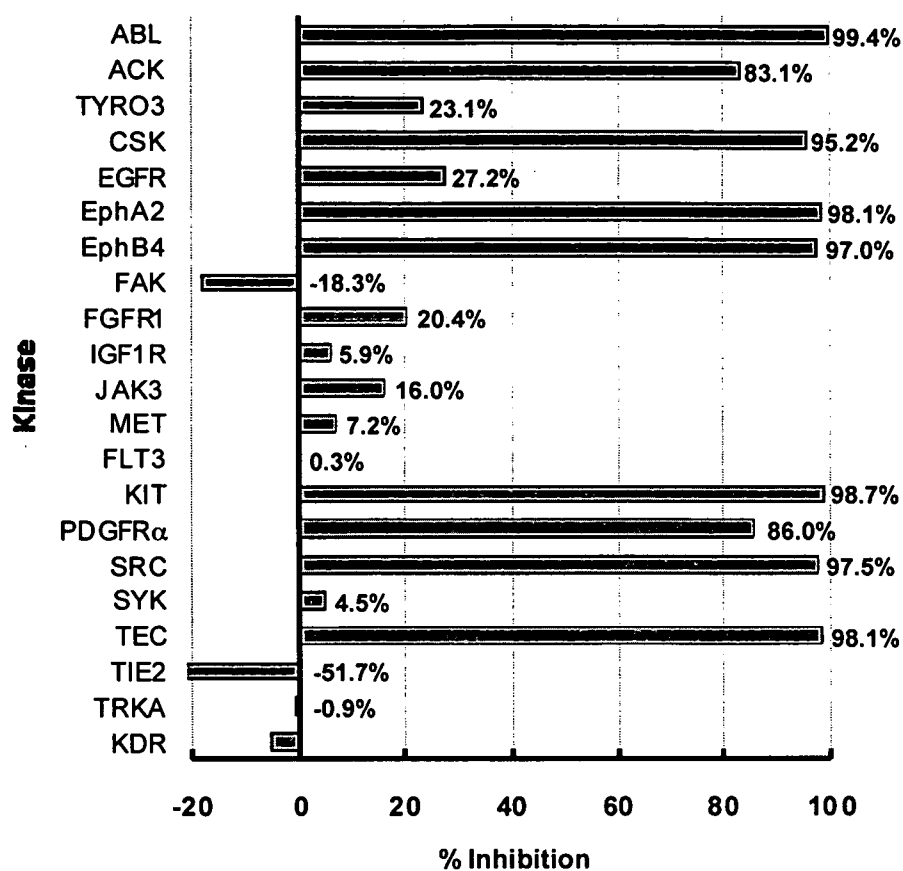


Fig. 5

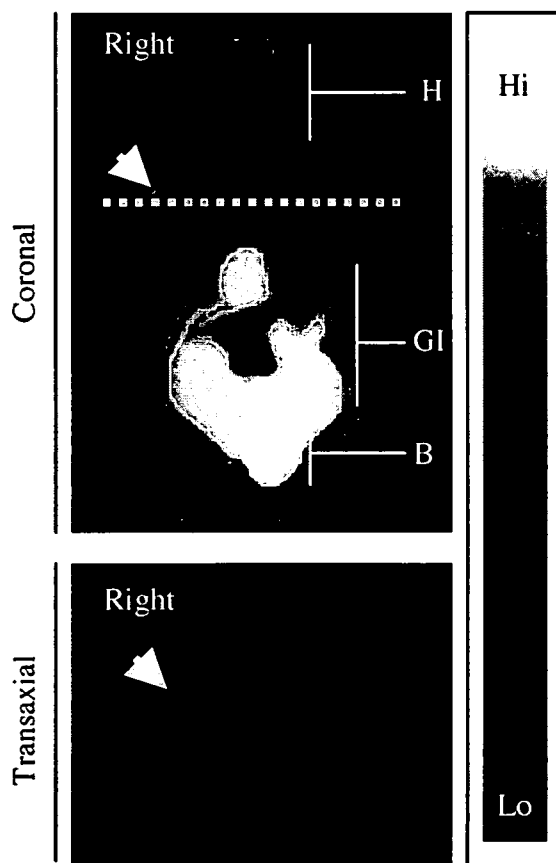


Fig. 6

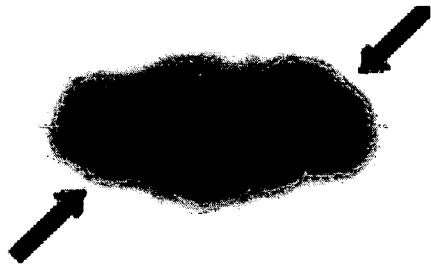


Fig. 7A



Fig. 7B

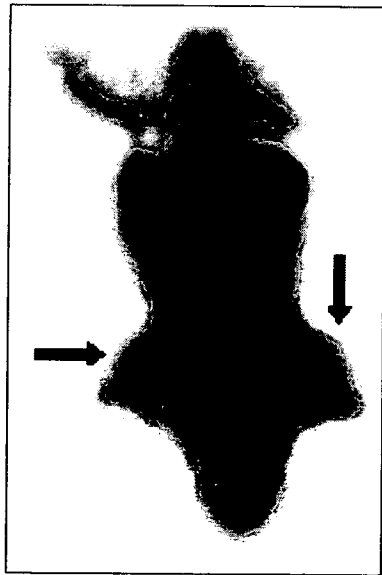


Fig. 7C



Fig. 7D