(12) INTERNATIONAL APPLICATION PUBLISHED UNDER THE PATENT COOPERATION TREATY (PCT)

(19) World Intellectual Property Organization
International Bureau

(43) International Publication Date
31 August 2017 (31.08.2017)

WIPO | PCT

(10) International Publication Number
WO 2017/145050 A1

(51) International Patent Classification:
C07D 213/69 (2006.01) *A61P 1/14* (2006.01)
A61K 31/444 (2006.01) *A61P 35/00* (2006.01)

(21) International Application Number:
PCT/IB2017/050981

(22) International Filing Date:
21 February 2017 (21.02.2017)

(25) Filing Language: English

(26) Publication Language: English

(30) Priority Data:
PCT/CN2016/074315
23 February 2016 (23.02.2016) CN

(71) Applicant: **GLAXOSMITHKLINE INTELLECTUAL PROPERTY DEVELOPMENT LIMITED** [GB/GB]; 980 Great West Road, Brentford Middlesex TW89GS (GB).

(72) Inventors: **DEMARTINO, Michael P.**; 1250 South Collegeville Road, Collegeville, Pennsylvania 19426 (US). **GUAN, Huiping Amy**; 288 Fute Zhong Road, Waigaoqiao, Shanghai (CN).

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

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

Declarations under Rule 4.17:

— *as to applicant's entitlement to apply for and be granted a patent (Rule 4.17(ii))*

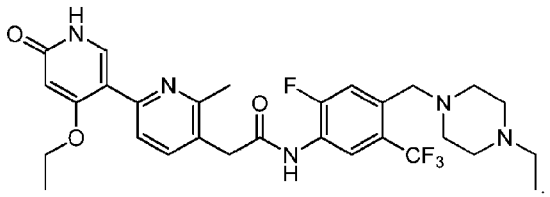
— *as to the applicant's entitlement to claim the priority of the earlier application (Rule 4.17(iii))*

— *of inventorship (Rule 4.17(iv))*

Published:

— *with international search report (Art. 21(3))*

(54) Title: PYRIDYLPYRIDONE DERIVATIVE USEFUL AS A RET KINASE INHIBITOR IN THE TREATMENT OF IBS AND CANCER



(I)

(57) Abstract: This invention relates to a novel compound which is an inhibitor of the Rearranged during Transfection (RET) kinase, to pharmaceutical compositions containing it, to processes for its preparation, and to its use in therapy, alone or in combination, for the normalization of gastrointestinal sensitivity, motility and/or secretion and/or abdominal disorders or diseases and/or treatment related to diseases related to RET dysfunction or where modulation of RET activity may have therapeutic benefit including but not limited to all classifications of irritable bowel syndrome (IBS) including diarrhea-predominant, constipation-predominant or alternating stool pattern, functional bloating, functional constipation, functional diarrhea, unspecified functional bowel disorder, functional abdominal pain syndrome, chronic idiopathic constipation, functional esophageal disorders, functional gastroduodenal disorders, functional anorectal pain, inflammatory bowel disease, proliferative diseases such as non-small cell lung cancer, hepatocellular carcinoma, colorectal cancer, medullary thyroid cancer, follicular thyroid cancer, anaplastic thyroid cancer, papillary thyroid cancer, brain tumors, peritoneal cavity cancer, solid tumors, other lung cancer, head and neck cancer, gliomas, neuroblastomas, Von Hippel-Lindau Syndrome and kidney tumors, breast cancer, fallopian tube cancer, ovarian cancer, transitional cell cancer, prostate cancer, cancer of the esophagus and gastroesophageal junction, biliary cancer, adenocarcinoma, and any malignancy with increased RET kinase activity. Formula (I)

WO 2017/145050 A1

PYRIDYLPYRIDONE DERIVATIVE USEFUL AS A RET KINASE INHIBITOR IN THE TREATMENT OF IBS AND CANCER

FIELD OF INVENTION

This invention relates to a novel compound which is an inhibitor of the Rearranged during Transfection (RET) kinase, to pharmaceutical compositions containing it, to processes for its preparation, and to its use in therapy, alone or in combination, for the normalization of gastrointestinal sensitivity, motility and/or secretion and/or abdominal disorders or diseases and/or treatment related to diseases related to RET dysfunction or where modulation of RET activity may have therapeutic benefit including but not limited to all classifications of irritable bowel syndrome (IBS) including diarrhea-predominant, constipation-predominant or alternating stool pattern, functional bloating, functional constipation, functional diarrhea, unspecified functional bowel disorder, functional abdominal pain syndrome, chronic idiopathic constipation, functional esophageal disorders, functional gastroduodenal disorders, functional anorectal pain, inflammatory bowel disease, proliferative diseases such as non-small cell lung cancer, hepatocellular carcinoma, colorectal cancer, medullary thyroid cancer, follicular thyroid cancer, anaplastic thyroid cancer, papillary thyroid cancer, brain tumors, peritoneal cavity cancer, solid tumors, other lung cancer, head and neck cancer, gliomas, neuroblastomas, Von Hippel-Lindau Syndrome and kidney tumors, breast cancer, fallopian tube cancer, ovarian cancer, transitional cell cancer, prostate cancer, cancer of the esophagus and gastroesophageal junction, biliary cancer and adenocarcinoma, and any malignancy with increased RET kinase activity.

BACKGROUND OF THE INVENTION

Irritable bowel syndrome (IBS) is a common illness affecting 10-20% of individuals in developed countries and is characterized by abnormal bowel habits, bloating and visceral hypersensitivity (Camilleri, M., N. Engl. J. Med., 2012, 367:1626-1635). While the etiology of IBS is unknown it is thought to result from either a disorder between the brain and gastrointestinal tract, a disturbance in the gut microbiome or increased inflammation. The resulting gastrointestinal changes affect normal bowel transit resulting in either diarrhea or constipation. Furthermore in a majority of IBS patients the sensitization of the peripheral nervous system results in visceral hypersensitivity or allodynia (Keszthelyi, D., Eur. J. Pain, 2012, 16:1444–1454).

While IBS does not directly alter life expectancy it has a considerable effect on a patient's quality of life. Moreover there is a significant financial cost for IBS associated healthcare and lost productivity due to worker absenteeism (Nellesen, D., et al., J. Manag. Care Pharm., 2013, 19:755-

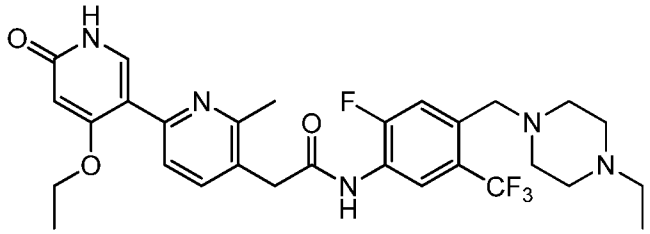
764). One of the most important symptoms that greatly affect an IBS patient's quality of life is visceral pain (Spiegel, B., et al., Am. J. Gastroenterol., 2008, 103:2536–2543). Molecular strategies that inhibit IBS associated visceral pain would greatly influence the IBS patient's quality of life and reduce associated costs.

Rearranged during transfection (RET) is a neuronal growth factor receptor tyrosine kinase that is activated upon binding one of four neurotrophic factors glial cell line-derived neurotrophic factor (GDNF), neurturin, artemin and persephin in combination with a co-receptor GDNF family receptor alpha-1, 2, 3, and 4 respectively (Plaza-Menacho, I., et al., Trends Genet., 2006, 22:627-636). RET is known to play an important role in the development and survival of afferent nociceptors in the skin and gut. RET kinase knock-out mice lack enteric neurons and have other nervous system anomalies suggesting that a functional RET kinase protein product is required during development (Taraviras, S. et al., Development, 1999, 126:2785-2797). Moreover population studies of patients with Hirschsprung's disease characterized by colonic obstruction due to lack of normal colonic enervation have a higher proportion of both familial and sporadic loss of function RET mutations (Butler Tjaden N., et al., Transl. Res., 2013, 162:1-15).

Similarly, aberrant RET kinase activity is associated with multiple endocrine neoplasia (MEN 2A and 2B), familial medullary thyroid carcinoma (FMTC), papillary thyroid carcinoma (PTC) and Hirschsprung's disease (HSCR) (Borello, M., et al., Expert Opin. Ther. Targets, 2013, 17:403-419). MEN 2A is a cancer syndrome resulting from a mutation in the extracellular cysteine-rich domain of RET leading to dimerization *via* a disulfide bond which causes constitutive activation of the tyrosine kinase activity (Wells Jr, S., et al., J. Clin. Endocrinol. Metab., 2013, 98:3149–3164). Individuals with this mutation may develop medullary thyroid carcinoma (MTC), parathyroid hyperplasia, and pheochromocytoma. MEN 2B is caused by a Met918Thr mutation in RET which changes the tyrosine kinase specificity. MEN 2B is similar to MEN 2A, but lacks the parathyroid hyperplasia and also leads to development of numerous mucosal ganglia of the lips, tongue, and intestinal tract. Chromosomal rearrangements linking the promoter and NH2-terminal domains or unrelated gene(s) to the COOH-terminus of RET kinase resulting in constitutively activated chimeric forms of the receptor (RET/PTC) are thought to be tumor initiating events in PTC (Viglietto, G. et al., Oncogene, 1995, 11:1207-1210). PTC's encompass about 80% of all thyroid carcinomas. These data indicate that inhibition of RET may be an attractive therapeutic strategy for the treatment of pain associated with IBS and other gastrointestinal disorders and for the treatment of cancers with constitutive RET kinase activity.

SUMMARY OF THE INVENTION

This invention relates to 2-(4'-ethoxy-6-methyl-6'-oxo-1',6'-dihydro-[2,3'-bipyridin]-5-yl)-*N*-(4-((4-ethylpiperazin-1-yl)methyl)-2-fluoro-5-(trifluoromethyl)phenyl)acetamide, represented by Formula (I):



(I),

or pharmaceutically acceptable salts thereof.

This invention also relates to a pharmaceutical composition comprising a compound of Formula (I), or a pharmaceutically acceptable salt thereof, and a pharmaceutically acceptable excipient.

This invention also relates to a method of treating irritable bowel syndrome comprising administering to a human in need thereof an effective amount of a compound of Formula (I), or a pharmaceutically acceptable salt thereof. This invention also relates to a method of treating cancer comprising administering to a human in need thereof an effective amount of a compound of Formula (I), or a pharmaceutically acceptable salt thereof.

This invention also relates to a compound of Formula (I), or a pharmaceutically acceptable salt thereof, for use in therapy. This invention also relates to a compound of Formula (I), or a pharmaceutically acceptable salt thereof, for use in the treatment of diseases mediated by RET. This invention also relates to a compound of Formula (I), or a pharmaceutically acceptable salt thereof, for use in the treatment of irritable bowel syndrome. This invention also relates to a compound of Formula (I), or a pharmaceutically acceptable salt thereof, for use in the treatment of cancer.

This invention also relates to the use of a compound of Formula (I), or a pharmaceutically acceptable salt thereof, in the manufacture of a medicament for the treatment of diseases mediated by RET. This invention also relates to the use of a compound of Formula (I), or a pharmaceutically acceptable salt thereof, in the manufacture of a medicament for the treatment of irritable bowel syndrome. This invention also relates to the use of a compound of Formula (I), or a pharmaceutically acceptable salt thereof, in the manufacture of a medicament for the treatment of cancer.

DETAILED DESCRIPTION OF THE INVENTION

This invention relates to a compound of Formula (I), or pharmaceutically acceptable salts thereof as defined above.

A person of ordinary skills in the art recognizes that the compound of the present invention may have alternative names when different naming software is used.

This invention also relates to a compound of Formula (I), or pharmaceutically acceptable salts thereof, for use in therapy, in particular, for use in therapy wherein the subject is a human. In particular, for use in the treatment of diseases mediated by RET: irritable bowel syndrome (IBS) including diarrhea-predominant, constipation-predominant or alternating stool pattern, functional bloating, functional constipation, functional diarrhea, unspecified functional bowel disorder, functional abdominal pain syndrome, chronic idiopathic constipation, functional esophageal disorders, functional gastroduodenal disorders, functional anorectal pain, inflammatory bowel disease, proliferative diseases such as non-small cell lung cancer, hepatocellular carcinoma, colorectal cancer, medullary thyroid cancer, follicular thyroid cancer, anaplastic thyroid cancer, papillary thyroid cancer, brain tumors, peritoneal cavity cancer, solid tumors, other lung cancer, head and neck cancer, gliomas, neuroblastomas, Von Hippel-Lindau Syndrome and kidney tumors, breast cancer, fallopian tube cancer, ovarian cancer, transitional cell cancer, prostate cancer, caner of the esophagus and gastroesophageal junction, biliary cancer and adenocarcinoma. In particular, this invention relates to a compound of Formula (I), or pharmaceutically acceptable salts thereof, for use in the treatment of irritable bowel syndrome (IBS) including diarrhea-predominant, constipation-predominant or alternating stool pattern, functional bloating, functional constipation, functional diarrhea, unspecified functional bowel disorder, functional abdominal pain syndrome, chronic idiopathic constipation, functional esophageal disorders, functional gastroduodenal disorders, functional anorectal pain, inflammatory bowel disease, non-small cell lung cancer, hepatocellular carcinoma, colorectal cancer, medullary thyroid cancer, follicular thyroid cancer, anaplastic thyroid cancer, papillary thyroid cancer, brain tumors, peritoneal cavity cancer, solid tumors, other lung cancer, head and neck cancer, gliomas, neuroblastomas, Von Hippel-Lindau Syndrome and kidney tumors, breast cancer, fallopian tube cancer, ovarian cancer, transitional cell cancer, prostate cancer, cancer of the esophagus and gastroesophageal junction, biliary cancer and adenocarcinoma.

This invention also relates to a compound of Formula (I), or pharmaceutically acceptable salts thereof, for use as a medicament. In another embodiment, the invention relates to the use of a compound of Formula (I), or pharmaceutically acceptable salts thereof, in the manufacture of a medicament for the treatment of diseases mediated by RET. This invention also relates to a compound of Formula (I), or pharmaceutically acceptable salts thereof, in the manufacture of a

medicament for the treatment of irritable bowel syndrome. This invention also relates to a compound of Formula (I), or pharmaceutically acceptable salts thereof, in the manufacture of a medicament for the treatment of cancer.

This invention also relates to the use of a compound of Formula (I), or pharmaceutically acceptable salts thereof, in therapy. The invention further includes the use of a compound of Formula (I), or pharmaceutically acceptable salts thereof, as an active therapeutic substance, in particular in the treatment of diseases mediated by RET. This invention also relates to the use of a compound of Formula (I), or pharmaceutically acceptable salts thereof, for the treatment of irritable bowel syndrome. This invention also relates to the use of a compound of Formula (I), or pharmaceutically acceptable salts thereof, for the treatment of cancer.

Because of their potential use in medicine, the salts of the compound of Formula (I) are preferably pharmaceutically acceptable. Suitable pharmaceutically acceptable salts include those described by Berge, Bighley, and Monkhouse, *J. Pharm. Sci.* (1977) 66, pp 1-19. Salts encompassed within the term "pharmaceutically acceptable salts" refer to non-toxic salts of the compound of Formula (I). Salts of the disclosed compound may be prepared by any suitable method known in the art, including treatment of the free base with an inorganic acid, such as hydrochloric acid, hydrobromic acid, sulfuric acid, nitric acid, phosphoric acid, and the like, or with an organic acid, such as acetic acid, trifluoroacetic acid, maleic acid, succinic acid, mandelic acid, fumaric acid, malonic acid, pyruvic acid, oxalic acid, glycolic acid, salicylic acid, pyranosidyl acid, such as glucuronic acid or galacturonic acid, alpha-hydroxy acid, such as citric acid or tartaric acid, amino acid, such as aspartic acid or glutamic acid, aromatic acid, such as benzoic acid or cinnamic acid, sulfonic acid, such as p-toluenesulfonic acid, methanesulfonic acid, ethanesulfonic acid or the like. Examples of pharmaceutically acceptable salts include sulfates, pyrosulfates, bisulfates, sulfites, bisulfites, phosphates, chlorides, bromides, iodides, acetates, propionates, decanoates, caprylates, acrylates, formates, isobutyrates, caproates, heptanoates, propiolates, oxalates, malonates succinates, suberates, sebacates, fumarates, maleates, butyne-1,4-dioates, hexyne-1,6-dioates, benzoates, chlorobenzoates, methylbenzoates, dinitrobenzoates, hydroxybenzoates, methoxybenzoates, phthalates, phenylacetates, phenylpropionates, phenylbutrates, citrates, lactates, γ-hydroxybutyrates, glycolates, tartrates mandelates, and sulfonates, such as xylenesulfonates, methanesulfonates, propanesulfonates, naphthalene-1-sulfonates and naphthalene-2-sulfonates.

Pharmaceutically acceptable salt may also be made with a base which affords a pharmaceutically acceptable cation, which includes alkali metal salts (especially sodium and potassium), alkaline earth metal salts (especially calcium and magnesium), aluminum salts and ammonium salts, as well as salts made from physiologically acceptable organic bases such as trimethylamine, triethylamine, morpholine, pyridine, piperidine, picoline, dicyclohexylamine, *N*,*N*'-dibenzylethylenediamine, 2-hydroxyethylamine, bis-(2-hydroxyethyl)amine, tri-(2-

hydroxyethyl)amine, procaine, dibenzylpiperidine, dehydroabietylamine, *N*,*N*'-bisdehydroabietylamine, glucamine, *N*-methylglucamine, collidine, choline, quinine, quinoline, and basic amino acid such as lysine and arginine.

Other salts, which are not pharmaceutically acceptable, may be useful in the preparation of the compound of Formula (I) and these should be considered to form a further aspect of the invention. These salts, such as trifluoroacetate, while not in themselves pharmaceutically acceptable, may be useful in the preparation of salts useful as intermediates in obtaining the compound of Formula (I) and its pharmaceutically acceptable salts.

If a compound of Formula (I) is isolated as a salt, the corresponding free base form of that compound may be prepared by any suitable method known to the art, including treatment of the salt with an inorganic or organic base, suitably an inorganic or organic base having a higher pK_a than the free base form of the compound.

The compound of Formula (I) may exist in a crystalline or noncrystalline form, or as a mixture thereof. The skilled artisan will appreciate that pharmaceutically acceptable solvates may be formed for crystalline or non-crystalline compounds. In crystalline solvates, solvent molecules are incorporated into the crystalline lattice during crystallization. Solvates may involve non-aqueous solvents such as, but not limited to, ethanol, isopropanol, DMSO, acetic acid, ethanolamine, or ethyl acetate, or they may involve water as the solvent that is incorporated into the crystalline lattice. Solvates wherein water is the solvent incorporated into the crystalline lattice are typically referred to as "hydrates." Hydrates include stoichiometric hydrates as well as compositions containing variable amounts of water. The invention includes all such solvates.

The skilled artisan will further appreciate that the compound of Formula (I), or a pharmaceutically acceptable salt thereof, that exists in crystalline form, including the various solvates thereof, may exhibit polymorphism (i.e. the capacity to occur in different crystalline structures). These different crystalline forms are typically known as "polymorphs." The invention includes all such polymorphs. Polymorphs have the same chemical composition but differ in packing, geometrical arrangement, and other descriptive properties of the crystalline solid state. Polymorphs, therefore, may have different physical properties such as shape, density, hardness, deformability, stability, and dissolution properties. Polymorphs typically exhibit different melting points, IR spectra, and X-ray powder diffraction patterns, which may be used for identification. The skilled artisan will appreciate that different polymorphs may be produced, for example, by changing or adjusting the reaction conditions or reagents, used in making the compound. For example, changes in temperature, pressure, or solvent may result in polymorphs. In addition, one polymorph may spontaneously convert to another polymorph under certain conditions.

Likewise, it is understood that a compound or salt of Formula (I) may exist in tautomeric forms other than that shown in the formula and these are also included within the scope of the

present invention. For example, while the compound of Formula (I) is depicted as containing a pyridin-2-one moiety, the corresponding 2-hydroxypyridine tautomer is also included within the scope of the present invention.

It will be appreciated by those skilled in the art that certain protected derivatives of a compound of Formula (I), which may be made prior to or following a final deprotection stage, may not possess pharmacological activity as such, but may, in certain instances, be administered orally or parenterally and thereafter metabolized in the body to form the compound of Formula (I) which is pharmacologically active. Such derivatives may therefore be described as "prodrugs". All protected derivatives and prodrugs of the compound of Formula (I) are included within the scope of the invention.

Examples of suitable pro-drugs for the compound of Formula (I) are described in Drugs of Today, Volume 19, Number 9, 1983, pp 499 - 538 and in Topics in Chemistry, Chapter 31, pp 306 - 316 and in "Design of Prodrugs" by H. Bundgaard, Elsevier, 1985, Chapter 1. It will further be appreciated by those skilled in the art, that certain moieties, known to those skilled in the art as "pro-moieties", for example as described by H. Bundgaard in "Design of Prodrugs" may be placed on appropriate functionalities when such functionalities are present within the compound of Formula (I). Preferred "pro-moieties" for the compound of Formula (I) include: ester, carbonate ester, hemi-ester, phosphate ester, nitro ester, sulfate ester, sulfoxide, amide, carbamate, azo-, phosphamide, glycoside, ether, acetal, and ketal derivatives of the compound of Formula (I).

Administration of a compound of Formula (I) as a prodrug may enable the skilled artisan to do one or more of the following: (a) modify the onset of the compound in vivo; (b) modify the duration of action of the compound in vivo; (c) modify the transportation or distribution of the compound in vivo; (d) modify the solubility of the compound in vivo; and (e) overcome a side effect or other difficulty encountered with the compound.

The subject invention also includes isotopically-labelled compounds, which are identical to the compound of Formula (I), but for the fact that one or more atoms are replaced by an atom having an atomic mass or mass number different from the atomic mass or mass number usually found in nature. Examples of isotopes that can be incorporated into the compound of Formula (I) and pharmaceutically acceptable salts thereof include isotopes of hydrogen, carbon, nitrogen, oxygen, phosphorous, sulphur, fluorine, iodine, and chlorine, such as ^{2}H, ^{3}H, ^{11}C, ^{13}C, ^{14}C, ^{15}N, ^{17}O, ^{18}O, ^{31}P, ^{32}P, ^{35}S, ^{18}F, ^{36}Cl, ^{123}I, and ^{125}I.

The compound of Formula (I) and pharmaceutically acceptable salts of said compound that contain the aforementioned isotopes and/or other isotopes of other atoms are within the scope of the present invention. Isotopically-labelled compounds of the present invention, for example those into which radioactive isotopes such as ^{3}H or ^{14}C are incorporated, are useful in drug and/or substrate tissue distribution assays. Tritiated, i.e., ^{3}H, and carbon-14, i.e., ^{14}C, isotopes are particularly

preferred for their ease of preparation and detectability. ^{11}C and ^{18}F isotopes are particularly useful in PET (positron emission tomography), and ^{125}I isotopes are particularly useful in SPECT (single photon emission computerized tomography), all useful in brain imaging. Further, substitution with heavier isotopes such as deuterium, i.e., ^{2}H, can afford certain therapeutic advantages resulting from greater metabolic stability, for example increased *in vivo* half-life or reduced dosage requirements and, hence, may be preferred in some circumstances. Isotopically labelled variants of the compound of Formula (I) can generally be prepared by carrying out the procedures disclosed in the Examples below, by substituting a readily available isotopically labelled reagent for a non-isotopically labelled reagent.

DEFINITIONS

Terms are used within their accepted meanings. The following definitions are meant to clarify, but not limit, the terms defined.

"Pharmaceutically acceptable" refers to those compounds, materials, compositions, and dosage forms which are, within the scope of sound medical judgment, suitable for use in contact with the tissues of human beings and animals without excessive toxicity, irritation, or other problem or complication, commensurate with a reasonable benefit/risk ratio.

As used herein, the term "pharmaceutically acceptable salts" refers to salts that retain the desired biological activity of the subject compound and exhibit minimal undesired toxicological effects. These pharmaceutically acceptable salts may be prepared *in situ* during the final isolation and purification of the compound, or by separately reacting the purified compound in its free acid or free base form with a suitable base or acid, respectively.

PHARMACEUTICAL COMPOSITIONS

The invention further provides a pharmaceutical composition (also referred to as pharmaceutical formulation) comprising a compound of Formula (I) or a pharmaceutically acceptable salt thereof, and one or more excipients (also referred to as carriers and/or diluents in the pharmaceutical arts). The excipients are pharmaceutically acceptable in the sense of being compatible with the other ingredients of the formulation and not deleterious to the recipient thereof (i.e., the patient).

Suitable pharmaceutically acceptable excipients include the following types of excipients: diluents, fillers, binders, disintegrants, lubricants, glidants, granulating agents, coating agents, wetting agents, solvents, co-solvents, suspending agents, emulsifiers, sweeteners, flavoring agents, flavor masking agents, coloring agents, anticaking agents, hemectants, chelating agents, plasticizers, viscosity increasing agents, antioxidants, preservatives, stabilizers, surfactants, and buffering agents. The skilled artisan will appreciate that certain pharmaceutically acceptable

excipients may serve more than one function and may serve alternative functions depending on how much of the excipient is present in the formulation and what other ingredients are present in the formulation.

Skilled artisans possess the knowledge and skill in the art to enable them to select suitable pharmaceutically acceptable excipients in appropriate amounts for use in the invention. In addition, there are a number of resources that are available to the skilled artisan which describe pharmaceutically acceptable excipients and may be useful in selecting suitable pharmaceutically acceptable excipients. Examples include Remington's Pharmaceutical Sciences (Mack Publishing Company), The Handbook of Pharmaceutical Additives (Gower Publishing Limited), and The Handbook of Pharmaceutical Excipients (the American Pharmaceutical Association and the Pharmaceutical Press).

The pharmaceutical compositions of the invention are prepared using techniques and methods known to those skilled in the art. Some of the methods commonly used in the art are described in Remington's Pharmaceutical Sciences (Mack Publishing Company).

In accordance with another aspect of the invention there is provided a process for the preparation of a pharmaceutical composition comprising mixing (or admixing) a compound of Formula (I) or a pharmaceutically acceptable salt thereof, with at least one excipient.

Pharmaceutical compositions may be in unit dose form containing a predetermined amount of active ingredient per unit dose. Such a unit may contain a therapeutically effective dose of the compound of Formula (I) or a pharmaceutically acceptable salt thereof, or a fraction of a therapeutically effective dose such that multiple unit dosage forms might be administered at a given time to achieve the desired therapeutically effective dose. Preferred unit dosage formulations are those containing a daily dose or sub-dose, as herein above recited, or an appropriate fraction thereof, of an active ingredient. Furthermore, such pharmaceutical compositions may be prepared by any of the methods well-known in the pharmacy art.

Pharmaceutical compositions may be adapted for administration by any appropriate route, for example, by oral (including buccal or sublingual), rectal, nasal, topical (including buccal, sublingual, or transdermal), vaginal, or parenteral (including subcutaneous, intramuscular, intravenous, or intradermal) routes. Such compositions may be prepared by any method known in the art of pharmacy, for example, by bringing into association the active ingredient with the excipient(s).

When adapted for oral administration, pharmaceutical compositions may be in discrete units such as tablets or capsules, powders or granules, solutions or suspensions in aqueous or non-aqueous liquids, edible foams or whips, oil-in-water liquid emulsions or water-in-oil liquid emulsions. The compound or salt thereof of the invention or the pharmaceutical composition of the

invention may also be incorporated into a candy, a wafer, and/or tongue tape formulation for administration as a "quick-dissolve" medicine.

For instance, for oral administration in the form of a tablet or capsule, the active drug component can be combined with an oral, non-toxic pharmaceutically acceptable inert carrier such as ethanol, glycerol, water, and the like. Powders or granules are prepared by comminuting the compound to a suitable fine size and mixing with a similarly comminuted pharmaceutical carrier such as an edible carbohydrate, as, for example, starch or mannitol. Flavoring, preservative, dispersing, and coloring agents can also be present.

Capsules are made by preparing a powder mixture, as described above, and filling formed gelatin or non-gelatinous sheaths. Glidants and lubricants such as colloidal silica, talc, magnesium stearate, calcium stearate, solid polyethylene glycol can be added to the powder mixture before the filling operation. A disintegrating or solubilizing agent such as agar-agar, calcium carbonate, or sodium carbonate can also be added to improve the availability of the medicine when the capsule is ingested.

Moreover, when desired or necessary, suitable binders, lubricants, disintegrating agents, and coloring agents can also be incorporated into the mixture. Suitable binders include starch, gelatin, natural sugars, such as glucose or beta-lactose, corn sweeteners, natural and synthetic gums such as acacia, tragacanth, sodium alginate, carboxymethylcellulose, polyethylene glycol, waxes, and the like. Lubricants used in these dosage forms include sodium oleate, sodium stearate, magnesium stearate, sodium benzoate, sodium acetate, sodium chloride, and the like. Disintegrators include, without limitation, starch, methylcellulose, agar, bentonite, xanthan gum, and the like.

Tablets are formulated, for example, by preparing a powder mixture, granulating or slugging, adding a lubricant and disintegrant, and pressing into tablets. A powder mixture is prepared by mixing the compound, suitably comminuted, with a diluent or base as described above, and optionally, with a binder such as carboxymethylcellulose, and aliginate, gelatin, or polyvinyl pyrrolidone, a solution retardant such as paraffin, a resorption accelerator such as a quaternary salt, and/or an absorption agent such as bentonite, kaolin, or dicalcium phosphate. The powder mixture can be granulated by wetting a binder such as a syrup, starch paste, acadia mucilage, or solutions of cellulosic or polymeric materials and forcing through a screen. As an alternative to granulating, the powder mixture can be run through a tablet machine, resulting in imperfectly formed slugs broken into granules. The granules can be lubricated to prevent sticking to the tablet forming dies by means of the addition of stearic acid, a stearate salt, talc, or mineral oil. The lubricated mixture is then compressed into tablets. The compound or salt of the present invention can also be combined with a free-flowing inert carrier and compressed into tablets directly without going through the granulating or slugging steps. A clear opaque protective coating consisting of a sealing coat of

shellac, a coating of sugar, or polymeric material, and a polish coating of wax can be provided. Dyestuffs can be added to these coatings to distinguish different dosages.

Oral fluids such as solutions, syrups, and elixirs can be prepared in dosage unit form so that a given quantity contains a predetermined amount of active ingredient. Syrups can be prepared by dissolving the compound or salt thereof of the invention in a suitably flavoured aqueous solution, while elixirs are prepared through the use of a non-toxic alcoholic vehicle. Suspensions can be formulated by dispersing the compound or salt of the invention in a non-toxic vehicle. Solubilizers and emulsifiers, such as ethoxylated isostearyl alcohols and polyoxyethylene sorbitol ethers, preservatives, flavor additives such as peppermint oil, natural sweeteners, saccharin, or other artificial sweeteners, and the like, can also be added.

Where appropriate, dosage unit formulations for oral administration can be microencapsulated. The formulation can also be prepared to prolong or sustain the release as, for example, by coating or embedding particulate material in polymers, wax, or the like.

In the present invention, tablets and capsules are preferred for delivery of the pharmaceutical composition.

As used herein, the term “treatment” refers to alleviating the specified condition, eliminating or reducing one or more symptoms of the condition, slowing or eliminating the progression of the condition, and preventing or delaying the reoccurrence of the condition in a previously afflicted or diagnosed patient or subject.

The present invention provides a method of treatment in a mammal, especially a human, suffering from irritable bowel syndrome (IBS) including diarrhea-predominant, constipation-predominant or alternating stool pattern, functional bloating, functional constipation, functional diarrhea, unspecified functional bowel disorder, functional abdominal pain syndrome, chronic idiopathic constipation, functional esophageal disorders, functional gastroduodenal disorders, functional anorectal pain, inflammatory bowel disease, proliferative diseases such as non-small cell lung cancer, hepatocellular carcinoma, colorectal cancer, medullary thyroid cancer, follicular thyroid cancer, anaplastic thyroid cancer, papillary thyroid cancer, brain tumors, peritoneal cavity cancer, solid tumors, other lung cancer, head and neck cancer, gliomas, neuroblastomas, Von Hippel-Lindau Syndrome and kidney tumors, breast cancer, fallopian tube cancer, ovarian cancer, transitional cell cancer, prostate cancer, caner of the esophagus and gastroesophageal junction, biliary cancer and adenocarcinoma or a combination thereof. Such treatment comprises the step of administering a therapeutically effective amount of a compound of Formula (I) or a pharmaceutically acceptable salt thereof, to said mammal, particularly a human. Treatment can also comprise the step of administering a therapeutically effective amount of a pharmaceutical composition containing a compound of Formula (I) or a pharmaceutically acceptable salt thereof, to said mammal, particularly a human.

As used herein, the term "effective amount" means that amount of a drug or pharmaceutical agent that will elicit the biological or medical response of a tissue, system, animal, or human that is being sought, for instance, by a researcher or clinician.

The term “therapeutically effective amount” means any amount which, as compared to a corresponding subject who has not received such amount, results in improved treatment, healing, prevention, or amelioration of a disease, disorder, or side effect, or a decrease in the rate of advancement of a disease or disorder. The term also includes within its scope amounts effective to enhance normal physiological function. For use in therapy, therapeutically effective amounts of a compound of Formula (I), as well as salts thereof, may be administered as the raw chemical. Additionally, the active ingredient may be presented as a pharmaceutical composition. While it is possible that, for use in therapy, a therapeutically effective amount of a compound of Formula (I) or a pharmaceutically acceptable salt thereof, may be administered as the raw chemical, it is typically presented as the active ingredient of a pharmaceutical composition or formulation.

The precise therapeutically effective amount of a compound or salt thereof of the invention will depend on a number of factors, including, but not limited to, the age and weight of the subject (patient) being treated, the precise disorder requiring treatment and its severity, the nature of the pharmaceutical formulation/composition, and route of administration, and will ultimately be at the discretion of the attending physician or veterinarian. Typically, a compound of Formula (I) or a pharmaceutically acceptable salt thereof, will be given for the treatment in the range of about 0.1 to 100 mg/kg body weight of recipient (patient, mammal) per day and more usually in the range of 0.1 to 10 mg/kg body weight per day. Acceptable daily dosages may be from about 0.1 to about 1000 mg/day, and preferably from about 1 to about 100 mg/day. This amount may be given in a single dose per day or in a number (such as two, three, four, five, or more) of sub-doses per day such that the total daily dose is the same. An effective amount of a salt thereof may be determined as a proportion of the effective amount of the compound of Formula (I) per se. Similar dosages should be appropriate for treatment of the other conditions referred herein for treatment. In general, determination of appropriate dosing can be readily arrived at by one skilled in medicine or the pharmacy art.

The compound of Formula (I), or a pharmaceutically acceptable salt thereof, may be used alone or in combination with one or more other therapeutic agents. Accordingly the present invention provides a combination comprising a compound of Formula (I), or a pharmaceutically acceptable salt thereof, and one or more other therapeutic agents. Such combinations may be presented individually (wherein each active is in separate composition) or the actives are presented in a combined composition.

The compound of Formula (I), or a pharmaceutically acceptable salt thereof, can be combined with or co-administered with other therapeutic agents, particularly agents that may

enhance the activity or time of disposition of the compounds. Combination therapies according to the invention comprise the administration of a compound of Formula (I), or a pharmaceutically acceptable salt thereof, and the use of at least one other treatment method. In one embodiment, combination therapies according to the invention comprise the administration of a compound of Formula (I), or a pharmaceutically acceptable salt thereof, and surgical therapy. In one embodiment, combination therapies according to the invention comprise the administration of a compound of Formula (I), or a pharmaceutically acceptable salt thereof, and radiotherapy. In one embodiment, combination therapies according to the invention comprise the administration of a compound of Formula (I), or a pharmaceutically acceptable salt thereof, and at least one supportive care agent (e.g., at least one anti-emetic agent). In one embodiment, combination therapies according to the present invention comprise the administration of a compound of Formula (I), or a pharmaceutically acceptable salt thereof, and at least one other chemotherapeutic agent. In one particular embodiment, the invention comprises the administration of a compound of Formula (I), or a pharmaceutically acceptable salt thereof, and at least one anti-neoplastic agent. In yet another embodiment, the invention comprises a therapeutic regimen where the RET inhibitors of this disclosure are not in and of themselves active or significantly active, but when combined with another therapy, which may or may not be active as a standalone therapy, the combination provides a useful therapeutic outcome.

By the term "co-administering" and derivatives thereof as used herein refers to either simultaneous administration or any manner of separate sequential administration of a RET inhibiting compound, as described herein, and a further active ingredient or ingredients, particularly those known to be useful in the treatment of cancer, including chemotherapy and radiation treatment. The term further active ingredient or ingredients, as used herein, includes any compound or therapeutic agent known to or that demonstrates advantageous properties when administered to a patient in need of treatment for cancer. Preferably, if the administration is not simultaneous, the compounds are administered in a close time proximity to each other. Furthermore, it does not matter if the compounds are administered in the same dosage form, e.g. one compound may be administered topically and another compound may be administered orally.

Typically, any anti-neoplastic agent that has activity versus a susceptible tumor being treated may be co-administered in the treatment of specified cancers in the present invention. Examples of such agents can be found in Cancer Principles and Practice of Oncology by V.T. Devita and S. Hellman (editors), 6th edition (February 15, 2001), Lippincott Williams & Wilkins Publishers. A person of ordinary skill in the art would be able to discern which combinations of agents would be useful based on the particular characteristics of the drugs and the cancer involved. Typical anti-neoplastic agents useful in the present invention include, but are not limited to, anti-microtubule agents such as diterpenoids and vinca alkaloids; platinum coordination complexes;

alkylating agents such as nitrogen mustards, oxazaphosphorines, alkylsulfonates, nitrosoureas, and triazenes; antibiotic agents such as anthracyclins, actinomycins and bleomycins; topoisomerase II inhibitors such as epipodophyllotoxins; antimetabolites such as purine and pyrimidine analogues and anti-folate compounds; topoisomerase I inhibitors such as camptothecins; hormones and hormonal analogues; DNA methyltransferase inhibitors such as azacitidine and decitabine; signal transduction pathway inhibitors; non-receptor tyrosine kinase angiogenesis inhibitors; immunotherapeutic agents; proapoptotic agents; and cell cycle signaling inhibitors.

Typically, any chemotherapeutic agent that has activity against a susceptible neoplasm being treated may be utilized in combination with a compound of Formula (I), or a pharmaceutically acceptable salt thereof, provided that the particular agent is clinically compatible with therapy employing a compound of Formula (I). Typical anti-neoplastic agents useful in the present invention include, but are not limited to: alkylating agents, anti-metabolites, antitumor antibiotics, antimitotic agents, nucleoside analogues, topoisomerase I and II inhibitors, hormones and hormonal analogues; retinoids, histone deacetylase inhibitors; signal transduction pathway inhibitors including inhibitors of cell growth or growth factor function, angiogenesis inhibitors, and serine/threonine or other kinase inhibitors; cyclin dependent kinase inhibitors; antisense therapies and immunotherapeutic agents, including monoclonals, vaccines or other biological agents.

Nucleoside analogues are those compounds which are converted to deoxynucleotide triphosphates and incorporated into replicating DNA in place of cytosine. DNA methyltransferases become covalently bound to the modified bases resulting in an inactive enzyme and reduced DNA methylation. Examples of nucleoside analogues include azacitidine and decitabine which are used for the treatment of myelodysplastic disorder. Histone deacetylase (HDAC) inhibitors include vorinostat, for the treatment of cutaneous T-cell lymphoma. HDACs modify chromatin through the deacetylation of histones. In addition, they have a variety of substrates including numerous transcription factors and signaling molecules. Other HDAC inhibitors are in development.

Signal transduction pathway inhibitors are those inhibitors which block or inhibit a chemical process which evokes an intracellular change. As used herein this change is cell proliferation or differentiation or survival. Signal transduction pathway inhibitors useful in the present invention include, but are not limited to, inhibitors of receptor tyrosine kinases, non-receptor tyrosine kinases, SH2/SH3 domain blockers, serine/threonine kinases, phosphatidyl inositol-3-OH kinases, myoinositol signaling, and Ras oncogenes. Signal transduction pathway inhibitors may be employed in combination with a compound of Formula (I), or a pharmaceutically acceptable salt thereof, in the compositions and methods described above.

Receptor kinase angiogenesis inhibitors may also find use in the present invention. Inhibitors of angiogenesis related to VEGFR and TIE-2 are discussed above in regard to signal transduction inhibitors (both are receptor tyrosine kinases). Other inhibitors may be used in

combination with a compound of Formula (I), or a pharmaceutically acceptable salt thereof. For example, anti-VEGF antibodies, which do not recognize VEGFR (the receptor tyrosine kinase), but bind to the ligand; small molecule inhibitors of integrin ($alpha_v$ $beta_3$) that inhibit angiogenesis; endostatin and angiostatin (non-RTK) may also prove useful in combination with a compound of Formula (I), or a pharmaceutically acceptable salt thereof. One example of a VEGFR antibody is bevacizumab (AVASTIN®).

Several inhibitors of growth factor receptors are under development and include ligand antagonists, antibodies, tyrosine kinase inhibitors, anti-sense oligonucleotides and aptamers. Any of these growth factor receptor inhibitors may be employed in combination with a compound of Formula (I), or a pharmaceutically acceptable salt thereof, in any of the compositions and methods/uses described herein. Trastuzumab (Herceptin®) is an example of an anti-erbB2 antibody inhibitor of growth factor function. One example of an anti-erbB1 antibody inhibitor of growth factor function is cetuximab (Erbitux™, C225). Bevacizumab (Avastin®) is an example of a monoclonal antibody directed against VEGFR. Examples of small molecule inhibitors of epidermal growth factor receptors include but are not limited to lapatinib (Tykerb®) and erlotinib (TARCEVA®). Imatinib mesylate (GLEEVEC®) is one example of a PDGFR inhibitor. Examples of VEGFR inhibitors include pazopanib (Votrient®), ZD6474, AZD2171, PTK787, sunitinib and sorafenib.

Anti-microtubule or anti-mitotic agents are phase specific agents active against the microtubules of tumor cells during M or the mitosis phase of the cell cycle. Examples of anti-microtubule agents include, but are not limited to, diterpenoids and vinca alkaloids.

Diterpenoids, which are derived from natural sources, are phase specific anti -cancer agents that operate at the G_2/M phases of the cell cycle. It is believed that the diterpenoids stabilize the β-tubulin subunit of the microtubules, by binding with this protein. Disassembly of the protein appears then to be inhibited with mitosis being arrested and cell death following. Examples of diterpenoids include, but are not limited to, paclitaxel and its analog docetaxel.

Paclitaxel, 5β,20-epoxy-1,2α,4,7β,10β,13α-hexa-hydroxytax-11-en-9-one 4,10-diacetate 2-benzoate 13-ester with (2*R*,3*S*)-*N*-benzoyl-3-phenylisoserine; is a natural diterpene product isolated from the Pacific yew tree *Taxus brevifolia* and is commercially available as an injectable solution TAXOL®. It is a member of the taxane family of terpenes. It was first isolated in 1971 by Wani et al. J. Am. Chem, Soc., 93:2325 (1971), who characterized its structure by chemical and X-ray crystallographic methods. One mechanism for its activity relates to paclitaxel's capacity to bind tubulin, thereby inhibiting cancer cell growth. Schiff et al., Proc. Natl, Acad, Sci. USA, 77:1561-1565 (1980); Schiff et al., Nature, 277:665-667 (1979); Kumar, J. Biol, Chem, 256: 10435-10441 (1981). For a review of synthesis and anticancer activity of some paclitaxel derivatives see: D. G. I. Kingston *et al.*, Studies in Organic Chemistry vol. 26, entitled “New trends in Natural Products

Chemistry 1986", Attaur-Rahman, P.W. Le Quesne, Eds. (Elsevier, Amsterdam, 1986) pp 219-235.

Paclitaxel has been approved for clinical use in the treatment of refractory ovarian cancer in the United States (Markman et al., Yale Journal of Biology and Medicine, 64:583, 1991; McGuire et al., Ann. Int. Med., 111:273,1989) and for the treatment of breast cancer (Holmes et al., J. Nat. Cancer Inst., 83:1797,1991.). It is a potential candidate for treatment of neoplasms in the skin (Einzig et. al., Proc. Am. Soc. Clin. Oncol., 20:46) and head and neck carcinomas (Forastire et. al., Sem. Oncol., 20:56, 1990). The compound also shows potential for the treatment of polycystic kidney disease (Woo et. al., Nature, 368:750. 1994), lung cancer and malaria. Treatment of patients with paclitaxel results in bone marrow suppression (multiple cell lineages, Ignoff, R.J. et. al, Cancer Chemotherapy Pocket Guide, 1998) related to the duration of dosing above a threshold concentration (50nM) (Kearns, C.M. et. al., Seminars in Oncology, 3(6) p.16-23, 1995).

Docetaxel, (2*R*,3*S*)- *N*-carboxy-3-phenylisoserine *N*-*tert*-butyl ester, 13-ester with 5β-20-epoxy-1,2α,4,7β,10β,13α-hexahydroxytax-11-en-9-one 4-acetate 2-benzoate, trihydrate; is commercially available as an injectable solution as TAXOTERE®. Docetaxel is indicated for the treatment of breast cancer. Docetaxel is a semisynthetic derivative of paclitaxel *q.v.*, prepared using a natural precursor, 10-deacetyl-baccatin III, extracted from the needle of the European Yew tree. The dose limiting toxicity of docetaxel is neutropenia.

Vinca alkaloids are phase specific anti-neoplastic agents derived from the periwinkle plant. Vinca alkaloids act at the M phase (mitosis) of the cell cycle by binding specifically to tubulin. Consequently, the bound tubulin molecule is unable to polymerize into microtubules. Mitosis is believed to be arrested in metaphase with cell death following. Examples of vinca alkaloids include, but are not limited to, vinblastine, vincristine, and vinorelbine.

Vinblastine, vincaleukoblastine sulfate, is commercially available as VELBAN® as an injectable solution. Although, it has possible indication as a second line therapy of various solid tumors, it is primarily indicated in the treatment of testicular cancer and various lymphomas including Hodgkin's Disease; and lymphocytic and histiocytic lymphomas. Myelosuppression is the dose limiting side effect of vinblastine.

Vincristine, vincaleukoblastine, 22-oxo-, sulfate, is commercially available as ONCOVIN® as an injectable solution. Vincristine is indicated for the treatment of acute leukemias and has also found use in treatment regimens for Hodgkin's and non-Hodgkin's malignant lymphomas. Alopecia and neurologic effects are the most common side effect of vincristine and to a lesser extent myelosupression and gastrointestinal mucositis effects occur.

Vinorelbine, 3',4'-didehydro-4'-deoxy-C'-norvincaleukoblastine [R-(R*,R*)-2,3-dihydroxybutanedioate (1:2)(salt)], commercially available as an injectable solution of vinorelbine tartrate (NAVELBINE®), is a semisynthetic vinca alkaloid. Vinorelbine is indicated as a single

agent or in combination with other chemotherapeutic agents, such as cisplatin, in the treatment of various solid tumors, particularly non-small cell lung, advanced breast, and hormone refractory prostate cancers. Myelosuppression is the most common dose limiting side effect of vinorelbine.

Platinum coordination complexes are non-phase specific anti-cancer agents, which are interactive with DNA. The platinum complexes enter tumor cells, undergo aquation and form intra- and interstrand crosslinks with DNA causing adverse biological effects to the tumor. Examples of platinum coordination complexes include, but are not limited to, cisplatin and carboplatin.

Cisplatin, cis-diamminedichloroplatinum, is commercially available as PLATINOL® as an injectable solution. Cisplatin is primarily indicated in the treatment of metastatic testicular and ovarian cancer and advanced bladder cancer. The primary dose limiting side effects of cisplatin are nephrotoxicity, which may be controlled by hydration and diuresis, and ototoxicity.

Carboplatin, platinum, diammine [1,1-cyclobutane-dicarboxylate(2-)-O,O'], is commercially available as PARAPLATIN® as an injectable solution. Carboplatin is primarily indicated in the first and second line treatment of advanced ovarian carcinoma. Bone marrow suppression is the dose limiting toxicity of carboplatin.

Alkylating agents are non-phase anti-cancer specific agents and strong electrophiles. Typically, alkylating agents form covalent linkages, by alkylation, to DNA through nucleophilic moieties of the DNA molecule such as phosphate, amino, sulfhydryl, hydroxyl, carboxyl, and imidazole groups. Such alkylation disrupts nucleic acid function leading to cell death. Examples of alkylating agents include, but are not limited to, nitrogen mustards such as cyclophosphamide, melphalan, and chlorambucil; alkyl sulfonates such as busulfan; nitrosoureas such as carmustine; and triazenes such as dacarbazine.

Cyclophosphamide, 2-[bis(2-chloroethyl)amino]tetrahydro-2H-1,3,2-oxazaphosphorine 2-oxide monohydrate, is commercially available as an injectable solution or tablets as CYTOXAN®. Cyclophosphamide is indicated as a single agent or in combination with other chemotherapeutic agents, in the treatment of malignant lymphomas, multiple myeloma, and leukemias. Alopecia, nausea, vomiting and leukopenia are the most common dose limiting side effects of cyclophosphamide.

Melphalan, 4-[bis(2-chloroethyl)amino]-L-phenylalanine, is commercially available as an injectable solution or tablets as ALKERAN®. Melphalan is indicated for the palliative treatment of multiple myeloma and non-resectable epithelial carcinoma of the ovary. Bone marrow suppression is the most common dose limiting side effect of melphalan.

Chlorambucil, 4-[bis(2-chloroethyl)amino]benzenebutanoic acid, is commercially available as LEUKERAN® tablets. Chlorambucil is indicated for the palliative treatment of chronic lymphatic leukemia, and malignant lymphomas such as lymphosarcoma, giant follicular

lymphoma, and Hodgkin's disease. Bone marrow suppression is the most common dose limiting side effect of chlorambucil.

Busulfan, 1,4-butanediol dimethanesulfonate, is commercially available as MYLERAN® TABLETS. Busulfan is indicated for the palliative treatment of chronic myelogenous leukemia. Bone marrow suppression is the most common dose limiting side effects of busulfan.

Carmustine, 1,3-[bis(2-chloroethyl)-1-nitrosourea, is commercially available as single vials of lyophilized material as BiCNU®. Carmustine is indicated for the palliative treatment as a single agent or in combination with other agents for brain tumors, multiple myeloma, Hodgkin's disease, and non-Hodgkin's lymphomas. Delayed myelosuppression is the most common dose limiting side effects of carmustine.

Dacarbazine, 5-(3,3-dimethyl-1-triazeno)-imidazole-4-carboxamide, is commercially available as single vials of material as DTIC-Dome®. Dacarbazine is indicated for the treatment of metastatic malignant melanoma and in combination with other agents for the second line treatment of Hodgkin's Disease. Nausea, vomiting, and anorexia are the most common dose limiting side effects of dacarbazine.

Antibiotic anti-neoplastics are non-phase specific agents, which bind or intercalate with DNA. Typically, such action results in stable DNA complexes or strand breakage, which disrupts ordinary function of the nucleic acids leading to cell death. Examples of antibiotic anti-neoplastic agents include, but are not limited to, actinomycins such as dactinomycin, anthrocyclins such as daunorubicin and doxorubicin; and bleomycins.

Dactinomycin, also known as Actinomycin D, is commercially available in injectable form as COSMEGEN®. Dactinomycin is indicated for the treatment of Wilm's tumor and rhabdomyosarcoma. Nausea, vomiting, and anorexia are the most common dose limiting side effects of dactinomycin.

Daunorubicin, (8S-cis-)-8-acetyl-10-[(3-amino-2,3,6-trideoxy-α-L-lyxo-hexopyranosyl)-oxy]-7,8,9,10-tetrahydro-6,8,11-trihydroxy-1-methoxy-5,12 naphthacenedione hydrochloride, is commercially available as a liposomal injectable form as DAUNOXOME® or as an injectable as CERUBIDINE®. Daunorubicin is indicated for remission induction in the treatment of acute nonlymphocytic leukemia and advanced HIV associated Kaposi's sarcoma. Myelosuppression is the most common dose limiting side effect of daunorubicin.

Doxorubicin, (8S,10S)-10-[(3-amino-2,3,6-trideoxy-α-L-lyxo-hexopyranosyl)oxy]-8-glycoloyl,7,8,9,10-tetrahydro-6,8,11-trihydroxy-1-methoxy-5,12 naphthacenedione hydrochloride, is commercially available as an injectable form as RUBEX® or ADRIAMYCIN RDF®. Doxorubicin is primarily indicated for the treatment of acute lymphoblastic leukemia and acute myeloblastic leukemia, but is also a useful component in the treatment of some solid tumors and lymphomas. Myelosuppression is the most common dose limiting side effect of doxorubicin.

Bleomycin, a mixture of cytotoxic glycopeptide antibiotics isolated from a strain of *Streptomyces verticillus*, is commercially available as BLENOXANE®. Bleomycin is indicated as a palliative treatment, as a single agent or in combination with other agents, of squamous cell carcinoma, lymphomas, and testicular carcinomas. Pulmonary and cutaneous toxicities are the most common dose limiting side effects of bleomycin.

Topoisomerase II inhibitors include, but are not limited to, epipodophyllotoxins.

Epipodophyllotoxins are phase specific anti-neoplastic agents derived from the mandrake plant. Epipodophyllotoxins typically affect cells in the S and G_2 phases of the cell cycle by forming a ternary complex with topoisomerase II and DNA causing DNA strand breaks. The strand breaks accumulate and cell death follows. Examples of epipodophyllotoxins include, but are not limited to, etoposide and teniposide.

Etoposide, 4'-demethyl-epipodophyllotoxin 9[4,6-0-(R)-ethylidene-β-D-glucopyranoside], is commercially available as an injectable solution or capsules as VePESID® and is commonly known as VP-16. Etoposide is indicated as a single agent or in combination with other chemotherapy agents in the treatment of testicular and non-small cell lung cancers. Myelosuppression is the most common side effect of etoposide. The incidence of leukopenialeukopenia tends to be more severe than thrombocytopenia.

Teniposide, 4'-demethyl-epipodophyllotoxin 9[4,6-0-(R)-thenylidene-β-D-glucopyranoside], is commercially available as an injectable solution as VUMON® and is commonly known as VM-26. Teniposide is indicated as a single agent or in combination with other chemotherapy agents in the treatment of acute leukemia in children. Myelosuppression is the most common dose limiting side effect of teniposide. Teniposide can induce both leukopenialeukopenia and thrombocytopenia.

Antimetabolite neoplastic agents are phase specific anti-neoplastic agents that act at S phase (DNA synthesis) of the cell cycle by inhibiting DNA synthesis or by inhibiting purine or pyrimidine base synthesis and thereby limiting DNA synthesis. Consequently, S phase does not proceed and cell death follows. Examples of antimetabolite anti-neoplastic agents include, but are not limited to, fluorouracil, methotrexate, cytarabine, mecaptopurine, thioguanine, and gemcitabine.

5-fluorouracil, 5-fluoro-2,4-(1H,3H)pyrimidinedione, is commercially available as fluorouracil. Administration of 5-fluorouracil leads to inhibition of thymidylate synthesis and is also incorporated into both RNA and DNA. The result typically is cell death. 5-fluorouracil is indicated as a single agent or in combination with other chemotherapy agents in the treatment of carcinomas of the breast, colon, rectum, stomach and pancreas. Myelosuppression and mucositis are dose limiting side effects of 5-fluorouracil. Other fluoropyrimidine analogs include 5-fluoro deoxyuridine (floxuridine) and 5-fluorodeoxyuridine monophosphate.

Cytarabine, 4-amino-1-β-D-arabinofuranosyl-2 (1*H*)-pyrimidinone, is commercially available as CYTOSAR-U® and is commonly known as Ara-C. It is believed that cytarabine exhibits cell phase specificity at S-phase by inhibiting DNA chain elongation by terminal incorporation of cytarabine into the growing DNA chain. Cytarabine is indicated as a single agent or in combination with other chemotherapy agents in the treatment of acute leukemia. Other cytidine analogs include 5-azacytidine and 2',2'-difluorodeoxycytidine (gemcitabine). Cytarabine induces leukopenialeukopenia, thrombocytopenia, and mucositis.

Mercaptopurine, 1,7-dihydro-6H-purine-6-thione monohydrate, is commercially available as PURINETHOL®. Mercaptopurine exhibits cell phase specificity at S-phase by inhibiting DNA synthesis by an as of yet unspecified mechanism. Mercaptopurine is indicated as a single agent or in combination with other chemotherapy agents in the treatment of acute leukemia. Myelosuppression and gastrointestinal mucositis are expected side effects of mercaptopurine at high doses. A useful mercaptopurine analog is azathioprine.

Thioguanine, 2-amino-1,7-dihydro-6H-purine-6-thione, is commercially available as TABLOID®. Thioguanine exhibits cell phase specificity at S-phase by inhibiting DNA synthesis by an as of yet unspecified mechanism. Thioguanine is indicated as a single agent or in combination with other chemotherapy agents in the treatment of acute leukemia. Myelosuppression, including leukopenialeukopenia, thrombocytopenia, and anemia, is the most common dose limiting side effect of thioguanine administration. However, gastrointestinal side effects occur and can be dose limiting. Other purine analogs include pentostatin, erythrohydroxynonyladenine, fludarabine phosphate, and cladribine.

Gemcitabine, 2'-deoxy-2', 2'-difluorocytidine monohydrochloride (β-isomer), is commercially available as GEMZAR®. Gemcitabine exhibits cell phase specificity at S-phase and by blocking progression of cells through the G1/S boundary. Gemcitabine is indicated in combination with cisplatin in the treatment of locally advanced non-small cell lung cancer and alone in the treatment of locally advanced pancreatic cancer. Myelosuppression, including leukopenialeukopenia, thrombocytopenia, and anemia, is the most common dose limiting side effect of gemcitabine administration.

Methotrexate, *N*-[4[[(2,4-diamino-6-pteridinyl)methyl]methylamino]benzoyl]-L-glutamic acid, is commercially available as methotrexate sodium. Methotrexate exhibits cell phase effects specifically at S-phase by inhibiting DNA synthesis, repair and/or replication through the inhibition of dyhydrofolic acid reductase which is required for synthesis of purine nucleotides and thymidylate. Methotrexate is indicated as a single agent or in combination with other chemotherapy agents in the treatment of choriocarcinoma, meningeal leukemia, non-Hodgkin's lymphoma, and carcinomas of the breast, head, neck, ovary and bladder. Myelosuppression

(leukopenia, thrombocytopenia, and anemia) and mucositis are expected side effect of methotrexate administration.

Camptothecins, including, camptothecin and camptothecin derivatives are available or under development as Topoisomerase I inhibitors. Camptothecins cytotoxic activity is believed to be related to its Topoisomerase I inhibitory activity. Examples of camptothecins include, but are not limited to irinotecan, topotecan, and the various optical forms of 7-(4-methylpiperazino-methylene)-10,11-ethylenedioxy-20-camptothecin described below.

Irinotecan HCl, (4S)-4,11-diethyl-4-hydroxy-9-[(4-piperidinopiperidino)carbonyloxy]-1H-pyrano[3',4',6,7]indolizino[1,2-b]quinoline-3,14(4H,12H)-dione hydrochloride, is commercially available as the injectable solution CAMPTOSAR®.

Irinotecan is a derivative of camptothecin which binds, along with its active metabolite SN-38, to the topoisomerase I – DNA complex. It is believed that cytotoxicity occurs as a result of irreparable double strand breaks caused by interaction of the topoisomerase I : DNA : irintecan or SN-38 ternary complex with replication enzymes. Irinotecan is indicated for treatment of metastatic cancer of the colon or rectum. The dose limiting side effects of irinotecan HCl are myelosuppression, including neutropenia, and GI effects, including diarrhea.

Topotecan HCl, (S)-10-[(dimethylamino)methyl]-4-ethyl-4,9-dihydroxy-1H-pyrano[3',4',6,7]indolizino[1,2-b]quinoline-3,14-(4H,12H)-dione monohydrochloride, is commercially available as the injectable solution HYCAMTIN®. Topotecan is a derivative of camptothecin which binds to the topoisomerase I – DNA complex and prevents religation of singles strand breaks caused by Topoisomerase I in response to torsional strain of the DNA molecule. Topotecan is indicated for second line treatment of metastatic carcinoma of the ovary and small cell lung cancer. The dose limiting side effect of topotecan HCl is myelosuppression, primarily neutropenia.

EXPERIMENTALS

The following examples illustrate the invention. These examples are not intended to limit the scope of the present invention, but rather to provide guidance to the skilled artisan to prepare and use the compounds, compositions, and methods of the present invention. While particular embodiments of the present invention are described, the skilled artisan will appreciate that various changes and modifications can be made without departing from the spirit and scope of the invention. Unless otherwise noted, reagents are commercially available or are prepared according to procedures in the literature. The symbols and conventions used in the descriptions of processes, schemes, and examples are consistent with those used in the contemporary scientific literature, for example, the Journal of the American Chemical Society or the Journal of Biological Chemistry.

In the Examples:

Chemical shifts are expressed in parts per million (ppm) units. Coupling constants (J) are in units of hertz (Hz). Splitting patterns describe apparent multiplicities and are designated as s (singlet), d (doublet), t (triplet), q (quartet), dd (double doublet), dt (double triplet), dq (double quartet), m (multiplet), br (broad).

Flash column chromatography was performed on silica gel.

The naming program used was ChemBioDraw® Ultra 12.0.

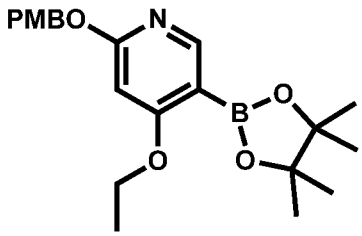
Abbreviations

aq.	aqueous
Cs_2CO_3	cesium carbonate
DCM	dichloromethane
DMF	*N*,*N*-dimethylformamide
ES-LCMS	electrospray liquid chromatography-mass spectrometry
EtOAc	ethyl acetate
g	gram(s)
h	hour(s)
HCl	hydrochloric acid
H_2O	water
HPLC	high performance liquid chromatography
H_2SO_4	sulfuric acid
KCN	potassium cyanide
LCMS	liquid chromatography-mass spectrometry
M	molar
MeCN	acetonitrile
MeOH	methanol
min	minute(s)
mL	milliliter(s)
mmol	millimole(s)
N_2	nitrogen gas
$NaBH_4$	sodium borohydride
$NaHCO_3$	sodium bicarbonate
NaOH	sodium hydroxide
Na_2SO_4	sodium sulfate
n-BuLi	*n*-butyllithium

NH_4Cl	ammonium chloride
NMR	nuclear magnetic resonance
PBr_3	phosphorus tribromide
$PdCl_2(dppf)$	1,1'-*bis*(diphenylphosphino)ferrocene]dichloropalladium(II)
PE	petroleum ether
PMB	*p*-methoxybenzyl
rt	room temperature
THF	tetrahydrofuran
TLC	thin layer chromotrography
$T_3P^®$	propylphosphonic anhydride

Preparation of Intermediates

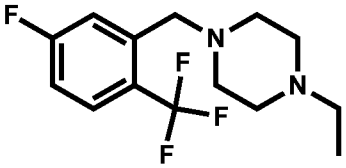
Intermediate 1: 4-ethoxy-2-((4-methoxybenzyl)oxy)-5-(4,4,5,5-tetramethyl-1,3,2-dioxaborolan-2-yl)pyridine



To a solution of 5-bromo-4-ethoxy-2-((4-methoxybenzyl)oxy)pyridine (50 g, 140 mmol, for a preparation see WO 2014/141187 A1) in THF (1 L) cooled to -78 °C was added *n*-BuLi (2.5 M in hexane) (0.073 L, 183 mmol) dropwise under a N_2 atmosphere. The mixture was then stirred at -78 °C for 0.5 h. To the mixture was added 2-isopropoxy- 4,4,5,5-tetramethyl-1,3,2-dioxaborolane (31.4 g, 169 mmol) and stirred at -78 °C for 1 h. The mixture was quenched with saturated aq. NH_4Cl solution (100 mL). The mixture was combined with another crude reaction mixture (performed at a scale of 5 g, 13.3 mmol of 5-bromo-4-ethoxy-2-((4-methoxybenzyl)oxy)pyridine using the same reagent and solvent stoichiometry as above) and worked up together. The crude mixture was extracted with EtOAc (200 mL x 2). The organic layer was dried over Na_2SO_4, filtered and concentrated to give 4-ethoxy-2-((4-methoxybenzyl)oxy)-5-(4,4,5,5-tetramethyl-1,3,2-dioxaborolan-2-yl)pyridine (60 g, 78 mmol, 50.9% combined yield) (purity: 50%) as a yellow oil. ^1H NMR (400 MHz, METHANOL-d_4) δ 8.19-8.13 (m, 1H), 7.34-7.32 (m, 2H), 6.91-6.86 (m, 2H), 6.30-6.27 (m, 1H), 5.23-5.18 (m, 2H), 4.09-4.06 (m, 2H), 3.78- 3.77 (m, 3H), 1.39-1.36 (m, 3H), 1.32-1.23 (s, 12H); ES-LCMS *m/z* 386.2 (M+H).

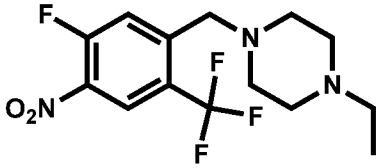
Intermediate 2: 4-((4-Ethylpiperazin-1-yl)methyl)-2-fluoro-5-(trifluoromethyl)aniline

Step 1: 1-Ethyl-4-(5-fluoro-2-(trifluoromethyl)benzyl)piperazine



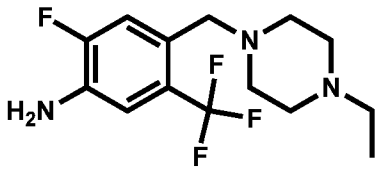
A solution of 5-fluoro-2-(trifluoromethyl)benzaldehyde (2 g, 10.41 mmol) and 1-ethylpiperazine (1.783 g, 15.62 mmol) in DCM (60 mL) was stirred at 20 °C. After 2 h, sodium triacetoxyborohydride (6.62 g, 31.2 mmol) was added. The resulting mixture was stirred at 20 °C overnight. After LCMS analysis showed the starting material disappeared, the mixture was dissolved in H_2O (30 mL) and adjusted to pH 8 with aq. $NaHCO_3$. The organic layer was washed with brine and dried over Na_2SO_4. After filtration, the filtrate was concentrated to give the crude product, which was purified by column chromatography (0-5% MeOH in DCM) to yield 1-ethyl-4-(5-fluoro-2-(trifluoromethyl)benzyl)piperazine (3 g, 8.74 mmol, 84% yield) as a yellow oil: ^{1}H NMR (400 MHz, CD_3OD) δ 7.77 (dd, J = 8.8, 5.2 Hz, 1H), 7.63 (dd, J = 10.0, 2.0 Hz, 1H), 7.21 (dt, J = 8.4, 2.4 Hz, 1H), 3.80 (s, 2H), 3.24 (br. s., 4H), 3.13 (q, J = 7.6 Hz, 2H), 2.77 (br. s., 4H), 1.34 (t, J = 7.2 Hz, 3H); ES-LCMS m/z: 291.1 (M+H).

Step 2: 1-Ethyl-4-(5-fluoro-4-nitro-2-(trifluoromethyl)benzyl)piperazine



To a solution of 1-ethyl-4-(5-fluoro-2-(trifluoromethyl)benzyl)piperazine (3 g, 10.33 mmol) in concentrated H_2SO_4 (6 mL, 113 mmol) was added nitric acid (0.716 g, 11.37 mmol). The resulting mixture was stirred at rt overnight. The mixture was then stirred at 50 °C for 2 h. After TLC analysis (PE/EtOAc = 10:1) showed the starting material disappeared, the mixture was adjusted to pH 8 by aq. NaOH and extracted by EtOAc (50 mL x 2). The organic layer was washed with H_2O (50 mL) and brine (50 mL), then dried over Na_2SO_4 and filtered. The filtrate was concentrated to give 1-ethyl-4-(5-fluoro-4-nitro-2-(trifluoromethyl)benzyl)-piperazine (2.2 g, 6.56 mmol, 63.5% yield) as a yellow oil: ^{1}H NMR (400 MHz, CD_3OD) δ 8.39 (d, J = 7.0 Hz, 1H), 7.92 (d, J = 12.0 Hz, 1H), 3.75 (s, 2H), 2.60 - 2.47 (m, 10H), 1.14 - 1.10 (m, 3H); ES-LCMS m/z 336.1 (M+H)

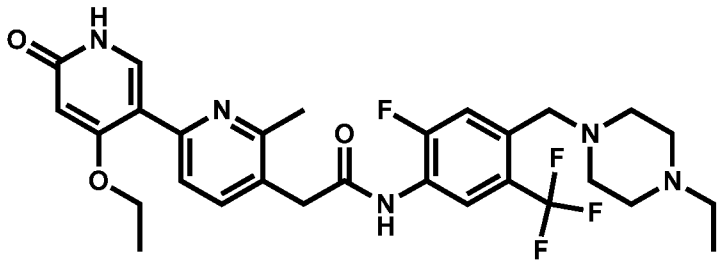
Step 3: 4-((4-Ethylpiperazin-1-yl)methyl)-2-fluoro-5-(trifluoromethyl)aniline



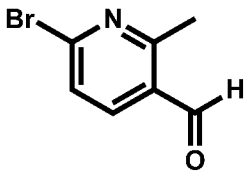
To a solution of 1-ethyl-4-(5-fluoro-4-nitro-2-(trifluoromethyl)benzyl)piperazine (2.2 g, 6.56 mmol) and zinc (4.29 g, 65.6 mmol) in MeOH (100 mL) was added NH_4Cl (3.51 g, 65.6 mmol) by portions. The resulting mixture was stirred at 20 °C for 12 h. After LCMS analysis showed the starting material disappeared, the mixture was filtered. The filtrate was concentrated to give the crude product, which was purified by preparative HPLC (Mobile phase A: H_2O with 0.05% $NH_3•H_2O$ solution/ Mobile phase B: MeCN/ Flow rate: 80 mL/min/ Detection: UV 220 nm / 254 nm/ Column: Phenomenex® Gemini C18 250*50 mm,10 μm/ Column temperature: RT/ Gradient Profile Description: 40-70 (B%)) to yield 4-((4-ethylpiperazin-1-yl)methyl)-2-fluoro-5-(trifluoromethyl)aniline (0.7 g, 2.265 mmol, 34.5% yield) as a yellow solid: ^1H NMR (400 MHz, CD_3OD) δ 7.31 (d, J = 12.6 Hz, 1H), 7.10 (d, J = 8.6 Hz, 1H), 3.51 (s, 2H), 2.74 - 2.15 (m, 10H), 1.10 (t, J = 7.3 Hz, 3H); ES-LCMS m/z: 306.1 (M+H).

Preparation of the compound of Formula (I)

Example 1: 2-(4'-ethoxy-6-methyl-6'-oxo-1',6'- dihydro-[2,3'-bipyridin]-5-yl)-N-(4-((4-ethylpiperazin-1-yl)methyl)-2-fluoro-5-(trifluoromethyl)phenyl)acetamide



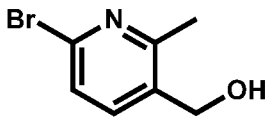
Step 1: 6-bromo-2-methylnicotinaldehyde



To a solution of 3,6-dibromo-2-methylpyridine (60 g, 239 mmol) in THF (600 mL) and was added *n*-BuLi (100 mL, 251 mmol) at -78 °C. The mixture was then stirred for 1 h at -78 °C under N_2 atmosphere. DMF (20.4 mL, 263 mmol) was added dropwise to the mixture. The mixture was then stirred for 1 h at -78 °C under N_2 atmosphere. The reaction mixture was warmed to 25 °C and 1 M aq. HCl solution (300 mL) was added. The reaction mixture was combined with five

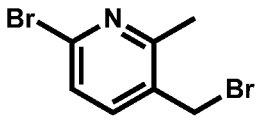
additional crude reaction mixtures (performed at scales of 30 g, 120 mmol and 4 x 50 g, 199 mmol of 3,6-dibromo-2-methylpyridine using the same reagent and solvent stoichiometry as above) and worked up together. The reaction mixture was extracted with EtOAc (800 mL x 4). The combined organic layers were washed with H_2O (300 mL x 3), dried over Na_2SO_4, filtered, and concentrated to give 6-bromo-2-methylnicotinaldehyde (260 g, 492 mmol, 42.5% combined yield) as a red oil. ^1H NMR (400 MHz, CHLOROFORM-d) δ 10.28 (s, 1H), 7.92 (d, *J*=7.9 Hz, 1H), 7.64 (s, 1H), 2.88 - 2.83 (m, 3H). LCMS $[M+H]^+$ = 200.1, 202.1.

Step 2: (6-bromo-2-methylpyridin-3-yl)methanol



To a solution of 6-bromo-2-methylnicotinaldehyde (80 g, 140 mmol) in MeOH (432 mL) was added $NaBH_4$ (15.13 g, 300 mmol) at 0 °C. The mixture was then stirred for 3 h at 25 °C under N_2 atmosphere. The reaction mixture was combined with three other crude reaction mixtures (performed at a scale of 3 x 60 g, 105 mmol of 6-bromo-2-methylnicotinaldehyde using the same reagent and solvent stoichiometry as above) and worked up together. The reaction mixture was concentrated. The residue was diluted with H_2O (500 mL) and extracted with EtOAc (500 mL x 3). The combined organic layers were washed with H_2O (200 mL x 3), dried over Na_2SO_4, filtered, and concentrated. The residue was purified by column chromatography (PE:EtOAc = 5:1, R_f = 0.2). The desired fractions were combined and concentrated to give (6-bromo-2-methylpyridin-3-yl)methanol (40 g, 164 mmol, 36.1% combined yield) as a red oil. ^1H NMR (400 MHz, CHLOROFORM-d) δ 7.59 - 7.56 (m, 1H), 7.30 (d, *J*=7.8 Hz, 1H), 4.66 (s, 2H), 2.46 (s, 3H), LCMS $[M+H]^+$ = 202.1, 204.1.

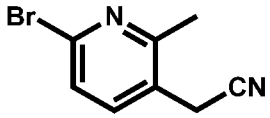
Step 3: 6-bromo-3-(bromomethyl)-2-methylpyridine



To a solution of (6-bromo-2-methylpyridin-3-yl)methanol (12 g, 59.4 mmol) in DCM (20 mL) was added PBr_3 (6.72 mL, 71.3 mmol) dropwise at 0 °C. The mixture was then stirred for 3 h at 25 °C under N_2 atmosphere. The reaction mixture was combined with two other crude reaction mixtures (performed at a scale of 1 g, 4.11 mmol and 12 g, 59.4 mmol (6-bromo-2-methylpyridin-3-yl)methanol using the same reagent and solvent stoichiometry as above) and worked up together. The mixture was washed with aq. $NaHCO_3$ (50 mL x 3) and brine (30 x 3 mL). The organic layer was dried over Na_2SO_4, filtered and concentrated. The residue was purified with column

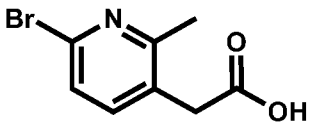
chromatography (PE:EtOAc = 5:1, R_f = 0.5). The desired fractions were combined and concentrated to give 6-bromo-3-(bromomethyl)-2-methylpyridine (15 g, 45.3 mmol, 35% combined yield) as a pale yellow solid. ^{1}H NMR (400 MHz, METHANOL-d_4) δ 7.63 (d, J = 7.9 Hz, 1H), 7.41 (d, J = 7.9 Hz, 1H), 4.58 (s, 2H), 2.56 (s, 3H); LCMS $[M+H]^+$ = 265.8, 267.8.

Step 4: 2-(6-bromo-2-methylpyridin-3-yl)acetonitrile



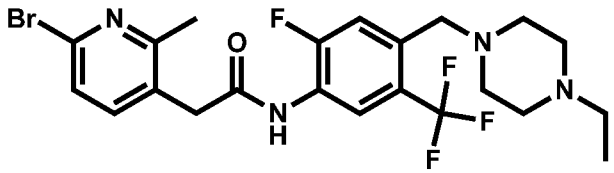
To a solution of 6-bromo-3-(bromomethyl)-2-methylpyridine (20 g, 60.4 mmol) in ethanol (200 mL) was added KCN (4.98 g 76 mmol). The mixture was then stirred for 12 h at 60 °C. Additional KCN (2.88 g 44.2 mmol) was added. The mixture was then stirred for 12 h at 60 °C. The reaction mixture was combined with another crude reaction mixture (performed at a scale of 0.65 g, 1.227 mmol of 6-bromo-3-(bromomethyl)-2-methylpyridine using the same reagent and solvent stoichiometry as above) and worked up together. The reaction mixture was concentrated. The residue was diluted with H_2O (200 mL) and extracted with DCM (250 mL x 3). The combined organic layers were washed with H_2O (100 mL x 3), dried over Na_2SO_4, filtered, and concentrated. The residue was purified by column chromatography (PE:EtOAc = 3:1, R_f = 0.5). The desired fractions were combined and concentrated to give 2-(6-bromo-2-methylpyridin-3-yl)acetonitrile (9 g, 42.2 mmol, 68.5% combined yield) as a yellow solid. ^{1}H NMR (400 MHz, METHANOL-d_4) δ 7.68 (d, *J*=8.0 Hz, 1H), 7.49 (d, *J*=8.0 Hz, 1H), 3.93 (s, 2H), 2.56 - 2.51 (m, 3H); LCMS $[M+H]^+$ = 211.0, 213.1.

Step 5: 2-(6-bromo-2-methylpyridin-3-yl)acetic acid



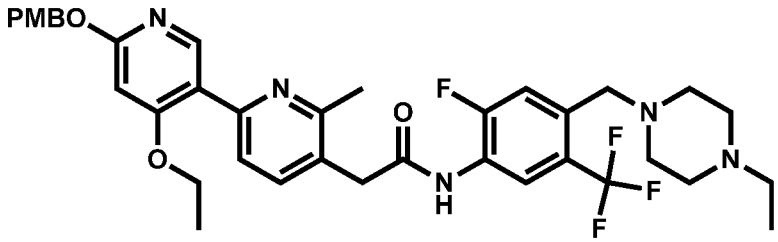
A solution of 2-(6-bromo-2-methylpyridin-3-yl)acetonitrile (8 g, 37.5 mmol) in concentrated H_2SO_4 (40 mL) and H_2O (40 mL) was stirred at 100 °C for 10 h. The mixture was dissolved in H_2O (200 mL) and adjusted to pH = 4 with an aq. NaOH solution. The organic layer was extracted by EtOAc (200 mL x 3). The combined organic layers were washed with brine (100 mL), dried over Na_2SO_4, filtered, and concentrated to yield 2-(6-bromo-2-methylpyridin-3-yl)acetic acid (8.2 g, 34.1 mmol, 91% yield) as an off-white solid: ^{1}H NMR (400 MHz, METHANOL-d_4) δ 7.51 (d, J=7.9 Hz, 1H), 7.38 (d, J=7.9 Hz, 1H), 3.67 (s, 2H), 2.47 (s, 3H); LCMS m/z $[M+H]^+$ = 230.1, 232.1 (M+2);

Step 6: 2-(6-bromo-2-methylpyridin-3-yl)-N-(4-((4-ethylpiperazin-1-yl)methyl)-2-fluoro-5-(trifluoromethyl)phenyl)acetamide



To a solution of 2-(6-bromo-2-methylpyridin-3-yl)acetic acid (7.8 g, 32.2 mmol) and 4-((4-ethylpiperazin-1-yl)methyl)-2-fluoro-5-(trifluoromethyl)aniline (11.57 g, 32.2 mmol) in pyridine (160 mL) was added $T_3P^{®}$ (50% in EtOAc) (40 mL, 32.2 mmol) dropwise. The mixture was stirred at 25 °C for 2 h. The reaction mixture was combined with another crude reaction (performed at a scale of 0.20 g, 0.826 mmol of 2-(6-bromo-2-methylpyridin-3-yl)acetic acid using the same reagent and solvent stoichiometry as above) and worked up together. H_2O (200 mL) was added to the residue which was then extracted with DCM/MeOH (10:1, 150 mL x 4). The combined organic layers were dried over Na_2SO_4, filtered, and concentrated to give 2-(6-bromo-2-methylpyridin-3-yl)-N-(4-((4-ethylpiperazin-1-yl)methyl)-2-fluoro-5-(trifluoromethyl)phenyl)acetamide (18 g, 29.9 mmol, 90.6% combined yield) as a yellow solid: ^{1}H NMR (400 MHz, METHANOL-d_4) δ 7.72 - 7.62 (m, 2H), 7.59 (d, J=8.4 Hz, 1H), 7.44 (d, J=7.9 Hz, 1H), 3.90 (s, 2H), 3.81 - 3.75 (m, 2H), 3.65 (d, J=10.6 Hz, 2H), 3.50 - 3.36 (m, 2H), 3.24 (q, J=7.1 Hz, 3H), 3.03 (br. s., 3H), 2.54 (s, 3H), 1.39 (t, J=7.1 Hz, 3H); ES-LCMS m/z 517.2, 519.2 (M+H).

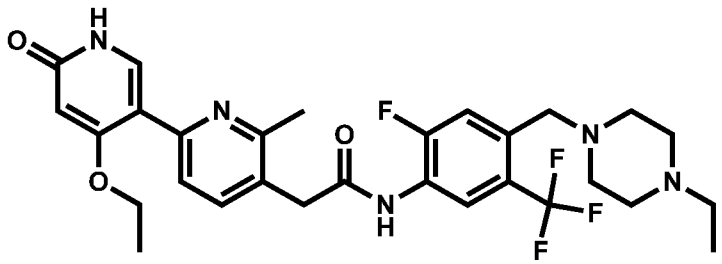
Step 7: 2-(4'-ethoxy-6'-((4-methoxybenzyl)oxy)-6-methyl-[2,3'-bipyridin]-5-yl)-N-(4-((4-ethylpiperazin-1-yl)methyl)-2-fluoro-5-(trifluoromethyl)phenyl)acetamide



To a mixture of 4-ethoxy-2-((4-methoxybenzyl)oxy)-5-(4,4,5,5-tetramethyl-1,3,2-dioxaborolan-2-yl)pyridine (25 g, 32.4 mmol) in 1,4-dioxane (200 mL) and H_2O (50 mL) was added 2-(6-bromo-2-methylpyridin-3-yl)-N-(4-((4-ethylpiperazin-1-yl)methyl)-2-fluoro-5-(trifluoromethyl)phenyl)acetamide (18 g, 29.9 mmol), $PdCl_2$(dppf) (2.4 g, 3.24 mmol), and Cs_2CO_3 (21.14 g, 64.9 mmol) under N_2 atmosphere. The mixture was stirred at 100 °C for 6 h. The mixture was concentrated and then the residue was diluted with DCM (500 mL) and washed with H_2O (100 mL x 3). The organic layer was dried over anhydrous Na_2SO_4. The solvent was removed under reduced pressure. The reaction mixture was combined with another crude reaction

(performed at a scale of 0.50 g, 0.649 mmol of 4-ethoxy-2-((4-methoxybenzyl)oxy)-5-(4,4,5,5-tetramethyl-1,3,2-dioxaborolan-2-yl)pyridine using the same reagent and solvent stoichiometry as above) and purified together. The crude product was purified by silica column chromatography (10% MeOH : 90% DCM, 120 g silica column). All fractions found to contain product by TLC (DCM : MeOH = 15:1, R_f = 0.5) were combined and concentrated to yield 2-(4'-ethoxy-6'-((4-methoxybenzyl)oxy)-6-methyl-[2,3'-bipyridin]-5-yl)-N-(4-((4-ethylpiperazin-1-yl)methyl)-2-fluoro-5-(trifluoromethyl)phenyl)acetamide (15 g, 13.28 mmol, 40.2% combined yield) as a dark red solid: ^{1}H NMR (400 MHz, METHANOL-d_4) δ 8.34 (d, J=7.5 Hz, 1H), 8.27 (s, 1H), 7.69 - 7.63 (m, 1H), 7.62 - 7.52 (m, 3H), 7.49 (dd, J=3.5, 7.1 Hz, 1H), 7.37 (d, J=8.4 Hz, 1H), 6.90 (d, J=8.8 Hz, 1H), 6.44 (s, 1H), 5.28 (s, 2H), 4.18 - 4.07 (m, 2H), 3.93 - 3.86 (m, 2H), 3.80 - 3.70 (m, 3H), 3.65 - 3.59 (m, 2H), 2.71 - 2.29 (m, 13H), 1.37 (t, J=6.8 Hz, 3H), 1.10 (t, J=7.3 Hz, 3H); LCMS: 696.2 (M+H).

Step 8: 2-(4'-ethoxy-6-methyl-6'-oxo-1',6'-dihydro-[2,3'-bipyridin]-5-yl)-N-(4-((4-ethylpiperazin-1-yl)methyl)-2-fluoro-5-(trifluoromethyl)phenyl)acetamide



To a mixture of 2-(4'-ethoxy-6'-((4-methoxybenzyl)oxy)-6-methyl-[2,3'-bipyridin]-5-yl)-N-(4-((4-ethylpiperazin-1-yl)methyl)-2-fluoro-5-(trifluoromethyl)phenyl)acetamide (12.8 g, 11.33 mmol) in EtOAc (120 mL) was added HCl (4 M in EtOAc) (50 mL, 200 mmol). The mixture was stirred at 25 °C for 1 h. The mixture was concentrated. The residue was diluted with EtOAc (60 mL) and adjusted to a pH of approximately 6-7 with $NaHCO_3$. The mixture was concentrated and the residue was purified by preparative HPLC (Instrument: V2; Column: Phenomenex® Gemini C18 250*50mm*10 μm; Mobile phase A-B: H_2O (0.05% ammonia hydroxide v/v) - Acetonitrile; Gradient: 40-70 (B%); Flowrate: 25 mL/min). The desired fractions were partially concentrated, and a solid was precipitated and filtered. The solid was collected and dried under vacuum to give 2-(4'-ethoxy-6-methyl-6'-oxo-1',6'-dihydro-[2,3'-bipyridin]-5-yl)-N-(4-((4-ethylpiperazin-1-yl)methyl)-2-fluoro-5-(trifluoromethyl)phenyl)acetamide (4.45 g, 7.73 mmol, 68.2% yield) as an off-white solid: ^{1}H NMR (400 MHz, METHANOL-d_4) δ 8.35-8.33 (d, J=8.0 Hz, 1H), 7.79 (s, 1H), 7.69 - 7.60 (m, 3H), 6.02 (s, 1H), 4.16 (q, J=6.9 Hz, 2H), 3.92 (s, 2H), 3.65 (s, 2H), 2.59 - 2.44 (m, 13H), 1.42 (t, J=6.8 Hz, 3H), 1.12 (t, J=7.3 Hz, 3H); ES-LCMS m/z 576.3 (M+H).

Biological Assays

The compound of the present invention was tested for RET kinase inhibitory activity in a RET kinase enzyme assay, a cell-based mechanistic assay and a cell-based proliferation assay.

RET Kinase Enzymatic Assay

Human RET kinase cytoplasmic domain (amino acids 658-1114 of accession number NP_000314.1) was expressed as an N-terminal GST-fusion protein using a baculovirus expression system. GST-RET was purified using glutathione sepharose chromatography. The RET kinase enzymatic assay was performed in a total volume of 10 uL with increasing concentrations of RET kinase inhibitor as a singlet in a 384 well format as follows: RET inhibitor compound plates are prepared by adding 100 nL of RET inhibitor at different concentrations to a 384-well plate. 5 µL/well of a 2X enzyme mix (50 mM HEPES (4-(2-hydroxyethyl)-1-piperazineethanesulfonic acid); 1 mM CHAPS (3-[(3-cholamidopropyl)dimethylammonio]-1-propanesulfonate); 0.1 mg/mL BSA (bovine serum albumin); 1 mM DTT (dithiothreitol); 0.2 nM RET kinase) was added to the 384-well plate and incubated for 30 minutes at 23°C. 5 µL/well of a 2X substrate mix (50 mM HEPES; 1 mM CHAPS; 0.1 mg/mL BSA; 20 µM adenosine triphosphate; 20 mM $MgCl_2$ and 1 µM biotinylated peptide substrate) was added and incubated for 1 hour at 23°C. 10 µL/well of 2X stop/detection mix (50 mM HEPES; 0.1 % BSA; 800 mM Potassium Fluoride; 50 mM EDTA (Ethylenediaminetetraacetic acid); 200 X dilution of Europium Cryptate labeled anti-phosphotyrosine antibody; 62.5 nM Streptavidin-XL665) incubated for 1 hour at 23°C and read on a Homogenous Time-Resolved Fluorescence reader. IC_{50}s were fitted using GraphPad Prism to a sigmoidal dose response.

RET Kinase Cell-Based Mechanistic Assay

The potency of the compound of Formula (I) was tested for its ability to inhibit constitutive RET kinase phosphorylation in cell-based assay. TT cells (ATCC CRL-1803), a medullary thyroid cancer cell line with constitutively activated RET kinase, were maintained in 150 cm^2 dishes in F12 Kaighn's medium, 10% fetal bovine serum, 1X Glutamax, 1X non-essential amino acids, 1X Pen/Strep antibiotics at 37 °C in 5 % carbon dioxide. 1.0E5 TT cells/well were plated in a 96-well cell culture plate and allowed to adhere overnight. TT cells were treated with different concentrations of RET inhibitor compounds for 2 h at 37 °C in 5 % carbon dioxide, washed with ice cold PBS (phosphate buffered saline) and lysed by adding 200 µL of 25 mM Tris HCl pH 7.5; 2 mM EDTA; 150 mM NaCl; 1 % sodium deoxycholate; 1 % Triton X-100; 50 mM sodium beta glycerophosphate; 1 mM sodium orthovanadate; 1X phosphatase inhibitor cocktail #2 (Sigma #P5726); 1X phosphatase inhibitor cocktail #3 (Sigma #P0044) and 1X complete mini EDTA free protease inhibitor cocktail (Roche #4693159001), incubation at -80 °C for 10 minutes and thawed

on ice. 100 µL of TT cell lysate was added to a 96-well plate overnight at 4 °C that had been coated overnight at 4 °C with 1:1,000 dilution of a rabbit anti-RET antibody (Cell Signaling #7032) blocked with 1X PBS; 0.05 % Tween-20; 1 % bovine serum albumin. Plates were washed 4X with 200 µL of 1X PBS; 0.05 % Tween-20 and then 100 µL of a 1:1,000 dilution of an anti-phosphotyrosine detection antibody (Cell Signaling #7034) was added and incubated for 1 hour at 37 °C. Plates were washed 4X with 200 µL of 1X PBS; 0.05 % Tween-20 and then 100 µL of a 1:1,000 dilution of an anti-mouse immunoglobulin horse radish peroxidase conjugate antibody (Cell Signaling #7034) was added and incubated for 30 minutes at 37 °C. Plates were washed 4X with 200 µL of 1X PBS; 0.05 % Tween-20, 100 µL of TMB (3,3', 5,5"-tetramethylbenzidine) substrate (Cell Signaling #7004) was added, incubated for 10 minutes at 37 °C, 100 µL of Stop solution (Cell Signaling #7002) was added and absorbance read on a spectrophotometer at 450 nm. IC_{50}s were fitted using GraphPad Prism to a sigmoidal dose response.

RET Kinase Cell-Based Proliferation Assay

The potency of compound of Formula (I) was tested for its ability to inhibit cell proliferation and cell viability. TT cells (ATCC CRL-1803), a medullary thyroid cancer cell line with constitutively activated RET kinase, were maintained in 150 cm^2 dishes in F12 Kaighn's medium, 10% fetal bovine serum, 1X Glutamax, 1X non-essential amino acids, 1X Pen/Strep antibiotics at 37 °C in 5 % carbon dioxide. 6.0E3 TT cells/well in 50 µL of media were added to a 96-well cell culture plate and allowed to adhere overnight. 50 µL of serially diluted RET inhibitor compounds were added to 96-well plate containing cultured TT cells and incubated at at 37 °C in 5 % carbon dioxide for eight days. 50 µL of CellTiter-Glo (Promega #G-7573) was added, contents mixed for 1 minute on shaker followed by 10 minutes in the dark at 23 °C and the luminescence read by EnVision (PerkinElmer). IC_{50}s were fitted using GraphPad Prism to a sigmoidal dose response.

Biological Data

The compound of Formula (I) was tested in the RET assays described above and was found to be an inhibitor of RET. Data for each assay is listed below in Table 1 as follows: + = 10 µM > IC_{50} > 100 nM; ++ = 100 nM ≥ IC_{50} > 10 nM; +++ = IC_{50} ≤ 10 nM.

Table 1

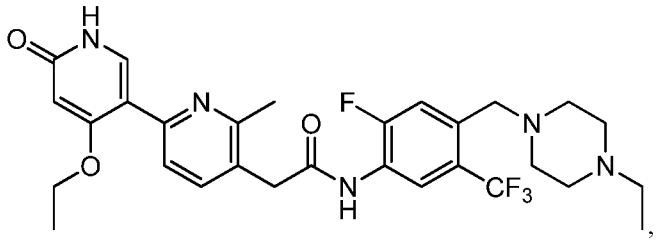
Example #	Human RET kinase enzymatic IC_{50}	Human RET kinase cell-based mechanistic IC_{50}	Human RET kinase cell-based proliferation IC_{50}
1	+++	+++	+++

***In vivo* Colonic Hypersensitivity Model**

The efficacy of RET kinase inhibitor compounds can be evaluated in an *in vivo* model of colonic hypersensitivity (Hoffman, J.M., et al., Gastroenterology, 2012, 142:844-854).

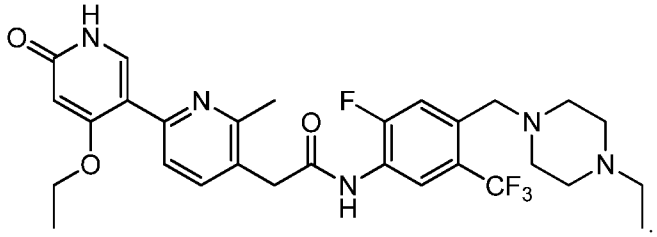
CLAIMS

1. A compound which is:



or a pharmaceutically acceptable salt thereof.

2. A compound which is:



3. A pharmaceutical composition comprising the compound or pharmaceutically acceptable salt according to claim 1 and a pharmaceutically acceptable excipient.

4. A pharmaceutical composition comprising the compound according to claim 2 and a pharmaceutically acceptable excipient.

5. A method of treating irritable bowel syndrome comprising administering to a human in need thereof an effective amount of the compound or pharmaceutically acceptable salt according to claim 1.

6. A method of treating irritable bowel syndrome comprising administering to a human in need thereof an effective amount of the compound according to claim 2.

7. A method of treating cancer comprising administering to a human in need thereof an effective amount of the compound or pharmaceutically acceptable salt according to claim 1.

8. A method of treating cancer comprising administering to a human in need thereof an effective amount of the compound according to claim 2.

9. The compound or pharmaceutically acceptable salt according to claim 1 or the compound according to claim 2 for use in therapy.

10. The compound or pharmaceutically acceptable salt according to claim 1 or the compound according to claim 2 for use in the treatment of irritable bowel syndrome.

11. The compound or pharmaceutically acceptable salt according to claim 1 or the compound according to claim 2 for use in the treatment of cancer.

12. The use of the compound or pharmaceutically acceptable salt according to claim 1 or the compound according to claim 2 in the manufacture of a medicament for the treatment of irritable bowel syndrome.

13. The use of the compound or pharmaceutically acceptable salt according to claim 1 or the compound according to claim 2 in the manufacture of a medicament for the treatment of cancer.

INTERNATIONAL SEARCH REPORT

International application No
PCT/IB2017/050981

A. CLASSIFICATION OF SUBJECT MATTER
INV. C07D213/69 A61K31/444 A61P1/14 A61P35/00
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)
C07D A61K A61P

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, WPI Data

C. DOCUMENTS CONSIDERED TO BE RELEVANT

Category*	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
Y	WO 2014/141187 A1 (GLAXOSMITHKLINE IP DEV LTD [GB]) 18 September 2014 (2014-09-18) cited in the application page 4, line 16 - line 22; claim 1	1-13
Y,P	WO 2016/037578 A1 (GLAXOSMITHKLINE IP DEV LTD [GB]; GLAXOSMITHKLINE CHINA R & D COMPANY L) 17 March 2016 (2016-03-17) claims 1,22	1-13

☐ 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

"L" document which may throw doubts on priority claim(s) or which is cited to establish the publication date of another citation or other special reason (as specified)

"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

"Y" document of particular relevance; the claimed invention cannot be considered to involve an inventive step when the document is combined with one or more other such documents, such combination being obvious to a person skilled in the art

"&" document member of the same patent family

Date of the actual completion of the international search	Date of mailing of the international search report
23 March 2017	05/04/2017

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	Johnson, Claire

INTERNATIONAL SEARCH REPORT

Information on patent family members

International application No
PCT/IB2017/050981

Patent document cited in search report	Publication date	Patent family member(s)	Publication date
WO 2014141187 A1	18-09-2014	AU 2014229233 A1	24-09-2015
		CA 2905627 A1	18-09-2014
		CL 2015002514 A1	05-02-2016
		CN 105051027 A	11-11-2015
		CR 20150490 A	20-11-2015
		DO P2015000244 A	31-12-2015
		EA 201591738 A1	29-01-2016
		EP 2970191 A1	20-01-2016
		JP 2016516026 A	02-06-2016
		LT 2970191 T	10-03-2017
		NZ 628388 A	30-09-2016
		PE 15382015 A1	18-11-2015
		PH 12015502044 A1	18-01-2016
		SG 11201506695U A	29-09-2015
		TW 201524958 A	01-07-2015
		US 2016002215 A1	07-01-2016
		US 2016271123 A1	22-09-2016
		UY 35465 A	31-10-2014
		WO 2014141187 A1	18-09-2014
WO 2016037578 A1	17-03-2016	NONE	