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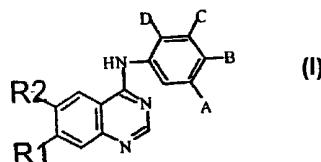
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MOGRAPHY

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(57) Abstract: A radiolabeled compound of formula (I): is described R1 and R2 are each independently selected from the group consisting of hydrogen, alkyl, hydroxy, alkoxy, halo, haloalkyl, carboxy, carbalkoxy and salts thereof; and A, B, C and D are each independently selected from the group consisting of a hydrogen and an electron withdrawing group, provided that at least one of A, B, C and D is [¹⁸] fluorine.

NOVEL EPIDERMAL GROWTH FACTOR RECEPTOR-BINDING
COMPOUNDS FOR POSITRON EMISSION TOMOGRAPHY

FIELD AND BACKGROUND OF THE INVENTION

The present invention relates to novel fluorinated Positron Emission
5 Tomography (PET) biomarkers, and, more particularly, to a fluorinated
biomarkers for quantification of epidermal growth factor receptor tyrosine
kinase.

Positron Emission Tomography (PET), a nuclear medicine imagine
technology which allows the three-dimensional, quantitative determination
10 of the distribution of radioactivity within the human body, is becoming an
increasingly important tool for the measurement of physiological,
biochemical, and pharmacological function at a molecular level, both in
healthy and pathological states. PET requires the administration to the
subject of a molecule labeled with a positron-emitting nuclide (radiotracer)
15 such as ^{15}O , ^{13}N , ^{11}C , and ^{18}F , which have half-lives of 2, 10, 20, and
110 minutes, respectively.

Polypeptides such as growth factors, differentiation factors, and
hormones often mediate their pleiotropic actions by binding to and
activating cell surface receptors with an intrinsic intracellular protein
20 tyrosine kinase activity. Epidermal growth factor receptor-tyrosine kinase
(EGFR-TK) is over expressed in breast cancer and other neoplasia. A
suitable radiotracer that binds to EGFR-TK might allow, through a nuclear
medicine imaging technique such as PET, the mapping and quantification of
this receptor-kinase. This would allow the study of changes in levels of
25 expression of this receptor, including the monitoring of response to
hormonal or other chemotherapy, and could lead to better patient
management and differentiation in regard to therapeutic course of action.

Recently, ^{99}mTc -labeled anti EGFR antibody was synthesized and
biodistribution and dosimetry studies were performed in humans [1, 2].

However this labeled antibody, similar to other protein radiopharmaceuticals, has high and prolonged retention of radioactivity in the liver which constitutes a major problem for clinical applications. Furthermore, these researchers found that it was difficult to obtain accurate 5 quantification of activity in tumors within normal organs because of varying background activities, particularly in lung lesions where fluid and atelectasis could not be differentiated from tumor.

EGF itself has been labeled for nuclear medicine imaging with gamma emitting nuclides including ^{99m}Tc [3, 4] and ^{111}In [5, 6], and the 10 positron-emitting nuclide ^{76}Br [7, 8]. The biodistribution in normal rats of the latter, [^{76}Br]EGF (murine), was reported [8], but no other in vivo studies in laboratory animals or humans have been reported.

4-Anilinoquinazolines have been shown to potently and selectively inhibit EGFR-TK activity by binding reversibly to an inner membrane ATP 15 binding site on EGFR-TK, the prototype for such compounds being the small-molecules PD 153035 [9] and AG 1478 [10] (see Table 1 below). A report of a radioiodinated analog of PD 153035 including in vitro binding studies in MDA-486 cells has been presented [11]. PD 153035 labeled with ^{11}C in the 6,7-methoxy groups has been evaluated in rats implanted with 20 human neuroblastoma xenografts (SH-SY5Y) but specific uptake was not determined in a blocking study [12]. PD 153035 was also labeled with ^{11}C specifically in the 7-methoxy position and biodistribution experiments were 25 performed in normal mice, but uptake specificity could not be demonstrated as administration of an enzyme-blocking dose of PD 153035 caused an increase in tracer uptake in the tissues studied [13]. The same abstract reported the labeling of the 7-(2-fluoroethoxy) PD 153035 analog with ^{18}F , but no biological experiments with this tracer were described. Additionally, the 2-[^{18}F]fluoroethyl group might be subject to a high rate of [^{18}F]hydrogen fluoride elimination to give the corresponding alkene ether,

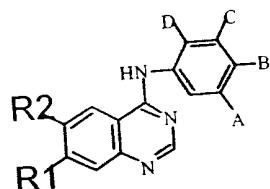
potentially resulting in high uptake of ^{18}F in bone, giving poor in vivo images. Further, these ultra potent ($\text{IC}_{50} < 30 \text{ pM}$) inhibitors may only measure flow or permeability surface area rather than biochemical changes [14]. And, PD 153035 has been shown to undergo metabolism to four 5 compounds, two of which have been identified as the 6- and 7-monodemethylated derivatives, compounds which maintain potency for EGFR-TK inhibition [15]. Thus labeling in the 6- or 7-alkoxy groups as described above, would lead to radioactive metabolites that do not bind EGFR-TK.

10 Another approach to small molecules as EGFR-TK PET tracers are 4-anilinoquinazoline derivatives labeled with ^{18}F on the aniline ring (see reference [16]). Assuming that metabolism of PD 153035 arylfluoro derivatives is similar to the metabolism of PD 153035 itself, in this approach the monodemethylated derivatives would retain the radiolabel and 15 should maintain affinity for EGFR-TK. While the presence in the blood of three compounds with potential to bind the target would complicate kinetic compartmental modeling, the probability of accumulation of radioactivity in the target would be increased. ^{18}F 's half life, five times longer than the half life of ^{11}C , affords a wider time-window for PET measurements than 20 ^{11}C does, possibly allowing the benefit of imaging after disappearance of blood radioactivity and washout of radiotracer nonspecific binding. Since PD 153035 plasma levels decrease from a maximum to 2 % of the maximum after approximately 3 hours [15], later imaging, perhaps an hour or more postinjection (virtually impossible with ^{11}C), may give a more pure 25 signal of EGFR-TK presence. In addition, labeling the aniline ring may result in a more inherently metabolically stable tracer that will lead to low nonspecific uptake of radioactivity in, e.g., bone. Additional related art is disclosed in U.S. Pat. No. 5,710,158; EP 566226B1 and CA 2,086,968.

There is thus a widely recognized need for, and it would be highly advantageous to have, novel fluorinated PET biomarkers devoid of the above limitations.

5 SUMMARY OF THE INVENTION

According to one aspect of the present invention there is provided a compound of a formulae:



10 wherein:

R1 and R2 are each independently selected from the group consisting of hydrogen, alkyl, hydroxy, alkoxy, halo, haloalkyl, carboxy, carbalkoxy and salts thereof; and

15 A, B, C and D are each independently selected from the group consisting of a hydrogen and an electron withdrawing group, provided that at least one of A, B, C and D is an electron withdrawing group.

According to still further features in the described preferred embodiments A and B are each a chlorine atom, C is a hydrogen atom and D is a fluorine atom.

20 According to still further features in the described preferred embodiments A is fluor atom, B and D are each a hydrogen atom, and C is a CF₃ group.

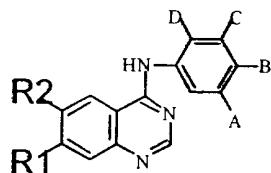
According to still further features in the described preferred embodiments A is a fluorine atom and B, C and D are each a hydrogen atom.

According to still further features in the described preferred embodiments B is a fluorine atom and A, C and D are each a hydrogen atom.

According to another aspect of the present invention there is 5 provided a method of inhibiting tyrosine kinase activity of epidermal growth factor receptor comprising the step of subjecting the epidermal growth factor receptor to the compound described above and which is further described and its activity exemplified in the following sections.

According to yet another aspect of the present invention there is 10 provided a method of treating a patient having impaired tyrosine kinase activity of epidermal growth factor receptor comprising the step of administering to the patient the compound described above and which is further described and its activity exemplified in the following sections.

According to still another aspect of the present invention there is 15 provided a radiolabeled compound of a formulae:



wherein:

R1 and R2 are each independently selected from the group consisting 20 of hydrogen, alkyl, hydroxy, alkoxy, halo, haloalkyl, carboxy, carbalkoxy and salts thereof; and

A, B, C and D are each independently selected from the group consisting of a hydrogen and an electron withdrawing group, provided that at least one of A, B, C and D is [¹⁸]fluorine.

According to further features in preferred embodiments of the invention described below, A and B are each a chlorine atom, C is a hydrogen atom and D is the [¹⁸]fluorine.

According to still further features in the described preferred 5 embodiments A is the [¹⁸]fluorine, B and D are each a hydrogen atom, and C is a CF₃ group.

According to still further features in the described preferred embodiments A is the [¹⁸]fluorine and B, C and D are each a hydrogen atom.

10 According to still further features in the described preferred embodiments B is the [¹⁸]fluorine and A, C and D are each a hydrogen atom.

According to further features in preferred embodiments of the invention described below, the electron withdrawing group is selected from 15 the group consisting of a halogen, SO₃H, NO₂, CN and CF₃.

According to still further features in the described preferred embodiments the halogen is selected from the group consisting of iodine, chlorine, bromine and fluorine.

According to yet an additional aspect of the present invention there is 20 provided a method of monitoring the level of epidermal growth factor receptor within a body of a patient comprising the steps of (a) administering to the patient the radiolabeled compound described above; and employing a nuclear imaging technique for monitoring a distribution of the compound within the body or within a portion thereof.

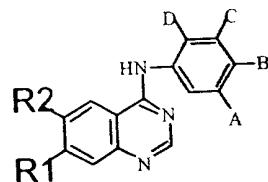
25 According to still further features in the described preferred embodiments the technique is positron emission tomography.

According to still further features in the described preferred embodiments the compounds described herein has IC₅₀ values for

inhibition of tyrosine kinase activity of epidermal growth factor receptor of between 0.1 and 120 nM.

According to an additional aspect of the present invention there is provided a pharmaceutical composition comprising as an active ingredient 5 any of the compounds described herein and a pharmaceutically acceptable carrier.

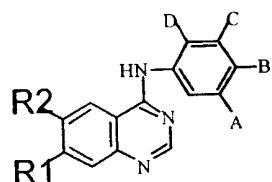
According to a further aspect of the present invention there is provided a method of synthesizing a compound of a general formulae:



10

wherein R1 and R2 are each independently selected from the group consisting of hydrogen, alkyl, hydroxy, alkoxy, halo, haloalkyl, carboxy, carbalkoxy and salts thereof; and A, B, C and D are each independently selected from the group consisting of a hydrogen and an electron withdrawing group, provided that at least one of A, B, C and D is an electron withdrawing group; the method comprising the step of coupling a -6-R1, 7-R2 derivatized 4-chloroquinazoline with an aniline derivatized by the A, B, C and D.

According to still a further aspect of the present invention there is 20 provided a method of synthesizing a radiolabeled compound of a general formulae:



wherein R1 and R2 are each independently selected from the group consisting of hydrogen, alkyl, hydroxy, alkoxy, halo, haloalkyl, carboxy, carbalkoxy and salts thereof; and A, B, C and D are each independently selected from the group consisting of a hydrogen and an electron withdrawing group, provided that at least one of A, B, C and D is a [18]fluorine; the method comprising the step of coupling a 6-R1, 7-R2 derivatized 4-chloroquinazoline with an aniline derivatized by the A, B, C and D.

The present invention successfully addresses the shortcomings of the presently known configurations by providing new biomarkers for PET.

BRIEF DESCRIPTION OF THE DRAWINGS

The invention is herein described, by way of example only, with reference to the accompanying drawings, wherein:

FIG. 1 demonstrates autophosphorylation inhibition curves for the four fluorinated compounds according to the present invention (compounds 1-4) and two reference compounds.

DESCRIPTION OF THE PREFERRED EMBODIMENTS

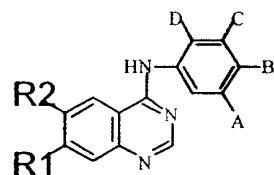
The present invention is of novel compounds which can be used as epidermal growth factor receptor tyrosine kinase inhibitors. Specifically, in their radiolabeled form, the novel compounds can be used as biomarkers for quantification of epidermal growth factor receptor tyrosine kinase using Positron Emission Tomography (PET).

The principles and operation of the present invention may be better understood with reference to the drawings and accompanying descriptions.

Before explaining at least one embodiment of the invention in detail, it is to be understood that the invention is not limited in its application to the details of construction and the arrangement of the components set forth in

the following description or illustrated in the drawings. The invention is capable of other embodiments or of being practiced or carried out in various ways. Also, it is to be understood that the phraseology and terminology employed herein is for the purpose of description and should not be
5 regarded as limiting.

According to one aspect of the present invention there is provided a compound of a formulae:



10 wherein:

R1 and R2 are each independently selected from the group consisting of hydrogen, alkyl, hydroxy, alkoxy, halo, haloalkyl, carboxy, carbalkoxy and salts thereof; and

15 A, B, C and D are each independently selected from the group consisting of a hydrogen and an electron withdrawing group, provided that at least one of A, B, C and D is an electron withdrawing group.

Several compounds corresponding to the above formulae where synthesized (see Table 1 below) and their IC₅₀ values with respect to epidermal growth factor receptor tyrosine kinase activity ranged between
20 0.1 and 120 nM. The present invention is therefore directed specifically at compounds having IC₅₀ values with respect to epidermal growth factor receptor tyrosine kinase activity in the range of 0.1 - 120 nM, preferably 0.1 - 60 nM, more preferably 0.1 - 30 nM, more preferably 0.1 - 15 nM, more preferably 0.1 - 10 nM, more preferably 0.1 - 5 nM, more preferably 0.1 - 1 nM, most preferably 0.1 - 0.5 nM, most preferably, below 0.3 nM.
25

In one compound thus prepared and which is referred to hereinbelow as compound **4**, A and B are each a chlorine atom, C is a hydrogen atom and D is a fluorine atom. The IC₅₀ of compound **4** toward epidermal growth factor receptor tyrosine kinase activity was measured to be 0.22±0.18 nM.

5 In another compound thus prepared and which is referred to hereinbelow as compound **3**, A is fluor atom, B and D are each a hydrogen atom, and C is a CF₃ group. The IC₅₀ of compound **3** toward epidermal growth factor receptor tyrosine kinase activity was measured to be 116±14 nM.

10 In yet another compound thus prepared and which is referred to hereinbelow as compound **2**, A is a fluorine atom and B, C and D are each a hydrogen atom. The IC₅₀ of compound **2** toward epidermal growth factor receptor tyrosine kinase activity was measured to be 19.8±8.2 nM.

15 In still another compound thus prepared and which is referred to hereinbelow as compound **1**, B is a fluorine atom and A, C and D are each a hydrogen atom. The IC₅₀ of compound **1** toward epidermal growth factor receptor tyrosine kinase activity was measured to be 76.1±3.2 nM.

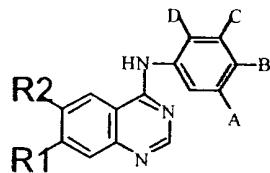
20 The R₁ and R₂ in compounds 1-4 are methoxy groups. It will however be appreciated by one ordinarily skilled in the art that compounds having other groups such as hydrogen, alkyl, hydroxy, alkoxy, halo, haloalkyl, carboxy, carbalkoxy and salts thereof can be readily prepared following the protocols described below, while changing the starting materials accordingly.

25 R₁ and R₂ selection influence the hydrophylicity of the compounds and affects their distribution in fat. Compounds which are less likely to be adsorbed in fat are presently preferred. Compounds having a pair of hydroxyls are less hydrophobic and are therefore expected to be less adsorbed in fat.

The compounds described herein can be used to inhibit epidermal growth factor receptor tyrosine kinase activity which typically results in autophosphorylation and activation in cases of impaired activity thereof. As such, these compounds can be used to treat cancers or other neoplasia 5 characterized by elevation in epidermal growth factor receptor tyrosine kinase activity, due, for example, to overexpression.

As used herein the term "treat" or its equivalent term "treating" includes substantially inhibiting, slowing or reversing the progression of a disease, substantially ameliorating clinical symptoms of a disease or 10 substantially preventing the appearance of clinical symptoms of a disease.

According to another aspect of the present invention there is provided a radiolabeled compound of a formulae:



15 wherein:

R1 and R2 are each independently selected from the group consisting of hydrogen, alkyl, hydroxy, alkoxy, halo, haloalkyl, carboxy, carbalkoxy and salts thereof;

A, B, C and D are each independently selected from the group 20 consisting of a hydrogen and an electron withdrawing group, provided that at least one of A, B, C and D is $[^{18}\text{F}]$ fluorine.

Several compounds corresponding to the above formulae where synthesized (see Table 1 below).

In one compound thus prepared and which is referred to hereinbelow 25 as $[^{18}\text{F}]$ compound 4, A and B are each a chlorine atom, C is a hydrogen atom and D is the $[^{18}\text{F}]$ fluorine.

In another compound thus prepared and which is referred to hereinbelow as [3'¹⁸F]compound 3, A is the [¹⁸F]fluorine, B and D are each a hydrogen atom, and C is a CF₃ group.

5 In yet another compound thus prepared and which is referred to hereinbelow as [¹⁸F]compound 2, A is the [¹⁸F]fluorine and B, C and D are each a hydrogen atom.

In still another compound thus prepared and which is referred to hereinbelow as [¹⁸F]compound 1, B is the [¹⁸F]fluorine and A, C and D are each a hydrogen atom.

10 For each of the compounds herein described the electron withdrawing group can be a halogen (iodine, chlorine, bromine and fluorine), SO₃H, NO₂, CN and CF₃. The number, type and position of the electron withdrawing group(s) affect the affinity, as determined, for example, by IC₅₀, of the compound to the receptor.

15 The radiolabeled compounds herein described can be used to effect a method of monitoring the level of epidermal growth factor receptor within a body of a patient by administering to the patient any of the radiolabeled compound described herein; and employing a nuclear imaging technique, e.g., positron emission tomography, for monitoring a distribution of the 20 compound within the body or within a portion thereof.

Any of the compounds described herein, both the non-labeled and the radiolabeled compounds, can be formulated into a pharmaceutical composition which can be used for treatment of a disease or for nuclear imaging. Such a composition includes as an active ingredient any of the 25 compounds, both radiolabeled or non-labeled, described herein and a pharmaceutically acceptable carrier.

Thus, for therapeutic or prophylactic treatment of diseases, disorders or medical conditions, or for nuclear imaging the compounds of the present invention can be formulated in a pharmaceutical composition, which may

include thickeners, carriers, buffers, diluents, surface active agents, preservatives, and the like, all as well known in the art. Pharmaceutical compositions may also include one or more active ingredients, such as, but not limited to, anti inflammatory agents, anti microbial agents, anesthetics and the like in addition to the compounds described herein.

The pharmaceutical composition may be administered in either one or more of ways depending on whether local or systemic treatment or administration is of choice, and on the area to be treated or diagnosed. Administration may be done topically (including ophtalmically, vaginally, 10 rectally, intranasally), orally, by inhalation, or parenterally, for example by intravenous drip or intraperitoneal, subcutaneous, intramuscular or intravenous injection.

Formulations for topical administration may include but are not limited to lotions, ointments, gels, creams, suppositories, drops, liquids, 15 sprays and powders. Conventional pharmaceutical carriers, aqueous, powder or oily bases, thickeners and the like may be necessary or desirable.

Compositions for oral administration include powders or granules, suspensions or solutions in water or non-aqueous media, sachets, capsules or tablets. Thickeners, diluents, flavorings, dispersing aids, emulsifiers or 20 binders may be desirable.

Formulations for parenteral administration may include, but are not limited to, sterile solutions which may also contain buffers, diluents and other suitable additives. Slow release compositions are envisaged for treatment.

25 Nuclear imaging dosing depend on the affinity of the compound to its receptor, the isotope employed and the specific activity of labeling. Persons ordinarily skilled in the art can easily determine optimum nuclear imaging dosages and dosing methodology.

Treatment dosing is dependent on severity and responsiveness of the condition to be treated, but will normally be one or more doses per day, with course of treatment lasting from several days to several months or until a cure is effected or a diminution of disease state is achieved. Persons 5 ordinarily skilled in the art can easily determine optimum dosages, dosing methodologies and repetition rates.

Additional objects, advantages, and novel features of the present invention will become apparent to one ordinarily skilled in the art upon examination of the following examples, which are not intended to be 10 limiting. Additionally, each of the various embodiments and aspects of the present invention as delineated hereinabove and as claimed in the claims section below finds experimental support in the following examples.

EXAMPLES

Reference is now made to the following examples, which together 15 with the above descriptions, illustrate the invention in a non limiting fashion.

Materials and Experimental Methods

4-Amino-6,7-dimethoxyquinazoline (AG 1477) [18] and 3-fluoro-5-trifluoromethylaniline [17] were prepared according to published 20 methods. All other chemicals were purchased from Sigma Chemical Co. (St. Louis, MO), Fisher Scientific (Pittsburgh, PA), Aldrich Co. (Milwaukee, WI) or Carlo Erba. Chemicals were used as supplied, except DMSO which was stored over activated molecular sieves for at least one day prior to use. Microwave heating was performed in a conventional oven 25 (BR 740XL, Brother) operating at 500 W (full power). Mass spectroscopy was performed in EI mode on an LKB 2091 gas chromatograph-mass spectrometer at the Hadassah-Hebrew University mass spectroscopy facility. ¹H-NMR spectra were obtained on a Bruker AMX 400 MHz, using

tetramethylsilane as internal standard. Elemental analysis was performed at the Hebrew University microanalysis laboratory.

[¹⁸F]Fluoride ion was produced by the ¹⁸O(p, n)¹⁸F nuclear reaction on ~350 μ l enriched [¹⁸O]water (97 % isotopic purity, Rotem, 5 Israel) as a target in the Hadassah-Hebrew University IBA 18/9 cyclotron (Belgium). Reactive organic [¹⁸F]fluoride ion was prepared by adding 10-50 μ L irradiated target water to Kryptofix®2.2.2 (10 mg, 27 μ l) and K₂CO₃ (1 mg) in water-acetonitrile. Azeotropic removal of water with acetonitrile was achieved by heating under a stream of nitrogen. The dried 10 Kryptofix®2.2.2 - potassium [¹⁸F]fluoride was then dissolved in 300 μ L anhydrous DMSO for use in radiolabeling.

HPLC was performed on a Varian 9012Q pump, a Varian 9050 variable wavelength UV detector operating at 254 nm, and a Bioscan Flow-Count radioactivity detector with a NaI crystal. Labeled compounds 15 were purified on a normal phase system using a silica column (5 μ m, 250 \times 10 mm, Primesphere, Phenomenex) and the following mobile phase system: hexane-dichloromethane-methanol, 50:48:2; at 10 minutes, gradient to 35:60:5 over 30 minutes; 5 mL/minutes. Eluent fractions (2.5 ml) were collected on a fraction collector (FC205, Gilson). Analysis of formulated 20 radiotracers was performed on a reversed phase system using a C18 column (5 μ m, 250 \times 4.6 mm, Econosil, Alltech) and the following mobile phase system: water-methanol, 20:80; 1 ml/minutes.

Radiotracer formulation was performed as follows: Selected 25 semi-preparative eluent fractions were transferred to a glass flask and the solution was concentrated in vacuo to dryness. The residue was dissolved in 0.5 ml EtOH and 0.5 ml isotonic saline. The solution was filtered through an EtOH-wetted Millex-FG filter (0.2 μ m, Millipore), and another 4 ml saline was used to rinse the flask and filter, providing a 5 ml, 10 % EtOH, 90 % saline formulation.

Synthesis of 4-[4-Fluorophenyl]amino]-6,7-dimethoxyquinazoline (compound 1):

4-Chloro-6,7-dimethoxyquinazoline (50 mg, 0.22 mmol) and 4-fluoroaniline (21 μ L, 0.22 mmol) were placed in a dry two-neck flask and a condenser was adjusted. DMF (6 mL) was added and the mixture was heated to 130 °C for 30 minutes. After cooling the precipitate was filtered washed with EtOH and dried in a vacuum oven (50 °C). The product was obtained as the hydrochloride salt in 94 % yield (70 mg). 1 H NMR [((CD₃)₂SO) δ 9.47(s, 1H), 8.42(s, 1H), 7.8(s, 1H), 7.77(m, 2H), 7.23(m, 2H), 7.17(s, 1H), 3.94(s, 3H), 3.91(s, 3H)]. MS, m/e: 300 (M⁺), 299 [(M-H)⁺]. Anal. Calcd. for C₁₆H₁₅FClN₃O₂: C, 57.14; H, 4.46; N, 12.50. Found: C, 57.16; H, 4.49; N, 12.38.

Synthesis of 4-[3-Fluorophenyl]amino]-6,7-dimethoxyquinazoline (compound 2):

Employing the same method used for compound 1, 4-chloro-6,7-dimethoxyquinazoline (113 mg, 0.5 mmol) and 3-fluoroaniline (48 μ L, 0.5 mmol) afforded compound 2 as the hydrochloride salt in 98 % yield (166 mg). 1 H NMR [((CD₃)₂SO) δ 11.59(s, 1H), 8.85(s, 1H), 8.43(s, 1H), 7.7(m, 1H), 7.6(m, 1H), 7.5(m, 1H), 7.4(s, 1H), 7.1(s, 1H), 4.1(s, 3H), 3.96(s, 3H)]. MS, m/e: 300 (M⁺), 299 [(M-H)⁺]. Anal. Calcd. for C₁₆H₁₅FClN₃O₂: C, 57.14; H, 4.46; N, 12.50. Found: C, 57.09; H, 4.53; N, 12.49.

Synthesis of 4-[3-Fluoro-5-trifluoromethylphenyl]amino]-6,7-dimethoxyquinazoline (compound 3):

4-Chloro-6,7-dimethoxyquinazoline (113.5 mg, 0.5 mmol) and 3-fluoro-5-trifluoromethylaniline [17] (93 mg, 0.52 mmol) were placed in a dry two-neck flask and a condenser was adjusted. EtOH (8 mL) was added and the mixture was refluxed for 60 minutes. After cooling the precipitate

was filtered washed with EtOH and dried in a vacuum oven (50 °C). The product was obtained as the hydrochloride salt in 78 % Yield (159.5 mg). ^1H NMR [((CD₃)₂SO) δ 8.71(s, 1H), 8.09(m, 1H), 7.64(s, 1H), 7.3(bs, 1H), 7.1(m, 1H), 7.0(m, 1H), 4.05(s, 3H), 3.90(s, 3H). MS, /e: 368 (M⁺). Anal. 5 Calcd. for C₁₇H₁₄F₄ClN₃O₂: C, 50.49; H, 3.46; N, 10.39. Found: C, 50.47; H, 3.59; N, 10.34.

Synthesis of 3,4-Dichloro-6-fluoroaniline (compound 8):

3,4-Dichloro-6-fluoronitrobenzene (compound 9, 474 mg) in 9:1 EtOH-water (7 mL) was added dropwise to a refluxing mixture of 500 μL 10 hydrazine hydrate, 60 mg Raney Nickel in 7 mL Ethanol-water 9:1. After the addition was completed, reflux was maintained for additional 25 minutes. After cooling to room temperature, the mixture was filtered and the solvent evaporated. Purification by silica flesh column chromatography gave 192.5 mg of pure compound 8. ^1H NMR [(CDCl₃) δ 7.1(d, J = 11 Hz, 1H), 6.8(d, J = 11 Hz, 1H). MS, m/e: 179 ([M-H]⁺). 15

Synthesis of 4-[(3,4-Dichloro-6-fluorophenyl)amino]-6,7-dimethoxyquinazoline (compound 4):

4-Chloro-6,7-dimethoxyquinazoline (128.3 mg, 0.56 mmol) and 20 3,4-dichloro-6-fluoroaniline (compound 8, 88.5 mg, 0.49 mmol) were placed in a dry two-neck flask and a condenser was adjusted. iPrOH (6 mL) was added and the mixture heated to 85 °C and treated with 5 μL of HCl (conc.). Reflux was maintained for 30 minutes. After cooling the precipitate was filtered washed with EtOH and dried in a vacuum oven (50 °C). The product was obtained as the hydrochloride salt in 84 % Yield 25 (167.5 mg). ^1H NMR [(CDCl₃) δ 8.91(d, J = 8Hz, 1H), 8.7(s, 1H), 7.29(d, J = 10Hz, 1H), 7.28(s, 1H), 6.9(s, 1H), 4.04(s, 3H), 4.02(s, 3H). MS, m/e: 369 (M⁺). Anal. Calcd. for C₁₆H₁₃FCl₃N₃O₂: C, 47.4; H, 3.21; N, 10.4. Found: C, 47.65; H, 3.21; N, 10.08.

Autophosphorylation Inhibition Experiments:

EGFR-TK source: As a source of EGFR-TK, A431 human epidermoid carcinoma cell lysate was used. A431 cells were grown in DMEM containing 10 % fetal calf serum and antibiotics (penicillin and streptomycin). After several days, the cells were removed from the flasks by incubation at 37 °C with PBS/1 mM EDTA buffer for 1 hour. The pellet obtained with centrifugation of the cell suspension (600 g x 5 minutes at room temperature) was then resuspended in lysis buffer (0.02 M Hepes pH 7.4, 0.125 M NaCl, 1 % Triton X-100, 10 % glycerol) and left in ice for 10 minutes. Cell lysate was obtained with a further centrifugation (10,000 rpm x 10 minutes at 4 °C), and the supernatant was collected and frozen at -70°C in aliquots.

ELISA assay: EGFR-TK autophosphorylation IC50 values were obtained by means an ELISA assay. All the following incubations were performed at room temperature and with constant shaking. After each step the plate was washed with water (5x) and TBST buffer (1x). The final volume for each well was 150 µl. A Corning 96 well ELISA plate was coated with monoclonal anti EGFR antibody m108 (Sugen Inc.) diluted in PBS (pH 8.2), and kept overnight at 4C°.

20 After removing the unbound m108, the plate was washed and PBS containing 5 % milk (1 % fat) was added for the blocking (25 minutes). One aliquot of A431 cell lysate was thawed and added to the plate. The amount of lysate was defined according to a previous test performed without inhibitors for the definition of the best ratio between the amount of 25 m108 and the amount of EGFR-TK in the A431 cell lysate.

After 25 minutes, seven different concentrations of each inhibitor were added, and for each case one well was left as a control. All inhibitors were diluted in TBS/DMSO and the final concentration of DMSO was 0.05 % in each well (including the controls).

After 25 minutes, and without washing the plate, ATP/MnCl₂ solution was added in each well. The final concentration was 3 µM ATP/5 mM MnCl₂. In this step the temperature was kept at 26 °C and the plate was under constant shaking. The incubation with ATP/MnCl₂ was for 5 minutes.

Then, to stop the phosphorylation reaction, EDTA was added (pH 8, final concentration in each well 20 mM) and after 1 minute all the plate was washed.

Afterward, polyclonal anti-phosphotyrosine serum (Sugen, Inc.) was added (dilution of antibody in TBST containing 5 % milk). The incubation was for 45 minutes.

For the colorimetric detection of phosphotyrosine in EGFR-TK, TAGO anti-rabbit peroxidase conjugate antibody (Sugen, Inc.) was added in TBST/5 % milk solution (45 minutes).

After washing, the colorimetric reaction was performed by adding ABTS/H₂O₂ in citrate-phosphate buffer. After 5-10 minutes the plate was read on Dynatec MR 5000 ELISA reader at 405 nm.

The analysis of the data was performed with "Regression" software.

Since the exact concentrations of m108 in the stock solution and EGFR-TK in the cell lysate were unknown, an experiment was performed to determine the optimal dilution of m108 stock and lysate. Stock m108 (approx. 0.2-0.5 µg/µl) was diluted 1:400, 1:600, 1:800 and 1:1000 with PBS, while A431 cell lysate was diluted 1:2, 1:4, 1:6, 1:8, and 1:10. At the end of the experiment, the chosen dilutions of m108 and A431 cell lysate were those in which optical densities were around 1.2-1.4 and 0.09-0.1 for the control group (performed with the same dilutions of m108 and lysate but without adding ATP for the phosphorylation reaction).

Synthesis *of*
4-[4-[¹⁸F]Fluorophenyl]amino]-6,7-dimethoxyquinazoline *(/¹⁸F]*
compound 1):

The Kryptofix®2.2.2 - potassium [¹⁸F]fluoride – DMSO solution described above was added to 2-3 mg 1,4-dinitrobenzene in a screw-top test tube (8 mL, Corning). The tube was capped, shaken and heated in a microwave for 3.5 minutes. After cooling in an ambient water bath, the vial contents were diluted with 10 mL water and loaded onto an activated (EtOH) and equilibrated (water) C18 Sep-Pak (classic, short body, Waters). The cartridge was washed with water (10 mL) and the desired intermediate, 4-[¹⁸F]fluoro-1-nitrobenzene, was eluted with EtOH (2 mL) into a small glass test tube. The reduction vessel was prepared by adding to a flat-bottomed glass vial (25 mL), sequentially, a few borosilicate glass beads, 100 µL 4:1 EtOH-water, 250 µL Raney® Nickel slurry, and 60 µL hydrazine monohydrate. After capping with a septum-equipped screw cap (vented with a large diameter needle) the vial was shaken and placed in a 40 °C heating block. The ethanolic 4-[¹⁸F]fluoro-1-nitrobenzene solution was diluted with 0.5 mL water and added slowly to the reduction vessel. After 5 minutes, the vessel was cooled in an ambient water bath, and the vial content was filtered through a 0.45 µm filter (Puradisc, polypropylene, Whatman) into another flat-bottomed 25 mL vial. To the filtered solution were added 8 mL water and 10 mL ether and by capping and inverting several times to mix, the reduction product, 4-[¹⁸F]fluoroaniline, was extracted into the ether layer. An 8 mL screw-top test tube was charged with 4-5 mg AG 1477 and 300 µL 2-propanol. The ethereal radioaniline solution was added to the tube by passing it through MgSO₄ (2 g) and a new 0.45 µm filter. The ether was removed under He, while warming the tube in an ambient water bath. Concentrated HCl (1 µL) was added and the capped tube was heated in a 110 °C oil bath for 15 minutes. After cooling

the tube in ambient water, the acid was neutralized and the free base liberated with the addition of 50 μ L 5 M NaOH. Dichloromethane (0.3 mL) and hexane (0.3 mL) were added to the tube and the solution was filtered through a 0.2 μ m filter (Acrodisc, nylon, Gelman) and injected onto the semi-preparative normal phase HPLC system described. $[^{18}\text{F}]$ compound 1 eluted with a t_R of 33.7 minutes and was formulated as described with a yield of 11 % from potassium $[^{18}\text{F}]$ fluoride. The formulation was then analyzed by reversed phase HPLC (t_R = 8.76 minutes; chemical purity = 89 %; radiochemical purity > 95 %). At formulation, $[^{18}\text{F}]$ compound 1 had a specific radioactivity of 363 Ci/mmol (13 GBq/ μ mol).

*Synthesis of
4-[$(3-[^{18}\text{F}]$ Fluoro-5-trifluoromethylphenyl)amino]-6,7-dimethoxyquinazoline ($[3'-^{18}\text{F}]$ compound 3):*

The general procedure was similar to that used to synthesize $[^{18}\text{F}]$ compound 1 described above, with the following exceptions: In place of 1,4-dinitrobenzene, 2-3 mg 3,5-dinitrobenzotrifluoride was used in the reaction with $[^{18}\text{F}]$ fluoride ion to provide 3-[^{18}F]fluoro-5-nitrobenzotrifluoride; in place of a liquid-liquid extraction following the reduction step, a second C18 Sep-Pak extraction was used, and elution of 3-[^{18}F]fluoro-5-trifluoromethylaniline was achieved with 2 ml ether. Normal phase semi-preparative HPLC (t_R = 36.4 minutes) provided ($[3'-^{18}\text{F}]$ compound 3). Formulated ($[3'-^{18}\text{F}]$ compound 3) (7 % overall yield) was analyzed by reversed phase HPLC (t_R = 11.6 minutes) indicating >95 % radiochemical purity, >95 % chemical purity and 460 Ci/mmol (17 GBq/ μ mol) specific radioactivity at formulation.

*Synthesis of
4-[$(3,4\text{-Dichloro-6-[}^{18}\text{F}]$ fluorophenyl)amino]-6,7-dimethoxyquinazoline ($[^{18}\text{F}]$ compound 4):*

The general procedure was similar to that used to synthesize $[^{18}\text{F}]$ compund 3 described above, with the following exceptions: In place of 3,5-dinitrobenzotrifluoride, 2-3 mg 1,2-dichloro-4,5-dinitrobenzene was used in the reaction with $[^{18}\text{F}]$ fluoride ion to provide 5 1,-dichloro-4- $[^{18}\text{F}]$ fluoro-5-nitrobenzene. Normal phase semi-preparative HPLC ($t_{\text{R}} = 31.7$ minutes) provided ($[^{18}\text{F}]$ compund 4). Formulated ($[^{18}\text{F}]$ compound 4) (4 % overall yield) was analyzed by reversed phase HPLC ($t_{\text{R}} = 9.1$ minutes) indicating >95 % radiochemical purity, ~90 % chemical purity and 430 Ci/mmol (16 GBq/ μmol) specific radioactivity.

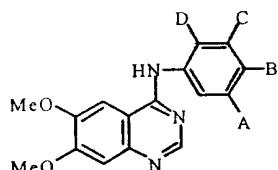
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Experimental Results

In a search for ^{18}F -labeled EGFR-TK PET tracers, four compounds, ^{18}F 1 – 4, were prepared as candidates for future ^{18}F -labeling (Table 1).

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Table 1
Structures and inhibition concentrations

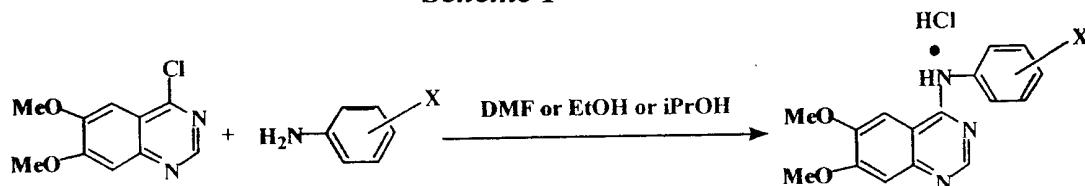


Compound	Substitution (ring position)				Autophosphorylation nM*	IC ₅₀
	A (3')	B (4')	C (5')	D (6')		
1	H	F	H	H	76.1 ± 3.2 (3)	
2	F	H	H	H	19.8 ± 8.2 (2)	
3	F	H	CF ₃	H	116 ± 14 (2)	
4	Cl	Cl	H	F	0.22 ± 0.18 (3)	
PD 153035	Br	H	H	H	0.174 ± 0.012 (2)	
AG 1478	Cl	H	H	H	0.79 ± 0.26 (7)	

*All values shown were determined using an identical method presented as average \pm SEM with the number of replicate analyses indicated in parentheses.

All four compounds were prepared by coupling 4-chloro-6,7-dimethoxyquinazoline with the corresponding aniline derivative in DMF, EtOH or acidic iPrOH (Scheme 1).

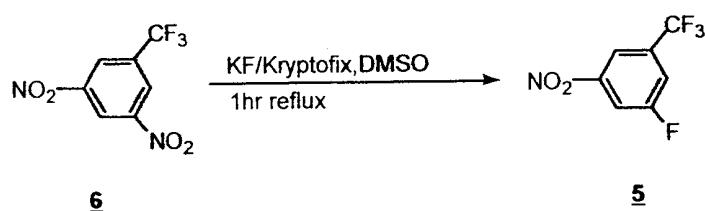
Scheme 1



After 30-60 minutes reflux, the final products were obtained as the hydrochloride salts in approximately 80-90 % yield. In the case of compound 3 3-fluoro-5-trifluoromethyl-nitrobenzene (compound 5) was initially prepared by reacting the dinitro derivative (compound 6) with potassium fluoride and Kryptofix®2.2.2 as phase transfer catalyst in DMSO solution (Scheme 2) [17].

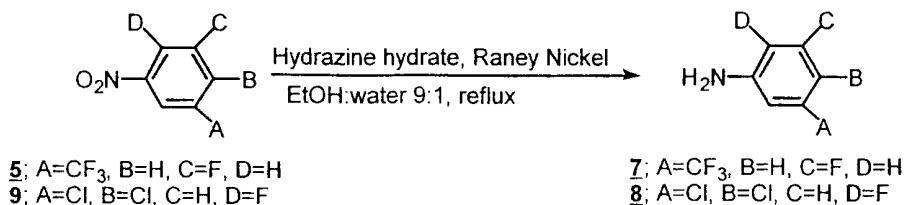
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Scheme 2



Synthon (compound 5) was then reduced in ethanolic solution of hydrazine hydrate and Raney® nickel to furnish the 3-fluoro-5-trifluoromethyl-aniline (compound 7) in 70 % overall yield (Scheme 3) [17].

Scheme 3

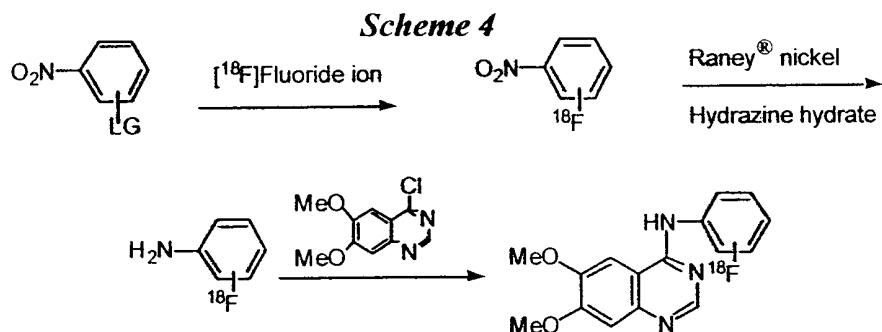


Compound 4 was chosen based on the high inhibition activity of 5 3'-chlorine-containing AG 1478 and because of the symmetrical nature of the starting material to be used in the radiosynthesis, 1,2-dichloro-4,5-dinitrobenzene, which would facilitate the incorporation of 18F. The aniline derivative (compound 8) was obtained from the corresponding nitrobenzene (compound 9) after reduction under the same 10 conditions as described above (Scheme 3).

EGFR-TK autophosphorylation IC₅₀ values were measured for the four fluorinated compounds in order to determine their potential as PET tracers. The method employed an ELISA assay based on an anti-EGFR antibody. Plots of example inhibition curves are shown in Figure 1 and the 15 results are summarized in Table 1 above. For compound 1, substitution of a fluorine atom at the para position on the aniline ring resulted in a moderate (relative to PD 153035 or AG 1478) IC₅₀ of 76 nM. When the fluorine atom was placed at the meta position the biological activity increased and IC₅₀ of 19.8 nM was measured for compound 2. However, this value is 20 higher by two orders of magnitude than the values for AG 1478 and PD 153035 where a heavier halogen such as chlorine or bromine is bonded to the meta position of the aniline ring. It is known that substitution of trifluoromethyl group on aryl moiety in biologically active compounds in most cases enhances the biological activity. However, in this particular case 25 the IC₅₀ measured for compound 3, which contains the trifluoromethyl group at the 5 position in addition to the fluorine atom at the meta position,

was higher than that of compound **2** (116 nM). Based on the autophosphorylation IC₅₀ values, compound **4** was found to be the most potent inhibitor with an IC₅₀ of 0.22 nM, reflecting the importance of the chlorine atom at the meta position. The addition of a chlorine atom at the 5 para position and fluorine at the 6 position contribute to the potency of compound **4** and resulted in an IC₅₀ value which is lower than the one obtained for AG 1478.

Each of compounds **1**, **2**, **3** and **4** was prepared according to Scheme 4, following the same synthetic strategy used to make the non-labeled 10 compounds.



An aryl dinitro derivative was reacted with organic [18F]fluoride ion in DMSO to give the corresponding aryl [18F]fluoronitro derivative in estimated yields of 30 % (compound **2**) or 60-80 % (compounds **1** and **3-4**). A conventional microwave oven was used to heat the reaction mixture. Following C18 solid phase extraction, the nitro residue was reduced to an amino residue in Raney® nickel at 40 °C. The moderate temperature and 15 short reaction time (5 minutes) were critical, as higher temperatures and longer times resulted in apparent over reduction of the [18F]fluoroaniline and loss of radioactivity on the Raney® nickel. The resulting [18F]fluoroaniline was then isolated by filtration and either C18 solid phase or ether-water liquid-liquid extraction (yields for compounds **1** and **3-4** averaged about 75 %, lower yields were obtained for compound **2**). The 20 final reaction mixture was prepared by adding the dried ethereal 25

radioaniline solution to an iPrOH solution of 4-chloro-6,7-dimethoxyquinazoline. The ether was removed by evaporation in a stream of helium gas at room temperature. Performing the ether evaporation in the presence of iPrOH was critical, as evaporation of the ether-radioaniline solution alone either in vacuo (using a rotary evaporator) or under helium gas either with or without heating, resulted in significant or complete co-volatilization of the radioaniline. After acidifying the reaction mixture, the reaction proceeded to the final labeled tracers, [¹⁸F]compound 1, [¹⁸F]compound 2, [^{3'-18}F]compound 3 and [¹⁸F]compound 4, in overall non-decay-corrected yields of about 1 % for compound 2 and 4-12 % for compounds 1 and 3-4 (from potassium [¹⁸F]fluoride and based on the final, formulated product). The entire process was completed within ~120-150 minutes of radionuclide production. In all cases, the products were radiochemically pure. The chemical purity of each formulation, measured by HPLC at 254 nm, remained high at 89 % to >95 %. Specific radioactivity averages, measured at formulation, ranged 363-460 Ci/mmol (13-17 GBq/ μ mol).

Thus, a method was developed for the synthesis of fluorinated EGFR-TK ATP-site inhibitors. Three were found to be of moderate potency and one, compound 4, was a very potent EGFR-TK autophosphorylation inhibitor. The four compounds, including highly potent compound 4, were successfully radiolabeled with ¹⁸F in yields suitable for further use as biological tracers. These compounds can therefore be used to measure differences in EGFR-TK expression and ATP binding site fractional occupancy in vitro and in vivo and be used as PET tracers in, for example, cancer diagnosis, staging and therapy protocol selection, e.g., in predicting which patients would benefit from EGF-directed therapeutic approaches such as those based on anti-EGF antibodies, EGF-directed fusion toxins, or EGFR-TK inhibitors.

Although the invention has been described in conjunction with specific embodiments thereof, it is evident that many alternatives, modifications and variations will be apparent to those skilled in the art.

5 Accordingly, it is intended to embrace all such alternatives, modifications and variations that fall within the spirit and broad scope of the appended claims.

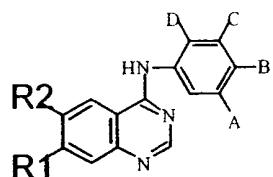
REFERENCES

1. Escobar, N. I.; Morales, A. M.; Ducong?, J.; Torres, I. C.; Fernandez, E.; Gomez, J. A. Pharmacokinetics, biodistribution and dosimetry of 99mTc-labeled anti-human epidermal growth factor receptor humanized monoclonal antibody R3 in rats. *Nucl. Med. Biol.* 1998, 25, 17-23.
2. Iznaga-Escobar, N.; Torres, L. A.; Morales, A.; Ramos, M.; Alvarez, I.; Perez, N.; Fraxedas, R.; Rodr?guez, O.; Rodriguez, N.; Perez, R.; Lage, A.; Stabin, M. G. *J. Nucl. Med.* 1998, 39, 15-23.
3. Capala, J.; Barth, R. F.; Bailey, M. Q.; Fenstermaker, R. A.; Marek, M. J.; Rhodes, B. A. Radiolabeling of epidermal growth factor with Tc and in vivo localization following intracerebral injection into normal and glioma-bearing rats. *Bioconjug. Chem.* 1997, 8, 289-295.
4. Holmberg, A.; Marquez, M.; Westlin, J.-E.; Nilsson, S. Labeling of polypeptides with technetium-99m using a dextran spacer. *Cancer Res.* 1995, 55, 5710s-5713s.
5. Remy, S.; Reilly, R. M.; Sheldon, K.; Gariepy, J. A new radioligand for the epidermal growth factor receptor: In labeled human epidermal growth factor derivatized with a bifunctional metal-chelating peptide. *Bioconjugate Chem.* 1995, 6, 683-690.
6. Reilly, R. M.; Gariepy, J. Investigation of factors influencing the sensitivity of tumor imaging with phantoms and a receptor binding radiopharmaceutical. *J. Nucl. Med.* 1996, 37 (supplement), 199P (abstract number 911).
7. Scott-Robson, S.; Capala, J.; Malmborg, P.; Lundqvist, H. Production of Br and its use in labeling proteins. *Acta Radiol. Suppl.* 1991, 376, 64.

8. Scott-Robson, S.; Capala, J.; Carlsson, J.; Malmborg, P.; Lundqvist, H. Distribution and stability in the rat of a Br/I-labeled polypeptide, epidermal growth factor. *Int. J. Appl. Instrum. [B]* 1991, 18, 241-246.
9. Fry, D. W.; Kraker, A. J.; McMichael, A.; Ambroso, L. A.; Nelson, J. M.; Leopold, W. R.; Connors, R. W.; Bridges, A. J. A specific inhibitor of the epidermal growth factor receptor tyrosine kinase. *Science* 1994, 265, 1093-1095.
10. Levitzki, A.; Gazit, A. *Science* 1995 267, 1782-1788.
11. Mulholland, G. K.; Winkle, W.; Mock, B. H.; Sledge, G. *J. Nucl. Med.* 1995, 36 (supplement), 71P.
12. Johnstrom P., Fredriksson A., Thorell J.-O., and Stone-Elander S. - *J. Labelled Cpd. Radiopharm.* 41 : 623 (1998).
13. Mulholland, G. K.; Zheng, Q.-H.; Winkle, W. L.; Carlson, K. A. *J. Nucl. Med.* 1997, 38, 141P (abstract number 529).
14. Eckelman, W. C. The application of receptor theory to receptor-binding and enzyme-binding oncologic radiopharmaceuticals. *Nucl. Med. Biol.* 1994, 21, 759-769.
15. Kunkel, M. W.; Hook, K. E.; Howard, C. T.; Przybranowski, S.; Roberts, B. J.; Elliot, W. L.; Leopold, W. R. Inhibition of the epidermal growth factor receptor tyrosine kinase by PD 153035 in human A431 tumors in athymic nude mice. *Invest. New Drugs* 1996, 13, 295-302.
16. Lim, J. K.; Riese, D. J.; Negash, K.; Hawkins, R. A.; VanBrocklin, H. F. Synthesis and in vitro evaluation of epidermal growth factor receptor tyrosine kinase inhibitors. *J. Nucl. Med.*, 1998, 39, 20P-21P (conference abstract)
17. Mishani E., Cristel M. E., Dence C. S., McCarthy T. J., and Welch M. J. - *Nucl. Med. Biol.* 24 : 269 (1997).
18. Gazit A., Chen J., App G., McMahon G., Hirth P., Chen I., Levitzki A. *Biorg. Med. Chem.* 8:1203-1207 (1996).

WHAT IS CLAIMED IS:

1. A compound of a formulae:



wherein:

R1 and R2 are each independently selected from the group consisting of hydrogen, alkyl, hydroxy, alkoxy, halo, haloalkyl, carboxy, carbalkoxy and salts thereof; and

A, B, C and D are each independently selected from the group consisting of a hydrogen and an electron withdrawing group, provided that at least one of A, B, C and D is an electron withdrawing group.

2. The compound of claim 1, wherein said electron withdrawing group is selected from the group consisting of a halogen, NH₂, SO₃H, NO₂, CN and CF₃.

3. The compound of claim 2, wherein said halogen is selected from the group consisting of iodine, chlorine, bromine and fluorine.

4. The compound of claim 1, wherein A and B are each a chlorine atom, C is a hydrogen atom and D is a fluorine atom.

5. The compound of claim 1, wherein A is fluor atom, B and D are each a hydrogen atom, and C is a CF₃ group.

6. The compound of claim 1, wherein A is a fluorine atom and B, C and D are each a hydrogen atom.

7. The compound of claim 1, wherein B is a fluorine atom and A, C and D are each a hydrogen atom.

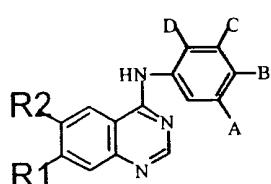
8. The compound of claim 1, having an IC₅₀ value for inhibition of tyrosine kinase activity of epidermal growth factor receptor between 0.1 and 120 nM.

9. A pharmaceutical composition comprising as an active ingredient the compound of claim 1 and a pharmaceutically acceptable carrier.

10. A method of inhibiting tyrosine kinase activity of epidermal growth factor receptor comprising the step of subjecting said epidermal growth factor receptor to the compound of claim 1.

11. A method of treating a patient having impaired tyrosine kinase activity of epidermal growth factor receptor comprising the step of administering to the patient the compound of claim 1.

12. A radiolabeled compound of a formulae:



wherein:

R1 and R2 are each independently selected from the group consisting of hydrogen, alkyl, hydroxy, alkoxy, halo, haloalkyl, carboxy, carbalkoxy and salts thereof; and

A, B, C and D are each independently selected from the group consisting of a hydrogen and an electron withdrawing group, provided that at least one of A, B, C and D is $[^{18}\text{F}]$ fluorine.

13. The compound of claim 12, wherein said electron withdrawing group is selected from the group consisting of a halogen, SO_3H , NO_2 , CN and CF_3 .

14. The compound of claim 13, wherein said halogen is selected from the group consisting of iodine, chlorine, bromine and fluorine.

15. The compound of claim 12, wherein A and B are each a chlorine atom, C is a hydrogen atom and D is said $[^{18}\text{F}]$ fluorine.

16. The compound of claim 12, wherein A is said $[^{18}\text{F}]$ fluorine, B and D are each a hydrogen atom, and C is a CF_3 group.

17. The compound of claim 12, wherein A is said $[^{18}\text{F}]$ fluorine and B, C and D are each a hydrogen atom.

18. The compound of claim 12, wherein B is said $[^{18}\text{F}]$ fluorine and A, C and D are each a hydrogen atom.

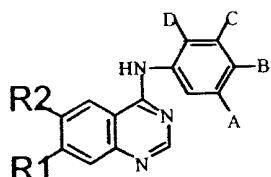
19. The compound of claim 12, having an IC₅₀ value for inhibition of tyrosine kinase activity of epidermal growth factor receptor of between 0.1 and 120 nM.

20. A pharmaceutical composition comprising as an active ingredient the compound of claim 12 and a pharmaceutically acceptable carrier.

21. A method of monitoring the level of epidermal growth factor receptor within a body of a patient comprising the steps of:

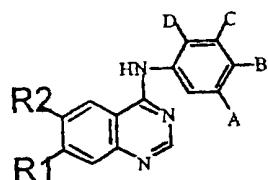
- (a) administering to the patient the compound of claim 12;
- (b) employing a positron emmision tomography for monitoring a distribution of the compound within the body or within a portion thereof.

22. A method of synthesizing a compound of a general formulae:



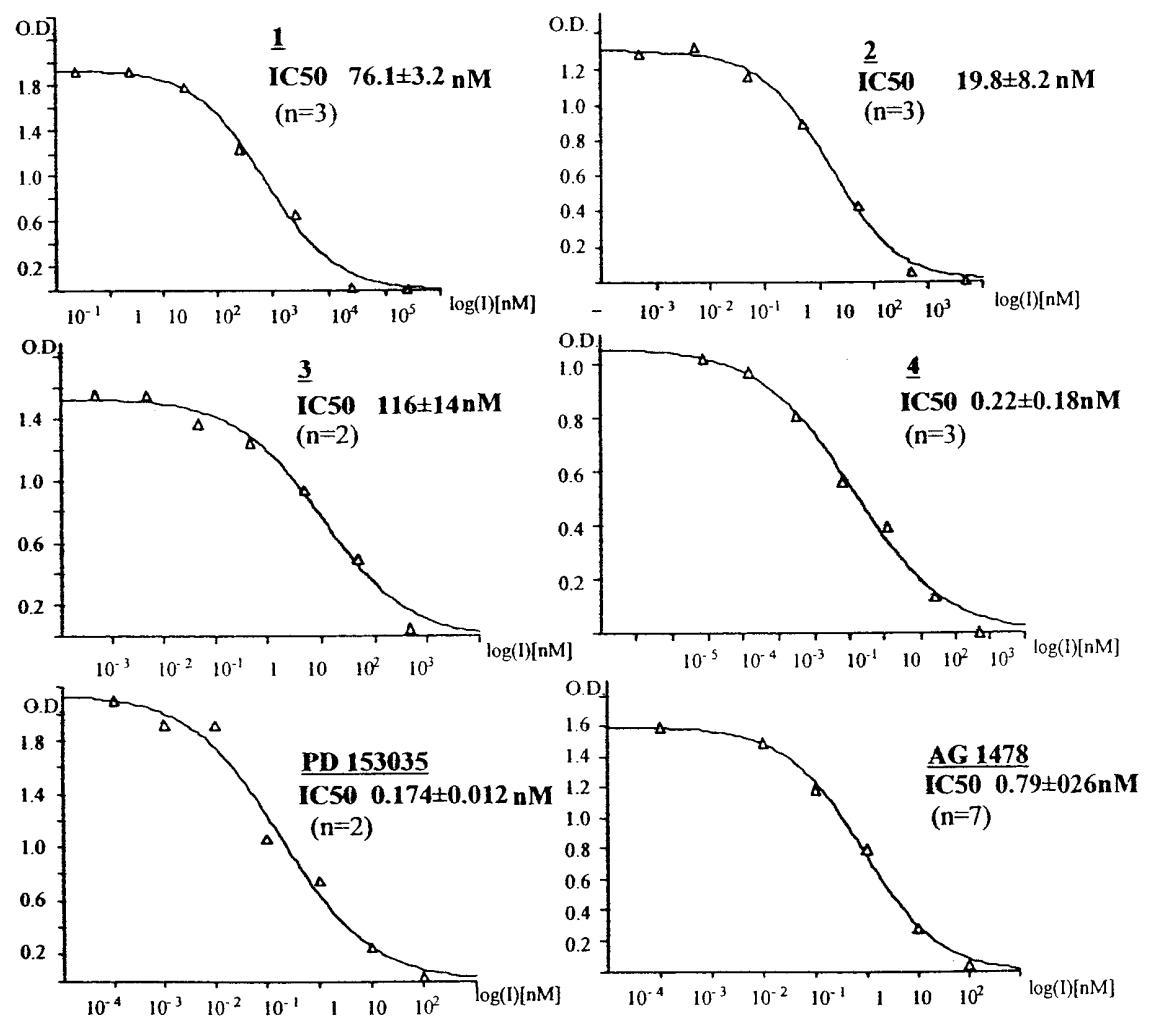
wherein R1 and R2 are each independently selected from the group consisting of hydrogen, alkyl, hydroxy, alkoxy, halo, haloalkyl, carboxy, carbalkoxy and salts thereof; and A, B, C and D are each independently selected from the group consisting of a hydrogen and an electron withdrawing group, provided that at least one of A, B, C and D is an electron withdrawing group; the method comprising the step of coupling a 6-R1, 7-R2 derivatized 4-chloroquinazoline with an aniline derivatized by said A, B, C and D.

23. A method of synthesizing a compound of a formula:



wherein R1 and R2 are each independently selected from the group consisting of hydrogen, alkyl, hydroxy, alkoxy, halo, haloalkyl, carboxy, carbalkoxy and salts thereof; and A, B, C and D are each independently selected from the group consisting of a hydrogen and an electron withdrawing group, provided that at least one of A, B, C and D is a [18]fluorine; the method comprising the step of coupling a 6-R1, 7-R2 substituted 4-chloroquinazoline with an aniline derivatized by said A, B, C and D.

FIGURE 1



INTERNATIONAL SEARCH REPORT

International application No.

PCT/US00/13749

A. CLASSIFICATION OF SUBJECT MATTER

IPC(7) : A61K 31/517; A61P 17/06, 35/00; C07D 239/94
US CL : 514/259; 544/293

According to International Patent Classification (IPC) or to both national classification and IPC

B. FIELDS SEARCHED

Minimum documentation searched (classification system followed by classification symbols)

U.S. : 514/259; 544/293

Documentation searched other than minimum documentation to the extent that such documents are included in the fields searched

Electronic data base consulted during the international search (name of data base and, where practicable, search terms used)
MEDLINE CASONLINE

C. DOCUMENTS CONSIDERED TO BE RELEVANT

Category *	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
X	WO 97/30035 A1 (ZENECA LIMITED) 21 August 1997 (21.08.1997), page 44, Example 8, Example 10, Example 11, Claim 3.	1-3, 8-11, & 22
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Y		4-7
X	US 5,457,105 A (BARKER ET AL) 10 October, 1995 (10.10.1995), column 25, Example 6, Compounds 1-3, column 35, Example 34, Compounds 5 and 7, column 50, Example 73, Compound 3, Claims 1-27.	1-3, 6-11, & 22
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Y		4 & 5
X	US 5,710,158 A (MYERS ET AL) 20 January 1998 (20.01.1998), column 17, first and seventh compounds, Claims 7-10.	1-3, 6, 8-11 & 22
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Y		4, 5, & 7
X	SINGH, P. ET AL. Inhibitors of the epidermal growth factor receptor protein tyrosine kinase. A quantitative structure-activity relationship analysis J. Enzyme Inhib. 1998, Vol. 13, No. 2, pages 125-134, especially compounds 7-56 in Table I on pages 126-127.	1-3, 6, 8-11, & 22
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Y		4, 5, & 7
X	JP 57-144266 A (SANKYO CO. LTD.) 06 September 1982 (06.09.1982), page 532, last paragraph on left and paragraph spanning pages 532-533.	1-3, 7-11, & 22
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Y		4-6

Further documents are listed in the continuation of Box C.

See patent family annex.

* Special categories of cited documents:		"T"	later document published after the international filing date or priority date and not in conflict with the application but cited to understand the principle or theory underlying the invention
"A"	document defining the general state of the art which is not considered to be of particular relevance	"X"	document of particular relevance; the claimed invention cannot be considered novel or cannot be considered to involve an inventive step when the document is taken alone
"E"	earlier application or patent published on or after the international filing date	"Y"	document of particular relevance; the claimed invention cannot be considered to involve an inventive step when the document is combined with one or more other such documents, such combination being obvious to a person skilled in the art
"L"	document which may throw doubts on priority claim(s) or which is cited to establish the publication date of another citation or other special reason (as specified)	"&"	document member of the same patent family
"O"	document referring to an oral disclosure, use, exhibition or other means		
"P"	document published prior to the international filing date but later than the priority date claimed		

Date of the actual completion of the international search

17 August 2000 (17.08.2000)

Date of mailing of the international search report

30 AUG 2000

Name and mailing address of the ISA/US

Commissioner of Patents and Trademarks
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INTERNATIONAL SEARCH REPORT

International application No.

PCT/US00/13749

Box I Observations where certain claims were found unsearchable (Continuation of Item 1 of first sheet)

This international report has not been established in respect of certain claims under Article 17(2)(a) for the following reasons:

1. Claim Nos.:
because they relate to subject matter not required to be searched by this Authority, namely:

2. Claim Nos.:
because they relate to parts of the international application that do not comply with the prescribed requirements to such an extent that no meaningful international search can be carried out, specifically:

3. Claim Nos.:
because they are dependent claims and are not drafted in accordance with the second and third sentences of Rule 6.4(a).

Box II Observations where unity of invention is lacking (Continuation of Item 2 of first sheet)

This International Searching Authority found multiple inventions in this international application, as follows:
Please See Continuation Sheet

1. As all required additional search fees were timely paid by the applicant, this international search report covers all searchable claims.
2. As all searchable claims could be searched without effort justifying an additional fee, this Authority did not invite payment of any additional fee.
3. As only some of the required additional search fees were timely paid by the applicant, this international search report covers only those claims for which fees were paid, specifically claims Nos.:

4. No required additional search fees were timely paid by the applicant. Consequently, this international search report is restricted to the invention first mentioned in the claims; it is covered by claims Nos.: 1-11 and 22

Remark on Protest The additional search fees were accompanied by the applicant's protest.
 No protest accompanied the payment of additional search fees.

INTERNATIONAL SEARCH REPORT

International application No.

PCT/US00/13749

BOX II. OBSERVATIONS WHERE UNITY OF INVENTION IS LACKING This application contains the following inventions or groups of inventions which are not so linked as to form a single general inventive concept under PCT Rule 13.1. In order for all inventions to be examined, the appropriate additional examination fees must be paid.

Group I, claim(s) 1-11 and 22, drawn to non-radiolabeled quinazolones.

Group II, claim(s) 12-21 and 23, drawn to ¹⁸F-quinazolones.

The inventions listed as Groups do not relate to a single general inventive concept under PCT Rule 13.1 because, under PCT Rule 13.2, they lack the same or corresponding special technical features for the following reasons:.

The inventions listed as Groups I and II do not relate to a single general inventive concept under PCT Rule 13.1 because, under PCT Rule 13.2, they lack the same or corresponding special technical features for the following reasons: the technical feature linking these claims is a 4-anilinoquinazolone with specifically claimed substituents for A, B, C, D, R₁, and R². This technical feature can not be a special technical feature because such compounds are known. For example, the compound with CAS registry number 202475-55-6 is disclosed in US 5710158 A (MYERS ET AL) 20 January 1998. It has B = C =D = hydrogen, A = fluorine, and R₁ = R₂ = methoxy. The compounds of Group I have a therapeutic utility, which is different from the diagnostic utility of the compounds of Group II.