

(19) World Intellectual Property Organization
International Bureau



(43) International Publication Date
9 March 2006 (09.03.2006)

PCT

(10) International Publication Number
WO 2006/024945 A1

- (51) International Patent Classification⁷: **A61K 47/02**, 47/26, 31/4184, A61P 35/00
- (21) International Application Number: PCT/IB2005/002734
- (22) International Filing Date: 29 August 2005 (29.08.2005)
- (25) Filing Language: English
- (26) Publication Language: English
- (30) Priority Data:
60/606,953 3 September 2004 (03.09.2004) US
- (71) Applicant (for all designated States except US): **PFIZER INC.** [US/US]; 235 East 42nd Street, New York, NY 10017 (US).
- (72) Inventors; and
- (75) Inventors/Applicants (for US only): **KHAMPHAVONG, Phennapha** [LA/US]; Pfizer Global Research and Development, Agouron/Pfizer La Jolla, 10777 Science Center Drive, San Diego, CA 92121 (US). **LA, Julie, Chengile** [CN/US]; Pfizer Global Research and Development, Agouron/Pfizer La Jolla, 10777 Science Center Drive, San Diego, CA 92121 (US). **QI, Hong** [CN/US]; Pfizer Global Research and Development, Agouron/Pfizer La Jolla, 10777 Science Center Drive, San Diego, CA 92121 (US). **TSAI, Yali, Jennifer** [CN/US]; Pfizer Global Research and Development, Agouron/Pfizer La Jolla, 10777 Science Center Drive, San Diego, CA 92121 (US).
- (74) Agent: **FULLER, Grover, F., Jr.**; c/o Keith S. Ruddock, Pfizer Research and Development, Ramsgate Road, Sandwich, Kent CT13 9NJ (GB).
- (81) Designated States (unless otherwise indicated, for every kind of national protection available): AE, AG, AL, AM, AT, AU, AZ, BA, BB, BG, BR, BW, BY, BZ, CA, CH, CN, CO, CR, CU, CZ, DE, DK, DM, DZ, EC, EE, EG, ES, FI, GB, GD, GE, GH, GM, HR, HU, ID, IL, IN, IS, JP, KE, KG, KM, KP, KR, KZ, LC, LK, LR, LS, LT, LU, LV, MA, MD, MG, MK, MN, MW, MX, MZ, NA, NG, NI, NO, NZ, OM, PG, PH, PL, PT, RO, RU, SC, SD, SE, SG, SK, SL, SM, SY, TJ, TM, TN, TR, TT, TZ, UA, UG, US, UZ, VC, VN, YU, ZA, ZM, ZW.
- (84) Designated States (unless otherwise indicated, for every kind of regional protection available): ARIPO (BW, GH, GM, KE, LS, MW, MZ, NA, SD, SL, SZ, TZ, UG, ZM, ZW), Eurasian (AM, AZ, BY, KG, KZ, MD, RU, TJ, TM), European (AT, BE, BG, CH, CY, CZ, DE, DK, EE, ES, FI, FR, GB, GR, HU, IE, IS, IT, LT, LU, LV, MC, NL, PL, PT, RO, SE, SI, SK, TR), OAPI (BF, BJ, CF, CG, CI, CM, GA, GN, GQ, GW, ML, MR, NE, SN, TD, TG).

Published:

— with international search report

For two-letter codes and other abbreviations, refer to the "Guidance Notes on Codes and Abbreviations" appearing at the beginning of each regular issue of the PCT Gazette.

(54) Title: PHARMACEUTICAL COMPOSITIONS COMPRISING A CDK INHIBITOR

(57) Abstract: The present invention features pharmaceutical and lyophilized compositions comprising {5-[3(4,6-Difluoro-1 H-benzimidazol-2-yl)-1 Hindazol-5-yl]-4-methyl-pyridin-3-ylmethyl}-ethyl-amine, an inhibitor of cyclin-dependent kinases (CDKs). The pharmaceutical and lyophilized compositions are suitable for parenteral administration, wherein the composition comprises {5-[3-(4,6-Difluoro-1 H benzimidazol-2-yl)-1 H-indazol-5-yl]-4-methyl-pyridin-3-ylmethyl}-ethyl-amine, a Solubilizing Agent, and a Stabilizing Agent as defined herein. Also disclosed are methods of making the compositions, and methods of treating cell-proliferative disorders by administration of the compositions.



WO 2006/024945 A1

- 1 -

PHARMACEUTICAL COMPOSITIONS COMPRISING A CDK INHIBITOR

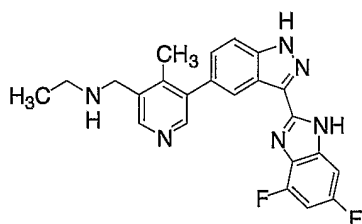
FIELD OF THE INVENTION

The instant invention provides pharmaceutical compositions containing a cyclin-dependent kinase ("CDK") inhibitor such as {5-[3-(4,6-Difluoro-1*H*-benzoimidazol-2-yl)-1*H*-indazol-5-yl]-4-methyl-pyridin-3-ylmethyl}-ethyl-amine. Also provided are methods of making and using the pharmaceutical compositions of the invention.

BACKGROUND OF THE INVENTION

The compound {5-[3-(4,6-Difluoro-1*H*-benzoimidazol-2-yl)-1*H*-indazol-5-yl]-4-methyl-pyridin-3-ylmethyl}-ethyl-amine (hereinafter referred to as Compound A or Active Ingredient in this specification) has the chemical structure shown below and is an inhibitor of cyclin-dependent kinases (CDKs) 1, 2 and 4. CDKs are serine-threonine protein kinases that play critical roles in regulating the transitions between different phases of the cell-cycle, such as the progression from a quiescent stage in G₁ to S, or the progression from G₂ to M phase, in which active mitosis and cell-division occurs. It is believed that inhibition of CDKs can in turn to inhibit progression of different phases of the cell-cycle, and consequently can provide a method in the treatment of cell proliferative disorders.

Compound A may be used for the treatment of patients suffering from cell-proliferative disorders such as cancer. Compound A is disclosed as Example 1 in co-pending U.S. Patent Application No. 10/866,059, the disclosure of which is incorporated herein by reference.



- 20 Medicaments for injections are usually formulated into emulsions, suspensions or solutions, as well as lyophilized preparations, which can be dissolved before use. For purposes of parenteral administration, it is desirable that the lyophilized composition be stable, preserved and easily reconstituted. Lyophilized compositions generally have the advantage of being easy to maintain as sterile since foreign insoluble matter is eliminated before storage.
- 25 Storage of a pharmaceutical composition of Compound A prepared by known methods may lead to degradation of its content and a gelling and worsening of its reconstitution properties, especially in the case of injections to be dissolved before use. It is difficult to provide a pharmaceutical composition of Compound A which has good stability and a good reconstitution property, especially for injections of Compound A to be dissolved before use, after long storage at room temperature. Specifically, Compound

- 2 -

A tends to gel or turn hazy when in solution form, which makes it desirable to obtain a stable injection solution and a processing solution.

Even if a clear, non-gelling solution of Compound A were obtained by changing conditions such as temperature and the like, degradation of a solution of Compound A and a clouding would occur after long storage, resulting in degradation and non-uniform contents of Compound A in the solution or the lyophilized injectable preparation of Compound A. It would be advantageous to keep Compound A physically stable in aqueous carriers.

Preparing lyophilized products by the methods of the prior art sometimes causes problems in quality. These problems include a sublimation of some ingredients coinciding with that of water, content of ingredients decreases in the lyophilized product, a partial solidifying of product, lyophilized preparations are cracked and shrunk and may have a thin layer on the top of the cake, adherence to the upper portion of vials and spatters, and non-uniform appearance of lyophilized cakes. Pharmacists and the like require injections to be dissolved in an infusion solution before use, consequently, a lyophilized injection should have good reconstitution properties.

It would be desirable to have pharmaceutical compositions comprising Compound A that have one or more desirable properties, such as improved storage stability, uniform appearance, and good reconstitution properties in aqueous medium, to give an injectable solution for treatment of cell-proliferative disorders.

SUMMARY OF THE INVENTION

Unless otherwise stated the following terms used in the specification and claims have the meanings discussed below:

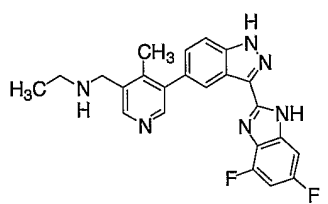
The pharmaceutical compositions of the invention exist in liquid, lyophilized or frozen form.

The term, "lyophilized composition(s)" refers to the solid freeze-dried composition of matter prepared by the process of this invention and comprising as essential ingredients: (1) {5-[3-(4,6-Difluoro-1H-benzoimidazol-2-yl)-1H-indazol-5-yl]-4-methyl-pyridin-3-ylmethyl}-ethyl-amine; (2) a Stabilizing Agent; and (3) a Solubilizing Agent.

As defined herein, the term "stable solution" refers to a solution free of gelling or haze as determined by visual inspection. The solutions of the invention are stable for a period of at least 24 hours.

The term, "Active Ingredient" (also called Compound A) refers to the compound) {5-[3-(4,6-Difluoro-1H-benzoimidazol-2-yl)-1H-indazol-5-yl]-4-methyl-pyridin-3-ylmethyl}-ethyl-amine, a compound represented by the formula:

- 3 -



In preferred embodiments of the invention, the formulations are effective in treating a cell proliferative disorder in a patient in need of such treatment. The patient is preferably a mammal and more preferably a human.

- 5 The term “treating” as used herein refers to the method of the invention having a therapeutic effect and at least partially alleviating or abrogating the abnormal condition in the organism (e.g., patient).

The term “therapeutic effect” as used herein refers to inhibition of the abnormal condition. The term “therapeutic effect” also refers to the inhibition of factors causing or contributing to the abnormal condition. A therapeutic effect relieves to some extent one or more of the symptoms of the abnormal condition.

- 10 The term “mammal” as used herein preferably refers to the organisms of the class known as “mammalia”, such as mice, rats, rabbits, guinea pigs, goats, sheep, horses, cows, dogs, cats, monkeys, apes, humans, and the like; more preferably dogs, cats, monkeys, apes, humans, and the like; and most preferably humans.

- The term “cell proliferative disorder” as used herein refers to a disorder where an excess cell proliferation of one or more subset of cells in a multicellular organism occurs resulting in harm (e.g., discomfort or decreased life expectancy) to the multicellular organism. The excess cell proliferation can be determined by reference to the general population and/or by reference to a particular patient (e.g., at an earlier point in the patient’s life). Hyper-proliferative cell disorders can occur in different types of animals and in humans, and produce different physical manifestations depending upon the affected cells. Hyper-proliferative cell disorders include without limitation cancers, blood vessel proliferative disorders, fibrotic disorders, autoimmune disorders, and the like. Cell proliferative disorders suitable for treatment in accordance with the present invention include without limitation:
- 15
- 20

- 25 - a variety of cancers, including, but not limited to, carcinoma, hematopoietic tumors of lymphoid lineage, hematopoietic tumors of myeloid lineage, tumors of mesenchymal origin, tumors of the central and peripheral nervous system and other tumors including melanoma, seminoma and Kaposi’s sarcoma and the like. Such cancers specifically include, but are not limited to erythroblastoma, glioblastoma, meningioma, astrocytoma, melanoma, myoblastoma, breast cancers, gastric cancers, ovarian cancers, renal cancers, hepatic cancers, pancreatic cancers, bladder cancers, thyroid cancers, prostate cancers, colorectal cancers, solid tumor
- 30 cancers, colon cancer, brain cancer, blood cancers, bone cancers, liver cancer, kidney cancer, stomach cancer, lung cancer, Kaposi’s sarcoma, non-small cell lung cancer, skin cancer, and the like, non-small cell lung cancers, and the like.

- 4 -

- a disease process which features abnormal cellular proliferation, e.g., benign prostatic hyperplasia, familial adenomatous polyposis, neuro-fibromatosis, atherosclerosis, pulmonary fibrosis, arthritis, psoriasis, glomerulonephritis, restenosis following angioplasty or vascular surgery, hypertrophic scar formation, inflammatory bowel disease, transplantation rejection, endotoxic shock, and fungal infections.
- defective apoptosis-associated conditions, such as cancers (including but not limited to those types mentioned hereinabove), viral infections (including but not limited to herpes virus, poxvirus, Epstein-Barr virus, Sindbis virus and adenovirus), prevention of AIDS development in HIV-infected individuals, autoimmune diseases (including but not limited to systemic lupus erythematosus, rheumatoid arthritis, psoriasis, autoimmune mediated glomerulonephritis, inflammatory bowel disease and autoimmune diabetes mellitus), neurodegenerative disorders (including but not limited to Alzheimer's disease, amyotrophic lateral sclerosis, retinitis pigmentosa, Parkinson's disease, AIDS-related dementia, spinal muscular atrophy and cerebellar degeneration), myelodysplastic syndromes, aplastic anemia, ischemic injury associated with myocardial infarctions, stroke and reperfusion injury, arrhythmia, atherosclerosis, toxin-induced or alcohol related liver diseases,
- hematological diseases (including but not limited to chronic anemia and aplastic anemia), degenerative diseases of the musculoskeletal system (including but not limited to osteoporosis and arthritis), aspirin-sensitive rhinosinusitis, cystic fibrosis, multiple sclerosis, kidney diseases and cancer pain.

In addition, Compound A, as an inhibitor of the CDKs, can modulate the level of cellular RNA and DNA synthesis and is therefore expected to be useful in the treatment of viral infections such as HIV, human papilloma virus, herpesvirus, Epstein-Barr virus, adenovirus, Sindbis virus, poxvirus and the like.

- 25 In reference to the treatment of abnormal conditions caused, in whole or in part, by a cell proliferative disorder, a therapeutic effect refers to one or more of the following: (a) reducing tumor size; (b) inhibiting (e.g, slowing or stopping) tumor metastasis; (c) inhibiting tumor growth; and (d) relieving to some extent one or more of the symptoms associated with the abnormal condition.

30 The term "collapse temperature" describes the glass transition temperature for amorphous solids or eutectic temperature for crystalline solids. Collapse temperature is that temperature above which the product is not completely frozen. Freeze dry microscopy enables measurement of the temperature at which frozen solutions begin to lose their rigid structure during a sublimation process. For pharmaceutical compositions comprising {5-[3-(4,6-Difluoro-1*H*-benzimidazol-2-yl)-1*H*-indazol-5-yl]-4-methyl-pyridin-3-ylmethyl}-ethyl-amine, a Stabilizing Agent and a Solubilizing Agent, the collapse temperature has been
35 measured to between about 0°C and -35°C.

The term "Stabilizing Agent" refers to a non-ionic agent such as a polysaccharide, a solid sugar or a sugar-alcohol that assists in stabilizing the Active Ingredient. An "effective amount of a Stabilizing Agent"

is a quantity of Stabilizing Agent that permits the Active Ingredient to form stable aqueous solutions for medical use.

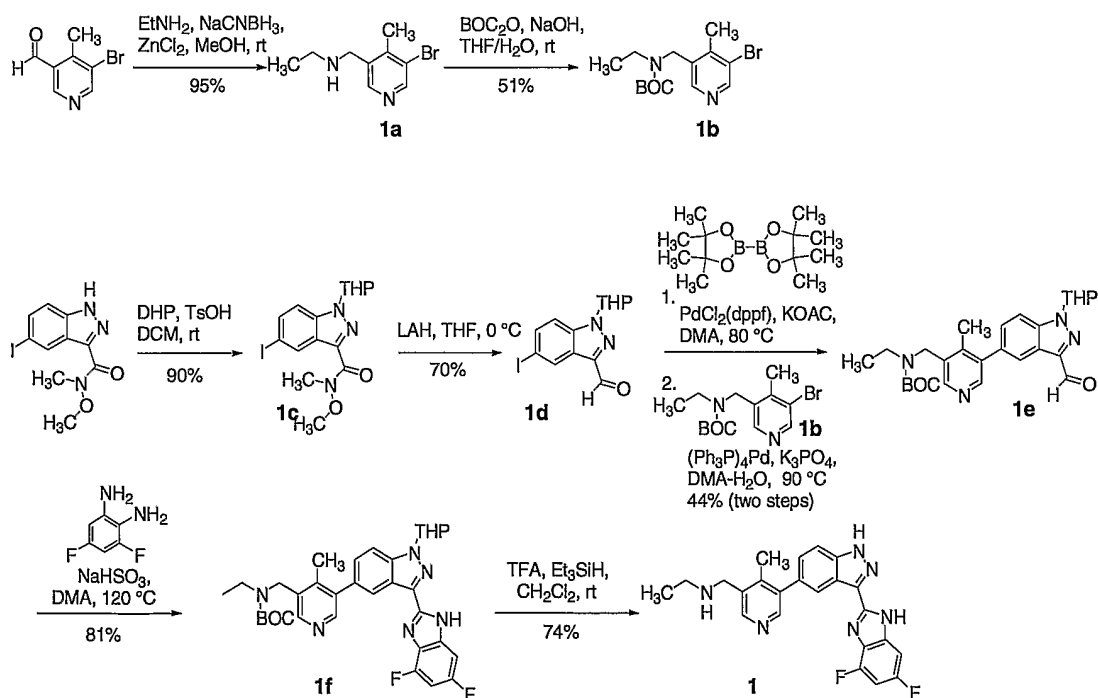
The term “Solubilizing Agent” herein refers to an acid, a co-solvent, a surfactant or a water-soluble polymer used to facilitate dissolution of the composition. An “effective amount of Solubilizing Agent” is a quantity of Solubilizing Agent that permits the composition to be readily dissolved to form aqueous solutions suitable for medical use.

The term “dose-concentrate” refers to a solution of pharmaceutical formulation. The dose-concentrate may be held in the container where it was formed by adding aqueous solvent to the pharmaceutical composition or it may be removed and held externally. The dose-concentrate may be used as is, or can be further diluted to a unit dosage concentration for administration to a mammal. The entire volume of the dose-concentrate or aliquots thereof may be used in preparing unit dose(s) for treatment by the method of this invention.

As used herein, the “weight %” or “(w/w)” of a given ingredient of the composition is measured by the relative weight of the ingredient to the total weight of the composition.

DETAILED DESCRIPTION OF THE INVENTION

Compound A to be used in the present invention can be used for the compositions and preparations of the present invention in any state, including crystal, amorphous, hydrate, solvate, or a mixture of such forms. An illustrative synthesis of Compound A is shown as follows:



- 6 -

(a) Intermediate **1a** – (5-Bromo-4-methyl-pyridin-3-ylmethyl)-ethyl-amine

5-Bromo-4-methyl-pyridine-3-carbaldehyde (6.74 g, 33.7 mmol) [for the preparation of this compound see: Reich, S. R.; Bleckman, T. M.; Kephart, S. E.; Romines, W. H.; Wallace, M. B., US patent 6,555,539, April 29, 2003.] was dissolved in methanol (290 mL) under a nitrogen atmosphere. A solution of ethylamine in methanol (2.0 M, 90 ml, 180 mmol) was added dropwise over 30 minutes. Stirring was continued at room temperature for 30 minutes further.

In a separate flask, sodium cyanoborohydride (2.33 g, 37.1 mmol) was dissolved in methanol (150 mL). Anhydrous zinc chloride (2.53 g, 18.5 mmol) was added and stirring continued at room temperature for 20 minutes. This solution (zinc/cyanoborohydride) was then slowly added to the above aldehyde/ethylamine solution. The reaction solution was acidified to pH 4 with 2.0 M HCl in methanol (120 mL), and then stirred at room temperature for 18 hours.

The solvents were removed by rotary evaporation and the residue partitioned between ethyl acetate and 10% aqueous sodium carbonate. The organic extracts were dried over magnesium sulfate and concentrated, affording crude amine **1a** (7.36 g, 95%) as an orange oil, which was used in the next step without further purification: ^1H NMR (CDCl_3) δ 8.53 (s, 1H), 8.31 (s, 1H), 3.77 (s, 2H), 2.67 (q, $J = 7.0$ Hz, 2 H), 2.42 (s, 3H), 1.11 (t, $J = 7.0$ Hz, 3H).

(b) Intermediate **1b** – (5-Bromo-4-methyl-pyridin-3-ylmethyl)-ethyl-carbamic acid tert-butyl ester

Di-tert-butyl dicarbonate (10.43 g, 47.8 mmol) was added to a solution of crude amine **1a** (7.36 g, 32.1 mmol) in THF (400 mL), followed by aqueous sodium hydroxide solution (1.0 M, 101 mL). The biphasic solution was stirred vigorously for 20 hours at room temperature. The solution was partitioned between water and ethyl acetate; the organic extracts were dried over magnesium sulfate, filtered, and concentrated. The crude yellow oil thus obtained was purified by silica gel chromatography (eluting with a gradient of 10% to 30% ethyl acetate in hexanes), yielded bromopyridine **1b** (5.37 g, 51%) as a yellow oil: ^1H NMR (CDCl_3) δ 8.58 (s, 1H), 8.22 (s, 1H), 4.47 (s, 2H), 3.17 (br s, 2H), 2.37 (s, 3H), 1.45 (s, 9H), 1.03 (t, $J = 7.2$ Hz, 3H).

(c) Intermediate **1c** – 5-Iodo-1-(tetrahydro-pyran-2-yl)-1*H*-indazole-3-carboxylic acid methoxy-methyl-amide

5-Iodo-1*H*-indazole-3-carboxylic acid methoxy-methyl-amide [for the preparation of this compound see: Reich, S. R.; Bleckman, T. M.; Kephart, S. E.; Romines, W. H.; Wallace, M. B., US patent 6,555,539 B2, April 29, 2003.] was alkylated with dihydropyran according to the method of Sun, et. al. [Sun, J.-H.; Teleha, C. A.; Yan, J.-S.; Rogers, J. D.; and Nugiel, D. A., *J. Org. Chem.* **1997**, *62*, 5627], affording amide **1c** (typically >90%) as an off-white powder: ^1H NMR ($\text{DMSO}-d_6$) δ 8.37 (s, 1H), 7.74 (dd, $J = 1.5, 8.8$ Hz, 1H), 7.68 (d, $J = 8.8$ Hz, 1H), 5.97 (dd, $J = 2.3, 9.0$ Hz, 1H), 3.88 (m, 2H), 3.79 (s, 3H), 3.42 (s, 3H), 2.35 (m, 1H), 2.03 (m, 2H), 1.75 (m, 1H), 1.58 (m, 2H).

(d) Intermediate **1d** – 5-Iodo-1-(tetrahydro-pyran-2-yl)-1*H*-indazole-3-carbaldehyde

Lithium aluminum hydride (1.2 equiv.) is added portionwise to a cooled (<5 °C) solution of amide **1c** (1.0 equiv.) in THF. Stirring is continued at <5 °C until the reaction is complete, typically 30 minutes. The reaction was quenched by the slow addition of ethyl acetate at <5 °C, and the whole mixture poured into 0.4 N NaHSO_4 . The organic layer was washed with brine, dried over magnesium sulfate, concentrated, and purified by silica gel chromatography to give aldehyde **1d** (typically ~70%) as an off-white powder: ^1H

- 7 -

NMR (CDCl₃) δ 10.15 (s, 1H), 8.47 (s, 1H), 7.82 (dd, J = 1.5, 8.7 Hz, 1H), 7.78 (d, J = 8.5 Hz, 1H), 6.04 (dd, J = 2.3, 9.28 Hz, 1H), 3.85 (m, 2H), 2.35 (m, 1H), 2.05 (m, 2H), 1.76 (m, 1H), 1.60 (m, 2H).

(e) Intermediate **1e** – Ethyl-[5-[3-formyl-1-(tetrahydro-pyran-2-yl)-1*H*-indazol-5-yl]-4-methyl-pyridin-3-ylmethyl]-carbamic acid tert-butyl ester

5 Iodoindazole **1d** (3.56 g, 10.0 mmol), bis(pinacolato)diboron (2.79 g, 11 mmol), potassium acetate (2.74 g, 30 mmol) and [1,1'-bis(diphenylphosphino)-ferrocene]

dichloropalladium(II) complex with dichloromethane (245 mg, 0.3 mmol) were dissolved in N,N-dimethylacetamide (60 mL). The solution was degassed by evacuating (until the solvent begins to bubble) and purging with Argon (3 cycles), then heated in an 80 °C oilbath for 2 hours. After cooling slightly (to ~50 °C), a solution of bromopyridine **1b** (3.62 g, 11 mmol) in N,N-dimethylacetamide (40 mL) was added, followed by deionized water (10 mL) and potassium phosphate (3.18 g, 15 mmol). The solution was degassed, tetrakis(triphenylphosphine) palladium (0) (347 mg, 0.3 mmol) added, and degassed again. The mixture was stirred in a 90 °C oilbath for 4.5 hours. After cooling to room temperature, the mixture was diluted with ethyl acetate (300 mL), washed with deionized water (150 mL), and saturated sodium chloride (100 mL). The organic layer was dried over magnesium sulfate, filtered, and concentrated to a crude red-black oil (9.43g). Purification by silica gel chromatography (eluting with 50-100% ethyl acetate in hexanes) afforded coupled product **1e** (2.9462 g) as an orange oil. ¹H NMR of this product showed it was contaminated with ~1 equivalent of pinacol. Trituration from hexanes afforded pure **1e** (2.0853 g, 44%) as a fine yellow powder: ¹H NMR (CDCl₃) δ 10.25 (s, 1H), 8.39 (s, 1H), 8.34 (s, 1H), 8.22 (s, 1H), 7.74 (d, J = 8.7 Hz, 1H), 7.38 (dd, J = 1.5, 8.5 Hz, 1H), 5.88 (dd, J = 2.8, 9.2 Hz, 1H), 4.53 (s, 2H), 4.03 (m, 1H), 3.81 (m, 1H), 3.24 (br s, 2H), 2.60 (m, 1H), 2.18 (s, 3H), 2.15 (m, 2H), 1.77 (m, 1H), 1.65 (m, 2H), 1.47 (s, 9H), 1.09 (t, J = 7.0 Hz, 1H).

(f) Intermediate **1f** – {5-[3-(4,6-Difluoro-1*H*-benzimidazol-2-yl)-1-(tetrahydro-pyran-2-yl)-1*H*-indazol-5-yl]-4-methyl-pyridin-3-ylmethyl}-ethyl-carbamic acid tert-butyl ester

25 Aldehyde **1e** (2.05 g, 4.28 mmol), 1,2-diamino-3,5-difluorobenzene (617 mg, 4.28 mmol) and sodium bisulfite (891 mg, 8.57 mmol) were dissolved in N,N-dimethylacetamide (43 mL) and heated in a 120 °C oilbath for 21 hours. After cooling to room temperature, the mixture was diluted with ethyl acetate (100 mL) and washed with half-saturated aqueous sodium chloride solution (75 mL, a 1:1 mixture of deionized water and saturated aqueous sodium chloride solution). The aqueous layer was back-extracted with ethyl acetate (2 x 100 mL). All the organic extracts were combined, dried over magnesium sulfate, and concentrated to a brown tar (3.39 g). This crude material was purified by silica gel chromatography (eluting with a gradient of 70% to 100% ethyl acetate in hexanes), to give benzimidazole product **1f** (2.11 g, 81%) as a tan foam: ¹H NMR (CD₃OD) δ 8.46 (s, 1H), 8.41 (s, 1H), 8.29 (s, 1H), 7.87 (d, J = 8.6 Hz, 1H), 7.46 (dd, J = 1.3, 8.6 Hz, 1H), 7.13 (m, 1H), 6.84 (m, 1H), 5.99 (dd, J = 2.3, 9.9 Hz, 1H), 4.60 (s, 2H), 4.01 (m, 1H), 3.86 (m, 1H), 3.32 (m, 2H, obscured by solvent peak) 2.67 (m, 1H), 2.28 (s, 3H), 2.18 (m, 2H), 1.89 (m, 1H), 1.73 (m, 2H), 1.47 (s, 9H), 1.13 (t, J = 7.1 Hz, 1H). Anal. (C₃₃H₃₆F₂N₆O₃•0.4 H₂O) C, H, N, F.

(g) Example 1 – {5-[3-(4,6-Difluoro-1*H*-benzimidazol-2-yl)-1*H*-indazol-5-yl]-4-methyl-pyridin-3-ylmethyl}-ethyl-amine

- 8 -

Triethyl silane (976 mg, 8.40 mmol) and trifluoroacetic acid (12.9 mL, 168 mmol) were added to a solution of **1f** (2.02 g, 3.36 mmol) in dichloromethane (12.9 mL). The mixture was stirred at room temperature for 3.5 hours. The volatiles were removed by rotary evaporation, and the residue treated with cyclohexane (10 mL) and aqueous ammonium hydroxide (2 N, 20 mL). After vigorous stirring for 15 minutes, a pink precipitate forms, which was collected by suction filtration. The filtrate was extracted with ethyl acetate (3 x 50 mL). The combined organic extracts were dried over magnesium sulfate, filtered, and concentrated to an orange solid (~0.4 g). This solid was added to the pink precipitate obtained above and purified by column chromatography (eluting with a mixture of 1% concentrated ammonium hydroxide to 19% absolute ethanol to 80% dichloromethane). The product thus obtained (1.23 g off-white solid) was further purified by trituration from cyclohexane to yield pure **1** (1.09 g, 74%) as an off-white solid: ¹H NMR (DMSO-*d*₆) δ 13.81 (very br s, 1H), 8.46 (s, 1H), 8.35 (s, 2H), 7.76 (d, *J* = 8.6 Hz, 1H), 7.44 (dd, *J* = 1.3, 8.6 Hz, 1H), 7.17 (m, 1H), 7.07 (t of d, *J*_t = 1.5 Hz, *J*_d = 10.6 Hz, 1H), 3.78 (s, 2H), 2.63 (q, *J* = 7.1 Hz, 2H), 2.25 (s, 3H), 1.07 (t, *J* = 7.1 Hz, 3H). HRMS [*M*+*H*]⁺ calc. 419.1791; found 419.1811. Anal. (C₂₃H₂₀F₂N₆•1.1 H₂O) C, H, N, F.

BIOCHEMICAL AND BIOLOGICAL EVALUATION

Cyclin-dependent kinase activity was measured by quantifying the enzyme-catalyzed, time-dependent incorporation of radioactive phosphate from [³²P]ATP or [³³P]ATP into a protein substrate. Unless noted otherwise, assays were performed in 96-well plates in a total volume of 50 μL, in the presence of 10 mM HEPES (N-[2-hydroxyethyl]piperazine-N'-[2-ethanesulfonic acid]) (pH 7.4), 10 mM MgCl₂, 25 μM adenosine triphosphate (ATP), 1 mg/mL ovalbumin, 5 μg/mL leupeptin, 1 mM dithiothreitol, 10 mM beta-glycerophosphate, 0.1 mM sodium vanadate, 1 mM sodium fluoride, 2.5 mM ethylene glycol-bis(β-aminoethyl ethKer)-N,N,N',N'-tetraacetic acid (EGTA), 2% (v/v) dimethylsulfoxide, and 0.03 - 0.4 μCi [³²/³³P]ATP per reaction. Reactions were initiated with enzyme, incubated at 30°C, and terminated after 20 minutes by the addition of ethylenediaminetetraacetic acid (EDTA) to 250 mM. The phosphorylated substrate was then captured on a nitrocellulose or phosphocellulose membrane using a 96-well filtration manifold, and unincorporated radioactivity was removed by repeated washing with 0.85% phosphoric acid. Radioactivity was quantified by exposing the dried membranes to a phosphorimager.

Apparent K_i values were measured by assaying enzyme activity in the presence of the CDK different inhibitor compound concentrations and subtracting the background radioactivity measured in the absence of enzyme. Inhibition data were fit to an equation for competitive inhibition using Kaleidagraph (Synergy Software), or were fit to an equation for competitive tight-binding inhibition using the software KineTic (BioKin, Ltd.).

Inhibition of CDK2/Cyclin A Retinoblastoma Kinase Activity

CDK2 was purified using published methodology (Rosenblatt *et al.*, "Purification and Crystallization of Human Cyclin-dependent Kinase 2," *J. Mol. Biol.*, vol. 230, 1993, pp. 1317-1319) from insect cells that had been infected with a baculovirus expression vector. Cyclin A was purified from *E. coli* cells expressing full-length recombinant cyclin A, and a truncated cyclin A construct was generated by limited proteolysis and purified as described previously (Jeffrey *et al.*, "Mechanism of CDK activation revealed by the structure of a cyclin A-CDK2 complex," *Nature*, vol. 376 (27 July 1995), pp. 313-320). A

- 9 -

complex of CDK2 and proteolyzed cyclin A was prepared and purified by gel filtration. The substrate for this assay was the same Rb substrate fragment used for the CDK4 assays, and the methodology of the CDK2/cyclin A and the CDK4/cyclin D3 assays was essentially the same, except that CDK2 was present at 150 nM or 5 nM. The K_i of Compound A against CDK2/CyclinA was measured to be 0.78 nM.

5 Inhibition of Cell Growth: Assessment of Cytotoxicity

Inhibition of cell growth was measured using the tetrazolium salt assay, which is based on the ability of viable cells to reduce 3-(4,5-dimethylthiazol-2-yl)-2,5-[2H]-diphenyltetrazolium bromide (MTT) to formazan (Mossman, Journal of Immunological Methods, vol. 65 (1983), pp. 55-58). The water-insoluble purple formazan product was then detected spectrophotometrically. The HCT 116 cell line was grown in 96-well plates. Cells were plated in the appropriate medium at a volume of 135 μ L/well in McCoy's 5A Medium. Plates were incubated for four hours before addition of inhibitor compounds. Different concentrations of inhibitor compounds were added in 0.5% (v/v) dimethylsulfoxide (15 μ L/well), and cells were incubated at 37°C (5% CO₂) for four to six days (depending on cell type). At the end of the incubation, MTT was added to a final concentration of 0.2 mg/mL, and cells were incubated for 4 hours more at 37°C. After centrifugation of the plates and removal of medium, the absorbance of the formazan (solubilized in dimethylsulfoxide) was measured at 540 nm. All results were compared to control cells treated only with 0.5% (v/v) dimethylsulfoxide. The concentration of inhibitor compound causing 50% inhibition of growth was determined from the linear portion of a semi-log plot of inhibitor concentration versus percentage inhibition. The IC₅₀ of Compound A in HCT116 cells was determined to be 120 nM.

20

PHARMACEUTICAL COMPOSITIONS

The present invention includes a pharmaceutical composition comprising an amount of a Solubilizing Agent effective to cause dissolution of Compound A. The Solubilizing Agent may be an acid, a co-solvent, a surfactant or a water-soluble polymer. Without any limitation being implied, exemplary co-solvents useful in the invention include polyethylene glycols (e.g. polyethylene glycols 300 and 400), propylene glycol, ethanol. Useful surfactants include, but are not limited to, polyoxyethylene sorbitan fatty acid esters such as polysorbate 80, phosphatides such as lecithin. Water-soluble polymers that are useful in the invention, include but are not limited to, dextrans, carboxymethylcellulose, polyvinylpyrrolidone or gelatin.

30 In one embodiment of the pharmaceutical compositions of the invention, the Solubilizing Agent is an acid capable of fixing the pH of the medium at a value below the pH of the blood plasma, and in particular at values at which the stability of Compound A is not affected, i.e. at values which do not entail any immediate or rapid degradation of Compound A. The Solubilizing Agent may be any pharmaceutically acceptable acid formed by, for example, preferably at least one compound selected from phosphoric acid, polyphosphoric acid and their salts, citric acid, edetic acid (e.g. disodium EDTA), acetic acid, lactic acid, amino acids, malic acid, ascorbic acid, glutamic acid, benzoic acid, glutaric acid, propionic acid, succinic acid, formic acid, maleic acid, aspartic acid, malonic acid, gluconic acid, glucoheptonic acid, hydrochloric acid. Examples of polyphosphoric acid and their salts include potassium polyphosphate, sodium citrate, including trisodium citrate pentahydrate, and trisodium citrate dihydrate. In one embodiment of the

- 10 -

present invention, the Solubilizing Agent is selected from phosphoric acid, citric acid, acetic acid or hydrochloric acid.

In pharmaceutical compositions where the Solubilizing Agent is an acid, the Solubilizing Agent is added in stoichiometric amounts to achieve a pH within the range of from about 2 to about 8. In one embodiment, the pH achieved is in the range of about 3.0 to about 4.5. In a further embodiment, the pH achieved by addition of the Solubilizing Agent is in the range of about 3.5 to about 4.1, and more particularly is about 3.8. In embodiments where the Solubilizing Agent is not an acid, the Solubilizing Agent is typically added in an amount sufficient to achieve dissolution of Compound A at 2 mg/ml.

The amount of Solubilizing Agent present in the pharmaceutical composition varies with the kind of Solubilizing Agent and the concentration of Compound A, and may be from about 0.1% to about 50% (w/w). In an embodiment where the Solubilizing Agent is an acid, the amount of acid required to achieve the target pH may be from about 0.1% to about 2%. In another embodiment, where the Solubilizing Agent is a water-soluble polymer such as cyclo-dextrin, the concentration range is from about 5% to about 30%. In yet another embodiment where the Solubilizing Agent is a co-solvent, the concentration range is from about 5% to about 50%. In yet a further embodiment where the Solubilizing Agent is a surfactant the concentration range is from about 1% to about 10%. For pharmaceutical compositions where the Solubilizing Agent is selected from phosphoric acid, citric acid, acetic acid or hydrochloric acid, the Solubilizing Agent is present at about 0.1% to about 0.5% (w/w). In a specific embodiment where the Solubilizing Agent is phosphoric acid, the Solubilizing Agent is present at about 0.15% (w/w).

The pharmaceutical composition also has an effective amount of a Stabilizing Agent. The Stabilizing Agent comprises at least one pharmaceutically acceptable non-ionic agent. The Stabilizing Agent can be selected from polysaccharides such as dextran, cellulose, alginate, hyaluronic acid, or from cryoprotective agents such as solid sugars and sugar-alcohols. Solid sugars and sugar-alcohols useful as Stabilizing Agents in the invention include, but are not limited to, sucrose, lactose, trehalose, glucose, fructose, maltose, mannitol, sorbitol, xylitol and combinations thereof. In one embodiment, the Solubilizing Agent is selected from mannitol, fructose, lactose or combinations thereof.

The amount of Stabilizing Agent present in the pharmaceutical compositions of the invention varies with the kind of Stabilizing Agent and the concentration of Compound A. In an embodiment where the Stabilizing Agent is a sugar or sugar-alcohol, the amount of Stabilizing Agent is present in a range from about 1% to about 15% (w/w), and more particularly in a range of about 2% to about 10% (w/w). In an embodiment where the Stabilizing Agent is selected from mannitol, sucrose, lactose or a combination thereof, the Stabilizing Agent is present at about 2.5% to about 5% (w/w).

Without departing from the object and scope of the present invention, other pharmaceutically acceptable adjuvants may optionally be added to the pharmaceutical compositions of the present invention. When the stabilized pharmaceutical composition contains a pharmaceutically acceptable adjuvant, the adjuvant is chosen from tonicity agents such as sugars, sugar-alcohol and sodium chloride, co-solvents, stabilizers, cryoprotective agents, fillers, and surfactants as previously above and as otherwise known in the art.

- 11 -

Where a solution according to the invention is prepared for injection, adjuvants such as an isotonicizing agent, preservatives such as sorbic acid, benzylchonium, polyparabins or other additives may be added thereto.

5 In one embodiment, the pharmaceutical compositions described above are salt-free except for the Active Ingredient, the Solubilizing Agent and the Stabilizing Agent.

In another embodiment, the pharmaceutical compositions described are lyophilized. The lyophilized composition can be prepared with an annealing step by employing the collapse temperature characteristics of the composition.

10 The lyophilized composition contains a Solubilizing Agent selected from an acid, a surfactant or a water-soluble polymer in an amount from about 0.1% to about 30% (w/w). In a lyophilized composition where the Solubilizing Agent is an acid, the lyophilized composition comprises an amount of acid from about 0.1% to about 2%. In another embodiment of the lyophilized composition, where the Solubilizing Agent is a water-soluble polymer such as cyclo-dextrin, the concentration range is from about 5% to about 30%. In another embodiment, where the Solubilizing Agent is a surfactant, the concentration range is from about 15 1% to about 10%. For lyophilized compositions where the Solubilizing Agent is selected from phosphoric acid, citric acid, acetic acid or hydrochloric acid, the Solubilizing Agent is present at about 0.1% to about 0.5% (w/w). In a specific embodiment where the lyophilized composition comprises phosphoric acid as the Solubilizing Agent, the Solubilizing Agent is present at about 0.15% (w/w).

20 The identity and proportions of Stabilizing Agent used in the lyophilized compositions of the invention are the same as those set out in the preceding section for the pharmaceutical compositions. In one embodiment, mannitol alone is used as the Stabilizing Agent ingredient of the lyophilized composition of the invention. In additional embodiments provided below, sucrose and lactose, as combined with or without mannitol, are used as the Stabilizing Agent of the lyophilized composition.

25 In the present invention, the solid lyophilized compositions of the invention are substantially salt-free, except for Compound A and the Solubilizing Agent and Stabilizing Agent contained therein.

The pharmaceutical or lyophilized composition is prepared by simultaneous or successive dissolution of the Active Ingredient, Solubilizing Agent and Stabilizing Agent in water, and/or addition of other pharmaceutically acceptable adjuvants, and where appropriate, lyophilization and/or freezing.

PREPARATION OF LYOPHILIZED COMPOSITION

30 The inventive compositions are more particularly prepared by dissolving the Active Ingredient in an aqueous solvent such as water that comprises an amount of Solubilizing Agent, followed by the Stabilizing Agent and, where appropriate, further adjusting the pH to within the range of about 3.5 to about 5. Where appropriate, they are lyophilized and/or frozen. The preparation and distribution of the solution are generally carried out between 0°C and room temperature. These stabilized pharmaceutical compositions 35 are optionally sterilized, in particular by sterilizing filtration.

- 12 -

The lyophilized composition can be prepared by freeze drying a solution containing Compound A, subjecting it to annealing, and then drying in a high vacuum for sublimating water. Such lyophilized preparations include lyophilized preparations for injection as mentioned above. The lyophilized preparation may be produced by conventional methods including tray lyophilization, spray lyophilization and vial lyophilization methods. Vial lyophilization is advantageous for preparing multi-dosage units of the invention, as described infra.

In order to obtain a solution of Compound A by the process of the present invention, Compound A, a Solubilizing Agent and a solvent are mixed and stirred until the mixture becomes clear. The solvent is preferably an aqueous solvent as described in the preceding paragraph. such as water, purified water, water for injection, or sugar solution for injection such as 5% dextrose injection solution. In one embodiment, the solvent is a salt-free aqueous solvent such as water, purified water, water for injection or sugar solutions for injection.

In order to obtain a lyophilized preparation of Compound A by the process of the present invention, first a processing solution prior to lyophilization is prepared. The processing solution before lyophilization is a solution prepared by mixing and stirring Compound A, a Solubilizing Agent and a solvent as set out above, and a Stabilizing Agent, until the mixture becomes clear. For the sequence of addition of the ingredients to the solvent it is highly preferred to first dissolve the Solubilizing Agent and Active Ingredient, and thereafter dissolve the Stabilizing Agent. The solvent is preferably an aqueous solvent such as previously set out above. The processing solution before lyophilization of Compound A may contain Compound A at a concentration of from about 0.01% to about 2%, and more particularly at a concentration of from about 0.5% to about 1% (w/w).

If desired, the processing solution before lyophilization may be subjected to a filtration process. The filtration process includes, for example in the case of injectable preparations, a sterilizing filtration and/or an ultra filtration of the processing solution before lyophilization to eliminate microorganisms or other contaminating matter from the processing solution before lyophilization.

If desired, the processing solution before lyophilization may be subjected to a distributing process. The distributing process includes, for example in the case of vial lyophilizations, a process distributing a suitable volume of the processing solution before lyophilization into vials taking the concentration of Compound A into consideration in order that vial products carry a desired amount of Compound A.

The lyophilization composition is prepared by a sequential heating and cooling process, comprising the steps of:

- (i) dissolving lyophilized composition ingredients, {5-[3-(4,6-Difluoro-1*H*-benzimidazol-2-yl)-1*H*-indazol-5-yl]-4-methyl-pyridin-3-ylmethyl}-ethyl-amine, a Solubilizing Agent, and a Stabilizing Agent in an aqueous solvent;
- (ii) cooling the processing solution of step (i) to a temperature below about -35°C;

- 13 -

(iii) heating the product of step (ii) to a temperature above about -25°C ;

(iv) cooling the product of step (iii) to a temperature below about -35°C ;

(v) heating the product of step (iv) to a temperature above about -25°C under subatmospheric pressure for a time sufficient to remove water from the aqueous solvent and yield a solid lyophilized product.

5 In one embodiment, step (i) is conducted by dissolving in an aqueous solvent {5-[3-(4,6-Difluoro-1*H*-benzoimidazol-2-yl)-1*H*-indazol-5-yl]-4-methyl-pyridin-3-ylmethyl}-ethyl-amine; a Solubilizing Agent present at about 0.1% to about 30% (w/w), and a Stabilizing Agent selected present at about 1% to about 15%. In an embodiment where the Solubilizing Agent is selected from phosphoric acid, citric acid, acetic acid or hydrochloric acid, the Solubilizing Agent is present from about 0.1% to about 0.5% (w/w). Where
10 the Stabilizing Agent is selected from mannitol, sucrose lactose or combinations thereof, the Stabilizing Agent is present at about 2% to about 10% (w/w). In a specific embodiment, the Solubilizing Agent is phosphoric acid at about 0.15% (w/w) and the Stabilizing Agent is mannitol at about 2.5% to about 5% (w/w). Each of steps (ii), (iii), (iv) and (v) is preferably conducted for a period of at least one-half hour,
15 and step (v) is performed at a subatmospheric pressure less than about 133 Pa (1000 milliTorr).

In a preferred parameter of the lyophilization process, a solution of Compound A, the Solubilizing Agent and the Stabilizing Agent are frozen by cooling to a range of about -35°C to about -45°C , and more particularly to a range of about -40°C . This cooling step is performed over the course of about 2 to 4 hours and is called the "first cooling step".

20 In a second step, the product of the first cooling step is warmed to about -5°C to about -25°C to allow the product to crystallize. In a further embodiment, the product of the first cooling step is warmed to about -10°C to about -20°C and more particularly, is warmed to about -15°C . This warming step is performed over the course of about 5 to 30 hours, more particularly in the range of about 10 to 20 hours, and is referred to as the "annealing step."

25 In a third step, the product of the annealing step is re-cooled to a range of about -35°C to about -45°C , and more particularly to a range of about -40°C . This second cooling step is again performed over the course of about 2 to 4 hours and is called the "re-cooling step".

Finally, the product of the re-cooling step is dried under a high vacuum by sublimating water according to methods known to those skilled in the art to obtain the lyophilized composition. In one embodiment, the
30 drying step is performed by warming to a temperature of about -10°C to about -25°C at a subatmospheric pressure of about 100 to about 200 milliTorr. This step is referred to as the "drying step." The drying step is performed over the course of about 20 hours, and more particularly is performed for at least 30 hours. Optionally, an additional step of drying, herein referred to as the "further drying step" may be performed where the temperature and degree of vacuum are varied for complete removal of water. In one
35 embodiment of the invention, the further drying step is performed over the course of about 10 hours by

- 14 -

further warming to a temperature of about 20°C to about 40°C at a subatmospheric pressure of about 100 to about 500 milliTorr.

The final lyophilized composition may contain some free water, typically in a range of from about 0.1% to about 5.0% (w/w) of the amount of equivalent base of Compound A. More particularly, the water content
5 ranges from about 0.8% to about 2.0% (w/w).

A dose-concentrate configuration as used in the invention is a sealed container holding an amount of lyophilized pharmaceutical formulation of the invention employed over a standard treatment interval such as 6 or 24 hours. The dose-concentrate configuration is prepared by placing lyophilized composition in a container (e.g., glass, bottles, vials, ampoules) in a sufficient amount to treat a mammal for a period
10 ranging from 1 hour to 1 several days. In one embodiment, the dose-concentrate provides sufficient lyophilized composition for about 4 to 12 hours. The container also should contain sufficient empty space to permit agitation and allow complete dissolution of the lyophilized composition in the added aqueous solvent. The container may be equipped with a penetrable top, e.g., a rubber seal, so that aqueous solvent may be added by penetrating the seal with a hypodermic syringe for removal of the concentrate.

15 An example of a dose-concentrate configuration is a glass vial having a capacity of from about 10 to about 100 milliliters containing 50 to 5000 milligrams of lyophilized pharmaceutical composition. A specific example is a 20 ml glass bottle with a rubber seal having lyophilized pharmaceutical composition containing 40 mg of Compound A, about 11 mg of phosphoric acid, and about 20 mg of mannitol. The empty space above the solid composition has ample space for adding solvent such as water for injection
20 plus enough space for agitation of the contents. The lyophilized composition can be stored for up to about 2 hours at a temperature of about 2 to 8°C.

The addition of the aqueous solvent to the dose-concentrate configuration results in a liquid concentrate which may then be conveniently used to form unit dosages of liquid pharmaceutical formulations by removing aliquot portions or entire contents for dilution as set out in the following section.

25 **INJECTABLE PREPARATIONS**

The lyophilized pharmaceutical formulation can be taken up, at the time of use, in any pharmaceutically acceptable injectable medium, e.g. water, water for injection, purified water, or other media, in particular in media such as sugar solutions, as for example, aqueous dextran solutions, or without any limitation being implied, with glucose solutions, polyvinylpyrrolidone solutions, or polysorbate solutions. For example, for
30 intravenous injection the compositions of the invention may be dissolved in a concentration of 2 mg/ml in a 5% dextrose solution

The formulations are taken up in a solution by passing via a concentrated solution of about 2 to about 5 mg/ml, to form the concentrate. The concentrate solution can be used as is or can be further diluted as the injectable medium for administration by infusion.

- 15 -

Frozen formulations can be frozen from solutions initially prepared, containing about 0.1 to about 10 mg/ml, and more particularly about 2 to about 5 mg/ml, or from diluted solutions, for the preparation of frozen bags, for example. The frozen formulations are thawed at the time of use and then diluted, if necessary.

- 5 The solutions presented in the liquid state contain about 0.1 to about 10 mg/ml of Active Ingredient.

The injectable medium comprising the pharmaceutical composition can be made at the time of injection or shortly before and should be used in approximately 4 to 12 hours from preparation. The addition of the water or sugar solution can be made directly in the infusion bag, into which the water or sugar solution will have been introduced first followed by introduction of the pharmaceutical composition, or optionally of its concentrate. Alternatively, the water or sugar solution can be introduced to a container or bag already containing the pharmaceutical composition or its concentrate. As an additional alternative, the combination can also be made by using two infusion bags, one containing a concentrate solution of the pharmaceutical composition in the injectable medium and the other containing further water or sugar solution.

- 10
15 When frozen bags already containing the dilute solution of the pharmaceutical composition are used, the bags can be thawed and the solution can optionally be re-diluted with any pharmaceutically acceptable injectable medium.

It is understood that the presentation kits for the inventive compositions also fall within the context of the present invention. Presentation kits of any form can be suitable, in particular, for example, presentations in the form of a bottle, an infusion bag, or an ampoule.

20 The injectable medium comprising the pharmaceutical or lyophilized composition is added to an intravenous (IV) container containing a suitable aqueous solvent. Useful solvents are standard solutions for injection as previously described (e.g., 5% dextrose or water for injection, etc). Typical unit dosage IV bags are conventional glass or plastic containers having inlet and outlet means and having standard (e.g., 250 ml and 500 ml) capacities. The concentrated solution of lyophilized pharmaceutical formulation is added to the unit dose IV bag in an amount to achieve a concentration of about 0.05 to about 2.0 mg of Compound per ml and more particularly from about 0.2 to about 1.5 mg per ml.

ADMINISTRATION

30 The doses of the pharmaceutical composition administered to a mammal are usually in the range from about 0.2 to about 40 mg/kg. In one embodiment where the mammal is a human, the range is from about 0.2 to about 4.0 mg/kg. In embodiments which include rats and other non-human mammals, the range of dose is from about 7.5 to about 40 mg/kg.

The pharmaceutical or lyophilized composition as diluted in injectable media, is sufficiently stable for extemporaneous administration by infusion. In particular, they are stable, without any appreciable degradation for a period of at least 24 hours at room temperature.

35

- 16 -

METHODS OF TREATMENT

The diluted formulations for this invention are given by injection into a vein. In one embodiment, the mode of delivery is by intravenous injection to the mammal, which offers the advantage of a quick effect and rapid access into the circulation system.

- 5 The pharmaceutical or lyophilized compositions of this invention are diluted with aqueous solvent suitable for injection and a liquid unit dosage form prepared (viz. IV Bag) for administration to a mammal. In a preferred embodiment of the invention, the mammal is a human patient. An IV bag for pediatric use may have a 100 ml capacity.

- 10 It may be necessary to make routine variations to the dosage depending on the age and condition and tolerance of the patient. The specific dose of a compound administered according to this invention to obtain therapeutic effects will, of course, be determined by the particular circumstances surrounding the case, including for example, the condition being treated. Typical daily doses for a patient will contain a Compound A dosage level of from about 0.2 mg/kg to about 4 mg/kg of of an Active Ingredient of this invention.

- 15 The pharmaceutical and lyophilized compositions of the present invention are contained in an amount sufficient to achieve the intended purpose; i.e., the modulation of cyclin-dependent kinase (CDK) activity or the treatment or prevention of a CDK-related disorder. The CDK-related disorder refers to a cell-proliferative disorder that would include cancers: squamous cell carcinoma, sarcomas such as Kaposi's sarcoma, astrocytoma, glioblastoma, lung cancer, bladder cancer, colorectal cancer, gastrointestinal
20 cancer, head and neck cancer, melanoma, ovarian cancer, prostate cancer, breast cancer, small-cell lung cancer, leukemia and glioma in a further aspect of this invention. Treatment or prevention of a CDK-related disorder may also include diabetes, a hyper-proliferation disorder, von Hippel-Lindau disease, restenosis, fibrosis, psoriasis, osteoarthritis, rheumatoid arthritis, an inflammatory disorder, mastocytosis and angiogenesis in yet another aspect of this invention. Additional disorders which may be treated or
25 prevented using the compounds of this invention are immunological disorders such as autoimmune disease (AIDS) and cardiovascular disorders such as atherosclerosis.

More specifically, a therapeutically effective amount means an amount of compound effective to prevent, alleviate or ameliorate symptoms of disease or prolong the survival of the subject being treated.

- 30 Determination of a therapeutically effective amount is well within the capability of those skilled in the art, especially in light of the detailed disclosure provided herein.

- For any compound used in the methods of the invention, the therapeutically effective amount or dose can be estimated initially from cell culture assays. Then, the dosage can be formulated for use in animal models so as to achieve a circulating concentration range that includes the IC_{50} as determined in cell culture (i.e., the concentration of the test compound which achieves a half-maximal inhibition of the PK
35 activity). Such information can then be used to more accurately determine useful doses in humans.

- 17 -

Toxicity and therapeutic efficacy of the compounds described herein can be determined by standard pharmaceutical procedures in cell cultures or experimental animals, e.g., by determining the IC_{50} and the LD_{50} (both of which are discussed elsewhere herein) for a subject compound. The data obtained from these cell culture assays and animal studies can be used in formulating a range of dosage for use in humans. The dosage may vary depending upon the dosage form employed and the route of administration utilized. The exact dosage can be chosen by the individual physician in view of the patient's condition. (See e.g., Fingl, et al., 1975, in "The Pharmacological Basis of Therapeutics", Ch. 1 p.1).

Therapeutic compounds should be more potent in inhibiting CDK activity than in exerting a cytotoxic effect. A measure of the effectiveness and cell toxicity of a compound can be obtained by determining the therapeutic index; i.e., IC_{50}/LD_{50} . IC_{50} , the dose required to achieve 50% inhibition, can be measured using standard techniques such as those described herein. LD_{50} , the dosage which results in 50% toxicity, can also be measured by standard techniques as well (Mossman, 1983, J. Immunol. Methods, 65:55-63), by measuring the amount of LDH released (Korzeniewski and Callewaert, 1983, J. Immunol. Methods, 64:313; Decker and Lohmann-Matthes, 1988, J. Immunol. Methods, 115:61), or by measuring the lethal dose in animal models. Compounds with a large therapeutic index are preferred. Thus, in one aspect of the invention, a preferred dosage of the compounds, agents, combinations, and pharmaceutical compositions contemplated for use in the invention requires the therapeutic index of each active component to be greater than 2, preferably at least 10, more preferably at least 50.

Dosage amount and interval may be adjusted individually to provide plasma levels of the active species, which are sufficient to maintain the kinase modulating effects. These plasma levels are referred to as minimal effective concentrations (MECs). The MEC will vary for each compound but can be estimated from in vitro data; e.g., the concentration necessary to achieve 50-90% inhibition of a kinase may be ascertained using the assays described herein. Dosages necessary to achieve the MEC will depend on individual characteristics and route of administration. HPLC assays or bioassays can be used to determine plasma concentrations.

Dosage intervals can also be determined using MEC value. Compounds should be administered using a regimen that maintains plasma levels above the MEC for 10-90% of the time, preferably between 30-90% and most preferably between 50-90%.

The decision to determine the length of therapy may be supported by standard clinical laboratory results from commercially available assays or instrumentation supporting the eradication of the symptoms defining the cell-proliferative disorder. The method of the invention may be practiced by continuously or intermittently administering a therapeutically effective dose of the solution prepared from the lyophilized pharmaceutical formulation for as long as deemed efficacious for the treatment of the cell-proliferative disorder.

The decision to end therapy by the method of the invention may be supported by standard clinical laboratory results from commercially available assays or instrumentation or the disappearance of clinical

- 18 -

symptoms characteristic of the cell-proliferative disorder. The therapy may be restarted upon a relapse of the cell-proliferative disorder.

In general, a "therapeutically effective amount" refers to that amount of an agent or its metabolite which is effective to prevent, alleviate, reduce or ameliorate symptoms of disease and/or the undesired side effects attributable to treatment of disease with another agent or its metabolite, or to prolong the survival of the patient being treated. More particularly, in reference to the treatment of cancer, a therapeutically effective amount refers to that amount which has the effect of (1) reducing the size of (or preferably eliminating) the tumor; (2) inhibiting (that is, slowing to some extent, preferably stopping) tumor metastasis; (3) inhibiting to some extent (that is slowing to some extent, preferably stopping) tumor growth; and/or, (4) relieving to some extent (or preferably eliminating) one or more symptoms associated with the cancer and/or one or more undesired side effects attributable to treatment of the cancer with another agent or its metabolite. Non-limiting examples of therapeutically effective amounts of particular agents and compounds contemplated for use in the present invention are further described below.

In addition to the above general definition, by a "therapeutically effective amount" of an agent is meant any amount administered in any manner and in any treatment regime as may be currently recognized in the medical arts or as may come about as the result of future developments regarding the use of these agents.

A "treatment regime" refers to specific quantities of the Active Ingredient contemplated for use in this invention) administered at set times in a set manner over an established time period.

When referring to "set times" of administration within a treatment regime, "consecutive days" means consecutive calendar days; i.e., Monday, Tuesday, Wednesday, etc. "Staggered" days means calendar days with other calendar days between them, e.g., without limitation, Monday, Wednesday, Saturday, etc.

Furthermore, with regard to a "therapeutically effective amount" of the Active Ingredient, the phrase refers to an amount of the compound sufficient to inhibit the growth, size and vascularization; i.e., angiogenesis and/or vasculogenesis, of tumors during the "recovery" periods, i.e., the periods in a treatment regime when no other chemotherapeutic agent is being administered to a patient.

The compounds of the preferred embodiments of the present invention may be administered in doses ranging from about 20 mg/m² to about 250 mg/m². Of course, the dose would depend on a number of factors, including patient specific factor, e.g., weight, dosing regimen (e.g., frequency of administration etc).

In a presently preferred embodiment, the composition dose is administered during rest periods when no other agent is being administered to a patient.

COMPOSITION EXAMPLES

Generally, the formulations of the preferred embodiments of the present invention are prepared by combining the Active Ingredient with one or more pharmaceutically acceptable Solubilizing Agents and

pharmaceutically acceptable Stabilizing Agents, as well as optional pharmaceutically acceptable adjuvants.

Examples 1-11:

- 5 The processing solution before lyophilization was prepared at about room temperature by charging the mixing vessel with half of the required water for injection. Approximately 80% of the 1M phosphoric acid is added into the mixing vessel. Compound A was added at 5 mg/ml. The mannitol, sucrose or lactose was subsequently added slowly at the weight % provided in Table 1 and mixed in until dissolved.

Table 1 lists Specific Lyophilized Compositions of the Invention, and the target pH:

10 Table 1:

Example #	Mannitol %	Sucrose %	Lactose %	Target pH
1	5	-	-	3.8
2	-	-	4	3.8
3	4	-	2	3.8
4	3	-	4	3.8
5	-	-	2	3.8
6	3	4	-	3.5
7	3	4	-	3.8
8	3	4	-	4.1
9	-	4	-	3.8
10	4	2	-	3.8
11	2.5	-	-	3.8

- 15 The pH of the solution was adjusted to the designated amount as shown in Table 1 by slowly adding the remaining 1M phosphoric acid. Water for injection was further added to batch weight, such that the final concentration of Compound A was about 5 mg/ml. The processing solution before lyophilization was subjected to a sterilizing filtration using a polyvinylidene fluoride (PVDF) membrane filter of which the pore size was 0.2 micrometer and then 8.5 ml was distributed into depyrogenated 20 ml glass vials. A stopper was then partially inserted into each vial followed by lyophilization.

- 20 A portion of vials from each of Examples 1-11 did not proceed to lyophilization and were stored for daily observation. It was noted that after approximately one week, all of Examples 1-11 showed signs of haze and gelling.

- 25 The lyophilization was performed as follows. The first cooling step was done for 8 hours at -40°C, followed by the annealing step for 24 hours at -15°C, and then the re-cooling step for 8 hours at about -40°C. The drying step was then performed by warming the product for 67 hours to -15°C, and backfilling the chamber with nitrogen at 0.5 Pa. A further drying step was also performed for 10 hours at 40°C at 100 to 500 milliTorr. The vials were then stored at about 2 to 8°C and protected from light.

- 20 -

Table 2 below indicates the appearance of the lyophilization cakes after 6 weeks at 40°C at 75% humidity. The lyophilized cakes have either no or very limited coloring, and do not have any cracked or shrunken parts.

- 5 The lyophilized product was reconstituted with a 21 ml of 5% dextrose solution to produce a clear, isotonic 2 mg/ml drug solution for intravenous administration. After 6 weeks under the above-noted conditions, the reconstitution time was measured to generally less than one minute for each of Examples 1-11. The reconstitution times of the lyophilized compositions were measured by adding 8 ml of water for injection to the vial and hand-shaking the resulting mixture. The period of time until the samples were completely
10 dissolved was then measured.

Content uniformity tests for Compound A in each of Examples 1- 11 were performed. The content of Compound A was measured by HPLC and is recorded below in Table 2.

- 15 Table 2 summarizes the data for a 6 week stability study at 40°C and 75% relative humidity, including the appearance of the lyophilized cakes, the reconstitution times, the appearance of the reconstituted solution and the Compound A content % for each of Examples 1-11.

Table 2:

Example #	Appearance of lyo cake	Reconstitution Time	Appearance of Reconstituted Solution	Compound A content (%)
1	white compact	~30 sec	clear	93.2
2	pale yellow compact	~30 sec	clear	101.8
3	white compact	~30 sec	clear	99.4
4	white compact	~30 sec	clear	97.7
5	Yellowish compact	~30 sec	clear	109.9
6	white compact	~30 sec	clear	89.9
7	white compact	~30 sec	clear	90.6
8	white compact	~30 sec	clear	90.4
9	white compact	~30 sec	clear	93.7
10	white compact	~30 sec	clear	91.8
11	white compact	~30 sec	clear	111.4

- 21 -

CLAIMS

What is claimed is:

1. A pharmaceutical composition comprising {5-[3-(4,6-Difluoro-1H-benzoimidazol-2-yl)-1H-indazol-5-yl]-4-methyl-pyridin-3-ylmethyl}-ethyl-amine as Active Ingredient, a Solubilizing Agent and a Stabilizing Agent.
5
2. The pharmaceutical composition of claim 1, wherein the Solubilizing Agent is selected from phosphoric acid, citric acid, acetic acid and hydrochloric acid.
3. The pharmaceutical composition of claim 1 or 2, wherein the Solubilizing Agent is phosphoric acid.
- 10 4. The pharmaceutical composition of claim 1, 2 or 3, wherein the Stabilizing Agent is selected from mannitol, sucrose, lactose and combinations thereof.
5. The pharmaceutical composition of any one of the preceding claims, wherein the Stabilizing Agent is mannitol.
6. The pharmaceutical composition of claim 1, wherein the amount of Solubilizing Agent is about
15 0.1% to about 50% (w/w).
7. The pharmaceutical composition of claim 1, wherein the amount of Solubilizing Agent is about 0.1% to about 0.5% (w/w).
8. The pharmaceutical composition of claim 1, wherein the amount of Stabilizing Agent is about 1% to about 15% (w/w).
- 20 9. The pharmaceutical composition of claim 1, wherein the amount of Stabilizing Agent is about 2.5% to about 5% (w/w).
10. A solution prepared from the lyophilized composition of claim 1, further comprising a 5% dextrose solution.
11. A method of treating a mammal for a cell-proliferative disorder, wherein the method comprises
25 parentally administering to said mammal an effective amount of a solution prepared from the composition of any of claims 1 or 9.
12. A method of inhibiting a cyclin-dependent kinase (CDK) comprising contacting the CDK with a therapeutically effective amount of a solution prepared from the composition of any of claims 1 or 9.
13. A container having the lyophilized composition of claim 12, said container further having a space
30 adequate for introduction of a volume of aqueous solvent sufficient to form a solution of said composition.

- 22 -

14. A unit dose IV container having a liquid for intravenous injection in the treatment of a cell-proliferative disorder, said liquid comprising an aqueous solution prepared from the composition of any of claims 1 or 9.
15. A method of making a pharmaceutical composition, comprising the steps of:
- 5 (i) dissolving {5-[3-(4,6-Difluoro-1H-benzimidazol-2-yl)-1H-indazol-5-yl]-4-methyl-pyridin-3-ylmethyl}-ethyl-amine, a Solubilizing Agent, and a Stabilizing Agent in an aqueous solvent to form a processing solution;
- (ii) cooling the processing solution of step (i) to a temperature below about -35°C ;
- (iii) heating the product of step (ii) to a temperature above about -25°C ;
- 10 (iv) cooling the product of step (iii) to a temperature below about -35°C ;
- (v) heating the product of step (iv) to a temperature above about -25°C under subatmospheric pressure for a time sufficient to remove water from the aqueous solvent and yield a solid lyophilized product.
16. A method of treating a cell-proliferative disorder in a mammal comprising administering to the
- 15 mammal a therapeutically effective amount of an aqueous solution comprising {5-[3-(4,6-Difluoro-1H-benzimidazol-2-yl)-1H-indazol-5-yl]-4-methyl-pyridin-3-ylmethyl}-ethyl-amine, phosphoric acid at about 0.15% (w/w), and mannitol at about 2.5% (w/w).

INTERNATIONAL SEARCH REPORT

International Application No
PCT/IB2005/002734

A. CLASSIFICATION OF SUBJECT MATTER

IPC 7 A61K47/02 A61K47/26 A61K31/4184 A61P35/00

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 7 A61K A61P

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

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

EPO-Internal, WPI Data, PAJ, BIOSIS, EMBASE, CHEM ABS Data, CANCERLIT, SCISEARCH

C. DOCUMENTS CONSIDERED TO BE RELEVANT

Category *	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
P, X	WO 2005/009997 A (PFIZER INC; KEPHART, SUSAN, ELIZABETH; MCALPINE, INDRAWAN, JAMES; REIC) 3 February 2005 (2005-02-03) page 9, lines 3-7 page 12, last paragraph - page 13, paragraph 1 page 20; example 1	1, 10-16
A	DAI YUN ET AL: "Cyclin-dependent kinase inhibitors." CURRENT OPINION IN PHARMACOLOGY, vol. 3, no. 4, August 2003 (2003-08), pages 362-370, XP002346558 ISSN: 1471-4892 the whole document	1-16

☐ Further documents are listed in the continuation of box C.

☒ Patent family members are listed in 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 document 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

27 October 2005

Date of mailing of the international search report

04/11/2005

Name and mailing address of the ISA

European Patent Office, P.B. 5818 Patentlaan 2
NL - 2280 HV Rijswijk
Tel. (+31-70) 340-2040, Tx. 31 651 epo nl,
Fax: (+31-70) 340-3016

Authorized officer

Rodriguez-Palmero, M

INTERNATIONAL SEARCH REPORT

International application No.
PCT/IB2005/002734

Box 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.:
because they relate to subject matter not required to be searched by this Authority, namely:

Although claims 11, 12 and 16 are directed to a method of treatment of the human/animal body, the search has been carried out and based on the alleged effects of the compound/composition.
2. ☐ Claims Nos.:
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:
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 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.
- ☐ No protest accompanied the payment of additional search fees.

INTERNATIONAL SEARCH REPORT

Information on patent family members

Interi

al Application No

PCT/IB2005/002734

Patent document cited in search report	Publication date	Patent family member(s)	Publication date
WO 2005009997 A	03-02-2005	NL 1026761 A1	01-02-2005