Cancer stem cell inhibition

Field of the Invention

The present invention relates generally to methods and compositions for inhibiting the growth of, and/or killing, cancer stem cells. Accordingly, also provided are methods and compositions for the treatment or prevention of cancer, in particular breast and colon cancer, wherein the growth of cancer stem cells is inhibited and/or cancer stem cells are killed.

Background of the Invention

The traditional model for carcinogenesis predicts that the processes of self-renewal versus differentiation occurs randomly and thus every cancer cell has an equal probability of retaining self-renewal capacity and the potential to form a new tumour. However, there is increasing evidence that cancers are clonal, and tumours are functionally heterogeneous with only a limited number of cells capable of initiating a tumour. There is increasing *in vitro* and clinical evidence supporting the theory that mutation accumulation of progenitor or adult stem cells triggers the transformation of normal progenitor or adult stem cells into cancer stem cells that subsequently grow and differentiate into primary tumours.

The existence of cancer stem cells was first demonstrated in acute myeloid leukemia, characterised by cell surface marker expression, namely $CD34^{+}CD38^{-}$ (Lapidot *et al.*, 1994; Bonnet and Dick, 1997). Subsequently, cancer stem cells have been identified in a range of haematopoietic malignancies, breast cancers, lung cancers, ovarian cancers, prostate cancers, gastric cancers, colorectal cancers and brain tumours. It is estimated that cancer stem cells constitute <5% of all tumour cells in these malignancies (Gil *et al.*, 2008).

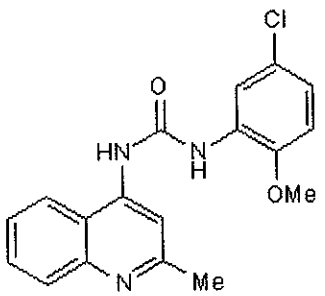
With the capacity of self-renewal, differentiation and proliferation as extensive as their normal counterparts, cancer stem cells play an important role in tumour initiation, growth and spread. First, cancer stem cells arise due to mutations accumulated in normal stem cells, and consequently grow and differentiate into primary tumours. Second, cancer stem cells survive conventional chemotherapy that targets the majority of cells in a primary tumour, leading to regrowth of the tumour causing a relapse. Third, cancer stem cells in the primary tumour may travel to distal sites and cause metastases. Therefore, therapeutic strategies that target cancer stem cells are attracting increasing interest.

Cancer stems cells may exist in a quiescent state for a long period of time (Guzman et al., 2005). Current cytotoxic chemotherapy regimens typically employ drugs that interfere with DNA replication and/or induce apoptosis in rapidly dividing cells. As such these chemotherapy approaches are not able to effectively target the important tumour-initiating population of cancer stem cells. As a result, it is likely that cancer stem cells survive current chemotherapies and thus may contribute to the re-emergence of cancer after chemotherapy. The development of therapies that specifically target cancer stem cells is therefore of particular importance to provide a more effective, sustainable and long-term means of combating cancer. Further, combining therapies that target both normal cancer cells and cancer stem cells may offer a new approach for effective and long-term cancer management.

One compound, salinomycin, has been shown to reduce the proportion of cancer stem cells in a breast cancer cell population by more than 100-fold relative to paclitaxel (Gupta *et al.*, 2009, *Cell* 138:1-15). Now described herein is the inventors' surprising finding that the compound PQ401 (*N*-(5-chloro-2-methoxyphenyl)-*N'*-(2-methyl-4-quinolinyl)urea) is a selective inhibitor of cancer stem cells, and moreover is significantly more potent as a selective cancer stem cell inhibitor than salinomycin against breast and colon cancer stem cells.

Summary of the Invention

According to a first aspect the present invention provides a method for inhibiting the growth and/or proliferation of cancer stem cells, the method comprising contacting cancer stem cells with an effective amount of the compound



or a pharmaceutically acceptable derivative or salt thereof.

The compound or pharmaceutically acceptable derivative or salt thereof typically selectively inhibits the growth and/or proliferation of the cancer stem cells.

The cancer stem cells contacted with the compound may reside in a subject in which case an effective amount of the compound is administered to the subject. The subject may have, or be

suspected of having, cancer. Alternatively, the cancer stem cells may be derived from a subject, wherein a biological sample comprising the cancer stem cells is isolated from the subject prior to be being contacted with the compound.

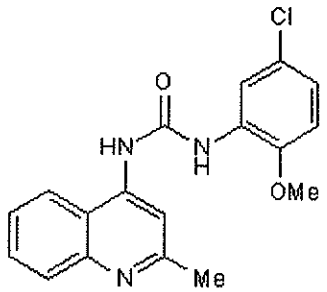
Typically the subject is a mammal. More typically the mammal is a human.

The subject may be diagnosed with cancer, may be at risk of developing cancer or may be susceptible to the development of cancer.

By way of example, the cancer may be one associated with activity or overexpression of insulin growth factor receptor I (IGFRI). For example, the cancer may be breast, prostate, lung or colon cancer.

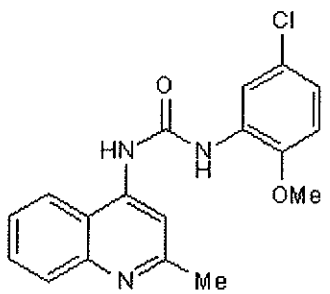
In particular embodiments the cancer is selected from breast cancer and colon cancer.

A further aspect of the invention provides the use of the compound



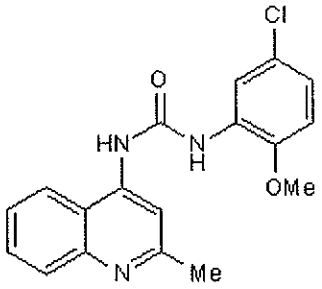
or a pharmaceutically acceptable derivative or salt thereof for the manufacture of a medicament for inhibiting the growth and/or proliferation of cancer stem cells.

A further aspect of the invention provides a composition for use in inhibiting the growth and/or proliferation of cancer stem cells, the composition comprising the compound



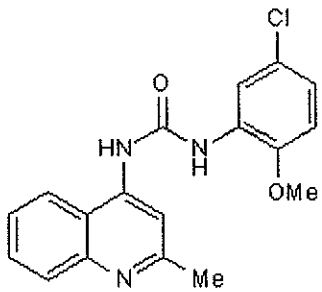
or a pharmaceutically acceptable derivative or salt thereof.

A further aspect of the invention provides a method for selectively inhibiting the growth and/or proliferation of cancer stem cells within a population of cancer cells, the method comprising contacting the cell population with an effective amount of the compound



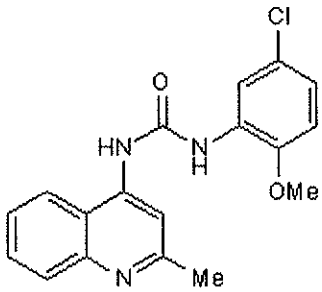
or a pharmaceutically acceptable derivative or salt thereof.

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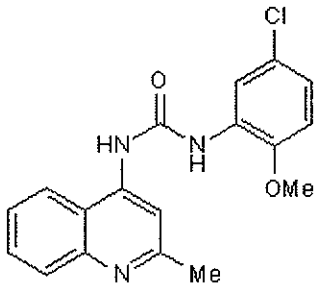
or a pharmaceutically acceptable derivative or salt thereof for the manufacture of a medicament for selectively inhibiting the growth and/or proliferation of cancer stem cells within a population of cancer cells.

A further aspect of the invention provides a composition for use in selectively inhibiting the growth and/or proliferation of cancer stem cells within a population of cancer cells, the composition comprising the compound

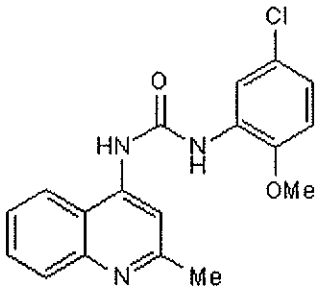


or a pharmaceutically acceptable derivative or salt thereof.

A further aspect of the invention provides a method for treating a subject having, or suspected of having, cancer comprising administering to the subject an effective amount of a compound that selectively inhibits growth and/or proliferation of cancer stem cells, or a pharmaceutically acceptable derivative or salt thereof, the compound having the formula

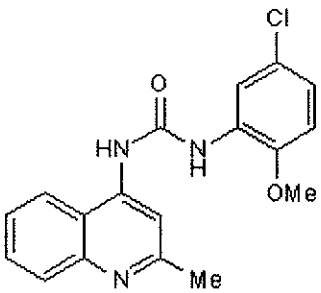


A further aspect of the invention provides the use of a compound having the formula



or a pharmaceutically acceptable derivative or salt thereof, for the manufacture of a medicament for treating subjects having, or suspected of having, cancer, wherein the compound selectively inhibits the growth and/or proliferation of cancer stem cells.

A further aspect of the invention provides a composition for use in the treatment of cancer, the composition comprising a compound that selectively inhibits the growth and/or proliferation of cancer stem cells, or a pharmaceutically acceptable derivative or salt thereof, the compound having the formula



In accordance with the above aspects and embodiments the derivative may be a conjugate agent in which the compound disclosed herein or a portion thereof is conjugated to at least one other compound. The at least one other compound may inhibit the growth and/or proliferation of cancer stem cells or non-cancer cells within a tumour or other population of cancer cells.

Thus, the present invention also contemplates methods for treating cancer in a subject and/or inhibiting the growth and/or proliferation of cancer stem cells in a subject, the method comprising administering to the subject an effective amount of a conjugate agent, wherein the conjugate compound comprises a compound of formula (I) as defined herein or a portion thereof, or a compound of formula (II) as defined herein or a portion thereof.

Also contemplated herein are combination therapies in which compounds and compositions described above are administered with additional anti-cancer agents. Such additional agents may be administered by the same or different route as the compounds and compositions described herein. The administration may be sequential or concomitant.

Brief Description of the Drawings

The present invention is further described herein, by way of non-limiting example only, with reference to the accompanying drawings.

Figure 1. PQ401 is a selective inhibitor of cancer stem cells (CSCs). PQ401 (25μg/ml) has significantly greater cytotoxic activity against cancer stem cells (breast CSC and breast CSC(parental)) than non-cancer stem cells (HepG2 and HeLa). Breast CSC(parental) is a mixed population of cancer stem cells and non-cancer stem cells.

Detailed Description of the Invention

The reference in this specification to any prior publication (or information derived from it), or to any matter which is known, is not, and should not be taken as an acknowledgment or admission or any form of suggestion that that prior publication (or information derived from it) or known matter forms part of the common general knowledge in the field of endeavour to which this specification relates.

It will be appreciated by persons skilled in the art that numerous variations and/or modifications may be made to the invention without departing from the spirit or scope of the invention as broadly described. The present embodiments are, therefore, to be considered in all respects as illustrative and not restrictive.

Throughout this specification and the claims which follow, unless the context requires otherwise, the word "comprise", and variations such as "comprises" or "comprising", will be understood to imply the inclusion of a stated integer or step or group of integers or steps but not the exclusion of any other integer or step or group of integers or steps.

The articles "a" and "an" are used herein to refer to one or to more than one (*i.e.*, to at least one) of the grammatical object of the article. By way of example, "an element" means one element or more than one element.

In the context of this specification, the term "about" is understood to refer to a range of numbers that a person of skill in the art would consider equivalent to the recited value in the context of achieving the same function or result.

The term "growth" as used herein in the context of the ability of compounds, agents and compositions to inhibit the growth of cancer stem cells refers to any and all aspects of the growth of such cells including the division, proliferation and differentiation of cells. Inhibition of the growth of cancer stem cells may occur as a result of cytotoxic activity of the compound, agent or composition. Hence inhibition of cancer stem cell growth encompasses killing cancer stem cells.

The term "inhibiting" and variations thereof such as "inhibition" and "inhibits" as used herein do not necessarily imply the complete inhibition of the "growth" of cancer stem cells. Rather, the inhibition may be to an extent, and/or for a time, sufficient to produce the desired effect. Inhibition may be prevention, retardation, reduction or otherwise hindrance of the cancer stem cell growth. Such inhibition may be in magnitude and/or be temporal in nature. Further, such inhibition by a compound as described herein may be direct or indirect. By indirect inhibition is meant that the extract or agent may effect the expression or activity of molecules which in turn regulate the growth of the cancer stem cells. Inhibition of cell growth can be assessed using methods well known in the art. Inhibition may be by at least or about 10%, 15%, 20%, 25%, 30%, 35%, 40%, 45%, 50%, 55%, 60%, 65%, 70%, 75%, 80%, 85%, 90%, 95% or more compared to cell growth in the absence of the agent.

As used herein the terms "treating", "treatment", "preventing" and "prevention" refer to any and all uses which remedy a condition or symptoms, prevent the establishment of a condition or disease, or otherwise prevent, hinder, retard, or reverse the progression of a condition or disease or other undesirable symptoms in any way whatsoever. Thus the terms "treating" and "preventing" and the like are to be considered in their broadest context. For example, treatment does not necessarily imply that a patient is treated until total recovery.

As used herein the term "effective amount" includes a non-toxic but sufficient amount or dose of an agent or compound to provide the desired effect. The exact amount or dose required will vary from subject to subject depending on factors such as the species being treated, the age and general condition of the subject, the severity of the condition being treated, the particular agent being administered and the mode of administration and so forth. Thus, it is not possible to specify an exact

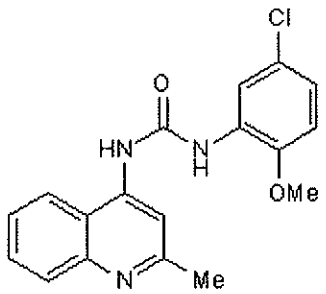
"effective amount". However, for any given case, an appropriate "effective amount" may be determined by one of ordinary skill in the art using only routine experimentation.

The term "pharmaceutically acceptable salt" refers to an organic or inorganic moiety that carries a charge and that can be administered in association with a pharmaceutical agent, for example, as a counter-cation or counter-anion in a salt. Pharmaceutically acceptable cations are known to those of skilled in the art, and include but are not limited to sodium, potassium, calcium, zinc and quaternary amine. Pharmaceutically acceptable anions are known to those of skill in the art, and include but are not limited to chloride, acetate, citrate, bicarbonate and carbonate.

The term "pharmaceutically acceptable derivative" includes within its scope a derivative of the active compound that upon administration to the recipient, is capable of providing directly or indirectly, the parent compound or metabolite, or that exhibits activity itself. Prodrugs are included within the scope of the present invention as "pharmaceutically acceptable derivatives".

As used herein, a "subject" includes human and non-human animals, including, for example livestock animals, companion animals and research or experimental model organisms. By way of example, such non-human animals include non-human primates, monkeys, mice, cows, sheep, goats, dogs, cats, horses, birds and pigs.

The diaryl urea compound PQ401 (*N*-(5-chloro-2-methoxyphenyl)-*N'*-(2-methyl-4-quinolinyl)urea) of formula



is an inhibitor of insulin growth factor receptor I (IGFRI). Now described and exemplified herein is the ability of PQ401 to selectively inhibit cancer stem cells more potently than current cancer stem cell inhibitors such as salinomycin.

Accordingly, provided herein is a method for inhibiting the growth and/or proliferation of cancer stem cells, the method comprising contacting cancer stem cells with an effective amount of PQ401 or a pharmaceutically acceptable derivative or salt thereof.

Also provided is a method for selectively inhibiting the growth and/or proliferation of cancer stem cells within a population of cancer cells, the method comprising contacting the cell population with an effective amount of PQ401 or a pharmaceutically acceptable derivative or salt thereof.

Also provided is a method for treating a subject having, or suspected of having, cancer comprising administering to the subject an effective amount of PQ401, or a pharmaceutically acceptable derivative or salt thereof.

Embodiments of the present invention provide methods for inhibiting the growth and/or proliferation of cancer stem cells, wherein the cancer stem cells are contacted in vitro or in vivo with an effective amount of PQ401 or a composition comprising PQ401, or a pharmaceutically acceptable derivative or salt thereof. Embodiments of the invention also provide uses of such compounds for the manufacture of medicaments for inhibiting the growth and/or proliferation of cancer stem cells.

Typically the compound or pharmaceutically acceptable salt or derivative to be employed for the inhibition of cancer stem cell growth and/or proliferation displays low or negligible cytotoxicity against non-cancerous cells, and typically also against cancerous cells that are not cancer stem cells.

Also provided herein are conjugate compounds including PQ401 or a pharmaceutically acceptable salt or derivative thereof, conjugated to another compound or molecule and to uses of such conjugate compounds in methods as described herein. Suitable compounds and molecule to which PQ401 or derivative may be conjugated include, but are not limited to, compounds and molecules that may enhance the efficacy or potency of PQ401, assist in targeting or delivering the compound to the cells to be inhibited, or assist in uptake of the compound by the target cells. Alternatively the additional compound or molecule may itself have activity in inhibiting the growth and/or proliferation of cancer stem cells or other cancerous cells. Conjugate compounds for use in accordance with embodiments of the invention will typically display low or negligible cytotoxicity against non-cancerous cells.

The cancer stem cells to be contacted in accordance with the embodiments of the present invention may be capable of giving rise to any cancer or tumour type. Such cancers and tumours include, but are not limited to, breast cancer, colon cancer, bladder cancer, ovarian cancer, prostate cancer, testicular cancer, thyroid cancer, lung cancer, gastrointestinal cancer, liver cancer, head and neck cancer, leukemia, melanoma, a sarcoma, a glioma, a neuroblastoma, an astrocytoma, a mesenchymal tumour, a retinoblastoma, a nasopharyngeal carcinoma and an esophageal carcinoma. Those skilled in the art will appreciate that any cancer or tumour associated with or arising from the growth, proliferation or differentiation of cancer stem cells is encompassed by the embodiments described herein. In particular embodiments the cancer stem cells are stem cells giving rise to or associated with cancers associated with activity or overexpression of insulin growth factor receptor I (IGFRI), such as, for example, breast cancer, colon cancer, prostate cancer and lung cancer.

Optionally, the compounds to be contacted with cancer stem cells in accordance with embodiments of the present invention may be administered to subjects in need of treatment in the form of pharmaceutical compositions, which compositions may comprise one or more pharmaceutically acceptable diluents, adjuvants and/or excipients. It will also be readily appreciated by those skilled in the art that the present invention contemplates the administration of one or more compounds as described and exemplified herein in conjunction with at least one additional therapeutic compound or agent.

The compounds contemplated by the present invention include all salts, such as acid addition salts, anionic salts and zwitterionic salts, and in particular include pharmaceutically acceptable salts as would be known to those skilled in the art. Pharmaceutically acceptable salts include those formed from: acetic, ascorbic, aspartic, benzoic, benzenesulphonic, citric, cinnamic, ethanesulphonic, fumaric, glutamic, glutaric, gluconic, hydrochloric, hydrobromic, lactic, maleic, malic, methanesulphonic, naphthoic, hydroxynaphthoic, naphthalenesulphonic, naphthalenedisulphonic, naphthaleneacrylic, oleic, oxalic, oxaloacetic, phosphoric, pyruvic, *p*-toluenesulphonic, tartaric, trifluoroacetic, triphenylacetic, tricarballylic, salicylic, sulphuric, sulphamic, sulphanilic and succinic acid.

Pharmaceutically acceptable derivatives include solvates, pharmaceutically active esters, prodrugs or the like. This also includes derivatives with physiologically cleavable leaving groups that can be cleaved *in vivo* to provide the active compound or active moiety. The leaving groups may include acyl, phosphate, sulfate, sulfonate, and typically are mono-, di- and per-acyl oxy-substituted compounds, where one or more of the pendant hydroxy groups are protected by an acyl group, preferably an acetyl group. Typically acyloxy substituted compounds of the invention are readily cleavable to the corresponding hydroxy substituted compounds.

According to the methods of present invention compounds and compositions comprising the compounds may be administered by any suitable route, either systemically, regionally or locally. The particular route of administration to be used in any given circumstance will depend on a number of factors, including the nature of the condition to be treated or prevented, the required dosage of the particular compound to be delivered and the potential side-effects of the compound. For example, in circumstances where it is required that appropriate concentrations of the desired compound are delivered directly to the site in the body to be treated, administration may be regional rather than systemic. Regional administration provides the capability of delivering very high local concentrations of the desired compound to the required site and thus is suitable for achieving the desired

therapeutic or preventative effect whilst avoiding exposure of other organs of the body to the compound and thereby potentially reducing side effects.

By way of example, administration according to embodiments of the invention may be achieved by any standard routes, including intracavitary, intravesical, intramuscular, intraarterial, intravenous, intraocular, subcutaneous, topical or oral. In particular embodiments, administration is oral.

In employing methods of the invention, compounds may be formulated in pharmaceutical compositions. Suitable compositions may be prepared according to methods that are known to those of ordinary skill in the art and may include a pharmaceutically acceptable diluent, adjuvant and/or excipient. The diluents, adjuvants and excipients must be "acceptable" in terms of being compatible with the other ingredients of the composition, and not deleterious to the recipient thereof. The diluent, adjuvant or excipient may be a solid or a liquid, or both, and may be formulated with the compound as a unit-dose, for example, a tablet, which may contain from 0.5% to 59% by weight of the active compound, or up to 100% by weight of the active compound. One or more active compounds may be incorporated in the formulations of the invention, which may be prepared by any of the well known techniques of pharmacy consisting essentially of admixing the components, optionally including one or more accessory ingredients.

Examples of pharmaceutically acceptable diluents are demineralised or distilled water; saline solution; vegetable based oils such as peanut oil, safflower oil, olive oil, cottonseed oil, maize oil, sesame oils such as peanut oil, safflower oil, olive oil, cottonseed oil, maize oil, sesame oil, arachis oil or coconut oil; silicone oils, including polysiloxanes, such as methyl polysiloxane, phenyl polysiloxane and methylphenyl polysolpoxane; volatile silicones; mineral oils such as liquid paraffin, soft paraffin or squalane; cellulose derivatives such as methyl cellulose, ethyl cellulose, carboxymethylcellulose, sodium carboxymethylcellulose or hydroxypropylmethylcellulose; lower alkanols, for example ethanol or iso-propanol; lower aralkanols; lower polyalkylene glycols or lower alkylene glycols, for example polyethylene glycol, polypropylene glycol, ethylene glycol, propylene glycol, 1,3-butylene glycol or glycerin; fatty acid esters such as isopropyl palmitate, isopropyl myristate or ethyl oleate; polyvinylpyrridone; agar; carrageenan; gum tragacanth or gum acacia, and petroleum jelly. Typically, the carrier or carriers will form from 1% to 99.9% by weight of the compositions.

Formulations suitable for oral administration may be presented in discrete units, such as capsules, sachets, lozenges, or tablets, each containing a predetermined amount of the active compound; as a powder or granules; as a solution or a suspension in an aqueous or non-aqueous liquid; or as an oil-in-water or water-in-oil emulsion. Such formulations may be prepared by any suitable method of

pharmacy which includes the step of bringing into association the active compound and a suitable carrier (which may contain one or more accessory ingredients as noted above). In general, the formulations of the invention are prepared by uniformly and intimately admixing the active compound with a liquid or finely divided solid carrier, or both, and then, if necessary, shaping the resulting mixture such as to form a unit dosage. For example, a tablet may be prepared by compressing or moulding a powder or granules containing the active compound, optionally with one or more accessory ingredients. Compressed tablets may be prepared by compressing, in a suitable machine, the compound of the free-flowing, such as a powder or granules optionally mixed with a binder, lubricant, inert diluent, and/or surface active/dispersing agent(s). Moulded tablets may be made by moulding, in a suitable machine, the powdered compound moistened with an inert liquid binder.

Solid forms for oral administration may contain binders acceptable in human and veterinary pharmaceutical practice, sweeteners, disintegrating agents, diluents, flavourings, coating agents, preservatives, lubricants and/or time delay agents. Suitable binders include gum acacia, gelatine, corn starch, gum tragacanth, sodium alginate, carboxymethylcellulose or polyethylene glycol. Suitable sweeteners include sucrose, lactose, glucose, aspartame or saccharine. Suitable disintegrating agents include corn starch, methylcellulose, polyvinylpyrrolidone, guar gum, xanthan gum, bentonite, alginic acid or agar. Suitable diluents include lactose, sorbitol, mannitol, dextrose, kaolin, cellulose, calcium carbonate, calcium silicate or dicalcium phosphate. Suitable flavouring agents include peppermint oil, oil of wintergreen, cherry, orange or raspberry flavouring. Suitable coating agents include polymers or copolymers of acrylic acid and/or methacrylic acid and/or their esters, waxes, fatty alcohols, zein, shellac or gluten. Suitable preservatives include sodium benzoate, vitamin E, alpha-tocopherol, ascorbic acid, methyl paraben, propyl paraben or sodium bisulphite. Suitable lubricants include magnesium stearate, stearic acid, sodium oleate, sodium chloride or talc. Suitable time delay agents include glyceryl monostearate or glyceryl distearate.

Liquid forms for oral administration may contain, in addition to the above agents, a liquid carrier. Suitable liquid carriers include water, oils such as olive oil, peanut oil, sesame oil, sunflower oil, safflower oil, arachis oil, coconut oil, liquid paraffin, ethylene glycol, propylene glycol, polyethylene glycol, ethanol, propanol, isopropanol, glycerol, fatty alcohols, triglycerides or mixtures thereof.

Formulations suitable for buccal (sublingual) administration include lozenges comprising the active compound in a flavoured base, usually sucrose and acacia or tragacanth; and pastilles comprising the compound in an inert base such as gelatin and glycerin or sucrose and acacia.

Compositions of the present invention suitable for parenteral administration typically conveniently comprise sterile aqueous preparations of the active compounds, which preparations may be isotonic with the blood of the intended recipient. These preparations are typically administered intravenously, although administration may also be effected by means of subcutaneous, intramuscular, or intradermal injection. Such preparations may conveniently be prepared by admixing the compound with water or a glycine buffer and rendering the resulting solution sterile and isotonic with the blood. Injectable formulations according to the invention generally contain from 0.1% to 60% w/v of active compound(s) and are administered at a rate of 0.1 ml/minute/kg or as appropriate.

Formulations for infusion, for example, may be prepared employing saline as the carrier and a solubilising agent such as a cyclodextrin or derivative thereof. Suitable cyclodextrins include α-cyclodextrin, β-cyclodextrin, γ-cyclodextrin, dimethyl-β-cyclodextrin, 2-hydroxyethyl-β-cyclodextrin, 2-hydroxypropyl-cyclodextrin, 3-hydroxypropyl-β-cyclodextrin and tri-methyl-β-cyclodextrin. More preferably the cyclodextrin is hydroxypropyl-β-cyclodextrin. Suitable derivatives of cyclodextrins include Captisol® a sulfobutyl ether derivative of cyclodextrin and analogues thereof as described in US 5,134,127.

Formulations or compositions suitable for topical administration to the skin may take the form of an ointment, cream, lotion, paste, gel, spray, aerosol, or oil. Carriers that may be used include vaseline, lanoline, polyethylene glycols, alcohols, and combination of two or more thereof. The active compound is generally present at a concentration of from 0.1% to 0.5% w/w, for example, from 0.5% to 2% w/w. Examples of such compositions include cosmetic skin creams.

Formulations suitable for inhalation may be delivered as a spray composition in the form of a solution, suspension or emulsion. The inhalation spray composition may further comprise a pharmaceutically acceptable propellant such as carbon dioxide or nitrous oxide or a hydrogen containing fluorocarbon such as 1,1,1,2-tetrafluoroethane, 1,1,1,2,3,3,3-heptafluoro-n-propane or mixtures thereof.

Formulations suitable for transdermal administration may be presented as discrete patches adapted to remain in intimate contact with the epidermis of the recipient for a prolonged period of time. Such patches suitably contain the active compound as an optionally buffered aqueous solution of, for example, 0.1 M to 0.2 M concentration with respect to the said active compound. Formulations suitable for transdermal administration may also be delivered by iontophoresis (see, for example, *Pharmaceutical Research* 3 (6), 318 (1986)) and typically take the form of an optionally buffered aqueous solution of the active compound. For example, suitable formulations may comprise citrate or bis/tris buffer (pH 6) or ethanol/water and contain from 0.1 M to 0.2 M active ingredient.

The active compounds may be provided in the form of food stuffs, such as being added to, admixed into, coated, combined or otherwise added to a food stuff. The term food stuff is used in its widest possible sense and includes liquid formulations such as drinks including dairy products and other foods, such as health bars, desserts, etc. Food formulations containing compounds of the invention can be readily prepared according to standard practices.

The effective dose level of the administered compound for any particular subject will depend upon a variety of factors including: the type of condition being treated and the stage of the condition; the activity of the compound employed; the composition employed; the age, body weight, general health, sex and diet of the patient; the time of administration; the route of administration; the rate of sequestration of compounds; the duration of the treatment; drugs used in combination or coincidental with the treatment, together with other related factors well known in medicine.

One skilled in the art would be able, by routine experimentation, to determine an effective, non-toxic dosage that would be required to treat applicable conditions. These will most often be determined on a case-by-case basis. By way of example only, an effective dosage may be expected to be in the range of about 0.0001mg to about 1000mg per kg body weight per 24 hours; typically, about 0.001mg to about 750mg per kg body weight per 24 hours; about 0.01mg to about 500mg per kg body weight per 24 hours; about 0.1mg to about 500mg per kg body weight per 24 hours; about 0.1mg to about 250mg per kg body weight per 24 hours; or about 1.0mg to about 250mg per kg body weight per 24 hours. More typically, an effective dose range is expected to be in the range of about 10mg to about 200mg per kg body weight per 24 hours.

Further, it will be apparent to those of ordinary skill in the art that the optimal quantity and spacing of individual dosages will principally be determined by the nature and extent of the condition being treated, the form, route and site of administration, and the individual being treated. Suitable conditions can be determined by conventional techniques.

It will also be apparent to those of ordinary skill in the art that the optimal course of treatment, such as, the number of doses of the composition given per day for a defined number of days, can be ascertained by those skilled in the art using conventional course of treatment determination tests.

In accordance with the methods of the invention, compounds or pharmaceutically acceptable derivatives or salts thereof can be co-administered with other active agents that do not impair the desired action, or with agents that supplement the desired action, such as chemotherapeutic agents. The particular agent(s) used will depend on a number of factors and will typically be tailored to the disease or disorder to be treated. The co-administration of agents may be simultaneous or sequential. Simultaneous administration may be effected by the compounds being formulated in a

single composition, or in separate compositions administered at the same or similar time. Sequential administration may be in any order as required.

The present invention will now be described with reference to the following specific examples, which should not be construed as in any way limiting the scope of the invention.

Examples

Example 1 – Comparative anti-cancer stem cell activity of PQ401

The inventors tested the cytotoxic activity of the diaryl urea compound PQ401 against breast and colon cancer stem cells (Celprogen) using a resazurin cell viability assay, and compared this activity to that of salinomycin, and paclitaxel (Sigma-Aldrich). Cells were plated in 96 well multi-well plates five days before the assay such to 20,000 cells per well. The test compound of interest was added to each well at a concentration of 12.5 μg/ml in total media of 200 ml with 1% v/v DMSO. Cells were incubated overnight. Media was then removed from the wells and 100 ml PBS added to each well. 20 ml resazurin was added to each well to a concentration of 8 μg/ml and the plates incubated at 37°C for 2.0 hrs before fluorescence measurement using a Tecan plate reader (emission wavelength: 590nm; excitation wavelength: 535nm; gain: 25).

The results are shown in Table 1 as IC_{50} values, demonstrating that PQ401 displays significantly greater cytotoxic activity against both breast and colon cancer stem cells than salinomycin and paclitaxel.

Table 1. IC_{50} values (μg/ml) for tested compounds against breast and colon cancer stem cells (CSCs)

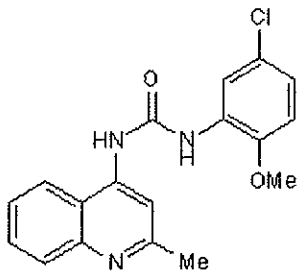
	PQ401	Salinomycin	Paclitaxel
Celprogen breast CSC (#36102-29), P20	61	92	255
Celprogen colon CSC (#36112-39), P20	63	273	>1000

Example 2 – PQ401 is a selective inhibitor of cancer stem cells

To assess the selectivity of the cytotoxic activity of PQ401 for cancer stem cells, the resazurin cell viability assay as described above was employed using breast cancer stem cells (see Table 1), a mixed population of breast cancer stem cells and non-stem cells (breast CSC(parental)), the non-stem cell liver cancer cell line HepG2 (ATCC CRL-10741) and the non-stem cell ovarian cancer cell line HeLa (ATCC CCL-2). The results shown in Figure 1 demonstrate that PQ401 displays greater cytotoxic activity against cancer stem cell lines than non-stem cell cancer cell lines.

Claims

1. A composition for use in inhibiting the growth and/or proliferation of cancer stem cells, the composition comprising the compound



or a pharmaceutically acceptable derivative or salt thereof.

2. A composition according to claim 1 wherein the compound or pharmaceutically acceptable derivative or salt thereof selectively inhibits the growth and/or proliferation of the cancer stem cells.

3. A composition according to any one of claims 1 to 2 wherein the cancer stem cells are derived from or give rise to a cancer associated with activity or overexpression of IGFRI.

4. A composition according to any one of claims 1 to 3 wherein the cancer stem cells are breast or colon cancer stem cells.

5. A composition according to any one of claims 1 to 4 wherein the compound is administered to a subject.

6. A composition according to claim 5 wherein the subject is diagnosed with cancer, suspected of having cancer, is at risk of developing cancer or is susceptible to the development of cancer.

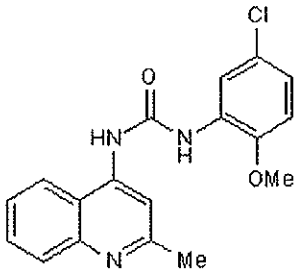
7. A composition according to claim 6 wherein the cancer is associated with activity of overexpression of IGFRI.

8. A composition according to any one of claim 6 or 7 wherein the cancer is selected from breast cancer, colon cancer, prostate cancer and lung cancer.

9. A composition according to claim 8 wherein the cancer is selected from breast cancer and colon cancer.

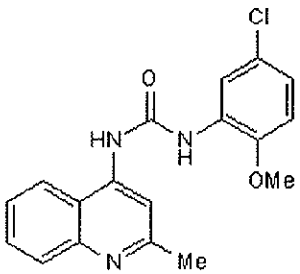
10. A composition according to any one of claims 1 to 4 wherein the cancer stem cells are derived from a subject, wherein a biological sample comprising the cancer stem cells is isolated from the subject prior to be being contacted with the compound.

11. Use of the compound



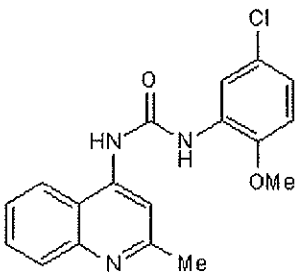
or a pharmaceutically acceptable derivative or salt thereof for the manufacture of a medicament for inhibiting the growth and/or proliferation of cancer stem cells.

12. Use of the compound



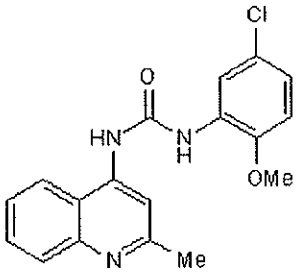
or a pharmaceutically acceptable derivative or salt thereof for the manufacture of a medicament for selectively inhibiting the growth and/or proliferation of cancer stem cells within a population of cancer cells.

13. A composition for use in selectively inhibiting the growth and/or proliferation of cancer stem cells within a population of cancer cells, the composition comprising the compound



or a pharmaceutically acceptable derivative or salt thereof

14. A method for inhibiting the growth and/or proliferation of cancer stem cells, the method comprising contacting cancer stem cells with an effective amount of the compound



or a pharmaceutically acceptable derivative or salt thereof.

15. A method according to claim 14 wherein the compound or pharmaceutically acceptable derivative or salt thereof selectively inhibits the growth and/or proliferation of the cancer stem cells.

16. A method according to any one of claims 14 to 15 wherein the cancer stem cells are derived from or give rise to a cancer associated with activity or overexpression of IGFRI.

17. A method according to any one of claims 14 to 16 wherein the cancer stem cells are breast or colon cancer stem cells.

18. A method according to any one of claims 14 to 17 wherein the compound is administered to a subject.

19. A method according to claim 18 wherein the subject is diagnosed with cancer, suspected of having cancer, is at risk of developing cancer or is susceptible to the development of cancer.

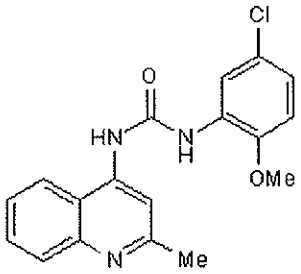
20. A method according to claim 19 wherein the cancer is associated with activity of overexpression of IGFRI.

21. A method according to claim 19 or 20 wherein the cancer is selected from breast cancer, colon cancer, prostate cancer and lung cancer.

22. A method according to claim 21 wherein the cancer is selected from breast cancer and colon cancer.

23. A method according to any one of claims 14 to 17 wherein the cancer stem cells are derived from a subject, wherein a biological sample comprising the cancer stem cells is isolated from the subject prior to be being contacted with the compound.

24. A method for selectively inhibiting the growth and/or proliferation of cancer stem cells within a population of cancer cells, the method comprising contacting the cell population with an effective amount of the compound



or a pharmaceutically acceptable derivative or salt thereof.