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(71) **Applicant**: **EXELIXIS, INC.** [US/US]; 210 East Grand Avenue, South San Francisco, CA 94080 (US).

(72) **Inventors**: **AFTAB, Dana, T.**; 736 Tamarack Drive, San Rafael, CA 94903 (US). **NAGANATHAN, Sriram**; 599 Curie Drive, San Jose, CA 95123 (US). **XU, Wei**; 327 Glasgow Circle, Danville, CA 94526 (US). **LACY, Steven**; 17 Aragon Blvd., San Mateo, CA 94402 (US).

(74) **Agent**: **BERVEN, Heidi**; Honigman Miller Schwartz and Cohn LLP, 350 East Michigan Avenue, Suite 300, Kalamazoo, MI 49007-3800 (US).

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(54) **Title**: DOSING OF CABOZANTINIB FORMULATIONS

(57) **Abstract**: The invention relates to administration of various pharmaceutical formulations of N- (4-{[6,7-bis(methyloxy)quinolin-4-yl]oxy}phenyl)-N'-(4-fluorophenyl)cyclopropane-1,1- dicarboxamide, (cabozantinib) a c-Met inhibitor, and its metabolites, to achieve desirable pharmacokinetic and pharmacodynamic effects.

Dosing of Cabozantinib Formulations

Priority Claim

[0001] This application claims priority to United States Application Serial No. 61/954,352, filed March 17, 2014. The entire contents of the aforementioned application are incorporated herein by reference.

Technical Field

[0002] The invention relates to administration of various pharmaceutical formulations of N-(4-{[6,7-bis(methyloxy)quinolin-4-yl]oxy}phenyl)-N'-(4-fluorophenyl)cyclopropane-1,1-dicarboxamide, (cabozantinib) a c-Met inhibitor, and its metabolites, to achieve desirable pharmacokinetic and pharmacodynamic effects.

Background

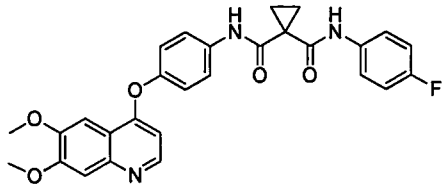
[0003] Traditionally, dramatic improvements in the treatment of cancer are associated with identification of therapeutic agents acting through novel mechanisms. One mechanism that can be exploited in cancer treatment is the modulation of protein kinase activity because signal transduction through protein kinase activation is responsible for many of the characteristics of tumor cells. Protein kinase signal transduction is of particular relevance in, for example, thyroid, gastric, head and neck, lung, breast, prostate, and colorectal cancers, as well as in the growth and proliferation of brain tumor cells.

[0004] Protein kinases can be categorized as receptor type or non-receptor type. Receptor-type tyrosine kinases are comprised of a large number of transmembrane receptors with diverse biological activity. For a detailed discussion of the receptor-type tyrosine kinases, see Plowman et al., DN&P 7(6): 334-339, 1994. Since protein kinases and their ligands play critical roles in various cellular activities, deregulation of protein kinase enzymatic activity can lead to altered cellular properties, such as uncontrolled cell growth associated with cancer. In addition to oncological indications, altered kinase signaling is implicated in numerous other pathological diseases, including, for example, immunological disorders, cardiovascular diseases, inflammatory diseases, and degenerative diseases. Therefore, protein kinases are attractive targets for small molecule drug discovery. Particularly attractive targets for small-molecule modulation with respect to antiangiogenic and antiproliferative activity include receptor type tyrosine kinases Ret, c-Met, and VEGFR2.

[0005] The kinase c-Met is the prototypic member of a subfamily of heterodimeric receptor tyrosine kinases (RTKs) which include Met, Ron, and Sea. The endogenous ligand for c-Met is the hepatocyte growth factor (HGF), a potent inducer of angiogenesis. Binding

for c-Met is the hepatocyte growth factor (HGF), a potent inducer of angiogenesis. Binding of HGF to c-Met induces activation of the receptor via autophosphorylation resulting in an increase of receptor dependent signaling, which promotes cell growth and invasion. Anti-HGF antibodies or HGF antagonists have been shown to inhibit tumor metastasis *in vivo* (See Maulik et al, Cytokine & Growth Factor Reviews, 2002, 13, 41-59). c-Met, VEGFR2, and/or Ret overexpression has been demonstrated on a wide variety of tumor types, including breast, colon, renal, lung, squamous cell myeloid leukemia, hemangiomas, melanomas, and astrocytic tumor (which includes glioblastoma, giant cell glioblastoma, gliosarcoma, and glioblastoma with oligodendroglial components). The Ret protein is a transmembrane receptor with tyrosine kinase activity. Ret is mutated in most familial forms of medullary thyroid cancer. These mutations activate the kinase function of Ret and convert it into an oncogenic form.

[0006] Accordingly, small-molecule compounds that specifically inhibit, regulate, and/or modulate the signal transduction of kinases, particularly including Ret, c-Met, and VEGFR2 described above, are particularly desirable as a means to treat or prevent disease states associated with abnormal cell proliferation and angiogenesis. One such small-molecule is XL184, known variously as N-(4-{[6,7-bis(methyloxy)quinolin-4-yl]oxy}phenyl)-N'-(4-fluorophenyl)cyclopropane-1,1-dicarboxamide, cabozantinib, and as COMETRIQ™ (the S-malate salt of cabozantinib). Cabozantinib has the chemical structure:



In November, 2012, cabozantinib achieved regulatory approval in the United States for the treatment of progressive metastatic medullary thyroid cancer. Other clinical trials of cabozantinib are ongoing.

[0007] WO 2005/030140, incorporated herein by reference, describes the synthesis of cabozantinib (Example 48) and also discloses the therapeutic activity of this molecule to inhibit, regulate, and/or modulate the signal transduction of kinases, (Assays, Table 4, entry 289). Example 48 is on paragraph [0353] in WO 2005/030140.

[0008] A need remains for identifying pharmaceutical formulations and dosing schedules for cabozantinib for the treatment of cancer.

Summary of the Invention

[0009] These and other needs are met by the present invention, which is directed to various of pharmaceutical formulations of N-(4-{[6,7-bis(methyloxy)quinolin-4-yl]oxy}phenyl)-N'-(4-fluorophenyl)cyclopropane-1,1-dicarboxamide, (cabozantinib) a c-Met inhibitor, and its metabolites, to achieve desired pharmacokinetic and pharmacodynamic effects.

[0010] In one aspect, the invention relates to a pharmaceutical formulation comprising a physiologically effective amount of cabozantinib, wherein oral administration of said pharmaceutical formulation to a selected human subject group produces in said selected human subject group:

an average cabozantinib plasma area under the curve (average AUC) of at least 60,000 ng·h/mL per each 140 mg dosage of cabozantinib delivered;

an average maximum cabozantinib blood plasma concentration (average C_{max}) of at least 1000 ng/mL per each 140 mg dosage of cabozantinib delivered; and

a ratio of AUC(cabozantinib) to the AUC of the sum of cabozantinib plus measured cabozantinib metabolites:

AUC (Cabozantinib) / AUC (Cabozantinib + Measured Metabolites)

of at least 0.25;

wherein the AUC is measured from time zero to the time of last measurable concentration.

[0011] In another aspect, the invention relates to a pharmaceutical formulation comprising a physiologically effective amount of cabozantinib, wherein oral administration of said pharmaceutical formulation to a selected human subject group produces in said selected human subject group:

a steady state area under the curve for cabozantinib (average AUC) of at least 35,000 ng·h/mL for a formulation comprising 100 mg of cabozantinib delivered once daily;

an average maximum cabozantinib blood plasma concentration (average C_{max}) of at least 2400 ng/mL per each 100 mg dosage of cabozantinib delivered;

an average minimum cabozantinib blood plasma concentration (average C_{max}) of at least 1100 ng/mL per each 100 mg dosage of cabozantinib delivered; and

a ratio of AUC(cabozantinib) to the AUC of the sum of cabozantinib plus measured cabozantinib metabolites:

AUC (Cabozantinib) / AUC (Cabozantinib + Measured Metabolites)

of at least 0.35;

wherein the AUC is measured on day 22.

[0012] In a further aspect, the invention relates to a method of treating cancer in a human patient, comprising orally administering to said patient a pharmaceutical formulation comprising a physiologically effective amount of cabozantinib at such a rate to achieve:

an average cabozantinib plasma area under the curve (average AUC) of at least 60,000 ng·h/mL per each 140 mg dosage of cabozantinib delivered;

an average maximum cabozantinib blood plasma concentration (average C_{max}) of at least 1000 ng/mL per each 140 mg dosage of cabozantinib delivered; and

a ratio of AUC(cabozantinib) to the AUC of the sum of cabozantinib plus measured cabozantinib metabolites:

AUC (Cabozantinib) / AUC (Cabozantinib + Measured Metabolites)

of at least 0.25;

wherein the AUC is measured from time zero to the time of last measurable concentration.

[0013] In another aspect, the invention relates to a method of treating cancer in a human patient, comprising orally administering to said patient a pharmaceutical formulation comprising a physiologically effective amount of cabozantinib at such a rate to achieve:

a steady state area under the curve for cabozantinib (average AUC) of at least 35,000 ng·h/mL for a formulation comprising 100 mg of cabozantinib delivered once daily;

an average maximum cabozantinib blood plasma concentration (average C_{max}) of at least 2400 ng/mL per each 100 mg dosage of cabozantinib delivered;

an average minimum cabozantinib blood plasma concentration (average C_{max}) of at least 1100 ng/mL per each 100 mg dosage of cabozantinib delivered; and

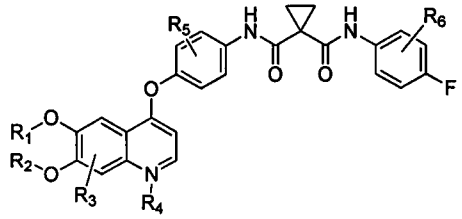
a ratio of AUC(cabozantinib) to the AUC of the sum of cabozantinib plus measured cabozantinib metabolites:

AUC (Cabozantinib) / AUC (Cabozantinib + Measured Metabolites)

of at least 0.35;

wherein the AUC is measured on day 22.

[0014] In these aspects, "metabolite" refers to a compound of formula Ia



Ia

having one or more of the following attributes:

a) one of R_1 or R_2 is H, SO_3H, or a glucuronic acid moiety, and the other is Me;

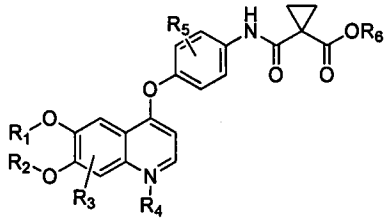
b) R_3 is OH or OSO_3H;

c) R_4 is O^-, provided that when R_4 is O^-, N is N^+;

d) R_5 is OH, or OSO_3H; and

e) R_6 is OH or OSO_3H.

[0015] "Metabolite" also refers to a compound of a compound of formula Ib



Ib

wherein:

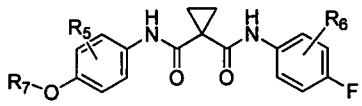
a) R_1 or R_2 are Me; or one of R_1 or R_2 is H, SO_3H, or a glucuronic acid moiety, and the other is Me;

b) R_3 is H, OH, or OSO_3H;

c) R_4 is absent or is O^-, provided that when R_4 is O^-, N is N^+; and

d) R_6 is H or Me.

[0016] "Metabolite" also refers to a compound of a compound of formula Ic



Ic

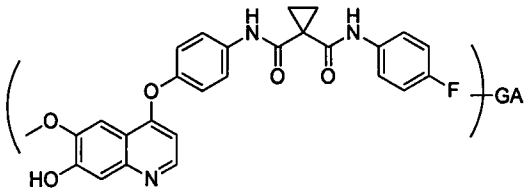
wherein:

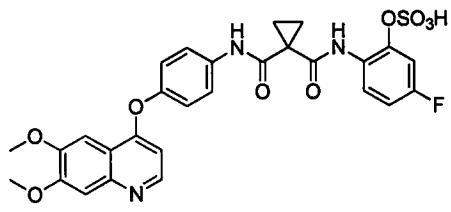
a) R_5 is is OH or OSO_3H; and

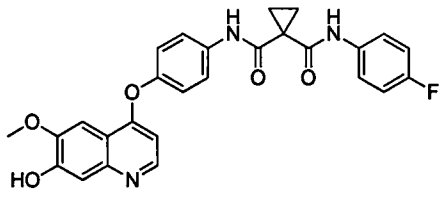
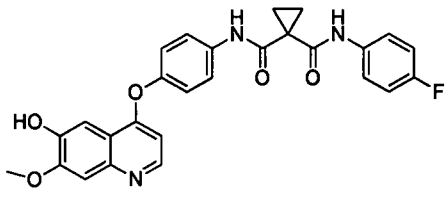
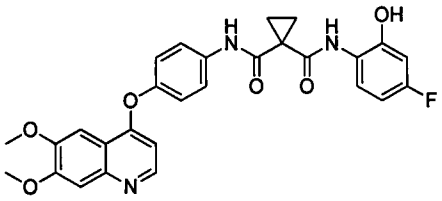
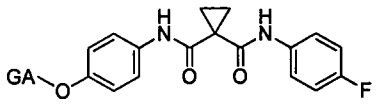
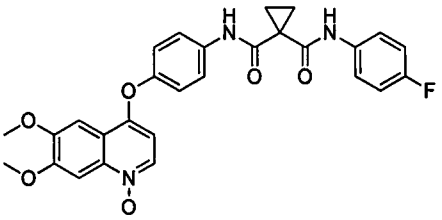
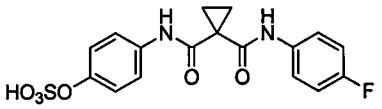
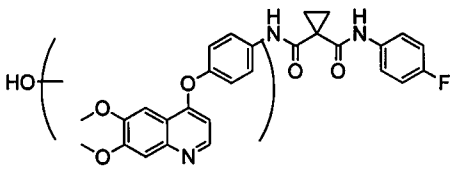
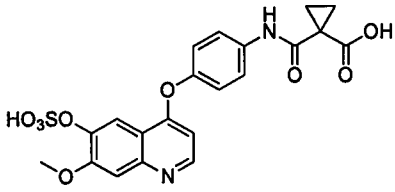
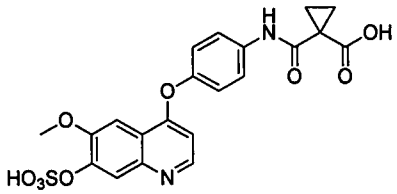
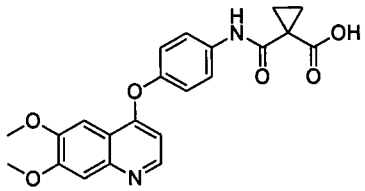
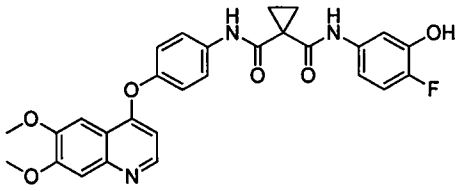
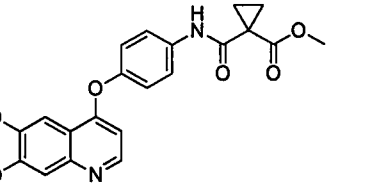
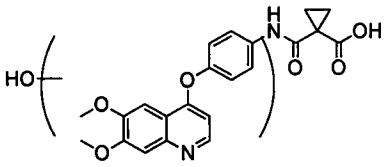
b) R_6 is OH or OSO_3H; and

c) R_7 is H, SO_3H, or a glucuronic acid moiety.

[0017] Specifically the metabolites include:



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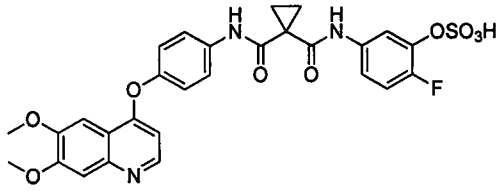
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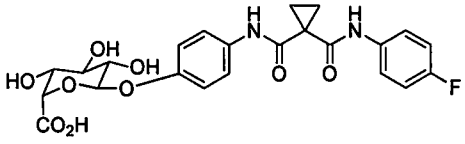
wherein GA is a glucuronic acid moiety such as in, for example,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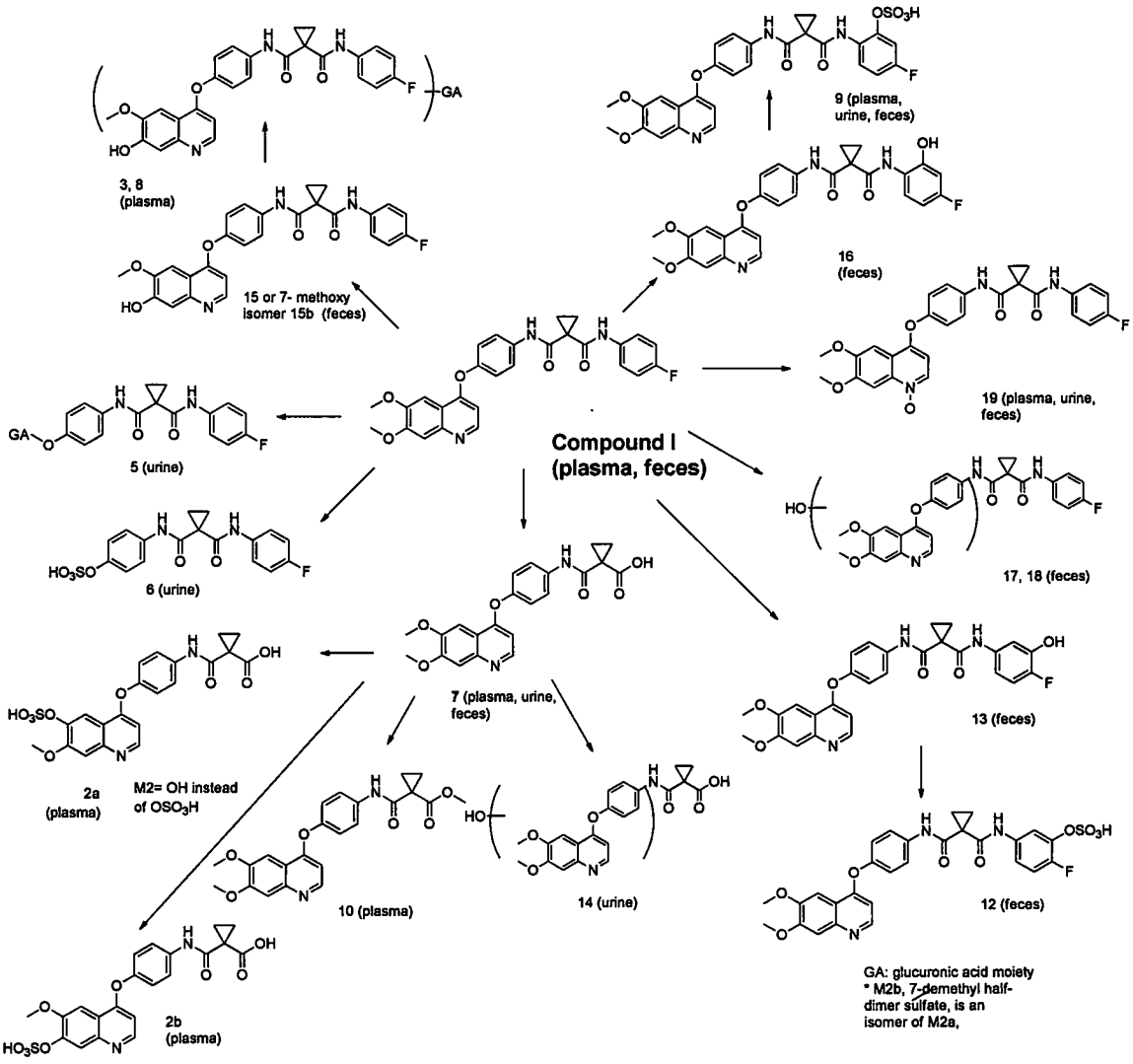
Detailed Description of the Invention

[0018] The metabolites described herein may be referred to hereinafter as "human metabolites." Human metabolites of cabozantinib include metabolites of cabozantinib that were formed in the bodies of human subjects after ingestion or application of cabozantinib according to clinical protocols regarding dosing and monitoring, including those described herein. In various embodiments, the term encompasses molecular species formed in vivo, whether or not the species is even detected or analyzed in a particular trial. It is also contemplated that some metabolites are unique to particular individuals, reflecting different genetic make-up and the presence and activity of various enzymes, including cytochrome P450 and UGT enzymes which are involved in metabolism. Thus, human metabolites cover all such metabolites formed in the human body.

[0019] Some human metabolites are depicted in Scheme 1. These human metabolites were identified during clinical studies of cabozantinib, which appears as compound **I** in Scheme 1, by metabolic profiling, particularly from human plasma, urine, and feces.

Scheme 1



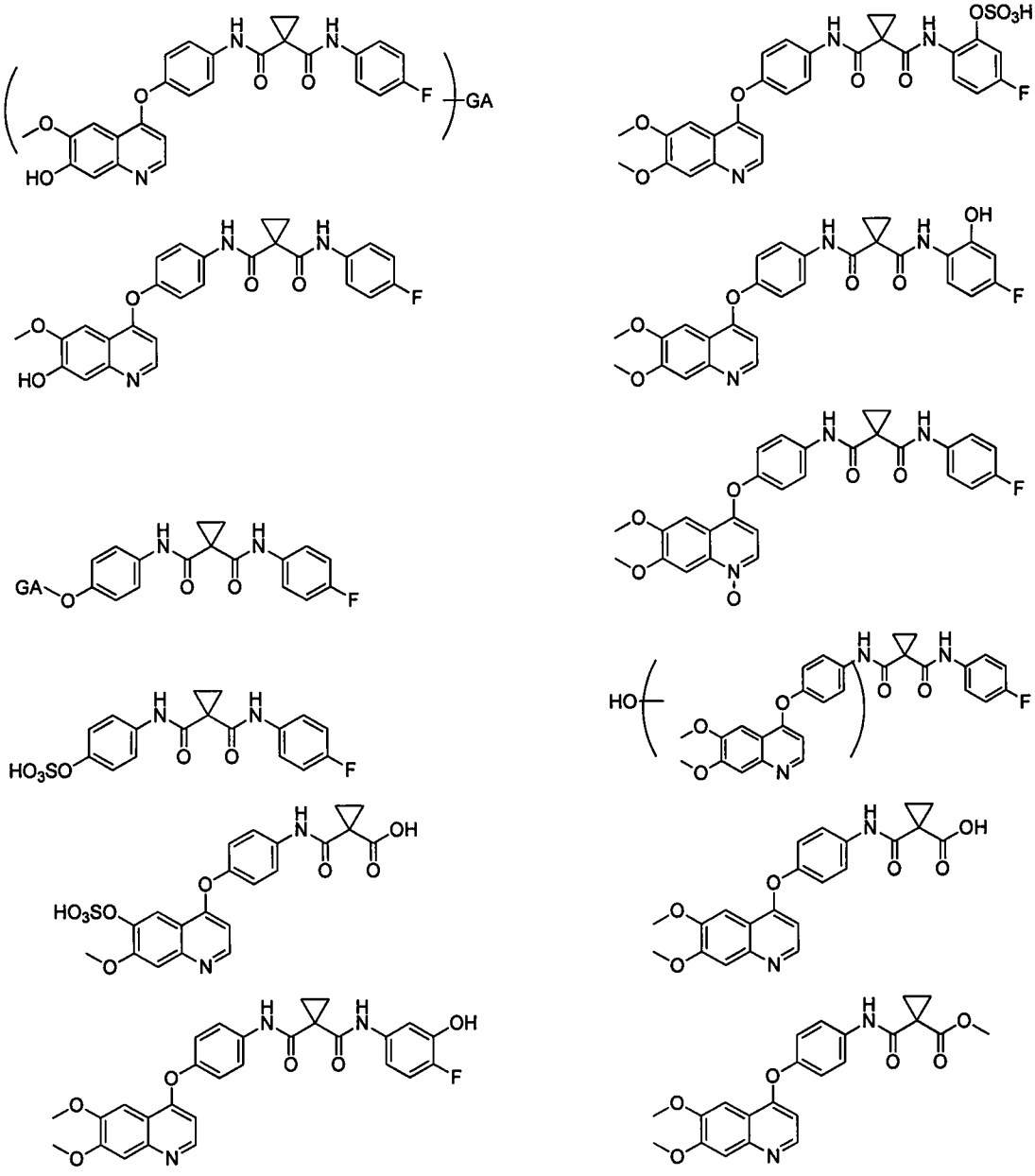
[0020] The cabozantinib metabolites described herein, including those depicted in Scheme 1, are isolated from body tissues and fluids, and/or are prepared synthetically according to methods available to the skilled artisan. A variety of separation processes can be carried out on tissue and fluid samples to provide samples for further analysis, such as nuclear magnetic resonance, gas chromatography (GC), liquid chromatography (LC), and mass spectrometry. In such samples, the metabolites are contained in compositions that are essentially lacking in the presence of any of the other metabolites. The presence of the metabolites can be quantified by physical methods, such as the measurement of nuclear decay from radioactive isotopes, measurement of index of refraction, flame ionization, ionization and deflection in magnetic fields, ultraviolet (UV absorption), and the like.

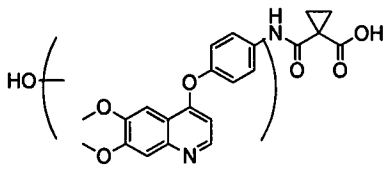
[0021] The human metabolites can be provided in crystalline or solution forms that have considerable degrees of purity. Organic synthetic routes are available for preparing the

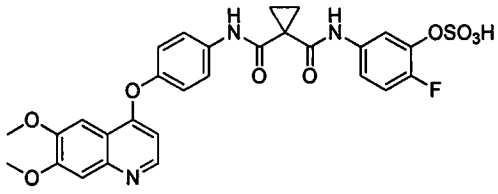
compounds in relative pure form, for example, in purities of 80 percent or greater, 90 percent or greater, 95 percent or greater, or 99 percent or greater. Recrystallization and other purification methods can be carried out to provide compounds that are essentially 100 percent pure. Such synthetic methods and purification techniques are known in the art.

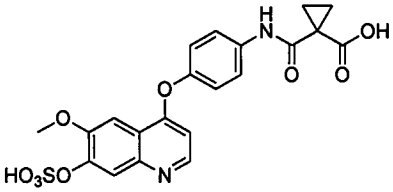
[0022] The metabolites can be provided in substantially pure form. "Substantially pure" means that metabolites are pure enough for FDA approval and contain essentially no contaminants or other materials. Alternatively, "substantially pure" means a level of impurity that does not adversely or unacceptably affect the properties of the compounds with respect to safety, effectiveness, stability, and other desirable properties.

[0023] The isolated metabolites depicted in Scheme 1include:

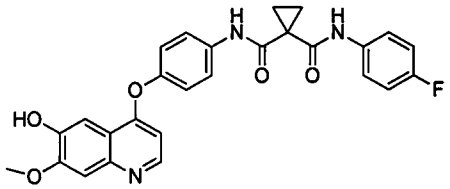








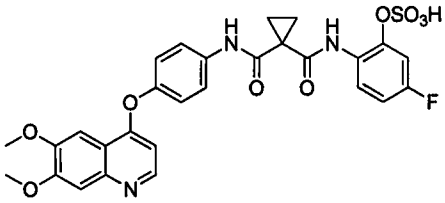
and

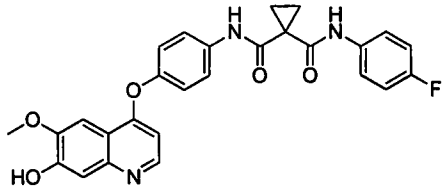


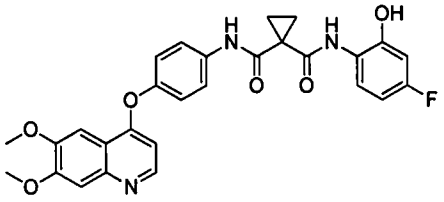
,

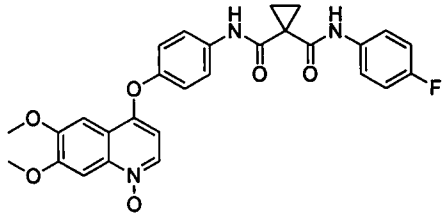
wherein GA is a glucuronic acid.

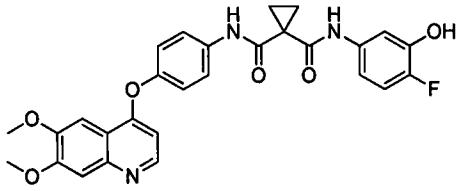
[0024] More particularly, the metabolites include:

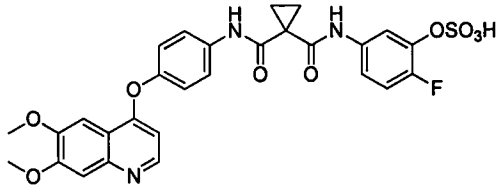


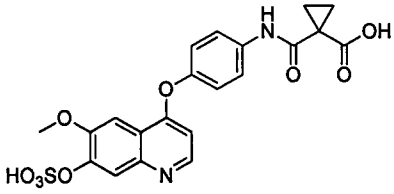












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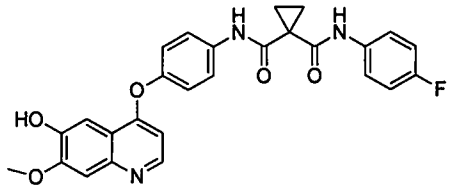
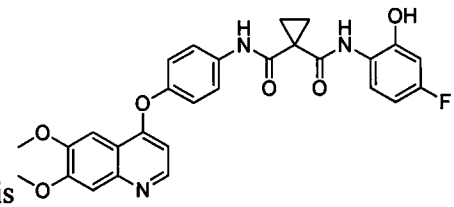
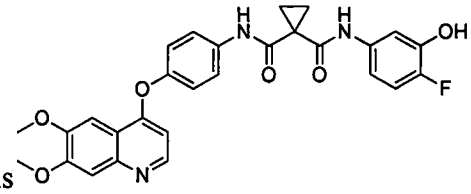


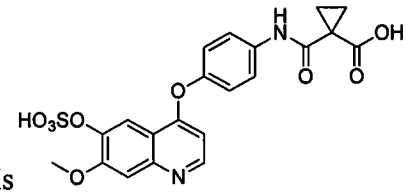
.

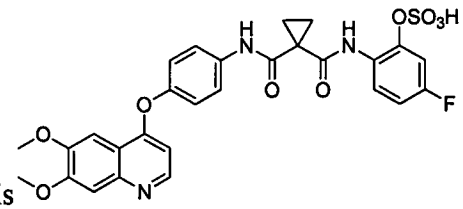
[0025] In particular, the isolated metabolite is

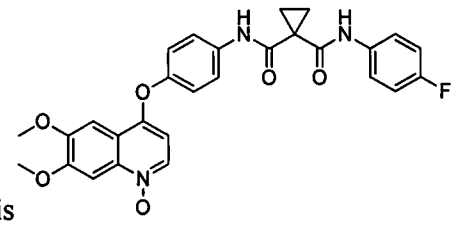


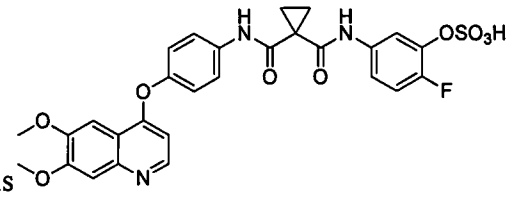
, or a pharmaceutically acceptable salt thereof.

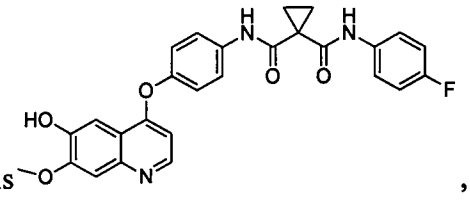
[0026] In particular, the isolated metabolite is  , or a pharmaceutically acceptable salt thereof.

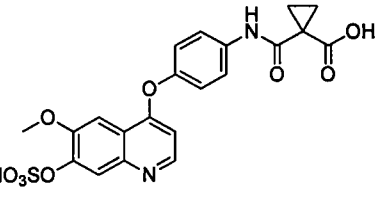
[0027] In particular, the isolated metabolite is  , or a pharmaceutically acceptable salt thereof.

[0028] In particular, the isolated metabolite is  , or a pharmaceutically acceptable salt thereof.

[0029] In particular, the isolated metabolite is  , or a pharmaceutically acceptable salt thereof.

[0030] In particular, the isolated metabolite is  , or a pharmaceutically acceptable salt thereof.

[0031] In particular, the isolated metabolite is  , or a pharmaceutically acceptable salt thereof.

[0032] In particular, the isolated metabolite is  , or a pharmaceutically acceptable salt thereof.

[0033] The pharmaceutical formulations contemplated in the invention can be selected from one of the following:

Ingredient	(% w/w)
Cabozantinib	31.68
Microcrystalline Cellulose	38.85
Lactose anhydrous	19.42
Hydroxypropyl Cellulose	3.00
Croscarmellose Sodium	3.00
Total Intra-granular	**95.95**
Silicon dioxide, Colloidal	0.30
Croscarmellose Sodium	3.00
Magnesium Stearate	0.75
Total	**100.00**

Ingredient	(% w/w)
Cabozantinib	25.0-33.3
Microcrystalline Cellulose	q.s
Hydroxypropyl Cellulose	3
Poloxamer	0-3
Croscarmellose Sodium	6.0
Colloidal Silicon Dioxide	0.5
Magnesium Stearate	0.5-1.0
Total	**100**

Ingredient	Theoretical Quantity (mg/unit dose)
Cabozantinib	100.0
Microcrystalline Cellulose PH-102	155.4
Lactose Anhydrous 60M	77.7
Hydroxypropyl Cellulose, EXF	12.0
Croscarmellose Sodium	24
Colloidal Silicon Dioxide	1.2
Magnesium Stearate (Non-Bovine)	3.0
Opadry Yellow	16.0
Total	**416**

Component	Weight/Weight Percent
Cabozantinib	25-29
Microcrystalline Cellulose	q.s.
Lactose Anhydrous	40-44

Hydroxypropyl Cellulose	2-4
Croscarmellose Sodium	2-8
Colloidal Silicon Dioxide	0.1-0.4
Magnesium Stearate	0.7-0.9
Total	**100**

Ingredient	**% w/w**
Cabozantinib	12.67
MCC	51.52
Lactose	25.76
Hydroxypropyl cellulose	3.0
Croscarmellose Sodium	6.0
Colloidal Silicon Dioxide	0.3
Magnesium Stearate	0.75
Total	**100**

Ingredient	**mg/unit dose**
Cabozantinib	25
Silicified Microcrystalline Cellulose	196.75
Croscarmellose sodium	12.5
Sodium starch glycolate	12.5
Fumed Silica	0.75
Stearic acid	2.5
Total Fill Weight	**250**

Ingredient	**mg/unit dose**
Cabozantinib	100
Silicified Microcrystalline Cellulose	75.40
Croscarmellose sodium	10.00
Sodium Starch Glycolate	10.00
Fumed silica	0.6
Stearic Acid	4.0
Total Fill Weight	**200**

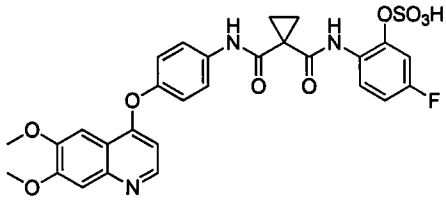
	mg/unit dose
Ingredient	**50mg**
Cabozantinib	63.35
Microcrystalline Cellulose	95.39
Croscarmellose sodium	9.05
Sodium starch glycolate	9.05
Fumed Silica	0.54
Stearic acid	3.62
Total Fill Weight	**181.00**

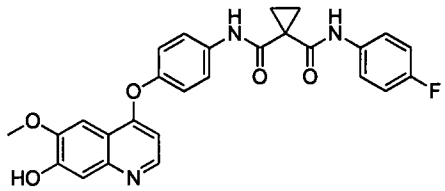
	mg/unit dose
Ingredient	**60mg**
Cabozantinib	73.95
Microcrystalline Cellulose	114.36
Croscarmellose sodium	10.85
Sodium starch glycolate	10.85

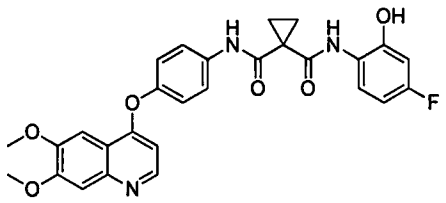
Fumed Silica	0.65
Stearic acid	4.34
Total Fill Weight	**217.00**

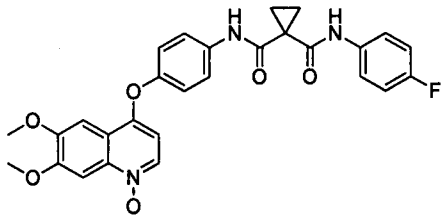
Ingredient	**% w/w**
Cabozantinib	31.7
Microcrystalline Cellulose (Avicel PH-102)	38.9
Lactose Anhydrous (60M)	19.4
Hydroxypropyl Cellulose (EXF)	3.0
Croscarmellose Sodium (Ac-Di-Sol)	6.0
Colloidal Silicon Dioxide,	0.3
Magnesium Stearate	0.75
Opadry Yellow Film Coating which includes: - HPMC 2910 /Hypromellose 6 cp - Titanium dioxide - Triacetin - Iron Oxide Yellow	4.00

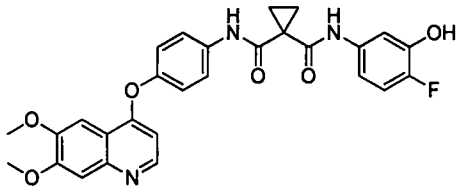
[0034] The invention also relates to a pharmaceutical formulation comprising one or more cabozantinib isolated metabolites that inhibits CMet or that have other benefical attributes. The isolated metabolite is selected from:

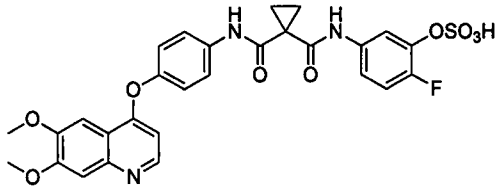


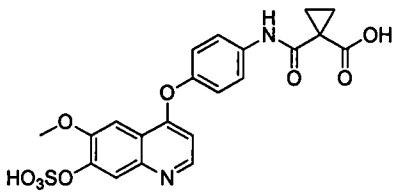




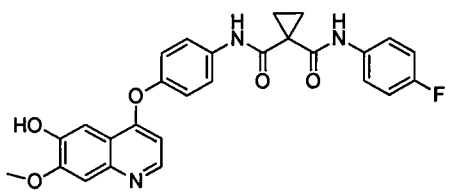








and



,

or a pharmaceutically acceptable salt thereof, and at least one pharmaceutically acceptable carrier.

[0035] The pharmaceutical formulations can be used to treat cancer when administered as described herein. In typical embodiments, administration is oral, once daily, but can be adjusted as required by the situation. Tablets or capsules containing different amounts of cabozantinib can be combined to achieve the desired dose. For example, a 140 mg dose of cabozantinib would require administration of one tablet or capsule containing 80 mg cabozantinib and three tablets or capsules containing 20 mg of cabozantinib. A 140 mg dose of cabozantinib would require administration of one tablet or capsule containing 80 mg cabozantinib and one tablet or capsule containing 20 mg of cabozantinib.

[0036] Thus, in a further aspect, the invention relates to a method of treating cancer in a human patient, comprising orally administering to said patient a pharmaceutical formulation comprising a physiologically effective amount of cabozantinib at such a rate to achieve:

an average cabozantinib plasma area under the curve (average AUC) of at least 60,000 ng·h/mL per each 140 mg dosage of cabozantinib delivered;

an average maximum cabozantinib blood plasma concentration (average C_{max}) of at least 1000 ng/mL per each 140 mg dosage of cabozantinib delivered; and

a ratio of AUC(cabozantinib) to the AUC of the sum of cabozantinib plus measured cabozantinib metabolites:

AUC (Cabozantinib) / AUC (Cabozantinib + Measured Metabolites)

of at least 0.25;

wherein the AUC is measured from time zero to the time of last measurable concentration.

[0037] In another aspect, the invention relates to a method of treating cancer in a human patient, comprising orally administering to said patient a pharmaceutical formulation comprising a physiologically effective amount of cabozantinib at such a rate to achieve:

a steady state area under the curve for cabozantinib (average AUC) of at least 35,000 ng·h/mL for a formulation comprising 100 mg of cabozantinib delivered once daily;

an average maximum cabozantinib blood plasma concentration (average C_{max}) of at least 2400 ng/mL per each 100 mg dosage of cabozantinib delivered;

an average minimum cabozantinib blood plasma concentration (average C_{max}) of at least 1100 ng/mL per each 100 mg dosage of cabozantinib delivered; and

a ratio of AUC(cabozantinib) to the AUC of the sum of cabozantinib plus measured cabozantinib metabolites:

AUC (Cabozantinib) / AUC (Cabozantinib + Measured Metabolites)

of at least 0.35;

wherein the AUC is measured on day 22.

[0038] "Cancer" includes tumor types such as tumor types including breast, colon, renal, lung, squamous cell myeloid leukemia, hemangiomas, melanomas, astrocytomas, and glioblastomas as well as other cellular-proliferative disease states, including but not limited to: Cardiac: sarcoma (angiosarcoma, fibrosarcoma, rhabdomyosarcoma, liposarcoma), myxoma, rhabdomyoma, fibroma, lipoma and teratoma; Lung: bronchogenic carcinoma (squamous cell, undifferentiated small cell, undifferentiated large cell, adenocarcinoma), alveolar (bronchiolar) carcinoma, bronchial adenoma, sarcoma, lymphoma, chondromatous hanlartoma, inesothelioma; Gastrointestinal: esophagus (squamous cell carcinoma, adenocarcinoma, leiomyosarcoma, lymphoma), stomach (carcinoma, lymphoma, leiomyosarcoma), pancreas (ductal adenocarcinoma, insulinorna, glucagonoma, gastrinoma, carcinoid tumors, vipoma), small bowel (adenocarcinorna, lymphoma, carcinoid tumors, Karposi's sarcoma, leiomyoma, hemangioma, lipoma, neurofibroma, fibroma), large bowel (adenocarcinoma, tubular adenoma, villous adenoma, hamartoma, leiomyoma); Genitourinary tract: kidney (adenocarcinoma, Wilm's tumor [nephroblastoma], lymphoma, leukemia, renal cell carcinoma), bladder and urethra (squamous cell carcinoma, transitional cell carcinoma, adenocarcinoma), prostate (adenocarcinoma, sarcoma, small cell carcinoma of the prostate), testis (seminoma, teratoma, embryonal carcinoma, teratocarcinoma, choriocarcinoma, sarcoma, interstitial cell carcinoma, fibroma, fibroadenoma, adenomatoid tumors, lipoma); Liver: hepatoma (hepatocellular carcinoma), cholangiocarcinoma, hepatoblastoma, angiosarcoma, hepatocellular adenoma, hemangioma; Bone: osteogenic sarcoma (osteosarcoma), fibrosarcoma, malignant fibrous histiocytoma, chondrosarcoma, Ewing's sarcoma, malignant lymphoma (reticulum cell sarcoma), malignant giant cell tumor chordoma, osteochronfroma (osteocartilaginous exostoses), benign chondroma, chondroblastoma, chondromyxofibroma, osteoid osteoma and giant cell tumors; Nervous system: skull (osteoma, hemangioma, granuloma, xanthoma, osteitis defornians), meninges (meningioma, meningiosarcoma, gliomatosis), brain (astrocytoma, medulloblastoma, glioma, ependymoma, germinoma [pinealoma], glioblastorna multiform, oligodendroglioma, schwannoma, retinoblastoma, congenital tumors), spinal cord neurofibroma, meningioma, glioma, sarcoma); Gynecological: uterus (endometrial carcinoma), cervix (cervical carcinoma, pre-tumor cervical dysplasia), ovaries (ovarian carcinoma [serous cystadenocarcinoma, mucinous cystadenocarcinoma, unclassified carcinoma], granulosa-thecal cell tumors, Sertoli

-Leydig cell tumors, dysgerminoma, malignant teratoma), vulva (squamous cell carcinoma, intraepithelial carcinoma, adenocarcinoma, fibrosarcoma, melanoma), vagina (clear cell carcinoma, squamous cell carcinoma, botryoid sarcoma (embryonal rhabdomyosarcoma], fallopian tubes (carcinoma); Hematologic: blood (myeloid leukemia [acute and chronic], acute lymphoblastic leukemia, chronic lymphocytic leukemia, myeloproliferative diseases, multiple myeloma, myelodysplastic syndrome), Hodgkin's disease, non-Hodgkin's lymphoma [malignant lymphoma]; Skin: malignant melanoma, basal cell carcinoma, squamous cell carcinoma, Karposi's sarcoma, moles dysplastic nevi, lipoma, angioma, dermatofibroma, keloids, psoriasis; and Adrenal glands: neuroblastoma; as well as cancers of the thyroid including medullary thyroid cancer. Thus, the term "cancerous cell," as provided herein, includes a cell afflicted by any one of the above-identified conditions.

[0039] In one embodiment, the cancer is selected from ovarian cancer, prostate cancer, lung cancer, medullary thyroid cancer, liver cancer, gastrointestinal cancer, pancreatic cancer, bone cancer, hematologic cancer, skin cancer, kidney cancer, breast cancer, colon cancer, and fallopian tube cancer.

[0040] In another embodiment, the disease or disorder is ovarian cancer.

[0041] In another embodiment, the disease or disorder is prostate cancer.

[0042] In another embodiment, the disease or disorder is lung cancer.

[0043] In another embodiment, the disease or disorder is medullary thyroid cancer.

[0044] In another embodiment, the disease or disorder is liver cancer.

[0045] In another embodiment, the disease or disorder is gastrointestinal cancer.

[0046] In another embodiment, the disease or disorder is pancreatic cancer.

[0047] In another embodiment, the disease or disorder is bone cancer.

[0048] In another embodiment, the disease or disorder is hematologic cancer.

[0049] In another embodiment, the disease or disorder is skin cancer.

[0050] In another embodiment, the disease or disorder is kidney cancer.

[0051] In another embodiment, the disease or disorder is breast cancer.

[0052] In another embodiment, the disease or disorder is colon cancer.

[0053] In another embodiment, the disease or disorder is fallopian cancer.

[0054] In another embodiment, the disease or disorder is liver cancer, wherein the liver cancer is hepatocellular carcinoma, cholangiocarcinoma, hepatoblastoma, angiosarcoma, hepatocellular adenoma, or hemagioma.

[0055] In another embodiment, the disease or disorder is gastrointestinal cancer, wherein the gastrointestinal cancer is cancer of the esophagous which is squamous cell carcinoma,

adenocarcinoma, or leiomyosarcoma; cancer of the stomach which is carcinoma, or lymphoma; cancer of the pancreas, which is ductal adenocarcinoma, insulinoma, gucagonoma, gastrinoma, carcinoid tumors, or vipoma; cancer of the small bowel, which is adenocarcinoma, lymphoma, carcinoid tumors, Karposi's sarcoma, leiomyoma, hemagioma, lipoma, or cancer of the large bowel, which is adenocarcinoma, tubular adenoma, villous adenoma, hamartoma, or leiomyoma.

[0056] In another embodiment, the disease or disorder is cancer of the pancreas, wherein the cancer of the pancreas is ductal adenocarcinoma, insulinoma, gucagonoma, gastrinoma, carcinoid tumors, or vipoma.

[0057] In another embodiment, the disease or disorder is bone cancer, wherein the bone cancer is osteosarcoma, fibrosarcoma, malignant fibrous histiocytoma, chondrosarcoma, Ewing's sarcoma, malignant reticulum cell sarcoma, multiple myeloma, malignant giant cell tumor chordoma, osteocartiliginous exostoses, chondroblastoma, chondromyxofibroma, or osteoid osteoma.

[0058] In another embodiment, the disease or disorder is hematologic cancer, wherein the hematologic cancer is myeloid leukemia, acute lymphoblastic leukemia, chronic lymphocytic leukemia, myeloproliferative diseases, multiple myeloma, or myelodysplastic syndrome.

[0059] In another embodiment, the disease or disorder is skin cancer, wherein the skin cancer is malignant melanoma, basal cell carcinoma, squamous cell carcinoma, or Karposi's sarcoma.

[0060] In another embodiment, the disease or disorder is a renal tumor or renal cell carcinoma.

[0061] In another embodiment, the disease or disorder is breast cancer.

[0062] In another embodiment, the disease or disorder is a colon cancer tumor.

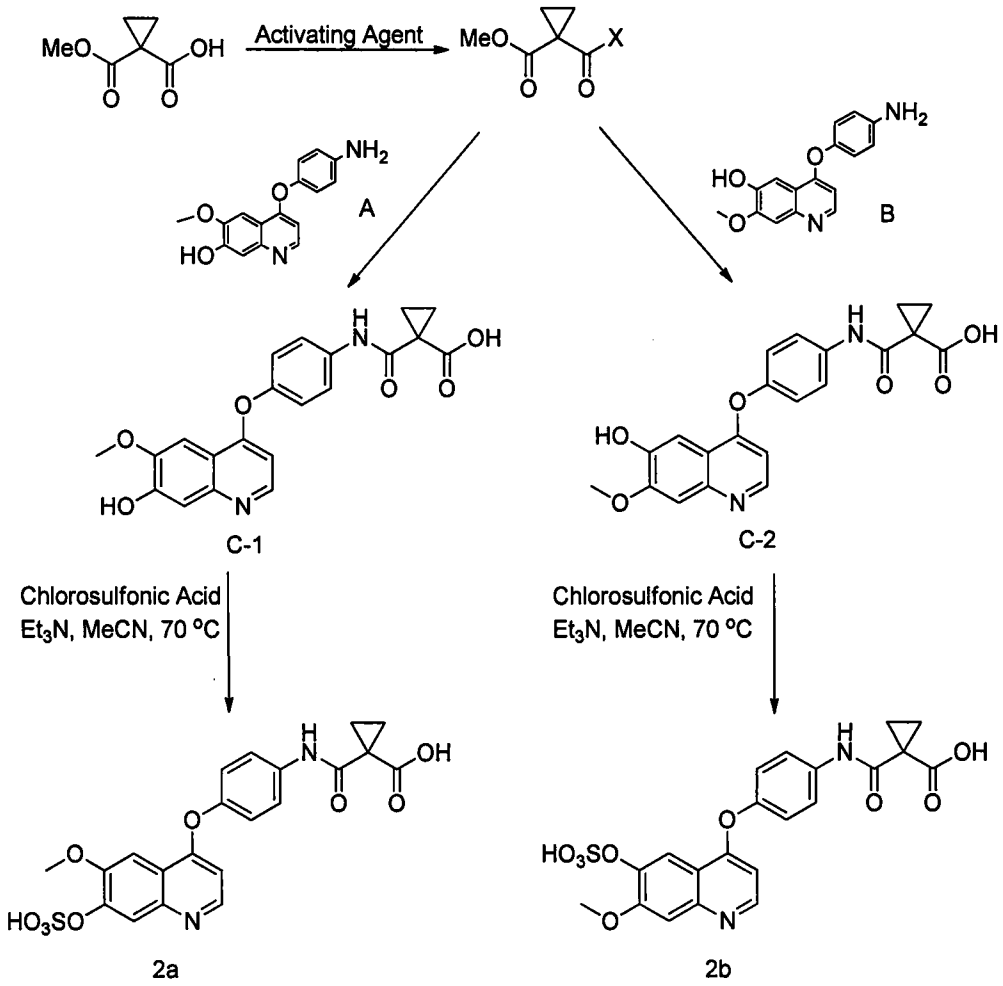
[0063] In another embodiment, the disease or disorder is fallopian tube carcinoma.

Preparation of Isolated Metabolites

[0064] The isolated metabolites disclosed herein can be made according to methods available to the skilled practitioner. For example, as depicted in Scheme 2, peptide chemistry can be used to make the phenols **C-1** and **C-2** from the corresponding amines and carboxylic acids. A variety of processes and reagents are available for achieving such transformations and are described, for instance, in Tetrahedron 61 (2005) 10827-10852. A representative example is depicted in Scheme 2, wherein the activating agent is thionyl chloride, oxalyl chloride, or the like. The corresponding acid chloride reacts with compound **A** or **B**, respectively, to provide phenol **C-1** or **C-2**. Subsequent reaction of phenol **C-1** or **C-2** with a

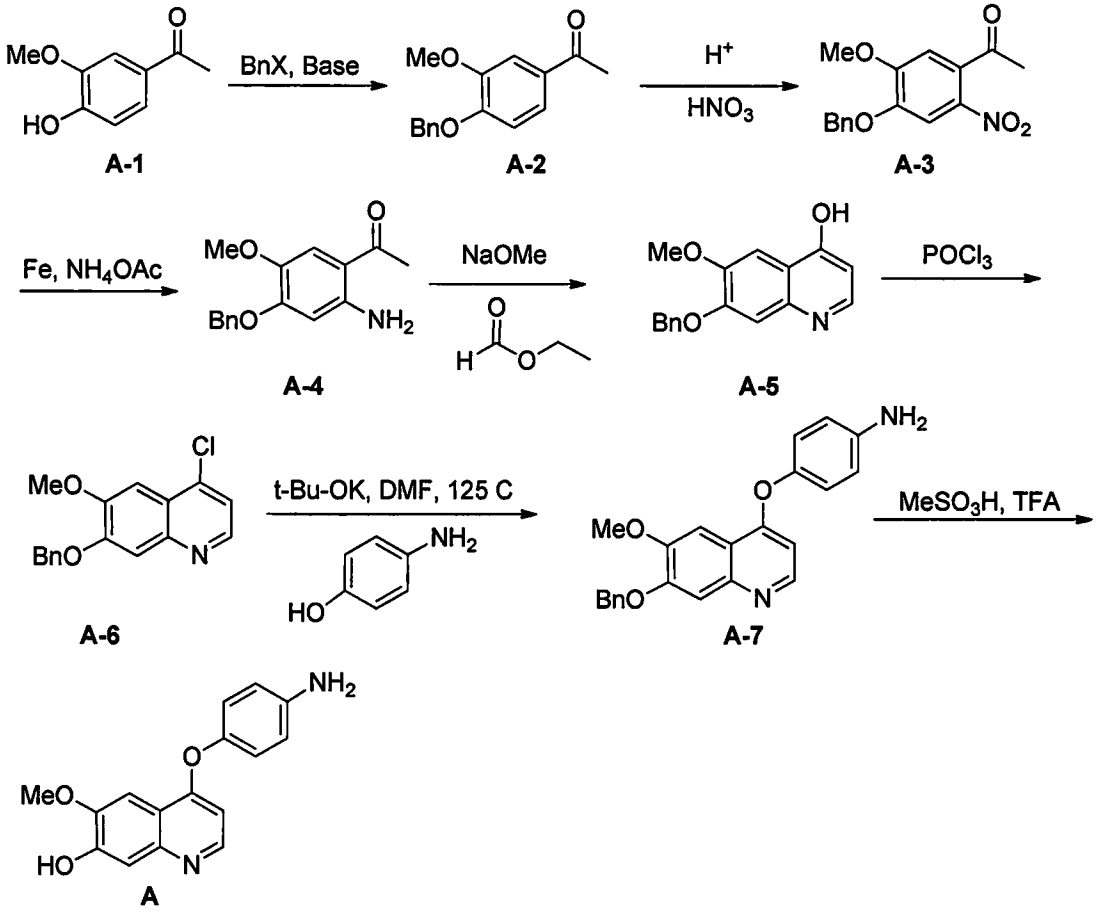
sulfating agent, such as chlorosulfonic acid or sulfur trioxide-trimethylamine complex, in the presence of a base, such as triethylamine, alkali metal hydroxide or the like, can provide the corresponding hydrogen sulfate **2b** or **2a**, respectively.

Scheme 2



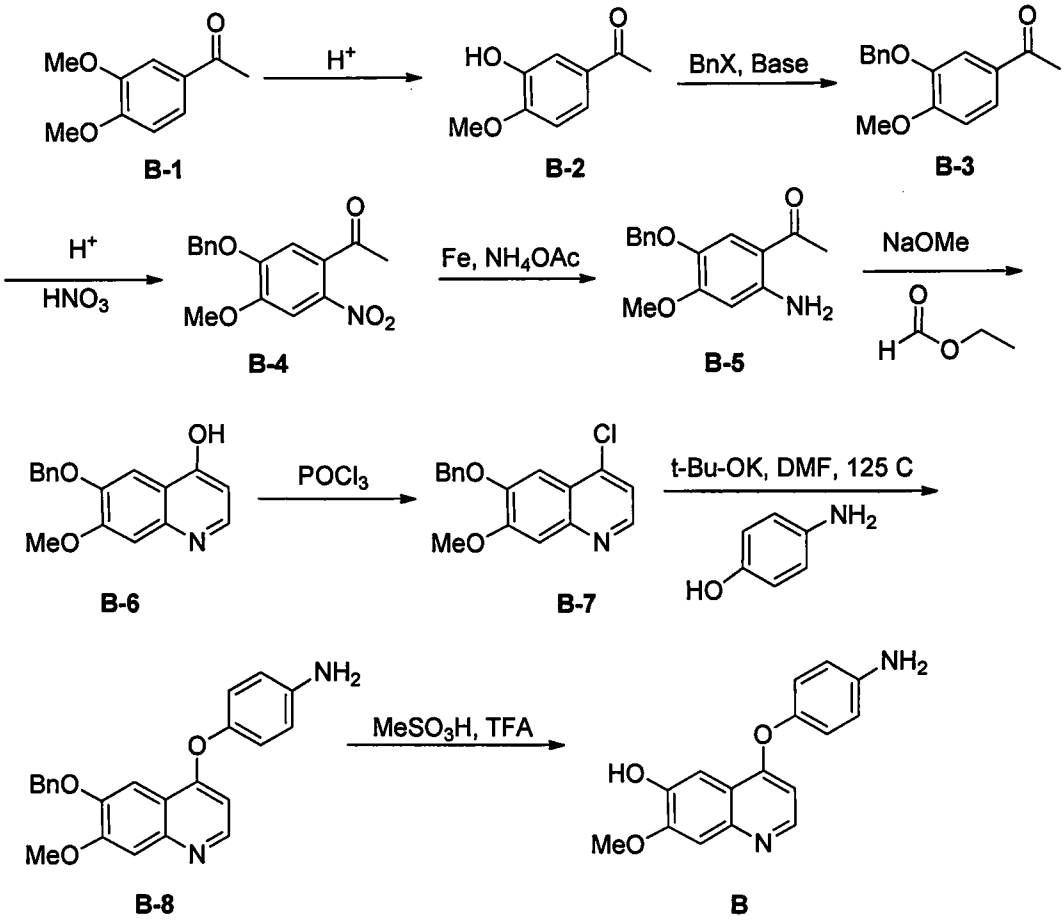
[0065] Compound **A** was prepared according to Scheme 3. Benzylation of **A-1** using a benzyl halide or the like (BnX, where X is Cl, Br, or I, methanesulofonate (OMs), trifluoromethansulfonate (OTf) or toluenesulfonate(OTs)) provides benzyl-protected **A-2**. Nitration of **A-2** using a mixture of nitric acid and sulfuric acid provides **A-3**. Reduction of the nitro group in **A-3** to the amine **A-4**, may be accomplished using standard nitro reduction conditions, such as iron and ammonium acetate. Cyclization of **A-4** with ethyl formate and an alkoxide such as sodium methoxide provides the **A-5**. Conversion of **A-5** to the corresponding chloride using phosphorous oxychloride provides **A-6**. Reaction of **A-6** with 4-amino phenol provides **A-7**, which is deprotected with methane sulfonic acid to provide compound **A**.

Scheme 3

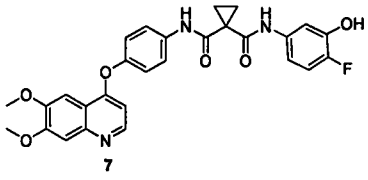


[0066] Similarly, compound **B** was prepared according to Scheme 4. Demethylation of **B-1** provides **B-2**. Benzylation of **B-2** using a benzyl halide or the like (BnX, where X is Cl, Br, or I, methanesulofonate (OMs), trifluoromethansulfonate (OTf) or toluenesulfonate(OTs)). Nitration of **B-3** using a mixture of nitric acid and sulfuric acid provides **B-4**. Reduction of nitro group in **B-4** to the amine **B-5**, may be accomplished using standard nitro reduction conditions, such as iron and ammonium acetate. Cyclization of **B-5** with ethyl formate and an alkoxide such as sodium methoxide provides the **B-6**. Conversion of **B-6** to the corresponding chloride using phosphorous oxychloride provides **B-7**. Reaction of **B-7** with 4-amino phenol provides **B-8,** which was deprotected with methane sulfonic acid to provide compound **B**.

Scheme 4

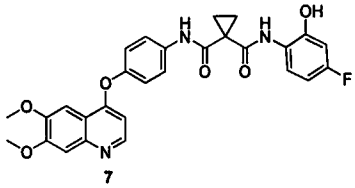


[0067] Phenols **13** and **16** can be similarly prepared from compound **7**, the synthesis of which is disclosed in WO 2005/030140 as Example 73. Thus, in Scheme 5, coupling of 7 with 2-amino-5-fluorophenol (CAS Reg. No. 53981-24-1) provides



7

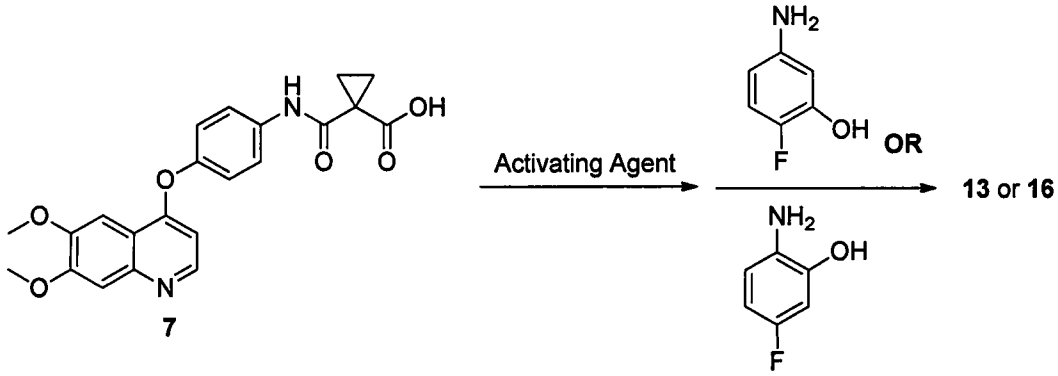
(Compound **13**). Coupling of **7** with 5-amino-2-fluorophenol (CAS Reg. No. 100367-48-4) provides



7

(Compound **16**).

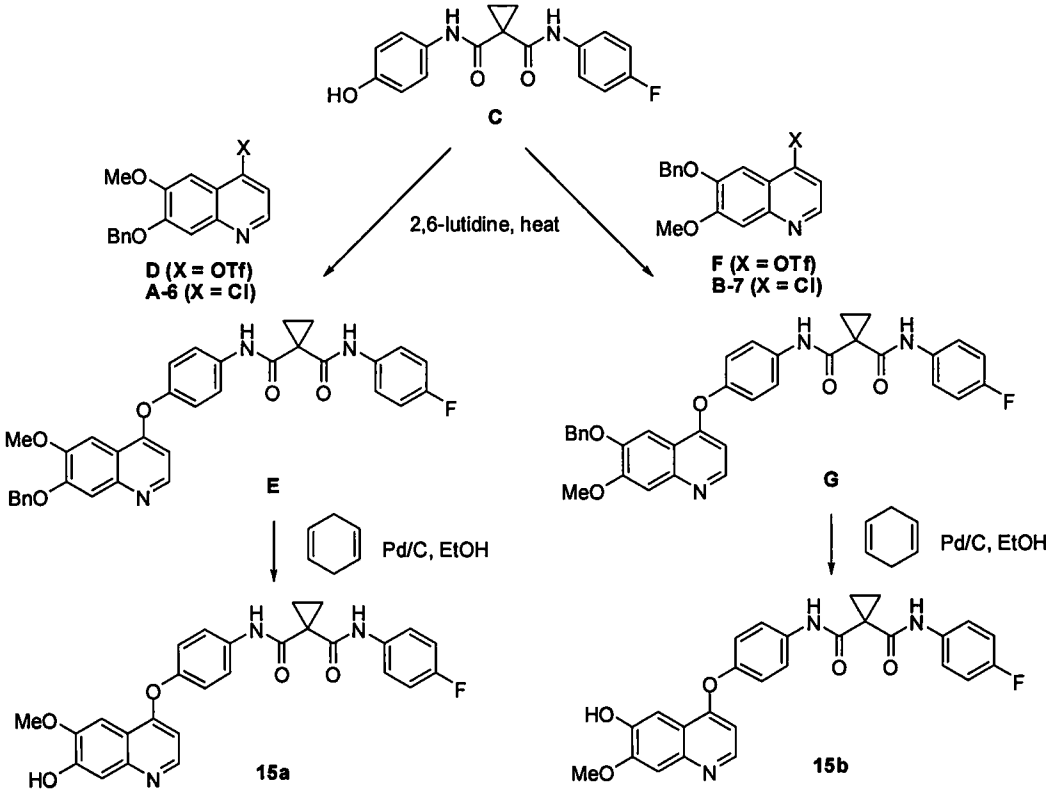
Scheme 5



[0068] Phenols **13** and **16** can be readily converted to the corresponding sulfates **9**, and **12** depicted in Scheme 1 using, for example, a sulfating agent, such as sulfur trioxide trimethyl amine complex, in the presence of a strong hydroxide, such as potassium hydroxide, sodium hydroxide, or the like, or using chlorosulfonic acid in the presence of an amine base such as triethylamine.

[0069] The phenols **15a** and **15b** can be prepared by employing the similar method that is disclosed in WO 2005/030140 for the preparation of Example 43. Thus, in Scheme 6, coupling of phenol **C** (Example 38 in WO 2005/030140) with triflate **D** (Example 33 in WO 2005/030140), or chloride **A-6** (Example 32 in WO 2005/030140) provides **E**, which is then deprotected under Pd-catalyzed hydrogenolysis condition to yield compound **15**. Similarly, reaction of phenol **C** with triflate **F** or chloride **B-7** provides **G**, which is subjected to O-benzyl deportection to provide compound **15b**.

Scheme 6



[0070] The N-oxide **19** can be prepared by the reaction of cabozantinib with an oxidizing agent, such as, for example a peroxide, a peracid, or the like. In one embodiment, the oxidizing agent is sodium perborate tetrahydrate.

[0071] The following non-limiting examples are meant to illustrate the invention.

Embodiments

[0072] **Embodiment 1.** A pharmaceutical formulation comprising a physiologically effective amount of cabozantinib, wherein oral administration of said composition to a selected human subject group produces in said selected human subject group:

an average cabozantinib plasma area under the curve (average AUC) of at least 60,000 ng·h/mL per each 140 mg dosage of cabozantinib delivered;

an average maximum cabozantinib blood plasma concentration (average C_{max}) of at least 1000 ng/mL per each 140 mg dosage of cabozantinib delivered; and

a ratio of AUC(cabozantinib) to the AUC of the sum of cabozantinib plus measured cabozantinib metabolites:

AUC (Cabozantinib) / AUC (Cabozantinib + Measured Metabolites)

of at least 0.25;

wherein the AUC is measured from time zero to the time of last measurable concentration.

[0073] **Embodiment 2.** A pharmaceutical formulation comprising a physiologically effective amount of cabozantinib, wherein oral administration of said pharmaceutical formulation to a selected human subject group produces in said selected human subject group:

a steady state area under the curve for cabozantinib (average AUC) of at least 35,000 ng·h/mL for a formulation comprising 100 mg of cabozantinib delivered once daily;

an average maximum cabozantinib blood plasma concentration (average C_{max}) of at least 2400 ng/mL per each 100 mg dosage of cabozantinib delivered;

an average minimum cabozantinib blood plasma concentration (average C_{max}) of at least 1100 ng/mL per each 100 mg dosage of cabozantinib delivered; and

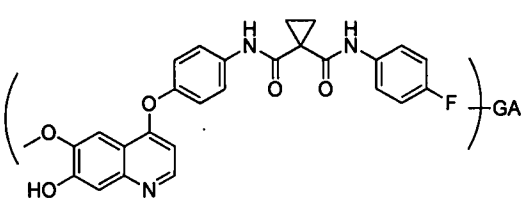
a ratio of AUC(cabozantinib) to the AUC of the sum of cabozantinib plus measured cabozantinib metabolites:

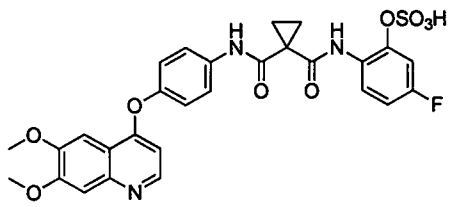
AUC (Cabozantinib) / AUC (Cabozantinib + Measured Metabolites)

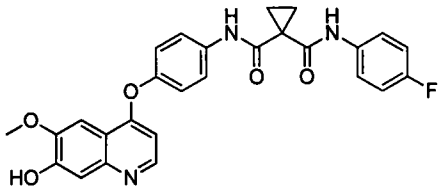
of at least 0.35;

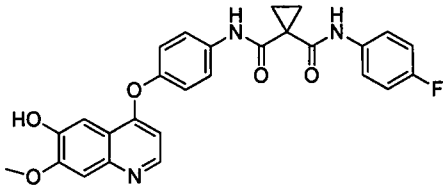
wherein the AUC is measured on day 22.

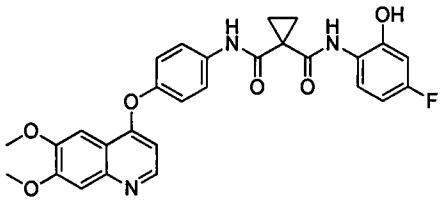
[0074] **Embodiment 3.** The pharmaceutical formulation of embodiments 1-2, wherein the measured metabolites are selected from the group consisting of:

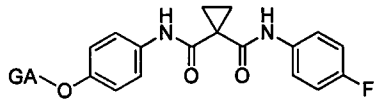


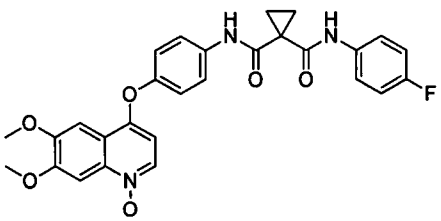


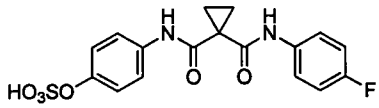


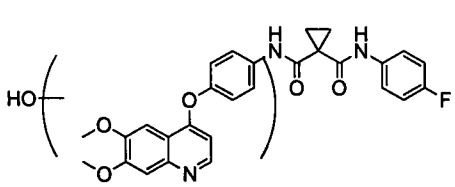
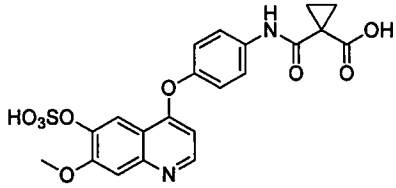
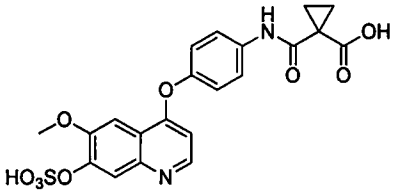
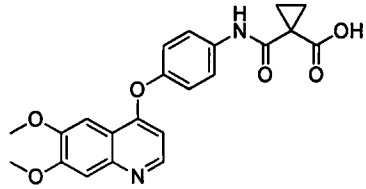
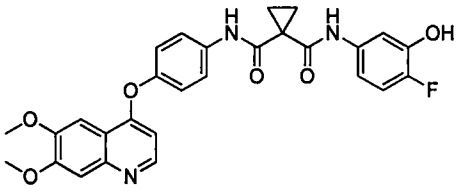
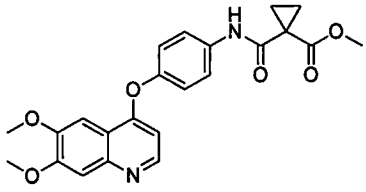
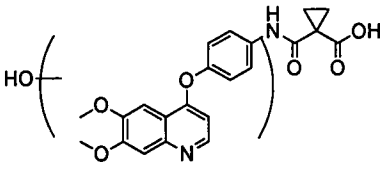








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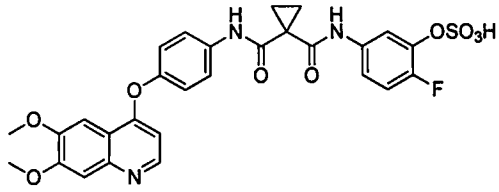
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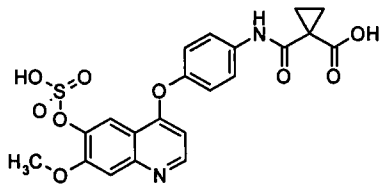
wherein GA is a glucuronic acid moiety,

[0075] **Embodiment** 4. The pharmaceutical formulation of claims 1-2, wherein the measured metabolites comprise one or more of: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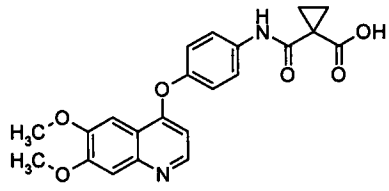
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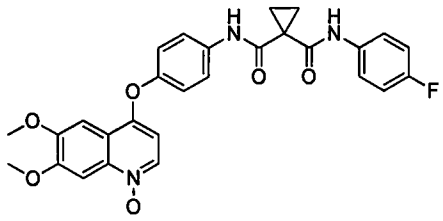
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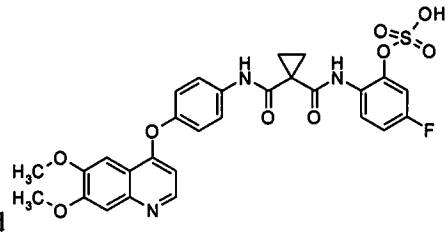
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[0076] **Embodiment 5.** A method of treating cancer in a human patient, comprising orally administering to said patient a pharmaceutical formulation comprising a physiologically effective amount of cabozantinib at such a rate to achieve:

an average cabozantinib plasma area under the curve (average AUC) of at least 60,000 ng·h/mL per each 140 mg dosage of cabozantinib delivered;

an average maximum cabozantinib blood plasma concentration (average C_{max}) of at least 1000 ng/mL per each 140 mg dosage of cabozantinib delivered; and

a ratio of AUC(cabozantinib) to the AUC of the sum of cabozantinib plus measured cabozantinib metabolites:

AUC (Cabozantinib) / AUC (Cabozantinib + Measured Metabolites)

of at least 0.25;

wherein the AUC is measured from time zero to the time of last measurable concentration.

[0077] **Embodiment 6.** A method of treating cancer in a human patient, comprising orally administering to said patient a pharmaceutical formulation comprising a physiologically effective amount of cabozantinib at such a rate to achieve:

a steady state area under the curve for cabozantinib (average AUC) of at least 35,000 ng·h/mL for a formulation comprising 100 mg of cabozantinib delivered once daily;

an average maximum cabozantinib blood plasma concentration (average C_{max}) of at least 2400 ng/mL per each 100 mg dosage of cabozantinib delivered;

an average minimum cabozantinib blood plasma concentration (average C_{max}) of at least 1100 ng/mL per each 100 mg dosage of cabozantinib delivered; and

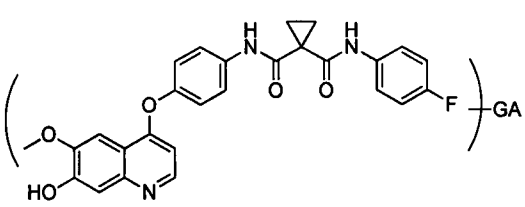
a ratio of AUC(cabozantinib) to the AUC of the sum of cabozantinib plus measured cabozantinib metabolites:

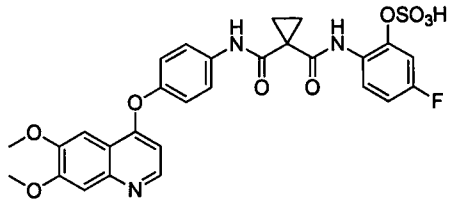
AUC (Cabozantinib) / AUC (Cabozantinib + Measured Metabolites)

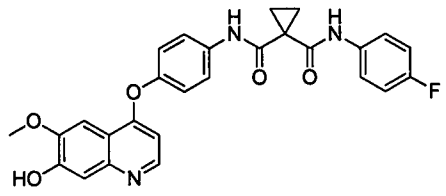
of at least 0.35;

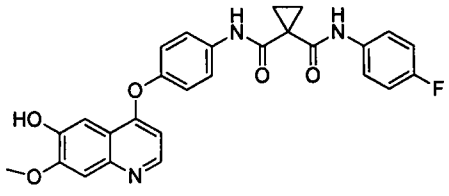
wherein the AUC is measured on day 22.

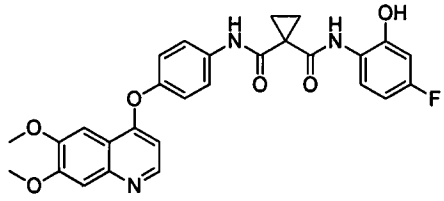
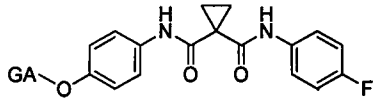
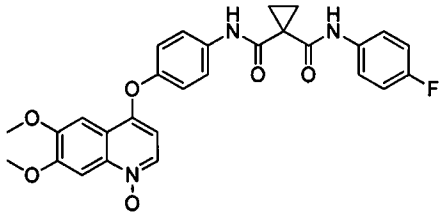
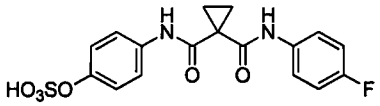
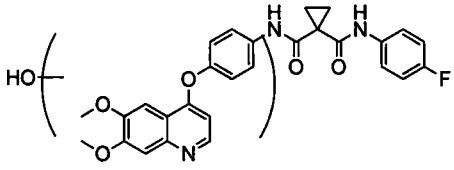
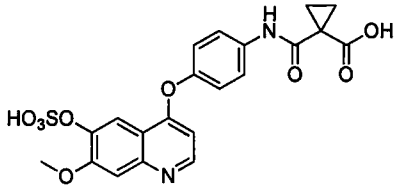
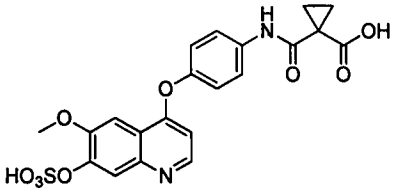
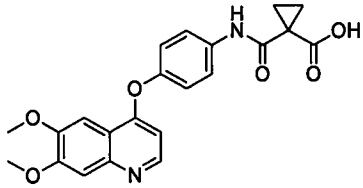
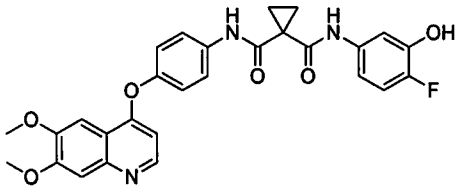
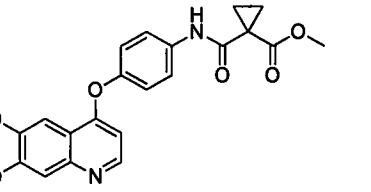
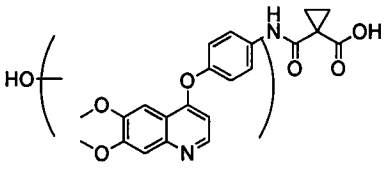
[0078] **Embodiment 7.** The method of embodiments 5-6, wherein the measured metabolites are selected from the group consisting of:







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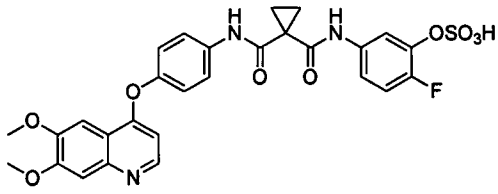
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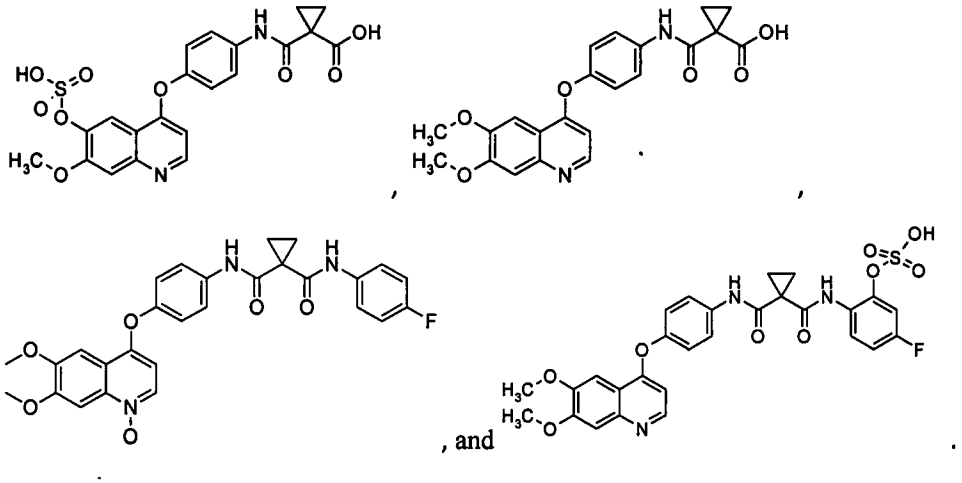
wherein GA is a glucuronic acid moiety,

[0079] **Embodiment 8.** The method of embodiments 5-7 wherein the measured metabolites comprise one or more of:



[0080] **Embodiment 9.** The method of embodiments 5-8, wherein the cancer is selected from ovarian cancer, lung cancer, medullary thyroid cancer, liver cancer, gastrointestinal cancer, pancreatic cancer, bone cancer, hematologic cancer, skin cancer, kidney cancer, breast cancer, colon cancer, and fallopian tube cancer.

[0081] **Embodiment 10.** The pharmaceutical formulation of embodiments 1-4 or the embodiments of claims 5-9, wherein the administration is once daily.

Examples

Identification of Cabozantinib Metabolites

[0082] The objective of this study was to profile and identify metabolites of cabozantinib in human plasma, urine, and feces. The plasma, urine and fecal samples were collected from a mass balance study of cabozantinib following a single 175 mg oral administration of cabozantinib (L-malate salt) containing [^{14}C] cabozantinib (100 μCi) in healthy male subjects.

Study Design and Plasma, Urine, and Feces Sampling

[0083] Eight male subjects participated in the study, and each subject received a single oral dose of 175 mg of cabozantinib (L-malate salt) containing [^{14}C]-cabozantinib (100 μCi). The plasma, urine, and fecal samples were collected from the 8 subjects for the metabolite profiling.

Plasma samples were collected at pre-dose, 0.5, 1, 2, 3, 4, 5, 8, 14, 24, 72, 168, 336, 504 and 648 hours post-dose; urine samples were collected at pre-dose, 0-8 hours, 8-24 hours, at 24-hour intervals to 480 hours post-dose, and at greater than 48-hour intervals from 504 to 1152 hours post-dose; and feces samples were collected at pre-dose, at 24-hour intervals to 480 hours post-dose, and at greater than 48-hour intervals from 504 to 1152 hours post-dose. All samples were shipped to QPS LLC (Newark, DE) and stored at -70 °C. HPLC/tandem MS

coupled with a radio flow-through detector (RFD) was used for metabolite profiling and identification for samples with sufficient levels of radioactivity.

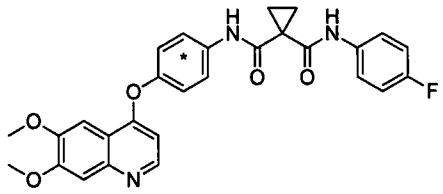
[0084] HPLC fraction collection followed by counting with TopCount NXT™ was used for radioquantitation of plasma samples with sufficient levels of radioactivity. Three (3) HPLC methods were used in this study to separate cabozantinib and its metabolites. HPLC Method 1 was used for the analysis of pooled urine and fecal samples and individual plasma samples from different time points. HPLC Method 2 was used for the analysis of plasma samples from a drug-drug interaction study to search for possible metabolites that may co-elute with cabozantinib sulfate. HPLC Method 3 was used for pooled plasma samples.

[0085] Selected samples for plasma, urine, and feces from 6 subjects were analyzed for cabozantinib and metabolites and reported.

[0086] Samples from 2 subjects were used for the investigation study.

Test Article

[0087] The test article for this study was a mixture of [^{14}C] cabozantinib and cabozantinib. The asterisk indicates the position of the [^{14}C] label. [^{14}C] labeled cabozantinib was prepared as provided in WO 2005/030140, except that [^{14}C] labeled 4-amino phenol was used instead of unlabeled 4-amino phenol. [^{14}C] labeled 4-amino phenol is commercially available as the hydrochloride salt, for instance, from Hartmann Analytic, American Radiolabeled Chemicals, or Fisher Scientific.



General Chemicals and Reference Standards

[0088] Formic acid and ammonium acetate were obtained from Sigma-Aldrich Chemical Co. (St. Louis, MO). Acetonitrile (B & J brand, carbonyl free, for applications sensitive to trace aldehyde and ketone), water (B & J brand, for GC, HPLC and spectrophotometry), and methanol (HPLC grade) were purchased from Fisher Scientific (Pittsburgh, PA). Type I water was generated using an Elgastat UHQ PS water purification system. Non-radiolabeled metabolite standards (fluoroaniline cleavage product, cabozantinib sulfate, and cabozantinib N-oxide) were provided by Exelixis, Inc.

Biological Sample Collection

[0089] The plasma, urine, and fecal samples were collected from a mass balance study of cabozantinib following a single 175 mg oral administration of cabozantinib (L-malate salt)

containing [^{14}C] cabozantinib (100 μCi) in healthy male subjects. Samples were shipped from Celerion (Lincoln, NE) to QPS LLC (Newark, DE) on dry ice and were stored at -70°C until analysis. Samples from 6 subjects were used for metabolite profiling, identification, and radio-quantitation. Plasma samples from 2 subjects were only used in a bridging study as part of investigation of co-eluting metabolites.

Sample Preparation and Radioactive Recovery for Human Plasma

[0090] For metabolite profiling, identification, and radio-quantitation, individual radiolabeled plasma samples collected at 0.5, 1, 2, 3, 4, 5, 8, 14, 24, 72, 168, and 336 hours post-dose were processed and analyzed for 6 subjects. For the investigation of co-eluting metabolites, nonradiolabeled plasma samples of six subjects were pooled, processed, and analyzed for pre-dose, 1-7, 8-96, and 120-336 hours post-dose. To bridge the metabolite data from non-radiolabeled to radiolabeled plasma samples from the human mass balance study, [^{14}C] plasma samples from 0-168 hours post-dose for each of the six subjects were also pooled using the Hamilton pooling method, processed, and analyzed by radio-quantitation. [^{14}C] Plasma samples from 1-168 hours post-dose for two subjects were pooled (equal volume), processed, and analyzed.

Initial Method for Plasma Extraction and Recovery

[0091] Two plasma samples from a subject (4 and 72 hours post-dose) were used for initial extraction and recovery determination. The total radioactivity for each plasma sample in mass balance study was provided by Exelixis, Inc., and was defined as 100%. After the samples were thawed under a biological hood, two 0.5 mL aliquots of each plasma sample were added to 3 volumes (1.5 mL) of MeOH:ACN (20:80, v/v) and vortexed (5 min). The mixtures were centrifuged at 2000 rpm for 10 minutes, and the supernatants were transferred to clean tubes. The pellets were extracted with two additional 3 volumes of MeOH:CAN (20:80, v/v). The mixtures were centrifuged, and the supernatants were combined. Aliquots were analyzed by a 2900 TR liquid scintillation counter (LSC) (Packard Instruments, Meridian, CT). The extraction recovery was calculated as the following:

Extraction Recovery (%) = (DPM in supernatant/DPM in plasma sample) x 100

[0092] The supernatants from the extraction were evaporated to dryness under a stream of nitrogen in an ambient water bath. The residues were then reconstituted in 0.35 – 0.5 mL of MeOH:ACN:water (10:20:70, v/v/v). The reconstituted samples were centrifuged at 15,000 rpm for 10 minutes and aliquots were analyzed by LSC for reconstitution recovery.

Reconstitution Recovery (%) = (DPM in reconstitution solution /DPM in supernatant) x 100

Plasma Sample Preparation

[0093] Radiolabeled and non-radiolabeled plasma samples were extracted employing the same method, using 1.0-2 mL plasma samples, depending on the volume available and radioactivity level of the samples. The supernatants were evaporated to dryness under a stream of nitrogen in an ambient water bath, and the residues were reconstituted in 0.35-0.5 mL of MeOH:ACN:water (10:20:70, v/v/v). The reconstituted samples were centrifuged at 15,000 rpm for 10 minutes. Aliquots of the supernatants were injected onto the HPLC system for analysis.

Sample Preparation and Radioactive Recovery for Human Urine

[0094] Pooled urine samples from a subject (0-72, 168-192, and 312-336 hours post dose) were lyophilized in triplicate (each 4 mL), and the residues were reconstituted in 1 mL of water:ACN:FA (80:20:0.1, v/v/v). The radioactivity in pooled urine and reconstituted solution was counted using LSC, and the reconstitution recovery calculated. For metabolite profiling, identification, and radio-quantitation, pre-dose and 3 pooled urine samples (0-72 hours, 168-192 hours, and 312-336 hours post-dose) from each of the six subjects were analyzed. Each pooled urine sample was lyophilized, the residue was reconstituted in water:ACN:FA (80:20:0.1, v/v/v), and the reconstituted sample was centrifuged at 15,000 rpm for 10 min before analysis.

Sample Preparation and Radioactive Recovery for Human Urine

[0095] To evaluate the extraction recovery of fecal samples, two fecal homogenate samples from a subject were thawed under a biological hood. Approximately 5.5-6 g fecal homogenate was accurately weighed out for the extraction. Fifteen mL ACN:MeOH (80:20) was added to the fecal homogenates. The mixtures were vortexed for 3 minutes and centrifuged at 3000 rpm for 10 minutes. The supernatants were transferred to clean tubes. The extraction procedure was repeated two more times. The supernatants from all three extractions were combined. The radioactivity in the combined supernatants was determined by LSC. The extraction recovery was calculated using the following formula:

Extraction Recovery (%) = (DPM in supernatant/DPM in fecal homogenate) x 100

[0096] The supernatant was concentrated under a nitrogen stream at ambient temperature, and the residues were reconstituted in MeOH:ACN:water (10:20:70). Aliquots of reconstitution solution were
counted with LSC for reconstitution recovery.

Reconstitution Recovery (%) = (DPM in reconstitution solution/DPM in supernatant) x 100

Overall Recovery (%) = Extraction Recovery (%) x Reconstitution Recovery (%)/100

[0097] For metabolite profiling, identification, and radio-quantitation, pre-dose and 3 pooled fecal samples (0-72, 168-192, and 312-336 hours post-dose) from each of the six subjects were extracted using the same procedures for extraction recovery. The supernatants were dried under a nitrogen stream, and the residues were reconstituted in water:ACN:FA (80:20:0.1, v/v/v). The reconstituted samples were centrifuged at 15,000 rpm for 10 min before analysis.

HPLC Column Recovery

[0098] HPLC column recovery was carried out to demonstrate that all radioactive components were effectively eluted from the column using HPLC Method 1. Aliquots of urine samples (Subject 1042, 24-48 hours post-dose) were injected onto the HPLC system with or without a column, and the eluents from 0-30 minutes were collected into clean 50 mL centrifuge tubes. The weights of eluent from each injection were obtained after collection, and duplicate aliquots (1 mL) were counted using LSC. The average value of the counts was used to calculate the total radioactivity contained in the collected eluent with or without a column installed.

Column Recovery (%) = (DPM in eluent with column/DPM in eluent without column) x 100

[0099] HPLC Method 3 was used for pooled plasma only, and the column recovery was not
performed due to limited sample volume available.

HPLC/MS/RFD and HPLC Radio-Analysis Systems

[00100] The system for metabolite profiling and identification (HPLC/MS/RFD) consisted of a HTC PAL autosampler, a Surveyor HPLC pump, a LTQ linear ion trap mass spectrometer, and a β-RAM Model 3 RFD. The data obtained by mass spectrometry and

RFD were processed by Xcalibur and Laura Lite 3 software, respectively. The HPLC eluent was split between the RFD and mass spectrometer with a ratio of 3 to 1. The following are the summary of the conditions for HPLC, mass spectrometer, and RFD.

HPLC/MS/RFD Method 1

HPLC	Surveyor HPLC pump		
• Column Type	Phenominex Synergi Polar RP, 4.6 x 250 mm, 4 μm		
• Mobile Phases	A: H_2O with 0.1% FA		
	B: ACN with 0.1% FA		
• Gradient Program	**Time (min)**	**A%**	**B%**
	0	80	20
	2	80	20
	22	30	70
	23	5	95
	27	5	95
	28	80	20
	34	80	20
• Flow Rate	800 μL/minutes		
• Analysis Time	34 minutes		
Mass Spectrometry:	Thermo Finnigan LTQ Linear Ion Trap		
• Sheath gas flow rate	50 unit		
• Auxiliary gas flow rate	20 unit		
• Sweep gas flow rate	10 unit		
• Ion spray voltage	5 kV for ESI+; 4.3 kV for ESI-		
• Capillary temperature	300 °C		
• Capillary voltage	22 V for ESI+; -9 V for ESI-		
• Tube lens voltage	80 V for ESI+; -96 V for ESI-		
• Ionization mode	ESI+, ESI-		
Radio Flow-through Detector:	β-RAM Model 3		
• Radionuclide	^{14}C		
• Cell Volume	400 μL		
• Scintillation Cocktail	Ultima-Flo M, Perkin Elmer		
• Cocktail/HPLC flow ratio	3:1		

HPLC/MS Method 2

HPLC	Surveyor HPLC pump		
• Column Type	Phenominex Synergi Polar RP, 4.6 x 250 mm, 4 μm		
• Mobile Phases	A: H_2O with 0.1% FA		
	B: ACN with 0.1% FA		
• Gradient Program	**Time (min)**	**A%**	**B%**
	0	80	20
	2	80	20
	40	35	65
	42	5	95
	47	5	95
	48	80	20
	55	80	20
• Flow Rate	800 μL/minutes		
• Analysis Time	55 minutes		

Mass Spectrometry:	Thermo Finnigan LTQ Linear Ion Trap
• Sheath gas flow rate	50 unit
• Auxiliary gas flow rate	20 unit
• Sweep gas flow rate	10 unit
• Ion spray voltage	5 kV
• Capillary temperature	300 °C
• Capillary voltage	22 V
• Tube lens voltage	80 V
• Ionization mode	ESI+

HPLC/MS Method 3

HPLC	Surveyor HPLC pump		
• Column Type	Waters Xbridge phenyl, 4.6 x 150 mm, 3.5 μm		
• Mobile Phases	A: H_2O with 0.1% FA		
	B: ACN with 0.15% FA		
• Gradient Program	**Time (min)**	**A%**	**B%**
	0	80	20
	2	80	20
	40	30	70
	42	5	95
	47	5	95
	48	80	20
	55	80	20
• Flow Rate	800 μL/minutes		
• Analysis Time	55 minutes		

Mass Spectrometry:	Thermo Finnigan LTQ Linear Ion Trap
• Sheath gas flow rate	50 unit
• Auxiliary gas flow rate	20 unit
• Sweep gas flow rate	10 unit
• Ion spray voltage	5 kV
• Capillary temperature	300 °C
• Capillary voltage	22 V
• Tube lens voltage	80 V
• Ionization mode	ESI+

[00101] The HPLC-MS system for high resolution MS consisted of a Michrom Bioresources Paradigm MS4B HPLC and a Thermo LTQ Orbitrap Discovery mass spectrometer. Chromatographic conditions and the ion source parameters were the same as HPLC method 1 for the LTQ system. Data were acquired with a resolution of 30000 in centroid mode.

[00102] An HPLC/TopCount NXT™ system was used for the radio-quantitation of plasma samples. The system consisted of an HTC PAL autosampler, two Shimadzu HPLC pumps, and a Foxy Jr. Fraction Collector (Isco, Lincoln, NE). HPLC fractions collected in a LumaPlate™ 96-well plate were dried using an EZ-2plus Personal Evaporator (Genevac, Valley Cottage, New York), and the dried samples were counted by TopCount NXT™ Microplate Scintillation & Luminescence Counter (PerkinElmer®). The data were processed using ProFSA (PerkinElmer®) software. The HPLC methods were the same as described above.

Metabolite Identification

[00103] Metabolites that represented greater than 5% of the total radioactivity or 5% of total AUC in the matrix were identified according to the following process. Mass spectra

(MS, MS/MS, and MS/MS/MS) of cabozantinib and its metabolite standards, provided by the Exelixis, Inc., were acquired on an ion trap mass spectrometer. Major fragment patterns were proposed. Identification of these metabolites was confirmed by matching mass spectra (MS and MS/MS) and retention times with authentic reference standards. For other unknown metabolites, molecular ions were searched on LC/MS chromatograms operating in full scan positive as well as negative ionization modes at the same retention times as those found on LC-radio chromatogram. Product ion mass spectra and high resolution mass spectra were then acquired for the corresponding molecular ions. Putative metabolite structures were proposed based on the analysis of their mass fragment patterns.

Quantitation of cabozantinib and its Metabolites

[00104] Quantitation of cabozantinib and its metabolites in pooled or individual samples from each matrix at different time points or time intervals was based on integration of the corresponding peaks found on their radio-chromatograms. For plasma samples, percent of total radioactivity in the sample for each peak at each time point was calculated and converted to ng/mL.

[00105] For quantification of cabozantinib and its metabolites in plasma:

ng/mL = (% of the total radioactivity) x (total ng equivalent/mL for the time point)/100

[00106] The values of total ng equivalent/mL were obtained from the results of the human mass balance study.

[00107] For the pooled urine samples, percent of total radioactivity in the pooled sample for each peak was calculated as the % of total non-parent in the pooled samples:

% of total non-parent in the pooled samples = (total radioactivity of the peak/total radioactivity of the non-parent peaks) x 100

[00108] For the pooled fecal samples, percent of total radioactivity in the pooled sample for each peak was calculated as the percent of total non-parent plus parent in the pooled samples:

% of total non-parent plus parent in the pooled samples = (total radioactivity of the peak/total radioactivity of the parent and non-parent peaks) x 100

[00109] The percent of total radioactivity in the pooled sample for each peak was converted to the percent of parent in the pooled samples:

% of parent in the pooled samples = (total radioactivity of the peak/total radioactivity of the parent peak) x 100

[00110] The limit of quantification for a radioactivity detector was defined as the ratio of signal to noise (3 to 1) on the radio-chromatogram. The low limits of quantification were 10 and 500 dpm for the TopCount and β-RAM radio flow-through detector, respectively.

Results and Discussion

Radioactive Recovery

[00111] The initial extraction recovery was determined using plasma samples from a subject at 4 hours and 72 hours post-dose with three volumes of MeOH:ACN (20:80) extracting three times. The mean extraction recoveries of radioactivity from 4 and 72 hour samples were 98.43% and 94.99%, respectively. After drying down and reconstitution into MeOH:ACN solution, the reconstitution recoveries were 92.73% and 85.90%, respectively. The overall recoveries were 91.27% and 81.60%, respectively.

[00112] Urine centrifugation recoveries determined using 0-8, 24-48, 72-96, and 120-144 hour post-dose samples from the subject ranged between 102% and 104%. Urine reconstitution recovery after lyophilization was 94.7% using pooled samples from a subject.

[00113] For pooled fecal samples from 0-48 hours post dose, the extraction, reconstitution, and overall recoveries were 98.48%, 88.80%, and 87.37%, respectively. For pooled fecal samples of 120-168 hours post dose, the extraction, reconstitution, and overall recoveries were 85.85%, 87.69%, and 75.24%, respectively.

[00114] The radioactivity recovery from HPLC column for urine sample was 97.05%.

[00115] No correction factor was applied to the plasma, urine, and fecal radio-quantitation to account for the recovery.

Metabolite Profiling

[00116] In a subject, cabozantinib, compound **9** (cabozantinib sulfate), and compound **19** (cabozantinib *N*-oxide) were the major peaks on the radio-chromatograms. Compound **2** (demethylated and sulfated fluoroaniline cleavage product) was the major metabolite in plasma samples after 72 hours post-dose. Metabolite compound 7 (fluoroaniline cleavage product) accounted for one of the minor peaks. Metabolite compounds **7**, **3** (demethyl

cabozantinib glucuronide B), **9**, and **10** (methyl ester of fluoroaniline cleavage product) co-eluted using HPLC Method 1.

[00117] Representative human urine metabolite profiles, the radio-chromatograms (using HPLC Method 1) of human urine samples from 0-72 hours, 144-192 hours, and 288-336 hours post-dose were collected from a subject. Metabolite compound **6** was the major metabolite in 0-72 hours, 144-192 hours, and 288-336 hours post dose pooled urine samples. In addition to compound **6**, metabolite compounds **1**, **4**, **5**, **7**, and **19** were observed in the pooled sample of 0-72 hours post dose. Metabolite compounds **1**, **4**, **5**, and **7** were observed in the pooled sample of 144-192 hours post dose. Metabolite compounds **1** and **5** were detected in the pooled sample of 288-336 hours post dose. Parent compound cabozantinib was not observed in urine samples.

[00118] Representative human fecal metabolite profiles, the radio-chromatograms (using HPLC Method 1) of human fecal samples from 0-72 hours, 144-192 hours, and 288-336 hours post-dose from a. Parent cabozantinib and metabolites compound **4**, **7**, **11**, and **15** (including compound **16**) were observed in the pooled sample of 0-72 hours post dose. Metabolite compounds **4**, **7**, **11**, **15**, **16**, and **18** were observed in the pooled sample of 144-192 hours post dose. Metabolite compounds **4** and **11** were observed in the pooled sample of 288-336 hours post dose.

Metabolite Identification Using HPLC/MS Analysis

[00119] HPLC/MS analysis of authentic standards using HPLC Method 1 showed that the retention times of cabozantinib, fluoroaniline cleavage product (compound **7**), sulfate (compound **9**), and *N*-oxide (compound **19**) were 20.3, 14.4, 16.5, and 23.1 minutes, respectively.

[00120] Plasma, urine, and fecal samples were next analyzed by HOPLC/MS, and the compounds were identified based on their protonated molecular ions and fragmentation patterns.

Metabolite Identification of Cabozantinib and its Metabolites in Human Plasma

[00121] The mass spectrum of the peak at approximately 19.1 minutes in the XIC showed the protonated molecular ions at m/z 502. Its product ion spectra showed major fragments at m/z 391, 323, and 297, which is consistent with those of cabozantinib standard. The MS data is summarized in Table 1 and 2.

Table 1. HPLC Radiochromatogram Retention Times of Metabolites in Samples from Single Oral Dose of [^{14}C] Cabozantinib

Compound	HPLC Method	Retention Time (min)
Standards		

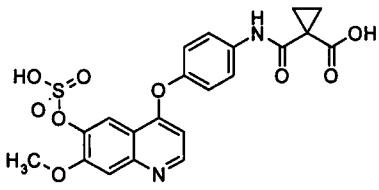
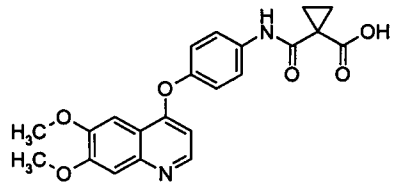
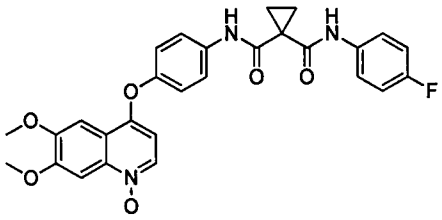
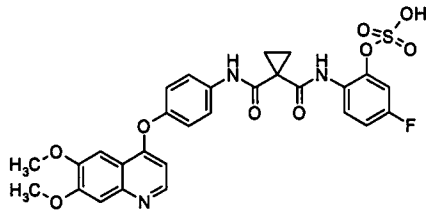
Compound	HPLC Method	Retention Time (min)
7	1	14.13
9	1	16.45
I	1	20.26
19	1	23.06
Plasma		
1	1	4.13
2a/2b	1	9.33
4	1	11.87
5	1	12.80
6	1	13.47
7	1	14.13
9	1	14.67
I	1	18.67
19	1	23.47
Urine		
1	1	4.13
4	1	11.87
5	1	12.80
6	1	13.47
7	1	14.13
19	1	23.47
Feces		
4	1	12.67
7	1	13.47
11	1	16.07
15	1	17.87
I	1	19.60
18	1	21.03
Hamilton Pooled Sample		
Plasma		
9	3	17.36
7	3	19.32
8	3	19.32 (shoulder)
19	3	25.20
I	3	37.52

Table 2. MS Data for Metabolites Using HPLC

Compound	HPLC Method	HPLC Retention Time	MS (m/z)
I	1	19.10	502
19	1	21.85	518
9	1	15.29	518 (loss of SO_3 from m/z molecular ion m/z at 598)
7	1	13.36	409
2a	1	10.70 (2a)	473, 395 (loss of SO_3 from

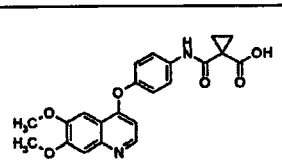
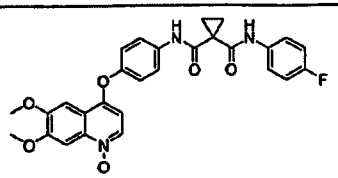
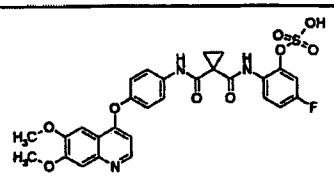
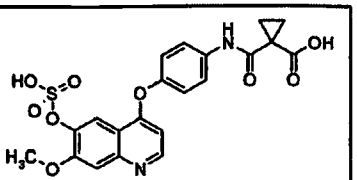
Compound	HPLC Method	HPLC Retention Time	MS (m/z)
			m/z molecular ion m/z at 473)
3	2	15.87	488
8	2	19.43	488
10	2	33.56	423
5	1	13.00	489
6	1	13.39	393
15	1	17.60	488
16	1	17.60	518
13	1	16.45	518
12	1	16.45	518
17	1	18.43	518

PK Parameters of Cabozanintinib and Metabolites

[00122] PK parameters for cabozantinib and various metabolites were measured in eight human subjects who were administered cabozantinib 140 mg/day. The metabolites that were observed included  ,  ,  , and  .

[00123] Parameters measured included maximum observed concentration (C_{max}) measured as ng/mL; Area under the concentration-time curve (AUC) measured as h · ng/mL at time 0 to 24 hours (AUC_{0-24}), at time 0 to 72 hours (AUC_{0-72}), at time 0 to the time of last measurable concentration (AUC_{0-t}). AUC Ratios were also calculated, including the ratio of AUC(cabozantinib) to the AUC of the sum of cabozantinib plus measured cabozantinib metabolites. Results are summarized in the following Table 3.

Table 3. Selected PK Parameters for Cabozantinib and Measured Metabolites

	Cabozantinib				
C_{max},ng/mL	1250±238 (19)	52.9±17.3 (33)	118±33.7 (28)	236±66.7 (28)	229±91 (40)
T_{max},h[a]	1.49 (1.00, 3.00)	18.99 (5.00, 24.10)	13.50 (2.00, 24.30)	24.00 (3.00, 48.00)	168 (72-240)
AUC_{0-24}, h·ng/mL	14300±2600 (18)	1080±341 (32)	2030±682 (34)	3970±1350 (34)	950±376 (40)
AUC_{0-72}, h·ng/mL	35000±6770 (19)	3120±976 (31)	5610±1940 (35)	12600±4180 (33)	.
AUC_{0-t}, h·ng/mL	67200±6880 (10)	6540±1680 (26)	10100±3210 (32)	28900±10700 (37)	99362±34386 (35)
Ratio [b], %	NA	9.93±3.20 (32)	15.0±3.80 (25)	42.9±14.4 (33)	150±51.3 (34)
Ratio [c], %	32±6.06 (19)	3.63±1.76 (48)	5.25±2.15 (41)	13.4±5.6(43)	46±11.2 (25)
AUC_{0-inf}, h·ng/mL	68000±6910 (10)	6770±1700 (25)	10300±3170 (31)	29500±10600 (36)	177682±62496(35)[d]
k_{el}, 1/h	0.00712±0.00176 (25)	0.00807±0.00218 (27)	0.00846±0.00256 (30)	0.00859±0.0022 (26)	.
$t_{1/2}$, h	102±23.3 (23)	91.8±25.4 (28)	89.2±29.2 (33)	86.0±24.3 (28)	494±99 (20)

a: median (range); [b]: ratio of AUC_{0-t} (metabolite)/AUC_{0-t} (parent);

c: ratio of AUC_{0-t} (each analyte)/ AUC_{0-t} (parent+4 measured metabolites); NA: Not Applicable;[d] >40% of AUCinf was extrapolated

C_{max}, maximum observed concentration; T_{max}, time of the maximum concentration; AUC_{0-t}, area under the concentration-time curve from time zero to the time of the last measurable concentration; AUC_{0-24}, area under the concentration-time curve from time zero to 24 hours post XL184 dose; AUC_{0-72}, area under the concentration-time curve from time zero to 72 hours post XL184 dose; AUC_{0-inf}, area under the concentration-time curve from time zero to infinity; k_{el}, apparent terminal elimination rate constant; $t_{1/2}$, apparent terminal elimination half-life; CL/F, apparent total body clearance; V/F, apparent total volume of distribution.

Kinase Activity of Cabozantinib Metabolites

Kinase Dilution

[00124] Kinase Activity was measured and profiled by by EMD Millipore according to the Kinase Profiler Service Assay Protocols Protocol Guide Volume 57. The results are summarized below in Table 4. Inhibition is indicated as IC_{50} with the following key: A = IC_{50} less than 50 nM, B = IC_{50} greater than 50 nM, but less than 500 nM, C = IC_{50} greater than 500 nM, but less than 5000 nM, and D = IC_{50} greater than 5,000 nM. Depending upon the functionality about the quinazoline or quinoline, exemplary compounds of the invention exhibit selectivity for any of c-Met, KDR, c-Kit, flt-3, and flt-4. Abbreviations for enzymes listed in Table 3 are defined as follows: c-Met refers to hepatocyte growth factor receptor kinase; RET refers to RET proto-oncogene kinase; KDR refers to kinase insert domain receptor tyrosine kinase; flt-1 alpha, flt-3, and flt-4, fms-like tyrosine kinases, representative of the FLK family of receptor tyrosine kinases; and Aurora B MP refers to Aurora B kinase. When a percentage is listed instead of an IC_{50} value, it indicates percent inhibition at 1 μM. Empty cells in the tables indicate lack of data only.

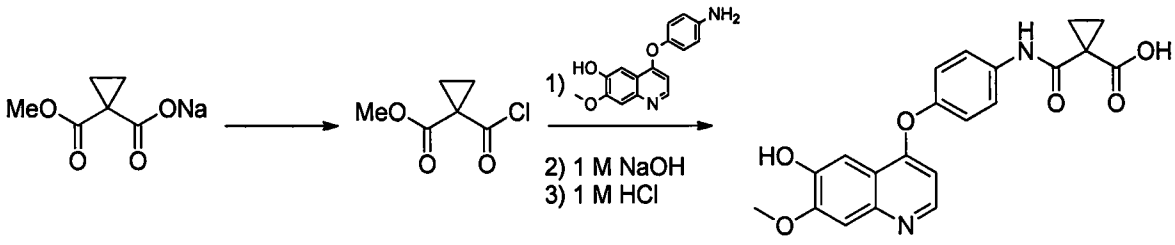
Table 4. Kinase Activity

Compound ID	MOLSTRUCTURE	c-Met Std (IC50) (nM)	RET Std (IC50) (nM)	KDR Std (IC50) (nM)	Flt-1 Alpha (IC50) (nM)	Flt-3 Std (IC50) (nM)	Flt-4 Std (IC50) (nM)	Aurora B MP 8pt Std (IC50) (nM)
Cabozantinib		A	A	A	A	A	A	
16		A		A	C	A		B
13		A		A	C	A		B
2a		≥ 50% @ 1μM	≤ 25% @ 1μM	≤ 25% @ 1μM	≤ 25% @ 1μM	≤ 25% @ 1μM	≥ 25% @ 1μM	≤ 25% @ 1μM

Compound ID	MOLSTRUCTURE	c-Met Std (IC50) (nM)	RET Std (IC50) (nM)	KDR Std (IC50) (nM)	Flt-1 Alpha (IC50) (nM)	Flt-3 Std (IC50) (nM)	Flt-4 Std (IC50) (nM)	Aurora B MP 8pt Std (IC50) (nM)
2b		Not Detected						
9		≥ 75% @ 1μM	≥ 75% @ 1μM	≤ 25% @ 1μM	≥ 50% @ 1μM	≥ 50% @ 1μM	≥ 75% @ 1μM	≥ 75% @ 1μM
19		B		B		C		C
7		D		D		C		C

Metabolite Synthesis and Structural Data

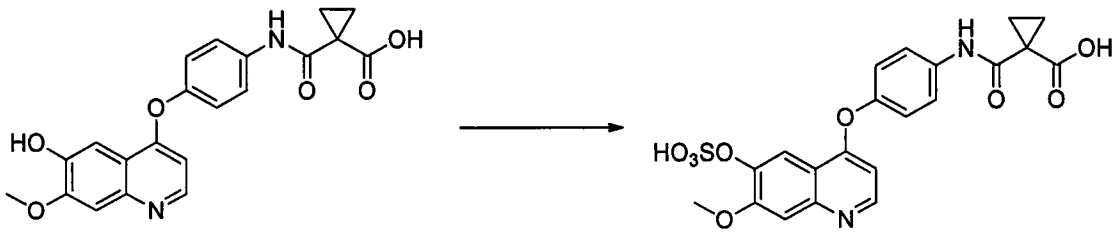
[00125] 6-Desmethyl Acid



6-Desmethyl Acid

[00126] In a vessel, cabozantinib (15.0 g; 53.3 mmol) and potassium carbonate (29.5 g; 213.3 mmol; 4 equiv) were suspended in THF (210 mL; 14 vol) and water (90 mL; 6 vol) at 20 °C. In a separate vessel, sodium 1-(methoxycarbonyl)cyclopropanecarboxylate (17.71 g; 106.6 mmol; 2 equiv.) was suspended in THF (90 mL; 6 vol). DMF (120 µL; 3 mol%) was added and cooled to less than 15 °C. Oxalyl chloride (9.34 mL; 106.6 mmol; 2 equiv.) was added over 90 minutes, and the reaction was aged 2 hours at 10-15 °C. The acid chloride slurry was added to the cabozantinib suspension over 2 hours at 20-25 °C and aged at least 3 hours, whereupon HPLC analysis showed greater than 99% conversion to a mixture of the mono- and biscarbonylated material. The reaction mixture was filtered over Celite®, washed with THF (30 mL; 2 vol), and the layers were separated. 1 M NaOH (150 mL; 10 vol) was added to the upper THF layer, and the mixture was heated at 40 °C for 1 hour whereupon HPLC analysis showed greater than 99% saponified product. The mixture was cooled to 25 °C, and the upper THF layer was removed. The aqueous layer was acidified to pH 3-4 with 1 M HCl to precipitate the product and was aged for 1 hour. The precipitate was filtered, washed with water (90 mL, 6 vol), and dried under vacuum (greater than 20 psig) with nitrogen bleed at 50 °C to give a grey to brown powder. ^{1}H-NMR (DMSO-d_6, 400 MHz) δ 10.8-11.0 (br s, 1H), 10.7 (s, 1H), 8.65 (d, J=6.9 Hz, 1H), 7.81 (d, J=9.3 Hz, 2H), 7.67 (s, 1H), 7.58 (s, 1H), 7.32 (d, J=9.3 Hz, 2H), 6.69 (d, J=6.9 Hz, 1H), 4.01 (s, 3H), 2.48-2.53 (m, 4H). MS (ESI-) m/z 393 $[M-H]^-$.

[00127] 6-Hydrogen Sulfate 6-Desmethyl Acid

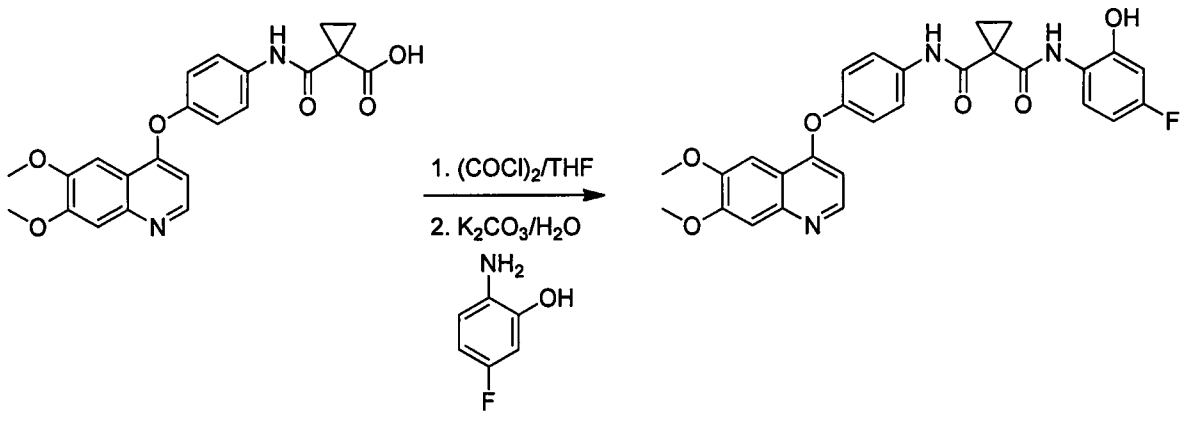


6-Desmethyl Acid **6-hydrogen sulfate**

[00128] 6-Desmethyl acid (120 mg; 0.30 mmol), potassium hydroxide (118 mg; 2.1 mmol; 7 equiv.), and sulfur trioxide trimethyl amine complex (292 mg; 2.1 mmol; 7 equiv.) was dissolved in water (3 mL; 25 vol) and heated to 70 °C for 2 hours whereupon analysis by HPLC showed greater than 99% conversion. The reaction mixture was then cooled in an ice bath and acidified dropwise with 1 N aq. H_2SO_4 to approximately pH 1. The slurry was aged at 25 °C for 1 hour, filtered, washed with water (3 mL; 25 vol), and deliquored. The wet cake was then washed with acetone (3 mL; 25 vol) and dried at 35 °C under vacuum (greater than 20 psig) with nitrogen bleed for 24 hours to give a beige powder.

[00129] Alternatively, 6-desmethyl acid (120 mg; 0.30 mmol) was suspended in MeCN (50 vol, 6 mL), and triethylamine (1.27 mL, 9.12 mmol, 30 equiv.) was added and then cooled in an ice bath. Chlorosulfonic acid (101 μL, 1.52 mmol, 5 equiv.) was added dropwise, and the reaction was then heated to 70 °C for 1 hour whereupon analysis by HPLC showed greater than 98 percent conversion. The reaction mixture was then cooled in an ice bath for 2 hours in which a precipitate was formed. The precipitate was removed with filtration, rinsing with cold MeCN (50 vol). The MeCN solution was then concentrated to approximately 20 vol (approximately 2 mL) and quenched with 100 vol 1N HCl and cooled in an ice bath to give a fine precipitate that was filtered, washed with 50 vol water and 50 vol acetone, and dried at 35 °C under vacuum (greater than 20 psig) with nitrogen bleed for 24 hours to give a beige powder. ^{1}H-NMR (DMSO-d_6, 400 MHz) δ 10.8 (s, 1H), 8.83 (d, *J*=5.9 Hz, 1H), 8.5 (s, 1H), 7.85 (d, *J*=8.5 Hz, 2H), 7.52 (s, 1H), 7.40 (d, *J*=8.5 Hz, 2H), 6.84 (d, *J*=5.9 Hz, 1H), 4.04 (s, 3H), 3.20-3.70 (br s, 1H), 1.39-1.48 (br s, 4H). MS (ESI-) m/z 473 $[M-H]^-$, 236.

[00130] Ortho-Hydroxy-Cabozantinib



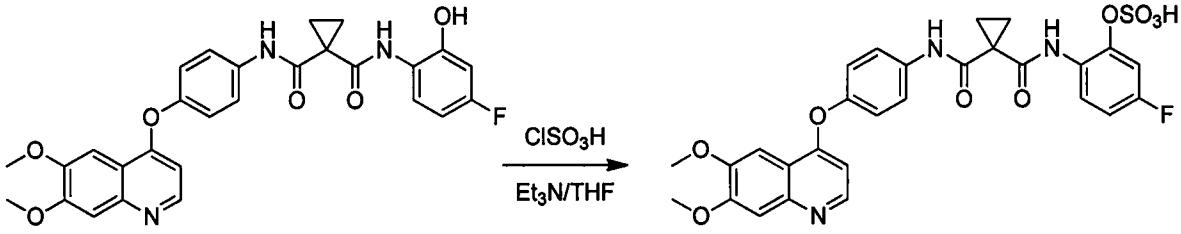
[00131] A flask was charged with the carboxylic acid (0.84 g; 2.1 mmol), THF (1.2 mL), and DMF (5 μL), and cooled to 15 °C. To this slurry was added oxalyl chloride (0.17 mL;

2.1 mmol) dropwise over approximately 20 minutes. After 2 hours, the acid chloride slurry was added to another vessel containing a stirred suspension of the aniline (0.2 g, 1.6 mmol), potassium carbonate (0.63 g, 4.6 mmol) in THF (2.8 mL), and water (1 mL) over approximately 15 minutes. After 3 hours, HPLC analysis showed complete conversion to the product. Stirring was stopped, the lower aqueous layer was removed, and water (30 mL) was added to precipitate the product. The product was then collected by filtration and washed with 1:1 THF-water solution (2 x 10 mL) to afford a pale grey solid. It was then further purified by flash chromatography on silica gel using methanol/dichloromethane as the mobile phase.

[00132] Alternatively, a suspension of the carboxylic acid (4.08 g; 10 mmol), aniline (1.52 g; 12 mmol), and triethylamine (2.7 mL; 20 mmol) in acetonitrile (100 mL) was treated with EDAC (2.30 g; 12 mmol) and HOBt (0.5 g; 3 mmol). The slurry was stirred overnight at room temperature, and the reaction progress was monitored by HPLC. At the end of the reaction, 150 mL of water was added ,and the precipitated product was collected by filtration, washed with water, and then purified by flash chromatography. ^{1}H-NMR (DMSO-d_6, 400 MHz) δ 10.46 (br s, 1H), 10.29 (br s, 1H), 10.0 (br s, 1H), 8.47 (d, 1H), 7.92 (dd, 1H), 7.73 (dd, 2H), 7.51 (s, 1H), 7.40 (s, 1H), 7.28 (dd, 2H), 6.68 (dd, 1H), 6.62 (dt, 1H), 6.45 (d, 1H), 3.95 (s, 3H), 3.94 (s, 3H), 1.60-1.55 (m, 4H). ^{13}C NMR (DMSO-d_6, 100 MHz) δ 169.82, 167.67, 159.91, 157.51, 152.58, 149.97, 149.35, 149.09, 148.98, 148.86, 146.49, 135.72, 123.00, 122.97, 122.91, 122.43, 121.30, 115.17, 107.86, 105.10, 104.87, 103.16, 102.43, 102.19, 99.08, 55.74, 55.71, 55.66, 30.02, 16.51.

MS (APCI+) m/z 518.3 $[M+H]^+$, 500.3.

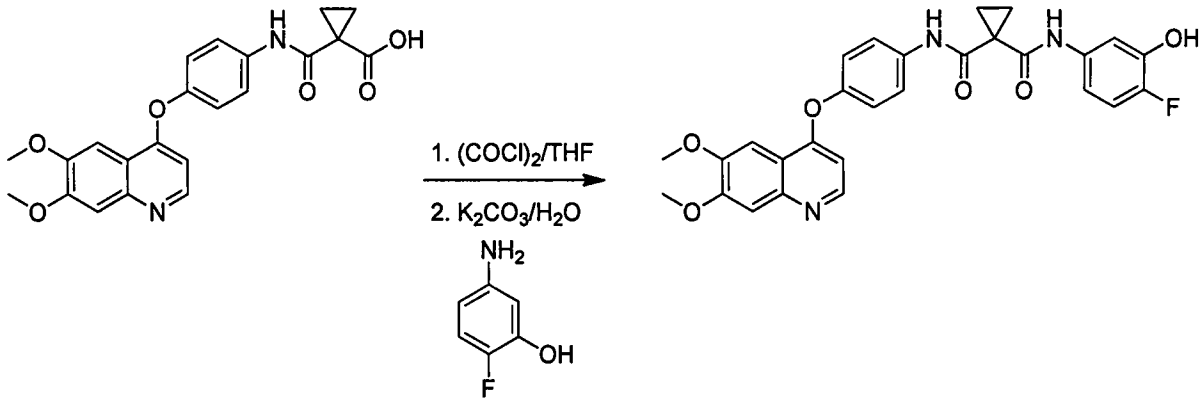
[00133] Cabozantinib-Hydroxysulfate



[00134] A suspension of the hydroxy-cabozantinib (0.95 g; 1.9 mmol) in THF (20 mL) was added triethylamine (5 mL; 36 mmol), and cooled to below 5 °C. Chlorosulfonic acid (1 mL; 15 mmol) was added dropwise such that the temperature remained below 10 °C, over approximately 15 minutes. After stirring overnight at room temperature, HPLC analysis showed approximately 5 percent of starting material remaining. The reaction mixture was

treated with aqueous 1 N HCl (25 mL). The precipitated product was collected by filtration, washed with water (4 x 25 mL), and dried under vacuum to yield an off-white solid (937 mg; 82 percent crude yield). Analysis by AN-HPLC showed that the product was 90.8% pure, the major impurity being the starting material. The product was purified to greater than 99 percent (AN-HPLC) by preparative HPLC on a C18 column, using aqueous ammonium acetate/acetonitrile mobile phase system. ^{1}H-NMR (DMSO-d_6, 400 MHz) δ 10.39 (s, 1H), 9.69 (s, 1H), 8.81 (d, 1H), 7.95 (dd, 1H), 7.85 (d, 2H), 7.77 (s, 1H), 7.51 (s, 1H), 7.11 (s, 1H), 7.08 (dd, 1H), 6.93 (dd, 1H), 6.45 (d, 1H), 4.05 (s, 3H), 4.04 (s, 3H), 1.53 (s, 4H). MS (ESI-) m/z 596.0 $[M-H]^-$.

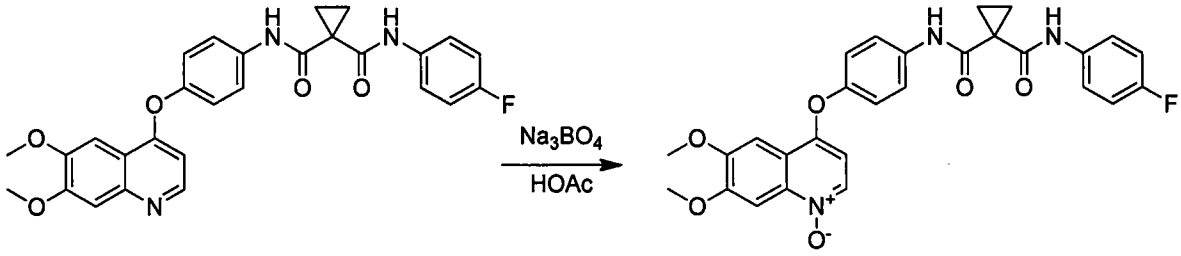
[00135] Meta-Hydroxy-Cabozantinib



[00136] A flask was charged with the carboxylic acid (0.84 g; 2.1 mmol), THF (1.2 mL), and DMF (5 μL), and cooled to 15 °C. To this slurry was added oxalyl chloride (0.17 mL; 2.1 mmol) dropwise over approximately 20 minutes. After 2 hours, the acid chloride slurry was added to another vessel containing a stirred suspension of the aniline (0.2 g, 1.6 mmol), potassium carbonate (0.63 g, 4.6 mmol) in THF (2.8 mL), and water (1 mL) over approximately 15 minutes. After 90 minutes, HPLC analysis showed complete conversion to the product. Stirring was stopped, and the lower aqueous layer was removed and extracted with ethyl acetate (15 mL). The organic layers were combined, dried over anhydrous $MgSO_4$, filtered, and concentrated to yield a brown solid. The solid was then further purified by flash chromatography on silica gel using ethyl acetate/heptane as the mobile phase. ^{1}H-NMR (DMSO-d_6, 400 MHz) δ 10.15 (br s, 1H), 9.96 (br s, 1H), 9.89 (br s, 1H), 8.46 (d, 1H), 7.76 (d, 1H), 7.50 (s, 1H), 7.41 (d, 2H), 7.39 (s, 1H), 7.22 (d, 2H), 7.07-6.98 (m, 2H), 6.42 (d, 1H), 3.94 (s, 3H), 3.93 (s, 3H), 1.46 (br s, 4H). ^{13}C NMR (DMSO-d_6, 100 MHz) δ 168.27, 167.95, 160.02, 152.56, 149.48, 149.33, 148.86, 148.56, 146.46, 146.21, 144.52, 144.39, 136.45, 135.33, 135.31, 122.23, 121.22, 115.63, 115.44, 115.15, 111.29, 111.23, 110.26, 107.85,

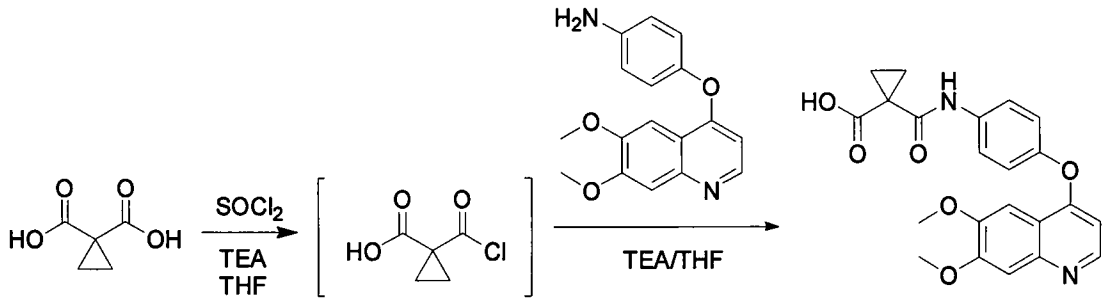
103.04, 99.08, 55.73, 55.71, 31.66, 15.40. MS (APCI+) m/z 518.3 $[M+H]^+$, 502.3.

[00137] Cabozantinib N-Oxide



[00138] A flask was charged with N-(4-{[6,7-bis(methyloxy)quinolin-4-yl]oxy}phenyl)-N'-(4-fluorophenyl)cyclopropane-1,1-dicarboxamide (3.21 g; 6.4 mmol), acetic acid (32.1 mL), and sodium perborate tetrahydrate (1.98 g, 12.8 mmol) and heated to 65 °C and stirred overnight. After 24 hours, HPLC analysis showed about 38:62 starting material: product. More oxidant (1.98 g; 12.8 mmol) was added, and heating continued overnight. Solvents were removed under vacuum, and the residue was purified by flash chromatography using dichloromethane-methanol gradient (dichloromethane to 10% methanol-dichloromethane) to obtain 0.95 g of the product as a white solid. ^{1}H-NMR (DMSO-d_6, 400 MHz) δ 10.20 (br s, 1H), 10.08 (br s, 1H), 8.28 (d, 1H), 7.90 (s, 1H), 7.74 (d, 2H), 7.64 (dd, 2H), 7.48 (s, 1H), 7.23 (d, 2H), 7.15 (t, 2H), 6.45 (d, 1H), 3.97 (s, 3H), 3.94 (s, 3H), 1.47 (br s, 4H). ^{13}C NMR (DMSO-d_6, 100 MHz) δ 172.11, 168.18, 168.13, 159.49, 157.09, 153.34, 150.72, 150.57, 149.98, 137.41, 136.32, 135.24, 135.21, 134.06, 122.44, 122.36, 122.19, 120.65, 117.23, 11.17, 114.95, 104.37, 100.34, 99.12, 56.09, 56.03, 31.59, 15.42. MS (APCI+) m/z 518.3 $[M+H]^+$.

[00139] 1-[4-(6,7-Dimethoxy-quinolin-4-yloxy)-phenylcarbamoyl]-cyclopropane carboxylic acid



[00140] To the cyclopropyl di-carboxylic acid (449 mg, 3.45 mmol) in THF (3.5 mL) was added TEA (485 μL, 3.45 mmol). The resulting solution was stirred at room temperature

under a nitrogen atmosphere for 40 minutes before adding thionyl chloride (250 μL, 3.44 mmol). The reaction was monitored by LCMS for the formation of mono acid chloride (quenched the sample with MeOH and looked for corresponding mono methyl ester). After 3 hours stirring at room temperature, 4-(6,7-dimethoxy-quinolin-4-yloxy)-phenylamine (1.02 g, 3.44 mmol) was added as a solid, followed by more THF (1.5 mL). The reaction continued to stir at room temperature for 16 hours. The resulting thick slurry was diluted with EtOAc (10 mL) and extracted with 1N NaOH. The biphasic slurry was filtered, and the aqueous phase was acidified with concentrated HCl to pH or approximately 6 and filtered. Both solids were combined and washed with EtOAc, then dried under vacuum. The desired product, 1-[4-(6,7-dimethoxy-quinolin-4-yloxy)-phenylcarbamoyl]-cyclopropanecarboxylic acid, was obtained (962 mg, 68.7 percent yield, 97 percent pure) as a white solid. ^{1}H NMR (D_2O/NaOH): 7.97 (d, 1H), 7.18 (d, 2H), 6.76 (m, 4H), 6.08 (d, 1H), 3.73 (s, 3H), 3.56 (s, 3H), 1.15 (d, 4H).

[00141] The foregoing disclosure has been described in some detail by way of illustration and example for purposes of clarity and understanding. The invention has been described with reference to various specific and preferred embodiments and techniques. However, it should be understood that many variations and modifications can be made while remaining within the spirit and scope of the invention. It will be obvious to one of skill in the art that changes and modifications can be practiced within the scope of the appended claims. Therefore, it is to be understood that the above description is intended to be illustrative and not restrictive. The scope of the invention should, therefore, be determined not with reference to the above description, but should instead be determined with reference to the following appended claims, along with the full scope of equivalents to which such claims are entitled. Unless otherwise stated all patent references cited herein are incorporated by reference.

Claims

1. A pharmaceutical formulation comprising a physiologically effective amount of cabozantinib, wherein oral administration of said pharmaceutical formulation to a selected human subject group produces in said selected human subject group:

an average cabozantinib plasma area under the curve (average AUC) of at least 60,000 ng·h/mL per each 140 mg dosage of cabozantinib delivered;

an average maximum cabozantinib blood plasma concentration (average C_{max}) of at least 1000 ng/mL per each 140 mg dosage of cabozantinib delivered; and

a ratio of AUC(cabozantinib) to the AUC of the sum of cabozantinib plus measured cabozantinib metabolites:

AUC (Cabozantinib) / AUC (Cabozantinib + Measured Metabolites)

of at least 0.25;

wherein the AUC is measured from time zero to the time of last measurable concentration.

2. A pharmaceutical formulation comprising a physiologically effective amount of cabozantinib, wherein oral administration of said pharmaceutical formulation to a selected human subject group produces in said selected human subject group:

a steady state area under the curve for cabozantinib (average AUC) of at least 35,000 ng·h/mL for a formulation comprising 100 mg of cabozantinib delivered once daily;

an average maximum cabozantinib blood plasma concentration (average C_{max}) of at least 2400 ng/mL per each 100 mg dosage of cabozantinib delivered;

an average minimum cabozantinib blood plasma concentration (average C_{max}) of at least 1100 ng/mL per each 100 mg dosage of cabozantinib delivered; and

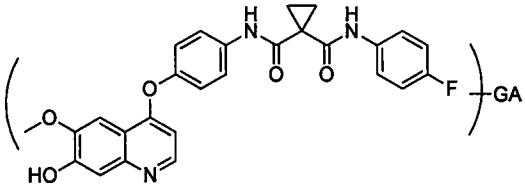
a ratio of AUC(cabozantinib) to the AUC of the sum of cabozantinib plus measured cabozantinib metabolites:

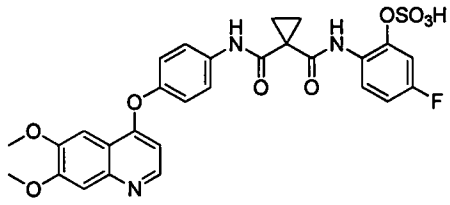
AUC (Cabozantinib) / AUC (Cabozantinib + Measured Metabolites)

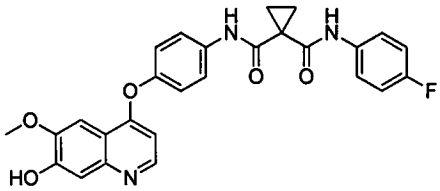
of at least 0.35;

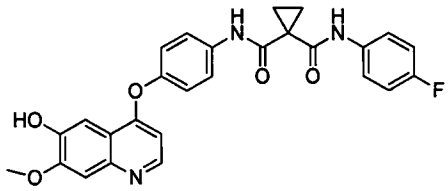
wherein the AUC is measured on day 22.

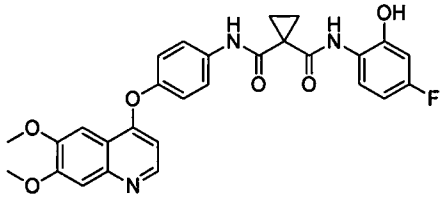
3. The pharmaceutical formulation of claims 1-2, wherein the measured metabolites are selected from the group consisting of:

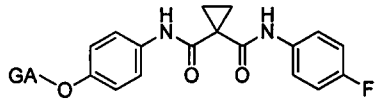


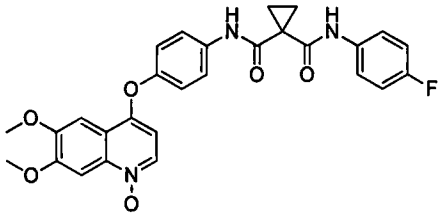


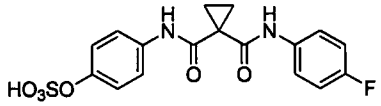


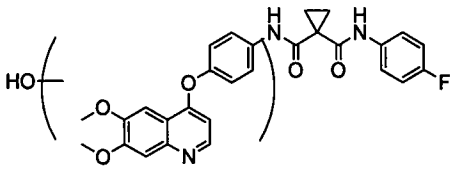


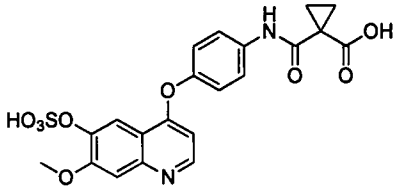


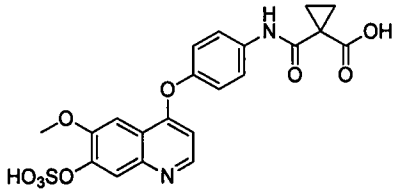


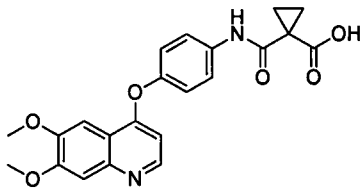


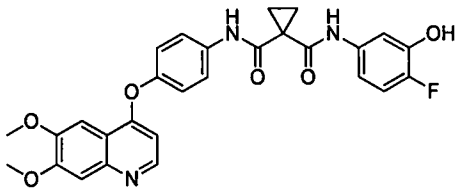


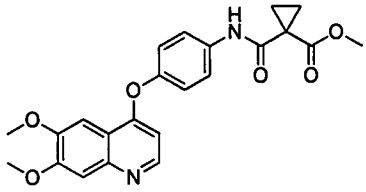


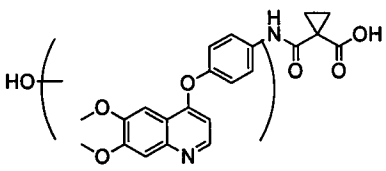












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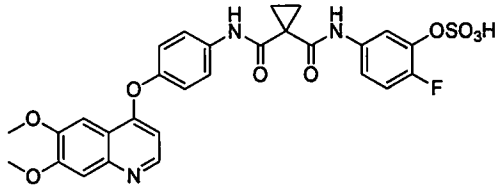

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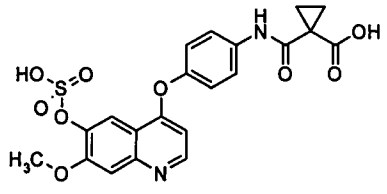
wherein GA is a glucuronic acid moiety,

4. The pharmaceutical formulation of claims 1-2, wherein the measured metabolites comprise one or more of:



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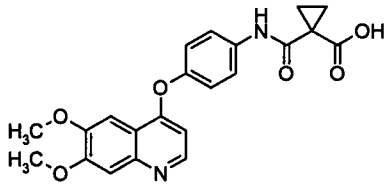

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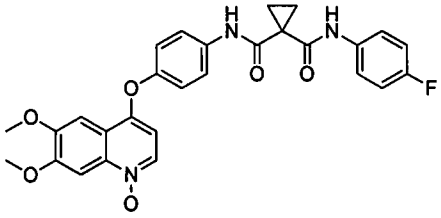

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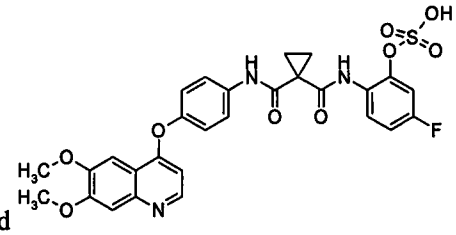

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5. A method of treating cancer in a human patient, comprising orally administering to said patient a pharmaceutical formulation comprising a physiologically effective amount of cabozantinib at such a rate to achieve:

an average cabozantinib plasma area under the curve (average AUC) of at least 60,000 ng·h/mL per each 140 mg dosage of cabozantinib delivered;

an average maximum cabozantinib blood plasma concentration (average C_{max}) of at least 1000 ng/mL per each 140 mg dosage of cabozantinib delivered; and

a ratio of AUC(cabozantinib) to the AUC of the sum of cabozantinib plus measured cabozantinib metabolites:

AUC (Cabozantinib) / AUC (Cabozantinib + Measured Metabolites)

of at least 0.25;

wherein the AUC is measured from time zero to the time of last measurable concentration.

6. A method of treating cancer in a human patient, comprising orally administering to said patient a pharmaceutical formulation comprising a physiologically effective amount of cabozantinib at such a rate to achieve:

a steady state area under the curve for cabozantinib (average AUC) of at least 35,000 ng·h/mL for a formulation comprising 100 mg of cabozantinib delivered once daily;

an average maximum cabozantinib blood plasma concentration (average C_{max}) of at least 2400 ng/mL per each 100 mg dosage of cabozantinib delivered;

an average minimum cabozantinib blood plasma concentration (average C_{max}) of at least 1100 ng/mL per each 100 mg dosage of cabozantinib delivered; and

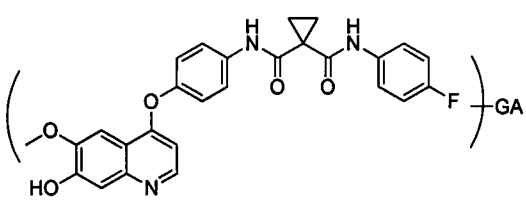
a ratio of AUC(cabozantinib) to the AUC of the sum of cabozantinib plus measured cabozantinib metabolites:

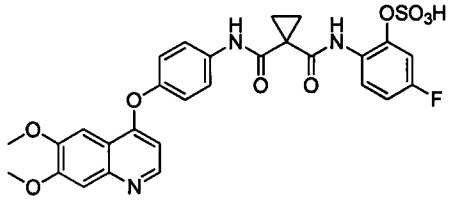
AUC (Cabozantinib) / AUC (Cabozantinib + Measured Metabolites)

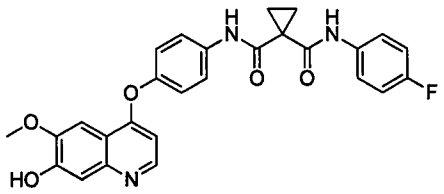
of at least 0.35;

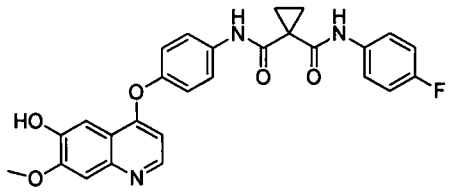
wherein the AUC is measured on day 22.

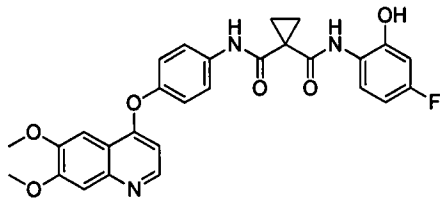
7. The method of claims 5-6, wherein the measured metabolites are selected from the group consisting of:

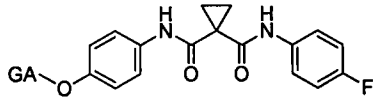


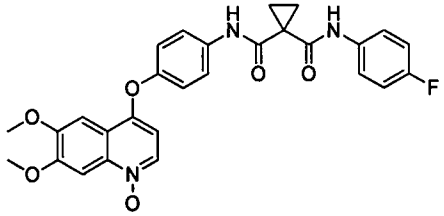


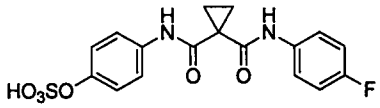


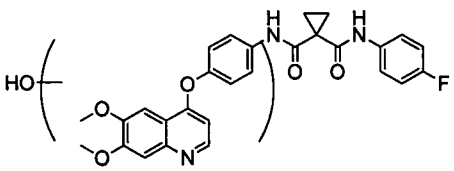


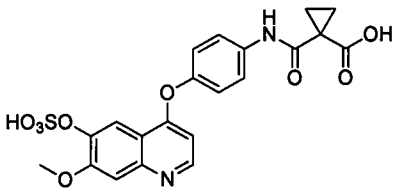


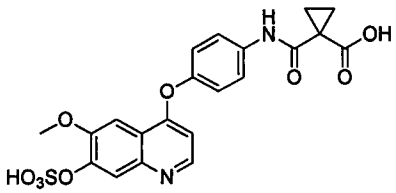


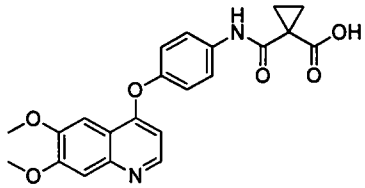


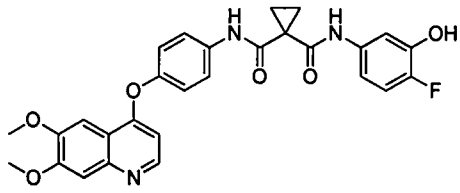


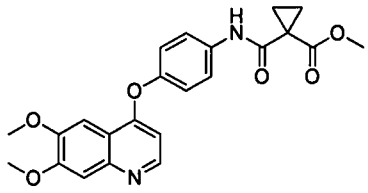


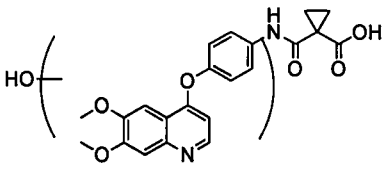




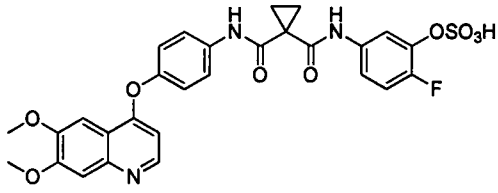






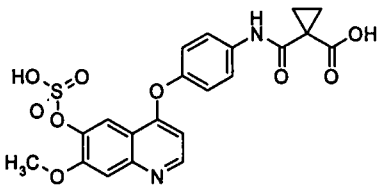


and

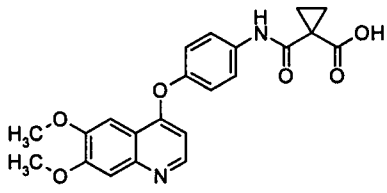


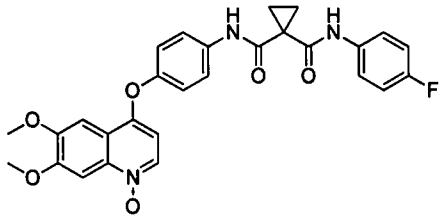
wherein GA is a glucuronic acid moiety,

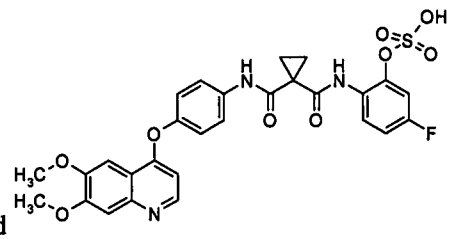
8. The method of wherein the measured metabolites comprise one or more of:



,







, and

9. The method of claims 5-8, wherein the cancer is selected from ovarian cancer, lung cancer, medullary thyroid cancer, liver cancer, gastrointestinal cancer, pancreatic cancer, bone cancer, hematologic cancer, skin cancer, kidney cancer, breast cancer, colon cancer, and fallopian tube cancer.

INTERNATIONAL SEARCH REPORT

International application No
PCT/US2015/021072

A. CLASSIFICATION OF SUBJECT MATTER
INV. A61P35/00 A61K31/47 C07D215/22 C07C305/24 C07C233/60
A61K9/00 A61K9/20 A61K9/48
ADD.
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)
A61K C07D C07C

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)
EPO-Internal, BIOSIS, CHEM ABS Data, EMBASE, WPI Data

C. DOCUMENTS CONSIDERED TO BE RELEVANT

Category*	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
X	R. ELISEI ET AL: "Cabozantinib in Progressive Medullary Thyroid Cancer", JOURNAL OF CLINICAL ONCOLOGY, vol. 31, no. 29, 3 September 2013 (2013-09-03), pages 3639-3646, XP055194909, ISSN: 0732-183X, DOI: 10.1200/JCO.2012.48.4659 abstract page 3640, left-hand column, paragraph 4 page 3640, right-hand column, last paragraph - page 3641, left-hand column, paragraph 2 ----- -/--	1,3-5, 7-9

[X] Further documents are listed in the continuation of Box C. [ ] See patent family annex.

* Special categories of cited documents :

"A" document defining the general state of the art which is not considered to be of particular relevance

"E" earlier application or patent but published on or after the international filing date

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"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

"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

"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

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"&" document member of the same patent family

Date of the actual completion of the international search	Date of mailing of the international search report
11 June 2015	19/06/2015

Name and mailing address of the ISA/	Authorized officer
European Patent Office, P.B. 5818 Patentlaan 2 NL - 2280 HV Rijswijk Tel. (+31-70) 340-2040, Fax: (+31-70) 340-3016	Gradassi, Giulia

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Form PCT/ISA/210 (second sheet) (April 2005)

C(Continuation). DOCUMENTS CONSIDERED TO BE RELEVANT

Category*	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
X	D. C. SMITH ET AL: "Cabozantinib in Patients With Advanced Prostate Cancer: Results of a Phase II Randomized Discontinuation Trial", JOURNAL OF CLINICAL ONCOLOGY, vol. 31, no. 4, 19 November 2012 (2012-11-19), pages 412-419, XP055122068, ISSN: 0732-183X, DOI: 10.1200/JCO.2012.45.0494 abstract page 2, left-hand column, paragraph 2 page 2, left-hand column, last paragraph - right-hand column, paragraph 1 page 3, right-hand column, last paragraph - page 5, left-hand column, paragraph 1	2-4,6-8
A	Anonymous: "NCT00940225 on 2012_05_30: ClinicalTrials.gov Archive", , 30 May 2012 (2012-05-30), XP055194913, Retrieved from the Internet: URL:https://clinicaltrials.gov/archive/NCT00940225/2012_05_30 [retrieved on 2015-06-10] pages 1-2	2-4,6-8