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(54) **Title**: A 6-OXO-1,6-DIHYDRO-PYRIDAZINE DERIVATIVE FOR THE USE FOR THE TREATMENT OF HEPATOCELLULAR CARCINOMA (HCC)

(57) **Abstract**: 3-(1-{3-[5-(1-methyl-piperidin-4-ylmethoxy)-pyrimidin-2-yl]-benzyl}-6-oxo-1,6-dihydro-pyridazin-3-yl)-benzonitrile or a pharmaceutically acceptable salt and/or solvate thereof for the use for the treatment of hepatocellular carcinoma (HCC).

A 6-oxo-1,6-dihydro-pyridazine derivative for the use for the treatment of hepatocellular carcinoma (HCC)

FIELD OF THE INVENTION

This invention relates to 3-(1-{3-[5-(1-methyl-piperidin-4-ylmethoxy)-pyrimidin-2-yl]-benzyl}-6-oxo-1,6-dihydro-pyridazin-3-yl)-benzonitrile or a pharmaceutically acceptable salt and/or solvate thereof for the use for the treatment of hepatocellular carcinoma (HCC).

BACKGROUND OF THE INVENTION

The invention had the object of finding novel pharmaceutical compositions having valuable properties, in particular those which can be used for the preparation of medicaments.

Moreover, aim of this invention are new compositions for the prevention and treatment of hepatocellular carcinoma.

It has been found that 3-(1-{3-[5-(1-methyl-piperidin-4-ylmethoxy)-pyrimidin-2-yl]-benzyl}-6-oxo-1,6-dihydro-pyridazin-3-yl)-benzonitrile according to the invention or a pharmaceutically acceptable salt and/or solvate thereof has very valuable pharmacological properties while being well tolerated.

HCC is the 5th most common malignancy worldwide, with 667,000 new cases worldwide and 17,500 in USA. 80% of patients present with advanced or unresectable disease at diagnosis. In Western countries, approx. 40% of patients are eligible for a potential curative treatment (resection, transplantation, local ablation) whereas approx. 20% are eligible for chemoembolization. In well-selected patients resection and transplantation provide 5-year survival rates of 70%, 50% of patients relapse within 3 years. Here we demonstrate that 3-(1-{3-[5-(1-methyl-piperidin-4-ylmethoxy)-

pyrimidin-2-yl]-benzyl}-6-oxo-1,6-dihydro-pyridazin-3-yl)-benzonitrile or a pharmaceutically acceptable salt and/or solvate thereof is active in HCC tumors with signs of active c-Met signaling.

Before the approval of sorafenib there was no effective systemic treatment increasing survival in HCC: conventional cytotoxic agents given as monotherapy or in combination regimen had low response rates and did not lead to survival advantage (Thomas MB, O'Beirne JP, Furuse J, Chan AT, Abou-Alfa G, Johnson P; Ann Surg Oncol. 2008 Apr;15(4):1008-14). However, although PFS times have been improved by sorafenib, PFS and overall survival remain limited. Secondary resistance occurs after several weeks of drug exposure. After progression there is currently no other therapeutic option. Due to the high unmet medical need in HCC alternative effective treatment options are needed.

PRIOR ART

3-(1-{3-[5-(1-Methyl-piperidin-4-ylmethoxy)-pyrimidin-2-yl]-benzyl}-6-oxo-1,6-dihydro-pyridazin-3-yl)-benzonitrile has been described in WO 2009/006959 A1.

3-(1-{3-[5-(1-Methyl-piperidin-4-ylmethoxy)-pyrimidin-2-yl]-benzyl}-6-oxo-1,6-dihydro-pyridazin-3-yl)-benzonitrile hydrochloride hydrate has been described in WO 2009/007074 A1.

SUMMARY OF THE INVENTION

The invention relates to 3-(1-{3-[5-(1-methyl-piperidin-4-ylmethoxy)-pyrimidin-2-yl]-benzyl}-6-oxo-1,6-dihydro-pyridazin-3-yl)-benzonitrile or a pharmaceutically acceptable salt and/or solvate thereof for the use for the treatment of hepatocellular carcinoma (HCC).

Moreover, the invention relates to 3-(1-{3-[5-(1-methyl-piperidin-4-ylmethoxy)-pyrimidin-2-yl]-benzyl}-6-oxo-1,6-dihydro-pyridazin-3-yl)-benzonitrile hydrochloride hydrate for the use for the treatment of hepatocellular carcinoma (HCC).

Moreover, the invention relates to 3-(1-{3-[5-(1-methyl-piperidin-4-ylmethoxy)-pyrimidin-2-yl]-benzyl}-6-oxo-1,6-dihydro-pyridazin-3-yl)-benzonitrile or a pharmaceutically acceptable salt and/or solvate thereof, wherein the compound is administered to a patient in an amount of 100 mg to 800 mg per day.

Moreover, the invention relates to 3-(1-{3-[5-(1-methyl-piperidin-4-ylmethoxy)-pyrimidin-2-yl]-benzyl}-6-oxo-1,6-dihydro-pyridazin-3-yl)-benzonitrile or a pharmaceutically acceptable salt and/or solvate thereof, wherein the compound is administered orally.

Moreover, the invention relates to the use of 3-(1-{3-[5-(1-methyl-piperidin-4-ylmethoxy)-pyrimidin-2-yl]-benzyl}-6-oxo-1,6-dihydro-pyridazin-3-yl)-benzonitrile or a pharmaceutically acceptable salt and/or solvate thereof for the manufacture of a medicament for the treatment of hepatocellular carcinoma (HCC).

Moreover, the invention relates to the use of 3-(1-{3-[5-(1-methyl-piperidin-4-ylmethoxy)-pyrimidin-2-yl]-benzyl}-6-oxo-1,6-dihydro-pyridazin-3-yl)-benzonitrile hydrochloride hydrate for the manufacture of a medicament for the treatment of hepatocellular carcinoma (HCC).

Moreover, the invention relates to the use as described above, wherein 3-(1-{3-[5-(1-methyl-piperidin-4-ylmethoxy)-pyrimidin-2-yl]-benzyl}-6-oxo-1,6-dihydro-pyridazin-3-yl)-benzonitrile or a pharmaceutically acceptable salt and/or solvate thereof or 3-(1-{3-[5-(1-methyl-piperidin-4-ylmethoxy)-pyrimidin-2-yl]-benzyl}-6-oxo-1,6-dihydro-pyridazin-3-yl)-benzonitrile hydrochloride hydrate, wherein the compound is administered to a patient in an amount of 100 mg to 800 mg per day, preferably in an amount of 200 mg to 700 mg per week, particularly preferably in an amount of 250 mg to 350 mg per day.

Moreover, the invention relates to the use as described above,

wherein 3-(1-{3-[5-(1-methyl-piperidin-4-ylmethoxy)-pyrimidin-2-yl]-benzyl}-6-oxo-1,6-dihydro-pyridazin-3-yl)-benzonitrile or a pharmaceutically acceptable salt and/or solvate thereof or
3-(1-{3-[5-(1-methyl-piperidin-4-ylmethoxy)-pyrimidin-2-yl]-benzyl}-6-oxo-1,6-dihydro-pyridazin-3-yl)-benzonitrile hydrochloride hydrate
wherein the compound is administered orally.

The therapy with 3-(1-{3-[5-(1-methyl-piperidin-4-ylmethoxy)-pyrimidin-2-yl]-benzyl}-6-oxo-1,6-dihydro-pyridazin-3-yl)-benzonitrile or a pharmaceutically acceptable salt and/or solvate thereof or
3-(1-{3-[5-(1-methyl-piperidin-4-ylmethoxy)-pyrimidin-2-yl]-benzyl}-6-oxo-1,6-dihydro-pyridazin-3-yl)-benzonitrile hydrochloride hydrate may include optionally further treatment with radiation. The invention relates furthermore to a new therapy form comprising the start of the administration of 3-(1-{3-[5-(1-methyl-piperidin-4-ylmethoxy)-pyrimidin-2-yl]-benzyl}-6-oxo-1,6-dihydro-pyridazin-3-yl)-benzonitrile or a pharmaceutically acceptable salt and/or solvate thereof prior to radiotherapy for the treatment of hepatocellular carcinoma (HCC).

The invention also relates to the optically active forms (stereoisomers), the enantiomers, the racemates, the diastereomers and the hydrates and solvates of the compound.
The invention also relates to the solvates of the salts of the compound e.g. the mono- or dihydrate of the hydrochloride.

The term solvates of the compound is taken to mean adductions of inert solvent molecules onto the compounds which form owing to their mutual attractive force. Solvates are, for example, mono- or dihydrates or alcoholates.

The expression "effective amount" denotes the amount of a medicament or of a pharmaceutical active ingredient which causes in a tissue, system, animal or

human a biological or medical response which is sought or desired, for example, by a researcher or physician.

In addition, the expression "therapeutically effective amount" denotes an amount which, compared with a corresponding subject who has not received this amount, has the following consequence:

improved treatment, healing, prevention or elimination of a disease, syndrome, condition, complaint, disorder or side-effects or also the reduction in the advance of a disease, complaint or disorder.

The expression "therapeutically effective amount" also encompasses the amounts which are effective for increasing normal physiological function.

Pharmaceutical salts and other forms

The said compounds according to the invention can be used in their final non-salt form. On the other hand, the present invention also encompasses the use of these compounds in the form of their pharmaceutically acceptable salts, which can be derived from various organic and inorganic acids and bases by procedures known in the art. Pharmaceutically acceptable salt forms of 3-(1-{3-[5-(1-methyl-piperidin-4-ylmethoxy)-pyrimidin-2-yl]-benzyl}-6-oxo-1,6-dihydro-pyridazin-3-yl)-benzonitrile and N-((S)-2,3-dihydroxy-propyl)-3-(2-fluoro-4-iodo-phenylamino)-isonicotinamide are for the most part prepared by conventional methods.

If a compound contains a carboxyl group, one of its suitable salts can be formed by reacting the compound with a suitable base to give the corresponding base-addition salt. Such bases are, for example, alkali metal hydroxides, including potassium hydroxide, sodium hydroxide and lithium hydroxide; alkaline earth metal hydroxides, such as barium hydroxide and calcium hydroxide; alkali metal alkoxides, for example potassium ethoxide and sodium propoxide; and various organic bases, such as piperidine, diethanolamine and N-methylglutamine. The aluminium salts of the compounds are likewise included. In the case of certain compounds acid-addition salts can be formed by treating these compounds with pharmaceutically acceptable organic and inorganic acids, for example hydrogen halides, such as

hydrogen chloride, hydrogen bromide or hydrogen iodide, other mineral acids and corresponding salts thereof, such as sulfate, nitrate or phosphate and the like, and alkyl- and monoarylsulfonates, such as ethanesulfonate, toluenesulfonate and benzenesulfonate, and other organic acids and corresponding salts thereof, such as acetate, trifluoroacetate, tartrate, maleate, succinate, citrate, benzoate, salicylate, ascorbate and the like. Accordingly, pharmaceutically acceptable acid-addition salts of the compounds include the following: acetate, adipate, alginate, arginate, aspartate, benzoate, benzenesulfonate (besylate), bisulfate, bisulfite, bromide, butyrate, camphorate, camphorsulfonate, caprylate, chloride, chlorobenzoate, citrate, cyclopentanepropionate, digluconate, dihydrogenphosphate, dinitrobenzoate, dodecylsulfate, ethanesulfonate, fumarate, galacterate (from mucic acid), galacturonate, glucoheptanoate, gluconate, glutamate, glycerophosphate, hemisuccinate, hemisulfate, heptanoate, hexanoate, hippurate, hydrochloride, hydrobromide, hydroiodide, 2-hydroxyethanesulfonate, iodide, isethionate, isobutyrate, lactate, lactobionate, malate, maleate, malonate, mandelate, metaphosphate, methanesulfonate, methylbenzoate, monohydrogenphosphate, 2-naphthalenesulfonate, nicotinate, nitrate, oxalate, oleate, palmoate, pectinate, persulfate, phenylacetate, 3-phenylpropionate, phosphate, phosphonate, phthalate, but this does not represent a restriction.

Furthermore, the base salts of the compounds according to the invention include aluminium, ammonium, calcium, copper, iron(III), iron(II), lithium, magnesium, manganese(III), manganese(II), potassium, sodium and zinc salts, but this is not intended to represent a restriction. Of the above-mentioned salts, preference is given to ammonium; the alkali metal salts sodium and potassium, and the alkaline earth metal salts calcium and magnesium. Salts of the compounds which are derived from pharmaceutically acceptable organic non-toxic bases include salts of primary, secondary and tertiary amines, substituted amines, also including naturally occurring substituted amines, cyclic amines, and basic ion exchanger resins, for example arginine, betaine, caffeine, chloroprocaine, choline, N,N'-dibenzylethylenediamine

(benzathine), dicyclohexylamine, diethanolamine, diethylamine, 2-diethylaminoethanol, 2-dimethylaminoethanol, ethanolamine, ethylenediamine, N-ethylmorpholine, N-ethylpiperidine, glucamine, glucosamine, histidine, hydrabamine, isopropylamine, lidocaine, lysine, meglumine, N-methyl-D-glucamine, morpholine, piperazine, piperidine, polyamine resins, procaine, purines, theobromine, triethanolamine, triethylamine, trimethylamine, tripropylamine and tris(hydroxymethyl)methylamine (tromethamine), but this is not intended to represent a restriction.

Compounds of the present invention which contain basic nitrogen-containing groups can be quaternised using agents such as (C_1-C_4)alkyl halides, for example methyl, ethyl, isopropyl and tert-butyl chloride, bromide and iodide; di(C_1-C_4)alkyl sulfates, for example dimethyl, diethyl and diamyl sulfate; (C_{10}-C_{18})alkyl halides, for example decyl, dodecyl, lauryl, myristyl and stearyl chloride, bromide and iodide; and aryl(C_1-C_4)alkyl halides, for example benzyl chloride and phenethyl bromide. Both water- and oil-soluble compounds according to the invention can be prepared using such salts.

The above-mentioned pharmaceutical salts which are preferred include acetate, trifluoroacetate, besylate, citrate, fumarate, gluconate, hemisuccinate, hippurate, hydrochloride, hydrobromide, isethionate, mandelate, meglumine, nitrate, oleate, phosphonate, pivalate, sodium phosphate, stearate, sulfate, sulfosalicylate, tartrate, thiomalate, tosylate and tromethamine, but this is not intended to represent a restriction.

Particular preference is given to hydrochloride, dihydrochloride, hydrobromide, maleate, mesylate, phosphate, sulfate and succinate.

The acid-addition salts of basic compounds are prepared by bringing the free base form into contact with a sufficient amount of the desired acid, causing the formation of the salt in a conventional manner. The free base can be regenerated by bringing the salt form into contact with a base and isolating the

free base in a conventional manner. The free base forms differ in a certain respect from the corresponding salt forms thereof with respect to certain physical properties, such as solubility in polar solvents; for the purposes of the invention, however, the salts otherwise correspond to the respective free base forms thereof.

As mentioned, the pharmaceutically acceptable base-addition salts of the compounds are formed with metals or amines, such as alkali metals and alkaline earth metals or organic amines. Preferred metals are sodium, potassium, magnesium and calcium. Preferred organic amines are N,N'-dibenzylethylenediamine, chloroprocaine, choline, diethanolamine, ethylenediamine, N-methyl-D-glucamine and procaine.

The base-addition salts of acidic compounds according to the invention are prepared by bringing the free acid form into contact with a sufficient amount of the desired base, causing the formation of the salt in a conventional manner. The free acid can be regenerated by bringing the salt form into contact with an acid and isolating the free acid in a conventional manner. The free acid forms differ in a certain respect from the corresponding salt forms thereof with respect to certain physical properties, such as solubility in polar solvents; for the purposes of the invention, however, the salts otherwise correspond to the respective free acid forms thereof.

With regard to that stated above, it can be seen that the expression "pharmaceutically acceptable salt" in the present connection is taken to mean an active ingredient which comprises a compound in the form of one of its salts, in particular if this salt form imparts improved pharmacokinetic properties on the active ingredient compared with the free form of the active ingredient or any other salt form of the active ingredient used earlier. The pharmaceutically acceptable salt form of the active ingredient can also provide this active ingredient for the first time with a desired pharmacokinetic property which it did not have earlier and can even have a positive influence on the

pharmacodynamics of this active ingredient with respect to its therapeutic efficacy in the body.

The invention furthermore relates to medicaments comprising at least one compound and/or pharmaceutically acceptable salts, solvates, tautomers and stereoisomers thereof, including mixtures thereof in all ratios, and optionally excipients and/or adjuvants.

Pharmaceutical formulations can be administered in the form of dosage units which comprise a predetermined amount of active ingredient per dosage unit. Such a unit can comprise, for example, 0.5 mg to 1 g, preferably 1 mg to 700 mg, particularly preferably 5 mg to 100 mg, of a compound according to the invention, depending on the condition treated, the method of administration and the age, weight and condition of the patient, or pharmaceutical formulations can be administered in the form of dosage units which comprise a predetermined amount of active ingredient per dosage unit. Preferred dosage unit formulations are those which comprise a daily dose or part-dose, as indicated above, or a corresponding fraction thereof of an active ingredient. Furthermore, pharmaceutical formulations of this type can be prepared using a process which is generally known in the pharmaceutical art.

Pharmaceutical formulations can be adapted for administration via any desired suitable method, 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) methods. Such formulations can be prepared using all processes known in the pharmaceutical art by, for example, combining the active ingredient with the excipient(s) or adjuvant(s).

Pharmaceutical formulations adapted for oral administration can be administered as separate units, such as, for example, capsules or tablets; powders or granules; solutions or suspensions in aqueous or non-aqueous liquids; edible

foams or foam foods; or oil-in-water liquid emulsions or water-in-oil liquid emulsions.

Thus, for example, in the case of oral administration in the form of a tablet or capsule, the active-ingredient component can be combined with an oral, non-toxic and pharmaceutically acceptable inert excipient, such as, for example, ethanol, glycerol, water and the like. Powders are prepared by comminuting the compound to a suitable fine size and mixing it with a pharmaceutical excipient comminuted in a similar manner, such as, for example, an edible carbohydrate, such as, for example, starch or mannitol. A flavour, preservative, dispersant and dye may likewise be present.

Capsules are produced by preparing a powder mixture as described above and filling shaped gelatine shells therewith. Glidants and lubricants, such as, for example, highly disperse silicic acid, talc, magnesium stearate, calcium stearate or polyethylene glycol in solid form, can be added to the powder mixture before the filling operation. A disintegrant or solubiliser, such as, for example, agar-agar, calcium carbonate or sodium carbonate, may likewise be added in order to improve the availability of the medicament after the capsule has been taken.

In addition, if desired or necessary, suitable binders, lubricants and disintegrants as well as dyes can likewise be incorporated into the mixture. Suitable binders include starch, gelatine, natural sugars, such as, for example, glucose or beta-lactose, sweeteners made from maize, natural and synthetic rubber, such as, for example, acacia, tragacanth or sodium alginate, carboxymethylcellulose, polyethylene glycol, waxes, and the like. The lubricants used in these dosage forms include sodium oleate, sodium stearate, magnesium stearate, sodium benzoate, sodium acetate, sodium chloride and the like. The disintegrants include, without being restricted thereto, starch, methylcellulose, agar, bentonite, xanthan gum and the like. The tablets are formulated by, for example, preparing a powder mixture, granulating or dry-pressing the mixture,

adding a lubricant and a disintegrant and pressing the entire mixture to give tablets. A powder mixture is prepared by mixing the compound comminuted in a suitable manner with a diluent or a base, as described above, and optionally with a binder, such as, for example, carboxymethylcellulose, an alginate, gelatine or polyvinylpyrrolidone, a dissolution retardant, such as, for example, paraffin, an absorption accelerator, such as, for example, a quaternary salt, and/or an absorbant, such as, for example, bentonite, kaolin or dicalcium phosphate. The powder mixture can be granulated by wetting it with a binder, such as, for example, syrup, starch paste, acadia mucilage or solutions of cellulose or polymer materials and pressing it through a sieve. As an alternative to granulation, the powder mixture can be run through a tabletting machine, giving lumps of non-uniform shape, which are broken up to form granules. The granules can be lubricated by addition of stearic acid, a stearate salt, talc or mineral oil in order to prevent sticking to the tablet casting moulds. The lubricated mixture is then pressed to give tablets. The compounds according to the invention can also be combined with a free-flowing inert excipient and then pressed directly to give tablets without carrying out the granulation or dry-pressing steps. A transparent or opaque protective layer consisting of a shellac sealing layer, a layer of sugar or polymer material and a gloss layer of wax may be present. Dyes can be added to these coatings in order to be able to differentiate between different dosage units.

Oral liquids, such as, for example, solution, syrups and elixirs, can be prepared in the form of dosage units so that a given quantity comprises a pre-specified amount of the compound. Syrups can be prepared by dissolving the compound in an aqueous solution with a suitable flavour, while elixirs are prepared using a non-toxic alcoholic vehicle. Suspensions can be formulated by dispersion of the compound in a non-toxic vehicle. Solubilisers and emulsifiers, such as, for example, ethoxylated isostearyl alcohols and polyoxyethylene sorbitol ethers, preservatives, flavour additives, such as, for example, peppermint oil or natural sweeteners or saccharin, or other artificial sweeteners and the like, can likewise be added.

The dosage unit formulations for oral administration can, if desired, be encapsulated in microcapsules. The formulation can also be prepared in such a way that the release is extended or retarded, such as, for example, by coating or embedding of particulate material in polymers, wax and the like.

The compounds and salts, solvates, tautomers and stereoisomers thereof can also be administered in the form of liposome delivery systems, such as, for example, small unilamellar vesicles, large unilamellar vesicles and multilamellar vesicles. Liposomes can be formed from various phospholipids, such as, for example, cholesterol, stearylamine or phosphatidylcholines.

The compounds and the salts, solvates, tautomers and stereoisomers thereof can also be delivered using monoclonal antibodies as individual carriers to which the compound molecules are coupled. The compounds can also be coupled to soluble polymers as targeted medicament carriers. Such polymers may encompass polyvinylpyrrolidone, pyran copolymer, polyhydroxypropylmethacrylamidophenol, polyhydroxyethylaspartamidophenol or polyethylene oxide polylysine, substituted by palmitoyl radicals. The compounds may furthermore be coupled to a class of biodegradable polymers which are suitable for achieving controlled release of a medicament, for example polylactic acid, poly-epsilon-caprolactone, polyhydroxybutyric acid, polyorthoesters, polyacetals, polydihydroxypyrans, polycyanoacrylates and crosslinked or amphipathic block copolymers of hydrogels.

Pharmaceutical formulations adapted for transdermal administration can be administered as independent plasters for extended, close contact with the epidermis of the recipient. Thus, for example, the active ingredient can be delivered from the plaster by iontophoresis, as described in general terms in Pharmaceutical Research, 3(6), 318 (1986).

Pharmaceutical compounds adapted for topical administration can be formulated as ointments, creams, suspensions, lotions, powders, solutions, pastes, gels, sprays, aerosols or oils.

Pharmaceutical formulations adapted for rectal administration can be administered in the form of suppositories or enemas.

Pharmaceutical formulations adapted for nasal administration in which the carrier substance is a solid comprise a coarse powder having a particle size, for example, in the range 20-500 microns, which is administered in the manner in which snuff is taken, i.e. by rapid inhalation via the nasal passages from a container containing the powder held close to the nose. Suitable formulations for administration as nasal spray or nose drops with a liquid as carrier substance encompass active-ingredient solutions in water or oil.

Pharmaceutical formulations adapted for administration by inhalation encompass finely particulate dusts or mists, which can be generated by various types of pressurised dispensers with aerosols, nebulisers or insufflators.

Pharmaceutical formulations adapted for parenteral administration include aqueous and non-aqueous sterile injection solutions comprising antioxidants, buffers, bacteriostatics and solutes, by means of which the formulation is rendered isotonic with the blood of the recipient to be treated; and aqueous and non-aqueous sterile suspensions, which may comprise suspension media and thickeners. The formulations can be administered in single-dose or multidose containers, for example sealed ampoules and vials, and stored in freeze-dried (lyophilised) state, so that only the addition of the sterile carrier liquid, for example water for injection purposes, immediately before use is necessary. Injection solutions and suspensions prepared in accordance with the recipe can be prepared from sterile powders, granules and tablets.

It goes without saying that, in addition to the above particularly mentioned constituents, the formulations may also comprise other agents usual in the art with respect to the particular type of formulation; thus, for example, formulations which are suitable for oral administration may comprise flavours.

A therapeutically effective amount of a compound depends on a number of factors, including, for example, the age and weight of the animal, the precise condition that requires treatment, and its severity, the nature of the formulation and the method of administration, and is ultimately determined by the treating doctor or vet. However, an effective amount of a compound according to the invention is generally in the range from 0.1 to 100 mg/kg of body weight of the recipient (mammal) per day and particularly typically in the range from 1 to 10 mg/kg of body weight per day. Thus, the actual amount per day for an adult mammal weighing 70 kg is usually between 70 and 700 mg, where this amount can be administered as a single dose per day or usually in a series of part-doses (such as, for example, two, three, four, five or six) per day, so that the total daily dose is the same. An effective amount of a salt, solvate, tautomer and stereoisomer thereof can be determined as the fraction of the effective amount of the compound according to the invention *per se*. It can be assumed that similar doses are suitable for the treatment of other conditions mentioned above.

The anti-cancer treatment defined herein may be applied as a sole therapy or may involve, in addition to the composition of the invention, conventional surgery or radiotherapy.

"Treating" as used herein, means an alleviation, in whole or in part, of symptoms associated with a disorder or disease, or slowing, or halting of further progression or worsening of those symptoms, or prevention or prophylaxis of the disease or disorder in a subject at risk for developing the disease or disorder.

The term "effective amount" in connection with a compound can mean an amount capable of alleviating, in whole or in part, symptoms associated with a disorder or disease, or slowing or halting further progression or worsening of those symptoms, or preventing or providing prophylaxis for the disease or disorder in a subject having or at risk for developing a disease disclosed herein, such as cancer,

The term "therapeutically effective" or "therapeutically effective amount" refers to an amount of a drug effective to treat a disease or disorder in a mammal. In the case of cancer, the therapeutically effective amount of the drug may reduce the number of cancer cells; reduce the tumor size; inhibit (i.e., slow to some extent and preferably stop) cancer cell infiltration into peripheral organs; inhibit (i.e., slow to some extent and preferably stop) tumor metastasis; inhibit, to some extent, tumor growth; and/or relieve to some extent one or more of the symptoms associated with the cancer. To the extent the drug may prevent growth and/or kill existing cancer cells, it may be cytostatic and/or cytotoxic. For cancer therapy, efficacy can, for example, be measured by assessing the time to disease progression (TTP) and/or determining the response rate (RR).

USE

3-(1-{3-[5-(1-methyl-piperidin-4-ylmethoxy)-pyrimidin-2-yl]-benzyl}-6-oxo-1,6-dihydro-pyridazin-3-yl)-benzonitrile hydrochloride hydrate is suitable as pharmaceutical active ingredient for mammals, especially for humans, in the treatment of hepatocellular carcinoma.

Experimental

Evaluation of 3-(1-{3-[5-(1-methyl-piperidin-4-ylmethoxy)-pyrimidin-2-yl]-benzyl}-6-oxo-1,6-dihydro-pyridazin-3-yl)-benzonitrile hydrochloride hydrate in the HCC xenograft model MHCC97H

Summary: 3-(1-{3-[5-(1-methyl-piperidin-4-ylmethoxy)-pyrimidin-2-yl]-benzyl}-6-oxo-1,6-dihydro-pyridazin-3-yl)-benzonitrile hydrochloride hydrate displays a greater activity than sorafenib in a hepatocarcinoma xenograft model and in HCC primary explants, all of which are characterized by high c-Met and/or HGF expression. While sorafenib led to substantial body weight loss in most HCC explant models (8/9) at all doses tested (50 mg/kg/5 out of 7 days and 60 mg/kg/qd), 3-(1-{3-[5-(1-methyl-piperidin-4-ylmethoxy)-pyrimidin-2-yl]-benzyl}-6-oxo-1,6-dihydro-pyridazin-3-yl)-benzonitrile hydrochloride hydrate was well tolerated in all mice as indicated by the lack of substantial weight loss of animals.

Data from pre-clinical in-house studies highlight the role of c-Met in maintenance and progression of HCC and indicate that c-Met inhibition might be an attractive treatment option for HCC. The MHCC97H cell line has been established from subcutaneous xenograft of a metastatic model of human HCC in nude mice (LCI-D20) and has a tendency to metastasize to the lungs (Wu FS, Zheng SS, Wu LJ, Teng LS, Ma ZM, Zhao WH, Wu W; Liver Int. 2007 Jun; 27(5):700-7).

MHCC97H cells co-express c-Met and HGF and also secrete alpha feto-protein (AFP), a fetal-specific glycoprotein antigen that is used as a tumor marker in the management of patients with HCC.

Treatment of established fast growing subcutaneous MHCC97H tumors with 3-(1-{3-[5-(1-methyl-piperidin-4-ylmethoxy)-pyrimidin-2-yl]-benzyl}-6-oxo-1,6-dihydro-pyridazin-3-yl)-benzonitrile hydrochloride hydrate completely inhibited growth and induced regressions. In comparison to 3-(1-{3-[5-(1-methyl-piperidin-4-ylmethoxy)-pyrimidin-2-yl]-benzyl}-6-oxo-1,6-dihydro-pyridazin-3-yl)-benzonitrile hydrochloride hydrate treatment of subcutaneous MHCC97H xenografts, administration of sorafenib resulted only in marginal anti tumor activity with no tumor regressions. To evaluate the effect of c-Met inhibition under more physiological conditions, MHCC97H cells were engrafted orthotopically in the liver of mice.

Oral administration of 3-(1-{3-[5-(1-methyl-piperidin-4-ylmethoxy)-pyrimidin-2-yl]-benzyl}-6-oxo-1,6-dihydro-pyridazin-3-yl)-benzonitrile hydrochloride hydrate starting one week after tumor fragment implantation exhibited significant anti-tumor activity, resulting in complete regression in all mice at the end of treatment at day 35. As a surrogate endpoint, body weight was followed throughout the study. Body weight loss in the vehicle group could be observed from day 18 onward, probably caused by increased tumor burden in the liver and/or lung metastasis. In contrast, for mice treated with the c-Met inhibitor, no body weight loss could be detected. At the end of the treatment period AFP levels in the circulation were analyzed. Whereas in the control group high AFP levels were detectable, no AFP was measurable in the mice treated with 3-(1-{3-[5-(1-methyl-piperidin-4-ylmethoxy)-pyrimidin-2-yl]-benzyl}-6-oxo-1,6-dihydro-pyridazin-3-yl)-benzonitrile hydrochloride hydrate.

Subcutaneous MHCC97H tumor model - comparison 3-(1-{3-[5-(1-methyl-piperidin-4-ylmethoxy)-pyrimidin-2-yl]-benzyl}-6-oxo-1,6-dihydro-pyridazin-3-yl)-benzonitrile hydrochloride hydrate and sorafenib monotherapy:

Method: Male BalB/c nude mice (6-8 week old) where subcutaneously injected with human MHCC97H liver tumor cells and were divided into treatment groups (ten animals in one group) after the tumors were established (ca. 500mm^3). Respective groups were administered orally with the 3-(1-{3-[5-(1-methyl-piperidin-4-ylmethoxy)-pyrimidin-2-yl]-benzyl}-6-oxo-1,6-dihydro-pyridazin-3-yl)-benzonitrile hydrochloride hydrate at different doses (10, 30 and 100 mg/kg) for 5 days on and 2 days on or daily with sorafenib (50mg/kg). At the end of treatment T/C values were calculated and tumor regrowth was observed.

Results: All doses of 3-(1-{3-[5-(1-methyl-piperidin-4-ylmethoxy)-pyrimidin-2-yl]-benzyl}-6-oxo-1,6-dihydro-pyridazin-3-yl)-benzonitrile hydrochloride hydrate showed significant anti-tumor activity inducing tumor regression with T/C ratios of -57%, -93% and -93%, respectively and a tumor growth delay (TGD, time to reach a tumor volume of 1000mm^3) of 24, 53 and more as 53 days,

respectively. Treatment of sorafenib showed less anti-tumor activity compared to MSC2156119J with a T/C value of 27%.

Orthotopic MHCC97H tumor model - 3-(1-{3-[5-(1-methyl-piperidin-4-ylmethoxy)-pyrimidin-2-yl]-benzyl}-6-oxo-1,6-dihydro-pyridazin-3-yl)-benzonitrile hydrochloride hydrate monotherapy:

Method: In male BalB/c nude mice (7-8 week old) MHCC97H tumor fragments (2-3 mm^3) where orthotopically implanted into the left lobe of the liver. After 1 week of intrahepatic implantation animals were divided into treatment groups (ten animals in one group). Respective groups were administered orally with 3-(1-{3-[5-(1-methyl-piperidin-4-ylmethoxy)-pyrimidin-2-yl]-benzyl}-6-oxo-1,6-dihydro-pyridazin-3-yl)-benzonitrile hydrochloride hydrate at 100 mg/kg/5 days on and 2 days off for 5 weeks. At the end of treatment, tumor size and tumor weight were measured, plasma AFP levels and lung metastases analyzed.

Results: Treatment with 3-(1-{3-[5-(1-methyl-piperidin-4-ylmethoxy)-pyrimidin-2-yl]-benzyl}-6-oxo-1,6-dihydro-pyridazin-3-yl)-benzonitrile hydrochloride hydrate induced significant anti-tumor activity resulting in primary tumor regression ($p<0.001$) and reduction lung metastases ($p<0.01$). AFP levels in plasma of mice analyzed at the end of treatment were also significantly reduced ($p<0.001$). Treatment of 3-(1-{3-[5-(1-methyl-piperidin-4-ylmethoxy)-pyrimidin-2-yl]-benzyl}-6-oxo-1,6-dihydro-pyridazin-3-yl)-benzonitrile hydrochloride hydrate was well tolerated.

Evaluation of 3-(1-{3-[5-(1-methyl-piperidin-4-ylmethoxy)-pyrimidin-2-yl]-benzyl}-6-oxo-1,6-dihydro-pyridazin-3-yl)-benzonitrile hydrochloride hydrate in primary HCC explants:

Summary: To study the therapeutic potential of 3-(1-{3-[5-(1-methyl-piperidin-4-ylmethoxy)-pyrimidin-2-yl]-benzyl}-6-oxo-1,6-dihydro-pyridazin-3-yl)-benzonitrile hydrochloride hydrate in HCC patients, the activity of 3-(1-{3-[5-(1-methyl-piperidin-4-ylmethoxy)-pyrimidin-2-yl]-benzyl}-6-oxo-1,6-dihydro-

pyridazin-3-yl)-benzonitrile hydrochloride hydrate was evaluated in a preclinical phase II type trial (PP2T trial) with human primary HCC explants. Treatment of nine subcutaneous, established primary explants resulted in 1/9 complete responses (CR), 2/9 stable diseases (SD) and marginal activity in one additional model. The activity of 3-(1-{3-[5-(1-methyl-piperidin-4-ylmethoxy)-pyrimidin-2-yl]-benzyl}-6-oxo-1,6-dihydro-pyridazin-3-yl)-benzonitrile hydrochloride hydrate correlated positive with the activation status of the c-Met receptor expressed in these models, as indicated by c-Met and HGF expression levels. None of the models with no or only low signs of detectable c-Met signaling (c-Met, phospho c-Met and/HGF levels) responded to 3-(1-{3-[5-(1-methyl-piperidin-4-ylmethoxy)-pyrimidin-2-yl]-benzyl}-6-oxo-1,6-dihydro-pyridazin-3-yl)-benzonitrile hydrochloride hydrate in monotherapy and no enhanced activity in combination with sorafenib has been observed.

Method: Male BalB/c nude mice (6-8 week old) where subcutaneously transplanted with a primary HCC tumor fragment. Animals were divided into treatment groups (12 animals in one group) after the tumors were established. Respective groups were administered orally with the 3-(1-{3-[5-(1-methyl-piperidin-4-ylmethoxy)-pyrimidin-2-yl]-benzyl}-6-oxo-1,6-dihydro-pyridazin-3-yl)-benzonitrile hydrochloride hydrate at 100mg/kg for 5 out of 7 days. At the end of treatment T/C values were calculated and tumor regrowth was observed.

Results: 3-(1-{3-[5-(1-methyl-piperidin-4-ylmethoxy)-pyrimidin-2-yl]-benzyl}-6-oxo-1,6-dihydro-pyridazin-3-yl)-benzonitrile hydrochloride hydrate (100 mg/kg/5 out of 7 days) significantly inhibited the growth of 4 out of the 9 models (LIM612, LIM801, LIM1098, LIM941; T/C values of 49% to -97%). Sorafenib (50 mg/kg/5 out of 7 days) displayed an anti-tumor activity in 7 out of 9 models (LIM348, LIM612, LIM941, LIM752, LIM1098, LIM801, LIM1081 with T/C values of 45% to -8%). 3-(1-{3-[5-(1-methyl-piperidin-4-ylmethoxy)-pyrimidin-2-yl]-benzyl}-6-oxo-1,6-dihydro-pyridazin-3-yl)-benzonitrile hydrochloride hydrate exhibited better anti-tumor activity than sorafenib monotherapy in LIM801 and LIM612, two models with strong signs of c-Met signaling. The combination of sorafenib with 3-(1-{3-[5-(1-methyl-piperidin-4-ylmethoxy)-pyrimidin-2-yl]-

benzyl}-6-oxo-1,6-dihydro-pyridazin-3-yl)-benzonitrile hydrochloride hydrate enhanced anti-tumor activity of best monotherapy in 2 out of 9 models (LIM1098 and LIM752 with T/C values of -32% and 4%, respectively).

Determination of c-Met phospho c-Met and HGF protein levels in primary explants:

Method: c-Met, phospho c-Met, and HGFalpha expression was studied with IHC in satellite animals of human primary tumor explants and xenografts (Table 1). The xenografts were excised, sectioned into few pieces, fixed in 4% buffered formaldehyde solution during 48 hrs at RT and embedded in paraffin. Sections of 3µm of formaldehyde fixed paraffin embedded (FFPE) tissue were mounted on positively charged SuperFrost®Plus slides (Menzel-Gläser, Braunschweig, Germany). The immunohistochemical staining procedure starting with the deparaffinization of sections was done with the staining instrument Discovery™ or the Discovery® XT (Ventana Medical Systems, Inc., Tucson, USA). After deparaffinization sections were heated for epitope retrieval in Tris-EDTA buffer pH 8. Endogenous peroxidase was blocked by incubation in 3% hydrogen peroxide (part of OmniMap™ Kit, Ventana Medical Systems). Sections were incubated with in PBS diluted antibodies. and then with the secondary antibody, the HRP conjugated polymers of the OmniMap Kit, for 16 min at 37°C. Horseradish peroxidase (HRP) catalyzes the 3,3'-diaminobenzidine tetrahydorchloride (DAB)/H2O2 reaction to produce an insoluble dark brown precipitate that can be visualized. Sections were counterstained with hematoxylin. Slides were washed in tap water, dehydrated, and mounted with glass coverslips in permanent mounting media Entellan® Neu (VWR, Germany). The detailed run protocols, generated by the staining instruments, are stored at Merck Serono, Darmstadt, Germany. Immunohistochemical stainings were scanned with the help of the MiraxSCAN (Zeiss) with a resolution x/y: 1 pixel = 0.23 x 0.23 µm2. The scannings were analyzed with the image analysis software Visiopharm Integrator System (VIS;

V 4.0.3.0; Visiopharm A/S, Denmark). Viable tissue area was outlined avoiding obvious necrotic areas and connective tissue. For the determination of the amount of antigen present, positive brown stained area was calculated as percent area of the viable tissue area. Antibody staining (arbitrary units) is calculated as Antibody staining (AU) = Positive area (%) * (255-Intensity)/100 of the brown colour.

Results: In explants of human hepatocellular carcinoma (HCC), high c-Met, phospho-Met and moderate HGFalpha expression could be detected in single explants with the help of immunohistochemistry (IHC). Out of 9 HCC explants, 8 were positive for c-Met. Two of the explants (LIM1098 and LIM612) showed high pTyr 1234/1235 Met and pTyr 1349 Met expression. Additional 3 explant tumors showed low to moderate pTyr1349-Met expression. Due to high background staining with the detection system for the HGF antibody clone B-3 (mouse IgG) in several explant xenografts, the tumors could not be analyzed with the help of image analysis. A semiquantitative scoring was performed (C. Wilm) by comparing the specific anti-HGF staining with the mouse IgG isotype control staining.

Scores are:

0 = negative

1 = low

2 = medium

3 = high

Two out of 9 HCC explants exhibited low to moderate HGF alpha expression. LIM612 with low HGF expression was highly positive for phospho-Met. LIM801 with moderate HGF expression was negative for phospho-Met.

Patent Claims

1. 3-(1-{3-[5-(1-methyl-piperidin-4-ylmethoxy)-pyrimidin-2-yl]-benzyl}-6-oxo-1,6-dihydro-pyridazin-3-yl)-benzonitrile or a pharmaceutically acceptable salt and/or solvate thereof for the use for the treatment of hepatocellular carcinoma (HCC).

2. 3-(1-{3-[5-(1-methyl-piperidin-4-ylmethoxy)-pyrimidin-2-yl]-benzyl}-6-oxo-1,6-dihydro-pyridazin-3-yl)-benzonitrile hydrochloride hydrate for the use for the treatment of hepatocellular carcinoma (HCC).

3. 3-(1-{3-[5-(1-methyl-piperidin-4-ylmethoxy)-pyrimidin-2-yl]-benzyl}-6-oxo-1,6-dihydro-pyridazin-3-yl)-benzonitrile according to claim 1 or 2, wherein the compound is administered to a patient in an amount of 100 mg to 800 mg per day.

4. 3-(1-{3-[5-(1-methyl-piperidin-4-ylmethoxy)-pyrimidin-2-yl]-benzyl}-6-oxo-1,6-dihydro-pyridazin-3-yl)-benzonitrile according to claim 1, 2 or 3, wherein the compound is administered orally.

5. Use of 3-(1-{3-[5-(1-methyl-piperidin-4-ylmethoxy)-pyrimidin-2-yl]-benzyl}-6-oxo-1,6-dihydro-pyridazin-3-yl)-benzonitrile or a pharmaceutically acceptable salt and/or solvate thereof for the manufacture of a medicament for the treatment of hepatocellular carcinoma (HCC).

6. Use of 3-(1-{3-[5-(1-methyl-piperidin-4-ylmethoxy)-pyrimidin-2-yl]-benzyl}-6-oxo-1,6-dihydro-pyridazin-3-yl)-benzonitrile hydrochloride hydrate for the manufacture of a medicament for the treatment of hepatocellular carcinoma (HCC).

7. Use according to claim 5 or 6, wherein the compound is administered to a patient in an amount of 100 mg to 800 mg per day.

8. Use according to claim 5, 6 or 7, wherein the compound is administered orally.

INTERNATIONAL SEARCH REPORT

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A. CLASSIFICATION OF SUBJECT MATTER
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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)
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Electronic data base consulted during the international search (name of data base and, where practicable, search terms used)
EPO-Internal, BIOSIS, EMBASE, PAJ

C. DOCUMENTS CONSIDERED TO BE RELEVANT

Category*	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
A	WO 2010/078897 A1 (MERCK PATENT GMBH [DE]; BECKER AXEL [DE]; KUEHN CLEMENS [DE]; SAAL CHR) 15 July 2010 (2010-07-15) Page 20, line 4; page 19, line 29 - page 20, line 16 -----	1-8

☐ 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

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INTERNATIONAL SEARCH REPORT

Information on patent family members

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Patent document cited in search report	Publication date	Patent family member(s)	Publication date
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		US 2011269767 A1	03-11-2011
		WO 2010078897 A1	15-07-2010

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权利要求书1页 说明书10页

(54)发明名称

用于治疗肝细胞癌(HCC)的6-氧代-1,6-二氢-哒嗪衍生物

(57)摘要

用于治疗肝细胞癌(HCC)的3-(1-{3-[5-(1-甲基-哌啶-4-基甲氧基)-嘧啶-2-基]-苄基}-6-氧代-1,6-二氢-哒嗪-3-基)-苄腈或其可药用盐和/或溶剂合物。

CN 104768553 A

1. 3-(1-{3-[5-(1- 甲基 - 哌啶 -4- 基甲氧基)- 嘧啶 -2- 基]- 苄基 }-6- 氧代 -1,6- 二氢 - 哒嗪 -3- 基)- 苄腈或其可药用盐和 / 或溶剂合物，其用于治疗肝细胞癌（HCC）。

2. 3-(1-{3-[5-(1- 甲基 - 哌啶 -4- 基甲氧基)- 嘧啶 -2- 基]- 苄基 }-6- 氧代 -1,6- 二氢 - 哒嗪 -3- 基)- 苄腈盐酸盐水合物，其用于治疗肝细胞癌（HCC）。

3. 根据权利要求 1 或 2 的 3-(1-{3-[5-(1- 甲基 - 哌啶 -4- 基甲氧基)- 嘧啶 -2- 基]- 苄基 }-6- 氧代 -1,6- 二氢 - 哒嗪 -3- 基)- 苄腈，其中所述化合物以每天 100 毫克至 800 毫克的量给药于患者。

4. 根据权利要求 1、2 或 3 的 3-(1-{3-[5-(1- 甲基 - 哌啶 -4- 基甲氧基)- 嘧啶 -2- 基]- 苄基 }-6- 氧代 -1,6- 二氢 - 哒嗪 -3- 基)- 苄腈，其中所述化合物口服给药。

5. 3-(1-{3-[5-(1- 甲基 - 哌啶 -4- 基甲氧基)- 嘧啶 -2- 基]- 苄基 }-6- 氧代 -1,6- 二氢 - 哒嗪 -3- 基)- 苄腈或其可药用盐和 / 或溶剂合物用于制备用于治疗肝细胞癌（HCC）的药物的用途。

6. 3-(1-{3-[5-(1- 甲基 - 哌啶 -4- 基甲氧基)- 嘧啶 -2- 基]- 苄基 }-6- 氧代 -1,6- 二氢 - 哒嗪 -3- 基)- 苄腈盐酸盐水合物用于制备用于治疗肝细胞癌（HCC）的药物的用途。

7. 根据权利要求 5 或 6 的用途，其中所述化合物以每天 100 毫克至 800 毫克的量给药于患者。

8. 根据权利要求 5、6 或 7 的用途，其中所述化合物口服给药。

用于治疗肝细胞癌(HCC)的 6- 氧代 -1,6- 二氢 - 哒嗪衍生物

发明领域

[0001] 本发明涉及用于治疗肝细胞癌(HCC)的 3-(1-{3-[5-(1- 甲基 - 哌啶 -4- 基甲氧基)- 嘧啶 -2- 基]- 苄基 }-6- 氧代 -1,6- 二氢 - 哒嗪 -3- 基)- 苄腈或其可药用盐和 / 或溶剂合物。

[0002] 发明背景

本发明的目的是找出具有有价值的性质的新型药物组合物,特别是可用于制备药物的那些。

[0003] 此外,本发明的目标是用于预防和治疗肝细胞癌的新型组合物。

[0004] 已经发现,本发明的 3-(1-{3-[5-(1- 甲基 - 哌啶 -4- 基甲氧基)- 嘧啶 -2- 基]- 苄基 }-6- 氧代 -1,6- 二氢 - 哒嗪 -3- 基)- 苄腈或其可药用盐和 / 或溶剂合物在耐受良好的同时具有非常有价值的药理性质。

[0005] HCC 是世界上第五常见的恶性肿瘤,全世界有 667,000 例新病例,在美国有 17,500 例。80% 的患者在诊断时呈现晚期或不可切除的疾病。在西方国家,大约 40% 的患者适合潜在根治疗法(切除、移植、局部消融),而大约 20% 适合化疗栓塞术。在精心挑选的患者中,切除和移植提供 70% 的 5 年存活率,50% 的患者在 3 年内复发。在此,我们证实 3-(1-{3-[5-(1- 甲基 - 哌啶 -4- 基甲氧基)- 嘧啶 -2- 基]- 苄基 }-6- 氧代 -1,6- 二氢 - 哒嗪 -3- 基)- 苄腈或其可药用盐和 / 或溶剂合物在具有活跃 c-Met 信号传导迹象的 HCC 肿瘤中有活性。

[0006] 在索拉非尼获批之前,没有有效的提高 HCC 存活率的全身疗法 :作为单一疗法或在联合方案中给予的传统细胞毒剂具有低响应率并且没有带来存活优势(Thomas MB, O'Beirne JP, Furuse J, Chan AT, Abou-Alfa G, Johnson P ;Ann Surg Oncol. 2008 Apr ;15(4):1008-14)。

[0007] 但是,尽管索拉非尼已经改善 PFS 时间,但 PFS 和总存活率仍有限。在药物暴露数周后出现继发耐药性。在进展后,目前没有其它治疗选择。由于 HCC 中高度未满足的医疗需求,需要替代性的有效治疗选择。

现有技术

[0008] 在 WO 2009/006959 A1 中已经描述了 3-(1-{3-[5-(1- 甲基 - 哌啶 -4- 基甲氧基)- 嘧啶 -2- 基]- 苄基 }-6- 氧代 -1,6- 二氢 - 哒嗪 -3- 基)- 苄腈。

[0009] 在 WO 2009/007074 A1 中已经描述了 3-(1-{3-[5-(1- 甲基 - 哌啶 -4- 基甲氧基)- 嘧啶 -2- 基]- 苄基 }-6- 氧代 -1,6- 二氢 - 哒嗪 -3- 基)- 苄腈盐酸盐水合物。

[0010] 发明概述

本发明涉及用于治疗肝细胞癌(HCC)的3-(1-{3-[5-(1- 甲基 - 哌啶 -4- 基甲氧基)- 嘧啶 -2- 基]- 苄基 }-6- 氧代 -1,6- 二氢 - 哒嗪 -3- 基)- 苄腈或其可药用盐和 / 或溶剂合物。

[0011] 此外，本发明涉及用于治疗肝细胞癌（HCC）的 3-(1-{3-[5-(1- 甲基 - 哌啶 -4- 基甲氧基)- 嘧啶 -2- 基]- 苄基 }-6- 氧代 -1,6- 二氢 - 哒嗪 -3- 基)- 苄腈盐酸盐水合物。

[0012] 此外，本发明涉及 3-(1-{3-[5-(1- 甲基 - 哌啶 -4- 基甲氧基)- 嘧啶 -2- 基]- 苄基 }-6- 氧代 -1,6- 二氢 - 哒嗪 -3- 基)- 苄腈或其可药用盐和 / 或溶剂合物，其中该化合物以每天 100 毫克至 800 毫克的量给药于患者。

[0013] 此外，本发明涉及 3-(1-{3-[5-(1- 甲基 - 哌啶 -4- 基甲氧基)- 嘧啶 -2- 基]- 苄基 }-6- 氧代 -1,6- 二氢 - 哒嗪 -3- 基)- 苄腈或其可药用盐和 / 或溶剂合物，其中该化合物口服给药。

[0014] 此外，本发明涉及 3-(1-{3-[5-(1- 甲基 - 哌啶 -4- 基甲氧基)- 嘧啶 -2- 基]- 苄基 }-6- 氧代 -1,6- 二氢 - 哒嗪 -3- 基)- 苄腈或其可药用盐和 / 或溶剂合物用于制备用于治疗肝细胞癌（HCC）的药物的用途。

[0015] 此外，本发明涉及 3-(1-{3-[5-(1- 甲基 - 哌啶 -4- 基甲氧基)- 嘧啶 -2- 基]- 苄基 }-6- 氧代 -1,6- 二氢 - 哒嗪 -3- 基)- 苄腈盐酸盐水合物用于制备用于治疗肝细胞癌（HCC）的药物的用途。

[0016] 此外，本发明涉及如上所述的用途，

其中 3-(1-{3-[5-(1- 甲基 - 哌啶 -4- 基甲氧基)- 嘧啶 -2- 基]- 苄基 }-6- 氧代 -1,6- 二氢 - 哒嗪 -3- 基)- 苄腈或其可药用盐和 / 或溶剂合物或 3-(1-{3-[5-(1- 甲基 - 哌啶 -4- 基甲氧基)- 嘧啶 -2- 基]- 苄基 }-6- 氧代 -1,6- 二氢 - 哒嗪 -3- 基)- 苄腈盐酸盐水合物，

其中所述化合物以每天 100 毫克至 800 毫克的量，优选以每周 200 毫克至 700 毫克的量，特别优选以每天 250 毫克至 350 毫克的量给药于患者。

[0017] 此外，本发明涉及如上所述的用途，

其中 3-(1-{3-[5-(1- 甲基 - 哌啶 -4- 基甲氧基)- 嘧啶 -2- 基]- 苄基 }-6- 氧代 -1,6- 二氢 - 哒嗪 -3- 基)- 苄腈或其可药用盐和 / 或溶剂合物或 3-(1-{3-[5-(1- 甲基 - 哌啶 -4- 基甲氧基)- 嘧啶 -2- 基]- 苄基 }-6- 氧代 -1,6- 二氢 - 哒嗪 -3- 基)- 苄腈盐酸盐水合物，

其中所述化合物口服给药。

[0018] 用 3-(1-{3-[5-(1- 甲基 - 哌啶 -4- 基甲氧基)- 嘧啶 -2- 基]- 苄基 }-6- 氧代 -1,6- 二氢 - 哒嗪 -3- 基)- 苄腈或其可药用盐和 / 或溶剂合物或 3-(1-{3-[5-(1- 甲基 - 哌啶 -4- 基甲氧基)- 嘧啶 -2- 基]- 苄基 }-6- 氧代 -1,6- 二氢 - 哒嗪 -3- 基)- 苄腈盐酸盐水合物治疗可包括任选进一步放射治疗。本发明还涉及一种用于治疗肝细胞癌（HCC）的新治疗形式，其包括在放射疗法之前开始给予 3-(1-{3-[5-(1- 甲基 - 哌啶 -4- 基甲氧基)- 嘧啶 -2- 基]- 苄基 }-6- 氧代 -1,6- 二氢 - 哒嗪 -3- 基)- 苄腈或其可药用盐和 / 或溶剂合物。

[0019] 本发明还涉及该化合物的旋光形式（立体异构体）、对映体、外消旋物、非对映体和水合物和溶剂合物。

[0020] 本发明还涉及该化合物的盐的溶剂合物，例如盐酸盐的一水合物或二水合物。

[0021] 术语“该化合物的溶剂合物”是指由于惰性溶剂分子与该化合物的相互吸引力而形成的惰性溶剂分子加合到该化合物上的加合物。溶剂合物是例如一水合物或二水合物或

醇化物。

[0022] 表述“有效量”是指在组织、系统、动物或人体中造成例如研究人员或医师追求或想要的生物或医疗响应的药物或药物活性成分的量。

[0023] 此外，表述“治疗有效量”是指与尚未接受这种量的相应对象相比具有下列后果的量：疾病、综合征、病症、不适、障碍或副作用的改善的治疗、治愈、预防或消除，或还减轻疾病、不适或障碍的进展。

[0024] 表述“治疗有效量”还包括有效提高正常生理机能的量。

[0025] 药用盐和其它形式

本发明的所述化合物可以以它们的最终非盐形式使用。另一方面，本发明还包括以可由各种有机和无机酸和碱通过本领域中已知的程序生成的它们的可药用盐形式使用这些化合物。主要通过常规方法制备 3-(1-{3-[5-(1- 甲基 - 哌啶 -4- 基甲氧基)- 嘧啶 -2- 基]- 苄基 }-6- 氧代 -1,6- 二氢 - 哒嗪 -3- 基)- 苄腈和 N-((S)-2,3- 二羟基 - 丙基)-3-(2- 氟 -4- 碘 - 苯基氨基)- 异烟酰胺的可药用盐形式。

[0026] 如果化合物含有羧基，可通过使该化合物与合适的碱反应产生相应的碱加成盐来形成其合适的盐之一。这样的碱是例如碱金属氢氧化物，包括氢氧化钾、氢氧化钠和氢氧化锂；碱土金属氢氧化物，如氢氧化钡和氢氧化钙；碱金属醇盐，例如乙醇钾和丙醇钠；和各种有机碱，如哌啶、二乙醇胺和 N- 甲基谷氨酰胺。还包括该化合物的铝盐。在某些化合物的情况中，可通过用可药用的有机和无机酸，例如氢卤酸，如盐酸、氢溴酸或氢碘酸，其它无机酸及其相应的盐，如硫酸盐、硝酸盐或磷酸盐等，和烷基 - 和单芳基磺酸盐，如乙磺酸盐、甲苯磺酸盐和苯磺酸盐，和其它有机酸及其相应的盐，如乙酸盐、三氟乙酸盐、酒石酸盐、马来酸盐、琥珀酸盐、柠檬酸盐、苯甲酸盐、水杨酸盐、抗坏血酸盐等处理这些化合物来形成酸加成盐。相应地，该化合物的可药用酸加成盐包括下列：乙酸盐、己二酸盐、藻酸盐、精氨酸盐、天冬氨酸盐、苯甲酸盐、苯磺酸盐、硫酸氢盐、亚硫酸氢盐、溴化物、丁酸盐、樟脑酸盐、樟脑磺酸盐、辛酸盐、氯化物、氯苯甲酸盐、柠檬酸盐、环戊烷丙酸盐、二葡糖酸盐、二氢磷酸盐、二硝基苯甲酸盐、十二烷基硫酸盐、乙磺酸盐、富马酸盐、半乳糖二酸盐(galacterate)(来自粘酸)、半乳糖醛酸盐、葡庚糖酸盐、葡糖酸盐、谷氨酸盐、甘油磷酸盐、半琥珀酸盐、半硫酸盐、庚酸盐、己酸盐、马尿酸盐、盐酸盐、氢溴酸盐、氢碘酸盐、2- 羟基乙磺酸盐、碘化物、羟乙基磺酸盐、异丁酸盐、乳酸盐、乳糖醛酸盐、苹果酸盐、马来酸盐、丙二酸盐、扁桃酸盐、偏磷酸盐、甲磺酸盐、甲基苯甲酸盐、磷酸一氢盐、2- 萘磺酸盐、烟酸盐、硝酸盐、草酸盐、油酸盐、palmoate、果胶酯酸盐、过硫酸盐、苯基乙酸盐、3- 苯基丙酸盐、磷酸盐、膦酸盐、邻苯二甲酸盐，但这不代表限制。

[0027] 此外，本发明的化合物的碱式盐包括铝盐、铵盐、钙盐、铜盐、铁 (III) 盐、铁 (II) 盐、锂盐、镁盐、锰 (III) 盐、锰 (II) 盐、钾盐、钠盐和锌盐，但这无意代表限制。在上述盐中，优选的是铵盐；碱金属盐钠盐和钾盐，和碱土金属盐钙盐和镁盐。衍生自可药用的有机无毒碱的化合物的盐包括伯胺、仲胺和叔胺、取代胺，也包括天然存在的取代胺、环胺和碱性离子交换树脂，例如精氨酸、甜菜碱、咖啡因、氯普鲁卡因、胆碱、N,N’- 二苄基乙二胺(苄星青霉素)、二环己基胺、二乙醇胺、二乙胺、2- 二乙基氨基乙醇、2- 二甲基氨基乙醇、乙醇胺、乙二胺、N- 乙基吗啉、N- 乙基哌啶、还原葡糖胺(glucamine)、氨基葡萄糖(glucosamine)、组氨酸、哈胺(hydrabamine)、异丙胺、利多卡因、赖氨酸、葡甲胺、N- 甲基 -D- 葡糖胺、吗啉、哌

嗪、哌啶、聚胺树脂、普鲁卡因、嘌呤、可可碱、三乙醇胺、三乙胺、三甲胺、三丙胺和三（羟甲基）甲基胺(氨丁三醇)的盐，但这无意代表限制。

[0028] 含有碱性含氮基团的本发明的化合物可以用例如以下试剂季铵化：(C_1-C_4)烷基卤化物，例如甲基、乙基、异丙基和叔丁基的氯化物、溴化物和碘化物；硫酸二(C_1-C_4)烷基酯，例如硫酸二甲酯、硫酸二乙酯和硫酸二戊酯；(C_{10}-C_{18})烷基卤化物，例如癸基、十二烷基、月桂基、十四烷基和十八烷基的氯化物、溴化物和碘化物；和芳基(C_1-C_4)烷基卤化物，例如苄基氯化物和苯乙基溴化物。本发明的水溶性和油溶性化合物都可以使用这样的盐制备。

[0029] 优选的上述可药用盐包括乙酸盐、三氟乙酸盐、苯磺酸盐、柠檬酸盐、富马酸盐、葡糖酸盐、半琥珀酸盐、马尿酸盐、盐酸盐、氢溴酸盐、羟乙基磺酸盐、扁桃酸盐、葡甲胺、硝酸盐、油酸盐、膦酸盐、特戊酸盐、磷酸钠、硬脂酸盐、硫酸盐、磺基水杨酸盐、酒石酸盐、硫代苹果酸盐、甲苯磺酸盐和氨丁三醇，但这无意代表限制。

[0030] 特别优选的是盐酸盐、二盐酸盐、氢溴酸盐、马来酸盐、甲磺酸盐、磷酸盐、硫酸盐和琥珀酸盐。

[0031] 通过使游离碱形式与足量的所需酸接触以致以常规方式形成盐来制备碱性化合物的酸加成盐。可通过使该盐形式与碱接触并以常规方式分离游离碱来再生游离碱。游离碱形式在某些方面，在某些物理性质，如在极性溶剂中的溶解度方面不同于其相应的盐形式；但对本发明的目的而言，该盐在其它方面相当于其各自的游离碱形式。

[0032] 如所提到的，用金属或胺，如碱金属和碱土金属或有机胺形成该化合物的可药用碱加成盐。优选的金属是钠、钾、镁和钙。优选的有机胺是N,N'-二苄基乙二胺、氯普鲁卡因、胆碱、二乙醇胺、乙二胺、N-甲基-D-葡糖胺和普鲁卡因。

[0033] 通过使游离酸形式与足量的所需碱接触以致以常规方式形成盐来制备本发明的酸性化合物的碱加成盐。可以通过使该盐形式与酸接触并以常规方式分离游离酸来再生游离酸。游离酸形式在某些方面，在某些物理性质，如在极性溶剂中的溶解度方面不同于其相应的盐形式；但对本发明的目的而言，该盐在其它方面相当于其各自的游离酸形式。

[0034] 根据上文可以看出，表述“可药用盐”在本文中是指包含其盐之一的形式的化合物的活性成分，特别是如果这种盐形式与该活性成分的游离形式或之前使用的该活性成分的任何其它盐形式相比为该活性成分提供改进的药代动力学性质的话。该活性成分的可药用盐形式还可首次为这种活性成分提供之前没有的并甚至就其体内治疗效力而言对这种活性成分的药效学具有积极影响的所需药代动力学性质。

[0035] 本发明还涉及包含至少一种化合物和/或其可药用盐、溶剂合物、互变异构体和立体异构体，包括它们的所有比率的混合物，和任选赋形剂和/或辅助剂的药剂。

[0036] 药物制剂可以以每剂量单位包含预定量的活性成分的剂量单位形式给药。这种单位可根据治疗的病症、给药方法和患者的年龄、体重和状况包含例如0.5毫克至1克，优选1毫克至700毫克，特别优选5毫克至100毫克的本发明的化合物，或药物制剂可以以每剂量单位包含预定量的活性成分的剂量单位形式给药。优选剂量单位制剂是包含如上指示的日剂量或分剂量或其相应分数的活性成分的那些。此外，可以使用制药领域中公知的方法制备这种类型的药物制剂。

[0037] 药物制剂可适于通过任何所需的合适方法给药，例如通过经口(包括口腔或舌

下)、直肠、经鼻、局部(包括口腔、舌下或经皮)、阴道或肠道外(包括皮下、肌内、静脉内或皮内)方法给药。可以使用制药领域中已知的所有方法通过例如将活性成分与一种或多种赋形剂或一种或多种辅助剂合并来制备这样的制剂。

[0038] 适合口服给药的药物制剂可作为独立单位,例如胶囊或片剂;粉剂或颗粒剂;在水性或非水液体中的溶液或悬浮液;可食用泡沫或泡沫食品;或水包油液体乳剂或油包水液体乳剂给药。

[0039] 因此,例如,在以片剂或胶囊形式口服给药的情况下,可以将活性成分组分与口服、无毒和可药用的惰性赋形剂,例如乙醇、甘油、水等合并。通过将该化合物研碎至合适的细粒度并将其与以类似方式研碎的药物赋形剂,例如可食用的碳水化合物,例如淀粉或甘露醇混合,制备粉剂。还可能存在香料、防腐剂、分散剂和染料。

[0040] 通过如上所述制备粉末混合物并用其填充成形明胶壳,制造胶囊。在填充操作之前,可以将助流剂和润滑剂,例如固体形式的高分散硅酸、滑石、硬脂酸镁、硬脂酸钙或聚乙二醇添加到该粉末混合物中。也可以添加崩解剂或增溶剂,例如琼脂、碳酸钙或碳酸钠以改进服用胶囊后药剂的利用率。

[0041] 此外,如果需要或必要,也可以将合适的粘合剂、润滑剂和崩解剂以及染料掺入该混合物中。合适的粘合剂包括淀粉、明胶、天然糖,例如葡萄糖或 β-乳糖,由玉米制成的甜味剂、天然和合成橡胶,例如阿拉伯树胶、黄蓍胶或藻酸钠、羧甲基纤维素、聚乙二醇、蜡等。这些剂型中所用的润滑剂包括油酸钠、硬脂酸钠、硬脂酸镁、苯甲酸钠、乙酸钠、氯化钠等。崩解剂包括,但不限于,淀粉、甲基纤维素、琼脂、膨润土、黄原胶等。例如通过制备粉末混合物、粒化或干压该混合物、添加润滑剂和崩解剂并将整个混合物压成片剂来配制片剂。通过将以合适方式粉碎的化合物与如上所述的稀释剂或基料和任选与粘合剂,例如羧甲基纤维素、藻酸盐、明胶或聚乙烯基吡咯烷酮,溶出阻滞剂,例如石蜡,吸收促进剂,例如季铵盐,和/或吸收剂,例如膨润土、高岭土或磷酸二钙混合,制备粉末混合物。可通过用粘合剂,例如糖浆、淀粉糊、acadia mucilage 或纤维素或聚合物材料的溶液润湿粉末混合物并将其压过筛子来粒化该粉末混合物。代替粒化,可以使该粉末混合物经过压片机,以产生形状不均匀的团块,将其打碎形成颗粒。可以通过添加硬脂酸、硬脂酸盐、滑石或矿物油来润滑颗粒以防止粘着到铸片模具上。然后将润滑的混合物压成片剂。本发明的化合物也可以与自由流动的惰性赋形剂合并,然后在不进行造粒或干压步骤的情况下直接压成片剂。可以存在由虫胶密封层、糖或聚合物材料层和蜡光泽层构成的透明或不透明保护层。可以将染料添加到这些涂层中以便能区分不同的剂量单位。

[0042] 口服液,例如溶液、糖浆和酏剂,可以以剂量单位形式制备以使所给的量包含预定量的该化合物。可以通过将该化合物溶解在含有合适香料的水溶液中来制备糖浆,而酏剂使用无毒醇类媒介物制备。可以通过将该化合物分散在无毒媒介物中来配制悬浮液。也可以加入增溶剂和乳化剂,例如乙氧基化异硬脂醇和聚氧乙烯山梨糖醇醚,防腐剂,香料添加剂,例如薄荷油,或天然甜味剂或糖精,或其它人工甜味剂等。

[0043] 如果需要,用于口服给药的剂量单位制剂可包封在微囊中。也可以以延长或延迟释放的方式制备该制剂,例如通过将微粒材料包衣或包埋在聚合物、蜡等中。

[0044] 该化合物及其盐、溶剂合物、互变异构体和立体异构体也可以以脂质体递送体系,例如小单层囊泡、大单层囊泡和多层囊泡的形式给药。脂质体可以由各种磷脂,例如胆固

醇、十八烷基胺或磷脂酰胆碱形成。

[0045] 该化合物及其盐、溶剂合物、互变异构体和立体异构体也可以使用单克隆抗体作为独立载体(该化合物分子偶联到其上)递送。该化合物也可以偶联到作为靶向药剂载体的可溶聚合物上。这样的聚合物可包括聚乙烯基吡咯烷酮、吡喃共聚物、聚羟丙基甲基丙烯酰胺基酚、聚羟乙基天冬酰胺基酚或聚环氧乙烷聚赖氨酸,其被棕榈酰基取代。该化合物还可偶联到适合实现药剂控释的一类可生物降解的聚合物上,例如聚乳酸、聚 ε - 己内酯、聚羟基丁酸、聚原酸酯、聚缩醛、聚二羟基吡喃、聚氰基丙烯酸酯和水凝胶的交联或两亲嵌段共聚物。

[0046] 适合经皮给药的药物制剂可作为独立的膏药给药以与接受者的表皮长时间密切接触。因此,例如,可以一般而言如 Pharmaceutical Research, 3(6), 318 (1986) 中所述通过离子电渗从膏药递送活性成分。

[0047] 适合局部给药的药物化合物可配制为软膏、乳膏、混悬剂、洗剂、粉剂、溶液、糊剂、凝胶、喷雾剂、气雾剂或油。

[0048] 适合直肠给药的药物制剂可以以栓剂或灌肠剂的形式给药。

[0049] 其中载体物质是固体的适合经鼻给药的药物制剂包括粒度为例如 20-500 微米的粗粉,其以鼻吸的方式给药,即通过经鼻腔通道从靠近鼻子放置的含有粉剂的容器中快速吸入。以液体作为载体物质的适合作为鼻喷剂或鼻滴剂给药的制剂包括在水或油中的活性成分溶液。

[0050] 适合通过吸入给药的药物制剂包含可由各种类型的含气雾剂的加压分配器、喷雾器或吹入器生成的细粒状粉或雾。

[0051] 适合肠道外给药的药物制剂包括包含抗氧化剂、缓冲剂、抑菌剂和溶质的水性和非水无菌注射液,借此使该制剂与被治疗的接受者的血液等渗 ;和可包含悬浮介质和增稠剂的水性和非水无菌悬浮液。该制剂可以在单剂或多剂容器,例如密封安瓿和管瓶中给药并以冷冻干燥(冻干)状态储存,以致只需在临使用前添加无菌载液,例如注射用水。可以由无菌粉剂、颗粒剂和片剂根据配方制备注射溶液和悬浮液。

[0052] 无需说明,除上文特别提到的成分外,该制剂还可包含本领域中根据制剂的特定类型常见的其它试剂 ;因此,例如,适合口服给药的制剂可包含香料。

[0053] 化合物的治疗有效量取决于许多因素,包括例如动物的年龄和体重、需要治疗的确切病症及其严重程度、制剂的性质和给药方法,并最终由治疗医生或兽医决定。但是,本发明的化合物的有效量通常为 0.1 至 100 毫克 / 公斤接受者(哺乳动物) 体重 / 天,特别通常为 1 至 10 毫克 / 公斤体重 / 天。因此,体重 70 公斤的成年哺乳动物每天的实际量通常为 70 至 700 毫克,其中这种量可作为每天单剂给药或通常以每天一系列分剂量(例如 2、3、4、5 或 6)给药,以使总日剂量相同。可作为本发明的化合物本身的有效量的分数确定其盐、溶剂合物、互变异构体和立体异构体的有效量。类似剂量被认为适用于治疗上文提到的其它病症。

[0054] 本文中定义的抗癌疗法可作为唯一疗法施用或除本发明的组合物外还可包括传统外科手术或放射疗法。

[0055] 本文所用的“治疗”是指完全或部分减轻与障碍或疾病有关的症状,或减慢或暂停这些症状的进一步发展或恶化,或在有发生该疾病或障碍的风险的对象中预防或防止该疾

病或障碍。

[0056] 关于化合物的术语“有效量”是指能够完全或部分减轻与障碍或疾病有关的症状，或减慢或暂停这些症状的进一步发展或恶化，或在患有或有风险发生本文中公开的疾病，如癌症的对象中预防或防止该疾病或障碍的量。

[0057] 术语“治疗有效”或“治疗有效量”是指有效治疗哺乳动物的疾病或障碍的药物量。就癌症而言，治疗有效量的该药物可减少癌细胞的数量；降低肿瘤尺寸；抑制（即在一定程度上减慢并优选停止）癌细胞浸润到外周器官中；抑制（即在一定程度上减慢并优选停止）肿瘤转移；在一定程度上抑制肿瘤生长；和/或在一定程度上减轻与癌症有关的一个或多个症状。就该药物可能阻止癌细胞生长和/或杀灭现有癌细胞而言，其可能是抑制细胞生长的和/或细胞毒性的。对于癌症疗法，可以例如通过评估疾病进展时间（TTP）和/或测定响应率（RR）来测量效力。

[0058] 用途

3-(1-{3-[5-(1-甲基-哌啶-4-基甲氧基)-嘧啶-2-基]-苄基}-6-氧代-1,6-二氢-哒嗪-3-基)-苄腈盐酸盐水合物适合作为哺乳动物，尤其是人类的肝细胞癌的治疗中的药物活性成分。

[0059] 实验

在HCC异种移植瘤模型MHCC97H中评估3-(1-{3-[5-(1-甲基-哌啶-4-基甲氧基)-嘧啶-2-基]-苄基}-6-氧代-1,6-二氢-哒嗪-3-基)-苄腈盐酸盐水合物

概述：3-(1-{3-[5-(1-甲基-哌啶-4-基甲氧基)-嘧啶-2-基]-苄基}-6-氧代-1,6-二氢-哒嗪-3-基)-苄腈盐酸盐水合物在肝癌异种移植瘤模型中和在HCC初代外植体（primary explants）中（它们都以高c-Met和/或HGF表达为特征）表现出比索拉非尼更高的活性。索拉非尼在所有受试剂量（50mg/kg/一周5天，和60mg/kg/qd）下在大多数HCC外植体模型(8/9)中造成显著的体重减轻，而3-(1-{3-[5-(1-甲基-哌啶-4-基甲氧基)-嘧啶-2-基]-苄基}-6-氧代-1,6-二氢-哒嗪-3-基)-苄腈盐酸盐水合物如动物体重没有显著减轻所示在所有小鼠中都耐受良好。

[0060] 来自临床前内部研究的数据强调c-Met在HCC的维持和进展中的作用并表明c-Met抑制可能是有吸引力的HCC治疗选择。已经由人HCC的转移模型在裸鼠中的皮下异种移植瘤（LCI-D20）建立MHCC97H细胞系并具有转移至肺部的倾向（Wu FS, Zheng SS, Wu LJ, Teng LS, Ma ZM, Zhao WH, Wu W；Liver Int. 2007 Jun；27(5):700-7）。

[0061] MHCC97H细胞共同表达c-Met和HGF并也分泌甲胎蛋白（AFP）——用作HCC患者管理中的肿瘤标记物的一种胎儿特异性糖蛋白抗原。

[0062] 用3-(1-{3-[5-(1-甲基-哌啶-4-基甲氧基)-嘧啶-2-基]-苄基}-6-氧代-1,6-二氢-哒嗪-3-基)-苄腈盐酸盐水合物治疗已建立的快速生长的皮下MHCC97H肿瘤完全抑制了生长并引发消退。与皮下MHCC97H异种移植瘤的3-(1-{3-[5-(1-甲基-哌啶-4-基甲氧基)-嘧啶-2-基]-苄基}-6-氧代-1,6-二氢-哒嗪-3-基)-苄腈盐酸盐水合物治疗相比，索拉非尼的给药仅带来微弱的抗肿瘤活性而没有肿瘤消退。为了评估c-Met抑制在更多生理状况下的作用，将MHCC97H细胞原位移植到小鼠的肝中。

[0063] 在肿瘤片段植入后一周开始口服给药3-(1-{3-[5-(1-甲基-哌啶-4-基甲氧基)-嘧啶-2-基]-苄基}-6-氧代-1,6-二氢-哒嗪-3-基)-苄腈盐酸盐水合物表现出

显著的抗肿瘤活性，在所有小鼠中在第 35 天治疗结束时造成完全消退。作为替代性终点，在研究全程追踪体重。从第 18 天起可观察到媒介物组中的体重减轻，可能由肝和 / 或肺转移中增加的肿瘤负荷造成。相反，对该 c-Met 抑制剂治疗的小鼠而言，没有检测到体重减轻。在治疗期结束时，分析循环中的 AFP 水平。在对照组中，可检测到高 AFP 水平，而在用 3-(1-{3-[5-(1- 甲基 - 哌啶 -4- 基甲氧基)- 嘧啶 -2- 基]- 苄基 }-6- 氧代 -1,6- 二氢 - 哒嗪 -3- 基)- 苄腈盐酸盐水合物治疗的小鼠中不可测得 AFP。

[0064] 皮下 MHCC97H 肿瘤模型 - 比较 3-(1-{3-[5-(1- 甲基 - 哌啶 -4- 基甲氧基)- 嘧啶 -2- 基]- 苄基 }-6- 氧代 -1,6- 二氢 - 哒嗪 -3- 基)- 苄腈盐酸盐水合物和索拉非尼单一疗法：

方法：雄性 BalB/c 裸鼠（6-8 周龄）皮下注入人 MHCC97H 肝肿瘤细胞并在肿瘤建立（大约 500 立方毫米）后分入治疗组（一组 10 只动物）。各组口服给药不同剂量（10、30 和 100 毫克 / 公斤）的 3-(1-{3-[5-(1- 甲基 - 哌啶 -4- 基甲氧基)- 嘧啶 -2- 基]- 苄基 }-6- 氧代 -1,6- 二氢 - 哒嗪 -3- 基)- 苄腈盐酸盐水合物 5 天，停 2 天（5 days on and 2 days on），或每天口服索拉非尼（50mg/kg）。在治疗结束时，计算 T/C 值并观察肿瘤再生。

[0065] 结果：所有剂量的 3-(1-{3-[5-(1- 甲基 - 哌啶 -4- 基甲氧基)- 嘧啶 -2- 基]- 苄基 }-6- 氧代 -1,6- 二氢 - 哒嗪 -3- 基)- 苄腈盐酸盐水合物表现出显著的抗肿瘤活性，诱发肿瘤消退，T/C 比分别为 -57%、-93% 和 -93% 以及肿瘤生长延迟（TGD，达到 1000 立方毫米肿瘤体积的时间）分别 24、53 和多于 53 天。索拉非尼治疗表现出比 MSC2156119J 低的抗肿瘤活性，具有 27% 的 T/C 值。

[0066] 原位 MHCC97H 肿瘤模型 - 3-(1-{3-[5-(1- 甲基 - 哌啶 -4- 基甲氧基)- 嘧啶 -2- 基]- 苄基 }-6- 氧代 -1,6- 二氢 - 哒嗪 -3- 基)- 苄腈盐酸盐水合物单一疗法：

方法：在雄性 BalB/c 裸鼠（7-8 周龄）中，将 MHCC97H 肿瘤片段（2-3 立方毫米）原位植入肝左叶中。在肝内移植 1 周后，将动物分入治疗组（一组 10 只动物）。各组口服给药 100 毫克 / 公斤的 3-(1-{3-[5-(1- 甲基 - 哌啶 -4- 基甲氧基)- 嘧啶 -2- 基]- 苄基 }-6- 氧代 -1,6- 二氢 - 哒嗪 -3- 基)- 苄腈盐酸盐水合物 5 天，停 2 天，持续 5 周。在治疗结束时，测量肿瘤尺寸和肿瘤重量，分析血浆 AFP 水平和肺转移。

[0067] 结果：用 3-(1-{3-[5-(1- 甲基 - 哌啶 -4- 基甲氧基)- 嘧啶 -2- 基]- 苄基 }-6- 氧代 -1,6- 二氢 - 哒嗪 -3- 基)- 苄腈盐酸盐水合物治疗诱发显著的抗肿瘤活性，造成原发肿瘤消退（$p<0.001$）和减轻肺转移（$p<0.01$）。在治疗结束时分析的小鼠血浆中的 AFP 水平也明显降低（$p<0.001$）。良好耐受 3-(1-{3-[5-(1- 甲基 - 哌啶 -4- 基甲氧基)- 嘧啶 -2- 基]- 苄基 }-6- 氧代 -1,6- 二氢 - 哒嗪 -3- 基)- 苄腈盐酸盐水合物的治疗。

[0068] *在初代 HCC 外植体中评估 3-(1-{3-[5-(1- 甲基 -4- 基甲氧基)- 嘧啶 -2- 基]- 苄基 }-6- 氧代 -1,6- 二氢 -3- 基)- 苄腈盐酸盐水合物：*

概述：为了研究 3-(1-{3-[5-(1- 甲基 - 哌啶 -4- 基甲氧基)- 嘧啶 -2- 基]- 苄基 }-6- 氧代 -1,6- 二氢 - 哒嗪 -3- 基)- 苄腈盐酸盐水合物在 HCC 患者中的治疗潜力，在临床前 II 期型试验（PP2T 试验）中用人初代 HCC 外植体评估 3-(1-{3-[5-(1- 甲基 - 哌啶 -4- 基甲氧基)- 嘧啶 -2- 基]- 苄基 }-6- 氧代 -1,6- 二氢 - 哒嗪 -3- 基)- 苄腈盐酸盐水合物的活性。九个皮下、已建立的初代外植体的治疗导致 1/9 完全响应（CR）、2/9 病情稳定（SD）和在另一模型中的微弱活性。3-(1-{3-[5-(1- 甲基 - 哌啶 -4- 基甲氧基)- 嘧

啶 -2- 基]- 苄基 }-6- 氧代 -1,6- 二氢 - 哒嗪 -3- 基)- 苄腈盐酸盐水合物的活性与这些模型中表达的 c-Met 受体的活化状态(如 c-Met 和 HGF 表达水平所示)正相关。没有或只有低的可检出 c-Met 信号传导(c-Met、phospho c-Met 和 /HGF 水平)迹象的模型无一响应 3-(1-{3-[5-(1- 甲基 - 哌啶 -4- 基甲氧基)- 嘧啶 -2- 基]- 苄基 }-6- 氧代 -1,6- 二氢 - 哒嗪 -3- 基)- 苄腈盐酸盐水合物单一疗法,并且在与索拉非尼联合时没有观察到增强的活性。

[0069] 方法 :雄性 BalB/c 裸鼠(6-8 周龄)皮下植入初代 HCC 肿瘤片段。在肿瘤建立后将动物分入治疗组(一组 12 只动物)。各组一周 5 天口服给药 100 毫克 / 公斤的 3-(1-{3-[5-(1- 甲基 - 哌啶 -4- 基甲氧基)- 嘧啶 -2- 基]- 苄基 }-6- 氧代 -1,6- 二氢 - 哒嗪 -3- 基)- 苄腈盐酸盐水合物。在治疗结束时,计算 T/C 值并观察肿瘤再生。

[0070] 结果 :3-(1-{3-[5-(1- 甲基 - 哌啶 -4- 基甲氧基)- 嘧啶 -2- 基]- 苄基 }-6- 氧代 -1,6- 二氢 - 哒嗪 -3- 基)- 苄腈盐酸盐水合物(100 毫克 / 公斤 / 一周 5 天)显著抑制 9 个模型中的 4 个的生长(LIM612、LIM801、LIM1098、LIM941 ;49% 至 -97% 的 T/C 值)。索拉非尼(50 毫克 / 公斤 / 一周 5 天)在 9 个模型中的 7 个中表现出抗肿瘤活性(LIM348、LIM612、LIM941、LIM752、LIM1098、LIM801、LIM1081,45% 至 -8% 的 T/C 值)。3-(1-{3-[5-(1- 甲基 - 哌啶 -4- 基甲氧基)- 嘧啶 -2- 基]- 苄基 }-6- 氧代 -1,6- 二氢 - 哒嗪 -3- 基)- 苄腈盐酸盐水合物在 LIM801 和 LIM612 (两个具有强 c-Met 信号传导迹象的模型)中表现出比索拉非尼单一疗法更好的抗肿瘤活性。索拉非尼与 3-(1-{3-[5-(1- 甲基 - 哌啶 -4- 基甲氧基)- 嘧啶 -2- 基]- 苄基 }-6- 氧代 -1,6- 二氢 - 哒嗪 -3- 基)- 苄腈盐酸盐水合物的联合在 9 个模型中的 2 个(LIM1098 和 LIM752, T/C 值分别为 -32% 和 4%)中增强最佳单一疗法的抗肿瘤活性。

[0071] *初代外植体中的 c-Met、phospho c-Met和 HGF蛋白质水平的测定 :*

方法 :在人原发肿瘤外植体和异种移植瘤的伴生动物(satellite animal)中用 IHC 研究 c-Met、phospho c-Met 和 HGFα 表达(表 1)。切除异种移植瘤,切成几片,在室温下在 4% 缓冲甲醛溶液中固定 48 小时并包埋在石蜡中。将 3 微米的甲醛固定石蜡包埋(FFPE)组织切片安置在带正电荷的 SuperFrost®Plus 载玻片(Menzel-Gläser, Braunschweig, Germany)上。用染色仪器Discovery™或Discovery® XT(Ventana Medical Systems, Inc., Tucson, USA)进行由切片的去石蜡化开始的免疫组织化学染色程序。在去石蜡化后,切片在 Tris-EDTA 缓冲剂 pH 8 中加热以修复抗原表位。通过在 3% 过氧化氢(OmniMap™ Kit 的一部分, Ventana Medical Systems)中孵育,阻断内源性过氧化物酶。用 PBS 稀释的抗体、然后用二抗(OmniMap Kit 的 HRP 缀合聚合物)在 37℃下孵育切片 16 分钟。辣根过氧化物酶(HRP)催化 3,3'- 二氨基联苯胺四盐酸盐(DAB)/H2O2 反应以产生可见的不可溶深褐色沉淀物。切片用苏木精复染。载玻片在自来水中洗涤,脱水并在永久封固剂(permanent mounting media)Entellan® Neu (VWR, Germany) 中用盖玻片封固。染色仪器生成的详细运行程序储存于 Merck Serono, Darmstadt, Germany。借助 MiraxSCAN (Zeiss) 以分辨率 x/y: 1 像素 = 0.23 x 0.23 μm2 扫描免疫组织化学染色。借助图像分析软件 Visiopharm Integrator System (VIS ;V 4.0.3.0 ;Visiopharm A/S, Denmark) 分析扫描结果。描画活组织区,避开明显坏死区和结缔组织。为了测定所存在的抗原量,以活组织区的面积百分比计算阳性的染成褐色的面积。如下计算抗体染色(任意单位):抗体染色 (AU) = 褐色的阳

性面积 (%) * (255- 强度)/100。

[0072] 结果 :在人肝细胞癌(HCC)的外植体中,可以借助免疫组织化学(IHC)在单一外植体中检测到高 c-Met、phospho-Met 和中等 HGFα 表达。在 9 个 HCC 外植体中,8 个为 c-Met 阳性。2 个外植体(LIM1098 和 LIM612)表现出高 pTyr 1234/1235 Met 和 pTyr 1349 Met 表达。另外 3 个外植体肿瘤表现出低至中等的 pTyr1349-Met 表达。由于在几个外植体异种移植瘤中由 HGF 抗体克隆 B-3 (小鼠 IgG)的检测体系造成的高背景染色,借助图像分析无法分析肿瘤。通过将特异性抗 -HGF 染色与小鼠 IgG 同种型对照染色比较,进行半定量计分(C. Wilm)。

[0073] 分数为 :

0 = 阴性

1 = 低

2 = 中等

3 = 高

9 个 HCC 外植体中的 2 个表现出低至中等的 HGF α 表达。具有低 HGF 表达的 LIM612 为 phospho-Met 高度阳性。具有中等 HGF 表达的 LIM801 为 phospho-Met 阴性。