



(51) **International Patent Classification:**  
C07C 251/22 (2006.01) A61P 43/00 (2006.01)  
A61K 31/135 (2006.01)

(21) **International Application Number:**  
PCT/EP2010/065337

(22) **International Filing Date:**  
13 October 2010 (13.10.2010)

(25) **Filing Language:** English

(26) **Publication Language:** English

(30) **Priority Data:**  
0917927.6 13 October 2009 (13.10.2009) GB

(71) **Applicant (for all designated States except US):** LUDWIG INSTITUTE FOR CANCER RESEARCH LTD [US/US]; 605 Third Avenue, New York, New York 10158 (US).

(72) **Inventors; and**

(75) **Inventors/Applicants (for US only):** ROEHRIG, Ute [CH/CH]; Ludwig Institute for Cancer Research, Lausanne Branch, CH-1015 Lausanne (CH). LOAY, Awad [CH/CH]; Ludwig Institute for Cancer Research, Lausanne Branch, CH-1015 Lausanne (CH). MICHIELIN, Olivier [CH/CH]; Ludwig Institute for Cancer Research, Lausanne Branch, CH-1015 Lausanne (CH). VAN DEN EYNDE, Benoit [BE/BE]; Cellular Genetics Unit, Institute of Cellular Pathology, Universite Catholique de Louvain, B-1200 Brussels (BE). PILOTTE, Luc [BE/BE]; Ludwig Institute for Cancer Research, Avenue Hippocrate 74, UCL 7459, B-1200 Brussels (BE). STROOBANT, Vincent [BE/BE]; Cellular Genetics Unit, Institute of Cellular Pathology, Universite Catholique de Louvain,

B-1200 Brussels (BE). LARRIEU, Pierre [FR/BE]; Cellular Genetics Unit, Institute of Cellular Pathology, Universite Catholique de Louvain, B-1200 Brussels (BE). VOGEL, Pierre [CH/CH]; Laboratory of Glycochemistry and Asymmetric Synthesis, Ecole Polytechnique Federale de Lausanne (EPFL), CH-1015 Lausanne (CH). ZOETE, Vincent [CH/CH]; Swiss Institute of Bioinformatics, Molecular Modeling Group, CH-1015 Lausanne (CH).

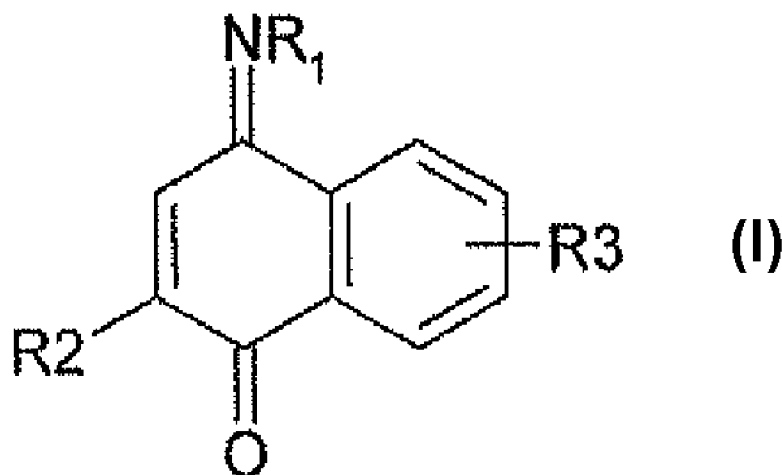
(74) **Agent:** OLDROYD, Richard Duncan; Elkington and Fife LLP, Prospect House, 8 Pembroke Road, Sevenoaks Kent TN13 1XR (GB).

(81) **Designated States (unless otherwise indicated, for every kind of national protection available):** AE, AG, AL, AM, AO, AT, AU, AZ, BA, BB, BG, BH, BR, BW, BY, BZ, CA, CH, CL, CN, CO, CR, CU, CZ, DE, DK, DM, DO, DZ, EC, EE, EG, ES, FI, GB, GD, GE, GH, GM, GT, HN, HR, HU, ID, IL, IN, IS, JP, KE, KG, KM, KN, KP, KR, KZ, LA, LC, LK, LR, LS, LT, LU, LY, MA, MD, ME, MG, MK, MN, MW, MX, MY, MZ, NA, NG, NI, NO, NZ, OM, PE, PG, PH, PL, PT, RO, RS, RU, SC, SD, SE, SG, SK, SL, SM, ST, SV, SY, TH, TJ, TM, TN, TR, TT, TZ, UA, UG, US, UZ, VC, VN, ZA, ZM, ZW.

(84) **Designated States (unless otherwise indicated, for every kind of regional protection available):** ARIPO (BW, GH, GM, KE, LR, LS, MW, MZ, NA, SD, SL, SZ, TZ, UG, ZM, ZW), Eurasian (AM, AZ, BY, KG, KZ, MD, RU, TJ, TM), European (AL, AT, BE, BG, CH, CY, CZ, DE, DK, EE, ES, FI, FR, GB, GR, HR, HU, IE, IS, IT, LT, LU, LV, MC, MK, MT, NL, NO, PL, PT, RO, RS, SE, SI, SK, SM, TR), OAPI (BF, BJ, CF, CG, CI, CM, GA, GN, GQ, GW, ML, MR, NE, SN, TD, TG).

[Continued on next page]

(54) **Title:** IDO INHIBITORS AND THERAPEUTIC USES THEREOF



(57) **Abstract:** Compounds of formula (I) in which R<sub>1</sub>, R<sub>2</sub> and R<sub>3</sub>, which may be the same or different, represent alkyl C<sub>1-6</sub>, cycloalkyl C<sub>3-7</sub>, aryl or NHR<sub>4</sub>, wherein R<sub>4</sub> may represent any of the values that any of R<sub>1</sub>, R<sub>2</sub> or R<sub>3</sub> may represent; additionally, R<sub>3</sub> may represent hydrogen, and pharmaceutically acceptable salts thereof, and their therapeutic use, eg in the treatment of cancer.



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**Published:**

— with international search report (Art. 21(3))

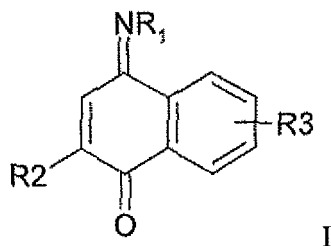
— before the expiration of the time limit for amending the claims and to be republished in the event of receipt of amendments (Rule 48.2(h))

**IDO Inhibitors and Therapeutic uses thereof**

This invention relates to inhibitors of indoleamine 2,3-dioxygenase (IDO) and their use in the treatment of cancer or infections, either alone or in combination with additional therapeutic agents.

The heme-containing enzyme indoleamine 2,3-dioxygenase (IDO, EC 1.13.11.52) has been implicated in the establishment of pathological immune tolerance by tumors. IDO catalyzes the initial and rate-limiting step in the catabolism of tryptophan (Trp) along the kynurenine pathway. By depleting Trp locally, IDO blocks the proliferation of T lymphocytes, which are extremely sensitive to Trp shortage. The observation that many human tumors constitutively express IDO introduced the hypothesis that its inhibition could enhance the effectiveness of cancer immunotherapy. Results from *in vitro* and *in vivo* studies suggest that the efficacy of therapeutic vaccination of cancer patients may indeed be improved by concomitant administration of an IDO inhibitor.

Accordingly, we provide the compounds of formula I



in which R<sub>1</sub>, R<sub>2</sub> and R<sub>3</sub>, which may be the same or different, represent alkyl C<sub>1-6</sub>, -cycloalkyl C<sub>3-7</sub>, aryl or NHR<sub>4</sub>, wherein R<sub>4</sub> may represent any of the values that any of R<sub>1</sub>, R<sub>2</sub> or R<sub>3</sub> may represent;  
additionally, R<sub>3</sub> may represent hydrogen,  
and pharmaceutically acceptable salts thereof.

We also provide the use of compounds I and pharmaceutically acceptable salts thereof as a pharmaceutical.

According to the invention, we further provide the use of the compounds of formula I, and pharmaceutically acceptable salts thereof, in the treatment of diseases in which

inhibition of IDO plays a therapeutic role, particularly those conditions mentioned herein.

We particularly prefer compounds of formula I in which  $R_1$  represents 2-propyl.

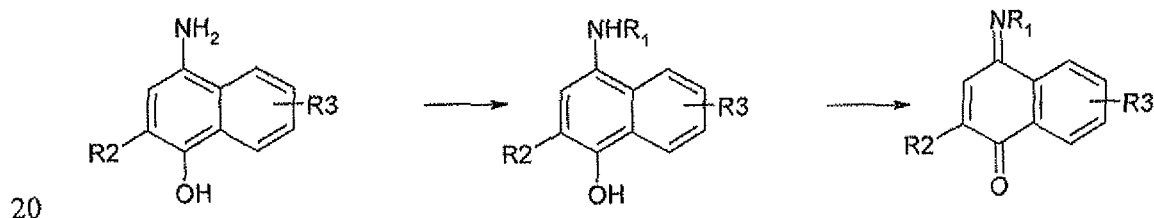
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We particularly prefer compounds of formula I in which  $R_2$  represents methyl.

We particularly prefer compounds of formula I in which  $R_3$  represents hydrogen.

- 10 Aryl groups that  $R_4$  may represent include benzene and 5- and 6-membered aromatic heterocycles, such as pyrrole, pyrazole and pyridine. The aryl group may be substituted, eg by one, two or three substituents, eg as defined for  $R_1$ ,  $R_2$  or  $R_3$  above

The compound of formula I can be obtained by oxidation of an appropriately substituted 1-amino-4 hydroxy-naphthalene derivative, eg using silver oxide in an inert solvent, such as chloroform. Substituted aminonaphthalenes may be prepared from the corresponding unsubstituted aminonaphthalene by reductive amination, using the appropriate aldehyde or ketone and a hydride reducing agent, such as  $\text{NaBH}(\text{OAc})_3$  in a polar aprotic solvent such as dichloroethane, as indicated in the following scheme:



in which  $R_1$ ,  $R_2$  and  $R_3$  are as defined above. An example of this scheme is given below.

- 25 Salts of compounds of formula I may be formed by reacting the free acid, or a salt thereof, with one or more equivalents of the appropriate base. The reaction may be carried out in a solvent or medium in which the salt is insoluble or in a solvent in which the salt is soluble, e.g. ethanol, tetrahydrofuran or diethyl ether, which may be removed in vacuo, or by freeze drying. The reaction may also be a metathetical process or it may
- 30 be carried out on an ion exchange resin.

Pharmaceutically acceptable salts of the compounds of formula I thereof include salts with strong acids, e.g., HCl, HBr, etc, and salts with weak acids, eg organic acids, for example carboxylic acids, such as acetic acid, benzoic acids, as well as sulphonic acids.

- 5 Non-toxic physiologically acceptable salts are preferred, although other salts are also useful, e.g. in isolating or purifying the product.

The compounds of formula I may be used alone or in combination with at least one additional therapeutic agent.

- 10 The at least one additional therapeutic agent may be an antineoplastic chemotherapy agent. Suitable antineoplastic chemotherapeutic agent is selected from the group consisting of cyclophosphamide, methotrexate, fluorouracil, doxorubicin, vincristine, ifosfamide, cisplatin, gemcytabine, busulfan, ara-C, and combinations thereof.

- 15 Alternatively, the at least one additional therapeutic agent may be radiation therapy. The radiation therapy may be localized radiation therapy delivered to the tumour or may be total body irradiation.

- The compounds of the invention may be used as an adjuvant to the therapeutic  
20 vaccination of various cancers. Cancers that may be mentioned include melanoma, colon cancer, pancreatic cancer, breast cancer, prostate cancer, lung cancer, leukemia, brain tumours, lymphoma, sarcoma, ovarian cancer, and Kaposi's sarcoma.

- Other cancers and tumours that may be mentioned include adrenocorticocancer, basal  
25 cell carcinoma, bladder cancer, bowel cancer, brain and CNS tumors, breast cancers, B-cell lymphoma, carcinoid tumours, cervical cancer, childhood cancers, chondrosarcoma, choriocarcinoma, chronic myeloid leukemia, rectal cancers, endocrine cancers, endometrial cancer, esophageal cancer, Ewing's sarcoma, eye cancer, gastric cancer or carcinoma, gastrointestinal cancers, genitourinary cancers, glioma,  
30 gynecological cancers, head and neck cancers, hepatocellular cancer, Hodgkins disease, hypopharynx cancer, islet cell cancer, kidney cancer, laryngeal cancer, liver cancer, lung cancer (including small-cell lung carcinoma and non-small-cell carcinoma),

lymphoma, male breast cancer, melanoma, mesothelioma, multiple myeloma, nasopharyngeal cancer, neuroblastoma, non-Hodgkins lymphoma, non-melanoma skin cancer, osteosarcoma, ovarian cancer, pancreas cancer, pituitary cancer, prostate cancer, renal cell carcinoma, retinoblastoma, rhabdomyosarcoma, sarcoma, skin cancer, squamous cell carcinoma, stomach cancer, testicular cancer or seminoma, thymus cancer, thyroid cancer, transitional cell cancer, trophoblastic cancer, uterine cancer, vaginal cancer, Waldenstrom's macroglobulinemia, and Wilm's tumor, colorectum, cervix, endometrium, ovary, testis, mesothelial lining, white blood cell (including lymphoma and leukemia) esophagus, muscle, connective tissue, adrenal gland, bone, glioblastoma, and cutaneous basocellular carcinoma.

In addition to cancers, IDO plays a role in several diseases, including *Clamydia psittaci* infection and *Streptococcus pyogenes* infection, systemic lupus erythematosus, rheumatoid arthritis, Alzheimer's disease, Huntington's disease, Parkinson's disease, Lyme neuroborreliosis, late Lyme encephalopathy, Tourette's syndrome, systemic sclerosis, multiple sclerosis, coronary heart disease, T-cell mediated immune diseases, chronic infections (viral, bacterial, fungal and microbial), depression, neurological disorders, cancer tumors, and cataracts. Inhibitors of IDO may be used to treat these diseases.

Other diseases that IDO inhibitors may be used to treat include, but are not limited to, human immunodeficiency virus (HIV) and AIDS-related cancers.

The compounds may also be used as adjuvants to bone marrow transplantation or peripheral blood stem cell transplantation and in immunotherapy by adoptive transfer.

Where the compounds are used in the treatment of an infection, the infection may be selected from the group consisting of a viral infection, infection with an intracellular parasite, and infection with an intracellular bacteria.

Particular viral infections include human immunodeficiency virus or cytomegalovirus.

Particular intracellular parasite infections may be selected from the group consisting of *Leishmania donovani*, *Leishmania tropica*, *Leishmania major*, *Leishmania aethiopica*, *Leishmania mexicana*, *Plasmodium falciparum*, *Plasmodium vivax*, *Plasmodium ovale*, and *Plasmodium malariae*.

5

Particular intracellular bacterial infections may be selected from the group consisting of *Mycobacterium leprae*, *Mycobacterium tuberculosis*, *Listeria monocytogenes*, and *Toxoplasma gondii*.

- 10 When the compounds are used in combination, the at least one additional therapeutic agent may be a vaccine, for example, an anti-viral vaccine, a vaccine against HIV, a vaccine against tuberculosis, a vaccine against malaria. The vaccine may also be a tumour vaccine or a melanoma vaccine. Preferably, the tumour vaccine comprises genetically modified tumour cells or genetically modified cell lines. In such cases,  
15 preferably the genetically modified tumour cells or genetically modified cell line has been transfected to express granulocyte-macrophage stimulating factor (GM-CSF).

- Alternatively, the vaccine may comprise one or more immunogenic peptides, preferably immunogenic peptides of cancer-testis antigens (CTAgS). Such CTAgS and  
20 immunogenic peptides thereof are well known in the art, see Scanlan et al. *Cancer Immun.* 2004; 4:1 and Simpson et al., *Nat Rev Cancer.* 2005; 5:615. CTAg proteins include MAGE, BAGE, GAGE, SSX, NY-ESO-1, LAGE, SCP, CTSP, CT7, CT8, CT9, CT10, CT11, SAGE, OY-TES-1, NY-SAR-35 and NY-BR-1. Several MAGE proteins are known, including MAGE-A1, A3, A4, A5, A6, A8, A9, A10, A12, B1, B2,  
25 B3, B4, C1, C2 and C3 proteins. Several SSX proteins exist, including SSX1, SSX2, SSX3 and SSX5.

Further, the tumour vaccine may comprise dendritic cells.

- 30 Further, the additional therapeutic agent may be a cytokine, for example a granulocyte-macrophage colony stimulating factor (GM-CSF) or flt3-ligand.

According to the invention we further provide a method of treating a subject receiving a bone marrow transplant or peripheral blood stem cell transplant comprising administering a therapeutically effective amount of compound of formula I or a pharmaceutically acceptable salt thereof to such a subject.

5

Preferably, the compound of formula I or a pharmaceutically acceptable salt thereof is administered in an amount effective to increase the delayed type hypersensitivity reaction to tumour antigen, delay the time to relapse of post-transplant malignancy, increase relapse free survival time post-transplant, and/or increase long-term post-transplant survival.

10

Preferably, the compound of formula I or a pharmaceutically acceptable salt thereof is administered prior to full hematopoietic reconstitution.

15 The compounds of formula I or a pharmaceutically acceptable salt thereof for use in the method will generally be administered in the form of a pharmaceutical composition.

Thus, according to a further aspect of the invention there is provided a pharmaceutical composition including preferably less than 80% w/w, more preferably less than 50% w/w, e.g. 0.1 to 20%, of the compound of formula I or a pharmaceutically acceptable salt thereof, in admixture with a pharmaceutically acceptable diluent or carrier.

20

We also provide a process for the production of such a pharmaceutical composition which comprises mixing the ingredients. Examples of pharmaceutical formulations which may be used, and suitable diluents or carriers, are as follows:

25

for intravenous injection or infusion - purified water or saline solution;

for inhalation compositions - coarse lactose;

for tablets, capsules and dragees - microcrystalline cellulose, calcium phosphate, diatomaceous earth, a sugar such as lactose, dextrose or mannitol, talc, stearic acid, starch, sodium bicarbonate and/or gelatin;

30

for suppositories - natural or hardened oils or waxes.

When the compounds of formula I or a pharmaceutically acceptable salt thereof is to be used in aqueous solution, e.g. for infusion, it may be necessary to incorporate other  
5 excipients. In particular there may be mentioned chelating or sequestering agents, antioxidants, tonicity adjusting agents, pH-modifying agents and buffering agents.

Solutions containing the compound of formula I or a pharmaceutically acceptable salt thereof may, if desired, be evaporated, e.g. by freeze drying or spray drying, to give a  
10 solid composition, which may be reconstituted prior to use.

When not in solution, the compound of formula I or a pharmaceutically acceptable salt thereof preferably is in a form having a mass median diameter of from 0.01 to 10µm. The compositions may also contain suitable preserving, stabilising and wetting agents,  
15 solubilisers, e.g. a water-soluble cellulose polymer such as hydroxypropyl methylcellulose, or a water-soluble glycol such as propylene glycol, sweetening and colouring agents and flavourings. Where appropriate, the compositions may be formulated in sustained release form.

20 The content of the compound of formula I or a pharmaceutically acceptable salt thereof in a pharmaceutical composition is generally about 0.01-about 99.9wt%, preferably about 0.1-about 50wt%, relative to the entire preparation.

The dose of the compound of formula I or a pharmaceutically acceptable salt thereof is  
25 determined in consideration of age, body weight, general health condition, diet, administration time, administration method, clearance rate, combination of drugs, the level of disease for which the patient is under treatment then, and other factors.

While the dose varies depending on the target disease, condition, subject of administration, administration method and the like, for oral administration as a therapeutic agent for the treatment of cancer in a patient suffering from such a disease is from 0.01 mg - 10 g, preferably 0.1 - 100 mg, is preferably administered in a single  
5 dose or in 2 or 3 portions per day. Where the compound of formula I or a pharmaceutically acceptable salt thereof is used in combination with other therapeutic agents, these may be used at their normal therapeutic doses, e.g., as set out in pharmacopoeias or prescribing guides, such as the Physicians' Desk Reference (PDR). In certain cases, the compound of formula I or a pharmaceutically acceptable salt  
10 thereof supplements the activity of the additional therapeutic agent(s) in a synergistic fashion, such that the additional therapeutic agent(s) can be administered at a lower dose than is normally used.

The potential activity of the compound of formula I or a pharmaceutically acceptable  
15 salt thereof in the treatment of cancer or infections has been demonstrated in the following predictive experiments, which demonstrate that the compound of formula I is an IDO inhibitor.

## Experimental Methods

### 20 Chemistry

Reagents were purchased from Acros, Fluka, Senn, Sigma-Aldrich or Merck and used without further purification. For extraction and chromatography all solvents were distilled prior to use. Anhydrous THF, diethyl ether, and toluene were distilled from sodium and benzophenone, dichloromethane from  $\text{CaH}_2$ , and methanol from  
25 magnesium. Reactions were monitored by thin layer chromatography (TLC) using silica gel plates (Merck 60 F254). Developed TLC plates were visualized with UV light (254 nm) or molybdic reagent (21 g of  $(\text{NH}_4)_6\text{Mo}_7\text{O}_{24} \cdot 4\text{H}_2\text{O}$ , 1 g of  $\text{Ce}(\text{SO}_4)_2$ , 31 ml of  $\text{H}_2\text{SO}_4$  and 470 ml of  $\text{H}_2\text{O}$ ). Flash chromatography (FC) was conducted using silica gel 60 Å, 230-400 mesh (Merck 9385). Melting points were measured with a Mettler FP52  
30 and are uncorrected. Optical rotations were measured with a JASCO DIP-370 digital polarimeter. UV spectra were recorded on a Kontron Uvikon 810 CW spectrophotometer. IR spectra were recorded on a Perkin Elmer Paragon 1000 FT-IR

spectrometer. Mass spectra were recorded on a Nermag R 10-10C in chemical ionisation mode. Electrospray mass analyses were recorded on a Finnigan MAT SSQ 710C spectrometer in positive ionisation mode.  $^1\text{H}$ -NMR spectra were recorded on a Bruker DPX-400 FT, Bruker ARX-400 FT, or Bruker AMX-600 spectrometer. All  $^1\text{H}$  signal assignments were confirmed by COSY spectra.  $^{13}\text{C}$ -NMR spectra were recorded on a Bruker DPX-400 FT (100.61 MHz) or on a Bruker ARX-400 FT (100.61 MHz) spectrometer. All  $^{13}\text{C}$  signal assignments were confirmed by HMQC spectra. Chemical shifts are given in ppm, relative to an internal standard such as residual solvent signals. Coupling constants are given in Hertz. High resolution mass spectra were recorded by ESI-TOF-HRMS or MALDI-TOF-HRMS.

**(E)-4-(Isopropylimino)-2-methylnaphthalen-1(4H)-one.**

(E)-4-(Isopropylimino)-2-methylnaphthalen-1(4H)-one, the compound of formula I in which  $\text{R}_1$  is isopropyl,  $\text{R}_2$  is methyl and  $\text{R}_3$  is hydrogen, was synthesized from vitamin K5 and acetone according to the reductive amination procedure followed by the oxidation reaction with  $\text{Ag}_2\text{O}$  to afford a yellow amorphous solid in 61% yield. TLC  $R_f$  = 0.65 (30% diethyl ether/pentane).  $^1\text{H}$  NMR (400 MHz,  $\text{CDCl}_3$ ):  $\delta$  8.40 (dd, 1H,  $^3J(\text{H-C}(8), \text{H-C}(7)) = 8.0$ ,  $^4J(\text{H-C}(8), \text{H-C}(6)) = 1.5$ , H-C(8)); 8.11 (dd, 1H,  $^3J(\text{H-C}(5), \text{H-C}(6)) = 8.0$ ,  $^4J(\text{H-C}(5), \text{H-C}(7)) = 1.5$ , H-C(5)); 7.62 (dt, 1H,  $^3J(\text{H-C}(7), \text{H-C}(8)) = 8.0$ ,  $^3J(\text{H-C}(7), \text{H-C}(6)) = 8.0$ ,  $^4J(\text{H-C}(7), \text{H-C}(5)) = 1.5$ , H-C(6)); 7.55 (dt, 1H,  $^3J(\text{H-C}(6), \text{H-C}(5)) = 8.0$ ,  $^3J(\text{H-C}(6), \text{H-C}(7)) = 8.0$ ,  $^4J(\text{H-C}(6), \text{H-C}(8)) = 1.5$ , H-C(6)); 7.38 (bs, 1H, H-C(3)); 4.32 (m, 1H,  $\text{NCH}(\text{CH}_3)_2$ ); 2.18 (s, 3H,  $\text{C}(2)\text{CH}_3$ ); 1.33 (d, 6H,  $^3J(\text{NCH}(\text{CH}_3)_2, \text{NCH}(\text{CH}_3)_2) = 6.5$ ,  $\text{NCH}(\text{CH}_3)_2$ ).  $^{13}\text{C}$  NMR (100.6 MHz,  $\text{DMSO-d}_6$ ):  $\delta$  186.1 (s, C(1)); 152.4 (s, C(4)); 141.0 (s, C(10)); 136.3 (s, C(2)); 132.5 (d,  $^1J(\text{C},\text{H}) = 161$ , C(3)); 131.0 (s, C(9)); 129.9 (d,  $^1J(\text{C},\text{H}) = 162$ , C(6)); 125.8 (d,  $^1J(\text{C},\text{H}) = 160$ , C(7)); 124.5 (d,  $^1J(\text{C},\text{H}) = 165$ , C(8)); 124.2 (d,  $^1J(\text{C},\text{H}) = 163$ , C(5)); 51.2 (t,  $^1J(\text{C},\text{H}) = 132$ ,  $\text{NCH}(\text{CH}_3)_2$ ); 24.4 (q,  $^1J(\text{C},\text{H}) = 127$ ,  $\text{NCH}(\text{CH}_3)_2$ ); 17.0 (q,  $^1J(\text{C},\text{H}) = 125$ ,  $\text{C}(2)\text{CH}_3$ ). ESI-TOF-HRMS  $m/z$  calcd. for (M + H)  $\text{C}_{14}\text{H}_{16}\text{NO}$  214.1232, found 214.1224.

### Protein expression and purification of recombinant human IDO

The coding region for human IDO (Ala2-Gly403) was cloned into a derivative of plasmid pET9 (Novagen). The recombinant plasmid, pETIDO, encodes a histidine tag at the N-terminus of IDO. The bacterial strain BL21 AI (Invitrogen) was used for overexpression of IDO and transformed with the plasmid pETIDO. The transformed cells were grown on a rotary shaker at 37 °C and 220 rpm to an OD<sub>600</sub> of 1.2, in LB medium supplemented with kanamycin 25 µg/ml, L-tryptophan 50 µg/ml and bovine hemin (Sigma) 10 µM. The culture was cooled in a water/ice bath and supplemented again with L-tryptophan 50 µg/ml, bovine hemin 10 µM. The expression of his-tagged IDO was induced by the addition of 1% (w/v) arabinose. Induced cells were grown at 20 °C, 60 rpm for 20 h. Cells (1 l culture) were collected by centrifugation, resuspended in 40 ml of Mes 25 mM, KCl 150 mM, imidazole 10 mM, protease inhibitors (complete EDTA free, Roche Applied Science), pH 6.5, and disrupted with a French press. The extract was clarified by centrifugation and filtration on a 0.22 µm filter. The enzyme was purified by IMAC using Ni<sup>2+</sup> as ligand and an IMAC HITRAP column (5 ml; GE Healthcare). Briefly, the extract was loaded on the column with buffer Mes 25 mM, KCl 150 mM, imidazole 10 mM, pH 6.5. The column was washed with 50 ml of the same buffer with imidazole adjusted to 100 mM. Finally, the protein was eluted with Mes 25 mM, KCl 150 mM, EDTA 50 mM, pH 6.5. The buffer was then exchanged to Mes 25 mM, KCl 150 mM, pH 6.5 using a HITRAP desalting column (GE Healthcare). The purity of the enzyme was estimated to be > 95% based on SDS PAGE gel and Coomassie blue staining. The ratio of absorbance 404 nm/280 nm of the protein was around 1.9.

### Enzymatic Assay

The enzymatic inhibition assays were performed as described by Takikawa *et al.* [Takikawa, O.; Kuroiwa, T.; Yamazaki, F.; Kido, R. *J Biol Chem*, 1988, 263, 2041–2048.] with some modifications. Briefly, the reaction mixture (100 µl) contained potassium phosphate buffer (100 mM, pH 6.5) ascorbic acid (20 mM), catalase (200 units/ml), methylene blue (10 µM), purified recombinant IDO (2 ng/µl), and L-Trp (200 µM). The inhibitors were serially diluted ranging from 0.1 to 1000 µM. The reaction was carried out at 37 °C for 60 min and stopped by the addition of 30% (w/v)

trichloroacetic acid (40  $\mu$ l). To convert the product of Trp dioxygenation by IDO, N-formylkynurenine, to spectroscopically detectable kynurenine, the tubes were incubated at 50 °C for 30 min, followed by a centrifugation at 10000g for 20 min. Lastly, 100  $\mu$ l of supernatant from each probe was transferred to another tube for HPLC analysis. The mobile phase for HPLC measurements consisted of 50% sodium citrate buffer (40 mM, pH 2.25) and 50% methanol with 400  $\mu$ M SDS. The flow rate through the S5-ODS1 column was chosen to be 1 ml/min, and kynurenine was detected at a wavelength of 365 nm.

## 10 Cellular Assay

### Cell Lines

A plasmid construct encoding murine IDO was transfected into mouse mastocytoma line P815B. Clone P185B-mIDO clone 6, [Uyttenhove et al, Nat. Med, 9(10) pp1269-1274 ((2003))] which over expresses IDO, was selected and used for the cellular assay.

15 Mouse IDO shares a 62% sequence identity with human IDO, and the active site residues mentioned in the Introduction are 100% conserved. For the assays evaluating inhibition of human IDO, a plasmid construct encoding human IDO was transfected into human HEK-293 cells, and clone 293-hIDO clone 17 [Uyttenhove et al] was selected and used in the assay.

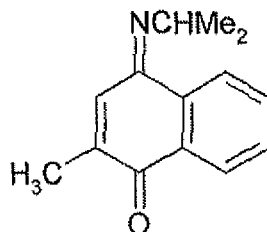
## 20 Assay

The assay was performed in 96-well flat bottom plates seeded with  $2 \times 10^5$  cells in a final volume of 200  $\mu$ l. To determine whether compounds were significant IDO inhibitors, the cells were incubated overnight at 37 °C in HBSS (Hanks Balanced Salt Solution, Invitrogen) supplemented with 50  $\mu$ M L-Trp and 2, 20 or 200  $\mu$ M of the compound. To determine the  $IC_{50}$ , the cells were incubated for 8 h at 37 °C in HBSS supplemented with 80  $\mu$ M L-Tryptophan and a titration of the compound ranging from 0.312 to 80  $\mu$ M or from 1.56 to 400  $\mu$ M. The plates were then centrifuged for 10 min at 300 g, and 150  $\mu$ l of the supernatant were collected. The supernatant was analyzed by HPLC to measure the concentration of tryptophan and kynurenine. For HPLC analysis, 30 50  $\mu$ l of supernatant were mixed with 500  $\mu$ l acetonitrile to precipitate the proteins. After centrifugation, the supernatant was collected, concentrated on a speedvac, resuspended in a final volume of 100  $\mu$ l water and injected in the HPLC (C18 column).

Trp was detected at an absorption wavelength of 280 nm, kynurenine at 360 nm. In the conditions used for  $IC_{50}$  estimation, about 50% of the initial amount of tryptophan was degraded in the absence of inhibitor, and an equimolar amount of kynurenine was produced. The percentages of inhibition of tryptophan degradation and kynurenine production by the compounds were calculated in reference to this maximal activity. The initial wells containing the cells in the remaining volume of 50  $\mu$ l were used to estimate cell viability in a classical MTT assay. To that end, 50  $\mu$ l of culture medium (Iscove medium with 10% FCS and amino acids) were added to the wells together with 50  $\mu$ l of MTT. After 3–4 h of incubation at 37 °C, 100  $\mu$ l of SDS/DMF were added to dissolve the crystals of formazan blue. The absorbance at 570 nm/650 nm was measured after overnight incubation at 37 °C.

### Experimental Results

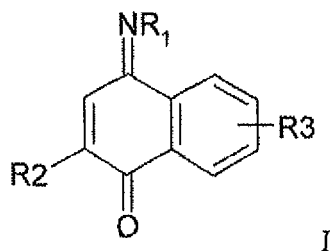
In the enzymatic model of IDO inhibition, the following activities were observed for the compound of formula I with the following structure,  $R_1$  = isopropyl,  $R_2$  = methyl and  $R_3$  = hydrogen



|    | <i>Cellular <math>IC_{50}(\mu M)</math></i> |             |             | <i>Enzymatic <math>IC_{50}(\mu M)</math></i> |
|----|---------------------------------------------|-------------|-------------|----------------------------------------------|
|    | <i>mIDO</i>                                 | <i>hIDO</i> | <i>mTDO</i> | <i>hIDO</i>                                  |
| 20 | 4.0                                         | >50         | 6.3         | 0.20                                         |

**Claims**

1. A compound of formula I



in which R<sub>1</sub>, R<sub>2</sub> and R<sub>3</sub>, which may be the same or different, represent alkyl C<sub>1-6</sub>, -  
5 cycloalkyl C<sub>3-7</sub>, aryl or NHR<sub>4</sub>, wherein R<sub>4</sub> may represent any of the values that any  
of R<sub>1</sub>, R<sub>2</sub> or R<sub>3</sub> may represent;

additionally, R<sub>3</sub> may represent hydrogen,  
and pharmaceutically acceptable salts thereof.

10 2. A compound according to claim 1 or a pharmaceutical salt thereof in combination  
with at least one additional therapeutic agent.

3. Use of a compound according to claim 1 or a pharmaceutical salt thereof as a  
pharmaceutical.

15 4. Use of a compound according to claim 1 or a pharmaceutical salt thereof as an  
adjuvant to the therapeutic vaccination of a cancer or tumour, including melanoma,  
colon cancer, pancreatic cancer, breast cancer, prostate cancer, lung cancer, leukemia,  
brain tumours, lymphoma, sarcoma, ovarian cancer, and Kaposi's sarcoma.

## INTERNATIONAL SEARCH REPORT

International application No  
PCT/EP2010/065337

## A. CLASSIFICATION OF SUBJECT MATTER

INV. C07C251/22 A61K31/135 A61P43/00  
ADD.

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)

C07C 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, BEILSTEIN Data, CHEM ABS Data

## C. DOCUMENTS CONSIDERED TO BE RELEVANT

| Category* | Citation of document, with indication, where appropriate, of the relevant passages                                                                                                                                                                                                                       | Relevant to claim No. |
|-----------|----------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------|-----------------------|
| X         | <p>F.J. BULLOCK: "Antiprotozoal Quinones. IV. 2-Amino-1,4-naphthoquinone Imines as Potential Antimalarials", JOURNAL OF MEDICINAL CHEMISTRY., vol. 13, no. 3, 1970, pages 550-552, XP002622028, USAMERICAN CHEMICAL SOCIETY. WASHINGTON. ISSN: 0022-2623 the whole document</p> <p>-----</p> <p>-/--</p> | 1,3                   |



Further documents are listed in the continuation of Box C.



See patent family annex.

## \* Special categories of cited documents :

"A" document defining the general state of the art which is not considered to be of particular relevance

"E" earlier 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

11 February 2011

Date of mailing of the international search report

21/02/2011

Name and mailing address of the ISA/

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

Authorized officer

Zervas, Brigitte

## INTERNATIONAL SEARCH REPORT

International application No

PCT/EP2010/065337

C(Continuation). DOCUMENTS CONSIDERED TO BE RELEVANT

| Category* | Citation of document, with indication, where appropriate, of the relevant passages                                                                                                                                                                                                                                                                                                                     | Relevant to claim No. |
|-----------|--------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------|-----------------------|
| X         | CARRENO M C ET AL: "Product Class 9: Benzo-1,2-, Benzo-1,4-, Naphtho-1,2-, and Naphtho-1,4-quinone Imines and Diimines", 1 January 2006 (2006-01-01), SCIENCE OF SYNTHESIS : HOUBEN-WEYL METHODS OF MOLECULAR TRANSFORMATIONS, STUTTGART [U.A.] : THIEME, DE, PAGE(S) 629 - 734, XP008132731, ISBN: 978-1-58890-460-7<br>page 717, compound 408; page 720, scheme 105; page 723, compound 444<br>----- | 1                     |
| X         | US 2 769 820 A (ENGLISH JACKSON P ET AL)<br>6 November 1956 (1956-11-06)<br>claims; examples<br>-----                                                                                                                                                                                                                                                                                                  | 1                     |
| X         | US 2 769 821 A (CLARK JOE H ET AL)<br>6 November 1956 (1956-11-06)<br>claims; examples<br>-----                                                                                                                                                                                                                                                                                                        | 1                     |
| X         | ALEXANDER R. FORRESTER ET AL.: "Nitroxide Radicals. Part 21. Spontaneous Decomposition of N-Aryl 1- and 2-Naphthyl Nitroxides", JOURNAL OF THE CHEMICAL SOCIETY, PERKIN TRANS. I, 1983, pages 1759-1764, XP002622029, GBCHEMICAL SOCIETY. LETCHWORTH. ISSN: 0368-1769<br>page 1760, compounds 7d, 7e; page 1763, column 1, line 17 - line 30<br>-----                                                  | 1                     |
| X         | JP 63 039862 A (OTSUKA PHARMA FACTORY INC OTSUKA PHARMA CO LTD)<br>20 February 1988 (1988-02-20)<br>* abstract<br>-----                                                                                                                                                                                                                                                                                | 1                     |
| X         | ROBERT LANTZ: "Réaction de l'ammoniaque et des amines sur un dérivé de l'hydroxy-2 naphthoquinone-1,4: l'hydroxy-2 N4-phényl naphthoquinone-1,4 imine-4 (anilino beta-naphthoquinone)", BULLETIN DE LA SOCIÉTÉ CHIMIQUE DE FRANCE, vol. 3, 1972, pages 1052-1054, XP002622030, page 1054, column 1, line 22 - column 2, line 27<br>-----                                                               | 1                     |
| A         | WO 2008/115804 A1 (LANKENAU INST FOR MEDICAL RES [US]; PRENDERGAST GEORGE C [US]; MALACHO)<br>25 September 2008 (2008-09-25)<br>claims; examples<br>-----<br>-/--                                                                                                                                                                                                                                      | 1-4                   |

## INTERNATIONAL SEARCH REPORT

International application No  
PCT/EP2010/065337

| C(Continuation). DOCUMENTS CONSIDERED TO BE RELEVANT |                                                                                                                                                                                                                                                                                                                                   |                       |
|------------------------------------------------------|-----------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------|-----------------------|
| Category*                                            | Citation of document, with indication, where appropriate, of the relevant passages                                                                                                                                                                                                                                                | Relevant to claim No. |
| A                                                    | WO 2008/052352 A1 (UNIV BRITISH COLUMBIA [CA]; ANDERSEN RAYMOND [CA]; LEBLANC MICHAEL [CA] 8 May 2008 (2008-05-08) claims; examples<br>-----                                                                                                                                                                                      | 1-4                   |
| X,P                                                  | WO 2009/127669 A2 (LUDWIG INST FOR CANCER RES LTD [US]; ROEHRIG UTE [CH]; LOAY AWAD [CH];) 22 October 2009 (2009-10-22) pages 7, 8, 20; claims<br>-----                                                                                                                                                                           | 1-4                   |
| X,P                                                  | UTE F. RÖHRIG ET AL.: "rational Design of Indoleamine 2,3-dioxygenase Inhibitors", JOURNAL OF MEDICINAL CHEMISTRY., vol. 53, 1 July 2010 (2010-07-01), pages 1172-1189, XP002622031, USAMERICAN CHEMICAL SOCIETY. WASHINGTON. ISSN: 0022-2623<br>page 1179, table 5, compound 55; page 1186, column 2, line 19 - line 39<br>----- | 1-4                   |

# INTERNATIONAL SEARCH REPORT

Information on patent family members

International application No

PCT/EP2010/065337

| Patent document<br>cited in search report |    | Publication<br>date | Patent family<br>member(s)        | Publication<br>date      |
|-------------------------------------------|----|---------------------|-----------------------------------|--------------------------|
| US 2769820                                | A  | 06-11-1956          | NONE                              |                          |
| US 2769821                                | A  | 06-11-1956          | NONE                              |                          |
| JP 63039862                               | A  | 20-02-1988          | JP 1857696 C<br>JP 5067141 B      | 27-07-1994<br>24-09-1993 |
| WO 2008115804                             | A1 | 25-09-2008          | EP 2137168 A1<br>US 2010076066 A1 | 30-12-2009<br>25-03-2010 |
| WO 2008052352                             | A1 | 08-05-2008          | NONE                              |                          |
| WO 2009127669                             | A2 | 22-10-2009          | NONE                              |                          |