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(54) **4- [3- (4-CYCLOPROPA NECARBO NYL-PIPERAZINE-I-CARBONYL) -4 - FLUORO-BENZYL] -2H-PHTHALAZ IN-1-ONE**

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(57) **ABSTRACT**

4-[3-(4-cyclopropanecarbonyl-piperazine-1-carbonyl)-4-fluoro-benzyl]-2H-phthalazin-1-one as crystalline Form L, methods of obtaining form L, pharmaceutical compositions comprising Form L and methods of using Form L and compositions comprising Form L.

8 Claims, 2 Drawing Sheets

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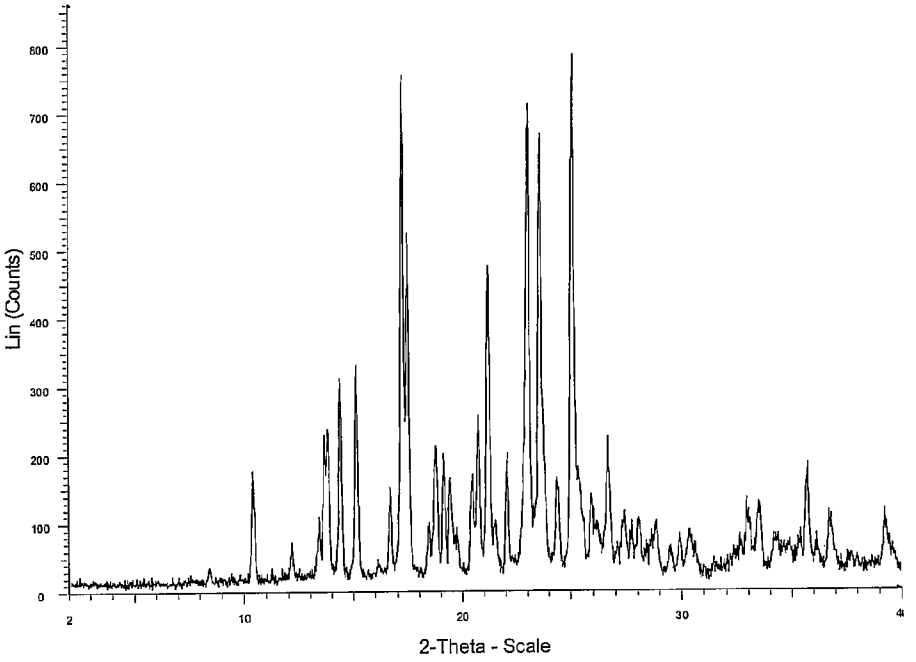


Figure 1

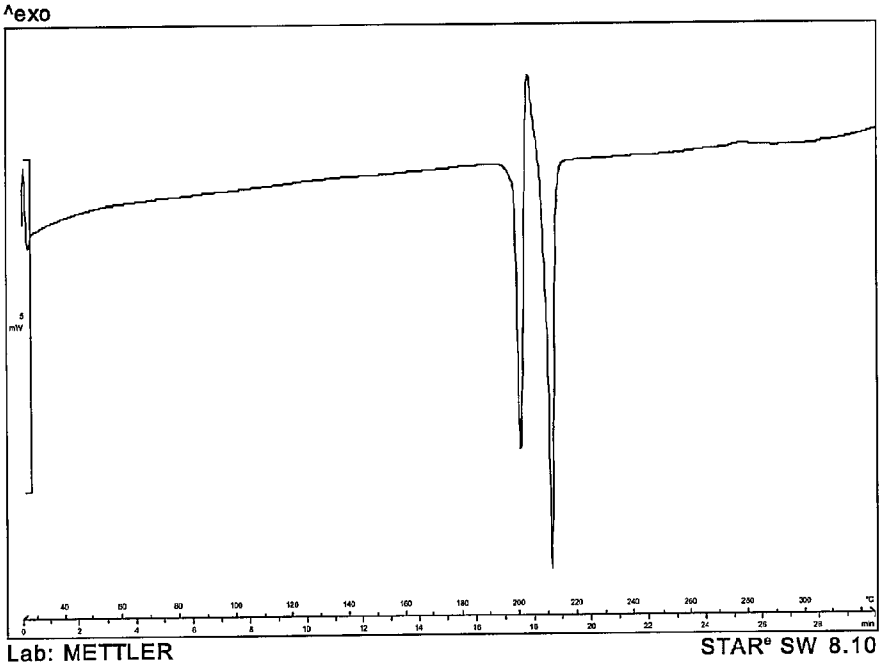


Figure 2

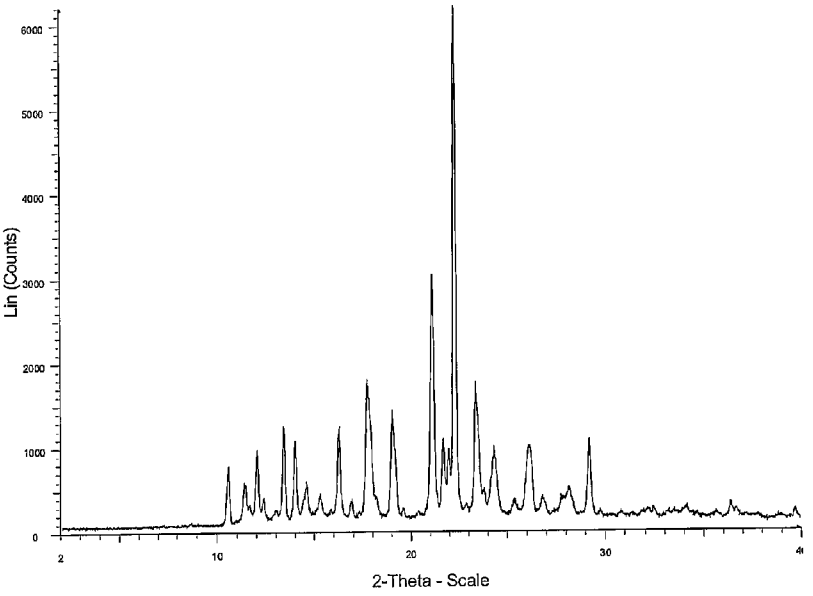


Figure 3

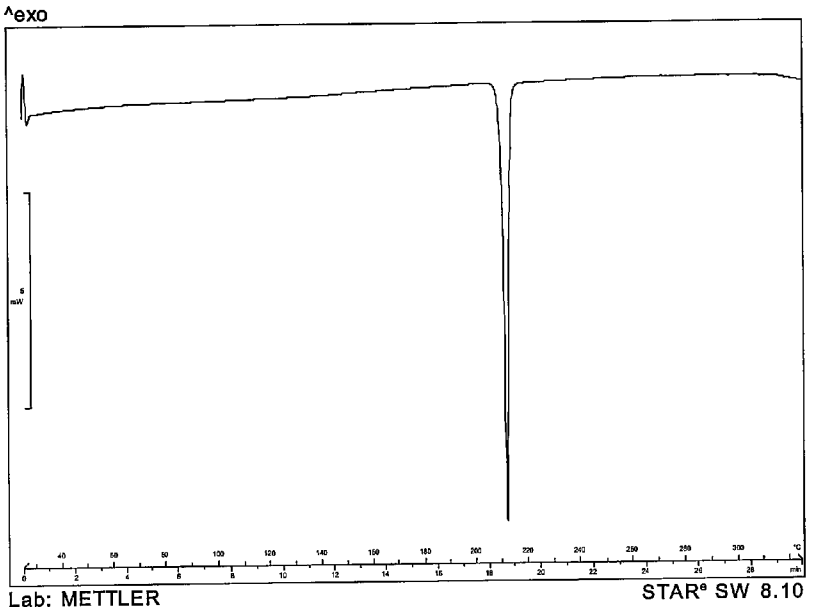


Figure 4

4- [3- (4-CYCLOPROPANECARBONYL-PIPERAZINE-I-CARBONYL) -4 - FLUORO-BENZYL] -2H-PHTHALAZ IN-1-ONE

CROSS-REFERENCE TO RELATED APPLICATIONS

This patent application is a national stage filing under 35 U.S.C. 371 of International Application No. PCT/GB2008/003510, filed on Oct. 17, 2008, which claims priority benefits to U.S. Provisional Application No. 60/980,508, filed on Oct. 17, 2007. These applications are incorporated herein by reference in their entireties.

The present invention relates to a crystalline form and pharmaceutical compositions and uses of the crystalline form.

The mammalian enzyme PARP (a 113-kDa multidomain protein) has been implicated in the signalling of DNA damage through its ability to recognize and rapidly bind to DNA single or double strand breaks (D'Amours, et al., *Biochem. J.,* 342, 249-268 (1999)).

Several observations have led to the conclusion that PARP participates in a variety of DNA-related functions including gene amplification, cell division, differentiation, apoptosis, DNA base excision repair and also effects on telomere length and chromosome stability (d'Adda di Fagagna, et al., *Nature Gen.,* 23(1), 76-80 (1999)).

Studies on the mechanism by which PARP modulates DNA repair and other processes has identified its importance in the formation of poly (ADP-ribose) chains within the cellular nucleus (Althaus, F. R. and Richter, C., ADP-Ribosylation of Proteins: Enzymology and Biological Significance, Springer-Verlag, Berlin (1987)). The DNA-bound, activated PARP utilizes NAD to synthesize poly (ADP-ribose) on a variety of nuclear target proteins, including topoisomerase, histones and PARP itself (Rhun, et al., *Biochem. Biophys. Res. Commun.,* 245, 1-10 (1998))

Poly (ADP-ribosyl)ation has also been associated with malignant transformation. For example, PARP activity is higher in the isolated nuclei of SV40-transformed fibroblasts, while both leukemic cells and colon cancer cells show higher enzyme activity than the equivalent normal leukocytes and colon mucosa (Miwa, et al., *Arch. Biochem. Biophys.,* 181, 313-321 (1977); Burzio, et al., *Proc. Soc. Exp. Biol. Med.,* 149, 933-938 (1975); and Hirai, et al., *Cancer Res.,* 43, 3441-3446 (1983)).

A number of low-molecular-weight inhibitors of PARP have been used to elucidate the functional role of poly (ADP-ribosyl)ation in DNA repair. In cells treated with alkylating agents, the inhibition of PARP leads to a marked increase in DNA-strand breakage and cell killing (Durkacz, et al., *Nature,* 283, 593-596 (1980); Berger, N. A., *Radiation Research,* 101, 4-14 (1985)).

Subsequently, such inhibitors have been shown to enhance the effects of radiation response by suppressing the repair of potentially lethal damage (Ben-Hur, et al., *British Journal of Cancer,* 49 (Suppl. VI), 34-42 (1984); Schlicker, et al., *Int. J. Radiat. Bioi.,* 75, 91-100 (1999)). PARP inhibitors have been reported to be effective in radio sensitising hypoxic tumour cells (U.S. Pat. No. 5,032,617; U.S. Pat. No. 5,215,738 and U.S. Pat. No. 5,041,653).

Furthermore, PARP knockout (PARP −/−) animals exhibit genomic instability in response to alkylating agents and γ-irradiation (Wang, et al., *Genes Dev.,* 9, 509-520 (1995); Menissier de Murcia, et al., *Proc. Natl. Acad. Sci. USA,* 94, 7303-7307 (1997)).

A role for PARP has also been demonstrated in certain vascular diseases, septic shock, ischaemic injury and neurotoxicity (Cantoni, et al., *Biochim. Biophys. Acta,* 1014, 1-7 (1989); Szabo, et al., *J. Clin. Invest.,* 100, 723-735 (1997)). Oxygen radical DNA damage that leads to strand breaks in DNA, which are subsequently recognised by PARP, is a major contributing factor to such disease states as shown by PARP inhibitor studies (Cosi, et al., *J. Neurosci. Res.,* 39, 38-46 (1994); Said, et al., *Proc. Natl. Acad. Sci. U.S.A.,* 93, 4688-4692 (1996)). More recently, PARP has been demonstrated to play a role in the pathogenesis of haemorrhagic shock (Liaudet, et al., *Proc. Natl. Acad. Sci. U.S.A.,* 97(3), 10203-10208 (2000)).

It has also been demonstrated that efficient retroviral infection of mammalian cells is blocked by the inhibition of PARP activity. Such inhibition of recombinant retroviral vector infections was shown to occur in various different cell types (Gaken, et al., *J. Virology,* 70(6), 3992-4000 (1996)). Inhibitors of PARP have thus been developed for the use in antiviral therapies and in cancer treatment (WO 91/18591).

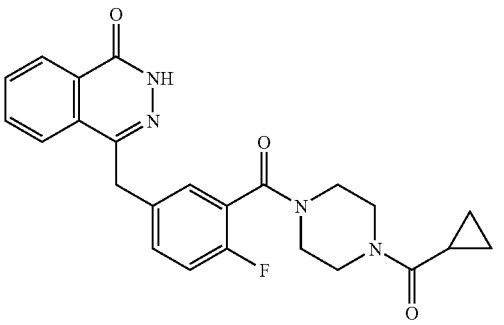
Moreover, PARP inhibition has been speculated to delay the onset of aging characteristics in human fibroblasts (Rattan and Clark, *Biochem. Biophys. Res. Comm.,* 201(2), 665-672 (1994)). This may be related to the role that PARP plays in controlling telomere function (d'Adda di Fagagna, et al., *Nature Gen.,* 23(1), 76-80 (1999)).

WO 2004/080976 discloses a number of phthalazinone derivatives, their activity in inhibiting PARP, and their consequential use in treating cancer, whether as an adjunct to radiotherapy or chemotherapy, or as a stand alone agent.

WO 2005/053662 describes the use of PARP inhibitors, in particular phthalazinone derivatives, as base excision repair (BER) inhibitors. The use of these inhibitors in the manufacture of medicaments for the treatment of cancers which are deficient in Homologous Recombination (HR) dependent DNA DSB repair activity, in particular for cancers which have a BRCA1 and/or a BRCA2 deficient phenotype, is described.

4-[3-(4-Cyclopropanecarbonyl-piperazine-1-carbonyl)-4-fluoro-benzyl]-2H-phthalazin-1-one (compound A) disclosed in WO 2004/080976:

A



is of particular interest.

A crystalline form of compound A (Form A) is disclosed in co-pending applications, which claim priority from U.S. 60/829,694, filed 17 Oct. 2006, entitled "Phthalazinone Derivative", including U.S. Ser. No. 11/873,671 and WO 2008/047082.

Particular forms of compound A may have advantageous properties, for example with regard to their solubility and/or their stability and/or their bioavailability and/or their impurity profile and/or their filtration characteristics and/or their

drying characteristics and/or their lack of hygroscopicity, and/or they may be easier to handle and/or micronise and/or form into tablets.

Accordingly, a first aspect of the present invention provides 4-[3-(4-cyclopropanecarbonyl-piperazine-1-carbonyl)-4-fluoro-benzyl]-2H-phthalazin-1-one (compound A) substantially as crystalline Form L.

"Substantially as crystalline Form L" as used above, means that at least 50% by weight of compound A as Form L, preferably at least 70% by weight, 80% or 90% by weight. In some embodiments, at least 95% by weight, 99% by weight or even 99.5% or more by weight may be in crystalline form.

Compound A as crystalline Form L has an X-ray diffraction pattern (λ=1.5418 Å) containing specific peaks at:

Peak	2θ° (±0.1°)
1	14.4
2	17.2
3	17.5
4	18.8
5	23.0

Compound A as crystalline Form L may also have the following additional peaks in an X-ray diffraction pattern (λ=1.5418 Å):

Peak	2θ° (±0.1°)
6	10.4
7	13.6
8	25.1

Compound A as crystalline Form L may also be characterised by any combination of three or more peaks selected from the list of 8 peaks above.

A representative powder XRD pattern of compound A in Form L is shown in FIG. **1**.

Form L of compound A is substantially free from solvent. The term "substantially free from solvent" as used herein refers to the form having only insignificant amounts of any solvent, e.g. a form with a total of 0.5% by weight or less of any solvent. The total amount of any solvent may be 0.25%, 0.1%, 0.05% or 0.025% by weight or less.

Form L of compound A may also be characterised using DSC. Form L of compound A when heated from 25° C. to 325° C. at 10° C. per minute will have an onset of melting at 198.5° C.±1° C. A representative DSC trace for compound A as Form L is shown in FIG. **2**. The second endotherm corresponds to melting of Form A, to which the melted Form L transforms.

The second aspect of the present invention provides a method of obtaining 4-[3-(4-cyclopropanecarbonyl-piperazine-1-carbonyl)-4-fluoro-benzyl]-2H-phthalazin-1-one (compound A) as crystalline Form L from compound A as crystalline Form A.

This method involves slurrying compound A as either Form A or a mixture of Forms A and L in an organic solvent that may contain up to 30% water v/v. In some embodiments, the mixture may contain no Form L, or 99%, 90% or 80% as Form A. In other embodiments, the amount of Form L may be up to 50, 70 or even 80% of the mixture. In some embodiments, the organic solvent may contain no water. In other embodiments, the organic solvent may contain up to 25 or 20% water. The solvent may be selected from a solvent which gives sufficient solubility for a transformation to occur. In some embodiments, the solvent is selected from the group consisting of: methanol, ethanol, acetone, acetonitrile and nitromethane.

The slurrying may take place at a temperature up to the boiling point of the solvent, but may more usually be carried out at a temperature lower than that. In some embodiments, the slurrying takes place at between 20° C. and 50° C., or even 20° C. and 40° C., or 20° C. and 30° C. With lower temperatures, the slurrying time may be increased. Typically, the slurrying is carried out for at least 3 hours, at least 6 hours or even at least 24 hours. In some embodiments, the slurrying is carried out for at least 3 days, 4 days, a week or even 3 weeks.

A third aspect of the present invention provides a pharmaceutical composition comprising a compound of the first aspect and a pharmaceutically acceptable carrier or diluent.

An fourth aspect of the present invention provides a compound of the first aspect for use in a method of treatment of the human or animal body.

A fifth aspect of the present invention provides the use of a compound as defined in the first aspect of the invention in the preparation of a medicament for inhibiting the activity of PARP.

Further aspects of the invention provide the use of a compound as defined in the first aspect of the invention in the preparation of a medicament for the treatment of: vascular disease; septic shock; ischaemic injury; neurotoxicity; haemorraghic shock; viral infection; or diseases ameliorated by the inhibition of the activity of PARP.

Another further aspect of the invention provides for the use of a compound as defined in the first aspect of the invention in the preparation of a medicament for use as an adjunct in cancer therapy or for potentiating tumour cells for treatment with ionizing radiation or chemotherapeutic agents.

Other further aspects of the invention provide for the treatment of disease ameliorated by the inhibition of PARP, comprising administering to a subject in need of treatment a therapeutically-effective amount of a compound as defined in the first aspect, preferably in the form of a pharmaceutical composition and the treatment of cancer, comprising administering to a subject in need of treatment a therapeutically-effective amount of a compound as defined in the first aspect in combination, preferably in the form of a pharmaceutical composition, simultaneously or sequentially with ionizing radiation or chemotherapeutic agents.

In further aspects of the present invention, the compounds may be used in the preparation of a medicament for the treatment of cancer which is deficient in Homologous Recombination (HR) dependent DNA DSB repair activity, or in the treatment of a patient of a cancer which is deficient in HR dependent DNA DSB repair activity, comprising administering to said patient a therapeutically-effective amount of the compound.

The HR dependent DNA DSB repair pathway repairs double-strand breaks (DSBs) in DNA via homologous mechanisms to reform a continuous DNA helix (K. K. Khanna and S. P. Jackson, Nat. Genet. 27(3): 247-254 (2001)). The components of the HR dependent DNA DSB repair pathway include, but are not limited to, ATM (NM_000051), RAD51 (NM_002875), RAD51L1 (NM_002877), RAD51C(NM_002876), RAD51L3 (NM_002878), DMC1 (NM_007068), XRCC2 (NM_005431), XRCC3 (NM_005432), RAD52 (NM_002879), RAD54L (NM_003579), RAD54B (NM_012415), BRCA1 (NM_007295), BRCA2 (NM_000059), RAD50 (NM_005732), MRE11A (NM_005590) and NBS1 (NM_002485). Other proteins involved in the HR dependent DNA DSB repair

pathway include regulatory factors such as EMSY (Hughes-Davies, et al., *Cell,* 115, pp 523-535). HR components are also described in Wood, et al., *Science,* 291, 1284-1289 (2001).

A cancer which is deficient in HR dependent DNA DSB repair may comprise or consist of one or more cancer cells which have a reduced or abrogated ability to repair DNA DSBs through that pathway, relative to normal cells i.e. the activity of the HR dependent DNA DSB repair pathway may be reduced or abolished in the one or more cancer cells.

The activity of one or more components of the HR dependent DNA DSB repair pathway may be abolished in the one or more cancer cells of an individual having a cancer which is deficient in HR dependent DNA DSB repair. Components of the HR dependent DNA DSB repair pathway are well characterised in the art (see for example, Wood, et al., *Science,* 291, 1284-1289 (2001)) and include the components listed above.

In some preferred embodiments, the cancer cells may have a BRCA1 and/or a BRCA2 deficient phenotype i.e. BRCA1 and/or BRCA2 activity is reduced or abolished in the cancer cells. Cancer cells with this phenotype may be deficient in BRCA1 and/or BRCA2, i.e. expression and/or activity of BRCA1 and/or BRCA2 may be reduced or abolished in the cancer cells, for example by means of mutation or polymorphism in the encoding nucleic acid, or by means of amplification, mutation or polymorphism in a gene encoding a regulatory factor, for example the EMSY gene which encodes a BRCA2 regulatory factor (Hughes-Davies, et al., *Cell,* 115, 523-535).

BRCA1 and BRCA2 are known tumour suppressors whose wild-type alleles are frequently lost in tumours of heterozygous carriers (Jasin M., *Oncogene,* 21(58), 8981-93 (2002); Tuft, et al., *Trends Mol. Med.,* 8(12), 571-6, (2002)). The association of BRCA1 and/or BRCA2 mutations with breast cancer is well-characterised in the art (Radice, P. J., *Exp Clin Cancer Res.,* 21(3 Suppl), 9-12 (2002)). Amplification of the EMSY gene, which encodes a BRCA2 binding factor, is also known to be associated with breast and ovarian cancer.

Carriers of mutations in BRCA1 and/or BRCA2 are also at elevated risk of cancer of the ovary, prostate and pancreas.

In some preferred embodiments, the individual is heterozygous for one or more variations, such as mutations and polymorphisms, in BRCA1 and/or BRCA2 or a regulator thereof. The detection of variation in BRCA1 and BRCA2 is well-known in the art and is described, for example in EP 699 754, EP 705 903, Neuhausen, S. L. and Ostrander, E. A., *Genet. Test,* 1, 75-83 (1992); Chappnis, P. O. and Foulkes, W. D., *Cancer Treat Res,* 107, 29-59 (2002); Janatova M., et al., *Neoplasma,* 50(4), 246-50 (2003); Jancarkova, N., *Ceska Gynekol.,* 68(1), 11-6 (2003)). Determination of amplification of the BRCA2 binding factor EMSY is described in Hughes-Davies, et al., *Cell,* 115, 523-535).

Mutations and polymorphisms associated with cancer may be detected at the nucleic acid level by detecting the presence of a variant nucleic acid sequenc or at the protein level by detecting the presence of a variant (i.e. a mutant or allelic variant) polypeptide.

BRIEF DESCRIPTION OF FIGURES

FIG. **1** shows a representative powder XRD pattern of compound A as Form L;

FIG. **2** shows a representative DSC trace of compound A as Form L obtained by heating from 25° C. to 325° C. at 10° C. per minute;

FIG. **3** shows a representative powder XRD pattern of compound A as Form A;

FIG. **4** shows a representative DSC trace of compound A as Form A obtained by heating from 25° C. to 325° C. at 10° C. per minute.

Use

The present invention provides compound A as Form L as an active compound, specifically, active in inhibiting the activity of PARP.

The term "active" as used herein, pertains to the compound which is capable of inhibiting PARP activity. One assay which may conveniently be used in order to assess the PARP inhibition offered by the compound is described in the examples below.

The present invention further provides a method of inhibiting the activity of PARP in a cell, comprising contacting said cell with an effective amount of the active compound, preferably in the form of a pharmaceutically acceptable composition. Such a method may be practised in vitro or in vivo.

For example, a sample of cells may be grown in vitro and the active compound brought into contact with said cells, and the effect of the compound on those cells observed. As examples of "effect", the amount of DNA repair effected in a certain time may be determined. Where the active compound is found to exert an influence on the cells, this may be used as a prognostic or diagnostic marker of the efficacy of the compound in methods of treating a patient carrying cells of the same cellular type.

The term "treatment", as used herein in the context of treating a condition, pertains generally to treatment and therapy, whether of a human or an animal (e.g. in veterinary applications), in which some desired therapeutic effect is achieved, for example, the inhibition of the progress of the condition, and includes a reduction in the rate of progress, a halt in the rate of progress, amelioration of the condition, and cure of the condition. Treatment as a prophylactic measure (i.e. prophylaxis) is also included.

The term "adjunct" as used herein relates to the use of the active compound in conjunction with known therapeutic means. Such means include cytotoxic regimes of drugs and/or ionising radiation as used in the treatment of different cancer types. In particular, the active compounds are known to potentiate the actions of a number of cancer chemotherapy treatments, which include the topoisomerase class of poisons (e.g. topotecan, irinotecan, rubitecan), most of the known alkylating agents (e.g. DTIC, temozolamide) and platinum based drugs (e.g. carboplatin, cisplatin) used in treating cancer.

The active compound may also be used as cell culture additives to inhibit PARP, for example, in order to sensitize cells to known chemotherapeutic agents or ionising radiation treatments in vitro.

The active compound may also be used as part of an in vitro assay, for example, in order to determine whether a candidate host is likely to benefit from treatment with the compound in question.

Administration

The active compound or pharmaceutical composition comprising the active compound may be administered to a subject by any convenient route of administration, whether systemically/peripherally or at the site of desired action, including but not limited to, oral (e.g. by ingestion); topical (including e.g. transdermal, intranasal, ocular, buccal, and sublingual); pulmonary (e.g. by inhalation or insufflation therapy using, e.g. an aerosol, e.g. through mouth or nose); rectal; vaginal; parenteral, for example, by injection, including subcutaneous, intradermal, intramuscular, intravenous, intraarterial,

intracardiac, intrathecal, intraspinal, intracapsular, subcapsular, intraorbital, intraperitoneal, intratracheal, subcuticular, intraarticular, subarachnoid, and intrasternal; by implant of a depot, for example, subcutaneously or intramuscularly.

The subject may be a eukaryote, an animal, a vertebrate animal, a mammal, a rodent (e.g. a guinea pig, a hamster, a rat, a mouse), murine (e.g. a mouse), canine (e.g. a dog), feline (e.g. a cat), equine (e.g. a horse), a primate, simian (e.g. a monkey or ape), a monkey (e.g. marmoset, baboon), an ape (e.g. gorilla, chimpanzee, orangutang, gibbon), or a human.

Formulations

While it is possible for the active compound to be administered alone, it is preferable to present it as a pharmaceutical composition (e.g., formulation) comprising the active compound, as defined above, together with one or more pharmaceutically acceptable carriers, adjuvants, excipients, diluents, fillers, buffers, stabilisers, preservatives, lubricants, or other materials well known to those skilled in the art and optionally other therapeutic or prophylactic agents.

Thus, the present invention further provides pharmaceutical compositions, as defined above, and methods of making a pharmaceutical composition comprising admixing the active compound, as defined above, together with one or more pharmaceutically acceptable carriers, excipients, buffers, adjuvants, stabilisers, or other materials, as described herein, such that active compound remains as crystalline Form L.

The term "pharmaceutically acceptable" as used herein pertains to compounds, materials, compositions, and/or dosage forms which are, within the scope of sound medical judgement, suitable for use in contact with the tissues of a subject (e.g. human) without excessive toxicity, irritation, allergic response, or other problem or complication, commensurate with a reasonable benefit/risk ratio. Each carrier, excipient, etc. must also be "acceptable" in the sense of being compatible with the other ingredients of the formulation.

Suitable carriers, diluents, excipients, etc. can be found in standard pharmaceutical texts. See, for example, "Handbook of Pharmaceutical Additives", 2nd Edition (eds. M. Ash and I. Ash), 2001 (Synapse Information Resources, Inc., Endicott, N.Y., USA), "Remington's Pharmaceutical Sciences", 20th edition, pub. Lippincott, Williams & Wilkins, 2000; and "Handbook of Pharmaceutical Excipients", 2nd edition, 1994.

The formulations may conveniently be presented in unit dosage form and may be prepared by any methods well known in the art of pharmacy. Such methods include the step of bringing into association the active compound with the carrier which constitutes one or more accessory ingredients. In general, the formulations are prepared by uniformly and intimately bringing into association the active compound with liquid carriers or finely divided solid carriers or both, and then if necessary shaping the product.

Formulations may be in the form of suspensions, tablets, granules, powders, capsules, cachets, pills or pastes.

Formulations suitable for oral administration (e.g., by ingestion) may be presented as discrete units such as capsules, cachets or tablets, each containing a predetermined amount of the active compound; as a powder or granules; as a suspension in an aqueous or non-aqueous liquid; or as a paste.

A tablet may be made by conventional means, e.g. compression or molding, optionally with one or more accessory ingredients. Compressed tablets may be prepared by compressing in a suitable machine the active compound in a free-flowing form such as a powder or granules, optionally mixed with one or more binders (e.g. povidone, gelatin, acacia, sorbitol, tragacanth, hydroxypropylmethyl cellulose); fillers or diluents (e.g. lactose, microcrystalline cellulose, calcium hydrogen phosphate); lubricants (e.g. magnesium stearate, talc, silica); disintegrants (e.g. sodium starch glycolate, cross-linked povidone, cross-linked sodium carboxymethyl cellulose); surface-active or dispersing or wetting agents (e.g., sodium lauryl sulfate); and preservatives (e.g., methyl p-hydroxybenzoate, propyl p-hydroxybenzoate, sorbic acid). Molded tablets may be made by molding in a suitable machine a mixture of the powdered compound moistened with an inert liquid diluent. The tablets may optionally be coated or scored and may be formulated so as to provide slow or controlled release of the active compound therein using, for example, hydroxypropylmethyl cellulose in varying proportions to provide the desired release profile. Tablets may optionally be provided with an enteric coating, to provide release in parts of the gut other than the stomach.

A capsule may include the active compound in suspension.

Formulations suitable for topical administration (e.g. transdermal, intranasal, ocular, buccal, and sublingual) may be formulated as a paste.

Formulations suitable for topical administration to the eye also include eye drops wherein the active compound is suspended in a suitable carrier, especially an aqueous solvent for the active compound.

Formulations suitable for nasal administration, wherein the carrier is a solid, include a coarse powder having a particle size, for example, in the range of about 20 to about 500 microns which is administered in the manner in which snuff is taken, i.e. by rapid inhalation through the nasal passage from a container of the powder held close up to the nose.

Formulations suitable for administration by inhalation include those presented as an aerosol spray from a pressurised pack, with the use of a suitable propellant, such as dichlorodifluoromethane, trichlorofluoromethane, dichoro-tetrafluoroethane, carbon dioxide, or other suitable gases.

Dosage

It will be appreciated that appropriate dosages of the active compound, and compositions comprising the active compound, can vary from patient to patient. Determining the optimal dosage will generally involve the balancing of the level of therapeutic benefit against any risk or deleterious side effects of the treatments of the present invention. The selected dosage level will depend on a variety of factors including, but not limited to, the activity of the particular compound, the route of administration, the time of administration, the rate of excretion of the compound, the duration of the treatment, other drugs, compounds, and/or materials used in combination, and the age, sex, weight, condition, general health, and prior medical history of the patient. The amount of compound and route of administration will ultimately be at the discretion of the physician, although generally the dosage will be to achieve local concentrations at the site of action which achieve the desired effect without causing substantial harmful or deleterious side-effects.

Administration in vivo can be effected in one dose, continuously or intermittently (e.g., in divided doses at appropriate intervals) throughout the course of treatment. Methods of determining the most effective means and dosage of administration are well known to those of skill in the art and will vary with the formulation used for therapy, the purpose of the therapy, the target cell being treated, and the subject being treated. Single or multiple administrations can be carried out with the dose level and pattern being selected by the treating physician.

In general, a suitable dose of the active compound is in the range of about 10 mg to about 600 mg per m^2 body area weight of the subject per day.

EXAMPLES

General Methods

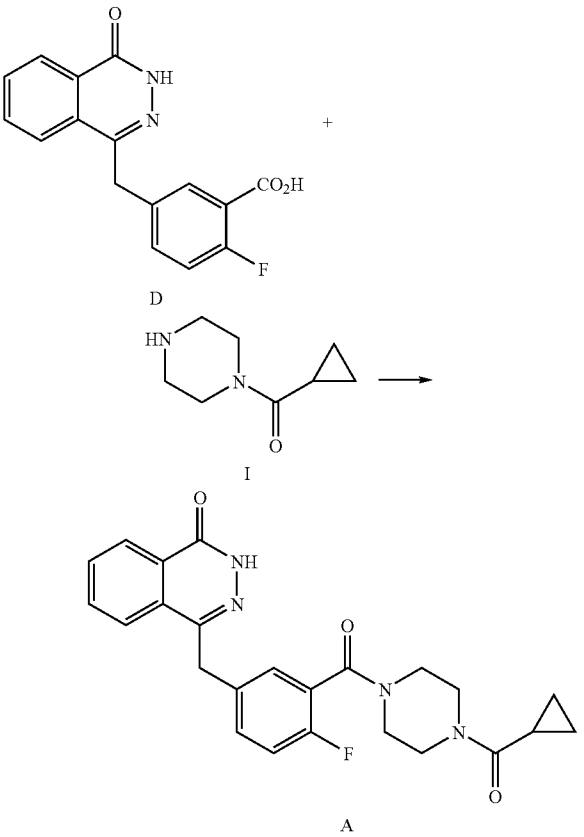
Powder XRD

Powder X-ray diffraction was recorded with a Bruker D5000 diffractometer (wavelength of X-rays 1.5418 Å Cu source, Voltage 40 kV, filament emission 40 mA). Samples were scanned from 2-40° 2θ using a 0.02° step width and a 1 second time count.

Differential Scanning Calorimetry (DSC)

DSC was recorded using a Mettler DSC820E with TS0801RO robotic system. Typically less than 5 mg of material, contained in a 40 μl aluminium pan fitted with a pierced lid, was heated over the temperature range 25° C. to 325° C. at a constant heating rate of 10° C. per minute. A nitrogen purge gas was used with flow rate 100 ml per minute.

Obtaining Compound A as Form A



NMR

^{1}H NMR spectra were recorded using Bruker DPX 400 spectrometer at 400 MHz. Chemical shifts were reported in parts per million (ppm) on the δ scale relative to tetramethylsilane internal standard. Unless stated otherwise all samples were dissolved in DMSO-d_6.

Mass Spectra

Mass spectra were recorded on an Agilent XCT ion trap mass spectrometer using tandem mass spectrometry (MS/MS) for structural confirmation. The instrument was operated in a positive ion electrospray mode.

(a) 4-[3-(4-Cyclopropanecarbonyl-piperazine-1-carbonyl)-4-fluoro-benzyl]-2H-phthalazin-1-one (Compound A)

2-Fluoro-5-[(4-oxo-3,4-dihydrophthalazin-1-yl)methyl] benzoic acid (D)(15.23 g, 51.07 mmol) was suspended with stirring under nitrogen in acetonitrile (96 ml). Diisopropylethylamine (19.6 ml, 112.3 mmol) was added followed by 1-cyclopropylcarbonylpiperazine (I)(9.45 g, 61.28 mmol) and acetonitrile (1 ml). The reaction mixture was cooled to 18° C. 0-Benzotriazol-1-yl-tetramethyluronium hexafluorophosphate (25.18 g, 66.39 mmol) was added over 30 minutes and the reaction mixture was stirred for 2 hours at room temperature. The reaction mixture was cooled to 3° C. and maintained at this temperature for 1 hour, before being filtered. The filter cake was washed with cold (3° C.) acetonitrile (20 ml) before being dried in vacuo at up to 40° C. to give the title compound as a pale yellow solid (20.21 g).

Mass Spectrum: MH+ 435

1H NMR (400 MHz, DMSO-d6) δ: 0.70 (m, 4H), 1.88 (br s, 1H), 3.20 (br s, 2H), 3.56 (m, 6H), 4.31 (s, 2H), 7.17 (t, 1H), 7.34 (dd, 1H), 7.41 (m, 1H), 7.77 (dt, 1H), 7.83 (dt, 1H), 7.92 (d, 1H), 8.25 (dd, 1H), 12.53 (s, 1H).

(b) Recrystallisation of Compound a from Ethanol

4-(3-{[4-(cyclopropylcarbonyl)piperazin-1-yl]carbonyl}-4-fluorobenzyl)phthalazin-1(2H)-one (compound A) (20.00 g, 44.66 mmol) was suspended in a mixture of water (50 ml) and ethanol (150 ml). The suspension was heated to reflux with stirring. The solution produced was then cooled to 70° C. and filtered. The filter pad was washed with a mixture of water (8 ml) and ethanol (22 ml).

The filtrate was cooled to 45° C. with stirring. 4-(3-{[4-(cyclopropylcarbonyl)piperazin-1-yl]carbonyl}-4-fluorobenzyl)phthalazin-1(2H)-one (Compound A) in Form A (0.08 g) was added in order to seed the mixture. The resulting suspension was cooled to 20° C. over 2.5 hours and was stirred at this temperature for a further 16 hours in order to establish crystallisation. Water (200 ml) was added over 5 hours maintaining the temperature at 20° C. At the end of the addition the suspension was held at 20° C. for 2 hours.

The suspension was filtered and the filter cake washed with a mixture of ethanol (24 ml) and water (56 ml). The isolated solid was discharged and dried under vacuum at 40-60° C., to give the compound A as Form A as an off white solid (18A g).

FIG. **3** shows a representative powder XRD pattern of compound A as Form A and FIG. **4** shows a representative DSC trace of compound A as Form A obtained by heating from 25° C. to 325° C. at 10° C. per minute. The XRD pattern was obtained as set out above, but with a 4 second time count.

Compound A as crystalline Form A has an X-ray diffraction pattern (λ=1.5418 Å) containing specific peaks at:

Peak	2θ° (±0.1°)
1	12.0
2	17.8
3	21.1
4	22.3
5	29.2

Compound A as crystalline Form A may also have the following additional peaks an X-ray diffraction pattern (λ=1.5418 Å):

Peak	2θ° (±0.1°)
6	10.5
7	14.0
8	21.7
9	24.3
10	26.1

Compound A as crystalline Form A may also be characterised by any combination of three or more peaks selected from the list of 10 peaks above.

Form A of compound A when heated from 25° C. to 325° C. at 10° C. per minute by DSC will have an onset of melting at 210.1° C.±1° C.

Example 1

23 mg of compound A as Form A was weighed into a vial. To this solid was added 0.25 mL of a solution made from 8.5 mL methanol and 1.5 mL water (i.e. 15% water v/v). The resulting slurry was heated to 70° C., filtered and allowed to cool slowly to ambient temperature in a capped vial. The resulting crystallisation yielded a solid which was dried by a vacuum oven. The product is compound A as Form L.

Example 2

Approximately 50 mg of compound A as Form A was added to a reaction tube and approximately 1 ml of ethanol/water (80:20 v/v) added. The mixture was slurried at 40° C. for 3 weeks. The sample was filtered and dried on the filter for 10 minutes before allowing to dry on the bench overnight. The product is compound A as Form L.

Example 3

In a similar manner to Example 2, compound A as Form A was slurried in an ethanol/water (80:20 v/v) mixture for 3 weeks at 60° C. to yield compound A as Form L.

Example 4

Approximately 30 mg of compound A as Form A was added to a reaction tube and about 1 ml of ethanol/water (80:20 v/v) added to form a saturated suspension of Form A. To this saturated suspension, 30 mg of compound A as Form L was added. The mixture was slurried at 25° C. for 3 days. The sample was filtered and dried on the filter for 10 minutes before allowing to dry on the bench overnight. The product is compound A as Form L, with no Form A.

Example 5

Example 4 was repeated but using the following solvents to slurry the mixture of Form A and Form L—for these experiments samples were slurried for 4 days.

Mixture	Solvent(s)
a	Acetone
b	Acetonitrile
c	Ethanol
d	Methanol
e	Nitromethane

In each case, the product is compound A as crystalline Form L, with no Form A.

Example 6

Inhibitory Action

In order to assess the inhibitory action of the active compound, the following assay was used to determine an IC_{50} value.

Mammalian PARP, isolated from Hela cell nuclear extract, was incubated with Z-buffer (25 mM Hepes (Sigma); 12.5 mM $MgCl_2$ (Sigma); 50 mM KCl (Sigma); 1 mM DTT (Sigma); 10% Glycerol (Sigma) 0.001% NP-40 (Sigma); pH 7.4) in 96 well FlashPlates (TRADE MARK) (NEN, UK) and varying concentrations of said inhibitors added. All compounds were diluted in DMSO and gave final assay concentrations of between 10 and 0.01 μM, with the DMSO being at a final concentration of 1% per well. The total assay volume per well was 40 μl.

After 10 minutes incubation at 30° C. the reactions were initiated by the addition of a 10 μl reaction mixture, containing NAD (5 μM), ^{3}H-NAD and 30mer double stranded DNA-oligos. Designated positive and negative reaction wells were done in combination with compound wells (unknowns) in order to calculate % enzyme activities. The plates were then shaken for 2 minutes and incubated at 30° C. for 45 minutes.

Following the incubation, the reactions were quenched by the addition of 50 μl 30% acetic acid to each well. The plates were then shaken for 1 hour at room temperature.

The plates were transferred to a TopCount NXT (TRADE MARK) (Packard, UK) for scintillation counting. Values recorded are counts per minute (cpm) following a 30 second counting of each well.

The % enzyme activity for the compound is then calculated using the following equation:

$$\%\ \text{Inhibition} = 100 - \left(100 \times \frac{(cpm\ \text{of unknowns} - \text{mean negative } cpm)}{(\text{mean positive } cpm - \text{mean neagative } cpm)}\right)$$

IC_{50} values (the concentration at which 50% of the enzyme activity is inhibited) were calculated, which are determined over a range of different concentrations, normally from 10 μM down to 0.001 μM. Such IC_{50} values are used as comparative values to identify increased compound potencies.

Compound A has an IC_{50} of about 5 nM.

Potentiation Factor

The Potentiation Factor (PF_{50}) for the active compound is calculated as a ratio of the IC_{50} of control cell growth divided by the IC_{50} of cell growth+PARP inhibitor. Growth inhibition curves for both control and compound treated cells are in the presence of the alkylating agent methyl methanesulfonate (MMS). The test compound was used at a fixed concentration of 0.2 micromolar. The concentrations of MMS were over a range from 0 to 10 μg/ml.

Cell growth was assessed using the sulforhodamine B (SRB) assay (Skehan, P., et al., (1990) New colorimetric cytotoxicity assay for anticancer-drug screening. J. Natl. Cancer Inst. 82, 1107-1112.). 2,000 HeLa cells were seeded into each well of a flat-bottomed 96-well microtiter plate in a volume of 100 μl and incubated for 6 hours at 37° C. Cells were either replaced with media alone or with media containing PARP inhibitor at a final concentration of 0.5, 1 or 5 μM. Cells were allowed to grow for a further 1 hour before the addition of MMS at a range of concentrations (typically 0, 1,

2, 3, 5, 7 and 10 μg/ml) to either untreated cells or PARP inhibitor treated cells. Cells treated with PARP inhibitor alone were used to assess the growth inhibition by the PARP inhibitor.

Cells were left for a further 16 hours before replacing the media and allowing the cells to grow for a further 72 hours at 37° C. The media was then removed and the cells fixed with 100 μl of ice cold 10% (w/v) trichloroacetic acid. The plates were incubated at 4° C. for 20 minutes and then washed four times with water. Each well of cells was then stained with 100 μl of 0.4% (w/v) SRB in 1% acetic acid for 20 minutes before washing four times with 1% acetic acid. Plates were then dried for 2 hours at room temperature. The dye from the stained cells was solubilized by the addition of 100 μl of 10 mM Tris Base into each well. Plates were gently shaken and left at room temperature for 30 minutes before measuring the optical density at 564 nM on a Microquant microtiter plate reader.

Compound A has a PF_{50} at 200 nM of at least 20.

The invention claimed is:

1. 4-[3-(4-cyclopropanecarbonyl-piperazine-1-carbonyl)-4-fluoro-benzyl]-2H-phthalazin-1-one as crystalline Form L.

2. The compound of claim **1** having the following characteristic peaks in a powder XRD:

Peak	2θ° (±0.1°) (λ = 1.5418 Å)
1	14.4
2	17.2
3	17.5
4	18.8
5	23.0.

3. The compound of claim **1** having the following characteristic peaks in a powder XRD:

Peak	2θ° (±0.1°) (λ = 1.5418 Å)
1	14.4
2	17.2
3	17.5
4	18.8
5	23.0
6	10.4
7	13.6
8	25.1.

4. The compound of claim **1**, which has an onset of melting at 198.5° C.±1° C. when heated from 25° C. to 325° C. at 10° C. per minute in DSC.

5. A method of obtaining 4-[3-(4-cyclopropanecarbonyl-piperazine-1-carbonyl)-4-fluoro-benzyl]-2H-phthalazin-1-one as crystalline Form L from 4-[3-(4-cyclopropanecarbonyl-piperazine-1-carbonyl)-4-fluoro-benzyl]-2H-phthalazin-1-one as crystalline Form A, comprising slurrying 4-[3-(4-cyclopropanecarbonyl-piperazine-1-carbonyl)-4-fluoro-benzyl]-2H-phthalazin-1-one as either Form A or a mixture of Forms A and L in an organic solvent selected from the group consisting of methanol, ethanol, acetone, acetonitrile and nitromethane; that optionally contains up to 30% water v/v.

6. A pharmaceutical composition comprising a compound having crystalline Form L according to claim **1** and a pharmaceutically acceptable carrier or diluent.

7. A compound having crystalline Form L according to claim **1** for use in a method of treatment of the human or animal body.

8. A compound having crystalline Form L according to claim **1** for the use in a method of inhibiting PARP in the treatment of the human or animal body.

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