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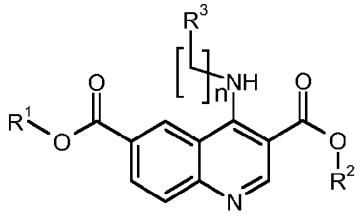
(54) **Title:** QUINOLINE DERIVATIVES ACTING AS TYROSINE KINASE INHIBITORS



(I)

(57) **Abstract:** A compound of formula (I) as well as pharmaceutically acceptable salts or prodrugs thereof, for use as a me- dicament. A pharmaceutical composition comprising a therapeutically effective amount of a compound of formula (I). Use of a compound of formula (I) for the manufacturing of a medicament for the treatment of a disorder selected from can- cer, diabetic retinopathy, age-related macular degeneration, chronic inflammation, stroke, ischemic myocardium, atherosclerosis, tumor growth, and macular edema.

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QUINOLINE DERIVATIVES ACTING AS TYROSINE KINASE INHIBITORS.

Field of the invention

The present invention relates to quinoline derivatives for use in therapy. More particularly, the present invention relates to quinoline derivatives for the treatment of cancer. Even more particularly, the present invention relates to compounds acting as tyrosine kinase inhibitors for the treatment and prevention of cell proliferative disorders or cell differentiation disorders, disorders that are associated with abnormal tyrosine kinase activities.

Background of the invention

Angiogenesis, the outgrowth of new capillaries from pre-existing vessels, is essential for embryonic development, organ formation, tissue regeneration, and remodeling (1). It also contributes to the development and progression of a variety of pathological conditions, including tumor growth and metastasis, cardiovascular diseases, diabetic retinopathy, rheumatoid arthritis, and psoriasis (2). Angiogenesis and vasculogenesis are complex multistep processes that include proliferation, migration and differentiation of endothelial cells, degradation of the extracellular matrix, tube formation, and sprouting of new capillary branches (3, 11). The complexity of the angiogenic processes suggests the existence of multiple controls of the system, which can be transiently switched on and off. A switch of the angiogenic phenotype in tissues is thought to depend on a local change of the balance between angiogenic stimulators and inhibitors (4).

Among many described angiogenic factors, vascular endothelial growth factor (VEGF)/ vascular permeability factor is one of the best-characterized positive regulator with its distinct specificity for vascular endothelial cells (5–7). The biological actions of VEGF include stimulation of endothelial cell proliferation, migration, differentiation, tube formation, increase of vascular permeability, and maintenance of vascular integrity (8–11, 28). The angiogenic responses induced by VEGF are mediated by two structurally related tyrosine kinase receptors, VEGFR-1 (Flt-1) and VEGFR-2 (KDR or Flk-1), both of which are expressed primarily on vascular cells of the endothelial lineage (8, 12, 13).

VEGFR-1 and VEGFR-2 belong to a class of enzymes that catalyze the transfer of the terminal phosphate of adenosine triphosphate to tyrosine residues in protein

substrates and therefore are called tyrosine kinases. Tyrosine kinases are believed, by the way of substrate phosphorylation, to play critical roles in signal transduction for a number of cell functions. Though the exact mechanism of signal transduction is still unclear, tyrosine kinases have been shown to be important contributing factors in cell proliferation, carcinogenesis and cell differentiation. Tyrosine kinases can be categorized as either the receptor type tyrosine kinases, that have an extracellular, a transmembrane, and an intracellular portion, or the non-receptor type tyrosine kinases that are wholly intracellular.

The receptor-type tyrosine kinases are comprised of a large number of transmembrane receptors with diverse biological activity, such as EGFR, HER2, HER3, HER4, TGF-α, amphiregulin, HB-EGF, betacellulin and heregulin, INS-R, IGF-IR, and IR-R, PDGF-α and β receptors, CSFIR, c-kit and FLK-II, VEGFR-1 (FLT-1), VEGFR-2 (KDR) and VEGFR-3 (FLT-3) (32). The non-receptor type of tyrosine kinases is also comprised of numerous subfamilies, including Src, Frk, Btk, Csk, Abl, Zap70, Fes/Fps, Fak, Jak, Ack, and LIMK. Each of these subfamilies is further sub-divided into varying groups (33).

As mentioned above VEGF binds to both the VEGFR-1 and VEGFR-2 tyrosine kinases. However, the biological responses appear to be quite different (14). For example, activation of VEGFR-2 leads to proliferation and migration of endothelial cells, whereas VEGFR-1 is unable to transduce such signals when stimulated with VEGF (14). VEGF is the prototype of an enlarging family of growth peptides that includes four other structurally related members. These recently identified VEGF-related molecules are placenta growth factor (PlGF) (15), VEGF-B/VEGF-related factor (16, 17), VEGF-C (18, 19), and c-*fos*-induced growth factor (FIGF/VEGF-D) (20, 29, 30). They show a striking similarity in their primary sequences, especially in the platelet-derived growth factor-like domain containing eight conserved cysteine residues. PlGF is predominantly expressed in the placenta and binds to VEGFR-1, but not to VEGFR-2 (21, 22). VEGF-B has been identified as a weak mitogen for endothelial cells and a robust expression is particularly detected in skeletal and cardiac muscle tissues (16). Both PlGF and VEGF-B modulate VEGF activity via formation of heterodimers (16, 23). VEGF-C is a ligand for two receptors, VEGFR-2 and VEGFR-3 (Flt-4) (18, 24). The latter differs from VEGFR-1 and VEGFR-2 by being predominantly expressed in lymphatic endothelial cells in adult tissues, but at low levels in most other vascular endothelial cells (25, 31). VEGF-C recently has been

characterized as being a fairly selective growth factor for lymphatic vessels (26, 27). In addition, proteolytic processing is involved in the regulation of VEGF-C activity (24). FIGF/VEGF-D, which is drastically induced by c-*fos* activation (20), also binds to VEGFR-2 and VEGFR-3 (30). Although these factors are believed to stimulate endothelial cell growth *in vitro*, their *in vivo* angiogenic effects have not yet been fully characterized.

Tyrosine kinases are without doubt interesting targets for finding methods for preventing or curing diseases that are developed due to overexpression of growth factors. Solid tumors can be treated by tyrosine kinase inhibitors since these tumors depend on angiogenesis for the formation of the blood vessels necessary to support their growth and, indeed, there are a number of tyrosine kinase inhibitors already in the clinic.

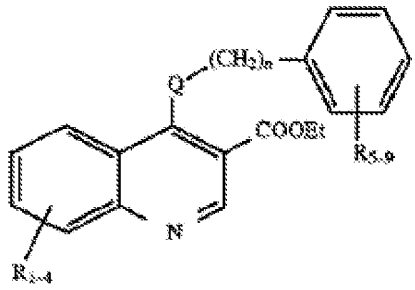
An important aspect of tyrosine kinase inhibitors is selectivity since, as mentioned herein above, many of the growth factors as well as the tyrosine kinases are very closely structurally related and also show many different biological responses. Many of the known kinase inhibitors of today are multitargeting and show different kinase inhibiting profiles and thus different biological responses. Thus, a "good kinase profile" will be important when developing a new drug with improved selectivity.

Recent research has established that blocking VEGFR-1 induces some unwanted side-effects such as bone marrow toxicity (37) and liver complications (38), which means that a selectivity between VEGFR-2 and VEGFR-1 is important and would be an advantageous feature of a medicament aimed at the treatment of disorders involving essentially the VEGFR-2 compared to the VEGFR-1. Accordingly, one object of the invention is to provide a compound which specifically inhibits, regulates and/or modulates the signal transduction of tyrosine kinases, in particular VEGFR-2, for use as a medicament.

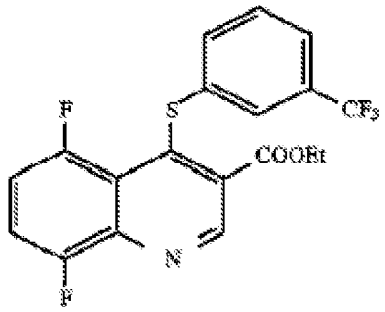
Quinoline derivatives have been described earlier in a biological context and also in a number of therapeutic areas. As an example, Bi et al (34) have described quinolines as extremely potent and selective PDE5 inhibitors, being potential agents for treatment of erectile dysfunction, Allais et al [44] describe 2-methyl/phenyl quinolines having anti-inflammatory activity. Hanifin et al [45] describe 4-anilino-3-quinolinecarboxylic acids and esters as diuretic and anti-depressant agents.

A number of quinoline derivatives also are known as tyrosine kinase inhibitors. As an example, Boscelli et al (35) have described quinoline-3-carbonitriles as Src Kinase inhibitors. Wissner et al (36) have described quinoline-3-carbonitrile as an inhibitor of epidermal growth factor receptor kinase. Quinoline-3-carbonitrile also has been described in Wissner et al [46] as not only EGFR inhibitor, but also as HER-2 inhibitor. Recently Kim et al described quinoline-3-carboxylic acid esters as DYRK1 inhibitors [47] for the treatment of learning and memory deficits of people with Down syndrome. Thomas et al [48] describe 3-fluoro/hydrogen quinolines as VEGF tyrosine kinase inhibitors.

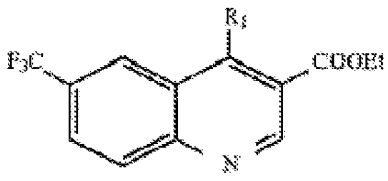
Tang et al [49] describe quinoline-3-carboxylic acid esters as general tyrosine kinase inhibitors. The compounds of [49] have the formula:



wherein Q is selected from the group consisting of NH and S; n is 0 or 1; and R1-9 are independently selected from the group consisting of halo, trihalomethyl, alkyl, nitro, hydroxy, alkoxy, sulphoxy, sulphonyl, amide, sulfonamide, carboxamide, amino, and hydrogen. Preferred compounds are said to be



and



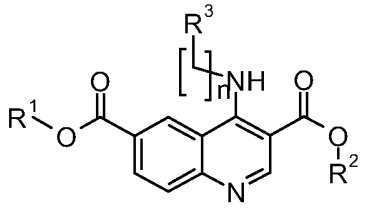
wherein R1 is selected from the group consisting of 3-bromophenylamino, 2-trifluoromethylbenzylamino, 3-trifluoromethylbenzylamino, 4-trifluoromethylbenzylamino, 2-trifluorophenylamino, 3-trifluorophenylamino, 4-trifluorophenylamino, 2-cyanophenylamino, 3-cyanophenylamino, 4-cyanophenylamino, 3,4-dimethoxyphenylamino, 4-bromophenylamino, 3-chlorophenylamino; and 4-chlorophenylamino. Only derivatives having the benzene moiety of the quinoline substituted either by trifluoro or by two halogen atoms are exemplified.

Sapelkin et al describe quinoline-3-carboxylic acid esters as CK2 inhibitors for treatment of cancer [50]. The compounds that in [50] are stated to act as CK2 inhibitors however were not found active towards CK2, when tested as CK2 kinase inhibitors (tests performed at Cerep, France). It might be added that the cancer in [50] is a type induced by virus, viz. not involving VEGF receptors.

Summary of the invention

The present inventors now have found quinoline-3-carboxylic acid esters with certain sidechain pattern that inhibit the VEGFR-2 tyrosine kinase with good potency and high selectivity, while being capable also of inhibiting other tyrosine kinases as well.

Consequently, according to one aspect, the present invention relates to a compound of formula (I)



(I)

wherein

R^1 and R^2 are the same or different and are independently selected from saturated or unsaturated, branched or unbranched C1-C10 alkyl and C3-C12 cycloalkyl;

n= 0-5;

R^3 is selected from substituted or non-substituted C6-C10 aryl or C1-C9 heteroaryl wherein the heteroatoms are independently selected from N, O and S; substituted

or non-substituted mono- or bicyclic C3-C12 cyclyl or C1-C9 heterocyclyl wherein the heteroatoms are independently selected from N, O and S;, saturated or unsaturated, branched or unbranched, substituted or non-substituted C1-C6 alkyl; and -O-CO-R^4 wherein R^4 is C3-C10 cycloalkyl or C1-C10 alkyl;
as well as pharmaceutically acceptable salts or prodrugs thereof, for use as a medicament.

According to a further aspect, the present invention provides the use of the compounds of formula (I) or pharmaceutically acceptable salts or prodrugs thereof for manufacturing a medicament for the treatment of diseases such as cancer, diabetic retinopathy, age-related macular degeneration, chronic inflammation, stroke, ischemic myocardium, atherosclerosis, tumor growth and macular edema.

According to another aspect, the invention provides a method of treatment of a disorder selected from cancer, diabetic retinopathy, age-related macular degeneration, chronic inflammation, stroke, ischemic myocardium, atherosclerosis, tumor growth and macular edema by administration of a therapeutically effective amount of a compound of formula (I) to a mammal in need of such treatment.

Further aspects of the invention are as defined in the claims.

Brief description of the drawings

Fig. 1 Fluorescence microscope image of cells fixed with paraformaldehyde, permeabilized with Triton X-100, and stained with rhodamine-phalloidin;
A PAE/VEGFR-2 cells; **B** PAE/VEGFR-2 cells treated with VEGF for 12 h; and
C PAE/VEGFR-2 cells treated with VEGF and inventive compound ACT-3673 for 12 h.

Fig. 2 Light microscope image of **A** PAE cells expressing VEGFR-2 without the presence of VEGF; **B** PAE cells expressing VEGFR-2 in the presence of VEGF; and **C** PAE cells expressing VEGFR-2 in the presence of VEGF and inventive compound ACT-3673.

Fig. 3 Schematic representation of chemotaxis assay performed in Boyden chamber with VEGF as attractant in the lower compartment.

Fig. 4 Bar chart representing the integrated density of migrated cells in the presence or not of the inventive compound ACT-3673.

Fig. 5 Growth rate of xenotransplanted murine T241 fibrosarcoma cells. Treatment began at the same day as inoculation of the tumor cells.

Fig. 6 Representative T241 fibrosarcoma tumor-bearing animals after 20 days of therapy: vehicle only (left) and ACT-3673 (right).

Fig. 7 Excised T241 fibrosarcoma tumor cells. Tumors in the ACT treated group were paler, smaller and harder compared to the red, fast growing control tumors.

Fig. 8 Weight of animals minus weight of tumor. The ACT-3673 treated animals gained in weight throughout the experiment while controls decreased in weight due to large tumor burden.

Fig. 9 Competition curve obtained with compound ACT-3673 at the VEGF receptor.

Fig. 10 Curve showing effects of compound ACT-3673 on VEGFR1-tyrosine kinase activity.

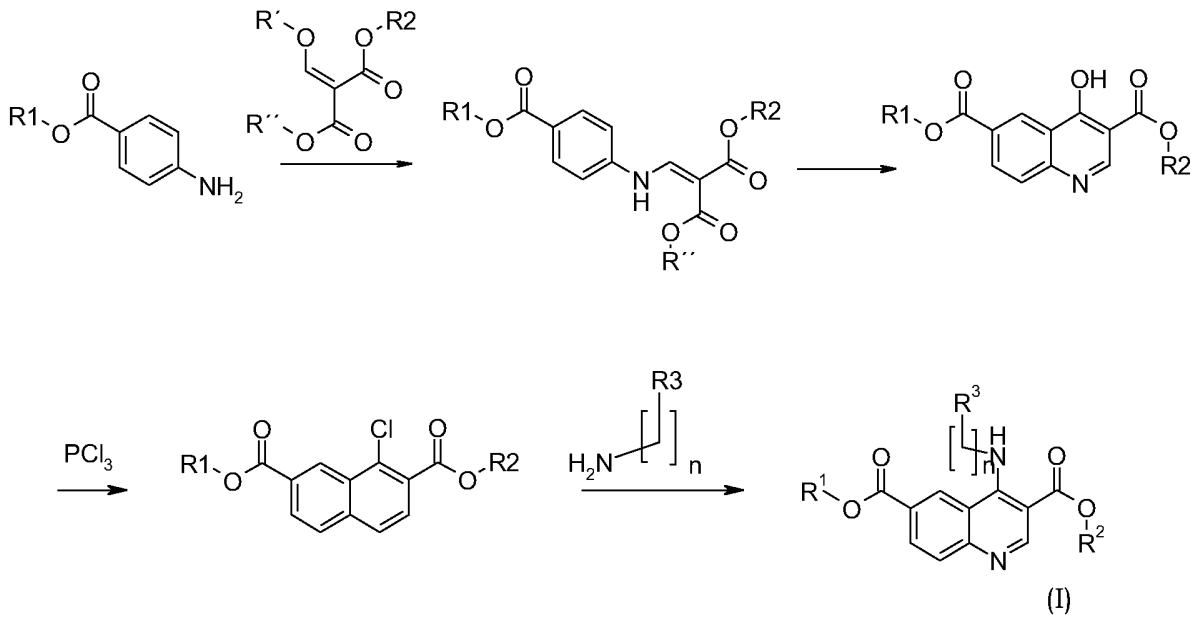
Fig. 11 Curve showing effects of compound ACT-3673 on VEGFR2-tyrosine kinase activity.

Detailed description of the invention

The present invention relates to quinoline-3-carboxylic acid ester derivatives which inhibit, regulate and/or modulate growth factor and tyrosine kinase signal transduction for use as a medicament, compositions which contain these derivatives, and methods of using them to treat growth factor and tyrosine kinase-dependent diseases and conditions such as cancer, diabetic retinopathy, age-related macular degeneration, chronic inflammation, stroke, ischemic myocardium, atherosclerosis, tumor growth, macular edema, and the like in mammals.

Quinoline-3-carboxylic acid esters of the present invention are commercially available. The preparation of the compounds of the invention also lies well within the capability of the person skilled in the art. As an example, a quinoline-3-carboxylic

acid ester derivative of the invention, may be formed in a four step procedure wherein, first, a suitable aniline derivative is reacted with a suitable diethylester, the formed intermediate is cyclized to give a quinoline-4-ol derivative, which is then converted to the corresponding halogen derivative and finally reacted with a suitable amine to form the selected quinoline-3-carboxylic acid ester derivative of formula (I) according to the invention. The entire synthesis is illustrated by the following reaction scheme:



With regard to the above reaction sequence, it is well within the capability of the person skilled in the art to select suitable reaction components as well as reaction conditions.

Unless otherwise specified, the alkyl groups that are considered useful in the compounds according to the invention generally may be selected from an unbranched or branched, cyclic, saturated or unsaturated (alkenyl or alkynyl) hydrocarbyl radical. Where cyclic, the alkyl group is preferably C3 to C12, more preferably C5 to C10, most preferably C5-C7. Where acyclic, the alkyl group is preferably C1 to C10, more preferably C1 to C6, more preferably methyl, ethyl, propyl (n-propyl, isopropyl), butyl (branched or unbranched) or pentyl, most preferably methyl.

As used herein, and unless otherwise specified, the term “aryl” means an aromatic group, such as phenyl or naphthyl.

As used herein, and unless otherwise specified, the term "functional groups" means in the case of unprotected: hydroxy-, thiolo-, aminofunction, carboxylic acid and in the case of protected: lower alkoxy, N-, O-, S- acetyl, carboxylic acid ester.

As used herein, and unless otherwise specified, the term "heteroaryl" means a mono-, bi-, or tricyclic heteroaromatic group containing one or ore heteroatom(s) preferably selected from N, O and S, such as pyridyl, pyrrolyl, quinolinyl, furanyl, thienyl, oxadiazolyl, thiadiazolyl, thiazolyl, oxazolyl, pyrazolyl, triazolyl, tetrazolyl, tetrahydroquinolinyl, thiochromanyl, isoxazolyl, isothiazolyl, isoquinolinyl, naphthyridinyl, imidazolyl, phenazinyl, phenothiazinyl, phthalazinyl, indolyl, pyridazinyl, quinazolinyl, quinolizinyl, quinoxalinyl, tetrahydroisoquinolinyl, pyrazinyl, indazolyl, indolinyl, pyrimidinyl, thiophenetyl, pyranyl, carbazolyl, chromanyl, cinnolinyl, acridinyl, benzimidazolyl, benzodioxanyl, benzodioxepinyl, benzodioxolyl, benzofuranyl, benzothiazolyl, benzobenzoxadiazolyl, benzoxazinyl, benzoxazolyl, benzomorpholinyl, benzoselenadiazolyl, benzothienyl, purinyl, pteridinyl and the like.

As used herein, and unless otherwise specified, the terms "non-aromatic heterocycle" and "heterocyclyl" mean a non-aromatic cyclic group containing one or more heteroatom(s) preferably selected from N, O and S, such as a aziridinyl, azetidinyl, dihydropyranyl, dihydropyridyl, dihydropyrrolyl, dioxolanyl, dioxanyl, dithianyl, dithiolanyl, imidazolidinyl, imidazolinyl, morpholinyl, oxetanyl, oxiranyl, pyrrolidinyl, pyrrolidinonyl, piperidyl, piperazinyl, piperidinyl, pyrazolidinyl, quinuclidinyl, sulfalonyl, 3-sulfolenyl, tetrahydrofuranyl tetrahydropyranyl, tetrahydropyridyl, thietanyl, thiiranyl, thiolanyl, thiomorpholinyl, trithianyl, tropanyl, monosaccharide and the like.

As used herein, and unless otherwise specified, the term "halogen" means a fluorine, chlorine, bromine or iodine.

As used herein, and unless specified otherwise, the term "substituted" means that the entity is substituted with at least one moiety selected from saturated or unsaturated, branched, unbranched or cyclic alkyl, or at least one functional group such as hydroxyl, amine, sulfide, silyl, carboxylic acid, halogen, aryl, etc.

The compounds according to formula (I) will be useful for treating various diseases such as cancer, diabetic retinopathy, age-related macular degeneration, chronic inflammation, stroke, ischemic myocardium, atherosclerosis, tumor growth and macular edema. The treatment may be preventive, palliative or curative.

Examples of pharmaceutically acceptable addition salts for use in the pharmaceutical compositions of the present invention include those derived from mineral acids, such as hydrochlorid, hydrobromic, phosphoric, metaphosphoric, nitric and sulphuric acids, and organic acids, such as tartaric, acetic, citric, malic, lactic, fumaric, benzoic, glycolic, gluconic, succinic, and arylsulphonic acids. The pharmaceutically acceptable excipients described herein, for example, vehicles, adjuvants, carriers or diluents, are well-known to those who are skilled in the art and are readily available to the public. The pharmaceutically acceptable carrier may be one that is chemically inert to the active compounds and that has no detrimental side effects or toxicity under the conditions of use. Pharmaceutical formulations are found e.g. in Remington: The Science and Practice of Pharmacy. A. R. Gennaro, Editor. Lippincott, Williams and Wilkins, 20th edition (2000).

Prodrugs of the compounds of formula (I) may be prepared by modifying functional groups present on the compound in such a way that the modifications are cleaved, in vivo when such prodrug is administered to a mammalian subject. The modifications typically are achieved by synthesizing the parent compound with a prodrug substituent. Prodrugs include compounds of formula (I) wherein a hydroxy, amino, sulfhydryl, carboxy or carbonyl group in a compound of formula (I) is bonded to any group that may be cleaved in vivo to regenerate the free hydroxyl, amino, or sulfhydryl group, respectively. Examples of prodrugs include, but are not limited to, esters and carbamates of hydroxy functional groups, esters groups of carboxyl functional groups, N-acyl derivatives and N-Mannich bases. General information on prodrugs may be found e.g. in Bundegaard, H. "Design of Prodrugs" pl-92, Elesevier, New York-Oxford (1985).

The composition according to the invention may be prepared for any route of administration, e.g. oral, intravenous, cutaneous or subcutaneous, nasal, intramuscular, or intraperitoneal. The precise nature of the carrier or other material will depend on the route of administration. For a parenteral administration, a parenterally acceptable aqueous solution is employed, which is pyrogen free and has requisite

pH, isotonicity and stability. Those skilled in the art are well able to prepare suitable solutions and numerous methods are described in the literature. A brief review of methods of drug delivery is also found in e.g. (43).

The dose administered to a mammal, particularly a human, in the context of the present invention should be sufficient to effect a therapeutic response in the mammal over a reasonable time frame. One skilled in the art will recognize that dosage will depend upon a variety of factors including the potency of the specific compound, the age, condition and body weight of the patient, as well as the stage/severity of the disease. The dose will also be determined by the route (administration form) timing and frequency of administration. In the case of oral administration the dosage can vary from about 0.01 mg to about 1000 mg per day of a compound of formula (I) or the corresponding amount of a pharmaceutically acceptable salt thereof.

The compounds of the present invention may be used or administered in combination with one or more additional drugs useful in the treatment of hyperproleferative diseases, e.g. a cytostatic agent. The components may be in the same formulation or in separate formulations for administration simultaneously or sequentially. The compounds of the present invention may also be used or administered in combination with other treatment such as irradiation for the treatment of cancer.

Examples of cytotstatic agents for use as indicated herein above are DNA alkylating compounds, topoisomerase I inhibitors, topoisomerase II inhibitors, compounds interfering with RNA and DNA synthesis, compounds polymerising the cytoskeleton, and compounds depolymerising the cytoskeleton.

Biological tests

Materials and Methods

Cells

A stable porcine aortic endothelial (PAE) cell line expressing VEGFR-2 was established as reported (14, 24) and maintained in Ham's F12 medium supplemented with glutamine, penicillin/streptomycin (PEST) and 10% fetal bovine serum (FBS).

Mouse fibrosarcoma cell line T241 was used to prepare conditioned medium contained recombinant human VEGF (cm/VEGF). The T241 cells were grown in DMEM medium supplemented with 5% FBS for 72 hours.

Cell Shape Assay

PAE/VEGFR-2 cells were grown in 48-well plates to about 50% confluence in Ham's F12 medium supplemented with Glutamine, PEST, 10%FBS. The medium was removed and replaced with fresh Ham's F12 medium containing 10% FBS and minimal concentration of cm/VEGF that activated cell shape change with or without test compound in a final concentration of 10μM/well. After 12 h of incubation, cells were fixed with 3% paraformaldehyde in phosphate buffer (pH 7.5) for 30 min at room temperature and rinsed three times with PBS. The cells were examined under light microscope (Fig. 2).

Actin Staining

PAE/VEGFR-2 cells were grown on coverslips in 6-well plates to about 50–70% confluence in Ham's F12 medium supplemented with 10% FBS. The medium was removed and replaced with fresh Ham's F12 medium containing 10% FBS and minimal concentration of cm/VEGF with or without test compound. After 12 h of incubation, cells were fixed with 3% paraformaldehyde in phosphate buffer (pH 7.5) for 30 min at room temperature. After rinsing three times with PBS, the cells were permeabilized with 0.5% Triton X-100 for 30 min. The cells then were washed three times with PBS and stained for 30 min at room temperature with 1mg/ml of rhodamine-phalloidin (Sigma) in PBS. After washing with PBS five times, the coverslips were mounted in a mixture of glycerol and PBS (90:10), and the cells were examined under light and fluorescence microscopes (Fig 1).

Chemotaxis assay

The motility response of cells to VEGF and test compounds was assayed by a modified Boyden chamber technique (39) as described (14, 40) by using micropore polycarbonate filters (PVPF, 8 micron) coated with 1% gelatine solution (Fig. 3). Cells were trypsinized and resuspended at a concentration of $6x10^5$ cells/ml in serum-free medium. The cells (30,000 cells per well) were placed in the upper chamber with or without test compound at a final concentration of 10μM/well and serum-free medium with or without VEGF in the lower chamber. After 6 h at 37°C, the medium was removed and cells attached to the filter were fixed in methanol and

stained with Giemsa solution. The number of cells migrating through the filter was counted and plotted as number of migrating cells per optic field (x32) or was detected by ImageJ software and plotted as integrated density of migrated cells (Fig. 4). All experiments were performed in triplicate.

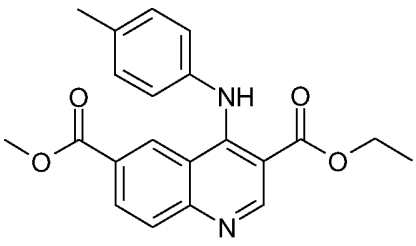
VEGF receptor binding assay

Testing for VEGF receptor binding was performed using *Cerep Kinase Profiling Service (www.cerep.com)*. HUV-EC-C cells were used for this analysis together with [^{125}I]VEGF (conc. 0.015 nM) and VEGF (non-specific, conc. 3 nM) as the ligand to be displaced by the inhibitor. Incubation was performed for 3 h at 4° C. The method of detection was scintillation counting.

The specific ligand binding to the receptors is defined as the difference between the total binding and the nonspecific binding determined in the presence of an excess of unlabelled ligand. The results are expressed as a percentage of control specific binding obtained in the presence of the tested compound.

The IC_{50} values (concentration causing a half-maximal inhibition of control specific binding) and Hill coefficients (n_H) were determined by non-linear regression analysis of the competition curves using Hill equation curve fitting. (Fig. 9).

As an example, a compound of the invention, viz.



termed ACT-3673 herein below, provided an IC_{50} of > 1.0E-04 M when tested in the receptor binding assay using VEGF (*h*).

Kinase binding assay

Testing for tyrosine kinase activity was performed using *Cerep Kinase Profiling Service (www.cerep.com)*. The VEGFR-1 and VEGFR-2 used were recombinant human proteins expressed in bacteria or in insect cells. They were assayed with appropriate

biotinylated substrates in the presence of 0.05-20 µM ATP (0.3 to 3 times the Km of the individual kinase) at 22 °C for 15 to 90 min, according to the kinase. Kinase activity was detected by an HTRF assay. HTRF (a trademark of CisBio International) is based on fluorescence transfer, using Europium (Eu3+) cryptate and XL665 as donor and acceptor respectively. When the biotinylated (or tagged) substrate is phosphorylated by the kinase, it cross-reacts with a cryptate-labeled phosphospecific antibody. Addition of Streptavidin-XL665, SA-XL665, (or anti-tag antibody XL665) causes the juxtaposition of the cryptate and XL665 fluorophore, resulting in FRET (fluorescence resonance energy transfer). FRET intensity depends on the amount of bounded cryptate antibody, which is proportional to the extent of substrate phosphorylation [41].

Table 1 and Figs. 10 and 11 show results obtained for compound ACT-3673 of the invention.

Table 1 Kinase assay - IC_{50} Determination: Summary Results

Assay Cerep Compound I.D.	Compound	IC_{50} (M)	n_H
FLT-1 kinase *(h)* (VEGFR-1)	ACT-3673	1.6E-05	1.0
KDR kinase *(h)* (VEGFR-2)	ACT-3673	2.5E-07	0.6

From Table 1 it appears that the effect of compound ACT-3673 on the VEGFR-1 tyrosine kinase activity, expressed as the IC_{50}, was 16 µM, while the effect of compound ACT-3673 on the VEGFR-2 tyrosine kinase activity was much higher, the IC_{50} being only 0.25 µM.

In-vivo analysis

T241 tumor fibrosarcoma cells

T241 tumor fibrosarcoma cells were kindly provided by Dr Yihai Cao, KI, Stockholm, Sweden. The cells were grown in Dulbecco´s modified essential medium supplemented with fetal calf serum, glutamine, and pencillin/streptomycin. The cells were grown in humidified air (95%) and CO_2 (5%) at 37 ˚C. The medium was changed twice a week and confluent cultures were subcultivated after treatment of with trypsin. For subcutaneous (sc) tumor injections, a single cell suspension was prepared, where cells were resuspended in medium and the viability and cell concentration were calculated after addition of trypan blue dye. The cell suspension

was kept on ice during the injection procedure. The culture was shown to be free from mycoplasm.

Animals

Female C57Bl/6J mice were used for xenografting at the age of 6-7 weeks (body weight 18-20 g). The mice were housed in an isolated room at 24 °C with a 12-h light, 12-h dark cycle. They were fed *ad libitum* with water and food pellets. The animal weight and general appearance were recorded every day throughout the experiment. The experiment was approved by regional ethics committee for animal research.

Xenografting

Tumor cells (1 x 10^6 in 0.1 ml medium) were implanted sc in the hindleg of the animal. Animals were anesthetized with isoflurane supplemented with oxygen. Tumor volume measurements began when the tumor became palpable (~ 0.1 ml) and were repeated every day using a calliper. The tumor volume was calculated by the formula: 0.52 x length x $width^2$.

Administration of drug

Treatment started at the same time as tumor implantation and all animals received treatment for 20 days. Control animals were given vehicle only. The compound ACT-3673 was given at a dose of 25 mg/kg/day sc in the neck.

Perfusion fixation and, autopsy

The animals were anesthetized by an i.p injection of 25 mg/kg of avertin. A cannula was inserted in the thoracic aorta, and the animal was perfused with PBS/heparin. The thoracic and abdominal viscera were examined for macroscopic metastases. The true tumor weight and volume were recorded at autopsy and correlated well to the calculated volume.

Tissue analyses

The dissected tumors were immersion-fixed in 4% formaldehyde for approximately 2 days before dehydration, and paraffin embedding. Sections were cut at 3μM and put on slides, dewaxed, rehydrated and stained immunohistochemically.

Immunohistochemistry

To quantify angiogenesis (vessel growth), Bandeira Simplicifolia-1 lectin histochemistry was used for highlighting endothelial cells.

Stereological quantification

A representative section from the geometrical center of each tumor was used. Structure was counted at x 400 with an eyepiece grid. The grid was placed at random at the upper left-hand corner of the section and systematically advanced every 1 to 3 mm, depending on tumor size, in both directions by use of the gonimeter stage of microscope. Vascular parameters from 25 to 35 grids were quantified from each tumor.

Results

It is known that the mature form of VEGF binds to the angiogenic receptor VEGFR-2 and mediates VEGF-stimulated biological responses including membrane ruffling, cell migration and proliferation (24). In the presence of VEGF the VEGFR-2 expressing cells acquire an elongated cell shape with distinct spindle-like processes and actin reorganization (Fig. 1B). The cell shape change appeared to be independent of the addition of bovine serum, because a similar change was observed in the presence and absence of serum. These data suggested that other serum factors were not required for the morphological effect (42).

The used assay comprised a culture of PAE/VEGFR-2 cells. Morphological changes of the cells were recorded microscopically after addition of the VEGF, followed by the test compound at final concentration 10μM. Growth inhibition of the PAE/VEGFFR-2 cells was detected in the presence of a number of compounds according to the invention at 10 μM. These compounds were retested in primary screening at lower concentration (1, 2.5 and 5μM) as well as higher concentration. The most active compounds were studied in a chemotaxis assay. Below, a number of compounds of the present invention are presented, together with the concentrations used for totally blocking the morphological shape (MIC, Minimum Inhibitory Concentration):

4-(ortho-Methylphenylamino)-quinoline-3,6-dicarboxylic acid 3-ethyl ester 6-methyl ester: 10 μM

4-(meta-Methylphenylamino)-quinoline-3,6-dicarboxylic acid 3-ethyl ester 6-methyl ester: 10 μM

4-(para-Methylphenylamino)-quinoline-3,6-dicarboxylic acid 3-ethyl ester 6-methyl ester: 10 μM

4-(4-Dimethylaminophenylamino)-quinoline-3,6-dicarboxylic acid 3-ethyl ester 6-methyl ester: 5 μM

4-(3-Chlorophenylamino)-quinoline-3,6-dicarboxylic acid 3-ethyl ester 6-methyl ester: 10 μM

4-(2-Methoxyphenylamino)-quinoline-3,6-dicarboxylic acid 3-ethyl ester 6-methyl ester: 10 μM

4-(3-Acetylphenylamino)-quinoline-3,6-dicarboxylic acid 3-ethyl ester 6-methyl ester: 2.5 μM

4-(3-Hydroxyphenylamino)-quinoline-3,6-dicarboxylic acid 3-ethyl ester 6-methyl ester: 10 μM

4-(3-Acetylphenylamino)-quinoline-3,6-dicarboxylic acid 3,6 diethyl ester: 2.5 μM

4-(2-Morpholin-4-yl-ethylamino)quinoline-3,6-dicarboxylic acid 3,6 diethyl ester: 20 μM

4-(2,2,6,6-Tetramethyl-piperidin-4-ylamino)quinoline-3,6-dicarboxylic acid 3,6 diethyl ester: 20 μM

4-(3-Methoxyphenylamino)-quinoline-3,6-dicarboxylic acid 3-ethyl ester 6-methyl ester: 5 μM

4-(4-Methoxyphenylamino)-quinoline-3,6-dicarboxylic acid 3-ethyl ester 6-methyl ester: 2.5 μM

4-(2,3-Dihydro-benzo[1,4]dioxin-6-ylamino)-quinoline-3,6-dicarboxylic acid 3-ethyl ester 6-methyl ester: 2.5 μM

4-(Phenylamino)-quinoline-3,6-dicarboxylic acid 3-ethyl ester 6-methyl ester: 10 μM

4-(4-hydroxyphenylamino)-quinoline-3,6-dicarboxylic acid 3-ethyl ester 6-methyl ester: 2.5 μM

4-(2-Hydroxy-propylamino)-quinoline-3,6-dicarboxylic acid 3,6 diethyl ester: 40 μM

4-(4-Acetylphenylamino)-quinoline-3,6-dicarboxylic acid 3-ethyl ester 6-methyl ester: 10 μM

4-[(Pyridin-3-ylmethyl)-amino]-quinoline-3,6-dicarboxylic acid diethyl ester: 20 μM

4-(3-Cyclohexanecarbonyloxy-propylamino)-quinoline-3,6-dicarboxylic acid 3,6 diethyl ester: 40 μM

Suppression of Primary Tumor Growth by Systemic Administration of compound ACT-3673.

Sc treatment with ACT-3673 potently suppressed tumor growth in T241 fibrosarcoma cell line. The treated/control quotient for T241 after 20 days of therapy was 0.13 ($p < 0.001$) (Figs. 5-7*)*. Neither toxicity nor metastases were seen and the weight increased/stayed the same throughout the experiment (Fig. 8). The ACT-3673 treated tumors were smaller, paler and harder than those of the control groups (Fig. 7).

The results of the stereological quantification of angiogenesis, expressed as number of vessels per grid, length of vessels per tumor volume, volume of vessels per tumor volume, and surface area of vessels per tumor volume, are shown in Table 2. It appears that at day 20 of therapy there was a significant reduction of the numbers of vessels per grid (63 % for ACT-3673). There was also a significant reduction in the length of vessels per tumor volume, the volume of vessels per tumor volume and the surface area of vessels per tumor volume in the ACT-3673 treated group, compared to controls.

Table 2 Quantification of vascular parameters at day 20 of therapy

Parameter	Controls, (n=8)	ACT-3673 25 mg/kg/day, (n=9)
Qv change (%)	124.3 +/- 23.5	46.2 +/- 12.6 - 63***
Lv (mm^{-2}) change (%)	164.8 +/- 19.2	58.9 +/- 10.9 - 64***
Vv (10^{-3}) change (%)	0.47 +/- 0.09	0.35 +/- 0.02 -26*
Sv (mm^{-1}) change (%)	9.3 +/- 1.1	3.9 +/-0.8 - 58***

Qv:number per vessels/grid, Lv:length of vessels per tumor volume, Vv: volume of vessels per tumor volume, Sv:surface area of vessels per tumor volume

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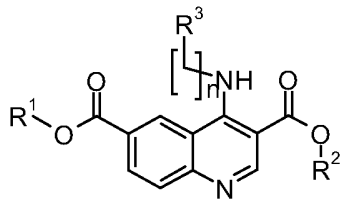
Claims

1. A compound of formula (I)



(I)

wherein

R^1 and R^2 are the same or different and are selected from saturated or unsaturated, branched or unbranched C1-C10 alkyl and C3-C12 cycloalkyl;

n= 0-5;

R^3 is selected from substituted or non-substituted C6-C10 aryl or C1-C9 heteroaryl wherein the heteroatoms are independently selected from N, O and S; substituted or non-substituted mono- or bicyclic C6-C10 cyclyl or C1-C9 heterocyclyl wherein the heteroatoms are independently selected from N, O and S; saturated or unsaturated, branched or unbranched, substituted or non-substituted C1-C6 alkyl; and -O-CO-R^4 wherein R^4 is C3-C10 cycloalkyl or C1-C10 alkyl;

as well as pharmaceutically acceptable salts or prodrugs thereof, for use as a medicament.

2. A compound according to claim 1, wherein R^1 and R^2 are selected from C1-C6 alkyl.

3. A compound according to claim 1, wherein R^1 and R^2 are selected from methyl and ethyl.

4. A compound according to any one of the claims 1-3, wherein the C6-C10 aryl is phenyl.

5. A compound according to any one of the claims 1-4, wherein R^3, the C1-C9 heteroaryl is moncyclic C1-C5 heteroaryl.

6. A compound according to claim 5, wherein the C1-C5 heteroaryl is pyridyl.

7. A compound according to any one of the claims 1-6, wherein R3 is substituted with at least one moiety selected from C1-C6 alkyl, more preferably C1-C3 alkyl; C1-C6 alkoxy, more preferably C1-C3 alkoxy; -CO-C1-C6 alkyl, more preferably -CO-C1-C3 alkyl; halogen and OH.

8. A compound according to claim 7, wherein the C1-C6 alkyl is methyl.

9. A compound according to claim 7 or 8, wherein the C1-C6 alkoxy is methoxy.

10. A compound according to any one of the claims 7-9, wherein the -CO-C1-C6 alkyl is acetyl.

11. A compound according to any one of the claims 7-10, wherein the halogen is Cl.

12. A compound according to any one of the claims 1-11 for the treatment of a disorder selected from cancer, diabetic retinopathy, age-related macular degeneration, chronic inflammation, stroke, ischemic myocardium, atherosclerosis, tumor growth, and macular edema.

13. A compound according to any one of the claims 1-12 for the treatment of a disorder related to VEGF over-expression.

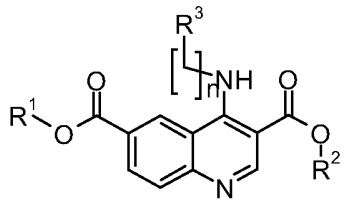
14. A compound according to any one of the claims 1-13, for use as a medicament having a VEGFR-2 tyrosine kinase inhibiting activity.

15. A pharmaceutical composition comprising a therapeutically effective amount of a compound according to any one of the claims 1-14, or a pharmaceutically acceptable salt or prodrug thereof, and at least one pharmaceutically acceptable excipient.

16. A pharmaceutical composition according to claim 15, comprising at least one further pharmaceutically active compound.

17. A pharmaceutical composition according to claim 16, wherein the further pharmaceutically active compound has an anti-tumor activity.

18. Use of a compound of formula (I)



(I)

wherein

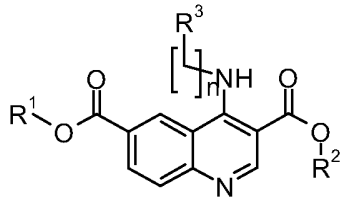
R^1 and R^2 are the same or different and are selected from saturated or unsaturated, branched or unbranched C1-C10 alkyl and C3-C12 cycloalkyl;

n= 0-5;

R^3 is selected from substituted or non-substituted C6-C10 aryl or C1-C9 heteroaryl wherein the heteroatoms are independently selected from N, O and S; substituted or non-substituted mono- or bicyclic C6-C10 cyclyl or C1-C9 heterocyclyl wherein the heteroatoms are independently selected from N, O and S, saturated or unsaturated, branched or unbranched, substituted or non-substituted C1-C6 alkyl; -O-CO-R^4 wherein R^4 is C3-C10 cycloalkyl or C1-C10 alkyl;

as well as pharmaceutically acceptable salts or prodrugs thereof, for the manufacturing of a medicament for the treatment of a disorder selected from cancer, diabetic retinopathy, age-related macular degeneration, chronic inflammation, stroke, ischemic myocardium, atherosclerosis, tumor growth, and macular edema.

19. A method of therapeutic treatment of a mammal comprising administering to said mammal a compound of formula (I)



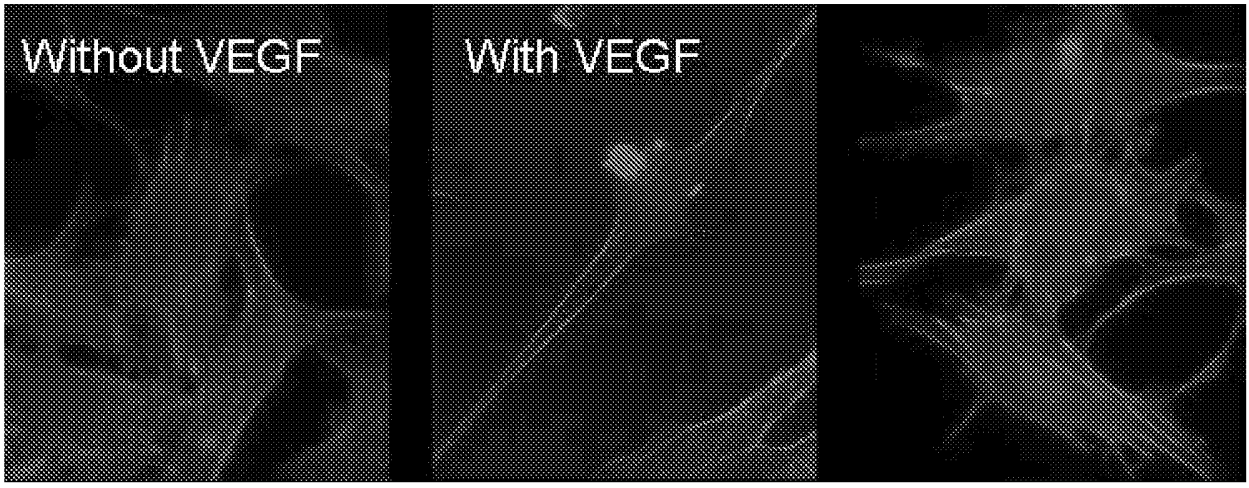
(I)

wherein

R^1 and R^2 are the same or different and are selected from saturated or unsaturated, branched or unbranched C1-C10 alkyl and C3-C12 cycloalkyl;

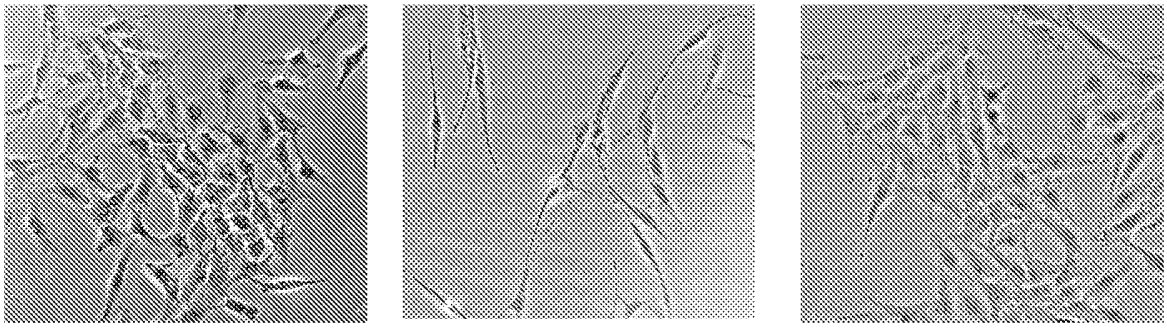
n= 0-5;

R^3 is selected from substituted or non-substituted C6-C10 aryl or C1-C9 heteroaryl wherein the heteroatoms are independently selected from N, O and S; substituted or non-substituted mono- or bicyclic C6-C10 cyclyl or C1-C9 heterocyclyl wherein the heteroatoms are independently selected from N, O and S, saturated or unsaturated, branched or unbranched, substituted or non-substituted C1-C6 alkyl; -O-CO-R^4 wherein R^4 is C3-C10 cycloalkyl or C1-C10 alkyl;
as well as pharmaceutically acceptable salts or prodrugs thereof



A B C

Figure 1



A B C

Figure 2

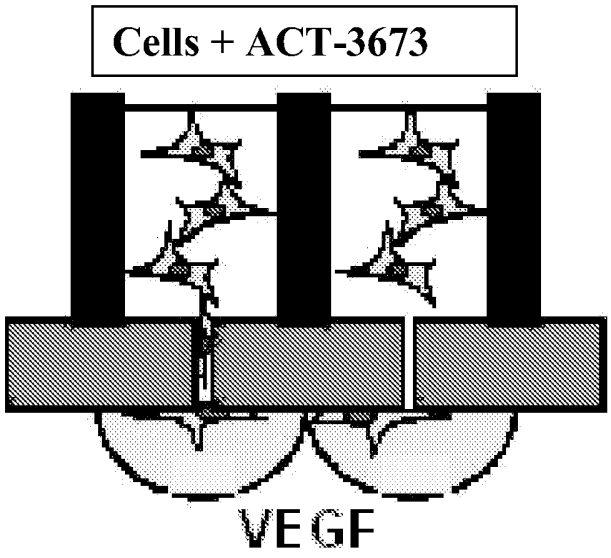


Figure 3

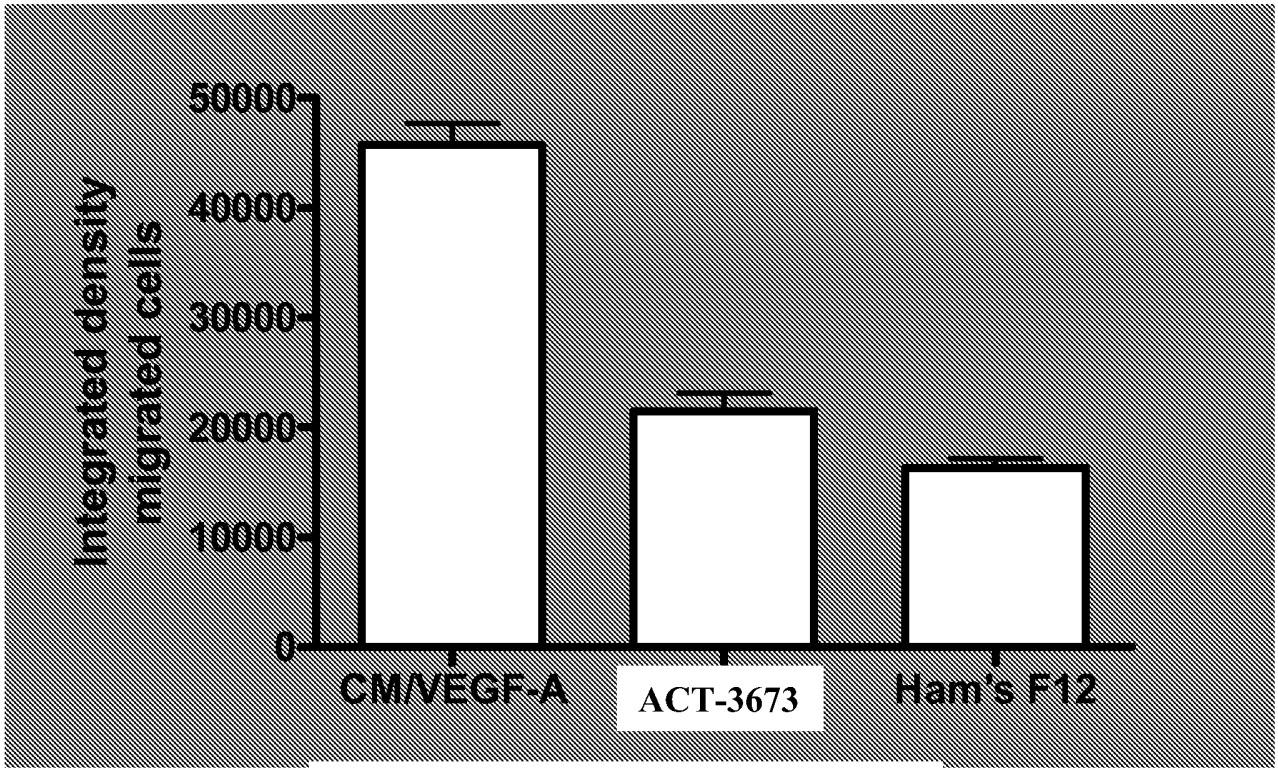


Figure 4

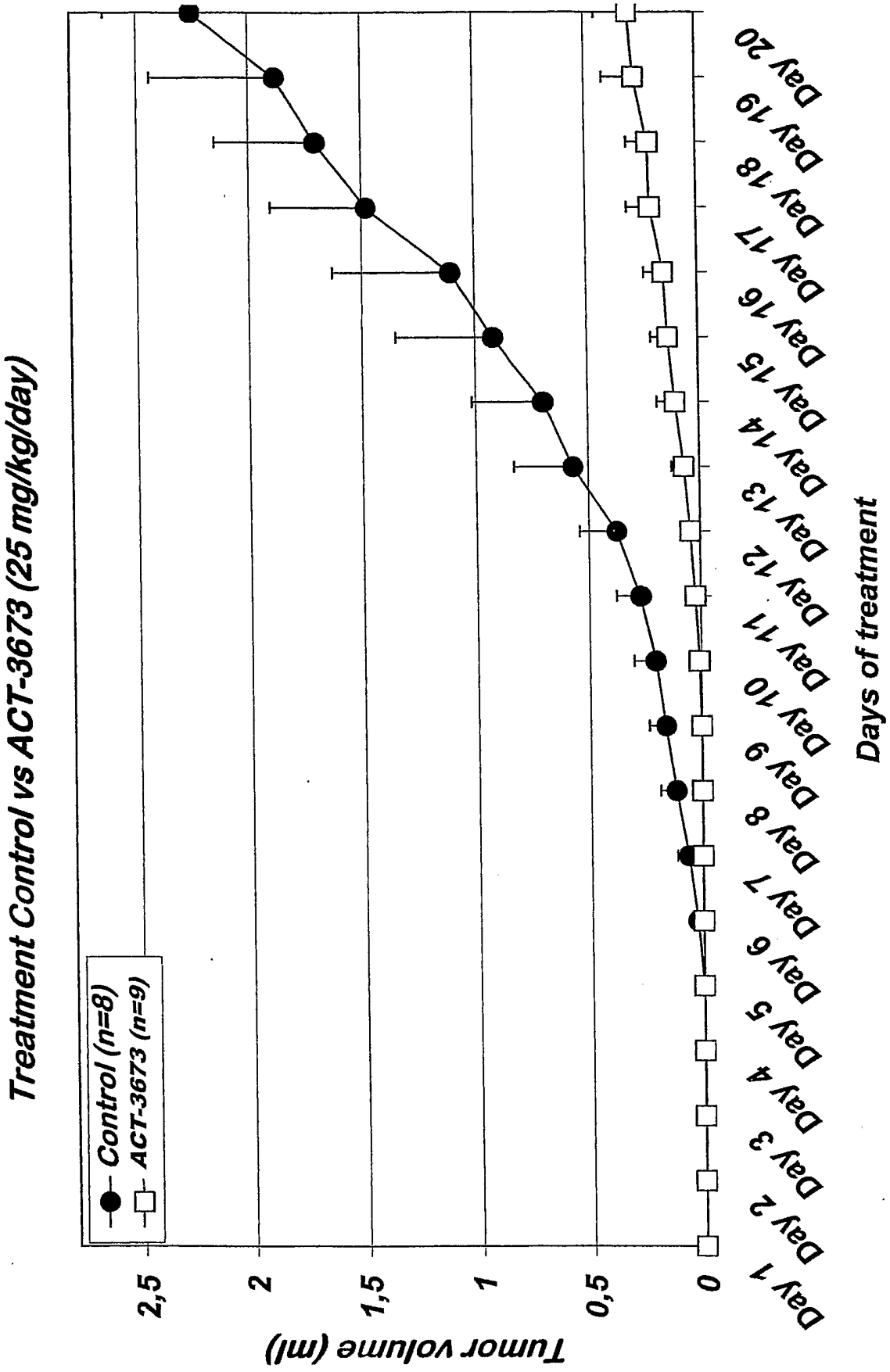
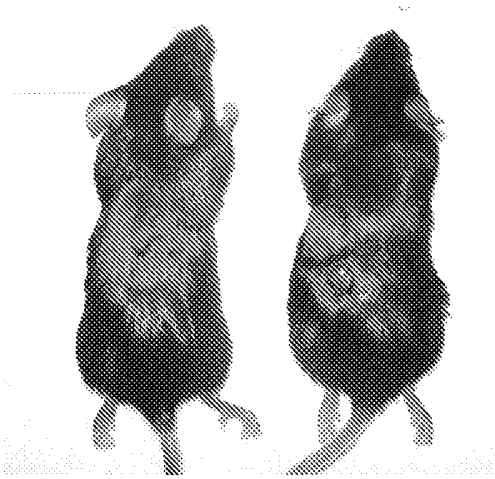
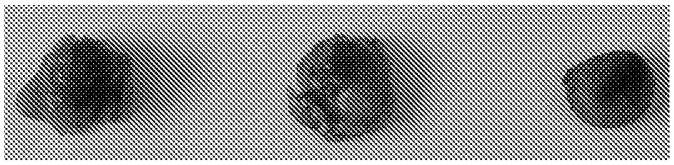

Treatment Control vs ACT-3673 (25 mg/kg/day)
Control (n=8)
ACT-3673 (n=9)
Tumor volume (ml)
2,5
2
1,5
1
0,5
0
Day 1
Day 2
Day 3
Day 4
Day 5
Day 6
Day 7
Day 8
Day 9
Day 10
Day 11
Day 12
Day 13
Day 14
Day 15
Day 16
Day 17
Day 18
Day 19
Day 20
Days of treatment

Figure 5

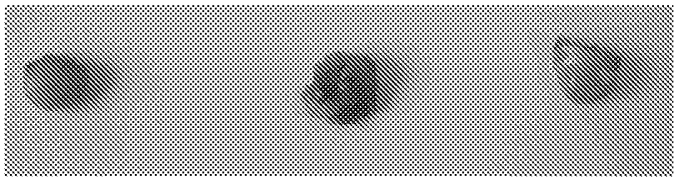


ACT-3673 Control

Figure 6



Controls



ACT-3673

Figure 7

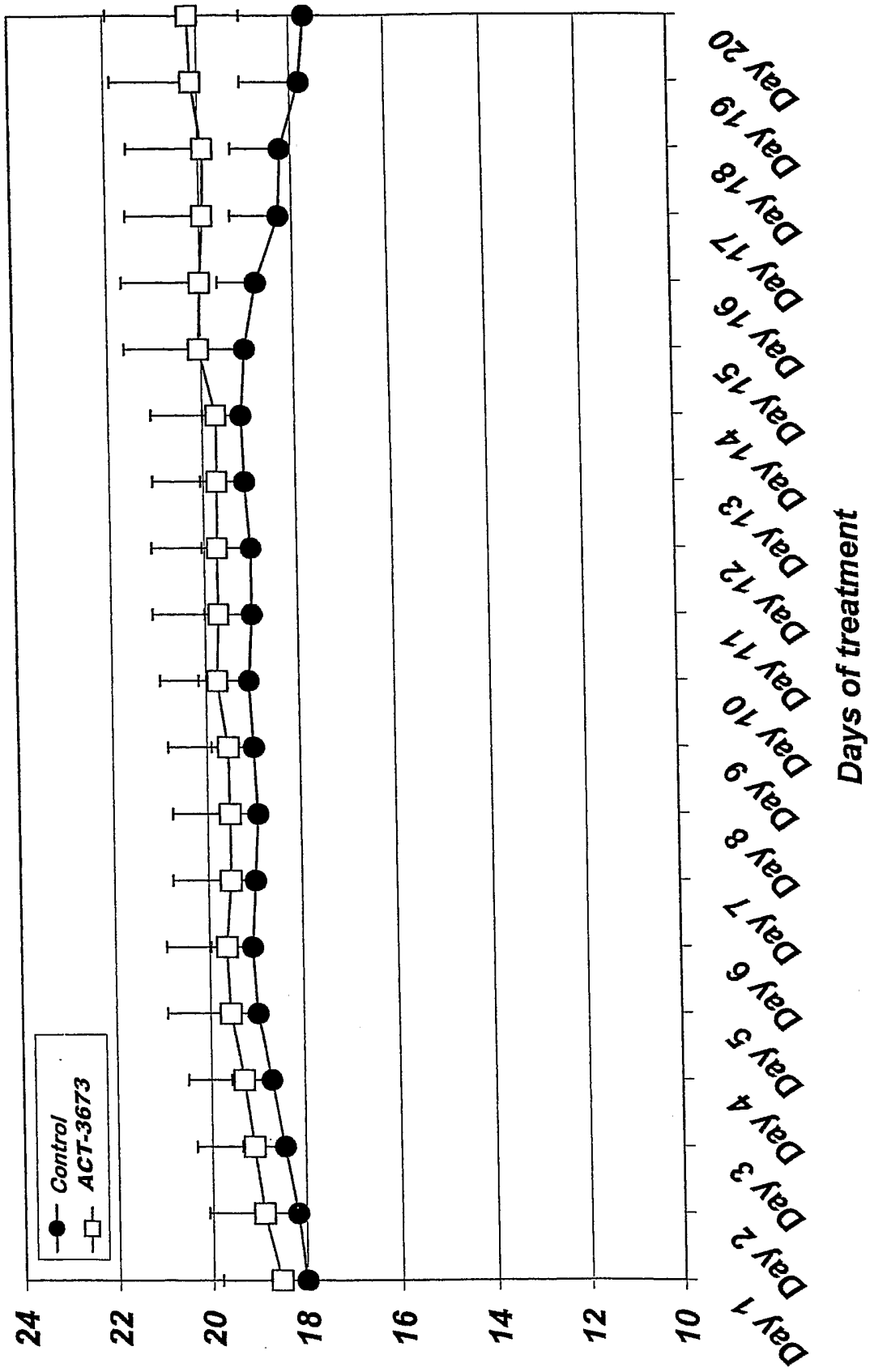

Control
ACT-3673
24
22
20
18
16
14
12
10
weight (g)
Day 1
Day 2
Day 3
Day 4
Day 5
Day 6
Day 7
Day 8
Day 9
Day 10
Day 11
Day 12
Day 13
Day 14
Day 15
Day 16
Day 17
Day 18
Day 19
Day 20
Days of treatment

Figure 8

IC50 > 1.0E-04 M

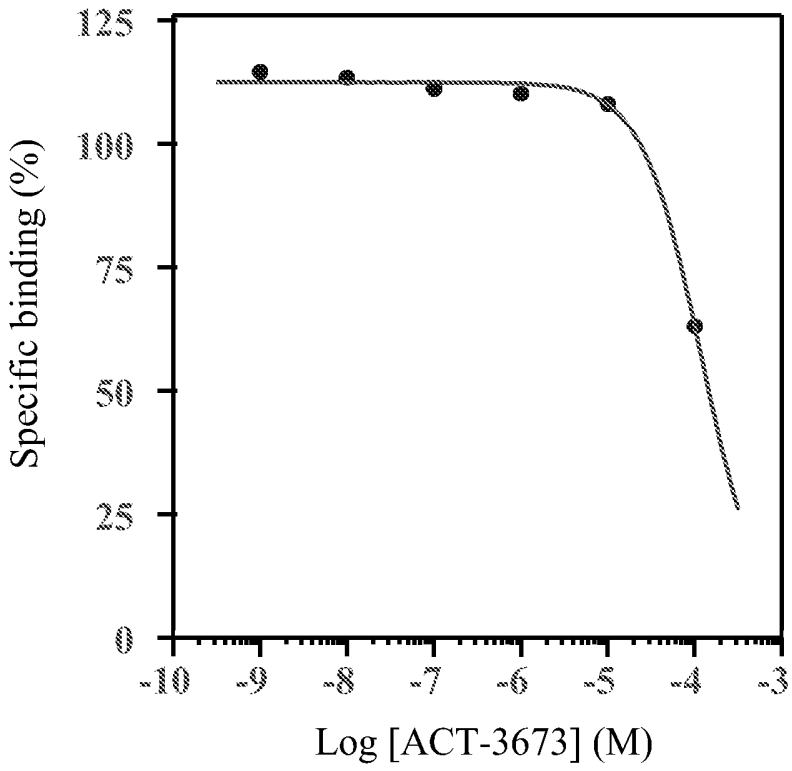


Figure 9

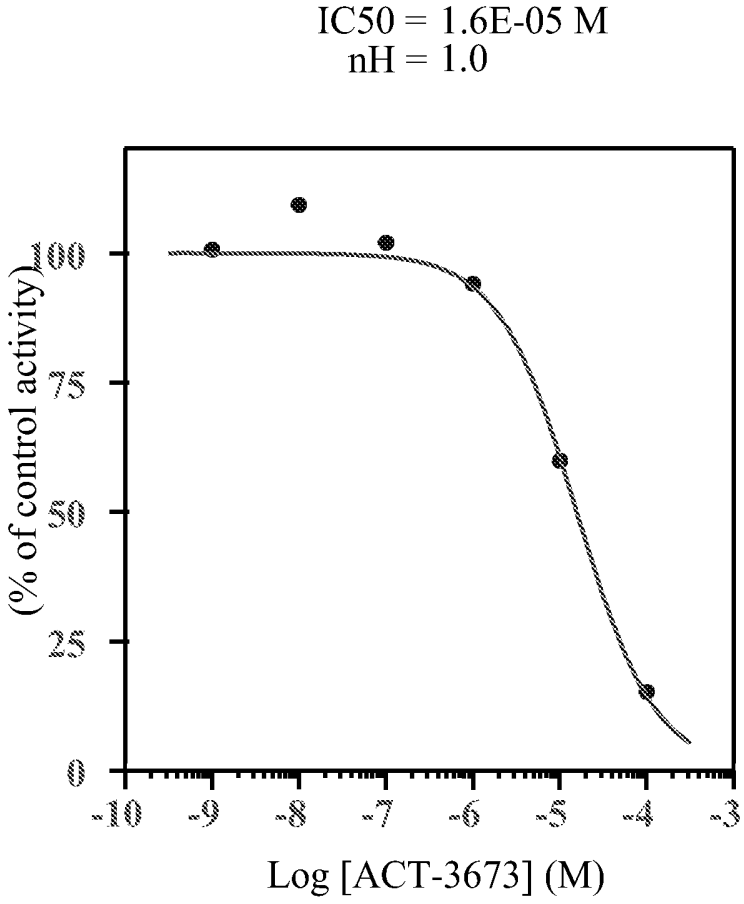

IC50 = 1.6E-05 M
nH = 1.0
(% of control activity)
100
75
50
25
0
-10
-9
-8
-7
-6
-5
-4
-3
Log [ACT-3673] (M)

Figure 10

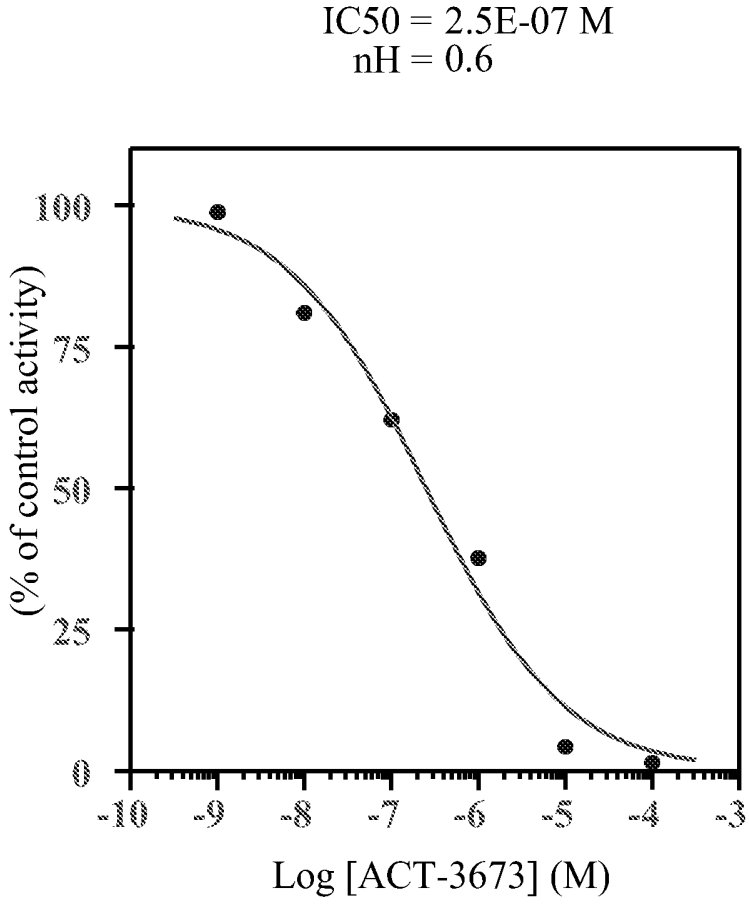

IC50 = 2.5E-07 M
nH = 0.6
100
75
50
25
0
(% of control activity)
-10
-9
-8
-7
-6
-5
-4
-3
Log [ACT-3673] (M)

Figure 11

INTERNATIONAL SEARCH REPORT

International application No.
PCT/SE2007/050358

A. CLASSIFICATION OF SUBJECT MATTER

IPC: see extra sheet
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)

IPC: C07D, A61K, A61P

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

SE,DK,FI,NO classes as above

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

EPO-INTERNAL, WPI DATA, PAJ, CHEMICAL ABSTRACTS

C. DOCUMENTS CONSIDERED TO BE RELEVANT

Category*	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
X	Sapelkin, V. M. et al; Screening for protein kinase CK2 inhibitors among 3-carboxy-4-aminoquinoline derivatives; Ukrainica Bioorganica Acta 2005, vol. 2, no. 1, 28-32, see page 30-31, abstract ---	1-4,7-8, 10-11
X	Kim, N. D. et al; Putative therapeutic agents for the learning and memory deficts of people with Down syndrome; Bioorganic & Medicinal Chemistry Letters 2006, vol. 16 no. 14, page 3772 - page 3776, see examples 4-6, abstract ---	1-4,7-8,10, 12-15

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

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

Date of the actual completion of the international search	Date of mailing of the international search report
2 October 2007	04-10-2007
Name and mailing address of the ISA/ Swedish Patent Office Box 5055, S-102 42 STOCKHOLM Facsimile No. +46 8 666 02 86	Authorized officer Anna Sjölund/EÖ Telephone No. +46 8 782 25 00

Form PCT/ISA/210 (second sheet) (April 2007)

INTERNATIONAL SEARCH REPORT

International application No.
PCT/SE2007/050358

C (Continuation). DOCUMENTS CONSIDERED TO BE RELEVANT

Category*	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
X	STN International, File REGISTRY; Registry Copyright 2002 ACS on STN 459418-98-5,459418-97-4, 459418-94-1, 459418-93-0, 459418-89-4, 459417-33-5, the whole document ---	1-11
X	US 5650415 A (TANG, PENG CHO ET AL), 22 July 1997 (22.07.1997), abstract, the whole document ---	1-18
A	WO 2004069250 A1 (ASTRAZENECA UK LIMITED), 19 August 2004 (19.08.2004), abstract ---	1-19
A	WO 02092571 A1 (ASTRAZENECA AB), 21 November 2002 (21.11.2002), abstract --- --------	1-19

International patent classification (IPC)

C07D 215/54 (2006.01)
A61K 31/4706 (2006.01)
A61K 31/4709 (2006.01)
A61P 29/00 (2006.01)
A61P 35/00 (2006.01)
A61P 9/10 (2006.01)
C07D 401/12 (2006.01)
C07D 407/12 (2006.01)

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Use the application number as username.
The password is **CDUIMCIERW**.

Paper copies can be ordered at a cost of 50 SEK per copy from PRV InterPat (telephone number 08-782 28 85).

Cited literature, if any, will be enclosed in paper form.

INTERNATIONAL SEARCH REPORT

International application No.
PCT/SE2007/050358

Box No. II Observations where certain claims were found unsearchable (Continuation of item 2 of first sheet)

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

1. ☒ Claims Nos.: 19
because they relate to subject matter not required to be searched by this Authority, namely:

Claim 19 relates to a method of treatment of the human or animal body by therapy, as well as diagnostic methods /Rule

.../...

2. ☒ Claims Nos.: 1-11
because they relate to parts of the international application that do not comply with the prescribed requirements to such an extent that no meaningful international search can be carried out, specifically:

The initial phase of the search revealed a very large number of compounds relevant to the issue of novelty. So many

.../...

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

Box No. III Observations where unity of invention is lacking (Continuation of item 3 of first sheet)

This International Searching Authority found multiple inventions in this international application, as follows:

1. ☐ As all required additional search fees were timely paid by the applicant, this international search report covers all searchable claims.

2. ☐ As all searchable claims could be searched without effort justifying an additional fee, this Authority did not invite payment of any additional fee.

3. ☐ As only some of the required additional search fees were timely paid by the applicant, this international search report covers only those claims for which fees were paid, specifically claims Nos.:

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

Remark on Protest

☐ The additional search fees were accompanied by the applicant's protest and, where applicable, the payment of a protest fee.

☐ The additional search fees were accompanied by the applicant's protest but the applicable protest fee was not paid within the time limit specified in the invitation.

☐ No protest accompanied the payment of additional search fees.

Box II.1

39.1(iv). Nevertheless, a search has been executed for this claim. The search has been based on the alleged effects of the compounds.

Box II.2

compounds were retrieved that it is impossible to determine which parts of the claims may be said to define subject-matter for which protection might legitimately be sought (Article 6 PCT). For these reasons, a meaningful search over the whole breadth of the claims is impossible. Consequently, the search has been restricted to:

For compounds, which are present in patents or other documents a full search has been done. However, as so many relevant compounds known only from STN file REGISTRY or CHEMCATS were retrieved, only a selection of such compounds has been given.

US	5650415	A	22/07/1997	NONE		
WO	2004069250	A1	19/08/2004	NONE		
WO	02092571	A1	21/11/2002	AT	284517 T	15/12/2004
				AU	8273901 A	13/02/2002
				BG	108325 A	30/11/2004
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				CA	2446717 A	21/11/2002
				CN	1286815 C	29/11/2006
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				EP	1387830 A	11/02/2004
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				SK	13712003 A	05/10/2004
				UA	76142 C	15/01/2004
				US	6830602 B	14/12/2004
				US	7037925 B	02/05/2006
				US	20040025483 A	12/02/2004
				US	20040248923 A	09/12/2004
				US	20060173034 A	03/08/2006
				ZA	200308350 A	27/01/2005