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SUPERINTENDENCIA DE INDUSTRJA Y C(111111 11111 IlIlIllIflI
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Referenda: Expediente No. 08.044.912
Solicitud de Patente de Invención titulada: "Combinaciones anti neoplasicas
con inhibidor de objetivo de rapamicina en mamiferos, herceptina, yb hki-
272"

Solicitante: Wyeth

S0LIcITuD DE EXAMEN DE PATENTABILIDAD SEGUN EL ARTICULO 44 DE LA DECISION
486. GACETA No. 602

ALVARO CORREA ORD61EZ, mayor de edad y vecino de Bogota D.C., identificado segi’ill
aparece al pie de mi firma, en ml condición de apoderado de la sciedad arriba mencionada,
solicito que se examine la invención de la referencia seguin los requisitos de patentabilidad Iprevistos en la DecisiOn 486 de la Comisión de La Comunidad Andina de Naciones. Adjunto . recibo de caja por valor de $452.000 para cubrir la tasa correspondiente segOn la Resolución 55819 del 26 de diciembre de 2008.

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Anexo: Recibo de caja No. 09-48,637 por valor de $452.000
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NOTA: Dentro del plazo de los seis (6) meses siguientes a partir de la fecha de publicaciôn en la gaceta de Propiedad Industrial, deberÆ solicitar y cancelar el examen de patentabilidad so pena de qua la solicitud caiga en abandono, de acuerdo con lo establecido en el ArtIculo 44 de la Decision 486/2000.

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V.

SE PRESENTARON OPOSICIONES FOR PARTE DE TERCEROS.

SI NO X

[CANCER RESEARCH 64, 3958-3965, June I 2004]

Antitumor Activity of HKL-272, an Orally Active, irreversible Inhibitor of the

HER-2 Tyrosine Kinase

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ABSTRACT

HER-2 belongs to the ErbB family of receptor tyrosine kinases, which
has been implicated in a variety of cancers. Overexpression of HER-2 is
seen in 25-30% of breast cancer patients and predicts a poor outcome in
patients with primary disease. Trastuzumab (Herceptin), a monoclonal
antibody to HER-2, is specifically approved for HER-2-positive breast cancer but is active only in a subset of these tumors. Blocking HER-2 function by a small molecule kinase inhibitor, therefore, represents an attractive alternate strategy to inhibit the growth of HER-2-positive tu-mors. lfKI-272 is a potent inhibitor of HER-2 and is highly active against LILR-2-overexpressing human breast cancer cell lines in vitro. It also

Shibits

the epidermal growth factor receptor (EGFR) kinase and the oliferation of EGFR-dependent cells. 111(1-272 reduces HER-2 receptor autophosphorylation in cells at doses consistent with inhibition of ecU proliferation and functions as an irreversible binding inhibitor, most likely by targeting a cysteine residue in the ATP-binding pocket of the receptor. In agreement with the predicted effects of HER-2 inactivation, 111(1-272 treatment of cells results in inhibition of downstream signal transduction events and cell cycle regulatory pathways. This leads to arrest at the G,-S (Gap 1/DNA synthesis)-phase transition of the cell division cycle, ultimately resulting in decreased cell proliferation. In vivo, HKI-272 is active in HER-2- and EGFR-dependent tumor xenograft models when dosed orally on a once daily schedule. On the basis of its favorable preclinical pharmacological profile, HKI-272 has been selected as a candidate for additional development as an antitumor agent in breast and other HER-2-dependent cancers.

INTRODUCTION

The ErbB family of receptor tyrosine kinases consists of four members: epidermal growth factor receptor (EGFR; also called HER- I. ErbB-I): HER-2 (neu. ErbB-2); HER-3 (ErbB-3); and HER-4 (ErbB-4) [reviewed in Refs. 1, 21. All four receptors are tyrosine-laotein kinases and consist of an extracellular ligand-binding domain, single membrane-spanning region, and intracellular tyrosine kinase and regulatory domains. A variety of peptide ligands bind to the receptors (except HER-2) with different affinities and overlapping specificity. Ligand-dependent receptor oligomerization (horno- or bet-erodimerization) results in autophosphorylation at the regulatory do-main and intracellular signal transduction, ultimately leading to in-creased cell proliferation. No ligand has been identified for I ILR-2; instead, this protein functions as a coreccptor by binding to other receptors in the family.
Deregulation of growth-factor signaling due to hyperactivation of the ErbB receptors (primarily EGFR and HER-2) is seen in several cancer types (2, 3). Activation of EGFR may be because of overex-pression, mutations resulting in constitutive activation, or autocrine expression of lig’and. In contrast, activation of HER-2 occurs mainly

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by overexpression, which leads to spontaneous homodimcrization and activation of downstream signaling events in a Iigand-independent manner (1--4). The role of HER-2 has been most thoroughly studied in breast cancer, where it is overexpressed in 25-30% of cases and is correlated with a poor prognosis (3-6). Overexpression occurs pri-marily as a result of gene amplification. IIER-2 overexpression is also seen in ovarian cancer (6), lung cancer (especially lung adenocarci-nomas; Refs. 7-9), and in hormone-refractory prostate cancer (lO, II).
The only agent currently approved for HER-2-positive breast can-cer treatment is trastuzumab (Heree tin), a humanized monoclonal antibody against the extracel ular domain of HER-2. Although tras-tuzumab treatment has rapidly become the standard of care for HER-2-positive breast cancer, it shows only a 15% response as mono-therapy and a 49% response in combination with paclitaxcl (12. 13). Optimal responses are observed in patients with HER-2 gene ampli-fication (14). Trastuzumab appears to exert its antitumor effect by accelerating the internalization and degradation ofthe IIER-2 receptor (15), antibody-dependent cell-mediated cytotoxicity (15), and antian-giogenie activity (16, 17). The goal of this study was to identify a small molecule HER-2 antagonist that, unlike trastuzumab. directly blocks HER-2 kinase activity and would offer an alternative approach to the treatment of HER-2-positive cancers. The ErbB family has been the target of drug discovery efforts at Wyeth and has resulted in the development of EK13-569, an irreversible-binding inhibitor of EGFR, currently in clinical trials for EGFR-dependent tumors. This com-pound is predicted to covalently modify a cysteine residue (cysteine-
773) within the ATP binding site of the kinase (18).EKB-569 shows poorer efficacy in HER-2-dependent tumor models than in EGFR-dependent models (19). Therefore, a compound that is more potent than EKB-569 in HER-2-expressing tumors will complement the activity of this compound in the clinic. Because lIER-2 is highly homologous to EGFR in the catalytic domain, with conservation of the targeted cysteine residue (as cysteine-805: Ref. 20), synthetic efforts were focused on the chemical scaffold of EKB-569 (4-anili-noquinoline-3 -carbonitrile), which was modified to improve the in-hibitory activity against HER-2. The biological properties of HKI-
272. the lead compound from this effort, are described.

MATERIALS AND METHODS

Cell Culture. Cells were maintained in RPMI 1640 (lnvutrogen-l...ufe Tech-nologies. Inc., Gaithersburg, MD) containing 10% FBS (Invitrogen) and 50 j.cglml gentamicin (Invitrogen) at 37°C in a humidified incubator, under 5-10% CO.,. A431, BT474, SK-Br-3. MDA-MB-435, and SW480 cells were obtained from the American Type Culture Collection (Manassas, VA); 3T3 and 313/nen cells were provided by Dr. Mary L. Disis (University of Wash-ington. Seattle, WA).
Purification of HER-2 and EGFR Cytoplasmic Domains. The cytoplas-mic domain of HER-2 (amino acids 676-1255) and EGFR (amino acids 645-1186) was clotted into baculovirus expression vectors [EGFR: pCRBac/Bac-N-Blue (Invitrogen); HER-2: pFastBacllTc (Invitrogen)j. Both constructs contain an Nil,,-terminal 6X-histidine tag to facilitate purification Sf9 insect (Invitrogen) were cultured in Grace’s insect medium (Invitrogen) sup-

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plemented with 10% FBS and 50 jig/ml gentamicin. For protein production, recombinant viral stock was used to infect St cells (multiplicity of infec-tion = 10) for 3 days. Infected cells were lysed in 50 mM HEPES (p1...1 7.5), 10 nmi NaCl, 1% Triton X-100, 10 rxs ammonium molybdate, 100 tkm sodium vanadate, 10 tkglml aprotinin, 10 ig/m1 leupeptin, 10 gg/ml pepstatin, 1 mm Pcfahloc SC, and 16 ig/ml benzamidine MCI (all chemical reagents obtained from Sigma-Aldrich, St. Louis, MO). Lysates were clarified by centrifugation and fractionated by nickel affinity chromatography (Nickel-NTA superfiow Qiagen. Valencia, CA). In brief, lysates were incubated with affinity resin in 50 mm sodium phosphate (pH 8), 300 mi NaCl, and 5 mri imidazole for 25 rnin at 4°C and packed into a column for washing and elution. Nonspecific proteins were removed by washing the column with buffer containing 100 mm imidazole. Specifically bound proteins were eluted with buffer containing 250 mM imidazole. Purified proteins were dialyzed against 20 mmvi HEPES (pH 7.5). 50 mm NaCI, 10% glycerol, and 1 g/ml protease inhibitors and stored at  80°C in single-use aliquots. Purity was determined by SDS-PAGE, followed by staining with Coomassie Blue R250 (Bio-Rad, Hercules. CA). Purity was estimated to be >80%.

Kinase Assays. Activity of HER-2 and E’GFR cytoplasmic domains was measured by an autophosphorylation assay using time-resolved fluorometiy (DELPHIA; Wallac/Perkin-Elmer Life Sciences, Boston, MA). Compounds were prepared as 10 mg/ml stocks in DMSO and diluted in 25 mm HEPES (pH 7.5: 0.002 ng/ml-20 jrg/mI). Enzyme [diluted in 100 mm HEPES (pEt 7.5) and

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temperature in 96-well ELISA plates (Maxisorb; Nalge None, Rochester, NY). The kinase reaction was initiated by the addition of 40 (LM ATP and 20 mm MgCl2 and allowed to proceed for 1 h at room temperature. Plates were washed, and phosphorylation was detected using Europium-labeled anti-phos-pho-tyrosine antibodies (IS ng/well; Wallac). Alter washing and enhancement steps according to the manufacturer’s recommendations, signal was detected using a Victor2 fluorescence reader (Wallac; excitation wavelength 340 min, emission wavelength 615 nm). The concentration of compound that inhibited receptor phosphorylation by 50% (IC50) was calculated from inhibition curves. Assays for other kinases were performed using recombinant enzymes ex-pressed in bacterial, insect, or human cell lines. All enzymes used were serine-threonine kinases, except c-Met, KDR, src (tyrosine kinases), and MEK I (dual specificity), Substrates used were peptides (Akt, IKK-2, MK2. PDKI, src, and Tpl2), proteins cyclin Dl/CDK4, cyclin E/CDK2, cyclin l.31/CDKI, and c-Rat), poly(glutamic acid4-tyrosine) (KDR), or the kinase itself (autophosphorylation; c-met). Phosphorylation was measured using TMB peroxidase substrate (Pierce, Rockford, IL) for cyclin/cyclin-dependent kinase (cdk), LabChip (Caliper Technologies. Mountain View, CA) for MK-2, or DELI>! hA/i.ANCE (Wallac) for all others.
Cell Proliferation Assays. Cells were plated in 96-well tissue culture 6,1tesa (3T3, 3T3/neu, 5000 cells/well; A43 I, SK-Br-3, BT474, MDA-MB-435, d SW620, 10,000 cells/well). The following day, dilutions of compound (0.5 ng/mI-5.rg/ml) were added, and cells were cultured for 2 days (6 days for BT474). Cell proliferation was determined using sulforhodamine B, a protein binding dye. Briefly, cells were fixed with 10% trichloroacetic acid and washed extensively with water. Cells were then stained with 0.1% sulforho-damine B (Sigma-Aldrich) and washed in 5% acetic acid. Protein-associated dye was solubilized in 10 mivt Tris, and absorbance was measured at 450 nM (Victor2). Inhibition of cell proliferation was calculated using the formula: percentage of inhibition = 100 - 100 (Td - T,>/T - T0), where Td is the absorbance of drug treated cells, T is the absorbance of untreated cells, and T0 is the absorbance at the time of drug addition. T0 values were determined by plating cells separately and fixing them at the time of drug addition. The concentration of compound which inhibits cell proliferation by 50% (IC50) was determined from inhibition curves.

Protein tinmunoblotting. For analysis of receptor phosphorylation. cells BT474 or A431 cells) were incubated with various concentrations of HKI-272 tbr 3 h. For A431 cells. EGF (100 ng/ml; R&D Systems, Minneapolis, MN) was added to the cells during the last 15 mm. In experiments designed to evaluate reversibility of inhibition, compound-containing medium was re-moved after 3 h and replaced with fresh medium without compound. Incuba-tion was then continued for 5 h, with two additional changes of medium during the incubation. For analysis of signal transduction and cell cycle regulatory pathways, cells were incubated with HKI-272 overnight. After treatment, cells

were washed twice in PBS (Invitrogen) and lysed in 50 mm. iris (pt I 7.6), 150 NaCl,nim 2 mnam EUfA, 1% NP40. 1 mM phenylmethylsulfonyl fluoride, 5 g/mI aprotinin, 5 g/ml leupeptin, and 0.2 mvt sodium vanadate. Alterna-tively, cells were lysed directly in NuPAGE sample buffer (Invitrogen). Lysates were clarified by centrifugation. and protein concentration was esti-mated using the Bio-Rad or Bio-Rad Dc protein assay. Proteins were separated by SDS-PAGE (Ready Gels; Bio-Rad or Novex; Invitrogen) or by WAGE (Invitrogen) and transferred to nitrocellulose (Bio-Rad). Nonspecific sites were blocked, and blots were incubated with antibody solutions,. After washing and incubation with secondary antibodies, blots were developed using enhanced chemiluminescence (Amersham Biosciences, Piscataway, NJ). The following antibodies/conjugates were used: phospho-tyrosine-horseradish peroxidase conjugate (BD Biosciences PharMingen, San Diego, CA), EGFR, HER-2, retinoblastoma gene product (Rb), cyclin DI (Santa Cruz Biotechnology, Santa Cruz, CA), phospho-mitogen-activated protein kinase (MAPK) and MAPK (Biosource, Camarillo. CA), phospho-Akt and Akt (Cell Signaling Technolo-gies, Beverly, MA). Blots were scanned and quantified using FloorS Multi-Image analyzer (Bio-Rad).
Binding Studies with 114CIUKL-272. Recombinant HER-2 cytoplasmic
domain (100 ng) was incubated with 1 tkM 14CIHKI-272 (specific activity 55 mCi/mmol: Amersham) in the absence or presence of 5 M unlabeled FIKI-272 for 15 min on ice. Samples were boiled in SOS sample buffer, and proteins were separated by SOS-PAGE. Gels were treated with autoradiographic en-hancer (En3Hance; NEN, Boston. MA), dried, and exposed to film. For labeling cells, 5 X 10 BT474 cells were treated with 1 jzM [14C]HKI-272 for 2 h at 37°C or pretreated with unlabeled HKI-272 (5 IztI) for I h at 37°C before addition of label. Cells were washed with PBS, boiled in SDS sample buffer. and analyzed by SDS-PAGE and fluorography. A separate sample of identi-cally labeled cells was lysed in 10 mm Tris (pH 7.5). 5 mm EDTA, 150 mM NaCl, 1% Triton X-100, 1% sodium deoxycholate, 0.1% SDS, 1 mM phenyl-methylsulfonyl fluoride, 10 cg/niI pepstatin, 10fig/ml Ieupeptin. It) zg/mI aprotinin, 2 mm sodium vanadate, and 100 mm sodium fluoride and immuno-precipitated with 4.rg of anti-HER-2 antibodies (Santa Cruz Biotechnology) for I h at 4°C. Immune complexes were collected on protein A-agarose heads (Invitrogen), boiled in SDS sample buffer, separated by SDS-PAGE, and detected by lluorography.
Cell Cycle Analysis. BT474 cells were incubated with HKI-272 (0-2 nvi) in 6-well clusters overnight. Immediately before collection, cells were pulse labeled for 30 min with 10 zM bromodeoxyuridine (Sigma-Aldrich). Cells were collected by trypsinization, washed, and fixed in 80% ethanol for 15 mm at 20°C. After acid denaturation, permeabilization, and neutralization, cells were stained with anti- bromodeoxyuridine-F ITC conjugates (BD Biosciences, San Jose, CA), counterstained with propidium iodide (Sigma-Aldrich), and analyzed by flow cytometry (FACSor1; BE) Biosciences). Data were analyzed using CEI,LQuest software (BD Biosciences).
Tumor Xenograft Studies. Tumor cells (maintained in tissue culture) or tumor fragments were implanted s.c. in the flanks of female athymtc (nude) mice (Charles River Laboratories., Wilmington, MA). For estrogen-dependent cell lines (BT474, MCF-7, and SK-OV-3), animals were implanted with hormone pellets (0.72 mg of 17-0 estradiol, 60-day release; Innovative Re-search of America, Sarasota, FL) 1 week before implantation of tumors. Additionally, SK-OV-3 cells were suspended in Matrigel basement membrane matrix (BE) Biosciences, Billerica, MA) for implantation. Treatment was initiated after tumors had reached a size of 90-200 mg, following random assignment of the animals to different treatment groups (staging, day 0). For 31’3/ncu xenografts, treatment was initiated the day after tumor implantation (day 0). HKJ-272 was formulated in 0.5% methocellulose-0,4% polysorbate-80 (Tween 80) and administered daily, p.o., by gavage. Tumor mass [(length X width2)/2] was determined every 7 days. Tumor outgrowth in all xenograft studies, except 3T3/neu, was expressed as relative tumor growth: the ratio of the mean tumor mass to the mean tumor mass on day 0. Inhibition of tumor growth was calculated relative to vehicle-treated controls. Statistical significance of inhibition was demonstrated using one-tailed Student’s / test (equal variance) after log transformation of the data.
HER-2 Phosphorylation in Xenografts. Aihymic female nude mice (5 animals/group) were implanted s.c. with BT474 tumor fragments (-30 m1113). When tumors reached 200-300 mg, animals were given a single oral dose (40 mg/kg) of HKI-272 in p11 2.0 water. Tumors from control and treated animals were excised at 1, 3, 6, and 24 h and minced. Tumor fragments were suspended 3959

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Fig. 1. Structure of HKI-272 (M, 55705)

Table I lnh:bi0on of ERR/3 kinases by HKI-272°

Enzyme IC.0 (nu)"
FIER-2 59 – 13 in = 5)
Epidermal growth factor receptor 92 – 17 5)
Purified recombinant COOFI-tenninal fragments of HER-2 and epidermal growth factor receptor were incubated with AT!’ in the absence or presence of a range of compound concentrations. Autophosphorvlation of the receptors was determined using phosptio-tyrosine antibodies. The concentration of compound that inhibits phosphoryla-lion by 50% shown.
Mean and SE with the number of independent determinations in parentheses.

in 10 mm Tris (pH 7.5), 5 mm EDTA, 150 mm NaCI, 1% Triton X-lOO, 1% sodium deoxycholate, 0.1% SDS, I mvt phenylmethylsulfonyl fluoride, 10 g!ml pepstatin, 10 gg/ml leupeptin, 10 jighril aprotinin, 2 mm sodium vanadate, and 100 mrsa sodium fluoride and lysed by homogenization on ice with a polytron. After clarification by centrifugation, protein concentration in lysates was estimated using the Bio-Rad Dc protein assay. Sixty.cg of lysate pooled from each group were analyzed by SDS-PAGE and immunoblotting with phospho-tyrosine-specific antibodies. Pooled extracts were also immuno-precipitated using 4 jig of anti-IIER-2 antibodies for I It at 4°C. Immune complexes were collected on protein A-agarose, washed, and analyzed by imniunoblotting using phospho-tyrosine-specific antibodies. Extracts from in-dividual tumors were analyzed to determine variability between animals.

RESULTS

Kinase Assays. In a cell-free autophosphorylation assay using the recombinant cytoplasmic domain of HER-2, fIKl-272 reduced kinase activity by 50% (IC5) at a concentration of 59 tIM (Fig. 1 and Table I). It also inhibited the kinase activity of the EGFR cytoplasmic domain under similar assay conditions at 92 rim. IIKI-272 did not  gniticantlY inhibit several serine-threonine kinases tested (Akt, cy-din D1/cdk4, cyclin EIcdk2, cyclin BI/cdkl, IKK-2, MK-2. PDKI, c-Rat’, and Tpl-2) or the tyrosine kinase, c-met (Table 2). It weakly inhibited two other tyrosine kinases tested. KDR and sre (IC’50 800 and 1400 ntt, respectively; Table 2), but was 14- and 24-fold less active against these kinases, compared with HER-2. HKI-272 therefore, a highly selective inhibitor of HER-2 and EGFR.
Cell Proliferation Assays. The activity of HKI-272 was evaluated in a panel of cell lines with varying levels of expression of EGFR and lIER-2 (Table 3). HKI-272 repressed the proliferation of a mouse fibroblast cell line (3T3) transfected with the JIER-2 oncogene (3’1’31 ,ieu) by 50% (IC at 3 nsi. This value was 230-fold lower than that obtained with the isogenic untransfected cells (IC50 700 nvi), demon-strating that 1-IKI-272 has a high degree of selectivity for this onco-genie pathway. HKI-272 also inhibited two other HER-2-overexpress-ing breast cancer cell lines, SK-Br-3 and BT474 (IC,, 2 nM), but was much less active on MDA-MB-435 and SW620 (a breast and a colon cancer cell line, respectively) that are EGFR- and HER-2-negative. Consistent with its activity against EGFR, 1-IKI-272 inhibited prolif-eration of the epidermal carcinoma cell line. A43 1. that overexpresses EGFR 0051 81 rim).

Receptor Phosphorylation. The ability of HKI-272 to inhibit the function of the target receptor was determined by measuring receptor autophosphorylation in intact cells exposed to the compound (Fig. 2 and Table 4). HKI-272 decreased ligand-independent receptor phos-phorylation by 50% (IC,,) at 5 nm in BT474 cells, consistent with its effects on cell proliferation. It also repressed EGF-dependent phos-phorylation of EGFR in A431 cells at a comparable dose (IC50 3 nM), which is lower than the IC, value in the cell proliferation assay (8 I nM; compare Table 4 with Table 3).
Irreversible Binding to HER-2. EKB-569 has been previously shown to form a covalent adduct with EGFR (19). This is most likely because of the interaction between the Michael acceptor functional group of EKB-569 with cysteine-773 within the ATP binding site of EGFR. On the basis of the presence of the same Michael acceptor

Table 2 Inhibition of kinases by HKI-272

Kinsse - - IC50 (nu)’
Akt >20,000
Cyclin DI/CDK4 >50,000
Cyclin E!CDK2 >18,000
CyclinB I /Cf3l< I >18,000
IKK-2 >9,000
KOR 800
-Met >35,000
MK2 >45,000
POKI >5000
c-Raf/MEK1 >18,000
src 1,400
Tpl-2 >18,000
"The concentration of HKI-272 that inhibits activity by 50% is shown

Table 3 EfJct of HK!-272 on cell proliferation"

Cell line HER-2 Epidermal growth factor receptor IC511 (nM)°
3T3 700 78 In 5)
3T3!neu 3 0.14 (ii"’ 5)
SK-Br-3 -s 1-4 2 0.18(0 4)
BT474 -++ 2–006(n=4)
A431 ++– 81 –9(n 5)
Mt)A-Mt3-435 -- 960 165 (n -" 2)
SW620 690 84 In 5)
"Cells were incubated with various concentrations of compound for 2 days (6 days for 131474). Cell survival was determined using a protein binding dye assay. The concentra-
tion of drug that inhibits cell proliferation by 50% shown. Relative expression of and
epidermal growth factor receptor and l-IER-2 in the cell lines are indicated.
Mean and SE are shown with the number of independent determinations in paren-theses.

BT 474 HKI-272 OM)

0 2 10 50 100 200

Anti-pY ....

Anti-HER-2

A431 HKI-272 (nM)

0 0 2 10 50 100

Fig. 2, Effect of HKl.272 on receptor pliosphorylation. BT474 and A431 cells were incubated with the indicated concentrations of HKI-272 for 3 It at 37°C. For A431 cells, epidermal growth factor (EGF TOO ngiml) was added to the Cultures during the last 15 mm, Protein extracts were analyzed by SOS-PAGE and minniunoblotting using ptiospho-tyrosine antibodies. Identical parallel blots were incubated with antibodies to HER-2 and EGF receptor (EGFR) to demonstrate equal protein loading.

ANTITUMOR ACTIVITY OF HKl.27

Table 4 ft)’rt qf HK1-272 on HER.2-s,gnalingpathways and cell cycle regulation’ Signaling marker IC-,(, (nM)
Phospho-HER-2 5
Phospho-EGFR" 3
Phospho-MAPK 2
l’hospho-Akt 2
Cyclin Dl 9
cells in S phase 2
"In HER-2 and EGFR phosphorylation assays, cells (BT474 and A43 1, respectively) were incubated with various concentrations of HKl-272 for 3 hat 37"C. For evaluation of dowistream signaling markers. BT474 cells were incubated with lIKI-272 for 12 16 h at 37’C. Protein extracts were analyzed by irnmnnohlottmg using phospho-tvrosise, phos-pho-MAPK, phospho-Akt, or cyclin Dl antibodies Antibodies to UER-2, EGFR, MAPK. and Akt were used to correct for protein loading. Actin antibodies were used to normalize loading for cyclin Dl. Blots were quantified by densitometric scanning. Concentration of llKI-272 (nM), which inhibits phosphorylation (protein levels for cyclin Dl) by 50%. is given. For cell cycle analysis, BT474 cells were treated with FtKI-272 for 12-16 h. Untreated and treated cells were pulse labeled for 30 min with bromodeoxyuridine to label cells in S phase. Uptake of label was quantified using brornodeoxyoridine antibodiesiFlTC conjugates by flow cytometly. The concentration of HKI-272, which decreased the number of cells in S phase by 50%, is given.
EGFR, epidermal growth factor receptor; MAPK, mitogen-activated protein kinase.

group in HKI-272 and conservation of a cysteine residue at an analogous position in HER-2 (cysteine-805; Ref. 20), it is expected

, at HKI-272 will irreversibly inhibit HER-2. To examine this, recep-r phosphorylation in cells was measured immediately after or 5 h after removal of HKI-272 from the medium (Fig. 14). If binding is irreversible, the inhibitor will remain bound to the kinasc and will continue to block phosphorylation of the receptor after withdrawal of drug. In BT474 cells, HKI-272 (1 M) inhibited lIER-2 receptor phosphorylation by 98% compared with untreated controls, and no increase in phosphorylation was observed 5 h after removal of com-pound from the medium (97% inhibition). Similar results were seen in A431 cells, where l{Kl-272 inhibited receptor phosphorylation by >99%, and no recovery of phosphorylation was detected after with-drawal of the compound (105010 inhibition). In contrast, a related EGFR inhibitor that lacks the Michael acceptor group (control) showed almost complete reversibility of inhibition (63 to 28% in BT474: 95 to 20% in A431 cells) within the same time period.
To directly demonstrate covalent interaction between HKI-272 and 11ER-2, I14C111KI_272 was incubated with the purified cytoplasmic domain of HER-2. After complete denaturation, the sample was analyzed by SDS-PAGE and fluorography (Fig. 3B). A single-labeled species, M 95,000, corresponding to the HER-2 cytoplasmic do-main was observed, which was decreased in the presence of a 5 -fold cess of unlabeled compound. The reduction in signal occurred whether the labeled and unlabeled compounds were added together or protein was preincubated with unlabeled compound before addition of radiolabeled drug. In similar experiments with BT474 cells incubated with 114clHKI-272. a prominent hand, Mr 185.000. was labeled, and the extent of labeling was decreased when cells were treated with 5 -fold excess of unlabeled compound before exposure to the label (Fig. 38). Immunoprecipitation of the lysate with HER-2 antibodies produced a single species, Mr " 185,000, consistent with the expected size off’IER-2 (Fig. 38).
HER-2-Mediated Signal Transduction. The phosphorylation of I IER-2 leads to activation of the MAI3K pathway and the Akt signal transduction pathways. lIKI-272 effectively repressed phosphoryla-tion of MAPK and Akt in BT474 cells at concentrations consistent with the inhibition of cell proliferation (1C30 2 nM Fig. 4A and Table 4). In contrast, although trastuzumab inhibited the growth of BT474 cells in vitro (IC30 0.4 p.g/ml; unpublished observations), it failed to completely inhibit HER-2 receptor phosphorylation or downstream signaling events at concentrations up to 30 g/ml (Fig. 4B).
Cell Cycle Progression. Activation of growth factor receptor sig-naling pathways ultimately result in cell proliferation because of

increased progression through the G1 -S-phase boundary of the cell cycle. The effect of HKI-272 on cell cycle regulatory proteins (Fig. 5) and cell cycle phase transitions (Tables 4 and 5) was examined. HKI-272 repressed cyclin Dl expression by 50% at 9 flM in BT474 cells (Table 4). HKI-272 also reduced the phosphorylation of the Rh-susceptibility gene product at a similar dose, as indicated by a shift from the slower migrating phosphorylated forms to the faster-migrat-ing unphosphorylated forms in SDS-PAGE (Fig. 5). The total amount of Rb was also reduced by HKI-272 treatment. A concomitant in-

A 100 BT474

80

60

40

CL 20

to 11

-20 EI-272 CONTROL

100 A431 Z

80

U60

20 U.
0’ ’ we
- 20’ HKI-272 CONTROL

B - + + unlabeled 141(1-272
+ - Pro-incubation

 115
- 89

HER-2 (cytoplasmic domain)

+ + Unlabeled HKl-272

J

--208 184
 105

-" Immuno-

Cell lysates precipitation

Fig. 3. Irreversible binding of 111(1-272. A. irreversibility, ofphosphorylatton inhibition, BT474 and A431 cells were incubated with I ssi of the indicated compound for 3 It. and protein extracts were prepared. In separate parallel cultures, treated cells were washed and incubated in the absence of compound for 5 h. Protein extracts were analyzed by SDS-PAGE and irnmunoblotling using anti-phospho.tyrosmne antibodies. Blots were quantified by densitometric scanning. Results were normalized for protein loading using anti HER-2 (for BT474) and anti-epidermal growth factor receptor (EGFR. for A431) antibodies, The control compound lacks the Michael acceptor functional group and is a reversible binding inhibitor, 0, phosphorylation of receptor at the end of incubation period with compound;, phosphorylation levels 5 h after withdrawal of compound. B. binding of l4CIHKI-272 to HER-2. Top, liCJUKI,272 was incubated with HER-2 (cytoplasmic domain) in the absence (-’) or presence 1’) of excess unlabeled compound. Compounds were either added together, or labeled HKI.272 was added after a 10-nun preincsbation with unlabeled compound, as indicated. Samples were denatured by boiling in SDS sample buffer and analyzed by SDS-PAGE and fluorography, lhilioni left. [14C]HKl-272 was incubated with BT474 cells in the absence () or presence (’5) of excess unlabeled compound. Samples were denatured in SDS sample buffer and directly analyzed by SDS-PAGE and fluorograplmy. Bottom right, a second sample of labeled cells was lysed and im’nmunoprecipitsted with tIER-2 antibodies. The position of molecular weight markers (Al,, in thousands) are shown on the right.

3961

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ANTITUMOR ACTIVITY OF FIKI.272

A B9 4

KKI-272 (nM) Herceptin (igIml)

0 (N in 04 001- CO) 0 N

Phospho-MAPK Phospho-Tyr

Fig. 4. Effect of HKI.272 and trastuzuinab on

signal transduction BT474 cells were incubated IMIAPIK ___ with HKI-272 (A) or Herccptin (MedWorld Phar- HER-2

macy, Valley Cottage, NY B) for 12....16 h at 37’C.

Protein extracts were analyzed by inununoblotting Phospho-Akt using phospho-tyrosine, phospho-ntitogen-activated Phospho-MAPK protein kinase (MAPK), or phospho-Akt antibodies, Akt Antibodies to HER-2, MAPK, and Akt were used to

demonstrate equal protein loading, MAPK.........

ease in p27, an inhibitor of cell cycle progression, was observed.
onsistent with the effects on cyclin Dl, p27, and Rh phosphoryla-
tion, HKI-272 blocked cell cycle progression, causing a G1 -S arrest
(Tables 4 and 5). A 50% decrease in the number of cells in the S
(I)NA synthesis) phase of the cell cycle was observed at a concen-
tration of 2 n (Table 4), consistent with the effects on cell prolifer-ation. This correlated with an increase in the number of cells in (i (Table 5). An increase in the number of cells with suh-G DNA content. indicative of apoptosis, was observed at concentrations > 2 nrvt (data not shown). No changes were observed at the G,-M (Gap2/mitosis) transition (Table 5).

HKI-272 (nM)

0 -

CychnDl 

p274060

. pRb

actin

Fig. 5. Effect of UKI-272 on cell cycle regulatory proteins. BT474 cells were incubated ssith FtKl-272 for 12 16 Ii at 37C. Protein extracts were analyzed by immunoblotting using cyclin Dl, p27, or retinoblasloina (Rb)-specific antibodies. Actin antibodies were used as control.

Table S EJfrcI of FIKI-272 on cell cvrk’ progression"

HKI-272 (nm) Sub-G1 G,-G, S S (BrdUrd)* G2M
o 8 61 21 26 Il
0.2 9 63 16 18 13
0.4 II 65 12 14 13
7 69 10 12 14
2 II 66 12 10 II
BT474 cells were treated with the indicated concentrations of i1Kl-272 for 12-16 Ii
at 37C Untreated and treated cells were pulse labeled for 30 min with bromodeoxyuri-dine (Brdljrd). Cells were stained with BrdUrd antibodies/FITC conjugates, counter-stained with propidiuns iodide, and analyzed by flow cytometry. The percentage of cells in sub-GI, G, S. and G. phases of the cell cycle are shown, as determined from the histogram of propidiuin iodide-stained cells. Additionally, the percentage of cells in S phase was confirmed by BrdtJrd uptake (-).’This data was use to calculate the lC shown it ’table 4.

Phospho-Akt - -

Akt

Activity in Tumor Xenografts. The in vivo activity of HKI-272 was studied in cells growing as xenografts in athymic (nude) mice. The activity of H.Kl-272 was first determined in xenografts of 3T3/neu cells. Treatment was initiated the day after tumor implantation in this model because of the rapid outgrowth of the tumors. IIKI-272 reduced tumor growth in a dose-dependent manner when administered orally between 20 mg/kg/day (53% inhibition, day 21) and 80 mg/kg! day (98% inhibition Fig. 6A). In a second independent test, HKI-272 showed antitumor effects between 10 mg/kg/day (34% inhibition, day
14) and 40 mg/kg/day (98% inhibition).
The effect of IKI-272 was next studied in xenografts of 11T474 (Fig. 6B). HKI-272 treatment repressed tumor growth when adminis-tered to animals between 10 mg/kg/day (67% inhibition, day 28) and 40 mg/kg/day (93% inhibition). In three other independent experi-ments, antitumor effects were observed between 5 mg/kg/day (70-- 82% inhibition, day 21) and 40 mg/kg/day (88-90% inhibition). To determine whether inhibition of tumor growth was correlated with inhibition of the target receptor, UER-2 phosphorylation was studied after administering a single oral dose (40 mg/kg) of HKT-272 to mice bearing 131474 xenografts (Fig. 7). Phosphorylation of HER-2 was inhibited by 84% within 1 li of administration. Inhibition was sus-tained at 6 h (97%) and decreased to 43% over 24 h.
HKI-272 was also effective against xenografts of SK-OV-3. a HER-2-overexpressing human ovarian carcinoma cell line (Fig. 6(7). HKI-272 repressed tumor growth in a dose-dependent manner, with maximum inhibition observed on day 21. Here, statistically significant inhibition of tumor growth was observed at doses between 5 mg/kg/day (31% inhibition) and 60 mg/kg/day (85% inhibition). Similar results were observed in two other independent experiments. Because HKI-272 inhibits EGFR kinase and the growth of EGFR-overexpressing cells in vitro, the effect of the compound in A431 xenografts was evaluated (Fig. 61)). Maximum inhibition of tumor growth was observed at the 40 mg/kg/day dose (76% inhibition on day 15). Inhibition was also observed at lower doses from S mg/kg/day (32%) to 20 mg/kg/day (44%). i’lowever, repression of growth in this tumor model was less than that seen with comparable doses in HER-2-dependent tumors. Therefore, HKI-272 is less potent against EGFR-dependent tumors than HER-2-dependent tumors in vivo, al-though it has equivalent activity against the two kinases in vitro.
i’o determine the specificity of the antitumor effects of HKJ-272 for cells expressing I IFR-2 or EGFR, the effect of the compound was
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ANTITUMOR ACTIVITY OF HKI.272

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- 9-VEHICLE

8 -4- HKI 272 (80) 19 HKl-272(40)

0
0 10 20 30
DAYS

studied in MCF-7 xenografts (Fig. 6E). This human breast cancer cell We expresses low levels of both receptors and is resistant to HKI-272 in vitro (IC/() 5 fLM). Treatment of animals with up to 80 mg/kg/day had only a marginal effect on tumor growth (28% inhibition, day 28), and no dose response was observed. HK1-272 was also inactive in xenografts of MX-1, a second human breast cancer cell line that expresses low levels of HER-2 and EGFR (data not shown).

DISCUSSION

Fig. 6. In vivo activity of llKl-272,.4, groups of 10 mice were implanted with 2 >< 106 31’3/neu cells. Animals were treated with the vehicle or HKI-272 on days I -ID (p.o.). beginning the day after implantation. IS 1/, mice were implanted with BT474 tumor fragments (30 mm’s; 8). 5 X 101 SK-OV-3 cells (C), 5 X 10° A431 cells (I)). or MCF.7 tumor fragments (30 mm’: 1/). Tumors were staged, and groups of 5 mice f1 for controls) were assigned to treatment groups. Animals were treated with the vehicle or HK1-272 orally, once daily for 20 days (10 days for A431 tumors). *. 1’ < 0,05 (Student’s I test). , 10 mg/kg/day; l, 20 mg/kg/day: A, 40 mg/kg/day; (>, 60 mg/kg/day; , SO mg/kg/day.

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HKI.272 Z
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Her-2 __

EIKI-272 is a potent inhibitor of the HER-2 and EGFR kinases in 20
vitro. It also blocks activity of IIER-4 (data not shown). In contrast, 01.
the structurally related molecule, EKB-569, is a potent inhibitor of 0 ’ 4 8 12 16 20 24 28 EGFR (IC51) 39 ovi in an autophosphorylation assay), whereas it is TIME (h)
substantially less active toward IIER-2 (1C 1255 nM Ref. 19). Fig 7 Inhibition of HER-2 phosphorytation in sno1-ratls B 1474 tumorbearing mnici,
l-IKI-272 was designed using a homology model for the catalytic s animals/group) were treated with vehicle or a single dose of itKl-272 Tumors from
domain of HER-2 kinase that was built using a combination of two control and treated mice were dissected at various times after compound administration.
closely related crystal structures as templates. fibroblast growth factor Protein extracts from each time point were pooled and analyzed by SDS-PAGE and

tmnntunobloltmng. Left, protein insmunoblots. Phosphorylatmon of HER2 was detected
receptor-I kinase for the NH..-terminal lobe and hematopoictic cell using pisospho-tyrosine antibodies. HER-2-specific antibodies were used as controls for
k indi. for the C0011terminal lohi. (18 21) The core structure of protein loading, Right, raphm il representation 01 the iritmmmnoblot data Blots were

quantified by scanning densitometry. Mean and SDs are shown. SDs were obtained by
111(1-272 is similar to the structure of LK13-569 but possesses a quantifying phosphorvlation of HER-2 in the individual tumors. Identical results were
different anilino headpiece (compare ’Fig. 1 with Ref. 19). The binding obtained when lysates were iminunoprecipmtated with anti -HER-2 antibodies.
3963

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ANTITUMOR ACTIVITY OF HKI-27

model for HKI-272 at the ATP site in HER-2 indicates that the aniline portion of the molecule fits into a long lipophilic pocket. Therefore, a lipophilic 2-pyridinylmethyl moiety was placed at the para-position of the aniline and a lipophilic chlorine atom at the meta-position. The nature and placement ofthese groups most likely gives this compound its improved HER-2 activity compared with EKB-569.
1lKl-272 interacts directly with its target enzyme and forms a covalent complex. This was suggested by the sustained inhibition of phosphorylation after withdrawal of drug from the medium and con-firmed by direct labeling of HER-2 by [14CJ1-lKl-272. This feature is expected to allow the compound to compete effectively with the high concentrations of cellular ATP and inhibit kinase activity for the extended periods of time needed to affect tumor growth. HKI-272 has a Michael acceptor functional group at the 6-position (Fig. I). Such groups are known to be reactive toward sulthydryl groups. On the basis of the binding model, cysteine-805. located within the catalytic cleft of FIER-2. is ideally positioned for covalent interaction with HKI-272 docked in the binding site (18). The binding model, as well as reactivity studies..suggest that the dimethylamino group present at the end of the Michael acceptor group can serve as an intramolecular catalyst for the addition of HKI-272 to the protein, which may

Wcropelerate the reaction between the hound drug and the protein. This

ic effect probably gives HKI-272 its ability to selectively react
with the target enzyme (18). Using peptide mapping and mass spec-troscopic analyses, PD 168393, a structurally related irreversible-binding Erbil inhibitor, has been shown to covalently bind to cysteine-773 of E431:R (22).
1lKl-272 inhibits the proliferation of cell lines that show high levels of HFR-2 (31-3Ineu. SK-[3r-3, and BT474) and is much less active in cell lines that express neither HER-2 nor EGFR (313, MDA-MB-435, and SW620). However, not all cells which show high levels of HER-2 are sensitive to this drug. MDA-MB-361 expresses -40% of the receptors expressed by SK-Br-3 and BT474 cells (Refs. 23, 24 and unpublished observations). These cells are -85-fold less sensitive to inhibition by HKI-272 relative to sensitive lines (190 nsi IC01 com-pared with 2 nm in BT474). Although the mechanism underlying this lack of sensitivity is unknown, it could be because of coexpression of other Erbil receptors or their ligands, which can modulate the sensi-tivity of cells to kinase inhibitors (25) or the expression of insulin-like growth factor I receptor, which has been reported to change the sensitivity of cells to trastuzumab (26). HKI-272 inhibits ligand-’dependent H ER-2 phosphorylation in B 1474 cells at a dose similar
6r1T,

r

that required to inhibit cell proliferation. However. EGF-dependent ECiFR phosphorylation was inhibited at a lower dose (IC,,, 3 nM), compared with the effects on cell proliferation (IC,,, 81 nNl). This may he because of a requirement for more complete inhibition of EGFR phosphorylation in A431 cells to affect cell proliferation or lateral signaling from other members ofthe EGFR family or their ligands, as discussed above.
HKI-272 treatment of BT474 cells results in the inhibition of MAPK and Akt phosphorylation, down-regulation of cyclin Dl lev-els, and induction of p27. The ras-Raf-MAPK pathway and the phosphotidylinositol-3 kinase/Akt pathway are the two major down-stream signaling pathways initiated as a consequence of HER-2 re-ceptor activation (27. 28). The two pathways collaborate in regulating the cell cycle. Specifically, they are involved in transcriptional induc-tion and stabilization of a key cell cycle regulator, cyclin Dl (29). Recruitment and activation of CDK4 by cyclin Dl results in phos-phorylation and inactivation of Rb, a negative regulator of the cell cycle (30). This process is antagonized by p27"°, a cyclin-dependent kinase inhibitor (31) the transcription and stability of which are negatively regulated by both pathways (32). Ultimately, modulation of these critical regulatory circuits by HKI-272 results in a cell cycle

arrest. The inhibition by HK1-272 of the phosphotidylinositol-3 ki-nase/Akt pathway. which also controls cell survival (33). may he responsible for the apoptosis observed at higher doses of the com-pound. UKI-272 treatment of 81474 cells completely inhibits phos-phorylation of FTER-2. MAPK. and Akt. whereas trastuzumab treat-ment causes only a weak, partial inhibition over a wide range of concentrations (0-30 g/ml). This is most likely caused by the different mechanisms of action of the two agents. Trastuzumab-mediated inhibition of phosphorylation and signal transduction are most likely caused by the internalization of the HER-2 receptor. followed by degradation (15). The duration and extent of down-modulation depend on the balance between removal ofthe cell surface receptor due to antibody-mediated internalization and the synthesis of new receptor. In contrast, the effects of HKI-272 are caused by the immediate inhibitory effect on the kinase activity of the receptor. Furthermore. because antibody-mediated receptor internalization is influenced by the density of antigens at the cell surface. lIER-2 kinase inhibitors may be more effective than trastuzumab at inhibiting tu-mors with lower levels of IIER-2.
HKI-272 inhibits the growth of HER-2-dependent tumors
The minimum dose, which causes a statistically significant inhibition of tumor growth. is estimated to be 5-10 mg/kg/day. In these xe-nograft studies, HKI-272 was well tolerated by the animals, and no weight loss or other compound-related toxicity was observed. In agreement with the antitumor effects of HKI-272, inhibition of HER-2 phosphorylation was also detected in xenografts. The sus-tained inhibition of phosphorylation seen in the xenograft studies is consistent with irreversible inhibition of the target because the termi-nal hall’-life of HKI-272 after a single oral dose (20 mg/kg) in nude mice is --4 h (data not shown). The prolonged duration of inhibition supports the once-daily oral dosing used in the xenograft models. Although HKI-272 blocks the kinase activity of EGFR and inhibits the proliferation of EGFR-dependent cell lines, it is less potent in A431 tumor xenograft models than EKB-569, the EGFR inhibitor (19). This is unlikely to he due to the pharmacokinetic properties of the compound because these are similar to EKB-569 (data not shown). and it is quite effective at inhibiting the growth of HER-2-dependent tumors. This suggests that the sensitivity to kinase inhibitors, of A431 cells growing as monolayers, is different from the sensitivity when grown as xenografts.
Several other small molecule inhibitors targeting Erbil receptors are in preclinical or clinical development (34-36). As with HKI-272 and EKB-569, all compounds are designed to block kinase activity by binding to the AlP site of the enzyme, an approach that has been validated by (ileevec (imatinib mesylate), an inhibitor of the bcr-ahl tyrosine kinase for the treatment of chronic myelogenous leukemia (37). Iressa (gefitinib; Ref. 38), a reversible EGFR inhibitor, was recently approved for lung cancer treatment. Although this compound is much less active against HER-2 in cell-free systems (38), it has been reported to inhibit the growth of HER-2-expressing cells in vitro and in vivo (24, 39). This may he because of cross-inhibition of IIER-2 by EGFR in cells expressing both receptors. ’l’arceva (cr10-tinib) is a second reversible-binding EGFR inhibitor in clinical trials (35, 40). As a consequence of the high degree of homology between the kinase domains of HER-2 and EGFR, some Erbil antagonists directly inhibit both EGFR and HER-2 function, similar to HKI-272. Among these are C1-1033 (41), GW2016 (42), and PKI-l66 (43). Of these, only Cl- i 033 has been shown to bind irreversibly to EGFR and HER-2 (22). CP-654577 has recently been reported as a selective HER-2 inhibitor (44).
In conclusion, HKI-272 is a promising new anticancer drug candi-date for the treatment of breast cancers and other HER-2-dependent cancers. Because it also inhibits the EGFR kinase with similar p0-

3964

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ANTITUMOR ACTIVITY OF HKI-272

tency. it may be useful to treat tumors that overexpress both HER-2 and EGFR and be more efficacious than a specific EGFR or HER-2

antagonist. The observed modulation of downstream signaling path-

ways and cell cycle regulatory proteins by 11KJ-272 identifies addi-

ional drug targets in the growth factor response pathways (MA K, Akt. and cyclin Dl/CDK4). Inhibitors of these proteins may be useful in combination therapy with a HER-2 inhibitor. These targets also

provide convenient pharmacodynarnic markers (biomarkers) to mon-

itor lIKI-272 activity in clinical trials.

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3965

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165
SUPERINTENDENCIA DE INDUSTRIA Y COMERCIO DIRECCION DE NUEVAS CREACIONES

2020
Bogota D.C.,

Abogado
ALVARO CORREA ORDOIJEZ

ASUNTO RadicaciOn 08 044 912
TrÆmite 011
Evento 378
Actuaciôn 519

Oficio -7
Folios 8

Patente de Invención[J

Patente de Modelode

Via Nacional [J

Via PCT (Fase Nacional) [} Capitulo I Capitulo ii P1
No. Solicitud Internacional PCT/US2006/042915
Fecha de Presentación Internacional 02/Noviembre/2006

Como resultado del Examen de Fondo realizado, con fundamento en el Articulo 45 de la DecisiOn 486 de la ComisiOn de la Comunidad Andina, se comunica:

1. Documentos estudiados

Descripcion Folios 42 a 85 02/Mayo/2008
Reivindicaciones: Folios 86 a 94 02/Mayo/2008
Folios 144 a 149 28/Septiembre/2009
Dibujos Folio 96 02/Mayo/2008
Prioridad US60/733,562 04/Noviembre/2005
US60/837,509 14/Agosto/2006

2. AnÆlisis dela Prioridad

Se acepta la reivindicaciOn de Prioridad porque esta solicitud fue presentada dentro del plazo establecido (Art. 9 D 486) y su contenido tØcnico corresponde con el de la prioridad reivindicada.

3. Objeto de la invenciôn

Titulo de la solicitud: "Combinaciones antineoplÆsticascon inhibidor de objeto de rampicina en mamiferos, herceptina, y/o HKI-272"

Expediente 08 044 912 ler Art 45

166

El problema planteado en esta solicitud es la necesidad de combinaciones herceptina con un inhibidor de mTOR y/ HK1272 Utiles en el tratamiento de neoplasmas.

Para resolver este problema tØcnico la solicitud en estudio proporciona un producto que contiene una temsirolimus y una herceptina como una preparaciôn combinada ü tiles en el tratamiento de un neoplasma en un mamifero.

El objeto de la presente Solicitud se relaciona con la Clasificaciôn Internacional de Patentes (CIP): A 61 K 39/395.

4. Excepcion a la Patentabilidad (Art 20 D 486 literal d)

Aunque las reivindicaciones I a 3 se pretenden presentar como producto que contiene temsirolimus y una herceptina (reivindicaciOn 1), rapamicina y un HKI-272 (reivindicación 2), y herceptina y un HKI-272 (reivindicaciOn 3), como una preparación combinada para uso simultaneo, separado o consecutivo en el tratamiento de un neoplasma en un mamIfero, dichas reivindicaciones tal y como se encuentran redactadas corresponden en realidad a un mØtodo de tratamiento humano o animal y constituyen una excepciôn a la Patentabilidad de conformidad con el ArtIculo 20 de la Decision 486, literal d). Teniendo en cuenta 10 anterior, se requiere que el solicitante retire las reivindicaciones antes mencionas y sus dependientes del capItulo reivindicatorio.

Este hecho se evidencia cuando se realiza el estudio del capItulo descriptivo actualmente presentado y los ejemplos alli ilustrados los cuales corresponden
Unicamente a mØtodos de tratamiento de neoplasmas.

5. De la Solicitud de Patente

Reivindicaciones (Art 30 D 486, Art 22 PCT)

El capItulo reivindicatorio no es consistente con la descripción de la solicitud bajo
estudio, toda vez que las reivindicaciones actualmente presentadas estÆndirigidas a
. composiciones que comprenden temsirolimus y una herceptina, o rapamicina y un HKI-272, o herceptina y un HKI-272, las cuales no encuentran un soporte adecuado
en el capItulo descriptivo. En este sentido, es importante resaltar que los ejemplos
ilustrados en la descripciOn estÆndirigidos Unicamente a mØtodos de tratamiento que
consisten en la administraciOn secuencial de los compuestos activos antes mencionados y en ninguno de los casos se hace referencia a una composiciôn que
los comprenda.

Las reivindicaciones 4 a 6 se refieren a un "paquete farmacØutico" que contiene temsirolimus y una herceptina (reMndicaciOn 4), rapamicina y an HKI-272 (reivindicaciOn 5), y herceptina y un HKI-272 (reivindicaciOn 6). Este "paquete farmacOutico" corresponde en realidad ünicamente a "uno o mÆscontenedores" tal y como se define en el capitulo descriptivo de la presente solicitud (pagina 32, lineas 17-22; folio 73), y dichos contenedores no pueden ser caracterizados por la composición que contiene o el uso al cual estÆn destinados, sino que deben ser definidos en funciOn de sus caracteristicas tØcnicas esenciales tales como la disposiciOn de los elementos que lo conforman. Es necesario entonces caracterizar el contenedor reclamado en las reivindicaciones 4-7 mencionando especificamente

Expediente 08 044 912 ler Art 45

167

las caracterIsticas tØcnicas esenciales que lo hacen novedoso e inventivo frente al estado de la tØcnica y que estØn debidamente sustentadas en el capitulo descriptivo, o de lo contrario, se sugiere eliminar dichas reivindicaciones y sus dependientes del pliego reivindicatorio.

Las reivindicaciones 7 a 9 mencionan composiciones que comprenden temsirolimus y una herceptina (reivindicaciOn 7), rapamicina y un HKI-272 (reivindicaciOn 8), y herceptina y un HKI-272 (reivindicación 9) y al menos un portadorfarmacØuticamente aceptable, sin embargo dichas reivindicaciones no mencionan las proporciones en las que se encuentra presente cada uno los componentes mencionados. Se le recuerda al solicitante que una composicion debe ser definida en función de sus caracterIsticas tØcnicas esenciales las cuales corresponden a sus ingredientes esenciales (en este caso, el nombre especifico del fÆrmaco,solventes y excipientes utilizados en la preparacion de la composición) y la proporciôn en la que se encuentra cada uno de ellos. Teniendo en cuenta lo anterior, se requiere que se modifique las reivindicaciones 7 a 9 y sus dependientes de forma tal que la composición reivindicada estØ definida en funciôn de sus caracteristicas tØcnicas esenciales.

La reivindicaciôn 10 no es clara porque caracteriza Ia composicion reivindicada usando la expresión "uno o mÆs de los componentes activos se provee en cantidades subterapeuticas efectivas" la cual es ambigua y no permite entender el alcance de la invenciôn. Se requiere que dicha expresión sea limitada a un nUmero especifico de componentes, y el nombre especifico de los componentes activos y la proporcion en la que se encuentra en la composición.

La reivindicaciôn 11 no es clara porque caracteriza la composición reivindicada en función del neoplasma que trata. Se requiere modificar esta reivindicaciôn de tal forma que la composiciOn estØ caracterizada en funciOn de los componentes que comprende y sus proporciones, o se sugiere eliminar la reivindicación del capitulo
reivindicatorio.

La reivindicaciOn 12 no es clara porque la expresión "uno o mÆs" presente en la definición de la composicion es ambigua y no permite entender el alcance de la invención. Se requiere limitar dicha expresión a un nümero especifico. Las expresiones "agentes de alquilacion antineoplÆsicos", "agentes antineoplÆsicos antimetabolito", "inmunomoduladores bioqulmicos", "imatinib", "inhibidores de EGFR" y "inhibidode multiples cinasas" son muy amplias y abarcan un nUmero indeterminado de compuestos que no tienen sustento en el capitulo descriptivo.- Ase requiere limitar las expresiones antes mencionadas al nombre especifico de los compuestos utilizados en la preparacion de la composición reivindicada.

Las reivindicaciones 12 - 15 no son claras porque mencionan una composicion que comprende antineoplÆsicos, agentes antineoplÆsicos antimetabolito, y inmunomoduladores bioquimicos, sin hacer referencia a la proporción en la que se encuentran dichos componentes. En necesario modificar las reivindicaciones 12 a 15 de forma tal que la composiciôn se defina indicando las proporciones en las que se encuentra cada uno de sus componentes.

La reivindicaciôn 16 noes clara porque menciona una composiciOn que comprende "Un taxano". Es necesario limitar esta expresiOn a los compuestos especIficos utilizados en la preparaciôn de la composición, asi como tambiØn es necesario especificar la proporciOn bien limitada en la que se encuentra dicho componente dentro de la composición. Un observaciôn similar se puede realizar en las Expediente 08 044 912 ler Art 45

168

reivindicaciones 17- 19 que mencionan la presencia de un compuesto de formula A, temsirolimus y 42-0-(2-hidroxi)etil rapamicina dentro de la composiciOn sin mencionar la proporciOn en la que se encuentran dichos compuestos.

Las reivindicaciones 20-25 caracterizan la composiciOn reivindicada en funciôn a su resultado a alcanzar y la forma de cOmo se debe administrarse. Se requiere modificar las reivindicaciones antes mencionadas de tal forma que la composiciOn reivindicada estØ definida en funciôn de los componentes especificos que comprende y las proporciones en las que se encuentran o de lo contrario se sugiere ehminar dichas reivindicaciones del pliego reivindicatorio.

6. Determinación del Estado de la TØcnica

El Estado de la TØcnica se determinó con base en Ia fecha de presentaciOn de la Solicitud de Prioridad: Noviembre 04 de 2005 y se encontraron los siguientes documentos que anticipan el objeto de esta solicitud:

Item Documento TItfflo Fecha de Publicación Combination of mtor inhibitor
Dl W02004004644A2 and a tyrosine kinase inhibitor I 5/EneroI2004
for the treatment of neoplasms

RABINDRAN S K; ETAL.
Cancer Research, 2004 Antitumor Activity of HKI-272,
0101 American an Orally Active, Irreversible
D2 Association for Cancer Inhibitor of the HER-2 Tyrosine 2004
Research, US ISSN Kinase
0008-5472. VoI:64, Nr: 11,
Page(s):3958 3965

Dl
httD://worldwide.esDacenet.com/searchResults?DBEPODOC&localeen EP&ST=advanced&compact =false&PN=W02004004644A2 revela una combinación que comprende: Inhibidor mTOR tal como Rapamicina (pagina 6, lInea 5, pagina 4, lineas 24-26), Inhibidores de tirosina quinasa tales como: trastuzumab (Herceptina), Imatinib, SU 101, ZD1 839, CI-1033, entre otros (pagina 4, linea 28 a pÆgina 5 Ilnea 4), y portadores farmacØuticamente aceptables (pÆgina 9, lineas 26-29).

D2 folios (157-164) enseæa menciona queel "HKI-272" es un potente inhibidorde HER2 (pagina 3958, resumen y columna izquierda; pagina 3965, columna izquierda).

7. Examen de Patentabilidad (Art 14 D486)

El anÆlisis depatentabilidad de las reivindicaciones 4-6 y sus dependientes se realiza teniendo en cuenta que el "paquete farmacØutico" reclamado corresponde en realidad Unicamente a uno o mÆscontenedores tal y como se define en el capitulo descriptivo de la presente solicitud (pÆgina 32, lineas 17-22; folio 73), y dichos contenedores no pueden ser caracterizados por Ia composiciOn que contienen o el uso al cual estÆn destinados, sino que deben ser definidos en funciOn de sus caracteristicas tØcnicas esenciales tales como la disposiciOn de los elementos que lo conforman.

Novedad (Art 16 D486)

La reivindicaciOn 7 actualmente presentada consiste en una composiciOn farmacØutica que comprende: (a) por lo menos una unidad de Temsirolimus (inhibidor mTOR, 42-ester de rapamicina 0 CCI-779), y (b) Por lo menos una unidad

Expediente 08 044 912 Ier Art 45

169

de Herceptina (trastuzumab), y por lo menos un portador farmacØuticamente aceptable.

ComparaciOn de las caracteristicas tØcnicas esenciales, con las del Estado de la TØcnica:

CaracterIsticas esenciales Dl

Composiciôn farmaceutica que comprende: CombinaciOn que comprende (pagina31,Ilneas 9-10):

(a) Por lo menos una unidad de Rapamicina CCI-779 (pªgina 4, lIneas Temsirolimus (inhibidor mTOR, 42- 24-28), entre otros. ester de rapamicina o CCI-779)

Anticuerpos de tirosina quinasa tales
(b) Por lo menos una unidad de Herceptina como: trastuzumab (Herceptina) (pagina
5, linea 3), entre otros.
Por lo menos un portador Portador farmacØuticamente aceptable
farmacØuticamente aceptable. I (pagina 9, lineas 26-29)

Las caracteristicas esenciales de la composición farmacØutica descrita en la reivindicaciOn 7 actualmente presentada ya habian sido divulgadas previamente por el documento Dl, el cual enseæa unacomposicion farmacØutica que comprende (pagina 31, lIneas 9-10): Rapamicina CCI-779 (pagina 4, lIneas 24-28), Anticuerpos de tirosina quinasa tales como: trastuzumab (Herceptina) (pagina 5, linea 3) y portadores farmacØuticamente aceptable (pagina 9, Ilneas 26-29).

En consecuencia, la reivindicaciôn 7 carece de novedad a la’ luz de las enseæanzas del documento Dl.

Las reivindicaciones dependientes 10-24 no mencionan alguna caracteristica que haga nueva a la composición de la reivindicación 7 y por tal razón tampoco se consideran nuevas.

Las reivindicaciones 4-6 mencionan "un paquete farmacØutico" que contiene composiciones que comprenden inhibidores mTOR y inhibidores tirosina quinasa.

El documento Dl ya habla anticipado paquete o contenedor que contiene composiciones que comprenden inhibidores mTOR y inhibidores tirosina quinasa.

En consecuencia, las reivindicaciones 4-6 carecen de novedad a la luz de las enseæanzasdel documento Dl.

Nivel Inventivo (Art18 D486)

La reivindicación 8 consiste en una composicion farmacØutica que comprende: (a) Por lo menos una unidad de un Rapamicina (Inhibidor mTOR), (b) por lo menos una unidad de HKI-272 (inhibidor de tirosina quinasa), y por lo menos un portador farmacØuticamente aceptable.

Comparación de las caracteristicas tØcnicas esenciales, con las del Estado de la TØcnica:

Caracteristicas esenciales Dl D2

Composiciôn farmacØutica Combinación que comprende: Inhibidores tirosina quinasa

que comprende:

(a) Por 10 menos una Inhibidor mTOR tal como unidad de un Rapamicina (pagina 6, linea 5, No se menciona Rapamicina (Inhibidor pÆgina4, llneas 24-26). mTOR).

(b) Por lo menos una I Inhibidores de tirosina quinasa tales I ’HKI-272" es un potente

Expediente 08 044 912 ler Art 45

170
unidad de HKI-272 como: trastuzumab (Herceptina) inhibidor de HER2 (pagina
(inhibidor de tirosina Imatinib, SUIOI, ZD1839, CI-1033, 3958, resumen columna
quinasa) entre otros (pagina 4, lInea 28 a izquierda; pagina 3965,
pagina 5 linea 4). columna izquierda).

Por lo menos un portador Portador farmacØuticamente No se menciona

farmacØuticamente aceptable (pÆgina 9, Ilneas26-29) aceptable.

Los documentos considerados como pertenecientes al estado de la tØcnica de la invenciôn descrita en la reivindicación 8 actualmente presentada corresponden a Dl y D2 porque:

El documento Dl revela una combinaciOn que comprende: Inhibidor mTOR tal como Rapamicina (pagina 6, linea 5, pagina 4, lineas 24-26), Inhibidores de tirosina
quinasa tales como: trastuzumab (Herceptina), Imatinib, SU101, ZD1839, CI-1033,
entre otros (pagina 4, linea 28 a pagina 5 linea 4), y portadores farmacØuticamente
aceptables (pagina 9, lineas 26-29). AdemÆs enseæa queeste tipo de combinaciones
tienen un efecto mejorado en comparaciôn del efecto que tienen los compuestos al
usarlos por separado (pagina 4, linea 12 - pagina 5, linea 20).

S La diferencia entre la invención definida en la reivindicación 8 y la composición

enseæada por Dlconsiste en la presencia especifica del inhibidor de tirosina quinasa
"HKl-272" en la composicion de la presente solicitud, mientras que dicho compuesto
no estÆpresente en la anterioridad Dl. No obstante, dicho documento si sugiere el
uso de varios tipos de inhibidores de tirosina quinasa en composiciones que comprenden ademÆsinhibidores "mTOR" tal como Rapamicina (pagina 6, linea 5,
pagina 4, lineas 24-26).

El efecto tØcnico que logra la diferencia antes mencionada no puede ser determinado toda vez que no existe alguna evidencia que sugiera que incluir el inhibidor de tirosina quinasa "HKI-272" en la composiciOn de la reivindicaciOn 8 otorgue a dicha
composiciôn una ventaja tØcnica frente a la composiciôn ya anticipada por Dl, la
cual tiene un efecto mejorado en comparación de cuando los compuestos se usan
de forma individual y superior al efecto esperado para combinación de los mismo
(pagina 4, linea 12 - pagina 5, linea 20).

Por lo tanto, el Problema TØcnico Objetivo a resolver en la presente solicitud

40 corresponde a proporcionar composiciones antineoplÆsicasque comprendan un inhibidor "mTOR" tal como Rapamicina, y un inhibidor de tirosina quinasa,
alternativas a las ya conocidas en la anterioridad Dl, y que ademÆscomprendan
"HKl-272".

Sin embargo, la utilizaciOn del inhibidor de tirosina quinasa "HKI-272" en
combinaciôn con otro tipo de inhibidores ya era conocida y enseæada por el
documento D2 el cual menciona que el "HKI-272" es un potente inhibidor de HER2 (pagina 3958, resumen y columna izquierda; pagina 3965, columna izquierda).

En consecuencia, la persona medianamente versada en la materia conociendo el problema tØcnico objetivo y las enseæanzasde las anterioridades Dl y D2, habria Ilegado de manera obvia y sin mucho esfuerzo de su parte a la composiciOn de la reivindicaciOn 8 mediante la adiciOn del inhibidor tirosina quinasa "HKI-272" enseæadopor D2 a la composicion revelada por la anterioridad Dl, toda vez que dicho documento ya sugerla composiciones que comprenden inhibidor tirosina quinasa e inhibidores "mTOR".

Expediente 08 044 912 ler Art 45

Iv
171

Las reivindicaciones 10 a 25 no mencionan caracterIsticas que hagan inventivo la composiciOn de la reivindicaciôn 8, y por tal razôn tampoco pueden considerarse
inventivas.

La reivindicaciôn 9 consiste en una composiciOn farmacØutica que comprende: (a) por lo menos una unidad de Herceptina, (b) por lo menos una unidad de HKI-272 (inhibidor de tirosina quinasa) y por 10 menos un portador farmacØuticamente aceptable.

ComparaciOn de las caracteristicas tØcnicas esenciales, con las del Estado de la TØcnica:

Caracteristicas esenciales Dl D2

Composición farmacØutica Combinación que comprende: Inhibidores tirosina quinasa

que comprende:

(a) Por lo menos una unidad Anticuerpos de tirosina quinasa trastuzumab (Herceptina)
de Herceptina tales como: trastuzumab (pagina 5, linea 3) (Herceptina) (pägina 5, linea 3)
Inhibidores de tirosina quinasa "HKI-272" es un potente

(b) Por lo menos una unidad tales como: trastuzumab inhibidor de HER2 (pagina

(Herceptina) Imatinib, SU10I, 3958, resumen columna

de HKl-272 (inhibidor de ZD1839, Cl-1033, entre otros izquierda; pagina 3965, tirosina quinasa) (pagina 4, linea 28 a pagina 5 columna izquierda).

lmnea 4).
Por lo menos un portador Portador farmacØuticamente No se menciona
farmacØuticamente aceptable. aceptable (pagina 9, lineas 26-29)

Los documentos considerados como pertenecientes al estado de la tØcnica de la invención descrita en la reivindicaciôn 9 actualmente presentada corresponden a Dl y D2 porque:

El docuemntoDl revela una combinación que comprende: I Anticuerpos de tirosina quinasa tales como: trastuzumab (Herceptina) (pagina 5, linea 3), otro tipo de inhibidores de de tirosina quinasa tales como: Imatinib, SUI01, ZD1839, CI-1033, entre otros (pagina 4, linea 28 a pagina 5 linea 4) y portadores farmacØuticamente aceptables (pagina 9, lIneas 26-29)

La diferencia entre la invención definida en la reivindicaciOn 9 y la composición enseæadapor DI consiste en la presencia especIfica del inhibidor de tirosina quinasa "HKI-272" en la composición de la presente solicitud, mientras que dicho compuesto no estÆpresente en la anterioridad Dl. No obstante, dicho documento Si sugiere el uso de varios tipos de inhibidores de tirosina quinasa en composiciones pagina 4, linea 28 a pagina 5 linea 4).

El efecto tØcnico que logra la diferencia antes mencionada no puede ser determinado toda vez que no existe alguna evidencia que sugiera que incluir el inhibidor de tirosina quinasa "HKI-272" en la composiciôn de la reivindicaciOn 9 otorgue a dicha composición una ventaja tØcnica frente a la composiciôn ya anticipada por DI, la cual tiene un efecto mejorado en comparaciôn de cuando los compuestos se usan de forma individual y superior al efecto esperado para combinación de los mismo (pagina 4, linea 12 - pagina 5, linea 20).

Por lo tanto, el Problema TØcnico Objetivo a resolver en la presente solicitud corresponde a proporcionar composiciones antineoplÆsicas que comprendan inhibidores de tirosina quinasa, alternativas a las ya conocidas en la anterioridad Dl, y que ademÆscomprendan "HKl-272".

Expediente 08 044 912 ler Art 4S

172

Sin embargo, la utilizaciôn del inhibidor de tirosina quinasa "HKI-272" en combinación con otro tipo de inhibidores ya era conocida y ensenada por el documento D2 el cual menciona que el "HKI-272" es un potente inhibidor de HER2 (pagina 3958, resumen y columna izquierda; pagina 3965, columna izquierda).

En consecuencia, la persona medianamente versada en la materia conociendo el problema tØcnico objetivo y las enseæanzas delas anterioridades Dl y D2, habria Ilegado de manera obvia y sin mucho esfuerzo de su parte a la composicion de la
reivindicación 9 mediante la adición del inhibidor tirosina quinasa "HKI-272"
ensenado por D2 a la composición revelada por la anterioridad Dl, toda vez que dicho documento ya sugerla composiciones que comprenden inhibidores tirosina quinasa y trastuzumab (Herceptina).

Las reivindicaciones 10 a 25 no mencionan caracterIsticas que hagan inventivo la composiciOn de la reivindicación 9, y por tal razôn tampoco pueden considerarse inventivas.

8. Conclusion

Los requisitos de Patentabilidad de la presente Solicitud estÆn afectadosast:

Item Documento Reivindicaciones afectadas Reguisito que afecta

4-7,10-25 Novedad Dl W02004004644A2 8-25 Nivel Inventivo
RABINDRAN S K; ET AL.
Cancer Research, 2004

D2 0101 American Association for 7-25 Nivel Inventivo Cancer Research, US ISSN
0008-5472. VoI:64, Nr:11,
Page(s):3958 3965

En cumplimiento del articulo 45 de la Decision 486 de la Comisiôn de la Comunidad Andina, se le indica que debe dar respuesta dentro del tØrmino de sesenta (60) dias contados a partir de la fecha de notificaciOn de la presente comunicación. En caso de no dar respuesta al presente requerimiento, 0 si a pesar de la respuesta subsistieran los impedimentos para la concesiOn, se denegarÆla patente.

NotifIquese el presente oficio conforme a lo dispuesto en el numeral 5.2, literal e), del capitulo quinto del tItulo primero de la Circular Unica No. 10 de 2001.

JOSÉ LUIS SALAZAR LOPEZ
Director de Nuevas Creaciones (C)

El anterior requerimiento fue notificado por fijaciôn en lista, de fecha
2p,

Elaborado por: Juan Carlos CortØs Garcia
Revisado por: Jacqueline Alfonso

Expediente 08 044 912 ler Art 45

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$UPERNTENDENCIA

REPUBLICA DE COLOMBIA SUPERINTENDENCIA DE INDUSTRIA Y COMERCIO

RESOLUCION No: 56091
Por la cual se deniega una patente de invenciôn
Rad. PCT/ 08-44912

EL SUPERINTENDENTE DE INDUSTRIA V COMERCIO

en ejerccio de sus facultades legales, en especial de las conferidas en el numeral 26 del artIcUlo 30 del Decreto 4886 de 2011, y

CONSIDERANDO:

PRIMERO: Que mediante esorito radicado en esta Superintendencia el dIa 2 de mayo de 2008 con el No 08-044912-00000-0000, por Ia sociedad WYETH, a travØs de apoderado, entró a fase nacional la soliqitud de patente de invenciOn titulada "COMBINACIONES ANTINEOPLASICAS CON i INHIBIDOR DE OBJETIVO DE RAPAMICINA EN MAMIFEROS, HERCEPTINAY/O HKI-272", que.corresponde ala solicitud internacional PCT/US2006/042915 del 2 de noviembre de 2006.

SEGUNDO: Que de conform, idad con lo dispuesto en el artIculo 27 del Tratado de Cooperación en Materia de Patentes (PCT) y la regla 51bis del Reglamento, que facultan a la Superintendencia sobre’ la aplicaciôn de la Decision 486 de la Comisión de la Comunidad Andina, se efectuó el examen de forma a la solicitud, de acuerdo con lo previsto en el artIculo 38 de lamencionada DecisiOn andina,

TERCERO: Que efectuado el examen de forma en cuanto a losrequisitos que debe Ilenar la solicitud y cumplidas las exigencias legales, se ordeno publicar el extracto sin que se hubieran presentado oposiciones por parte de terceros.

CUARTO: Que mediante escrito radicado en esta Superintendencia el dIa 28 de septiembre de 2009, el solicitante presento peticiOn de examende patentabilidad, en los tØrminos establecidos en eCl artIculo 44 de la Decision 486 de la ComisiOn de la

QUINTO: Que el artIculo 45 ce la DecisiOn 486 de la ComisiOn de la Comunidad Andina estabtece que: "Si ía oficina nacionai cornpetente encontrara que Ia invenciOn no es patentable o que no cumple con alguno de los requisitos establecidos en esta DccisiOn para la concesion de la patente, /0 notificara a! solicitante Este deberÆ respondera ía notificacion dentro del plazo de sesenta dIas contados a partir de ía fecha de ía notificaciOn. Este plazo podrÆ ser prorrogadopor una sola vez por un perfodo de treinta dIas adicionales. (...) Si el solicitante no respondiera a Ia notiflcación dentro del plazo seæalado, asi a pesai de la respuesta subsistieran los impedimentos para ía concesion, ía oficina nacional competente denegara Ia patente’

SEXTO: Que de conformidad con lo dispuesto en el artIculo 45 antes mencionado, se realizo el examen de fondo y se requirio al solicitante mediante Oficio No.9270, notificado el 1 de junio del 2012, para que se pronunciaa sobre las objeciones efectuadas a la solicitud en cuanto a excepciones a la patent.bilidad, falta de claridad, concision y sustento de las reivindicaciones, carencia de novedad y nivel inventivo, de
PÆginalde2

Al contester favor indique el nOmero S Sede Centro: Carrera 13 No. 2?-00 pisos 2, 5, 7 y 10 PBX: 5870000
de radicación que se indica a continuaciOn: Sede CAN: Av. Carrera 50 No. 26- 55 mt. 2 Tel 5737020
Cod Verificación: 1181368833 Call Center 5920400. Linea gratuita Nacional 01800-910165
Web: e-mail: contaetenos@sic.gov.co
Bogota D.C. Colombia

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REPUBLICA DE COLOMBIA SUPERINTENDENCIA DE INDUSTRIA Y COMERCJO

RESOLUCION No: 56091
Por la cup] se deniega una patente de invenciôn Rad. POT! 08-44912

acuerdo con las exgencias d los artIculos 16, 18, 20 d) y 30 de la Decision 486.

Transcurrido el plazo establØcido no se dio respuesta al requerimiento y sugerencias
arriba indicados.

RESUELVE:

ARTICULO PRIMERO: DenØgar la patente de lnvención titulada "COMBINACIONES ANTINEOPLASICAS CON I INHIBIDOR DE OBJETIVO 5DE RAPAMICINA EN MAMIFEROS, HERCEPTJNI Y/O HKI-272", que entrO en fase nacional en virtud del Tratado de CooperaciOn en Materia de Patentes (PCT).

ARTICULO SEGUNDO: Notificar el contenido de la presente resolución al doctor Alvaro Correa OrdOæez, en su calid;ad de apoderado de WYETH, oa quien haga sus veces, entregÆndole copia de la misma y advirtiØndole que contra ella procede el recurso de reposición, ante el Superintendente de Industria y Comercio, elcual podrÆser interpuesto en el momento de la notificaciön o dentro de los cinco 5 dIas hÆbilessiguientes a ella.

NOTIFIQUESE Y CUMPLASE
Dada en Bogota, D.C., el 26 Sep 2012

EL SUPERINTENDENTE DE INDUS RIA Y COMERCIO,

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Doctor
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Elaboró: Juan Carlos Chaparro Velez 00
RevsO: Jose Luis Salazar Lopez b
Aprobó: Jose Luis Londo10 Fernandez

Pàgina2de2

Al contestar favor indique el nümro
de radicaciOn que se indica a continuaciOn
Cod Verificación: 118136883311