TRICYCLIC PARP INHIBITORS

This invention relates to a series of compounds which are derivatives of tricyclic lactam indoles and tricyclic lactam benzimidazoles and which inhibit poly (ADP-ribose) polymerase (PARP), and more particularly to their use in the treatment of cancer, in particular breast cancer.

Homologous recombination (HR) has been shown to play an important role in repair of damage occurring at DNA replication forks in mammalian cells (2). Thus, cells deficient in HR show retarded growth and exhibit higher levels of genetic instability. It is believed that genetic instability due to loss of HR repair in human cancers significantly contributes to the development of cancer in these cells (1).

Post transcriptional modification of nuclear proteins by poly (ADP-ribosyl)ation in response to DNA strand breaks plays an important role in DNA repair, regulation of apoptosis, and maintenance of genomic stability.

Poly (ADP-ribose) polymerase (PARP-1) is the principal member of the PARP enzyme family and is an abundant nuclear protein in mammalian cells. PARP-1 catalyses the formation of poly (ADP-ribose) (PAR) polymers using NAD^+ as substrate. Upon DNA damage, PARP-1 binds rapidly to a DNA single-strand break (SSB) and catalyses the addition of negatively charged PAR chains to itself (automodification) and other proteins [see (3,4) for reviews]. The binding of PARP-1 to SSBs is believed to protect DNA lesions from further processing until PARP-1 is

dissociated from the break by the accumulated negative charge resulting from PAR polymers (5, 6).

Although PARP-1 has been implicated in several nuclear processes, such as modulation of chromatin structure, DNA-replication, DNA repair and transcription, PARP-1 knockout mice develop normally (7). Cells isolated from these mice exhibit a hyper recombination phenotype and genetic instability in the form of increased levels of sister chromatic exchanges (SCE) micronuclei and tetraploidy (8, 10). Genetic instability may also occur in these PARP-1 knockout mice through telomere shortening, increased frequency of chromosome fusion and aneuploid (11), although all these results could not be repeated in another set of PARP-1 knockout mice (12). In the former mice knockout, PARP-1 null mutation rescued impaired V (D) J recombination in SCID mice (13).

These results support the view suggested by Lindahl and co-workers that PARP-1 has a protective role against recombination (5). It was proposed that binding of PARP-1 to ssDNA breaks prevents the recombination machinery from recognising and processing DNA lesions or, alternatively that the negative charges accumulated following poly (ADP-ribosyl)ation repel adjacent recombinogenic DNA sequences. Only the latter model is consistent with inhibition of PARP-1 itself and expression of a dominant negative mutant PARP-1, including SCE, gene amplification and homologous recombination (14-18).

Studies based on treating cells with inhibitors of PARP-1 or cells derived from PARP-1 knockout mice indicate that the suppression of PARP-1 activity increases cell susceptibility to DNA damaging agents and inhibits strand break rejoining (3, 4, 8 – 11, 19, 20).

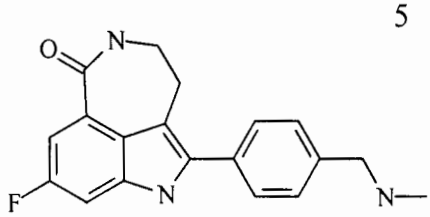
Inhibitors of PARP-1 activity have been used in combination with traditional cancer treatment regimes such as radio-therapy and chemotherapy (21). When the inhibitors were used in combination with methylating agents, topoisomerase poisons and ionising radiations they were found to enhance the effectiveness of these forms of treatment. However, such treatments are non-selective and as such cause damage and death to non-cancerous or 'healthy' cells. Furthermore, such treatments are known to give rise to unpleasant side effects.

Therefore, it is highly desirable to provide a treatment for cancer that is both effective and selective in the killing of cancer cells and which does not need to be administered in combination with radio-therapy or chemotherapy treatments.

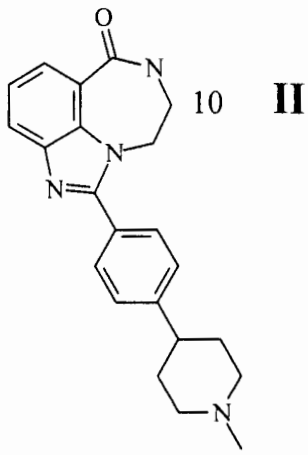
Surprisingly it has been found that cells deficient in homologous recombination (HR) are hypersensitive to PARP inhibitors relative to wild type cells.

Thus, according to a first aspect of the present invention there is provided the use of a therapeutic amount of a compound of formula I, II or III, or a pharmaceutically acceptable salt thereof, in the manufacture of a medicament for the treatment of a

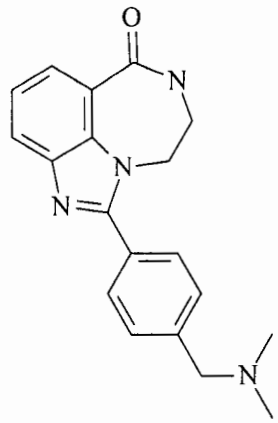
disease or condition that is caused by a genetic defect in a gene that mediates homologous recombination.



I



II



III

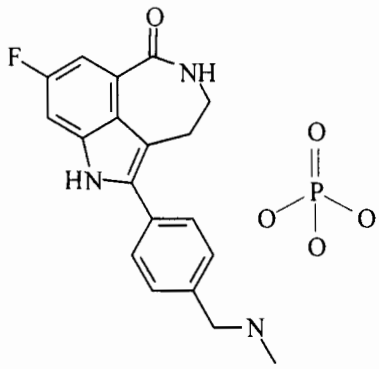
The compounds described herein can be prepared by synthetic routes based on those disclosed in WO 00/42040 and WO 01/16136.

It will be understood that where reference is made in this specification to compounds of formulas I to III the reference should be construed as extending also to their pharmaceutically acceptable salts and to other pharmaceutically acceptable bioprecursors (prodrug forms) where relevant. The term "prodrug" is used in the present specification to denote modified forms or derivatives of a pharmacologically active compound which biodegrade or are modified in vivo so as to become converted into said active compound after administration, especially oral or intravenous administration, in the course of therapeutic treatment of a mammal. Such prodrugs are commonly chosen because of an enhanced solubility in aqueous media which helps to overcome formulation problems, and also in some cases to give a relatively slow or controlled release of the active agent.

As referred to herein pharmaceutically acceptable salts include metal salts, phosphates and quaternary amines. The metal salts may be formed with alkali metals such as lithium, sodium or potassium.

Preferably, formula I, above, is administered in the form of a pharmaceutically acceptable phosphate salt having the following formula:

Formula I – phosphate



Thus, the present invention relates to the therapeutic utility of the compounds described herein.

Diseases and conditions which are caused by a genetic defect in a gene that mediates homologous recombination include, but are not limited to cancer, in particular breast cancer.

As referred herein "cancer" or "tumour" includes, but is not limited to, cancer of the lung, colon, pancreas, stomach, ovary, cervix, breast, prostate bone, brain or skin.

The use of PARP inhibitors is particularly suitable in the treatment of cancer which is caused by a genetic defect in a gene wherein the said gene mediates homologous recombinations. Cancer cells of this type tend to be HR defective.

The specific sensitivity of HR defective tumours to PARP inhibition means that normally dividing "healthy" cells in patients which have adequate amounts of HR will be largely unaffected by the treatment.

A further advantage of treatment using PARP inhibitors is that the PARP inhibitors do not need to be administered as a combination therapy along with conventional radiotherapy or chemotherapy treatments thereby avoiding the side effects associated with these conventional forms of treatment.

A defect in a gene that mediates homologous recombination may be due to a mutation in, the absence of, or defective expression of, a gene encoding a protein involved in HR.

According to a further aspect of the present invention there is provided the use of a therapeutic amount of a compound of formula I, II or III, or a pharmaceutically acceptable salt thereof, in the manufacture of a medicament for inducing apoptosis of cells defective in a gene that mediates HR.

Cancer cells suitable for treatment with the compounds described herein may be partially or totally deficient in HR. Preferably, the cells are totally deficient in HR.

The compounds described herein may be used to treat an inherited form of cancer wherein the patient to be treated has a familial predisposition to the cancer. However the said compounds are particularly suitable for the treatment of gene-linked hereditary cancer, and most particularly gene-linked hereditary breast cancer.

In a preferred aspect, the PARP inhibitor is useful in the treatment of cancer cells defective in the expression of a gene involved in HR. Genes with suggested function in HR include XRCC1, ADPRT (PARP-1), ADPRTL2, (PARP02) CTPS, RPA, RPA1, RPA2, RPA3, XPD, ERCC1, XPF, MMS19, RAD51, RAD51β, RAD51C, RAD51D, DMC1, XRCCR, XRCC3, BRCA1, BRCA2, RAD52, RAD54, RAD50, MRE11, NB51, WRN, BLM KU70, RU80, ATM, ATR CHK1, CHK2, FANCA, FANCB, FANCC, FANCD1, FANCD2, FANCE, FANCF, FANCG, FANCC, FANCD1, FANCD2, FANCE, FANCF, FANCG, RAD1, RAD9 [See (2, 3, 5, 22-28) for reviews].

A gene involved in HR may be a tumour suppressor gene. The invention thus provides for the treatment of cancer cells defective in the expression of a tumour suppressor gene. Preferably, the tumour suppressor gene is BRCA1 or BRCA2.

Breast cancer is the most common type of cancer among women in the Western World. Certain families have a strong predisposition for breast cancer, which is often owing to an inherited mutation in one allele of either BRCA1 or BRCA2. However, one functional allele is maintained. Thus, individuals possessing the said mutation develop normally and have no phenotypic consequence from this mutation. However,

in one cell, the functional allele might be lost, making this cell cancerous and at the same time deficient in HR. This step is critical for the onset of a tumour (1).

Therefore, according to a still further aspect of the invention there is provided the use of a therapeutic amount of a compound of formula I, II or III, or a pharmaceutically acceptable salt threof, in the manufacture of a medicament for the treatment of cancer cells defective in BRCA1 and/or BRCA2 expression.

The cancer cells to be treated may be partially or totally deficient in BRCA1 or BRCA2 expression. Such deficiencies can be identified using multiplex PCR techniques array techniques (29, 30) or using other screens known to the skilled person. Particularly useful techniques include real-time quantitiative RT-PCR, Northern blot, immunohistochemistry and Western Blot (31, 32).

Accordingly, the compounds of the present invention are of particular interest for the treatment of a range of selected cancer tumours, and the medicaments are useful in methods of treating a patient suffering from cancer.

The compounds described herein may be administered in a therapeutically effective non-toxic amount via any suitable route for effectively targeting cancer cells. Suitable administration routes include, but are not limited to, any of the following: oral, intravenous, intramuscular, intradermal, intranasal, or topical.

A therapeutically effective amount of the compounds described herein is typically one which is sufficient to achieve the desired effect and may vary according to the nature and severity of the disease condition, and the potency of the compound. It will be appreciated that different concentrations may be employed for prophylaxis than for treatment of an active disease.

For administration to mammals, and particularly humans, it is expected that the daily dosage level of the active agent will be from 0.01 to 50 mg/kg in mice and 0.01 mg/m^2 to 50 mg/m^2 body surface area in humans. Ultimately, however, the amount of active ingredient administered and the frequency of administration will be at the discretion of a physician.

Advantageously, only very low doses of PARP inhibiting compounds are needed to have a therapeutic effect in treating cancer thereby reducing systemic build up of the compounds and thus minimising any associated toxic effects.

While it may be possible for the compounds described herein to be administered alone as the 'raw' compound , it is preferable to present the compounds in a pharmaceutical composition.

All methods of formulation in making up such pharmaceutical compositions will generally include the step of bringing one of the compounds described herein into association with a carrier which constitutes one or more accessory ingredients. Usually, the formulations are prepared by uniformly and intimately bringing the compound of formula I into association with a liquid carrier or with a finely divided solid carrier or with both and then, if necessary, shaping the product into desired formulations.

Formulations suitable for oral administration may be presented as discrete units such as capsules, cachets, tablets or lozenges, each containing a predetermined amount of one of the compounds describe herein; as a powder or granules; or a suspension in an aqueous liquid or non-aqueous liquid such as a syrup, an elixir, an emulsion or a draught. Any one of the compounds described herein may also be presented as a bolus, electuary or paste.

A tablet may be made by compression or moulding, optionally with one or more accessory ingredients. Compressed tablets may be prepared by compressing, in a suitable machine, any one of the compounds described herein in a free-flowing form such as a powder or granules, optionally mixed with a binder, lubricant, inert diluent,

surface active or dispersing agent. Moulded tablets may be made by moulding, in a suitable machine, a mixture of any one of the powdered compound described herein with any suitable carrier.

A syrup may be made by adding any one of the compounds described herein to a concentrated, aqueous solution of a sugar, for example sucrose, to which may be added any desired accessory ingredient. Such accessory ingredient(s) may include flavourings, an agent to retard crystallisation of the sugar or an agent to increase the solubility of any other ingredient, such as a polyhydric alcohol, for example glycerol or sorbitol.

Formulations for rectal administration may be presented as a suppository with a usual carrier such as cocoa butter.

Formulations suitable for parenteral administration conveniently comprise a sterile aqueous preparation of any one of the compounds describe herein which is preferably isotonic with the blood for the recipient.

In addition to the aforementioned ingredients, formulations, for example ointments, creams and the like, may include one or more accessory ingredients, for example a diluent, buffer, flavouring agent, binder, surface active agent, thickener, lubricant and/or a preservative (including an antioxidant) or other pharmaceutically inert excipient.

The compounds of this invention may also be made up for administration in liposomal formulations which can be prepared by methods well-known in the art.

The medicament may take the form of a pharmaceutical composition comprising a compound of formula I, II or III, or a pharmaceutically acceptable salt thereof, as an active agent.

The pharmaceutical composition may further comprise at least one other ingredient providing a compatible pharmaceutically acceptable additive, carrier diluent carrier or excipient and may be presented in unit dosage form.

The carrier(s) must be pharmaceutically acceptable in the sense of being compatible with the other ingredients of the formulation and not deterious to the recipient thereof.

The possible formulations include those suitable for oral, rectal, topical and parenteral (including subcutaneous, intramuscular and intravenous) administration or for administration to the lung or another absorptive site such as the nasal passages.

The compounds referred to herein may be administered in combination with other anti-cancer compounds.

The medicaments are useful in methods of treating cancer in mammals by administering the compounds described herein and their pharmaceutically acceptable salts.

The present invention will now be described by way of example only with reference to the accompanying figures wherein:

Figure 1 is a graph showing cell survival in the presence of PARP inhibitor of formula III in AA8 cell line, IsrISF cell line and CxR3 cell line;

Figure 2 is a graph showing cell survival in the presence of PARP inhibitor of formula III in V79 cell line, VC8 cell line and VC8B2 cell line;

Figure 3 is a graph showing cell survival in the presence of PARP inhibitor of formula I in V79 cell line, VC8 cell line and VC8B2 cell line;

Figure 4 is a bar chart showing PARP activity in VC8, V79, VC8#13 and VC8, VC8#13 and VC8+B2 cell lines in the presence of PARP inhibitor of formula III;

Figure 5 is a pair of graphs showing inhibition of cellular PARP activity in the presence of PARP inhibitor of formula I and III in permeabilised (upper graph) and intact (lower graph) L1210 cells;

Figure 6 is a pair of bar charts showing the blood and tumour pharmacokinetics and pharmacodynamics with formula I-phosphate at 1 mg/kg (upper) and 10 mg/kg (lower) in mice bearing SW620 xenografts;

Figure 7 is a bar chart showing the pharmacokinetics and pharmacodynanics with formula III in mice bearing SW620 xenografts;

Figure 8 is a graph showing the tumour growth (median relative tumour volume) in mice bearing SW620 xenografts following treatment with formula III in combination with temozolomide (TMZ) and with TMZ alone;

Figure 9 is a graph showing the tumour growth (median relative tumour volume) of mice bearing SW620 xenografts following treatment with formula I-phosphate in combination with temozolomide (TMZ) and with formula I-phosphate and TMZ alone;

Fig. 1 shows the percentage survival of AA8, IrS ISF and CxR3 cell lines when treated with various concentrations of the compound of formula III. Formula III was found to be most active against IrS ISF, which lacks XRCC3, having an LC_{50} (the concentration of the active component that kills 50% of the cells) of 100 nM.

Fig. 2 shows the percentage survival of V79-Z, VC8 and VC8B2 cell lines when treated with various concentrations of the compound of formula III. Formula III was found to be most effective against the VC8 cell line, which lacks BRCA2, having an LC_{50} value of 43 nM and an LC_{90} (concentration of active component that kills 90% of the cells) was 1200 nM.

Fig. 3 shows the percentage survival of V79-Z, VC8 and VC8B2 cell lines when treated with various concentrations of the compound of formula I. Formula I was found to be most effective against the VC8 cell line, which lacks BRCA2, having an LC_{50} value of 12 nM, LC_{90} was 27 nM

Fig. 4 shows PARP activity of various cell lines when treated with various concentrations of the compound of formula III. The graph of Fig. 3 is divided into four result sets for each respective cell line. The first bar of each set shows the background PARP activity (no oligo present, so PARP activity is dependent upon endogenous DNA breaks), the second bar is total stimulatable (by oligo) PARP activity and the third and fourth bars show the PARP activity in the presence of the compound of formula III.

Fig. 5 shows the effect of Compounds Formula I and III on PARP activity.

Cells used to obtain the results shown in Fig. 5 were either permeabilised with digitonin and then assayed for total stimulatable (by oligo) PARP activity in the presence and absence of PARP inhibitor of formula I and formula III or exposed to one of said PARP inhibitors for 20 minutes prior to permeabilisation and assayed for total stimulatable PARP activity.

There was no difference in the PARP inhibitory activity of the compounds of formula I and formula III when the cells were permeabilised prior to adding the inhibitor

compound but the compound of formula I was more potent in intact cells, possibly because it accumulates within cells to a higher degree.

Fig. 6 shows the plasma and tumour concentrations of the compound of formula I, and its pharmacokinetic effect on mouse peripheral blood lymphocytes (pbl parp) and SW620 xenografts (tumour PARP), at various times following intraperitoneal administration of the phosphate salt of compound of formula I. The phosphate salt of the compound of formula I increases the solubility of formula I. However, on administration to an animal (including human) plasma phosphatases break the phosphate salt of formula I (formula I-phosphate) down to the parent compound i.e. formula I.

It is evident form fig. 6 that thirty minutes after administration of formula I-phosphate at 10 mg/kg highlevels of the parent compound were detected in both plasma and tumour. The concentration of formula 1 decreased with time more rapidly in the plasma than in the tumour and at 24 hr after administration significant levels were detectable in the tumour but none could be detected in the plasma. There was a profound and sustained inhibition of PARP activity in both pbls and tumour: <50% control up to 24 hr.

After administration of formula I-phosphate at 1 mg/kg lower levels of the compound of formula I can be found in both the plasma and tumour and consequently there was a less pronounced effect on PARP activity.

Fig. 7 shows the plasma and tumour concentrations of the compound of formula III, and its pharmacokinetic effect on SW620 xenografts (tumour PARP act), at various times following intraperitoneal administration of the 10 mg/kg of compound of formula III. This compound also distributes well to the tumour and is preferentially retained with time and similarly inhibits PARP activity in the tumour.

Fig. 8 shows that for 20 days from administration of temolozomide (68 mg/kg daily x5) the tumour xenograft has progressively reduced in size. However, shortly after this time the tumour size begins to increase. When a compound of formula III (5 mg/kg daily x 5) is administered in conjunction with temozolomide the tumour shrinks significantly for around 15 days, to an undetectable size, the tumour size remains undetectable for a further 50 days thereafter when it begins to increase in size. When a larger dose of formula III (15 mg/kg daily x 5) is administered the tumour size remains undetectable for a further 80 days until the end of the experiment when no tumour was detectable at autopsy i.e. complete tumour regression.

Fig. 9 shows a similar pattern to that seen in fig. 8 following the administration of formula I-phosphate (at 0.1 mg/kg and 1.0 mg/kg) in combination with temolozomide.

Table 1. Genotype and origin of cell lines used in this study.

Cell line	Genotype	Defect	Origin	Reference	Comments
AA8	wt	wt	CHO	[41]	Chinese hamster ovary cell line
irs1SF	*XRCC3*$^-$	XRCC3$^-$, deficient in HR	AA8	[41]	Radiation-sensitive cell line derived from AA8 which lacks XRCC3 a component of HR pathway
CXR3	*XRCC3*- + hXRCC3	wt	irs1SF	[41]	irs1SF transfected with hXRCC3 gene
V79-Z	wt	wt	V79	[42]	V79 are hamster lung fibroblasts
VC8	*BRCA2*-	BRCA2$^-$, deficient in HR	V79-Z	[42]	VC8 are radiation sensitive derivatives of V79 which are deficient in BRCA2
VC8#13	*BRCA2*$^-$ *+hBRCA2*	wt	VC8	[42]	VC8 with chromosome 13 containing *hBRCA2*
VC8+B2	*BRCA2*$^-$ *+hBRCA2*	wt	VC8	[42]	VC8 transfected *with hBRCA2*

Materials and Methods

Cytotoxicity of PARP inhibitors to cells deficient in HR (XRCC3 or BRCA2)

Cell culture

The AA8, irs1SF and CXR3 cell lines were provided by Larry Thompson [41].

The VC-8, VC-8+B2, VC-8#13 were a gift from Malgorzata Zdienicka [42]. All cell lines in this study were grown in Dulbecco's modified Eagle's Medium (DMEM) with 10% Foetalbovine serum and penicillin (100 U/ml) and steptomycin sulphate (100 μg/mL) at 37°C under an atmosphere containing 5% CO_2.

Toxicity assay – clonogenic survival assay

Exponentially growing cells in 6-well plates were exposed to the compound of formula III at the concentrations indicated in Fig 2 in 1% DMSO or 1% DMSO alone in medium for 24 hours.

The cells were harvested by trypsinisation, counted and seeded at varying densities in 10 cm dishes in fresh medium in the absence of drug for colony formation.

7-10 days later the dishes were fixed with methanol:acetic acid 3:1 and stained with 0.4% crystal violet.

Colonies were counted and the survival relative to 1%DMSO control treated cells calculated.

PARP activity assay

Exponentially growing cells were exposed to 1% DMSO in culture medium (control) or a compound of formula I or III in 1% DMSO at the concentrations indicated in Fig. 4 to cells permeabilised with digitonin, or intact cells for 20 minutes prior to washing and digitonin-permeabilization. PARP activity was measured by incorporation of a

[32P] labelled NAD+ substrate into TCA precipitateble polymers after stimulation by the addition of a blunt-ended oligonucleotide and compared with non-oligonucleotide-stimulated cells. PARP activity in tumour homogenates (1 in 40 in isotonic buffer) from formula III-treated mice was measured in the same way. PARP activity in pbls and tumour homogenates from fomula I-phospate treated mice was measured by immunological detection of polymer using the 10H antibody. Briefly, tumour homogenates diluted to up to 1:1000 in isotonic buffer were incubated with 350 μM NAD for 6 min and blotted onto nitrocellulose membrane. The poly(ADP-ribose) (PAR) polymer formation was quantified by chemiluminescence detection using a Fuji LAS3000 UV Illuminator by reference to serial dilutions of a PAR standard, following incubation with 10H antibody to PAR and a secondary anti-mouse antibody. The results were standardised by reference to the measured protein content of the homogenate.

It is of course to be understood that the invention is not intended to be restricted to the details of the above embodiments which are described by way of example only.

REFERENCES:

[1] C. Lundin, K. Erixon, C. Arnaudeau, N. Schultz, D. Jenssen, M. Meuth and T. Helleday Different roles for nonhomologous end joining and homologous recombination following replication arrest in mammalian cells, Mol Cell Biol 22 (2002) 5869-5878.

[2] A.R. Venkitaraman Cancer susceptibility and the functions of BRCA1 and BRCA2, Cell 108 (2002) 171-182.

[3] D. D'Amours, S. Desnoyers, I. D'Silva and G.G. Poirier Poly(ADP-ribosyl)ation reactions in the regulation of nuclear functions, Biochem J 342 (1999) 249-268.

[4] Z. Herceg and Z.Q. Wang Functions of poly(ADP-ribose) polymerase (PARP) in DNA repair, genomic integrity and cell death, Mutat Res 477 (2001) 97-110.

[5] T. Lindahl, M.S. Satoh, G.G. Poirier and A. Klungland Post-translational modification of poly(ADP-ribose) polymerase induced by DNA strand breaks, Trends Biochem Sci 20 (1995) 405-411.

[6] M.S. Satoh and T. Lindahl Role of poly(ADP-ribose) formation in DNA repair, Nature 356 (1992) 356-358.

[7] S. Shall and G. de Murcia Poly(ADP-ribose) polymerase-1: what have we learned from the deficient mouse model?, Mutat Res 460 (2000) 1-15.

[8] Z.Q. Wang, L. Stingl, C. Morrison, M. Jantsch, M. Los, K. Schulze-Osthoff and E.F. Wagner PARP is important for genomic stability but dispensable in apoptosis, Genes Dev 11 (1997) 2347-2358.

[9] C.M. Simbulan-Rosenthal, B.R. Haddad, D.S. Rosenthal, Z. Weaver, A. Coleman, R. Luo, H.M. Young, Z.Q. Wang, T. Ried and M.E. Smulson Chromosomal aberrations in PARP(-/-) mice: genome stabilization in immortalized cells by reintroduction of poly(ADP-ribose) polymerase cDNA, Proc Natl Acad Sci U S A 96 (1999) 13191-13196.

[10] J.M. de Murcia, C. Niedergang, C. Trucco, M. Ricoul, B. Dutrillaux, M. Mark, F.J. Oliver, M. Masson, A. Dierich, M. LeMeur, C. Walztinger, P. Chambon and G. de Murcia Requirement of poly(ADP-ribose) polymerase in recovery from DNA damage in mice and in cells, Proc Natl Acad Sci U S A 94 (1997) 7303-7307.

[11] F. d'Adda di Fagagna, M.P. Hande, W.M. Tong, P.M. Lansdorp, Z.Q. Wang and S.P. Jackson Functions of poly(ADP-ribose) polymerase in controlling telomere length and chromosomal stability, Nat Genet 23 (1999) 76-80.

[12] E. Samper, F.A. Goytisolo, J. Menissier-de Murcia, E. Gonzalez-Suarez, J.C. Cigudosa, G. de Murcia and M.A. Blasco Normal telomere length and chromosomal end capping in poly(ADP-ribose) polymerase-deficient mice

and primary cells despite increased chromosomal instability, J Cell Biol 154 (2001) 49-60.

[13] C. Morrison, G.C. Smith, L. Stingl, S.P. Jackson, E.F. Wagner and Z.Q. Wang Genetic interaction between PARP and DNA-PK in V(D)J recombination and tumorigenesis, Nat Genet 17 (1997) 479-482.

[14] V. Schreiber, D. Hunting, C. Trucco, B. Gowans, D. Grunwald, G. De Murcia and J.M. De Murcia A dominant-negative mutant of human poly(ADP-ribose) polymerase affects cell recovery, apoptosis, and sister chromatid exchange following DNA damage, Proc Natl Acad Sci U S A 92 (1995) 4753-4757.

[15] J.H. Kupper, M. Muller and A. Burkle Trans-dominant inhibition of poly(ADP-ribosyl)ation potentiates carcinogen induced gene amplification in SV40-transformed Chinese hamster cells, Cancer Res 56 (1996) 2715-2717.

[16] J. Magnusson and C. Ramel Inhibitor of poly(ADP-ribose)transferase potentiates the recombinogenic but not the mutagenic action of alkylating agents in somatic cells in vivo in Drosophila melanogaster, Mutagenesis 5 (1990) 511-514.

[17] A.S. Waldman and B.C. Waldman Stimulation of intrachromosomal homologous recombination in mammalian cells by an inhibitor of poly(ADP-ribosylation), Nucleic Acids Res 19 (1991) 5943-5947.

[18] A. Semionov, D. Cournoyer and T.Y. Chow Inhibition of poly(ADP-ribose)polymerase stimulates extrachromosomal homologous recombination in mouse Ltk-fibroblasts, Nucleic Acids Res 27 (1999) 4526-4531.

[19] F. Dantzer, V. Schreiber, C. Niedergang, C. Trucco, E. Flatter, G. De La Rubia, J. Oliver, V. Rolli, J. Menissier-de Murcia and G. de Murcia Involvement of poly(ADP-ribose) polymerase in base excision repair, Biochimie 81 (1999) 69-75.

[20] F. Dantzer, G. de La Rubia, J. Menissier-De Murcia, Z. Hostomsky, G. de Murcia and V. Schreiber Base excision repair is impaired in mammalian cells lacking Poly(ADP-ribose) polymerase-1, Biochemistry 39 (2000) 7559-7569.

[21] L. Tentori, I. Portarena and G. Graziani Potential clinical applications of poly(ADP-ribose) polymerase (PARP) inhibitors, Pharmacol Res 45 (2002) 73-85.

[22] T. Lindahl and R.D. Wood Quality control by DNA repair, Science 286 (1999) 1897-1905.

[23] K.W. Caldecott DNA single-strand break repair and spinocerebellar ataxia, Cell 112 (2003) 7-10.

[24] D. D'Amours and S.P. Jackson The Mre11 complex: at the crossroads of dna repair and checkpoint signalling, Nat Rev Mol Cell Biol 3 (2002) 317-327.

[25] A.D. D'Andrea and M. Grompe The Fanconi anaemia/BRCA pathway, Nat Rev Cancer 3 (2003) 23-34.

[26] S.P. Jackson Sensing and repairing DNA double-strand breaks, Carcinogenesis 23 (2002) 687-696.

[27] R. Kanaar, J.H. Hoeijmakers and D.C. van Gent Molecular mechanisms of DNA double strand break repair, Trends Cell Biol 8 (1998) 483-489.

[28] D.C. van Gent, J.H. Hoeijmakers and R. Kanaar Chromosomal stability and the DNA double-stranded break connection, Nat Rev Genet 2 (2001) 196-206.

[29] S.L. Neuhausen and E.A. Ostrander Mutation testing of early-onset breast cancer genes BRCA1 and BRCA2, Genet Test 1 (1997) 75-83.

[30] G. Kuperstein, W.D. Foulkes, P. Ghadirian, J. Hakimi and S.A. Narod A rapid fluorescent multiplexed-PCR analysis (FMPA) for founder mutations in the BRCA1 and BRCA2 genes, Clin Genet 57 (2000) 213-220.

[31] Vissac-Sabatier C, Coxam V, Dechelotte P, Picherit C, Horcajada M-N, Davicco M-J, Lebecque P, Bignon Y-J, and Bernard-Gallon D. Phytoestrogen-rich diets modulate expression of BRCA1 and BRCA2 tumour suppressor genes in mammary glands of female Wistar rats. Cancer Research vol 63 pp 6607-6612 (2003).

[32] Wu K, Jiang S-W and Couch FJ. p53 mediates repression of the BRCA2 promoter and down regulation of BRCA2 mRNA and protein levels in response to DNA damage. J. Biol. Chem. Vol 278 pp 15652-15660 (2003).

[33] A. Chiarugi Poly(ADP-ribose) polymerase: killer or conspirator? The 'suicide hypothesis' revisited, Trends Pharmacol Sci 23 (2002) 122-129.

[34] C.R. Calabrese, M.A. Batey, H.D. Thomas, B.W. Durkacz, L.Z. Wang, S. Kyle, D. Skalitzky, J. Li, C. Zhang, T. Boritzki, K. Maegley, A.H. Calvert, Z. Hostomsky, D.R. Newell and N.J. Curtin Identification of Potent Nontoxic

Poly(ADP-Ribose) Polymerase-1 Inhibitors: Chemopotentiation and Pharmacological Studies, Clin Cancer Res 9 (2003) 2711-2718.

[35] D. Ferraris, Y.S. Ko, T. Pahutski, R.P. Ficco, L. Serdyuk, C. Alemu, C. Bradford, T. Chiou, R. Hoover, S. Huang, S. Lautar, S. Liang, Q. Lin, M.X. Lu, M. Mooney, L. Morgan, Y. Qian, S. Tran, L.R. Williams, Q.Y. Wu, J. Zhang, Y. Zou and V. Kalish Design and synthesis of poly ADP-ribose polymerase-1 inhibitors. 2. Biological evaluation of aza-5[H]-phenanthridin-6-ones as potent, aqueous-soluble compounds for the treatment of ischemic injuries, J Med Chem 46 (2003) 3138-3151.

[36] K.J. Dillon, G.C. Smith and N.M. Martin A FlashPlate assay for the identification of PARP-1 inhibitors, J Biomol Screen 8 (2003) 347-352.

[37] A.J. Pierce, R.D. Johnson, L.H. Thompson and M. Jasin XRCC3 promotes homology-directed repair of DNA damage in mammalian cells, Genes Dev 13 (1999) 2633-2638.

[38] R.D. Johnson, N. Liu and M. Jasin Mammalian XRCC2 promotes the repair of DNA double-strand breaks by homologous recombination, Nature 401 (1999) 397-399.

[39] G.M. Shah, D. Poirier, S. Desnoyers, S. Saint-Martin, J.C. Hoflack, P. Rong, M. ApSimon, J.B. Kirkland and G.G. Poirier Complete inhibition of poly(ADP-ribose) polymerase activity prevents the recovery of C3H10T1/2 cells from oxidative stress, Biochim Biophys Acta 1312 (1996) 1-7.

[40] R.J. Griffin, S. Srinivasan, K. Bowman, A.H. Calvert, N.J. Curtin, D.R. Newell, L.C. Pemberton and B.T. Golding Resistance-modifying agents. 5. Synthesis and biological properties of quinazolinone inhibitors of the DNA repair enzyme poly(ADP-ribose) polymerase (PARP), J Med Chem 41 (1998) 5247-5256.

[41] S. Boulton, L.C. Pemberton, J.K. Porteous, N.J. Curtin, R.J. Griffin, B.T. Golding and B.W. Durkacz Potentiation of temozolomide-induced cytotoxicity: a comparative study of the biological effects of poly(ADP-ribose) polymerase inhibitors, Br J Cancer 72 (1995) 849-856.

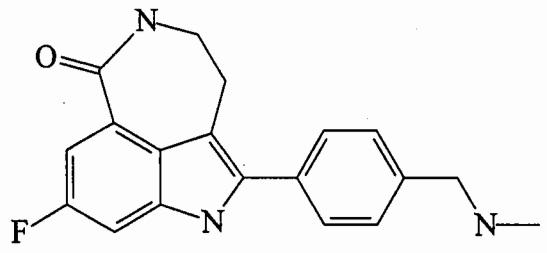
[42] C.S. Griffin, P.J. Simpson, C.R. Wilson and J. Thacker Mammalian recombination-repair genes XRCC2 and XRCC3 promote correct chromosome segregation, Nat Cell Biol 2 (2000) 757-761.

[43] R.S. Tebbs, Y. Zhao, J.D. Tucker, J.B. Scheerer, M.J. Siciliano, M. Hwang, N. Liu, R.J. Legerski and L.H. Thompson Correction of chromosomal instability and sensitivity to diverse mutagens by a cloned cDNA of the XRCC3 DNA repair gene, Proc Natl Acad Sci U S A 92 (1995) 6354-6358.

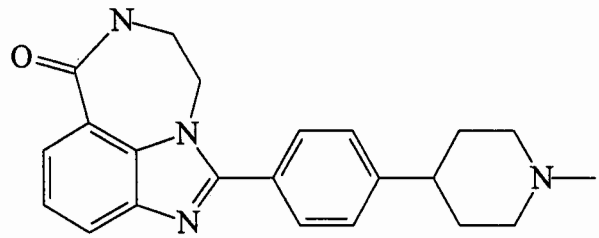
[44] M. Kraakman-van der Zwet, W.J. Overkamp, R.E. van Lange, J. Essers, A. van Duijn-Goedhart, I. Wiggers, S. Swaminathan, P.P. van Buul, A. Errami, R.T. Tan, N.G. Jaspers, S.K. Sharan, R. Kanaar and M.Z. Zdzienicka Brca2 (XRCC11) deficiency results in radioresistant DNA synthesis and a higher frequency of spontaneous deletions, Mol Cell Biol 22 (2002) 669-679.

CLAIMS

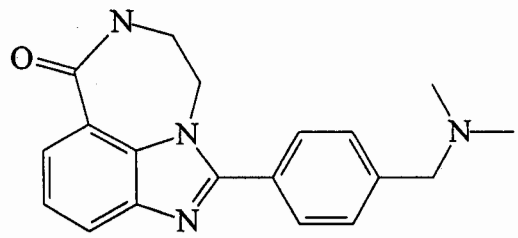
1. The use of a therapeutic amount of a compound of Formula I, II or III, or a pharmaceutically acceptable salt thereof, in the manufacture of a medicament for the treatment of a disease or condition that is caused by a genetic defect in a gene that mediates homologous recombination.



I



II



III

2. The use according to claim 1, wherein the compound is the compound of Formula I.

3. The use according to claim 2, wherein the compound of Formula I is in the form of a phosphate salt.

4. The use according to any one of claims 1 to 3, wherein the disease is cancer.

5. The use according to claim 4, wherein the cancer is breast cancer.

6. The use according to any one of claims 1 to 3, wherein the genetic defect is the absence of a gene encoding a protein involved in homologous recombination.

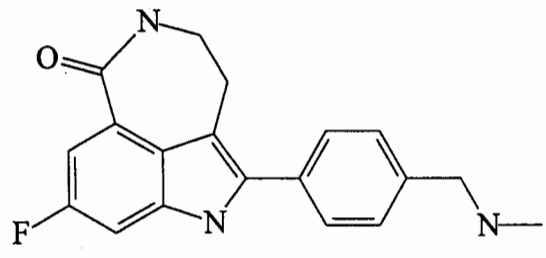
7. The use according to any one of claims 1 to 3, wherein the genetic defect is in the expression of a gene encoding a protein involved in homologous recombination.

8. The use according to claim 7, wherein the gene that mediates homologous recombination is selected from the group consisting of XRCC1, ADPRT (PARP-1), ADPRTL2 (PARP02), CTPS, RPA, RPA1, RPA2, RPA3, XPD, ERCC1, XPF, MMS19, RAD51, RAD51β, RAD51C, RAD51D, DMC1, XRCCR, XRCC3, BRCA1, BRCA2, RAD52, RAD54, RAD50, MRE11, NB51, WRN, BLM KU70, RU80, ATM, ATR CHK1, CHK2, FANCA, FANCB, FANCC, FANCD1, FANCD2, FANCE, FANCF, FANCG, FANCC, FANCD1, FANCD2, FANCE, FANCF, FANCG, RAD1, RAD9 and combinations thereof.

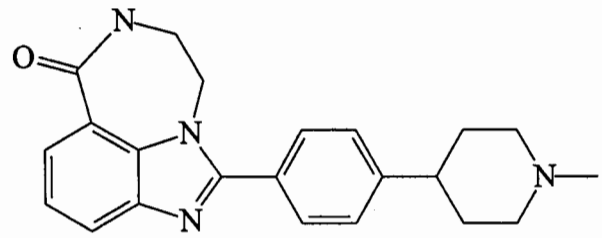
9. The use according to either of claim 7 or claim 8, wherein the gene that mediates homologous recombination is a tumour suppressor gene.

10. The use according to claim 9, wherein the tumour suppressor gene is BRCA1 and/or BRCA2.

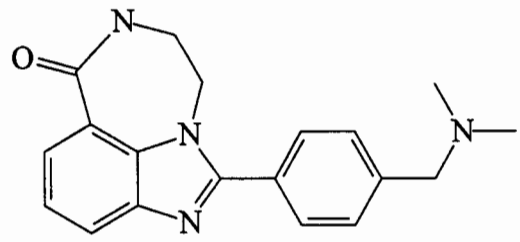
11. The use of a therapeutic amount of a compound of Formula I, II or III, or a pharmaceutically acceptable salt thereof, in the manufacture of a medicament for inducing apoptosis of cells defective in a gene that mediates homologous recombination.



I

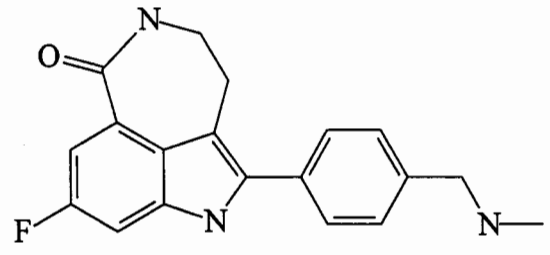


II

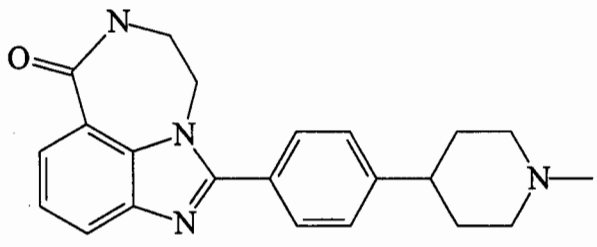


III

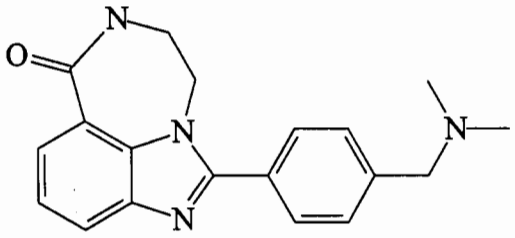
12. The use of a therapeutic amount of a compound of Formula I, II or III, or a pharmaceutically acceptable salt thereof, in the manufacture of a medicament for the treatment of cancer cells defective in BRCA1 and/or BRCA2 expression.



I



II



III

13. The use according to claim 12, wherein the cancer cells to be treated are partially or totally deficient in BRCA1 and/or BRCA2 expression.

Sequence Listings

Sequence 1 – Human cDNA sequence of PARP-1

1 cgcccgccca gccccggggg cagggaaagc ctaaattacg gaattaccgc gagcaaggag
61 cgcggaatcg gggagcgtcc ggagctagct ggatcctcta ggcaggatgg tgatgggaat
121 ctttgcaaat tgtatcttct gtttgaaagt gaagtactta cctcagcagc agaagaaaaa
181 gctacaaact gacattaagg aaaatggcgg aaagttttcc ttttcgttaa atcctcagtg
241 cacacatata atcttagata atgctgatgt tctgagtcag taccaactga attctatcca
301 aaagaaccac gttcatattg caaacccaga ttttatatgg aaatctatca gagaaaagag
361 actcttggat gtaaagaatt atgatcctta taagcccctg gacatcacac cacctcctga
421 tcagaaggcg agcagttctg aagtgaaaac agaaggtcta tgcccggaca gtgccacaga
481 ggaggaagac actgtggaac tcactgagtt tggtatgcag aatgttgaaa ttcctcatct
541 tcctcaagat tttgaagttg caaaatataa caccttggag aaagtgggaa tggagggagg
601 ccaggaagct gtggtggtgg agcttcagtg ttcgcgggac tccagggact gtcctttcct
661 gatatcctca cacttcctcc tggatgatgg catggagact agaagacagt ttgctataaa
721 gaaaacctct gaagatgcaa gtgaatactt tgaaaattac attgaagaac tgaagaaaca
781 aggatttcta ctaagagaac atttcacacc tgaagcaacc caattagcat ctgaacaatt
841 gcaagcattg cttttggagg aagtcatgaa ttcaagcact ctgagccaag aggtgagcga
901 tttagtagag atgatttggg cagaggccct gggccacctg gaacacatgc ttctcaagcc
961 agtgaacagg attagcctca acgatgtgag caaggcagag gggattctcc ttctagtaaa
1021 ggcagcactg aaaaatggag aaacagcaga gcaattgcaa aagatgatga cagagtttta
1081 cagactgata cctcacaaag gcacaatgcc caaagaagtg aacctgggac tattggctaa
1141 gaaagcagac ctctgccagc taataagaga catggttaat gtctgtgaaa ctaatttgtc
1201 caaacccaac ccaccatccc tggccaaata ccgagctttg aggtgcaaaa ttgagcatgt
1261 tgaacagaat actgaagaat ttctcagggt tagaaaagag gttttgcaga atcatcacag
1321 taagagccca gtggatgtct tgcagatatt tagagttggc agagtgaatg aaaccacaga
1381 gtttttgagc aaacttggta atgtgaggcc cttgttgcat ggttctcctg tacaaaacat
1441 cgtgggaatc ttgtgtcgag ggttgctttt acccaaagta gtggaagatc gtggtgtgca
1501 aagaacagac gtcggaaacc ttggaagtgg gatttatttc agtgattcgc tcagtacaag
1561 tatcaagtac tcacacccgg gagagacaga tggcaccaga ctcctgctca tttgtgacgt
1621 agccctcgga aagtgtatgg acttacatga gaaggacttt tccttaactg aagcaccacc
1681 aggctacgac agtgtgcatg gagtttcaca aacagcctct gtcaccacag actttgagga
1741 tgatgaattt gttgtctata aaaccaatca ggttaaaatg aaatatatta ttaaattttc
1801 catgcctgga gatcagataa aggactttca tcctagtgat catactgaat tagaggaata
1861 cagacctgag ttttcaaatt tttcaaaggt tgaagattac cagttaccag atgccaaaac
1921 ttccagcagc accaaggccg gcctccagga tgcttctggg aacttggttc ctctggagga
1981 tgtccacatc aaagggagaa tcatagacac tgtagcccag gtcattgttt ttcagacata
2041 cacaaataaa agtcacgtgc ccattgaggc aaaatatatc tttcctttgg atgacaaggc
2101 cgctgtgtgt ggcttcgaag ccttcatcaa tgggaagcac atagttggag agattaaaga
2161 gaaggaagaa gcccagcaag agtacctaga agccgtgacc cagggccatg gcgcttacct
2221 gatgagtcag gatgctccgg acgtttttac tgtaagtgtt ggaaacttac cccctaaggc
2281 taaggttctt ataaaaatta cctacatcac agaactcagc atcctgggca ctgttggtgt
2341 ctttttcatg cccgccaccg tagcaccctg gcaacaggac aaggctttga atgaaaacct
2401 tcaggataca gtagagaaga tttgtataaa agaaatagga acaaagcaaa gcttctcttt

2461 gactatgtct attgagatgc cgtacgtgat tgaattcatt ttcagtgata ctcatgaact
2521 gaaacaaaag cgcacagact gcaaagctgt cattagcacc atggaaggca gctccttaga
2581 cagcagtgga ttttctctcc acatcggttt gtctgctgcc tatctcccaa gaatgtgggt
2641 tgaaaaacat ccagaaaaag aaagcgaggc ttgcatgctt gtctttcaac ccgatctcga
2701 tgtcgacctc cctgacctag ccaatgagag cgaagtgatt atttgtcttg actgctccag
2761 ttccatggag ggtgtgacat tcttgcaagc caaggaaatc gccttgcatg cgctgtcctt
2821 ggtgggtgag aagcagaaag taaatattat ccagttcggc acaggttaca aggagctatt
2881 ttcgtatcct aagcatatca caagcaatac cgcggcagca gagttcatca tgtctgccac
2941 acctaccatg gggaacacag acttctggaa aacactccga tatcttagct tattgtaccc
3001 tgctcgaggg tcacggaaca tcctcctggt gtctgatggg cacctccagg atgagagcct
3061 gacattacag ctcgtgaaga ggagccgccc gcacaccagg ttattcgcct gcggtatcgg
3121 ttctacagca aatcgtcacg tcttaaggat tttgtcccag tgtggtgccg gagtatttga
3181 atattttaat gcaaaatcca agcatagttg gagaaaacag atagaagacc aaatgaccag
3241 gctatgttct ccgagttgcc actctgtctc cgtcaaatgg cagcaactca atccagatgc
3301 gcccgaggcc ctgcaggccc cagcccaggt gccatccttg tttcgcaatg atcgactcct
3361 tgtctatgga ttcattcctc actgcacaca ggcaactctg tgtgcactaa ttcaagagaa
3421 agaattttgt acaatggtgt cgactactga gcttcagaag acaactggaa ctatgatcca
3481 caagctggca gcccgagctc taatcagaga ttatgaagat ggcattcttc acgaaaatga
3541 aaccagtcat gagatgaaaa aacaaacctt gaaatctctg attattaaac tcagtaaaga
3601 aaactctctc ataacacaat ttacaagctt tgtggcagtt gagaaaaggg atgagaatga
3661 gtcacctttt cctgatattc caaaagtttc tgaacttatt gccaaagaag atgtagactt
3721 cctgccctac atgagctggc agggggaacc ccaagaagcc gtcaggaacc agtctctttt
3781 agcatcctct gagtggccag aattacgttt atccaaacga aaacatagga aaattccatt
3841 ttccaaaaga aaaatggaat tatctcagcc agaagtttct gaagattttg aagaggatgc
3901 cttaggtgta ctaccagctt tcacatcaaa tttggaacgt ggacgtgtgg aaaagctatt
3961 ggatttaagt tggacagagt catgtaaacc aacagcaact gaaccactat ttaagaaagt
4021 cagtccatgg gaaacatcta cttctagctt ttttcctatt ttggctccgg ccgttggttc
4081 ctatcttacc ccgactaccc gcgctcacag tcctgcttcc ttgtcttttg cctcatatcg
4141 tcaggtagct agtttcggtt cagctgctcc tcccagacag tttgatgcat ctcaattcag
4201 ccaaggccct gtgcctggca cttgtgctga ctggatccca cagtcggcgt cttgtcccac
4261 aggacctccc cagaacccac cttctgcacc ctattgtggc attgtttttt cagggagctc
4321 attaagctct gcacagtctg ctccactgca acatcctgga ggctttacta ccaggccttc
4381 tgctggcacc ttccctgagc tggattctcc ccagcttcat ttctctcttc ctacagaccc
4441 tgatcccatc agaggttttg ggtcttatca tccctctgct tactctcctt ttcattttca
4501 accttccgca gcctctttga ctgccaacct taggctgcca atggcctctg ctttacctga
4561 ggctctttgc agtcagtccc ggactacccc agtagatctc tgtcttctag aagaatcagt
4621 aggcagtctc gaaggaagtc gatgtcctgt ctttgctttt caaagttctg acacagaaag
4681 tgatgagcta tcagaagtac ttcaagacag ctgcttttta caaataaaat gtgatacaaa
4741 agatgacagt atcccgtgct ttctggaagt aaaagaagag gatgaaatag tgtgcacaca
4801 acactggcag gatgctgtgc cttggacaga actcctcagt ctacagacag aggatggctt
4861 ctggaaactt acaccagaac tgggacttat attaaatctt aatacaaatg gtttgcacag
4921 ctttcttaaa caaaaaggca ttcaatctct aggtgtaaaa ggaagagaat gtctcctgga
4981 cctaattgcc acaatgctgg tactacagtt tattcgcacc aggttggaaa aagagggaat
5041 agtgttcaaa tcactgatga aaatggatga cccttctatt tccaggaata ttccctgggc
5101 ttttgaggca ataaagcaag caagtgaatg ggtaagaaga actgaaggac agtacccatc
5161 tatctgccca cggcttgaac tggggaacga ctgggactct gccaccaagc agttgctggg
5221 actccagccc ataagcactg tgtcccctct tcatagagtc ctccattaca gtcaaggcta

5281 agtcaaatga aactgaattt taaacttttt gcatgcttct atgtagaaaa taatcaaatg
5341 ataatagata cttataatga aacttcatta aggtttcatt cagtgtagca attactgtct
5401 ttaaaaatta agtggaagaa gaattacttt aatcaactaa caagcaataa taaaatgaaa
5461 cttaaaat

Sequence 2 – Human cDNA sequence of PARP-2

1 ctagaattca gcggccgctg aattctaggc ggcgcggcgg cgacggagca ccggcggcgg
61 cagggcgaga gcattaaatg aaagcaaaag agttaataat ggcaacacgg ctccagaaga
121 ctcttcccct gccaagaaaa ctcgtagatg ccagagacag gagtcgaaaa agatgcctgt
181 ggctggagga aaagctaata aggacaggac agaagacaag caagatggta tgccaggaag
241 gtcatgggcc agcaaaaggg tctctgaatc tgtgaaggcc ttgctgttaa agggcaaagc
301 tcctgtggac ccagagtgta cagccaaggt ggggaaggct catgtgtatt gtgaaggaaa
361 tgatgtctat gatgtcatgc taaatcagac caatctccag ttcaacaaca acaagtacta
421 tctgattcag ctattagaag atgatgccca gaggaacttc agtgtttgga tgagatgggg
481 ccgagttggg aaaatgggac agcacagcct ggtggcttgt tcaggcaatc tcaacaaggc
541 caaggaaatc tttcagaaga aattccttga caaaacgaaa aacaattggg aagatcgaga
601 aaagtttgag aaggtgcctg gaaaatatga tatgctacag atggactatg ccaccaatac
661 tcaggatgaa gaggaaacaa aaaaagagga atctcttaaa tctcccttga agccagagtc
721 acagctagat cttcgggtac aggagttaat aaagttgatc tgtaatgttc aggccatgga
781 agaaatgatg atggaaatga agtataatac caagaaagcc ccacttggga agctgacagt
841 ggcacaaatc aaggcaggtt accagtctct taagaagatt gaggattgta ttcgggctgg
901 ccagcatgga cgagctctca tggaagcatg caatgaattc tacaccagga ttccgcatga
961 ctttggactc cgtactcctc cactaatccg gacacagaag gaactgtcag aaaaaataca
1021 attactagag gctttgggag acattgaaat tgctattaag ctggtgaaaa cagagctaca
1081 aagcccagaa cacccattgg accaacacta tagaaaccta cattgtgcct tgcgcccccc
1141 tgaccatgaa agttacgagt tcaaagtgat ttcccagtac ctacaatcta cccatgctcc
1201 cacacacagc gactatacca tgaccttgct ggatttgttt gaagtggaga aggatggtga
1261 gaaagaagcc ttcagagagg accttcataa caggatgctt ctatggcatg gttccaggat
1321 gagtaactgg gtgggaatct tgagccatgg gcttcgaatt gcccaccctg aagctcccat
1381 cacaggttac atgtttggga aaggaatcta ctttgctgac atgtcttcca agagtgccaa
1441 ttactgcttt gcctctcgcc taaagaatac aggactgctg ctcttatcag aggtagctct
1501 aggtcagtgt aatgaactac tagaggccaa tcctaaggcc gaaggattgc ttcaaggtaa
1561 acatagcacc aaggggctgg gcaagatggc tcccagttct gcccacttcg tcaccctgaa
1621 tgggagtaca gtgccattag gaccagcaag tgacacagga attctgaatc cagatggtta
1681 taccctcaac tacaatgaat atattgtata taacccccaac caggtccgta tgcggtacct
1741 tttaaaggtt cagtttaatt tccttcagct gtggtgaatg ttgatcttaa ataaaccaga
1801 gatctgatct tcaagcaaga aaataagcag tgttgtactt gtgaattttg tgatatttta
1861 tgtaataaaa actgtacagg tctaaaaaaa aaaaaaaaaa aaaaaaaaaa

Sequence 3 – Human cDNA sequence of PARP-3

1 tgggactggt cgcctgactc ggcctgcccc agcctctgct tcaccccact ggtggccaaa
61 tagccgatgt ctaatccccc acacaagctc atccccggcc tctgggattg ttgggaattc
121 tctccctaat tcacgcctga ggctcatgga gagttgctag acctgggact gccctgggag
181 gcgcacacaa ccaggccggg tggcagccag gacctctccc atgtccctgc ttttcttggc
241 catggctcca aagccgaagc cctgggtaca gactgagggc cctgagaaga agaagggccg
301 gcaggcagga agggaggagg acccccttcg ctccaccgct gaggccctca aggccatacc
361 cgcagagaag cgcataatcc gcgtggatcc aacatgtcca ctcagcagca accccgggac
421 ccaggtgtat gaggactaca actgcaccct gaaccagacc aacatcgaga acaacaacaa
481 caagttctac atcatccagc tgctccaaga cagcaaccgc ttcttcacct gctggaaccg
541 ctggggccgt gtgggagagg tcggccagtc aaagatcaac cacttcacaa ggctagaaga
601 tgcaaagaag gactttgaga agaaatttcg ggaaaagacc aagaacaact gggcagagcg
661 ggaccacttt gtgtctcacc cgggcaagta cacacttatc gaagtacagg cagaggatga
721 ggcccaggaa gctgtggtga aggtggacag aggcccagtg aggactgtga ctaagcgggt
781 gcagccctgc tccctggacc cagccacgca gaagctcatc actaacatct tcagcaagga
841 gatgttcaag aacaccatgg ccctcatgga cctggatgtg aagaagatgc ccctgggaaa
901 gctgagcaag caacagattg cacggggttt cgaggccttg gaggcgctgg aggaggccct
961 gaaaggcccc acggatggtg gccaaagcct ggaggagctg tcctcacact tttacaccgt
1021 catcccgcac aacttcggcc acagccagcc cccgcccatc aattcccctg agcttctgca
1081 ggccaagaag gacatgctgc tggtgctggc ggacatcgag ctggcccagg ccctgcaggc
1141 agtctctgag caggagaaga cggtggagga ggtgccacac cccctggacc gagactacca
1201 gcttctcaag tgccagctgc agctgctaga ctctggagca cctgagtaca aggtgataca
1261 gacctactta gaacagactg gcagcaacca caggtgccct acacttcaac acatctggaa
1321 agtaaaccaa gaaggggagg aagacagatt ccaggcccac tccaaactgg gtaatcggaa
1381 gctgctgtgg catggcacca acatggccgt ggtggccgcc atcctcacta gtgggctccg
1441 catcatgcca cattctggtg ggcgtgttgg caagggcatc tactttgcct cagagaacag
1501 caagtcagct ggatatgtta ttggcatgaa gtgtggggcc caccatgtcg gctacatgtt
1561 cctgggtgag gtggccctgg gcagagagca ccatatcaac acggacaacc ccagcttgaa
1621 gagcccacct cctggcttcg acagtgtcat tgcccgaggc cacaccgagc ctgatccgac
1681 ccaggacact gagttggagc tggatggcca gcaagtggtg gtgccccagg gccagcctgt
1741 gccctgccca gagttcagca gctccacatt ctcccagagc gagtacctca tctaccagga
1801 gagccagtgt cgcctgcgct acctgctgga ggtccacctc tgagtgcccg ccctgtcccc
1861 cggggtcctg caaggctgga ctgtgatctt caatcatcct gcccatctct ggtaccccta
1921 tatcactcct ttttttcaag aatacaatac gttgttgtta actatagtca ccatgctgta
1981 caagatccct gaacttatgc ctcctaactg aaattttgta ttctttgaca catctgccca
2041 gtccctctcc tcccagccca tggtaaccag catttgactc tttacttgta taagggcagc
2101 ttttataggt tccacatgta agtgagatca tgcagtgttt gtctttctgt gcctggctta
2161 tttcactcag cataatgtgc accgggttca cccatgtttt cataaatgac aagatttcct
2221 cctttaaaaa aaaaaaaaaa aaaaaaaaaa aaaaaaaaaa aaa

Sequence 4 – Human gDNA sequence of Tankyrase 1

1 cgaagatggc ggcgtcgcgt cgctctcagc atcatcacca ccatcatcaa caacagctcc
61 agcccgcccc aggggcttca gcgccgccgc cgccacctcc tcccccactc agccctggcc
121 tggcccccggg gaccacccca gcctctccca cggccagcgg cctggccccc ttcgcctccc
181 cgcggcacgg cctagcgctg ccggaggggg atggcagtcg ggatccgccc gacaggcccc
241 gatccccgga cccggttgac ggtaccagct gttgcagtac caccagcaca atctgtaccg
301 tcgccgccgc tcccgtggtc ccagcggttt ctacttcatc tgccgctggg gtcgctccca
361 acccagccgg cagtggcagt aacaattcac cgtcgtcctc ttcttccccg acttcttcct
421 catcttcctc tccatcctcc cctggatcga gcttggcgga gagccccgag gcggccggag
481 ttagcagcac agcaccactg gggcctgggg cagcaggacc tgggacaggg gtcccagcag
541 tgagcggggc cctacgggaa ctgctggagg cctgtcgcaa tggggacgtg tcccgggtaa
601 agaggctggt ggacgcggca aacgtaaatg caaaggacat ggccggccgg aagtcttctc
661 ccctgcactt cgctgcaggt tttggaagga aggatgttgt agaacactta ctacagatgg
721 gtgctaatgt ccacgctcgt gatgatggag gtctcatccc gcttcataat gcctgttctt
781 ttggccatgc tgaggttgtg agtctgttat tgtgccaagg agctgatcca aatgccaggg
841 ataactggaa ctatacacct ctgcatgaag ctgctattaa agggaagatc gatgtgtgca
901 ttgtgctgct gcagcacgga gctgacccaa acattcggaa cactgatggg aaatcagccc
961 tggacctggc agatccttca gcaaaagctg tccttacagg tgaatacaag aaagacgaac
1021 tcctagaagc tgctaggagt ggtaatgaag aaaaactaat ggctttactg actcctctaa
1081 atgtgaattg ccatgcaagt gatgggcgaa agtcgactcc tttacatcta gcagcgggct
1141 acaacagagt tcgaatagtt cagcttcttc ttcagcatgg tgctgatgtt catgcaaaag
1201 acaaaggtgg acttgtgcct cttcataatg catgttcata tggacattat gaagtcacag
1261 aactgctact aaagcatgga gcttgtgtta atgccatgga tctctggcag tttactccac
1321 tgcacgaggc tgcttccaag aaccgtgtag aagtctgctc tttgttactt agccatggcg
1381 ctgatcctac gttagtcaac tgccatggca aaagtgctgt ggatatggct ccaactccgg
1441 agcttaggga gagattgact tatgaattta aaggtcattc tttactacaa gcagccagag
1501 aagcagactt agctaaagtt aaaaaaacac tcgctctgga aatcattaat ttcaaacaac
1561 cgcagtctca tgaaacagca ctgcactgtg ctgtggcctc tctgcatccc aaacgtaaac
1621 aagtgacaga attgttactt agaaaaggag caaatgttaa tgaaaaaaat aaagatttca
1681 tgactcccct gcatgttgca gccgaaagag cccataatga tgtcatggaa gttctgcata
1741 agcatggcgc caagatgaat gcactggaca cccttggtca gactgctttg catagagccg
1801 ccctagcagg ccacctgcag acctgccgcc tcctgctgag ttacggctct gacccctcca
1861 tcatctcctt acaaggcttc acagcagcac agatgggcaa tgaagcagtg cagcagattc
1921 tgagtgtgag ttacggctct gacccctcca tcatctcctt acaaggcttc acagcagcac
1981 agatgggcaa tgaagcagtg cagcagattc tgagtggtca ttcgtagata gtgatcattc
2041 tacttcagcc ttaatggtga tcttgagacg ggaagattta gaaggaaatc tatccagcat
2101 gtcttcactg tcaacatgaa gagtacacct atacgtactt ctgatgttga ttatcgactc
2161 ttagaggcat ctaaagctgg agacttggaa actgtgaagc aactttgcag ctctcaaaat
2221 gtgaattgta gagacttaga gggccggcat tccacgccct tacacttcgc agcaggctac
2281 aacagagtac acctatacgt acttctgatg ttgattatcg actcttagag gcatctaaag
2341 ctggagactt ggaaactgtg aagcaacttt gcagctctca aaatgtgaat tgtagagact
2401 tagagggccg gcattccacg cccttacact tcgcagcagg ctacaaccgc gtgtctgttg
2461 tagagtacct gctacaccac ggtgccgatg tccatgccaa agacaagggt ggcttggtgc
2521 cccttcataa tgcctgttca tatggacact atgaggtggc tgagctttta gtaaggcatg
2581 gggcttctgt caatgtggcg gacttatgga aatttacccc tctccatgaa gcagcagcta

2641 aaggaaagta tgaaatctgc aagctccttt taaaacatgg agcagatcca actaaaaaga
2701 acagagatgg aaatacacct ttggatttgg taaaggaagg agacacagat attcaggact
2761 tactgaaagg ggatgctgct ttgttggatg ctgccaagaa gggctgcctg gcaagagtgc
2821 agaagctctg taccccagag aatatcaact gcagagacac ccagggcaga aattcaaccc
2881 ctctgcacct ggcagcaggc tataataacc tggaagtagc tgaatatctt ctagagcatg
2941 gagctgatgt taatgcccag gacaagggtg gtttaattcc tcttcataat gcggcatctt
3001 atgggcatgt tgacatagcg gctttattga taaaatacaa cacgtgtgta aatgcaacag
3061 ataagtgggc gtttactccc ctccatgaag cagcccagaa aggaaggacg cagctgtgcg
3121 ccctcctcct agcgcatggt gcagacccca ccatgaagaa ccaggaaggc cagacgcctc
3181 tggatctggc aacagctgac gatatcagag ctttgctgat agatgccatg cccccagagg
3241 ccttacctac ctgttttaaa cctcaggcta ctgtagtgag tgcctctctg atctcaccag
3301 catccacccc ctcctgcctc tcggctgcca gcagcataga caacctcact ggccctttag
3361 cagagttggc cgtaggagga gcctccaatg caggggatgg cgccgcggga acagaaagga
3421 aggaaggaga agttgctggt cttgacatga atatcagcca atttctaaaa agccttggcc
3481 ttgaacacct tcgggatatc tttgaaacag aacagattac actagatgtg ttggctgata
3541 tgggtcatga agagttgaaa gaaataggca tcaatgcata tgggcaccgc cacaaattaa
3601 tcaaaggagt agaaagactc ttaggtggac aacaaggcac caatccttat ttgacttttc
3661 actgtgttaa tcagggaacg attttgctgg atcttgctcc agaagataaa gaatatcagt
3721 cagtggaaga agagatgcaa agtactattc gagaacacag agatggtggt aatgctggcg
3781 gcatcttcaa cagatacaat gtcattcgaa ttcaaaaagt tgtcaacaag aagttgaggg
3841 agcggttctg ccaccgacag aaggaagtgt ctgaggagaa tcacaaccat cacaatgagc
3901 gcatgttgtt tcatggttct cctttcatta atgccattat tcataaaggg tttgatgagc
3961 gacatgcata cataggagga atgtttgggg ccgggattta ttttgctgaa aactcctcaa
4021 aaagcaacca atatgtttat ggaattggag gaggaacagg ctgccctaca cacaaggaca
4081 ggtcatgcta tatatgtcac agacaaatgc tcttctgtag agtgaccctt gggaaatcct
4141 ttctgcagtt tagcaccatg aaaatggccc acgcgcctcc agggcaccac tcagtcattg
4201 gtagaccgag cgtcaatggg ctggcatatg ctgaatatgt catctacaga ggagaacagg
4261 catacccaga gtatcttatc acttaccaga tcatgaagcc agaagcccct tcccagaccg
4321 caacagccgc agagcagaag acctagtgaa tgcctgctgg tgaaggccag atcagatttc
4381 aacctgggac tggattacag aggattgttt ctaataacaa catcaatatt ctagaagtcc
4441 ctgacagcct agaaataagc tgtttgtctt ctataaagca ttgctatagt g

Sequence 5 – Human mRNA sequence of Tankyrase 2

1 cgcgccgcct cgctagccga aacctgccca gccggtgccc ggccactgcg cacgcgcggg
61 acgacgtcac gtgcgctccc ggggctggac ggagctggca ggaggggcct tgccagcttc
121 cgccgccgcg tcgtttcagg acccggacgg cggattcgcg ctgcctccgc cgccgcgggg
181 cagccggggg gcagggagcc cagcgagggg cgcgcgtggg cgcggccatg ggactgcgcc
241 ggatccggtg acagcaggga gccaagcggc ccgggccctg agcgcgtctt ctccgggggg
301 cctcgccctc ctgctcgcgg ggccggggct cctgctccgg ttgctggcgc tgttgctggc
361 tgtggcggcg gccaggatca tgtcgggtcg ccgctgcgcc ggcgggggag cggcctgcgc
421 gagcgccgcg gccgaggccg tggagccggc cgcccgagag ctgttcgagg cgtgccgcaa
481 cggggacgtg gaacgagtca agaggctggt gacgcctgag aaggtgaaca gccgcgacac
541 ggcgggcagg aaatccaccc cgctgcactt cgccgcaggt tttgggcgga aagacgtagt
601 tgaatatttg cttcagaatg gtgcaaatgt ccaagcacgt gatgatgggg gccttattcc
661 tcttcataat gcatgctctt ttggtcatgc tgaagtagtc aatctccttt tgcgacatgg
721 tgcagacccc aatgctcgag ataattggaa ttatactcct ctccatgaag ctgcaattaa
781 aggaaagatt gatgtttgca ttgtgctgtt acagcatgga gctgagccaa ccatccgaaa
841 tacagatgga aggacagcat tggatttagc agatccatct gccaaagcag tgcttactgg
901 tgaatataag aaagatgaac tcttagaaag tgccaggagt ggcaatgaag aaaaaatgat
961 ggctctactc acaccattaa atgtcaactg ccacgcaagt gatggcagaa agtcaactcc
1021 attacatttg gcagcaggat ataacagagt aaagattgta cagctgttac tgcaacatgg
1081 agctgatgtc catgctaaag ataaaggtga tctggtacca ttacacaatg cctgttctta
1141 tggtcattat gaagtaactg aacttttggt caagcatggt gcctgtgtaa atgcaatgga
1201 cttgtggcaa ttcactcctc ttcatgaggc agcttctaag aacagggttg aagtatgttc
1261 tcttctctta agttatggtg cagacccaac actgctcaat tgtcacaata aaagtgctat
1321 agacttggct cccacaccac agttaaaaga aagattagca tatgaattta aaggccactc
1381 gttgctgcaa gctgcacgag aagctgatgt tactcgaatc aaaaaacatc tctctctgga
1441 aatggtgaat ttcaagcatc ctcaaacaca tgaaacagca ttgcattgtg ctgctgcatc
1501 tccatatccc aaaagaaagc aaatatgtga actgttgcta agaaaaggag caaacatcaa
1561 tgaaaagact aaagaattct tgactcctct gcacgtggca tctgagaaag ctcataatga
1621 tgttgttgaa gtagtggtga aacatgaagc aaaggttaat gctctggata atcttggtca
1681 gacttctcta cacagagctg catattgtgg tcatctacaa acctgccgcc tactcctgag
1741 ctatgggtgt gatcctaaca ttatatccct tcagggcttt actgctttac agatgggaaa
1801 tgaaaatgta cagcaactcc tccaagaggg tatctcatta ggtaattcag aggcagacag
1861 acaattgctg gaagctgcaa aggctggaga tgtcgaaact gtaaaaaaac tgtgtactgt
1921 tcagagtgtc aactgcagag acattgaagg gcgtcagtct acaccacttc attttgcagc
1981 tgggtataac agagtgtccg tggtggaata tctgctacag catggagctg atgtgcatgc
2041 taaagataaa ggaggccttg tacctttgca caatgcatgt tcttatggac attatgaagt
2101 tgcagaactt cttgttaaac atggagcagt agttaatgta gctgatttat ggaaatttac
2161 acctttacat gaagcagcag caaaaggaaa atatgaaatt tgcaaacttc tgctccagca
2221 tggtgcagac cctacaaaaa aaaacaggga tggaaatact cctttggatc ttgttaaaga
2281 tggagataca gatattcaag atctgcttag gggagatgca gctttgctag atgctgccaa
2341 gaagggttgt ttagccagag tgaagaagtt gtcttctcct gataatgtaa attgccgcga
2401 tacccaaggc agacattcaa caccttaca tttagcagct ggttataata atttagaagt
2461 tgcagagtat ttgttacaac acggagctga tgtgaatgcc caagacaaag gaggacttat
2521 tcctttacat aatgcagcat cttacgggca tgtagatgta gcagctctac taataaagta
2581 taatgcatgt gtcaatgcca cggacaaatg ggctttcaca cctttgcacg aagcagccca

2641 aaagggacga acacagcttt gtgctttgtt gctagcccat ggagctgacc cgactcttaa
2701 aaatcaggaa ggacaaacac ctttagattt agtttcagca gatgatgtca gcgctcttct
2761 gacagcagcc atgcccccat ctgctctgcc ctcttgttac aagcctcaag tgctcaatgg
2821 tgtgagaagc ccaggagcca ctgcagatgc tctctcttca ggtccatcta gcccatcaag
2881 cctttctgca gccagcagtc ttgacaactt atctgggagt ttttcagaac tgtcttcagt
2941 agttagttca agtggaacag agggtgcttc cagtttggag aaaaaggagg ttccaggagt
3001 agattttagc ataactcaat tcgtaaggaa tcttggactt gagcacctaa tggatatatt
3061 tgagagagaa cagatcactt tggatgtatt agttgagatg gggcacaagg agctgaagga
3121 gattggaatc aatgcttatg gacataggca caaactaatt aaaggagtcg agagacttat
3181 ctccggacaa caaggtctta acccatattt aactttgaac acctctggta gtggaacaat
3241 tcttatagat ctgtctcctg atgataaaga gtttcagtct gtggaggaag agatgcaaag
3301 tacagttcga gagcacagag atggaggtca tgcaggtgga atcttcaaca gatacaatat
3361 tctcaagatt cagaaggttt gtaacaagaa actatgggaa agatacactc accggagaaa
3421 agaagtttct gaagaaaacc acaaccatgc caatgaacga atgctatttc atgggtctcc
3481 ttttgtgaat gcaattatcc acaaaggctt tgatgaaagg catgcgtaca taggtggtat
3541 gtttggagct ggcatttatt ttgctgaaaa ctcttccaaa agcaatcaat atgtatatgg
3601 aattggagga ggtactgggt gtccagttca caaagacaga tcttgttaca tttgccacag
3661 gcagctgctc ttttgccggg taaccttggg aaagtctttc ctgcagttca gtgcaatgaa
3721 aatggcacat tctcctccag gtcatcactc agtcactggt aggcccagtg taaatggcct
3781 agcattagct gaatatgtta tttacagagg agaacaggct tatcctgagt atttaattac
3841 ttaccagatt atgaggcctg aaggtatggt cgatggataa atagttattt taagaaacta
3901 attccactga acctaaaatc atcaaagcag cagtggcctc tacgttttac tcctttgctg
3961 aaaaaaaatc atcttgccca caggcctgtg gcaaaaggat aaaaatgtga acgaagttta
4021 acattctgac ttgataaagc tttaataatg tacagtgttt tctaaatatt tcctgttttt
4081 tcagcacttt aacagatgcc attccaggtt aaactgggtt gtctgtacta aattataaac
4141 agagttaact tgaacctttt atatgttatg cattgattct aacaaactgt aatgccctca
4201 acagaactaa ttttactaat acaatactgt gttctttaaa acacagcatt tacactgaat
4261 acaatttcat ttgtaaaact gtaaataaga gcttttgtac tagcccagta tttatttaca
4321 ttgctttgta atataaatct gttttagaac tgcagcggtt tacaaaattt tttcatatgt
4381 attgttcatc tatacttcat cttacatcgt catgattgag tgatctttac atttgattcc
4441 agaggctatg ttcagttgtt agttgggaaa gattgagtta tcagatttaa tttgccgatg
4501 ggagccttta tctgtcatta gaaatctttc tcatttaaga acttatgaat atgctgaaga
4561 tttaatttgt gataccttg tatgtatgag acacattcca aagagctcta actatgatag
4621 gtcctgatta ctaaagaagc ttctttactg gcctcaattt ctagctttca tgttggaaaa
4681 ttttctgcag tccttctgtg aaaattagag caaagtgctc ctgtttttta gagaaactaa
4741 atcttgctgt tgaacaatta ttgtgttctt ttcatggaac ataagtagga tgttaacatt
4801 tccagggtgg gaagggtaat cctaaatcat ttcccaatct attctaatta ccttaaatct
4861 aaaggggaaa aaaaaaatca caaacaggac tgggtagttt tttatcctaa gtatattttt
4921 tcctgttctt tttacttggt tttattgctg tatttatagc caatctatac atcatgggta
4981 aacttaaccc agaactataa aatgtagttg tttcagtccc cttcaggcct cctgaatggg
5041 caagtgcagt gaaacaggtg cttcctgctc ctgggttttc tctccatgat gttatgccca
5101 attggaaata tgctgtcagt ttgtgcacca tatggtgacc acgcctgtgc tcagtttggc
5161 agctatagaa ggaaatgctg tcccataaaa tgccatccct atttctaata taacactctt
5221 ttccaggaag catgcttaag catcttgtta cagagacata catccattat ggcttggcaa
5281 tctcttttat ttgttgactc tagctccctt caaagtcgag gaaagatctt tactcactta
5341 atgaggacat tccccatcac tgtctgtacc agttcacctt tattttacgt tttattcagt
5401 ctgtaaatta actggccctt tgcagtaact tgtacataaa gtgctagaaa atcatgttcc

5461 ttgtcctgag taagagttaa tcagagtaag tgcatttctg gagttgtttc tgtgatgtaa
5521 attatgatca ttatttaaga agtcaaatcc tgatcttgaa gtgcttttta tacagctctc
5581 taataattac aaatatccga aagtcatttc ttggaacaca agtggagtat gccaaatttt
5641 atatgaattt ttcagattat ctaagcttcc aggttttata attagaagat aatgagagaa
5701 ttaatggggt ttatatttac attatctctc aactatgtag cccatattac tcaccctatg
5761 agtgaatctg gaattgcttt tcatgtgaaa tcattgtggt ctatgagttt acaatactgc
5821 aaactgtgtt attttatcta aaccattgct taatgagtgt gtttttccat gaatgaatat
5881 accgtggttc atatgttagc atggcagcat tttcagatag ctttttgttt gttgggaagt
5941 tggggttttg gggggagggg gagtattagt acgttgcatg gaatagccta ctttataatg
6001 atgggaatgc ttttttcttt gttttgggat ttttttttt gaagtgaaat ttaacttttt
6061 gtgccagtag tactattata cccatcttca gtgtcttact tgtactgtat caaattccat
6121 accctcattt aattcttaat aaaactgttc acttgtaaaa aaaaaaaaaa aaaaaaaaaa
6181 aaaaaaaaa

Sequence 6– Human mRNA sequence of VPARP

1 cgcccgccca gccccggggg cagggaaagc ctaaattacg gaattaccgc gagcaaggag
61 cgcggaatcg gggagcgtcc ggagctagct ggatcctcta ggcaggatgg tgatgggaat
121 ctttgcaaat tgtatcttct gtttgaaagt gaagtactta cctcagcagc agaagaaaaa
181 gctacaaact gacattaagg aaaatggcgg aaagttttcc ttttcgttaa atcctcagtg
241 cacacatata atcttagata atgctgatgt tctgagtcag taccaactga attctatcca
301 aaagaaccac gttcatattg caaacccaga ttttatatgg aaatctatca gagaaaagag
361 actcttggat gtaaagaatt atgatcctta taagcccctg gacatcacac cacctcctga
421 tcagaaggcg agcagttctg aagtgaaaac agaaggtcta tgcccggaca gtgccacaga
481 ggaggaagac actgtggaac tcactgagtt tggtatgcag aatgttgaaa ttcctcatct
541 tcctcaagat tttgaagttg caaaatataa caccttggag aaagtgggaa tggagggagg
601 ccaggaagct gtggtggtgg agcttcagtg ttcgcgggac tccagggact gtcctttcct
661 gatatcctca cacttcctcc tggatgatgg catggagact agaagacagt ttgctataaa
721 gaaaacctct gaagatgcaa gtgaatactt tgaaaattac attgaagaac tgaagaaaca
781 aggatttcta ctaagagaac atttcacacc tgaagcaacc caattagcat ctgaacaatt
841 gcaagcattg cttttggagg aagtcatgaa ttcaagcact ctgagccaag aggtgagcga
901 tttagtagag atgatttggg cagaggccct gggccacctg gaacacatgc ttctcaagcc
961 agtgaacagg attagcctca acgatgtgag caaggcagag gggattctcc ttctagtaaa
1021 ggcagcactg aaaaatggag aaacagcaga gcaattgcaa aagatgatga cagagtttta
1081 cagactgata cctcacaaag gcacaatgcc caaagaagtg aacctgggac tattggctaa
1141 gaaagcagac ctctgccagc taataagaga catggttaat gtctgtgaaa ctaatttgtc
1201 caaacccaac ccaccatccc tggccaaata ccgagctttg aggtgcaaaa ttgagcatgt
1261 tgaacagaat actgaagaat ttctcagggt tagaaaagag gttttgcaga atcatcacag
1321 taagagccca gtggatgtct tgcagatatt tagagttggc agagtgaatg aaaccacaga
1381 gtttttgagc aaacttggta atgtgaggcc cttgttgcat ggttctcctg tacaaaacat
1441 cgtgggaatc ttgtgtcgag ggttgctttt acccaaagta gtggaagatc gtggtgtgca
1501 aagaacagac gtcggaaacc ttggaagtgg gatttatttc agtgattcgc tcagtacaag
1561 tatcaagtac tcacacccgg gagagacaga tggcaccaga ctcctgctca tttgtgacgt
1621 agccctcgga aagtgtatgg acttacatga gaaggacttt cccttaactg aagcaccacc
1681 aggctacgac agtgtgcatg gagtttcaca aacagcctct gtcaccacag actttgagga
1741 tgatgaattt gttgtctata aaaccaatca ggttaaaatg aaatatatta ttaaattttc
1801 catgcctgga gatcagataa aggactttca tcctagtgat catactgaat tagaggaata
1861 cagacctgag ttttcaaatt tttcaaaggt tgaagattac cagttaccag atgccaaaac
1921 ttccagcagc accaaggccg gcctccagga tgcctctggg aacttggttc ctctggagga
1981 tgtccacatc aaagggagaa tcatagacac tgtagcccag gtcattgttt ttcagacata
2041 cacaaataaa agtcacgtgc ccattgaggc aaaatatatc tttcctttgg atgacaaggc
2101 cgctgtgtgt ggcttcgaag ccttcatcaa tgggaagcac atagttggag agattaaaga
2161 gaaggaagaa gcccagcaag agtacctaga agccgtgacc cagggccatg gcgcttacct
2221 gatgagtcag gatgctccgg acgtttttac tgtaagtgtt ggaaacttac cccctaaggc
2281 taaggttctt ataaaaatta cctacatcac agaactcagc atcctgggca ctgttggtgt
2341 ctttttcatg cccgccaccg tagcaccctg gcaacaggac aaggctttga atgaaaacct
2401 tcaggataca gtagagaaga tttgtataaa agaaatagga acaaagcaaa gcttctcttt
2461 gactatgtct attgagatgc cgtatgtgat tgaattcatt ttcagtgata cacatgaact
2521 gaaacaaaag cgcacagact gcaaagctgt cattagcacc atggaaggca gctccttaga

2581 cagcagtgga ttttctctcc acatcggttt gtctgctgcc tatctcccaa gaatgtggg
2641 tgaaaaacat ccagaaaaag aaagcgaggc ttgcatgctt gtctttcaac ccgatctcga
2701 tgtcgacctc cctgacctag ccagtgagag cgaagtgatt atttgtcttg actgctccag
2761 ttccatggag ggtgtgacat tcttgcaagc caagcaaatc accttgcatg cgctgtcctt
2821 ggtgggtgag aagcagaaag taaatattat ccagttcggc acaggttaca aggagctatt
2881 ttcgtatcct aagcatatca caagcaatac cacggcagca gagttcatca tgtctgccac
2941 acctaccatg gggaacacag acttctggaa aacactccga tatcttagct tattgtaccc
3001 tgctcgaggg tcacggaaca tcctcctggt gtctgatggg cacctccagg atgagagcct
3061 gacattacag ctcgtgaaga ggagccgccc gcacaccagg ttattcgcct gcggtatcgg
3121 ttctacagca aatcgtcacg tcttaaggat tttgtcccag tgtggtgccg gagtatttga
3181 atattttaat gcaaaatcca agcatagttg gagaaaacag atagaagacc aaatgaccag
3241 gctatgttct ccgagttgcc actctgtctc cgtcaaatgg cagcaactca atccagatgc
3301 gcccgaggcc ctgcaggccc cagcccaggt gccatccttg tttcgcaatg atcgactcct
3361 tgtctatgga ttcattcctc actgcacaca agcaactctg tgtgcactaa ttcaagagaa
3421 agaattttgt acaatggtgt cgactactga gcttcagaag acaactggaa ctatgatcca
3481 caagctggca gcccgagctc taatcagaga ttatgaagat ggcattcttc acgaaaatga
3541 aaccagtcat gagatgaaaa aacaaacctt gaaatctctg attattaaac tcagtaaaga
3601 aaactctctc ataacacaat ttacaagctt tgtggcagtt gagaaaaggg atgagaatga
3661 gtcgcctttt cctgatattc caaaagtttc tgaacttatt gccaaagaag atgtagactt
3721 cctgccctac atgagctggc agggggagcc ccaagaagcc gtcaggaacc agtctctttt
3781 agcatcctct gagtggccag aattacgttt atccaaacga aaacatagga aaattccatt
3841 ttccaaaaga aaaatggaat tatctcagcc agaagtttct gaagattttg aagaggatgg
3901 cttaggtgta ctaccagctt tcacatcaaa tttggaacgt ggaggtgtgg aaaagctatt
3961 ggatttaagt tggacagagt catgtaaacc aacagcaact gaaccactat ttaagaaagt
4021 cagtccatgg gaaacatcta cttctagctt ttttcctatt ttggctccgg ccgttggttc
4081 ctatcttacc ccgactaccc gcgctcacag tcctgcttcc ttgtcttttg cctcatatcg
4141 tcaggtagct agtttcggtt cagctgctcc tcccagacag tttgatgcat ctcaattcag
4201 ccaaggccct gtgcctggca cttgtgctga ctggatccca cagtcggcgt cttgtcccac
4261 aggacctccc cagaacccac cttctgcacc ctattgtggc attgtttttt cagggagctc
4321 attaagctct gcacagtctg ctccactgca acatcctgga ggctttacta ccaggccttc
4381 tgctggcacc ttccctgagc tggattctcc ccagcttcat ttctctcttc ctacagaccc
4441 tgatcccatc agaggttttg ggtcttatca tccctctgct tactctcctt ttcattttca
4501 accttccgca gcctctttga ctgccaacct taggctgcca atggcctctg ctttacctga
4561 ggctctttgc agtcagtccc ggactacccc agtagatctc tgtcttctag aagaatcagt
4621 aggcagtctc gaaggaagtc gatgtcctgt ctttgctttt caaagttctg acacagaaag
4681 tgatgagcta tcagaagtac ttcaagacag ctgcttttta caaataaagt gtgatacaaa
4741 agatgacagt atcccgtgct ttctggaatt aaaagaagag gatgaaatag tgtgcacaca
4801 acactggcag gatgctgtgc cttggacaga actcctcagt ctacagacag aggatggctt
4861 ctggaaactt acaccagaac tgggacttat attaaatctt aatacaaatg gtttgcacag
4921 ctttcttaaa caaaaaggca ttcaatctct aggtgtaaaa ggaagagaat gtctcctgga
4981 cctaattgcc acaatgctgg tactacagtt tattcgcacc aggttggaaa aagagggaat
5041 agtgttcaaa tcactgatga aaatggatga cccttctatt tccaggaata ttccctgggc
5101 ttttgaggca ataaagcaag caagtgaatg ggtaagaaga actgaaggac agtacccatc
5161 tatctgccca cggcttgaac tggggaacga ctgggactct gccaccaagc agttgctggg
5221 actccagccc ataagcactg tgtcccctct tcatagagtc ctccattaca gtcaaggcta
5281 agtcaaatga aactgaattt taaacttttt gcatgcttct atgtagaaaa taatcaaatg
5341 ataatagata attataatga aacttcatta aggtttcatt cagtgtagca attactgtct

5401 ttaaaaatta agtggaagaa gaattacttt aatcaactaa caagcaataa taaaatgaaa
5461 cttaaaataa aaaaaaaaaa aaaaaaaaaa