

# Poly(ADP-Ribose) Polymerase-1 (PARP-1) Inhibitors in Cancer Chemotherapy

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**Abstract:** Poly(ADP-ribose) polymerases (PARPs) are defined as a family of cell signaling enzymes present in eukaryotes, which are involved in poly(ADP-ribosylation) of DNA-binding proteins. The best studied of these enzymes (PARP-1) is involved in the cellular response to DNA damage so that in the event of irreparable DNA damage overactivation of PARP-1 leads to necrotic cell death. Inhibitors of PARP-1 activity in combination with DNA-binding antitumor drugs may constitute a suitable strategy in cancer chemotherapy. When DNA is moderately damaged, PARP-1 participates in the DNA repair process and the cell survives. However, in the case of extensive DNA damage PARP-1 overactivation induces a decrease of NAD<sup>+</sup> and ATP levels leading to cell dysfunction or even to necrotic cell death. So, due to PARP-1 involvement in cell death, pharmacological inhibition of PARP-1 activity by PARP-1 inhibitors may constitute a suitable target to enhance the activity of antitumor drugs through inhibition of necrosis and activation of apoptosis. PARP-1 inhibitors such as 3-aminobenzamide, 1,5-dihydroxyisoquinolinone and the recently patented tricyclic benzimidazoles have shown potent inhibitory effects of PARP-1 activity in tumor cells. The present review gives an update of the state-of-the-art of inhibition of PARP-1 activity as adjuvant therapy in cancer treatment.

**Keywords:** PARP-1, inhibitors, cancer, chemotherapy.

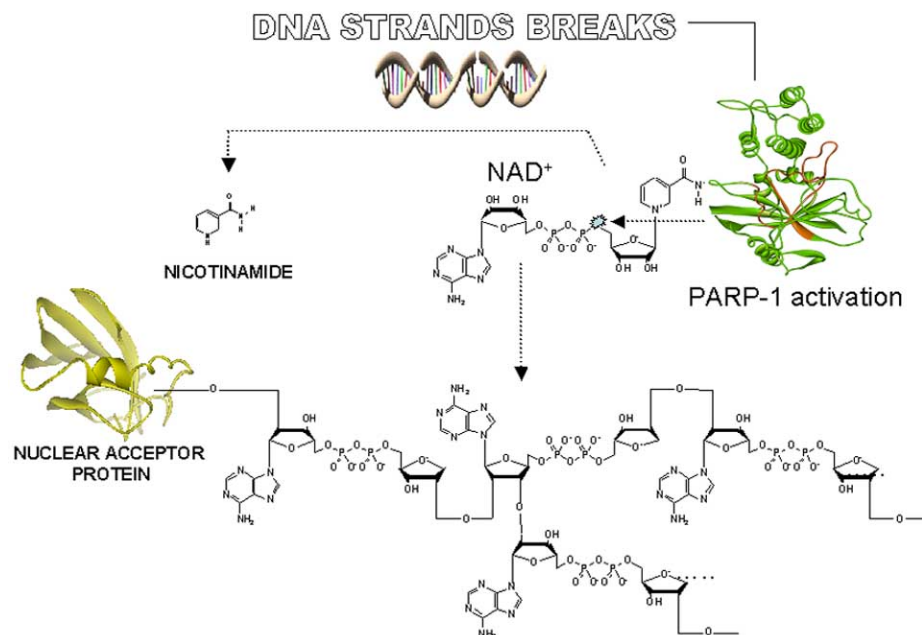
## 1. INTRODUCTION

Poly(ADP-ribose) polymerases (PARPs enzymes), also known as poly(ADP-ribose) synthetases and poly(ADP-ribose) transferases, constitute a family of cell signalling enzymes (e.g. PARPs; Vault PARP and Tankyrases) present in eukaryotes, which catalyze poly(ADP-ribosylation) of DNA-binding proteins [1-3]. These enzymes have emerged as critical regulatory components of the immediate cellular response to DNA damage. In particular, PARP-1 forms together with DNA ligase III, XRCC protein and DNA polymerase the DNA base scission repair multiprotein complex (BER) [4-6]. In response to DNA damage induced by ionizing radiation, oxidative stress and DNA-binding antitumor drugs, PARPs enzymes add ADP-ribose units to carboxylate groups of aspartic and glutamic residues of target proteins [2, 3]. This poly (ADP ribosylation) activity of PARPs is a post-translational modification that triggers the inactivation of the acceptor protein through the attachment of a complex branched polymer of ADP-ribose units [7].

Poly(ADP-ribose) polymerase 1 (PARP-1), is the first characterized and the best known member of the PARP family. PARP-1, encoded by the ADPRT (ADP-ribosyl transferase) gene, is an abundant and highly conserved chromatin bound enzyme which binds to nicked DNA as a

homodimer (MW= 2 x 113 kDa) and mediates protection against DNA damage. As shown in Fig. (1), upon binding to DNA breaks, activated PARP-1 cleaves NAD<sup>+</sup> into nicotinamide and ADP-ribose moieties (ADPR) and polymerizes the latter through surface accessible glutamate residues onto nuclear acceptor proteins, and PARP-1 itself [8]. Poly(ADP-ribose) polymers have a short half life because of their rapid hydrolysis by the enzyme poly(ADP-ribose) glycohydrolase (PARG). When DNA is mildly damaged, PARP-1 is activated and participates in the DNA repair process so that the cell survives. However, in the case of extensive DNA damage, PARP-1 is overactivated and induces a depletion of cellular NAD<sup>+</sup> and ATP levels leading to cell dysfunction or even to necrotic cell death [9]. So, overactivation of PARP-1 has been involved in the pathogenesis of several diseases including stroke, myocardial infarction, diabetes, shock, neurodegenerative disorder, allergy and several other inflammatory processes [10]. Due to the dual response of PARP-1 to DNA damage and its involvement in cell death, pharmacological modulation of PARP-1 activity may constitute a useful tool to increase the activity of DNA-binding antitumor drugs. In fact, various adjuvant strategies directed to modulate PARP-1 activity including the use of PARP-1 inhibitors [11] or the use of ATP-depleting agents [9] have been reported in recent years. In addition, PARP-1 inhibitors may be also useful to restore cellular functions in several pathophysiological states and diseases. This review gives an update of the use of PARP-1 inhibitors in cancer chemotherapy including recent important patents and also tries to shed light on further developments in this research area.

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**Fig. (1).** Poly (ADP-ribosylating) function of poly(ADP-ribose) polymerase-1. PARP-1 is activated by DNA strand breaks induced by several events including DNA repair, replication, recombination, oxidative stress and binding of drugs to DNA. Subsequently, PARP-1 catalyzes the cleavage of  $\text{NAD}^+$  into nicotinamide and ADP-ribose moieties and then uses the latter to synthesize ADP-ribose covalently bound to amino acid residues of acceptor proteins ('initiation reaction'). ADP-ribose may be also bound to other ADP-ribosyl moieties already transfer to a protein ('elongation reaction').

## 2. STRUCTURES OF PARP-1

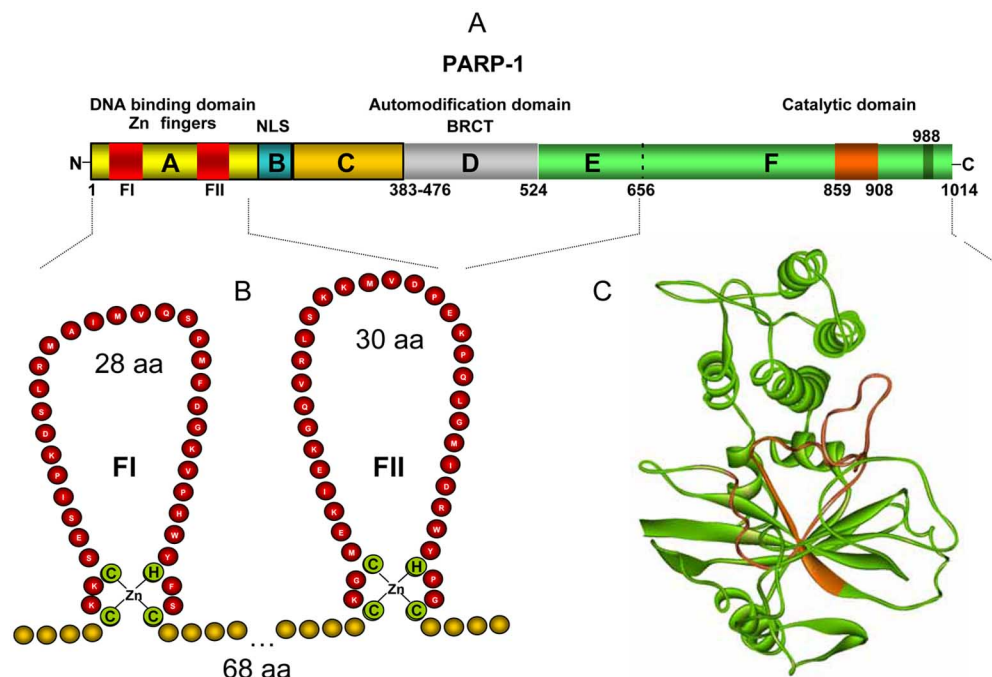
PARP-1 binds to nicked DNA as a homodimeric protein of MW= 2×113 kDa. PARP-1 binding to DNA leads to PARP-1 activation. The polypeptide possesses a highly conserved organization consisting of three main domains Fig. (2A). The N-terminal DNA-binding domain (DBD; MW= 46 kDa), which contains two zinc finger motifs F1 and F2 Fig. (2B), the automodification domain (AMD; MW= 22 kDa); and the C-terminal region (catPARP, MW= 54 kDa) accommodating the catalytic center Fig. (2C) [12-14].

The DBD domain acts as a DNA nick sensor, which contains the two zinc fingers and a nuclear localization signal (NLS) included in the caspase-cleavage site (DEVVD). Within the zinc fingers, the Zn(II) coordinates to cysteine and histidine residues Fig. (2B). It is interesting to note that human PARP-1 zinc fingers are not equally required for the recognition of DNA breaks and for the activation of the enzyme. Hence, damage on F1 Zn(II) finger results in complete loss of the enzymatic activity independently from the type of DNA breaks, whereas destruction of F2 Zn(II) finger only avoids PARP-1 activation in response to single strand DNA breaks. This asymmetric functionality of the zinc fingers has inspired the design of a specific class of PARP inhibitors [15].

The central portion of PARP-1 contains the automodification domain (AMD), which includes five conserved glutamate residues that function as poly(ADP-ribose) acceptors. AMD is considered a regulator segment because it contains regions for dimerization, which may modulate the interaction of PARP-1 with DNA and with proteins. This domain also has the terminus motif of breast cancer susceptibility protein C (BRCT) Fig. (2A), very common in many DNA repair and cell cycle proteins, which

acts in several protein-protein interactions [5] as well as 15 glutamic acid residues, which act as acceptor sites for the initiation reaction [16].

The C-terminal region (MW= 54 kDa), is the most strictly conserved part of the enzyme [12-14], and can be cut down to a C-terminal polypeptide (PARP-CF) without losing the basal catalytic activity [17]. Catalytic activities of this domain are related to the synthesis of the polymer and its binding to target proteins and consist of  $\text{NAD}^+$  hydrolysis, initiation, elongation, branching and termination of the (ADP-ribose) polymer [18], [15]. Interestingly, there is high homology in the primary structure of PARP-1 enzyme between different species, with the catalytic domain (the PARP catalytic fragment, PARP-CF) showing the highest degree of amino acid sequence similarity. PARP-CF is a monomer of 40 kDa that comprises residues 656-1014 Fig. (2A). This domain was expressed in insect cells, purified, crystallized and its tridimensional structure was elucidated in the presence and absence of PARP-1 inhibitor PD 128763 (8). The active site also known as "the PARP signature" or "cat PARP" is formed by a sequence of 50 amino acids and can be generally divided into two sites: the acceptor site (adenosine site) and the donor site (nicotinamide site). The acceptor site (N terminal) is occupied by the ADP moiety of the poly(ADP-ribose) chain, whereas the donor site is taken by  $\text{NAD}^+$  (C terminal). This donor site comprises three subsites: the nicotinamide-ribose binding site (NI site), the phosphate binding site (PH site) and the adenine-ribose binding site (AD site) [19]. The two sites at PARP-CF are functionally and structurally different. Thus, the acceptor site is formed by a purely  $\alpha$ -helical N-terminal domain from residue 662 to residue 784, and the donor site is a C-terminal domain from residue 785 to residue 1010. The N-terminal domain is formed by an  $\alpha$ -helical motif in which the



**Fig. (2).** (A). PARP-1 has three domain structure: the NH<sub>2</sub>-terminal DNA binding domain (DBD), the automodification domain (AMD), and the COOH-terminal catalytic domain. (B). The DBD domain contains two zinc fingers, which are responsible for DNA binding and protein-protein interactions. The DBD also contains a nuclear localization signal (NLS) in which the caspase-cleavage site is found. The AMD domain shows a breast cancer susceptibility protein C terminus (BRCT) motif, which is very common in repair and cell cycle proteins. PARP-1 binds to some proteins through the BRCT motif. (C) Ribbon diagram of the catalytic domain of PARP-1 (aminoacids 656 to 1014). The catalytic domain contains the active site of PARP-1, also called as the 'PARP signature', which is highly conserved in eukaryotes and consists of a 50-amino acids sequence (859 to 908).

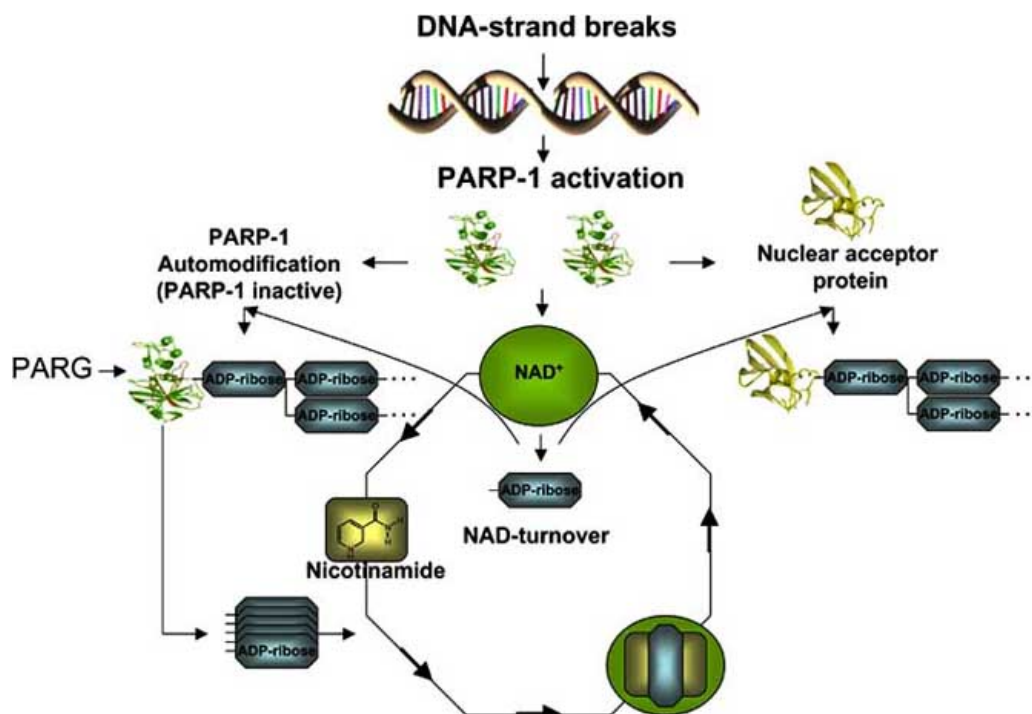
connections are 9 to 14 residues long. The C-terminal domain belongs to the ARTase family with its characteristic  $\beta$  fold, including the NAD<sup>+</sup> binding site [20]. The core of this region consists of a five-stranded antiparallel  $\beta$ -sheet and four-stranded mixed  $\beta$ -sheet. The two sheets are consecutive, connected via a single pair of hydrogen bonds between two strands that run at an angle of 90°, and surrounded by five  $\alpha$ -helices, three 3<sub>10</sub>-helices, and by a three- and a two-stranded  $\beta$ -sheet in a 37-residue stretch between two central  $\beta$ -strands [5]. The block of 50 amino acids (residues 859-908) of the C-terminal domain that are identical for all PARP-1 sequences of vertebrates is contained in a segment formed by a  $\beta$ -sheet, an  $\alpha$ -helix, a 3<sub>10</sub>-helix, a  $\beta$ -sheet and a  $\beta$ -helix, consecutively. Both His-862 on a  $\beta$ -strand as well as Glu-988 on another  $\beta$ -strand are involved in NAD<sup>+</sup>-binding or catalysis [21, 22]. It has been suggested that all ARTases have a similar ADP-ribosylating mechanism, due to their common catalytically competent glutamate (Glu-988 in PARP-1), and their analogous binding to the substrate. The donor NAD<sup>+</sup> is bound to the enzyme with the nicotinamide ribose in 3'-endo conformation. Then, Glu-988 forms hydrogen bonds to the acceptor nucleophile and to the donor NAD<sup>+</sup> polarizing both of them, so that the nucleophilicity of the acceptor increases and, therefore, the oxocarbenium in the donor ribose is stabilized. The internal strain of the attached substrate helps to lead the reaction because it is relieved after breaking the  $\beta$ -glycosidic bond. Most PARP-1 inhibitors bind to the catalytic site of the enzyme, particularly to the NAD<sup>+</sup> binding site, in a competitive way. As PARP-1 modifies a variety of proteins,

no specific protein docking surface appears to be required. In contrast to PARP, the other ARTases have high acceptor specificities [20]. Since the initial discovery of PARP-1, other PARP enzymes have been found (PARP-2; PARP-3; Vault PARP -vPARP-; Tankyrases -TANK1 and -2), which show differences in their domain structures [14]. These lately described forms of PARP show high homologies at their PARP-1 C-terminal catalytic domain. Therefore, it is very important to determine the selectivity of PARP inhibitors in relation to all these PARP enzymes. For instance, it has been recently reported that a new corn-shaped hydrophobic subsite emerges from the active site of PARP-1 when complexed with the inhibitor FR 257517 ("see below as (17)"), [19]. Under undamaging conditions, catalytically inactive PARP-1 resides in the nucleoplasm [23]. PARP-1 and other poly(ADP-ribosyltransferases) are also found within mitochondria [24]. A plausible explanation for the localization of PARP-1 in mitochondria and in the nucleus may be the direct role of mitochondrial poly(ADP-ribosylation) in cell death in connection with loss of mitochondrial transmembrane potential ( $\psi_m$ ), mitochondrial dysfunction, and release of apoptosis-inducing factors (AIFs). These data do not refute previous hypotheses suggesting that overactivation of nuclear PARP-1 consumes total cellular ATP stores contributing to mitochondrial dysfunction and cell death. However, these data do represent a paradigm shift, where poly(ADP-ribosylation) compartmentalized within the mitochondria contributes to AIFs release and cell death in the event of cellular energy failure [24].

### 3. PARP-1 ACTIVATION AND CATABOLISM

PARP-1 is activated in response to transient and localized DNA strand breaks caused by various biological processes like DNA repair, replication, recombination and gene rearrangement, as well as oxidative stress and DNA-binding drugs [25]. As shown in Fig. (3), the active PARP-1 enzyme rapidly transfers ADP-ribose moieties from  $\text{NAD}^+$  to itself (automodification) and to a limited number of nuclear DNA-binding proteins (heteromodification) such as histones, adaptor factors and DNA repair effectors [1, 5]. PARP-1 automodification reduces the affinity of the enzyme for damaged DNA, helping its dissociation and allowing repair enzymes access to the strand break [8]. In fact, the presence of these polymers with high negative charge in the neighbourhood of the DNA nick may cause electrostatic repulsion of PARP-1 from DNA, facilitating the recruitment of the base excision repair complex [19]. Once activated, the enzyme catalyses three reactions: (i) hydrolysis of  $\text{NAD}^+$  into ADP-ribose and nicotinamide,  $\text{NAD}^+ + \text{X} \rightarrow \text{X}-1\text{-ribose-5'-ADP} + \text{nicotinamide}$ , (ii) formation of an ester bond between the acceptor X glutamate in the target protein and ADP-ribose (initiation), and (iii) formation of a glycosidic bond between ADP-ribose molecules (chain elongation and branching). The result is the formation of covalent protein-bound linear and branched homo-ADP-ribose polymers [25]. The branching/elongation ratio is about 2% [26], and the size of the branched polymer varies from a few to up to 200 ADP-ribose units. If DNA damage is repaired, then the cell survives. However, in the event of unreparable DNA damage, PARP-1 is overactivated

producing depletion of  $\text{NAD}^+$  and ATP stores, and then the cell may die through necrosis. As the main mode of  $\text{NAD}^+$  catabolism occurs via cleavage by PARP-1, it appears that the cellular levels of the enzyme are critical in the maintenance of  $\text{NAD}^+$  stocks [27]. All cellular NADH,  $\text{NADP}^+$ , and  $\text{NADPH}$  are synthesised from  $\text{NAD}^+$ , which is also required for different metabolic pathways within the cell. A severe reduction of  $\text{NAD}^+$  leads to depletion of ATP, because its biosynthesis involves the hydrolysis of 2 M of ATP per 1 M of  $\text{NAD}^+$  generated. Inhibition of PARP-1 activity after DNA damage is necessary to avoid the reduction of  $\text{NAD}^+$  and ATP stores [16]. Two enzymes, poly(ADP-ribose) glycohydrolase (PARG) and ADP-ribosyl lyase, are involved in the catabolism of PARP-1. Poly(ADP-ribose) polymers are degraded by PARG Fig. (3), which cleaves ribose-ribose bonds of both linear and branched portions of poly(ADP-ribose). The proximal ADP-ribose monomer portion is removed by the lyase [28]. PARP-1 activation leads in last term to autoinhibition through poly(ADP-ribosylation). Hence, to reactivate PARP, removal of inhibitory poly(ADP-ribose) units by PARG from the automodification domain is required Fig. (3) [28]. Consequently, two forms of PARP can be found: (i) the inactive monomeric and poly(ADP-ribosylated) form, and (ii) the active homodimer or heterodimer formed after the degradation of the poly(ADP-ribose) polymer by PARG. Poly(ADP-ribose) glycohydrolase is a 110 kDa protein containing leucine zipper-like dimerization domains that allows the formation of stable dimers [29, 30]. PARG shares no homology with other proteins, and contains both a nuclear localization signal (NLS) and a nuclear export signal (NES).



**Fig. (3).** Poly(ADP-ribose) glycohydrolase (PARG) reactivates PARP-1 and allows  $\text{NAD}^+$  turnover. DNA nicks induce PARP-1 activation and subsequent cleavage of  $\text{NAD}^+$  into nicotinamide and ADP-ribose. ADP-ribose units are polymerized onto nuclear acceptor proteins (heteromodification). PARP-1 activation also leads to automodification of PARP-1 (homomodification) by ADP-ribosylation, which leads to PARP inhibition. By removing poly(ADP-ribose) from PARP-1, PARG reactivates PARP-1 and allows  $\text{NAD}^+$  turnover.

The presence of such signals provides support for the idea that PARG may shuttle between the nucleus and the cytoplasm [30]. This location of PARG may indicate regulatory functions and may also allow the enzyme to participate in the digestion of poly(ADP-ribose) synthesized by cytoplasmic PARP enzymes [14]. Poly(ADP-ribose) glycohydrolase is able to hydrolyse both terminal ADP-ribose units from poly(ADP-ribose) polymers *via* exoglycosidic activity and to remove larger oligo(ADP-ribose) fragments through endoglycosidic cleavage [28, 31]. The  $K_m$  value of PARG is much lower for larger (ADP-ribose)<sub>n</sub> polymers than for smaller ones [32]. So, it is likely that the enzyme removes and catabolizes bigger poly(ADP-ribose) fragments first. PARG then switches to its exoglycosidic mode and removes ADP-ribose units one by one.

#### 4. SUBSTRATES OF PARP-1

Poly(ADP-ribose) polymerase is able to interact with several nuclear proteins that are generally involved in the control of DNA metabolism and conformation. Their function result dramatically affected by the covalent attachment of the highly negative charged ADP-ribose polymer. As an example, ADP-ribosylation of histones H1 and H2B by PARP-1 leads to decondensation of chromosomes. In addition, auto-poly(ADP-ribosylation) triggers its dissociation from the DNA breaks due to the negative charges, and favours the access of the base excision repair complex (BER) to DNA [12, 33-36]. Target proteins of PARP-1 include histones, topoisomerases I and II, DNA and RNA polymerases, DNA ligases, p53, and Ca<sup>2+</sup>/Mg<sup>2+</sup>-dependent endonucleases [37]. Furthermore, PARP may interact with the polymerase  $\delta$ -primase complex and bind to a protein of the base excision repair pathway, which in turn interacts with DNA ligase II and DNA polymerase  $\beta$ . So, poly(ADP-ribosylation) modulates the activity of all these substrates, influencing DNA replication [38], transcription [39], and repair [6, 36, 40-43].

#### 5. PARP-1 INVOLVEMENT IN CELL DEATH

Because poly(ADP-ribose) polymerase-1 was one of the first identified substrates of caspases, the enzymes that execute apoptosis [44], cell biologists used in the 1990's the expression "death substrate" to name PARP-1 [45]. This finding led to suggest a role for PARP-1 in the regulation of apoptosis. However, more recent data rather suggest the involvement of PARP-1 in necrosis. Therefore, it is important to address the role that PARP-1 plays in these two main cell death pathways.

##### 5.1. Role of PARP-1 in Apoptotic Pathways

Apoptosis is a biochemically controlled pathway of cell demise, also called "programmed cell death" or "cell suicide". The apoptotic process requires ATP and is generally divided in three main phases (initiation, effector and execution) [46]. Caspases are cysteine proteases that digest cellular proteins in the last phase of apoptosis, in which the cell is dismantled through the formation of apoptotic bodies, and nuclear DNA is cleaved into oligonucleosomal fragments by an endonuclease. The most important substrates of caspases during the apoptotic process are pro-caspase-3 and pro-caspase-7, Bcl-2 family of

proteins (Bid, Bcl-X<sub>L</sub> and Bcl2), structural proteins (actin, cyokeratin and focal adhesion kinase), neural cell adhesion molecules (NCAMs) signal-transduction proteins (e.g., Ca<sup>2+</sup>/calmodulin-dependent protein kinase), and DNA-repair and cell cycle regulatory proteins (PARP-1, DNA polymerase  $\beta$ , cyclin D and p53 [47]. During the execution phase of apoptosis, the caspase-3,-6,-7 complex recognizes the DEVD motif in the nuclear localization signal (NLS) of PARP-1 [40] and cleaves the enzyme into two fragments p89 and p24 [48], separating the DNA binding domain (DBD) from the catalytic domain. This cleavage leads to the inactivation of PARP-1 because of the inhibition of homoassociation and DNA binding functions of intact PARP-1 by p89 and p24, respectively [49, 50]. It seems that the meaning of PARP-1 cleavage is to prevent the activation of the enzyme by fragmented DNA so that cellular energy is preserved for certain ATP-sensitive steps of apoptosis [14]. Thus, blockage of PARP-1 activation is crucial for the proper function of the apoptotic machinery.

##### 5.2. Role of PARP-1 in Necrotic Cell Death

It is important to consider that necrosis may not be simply regarded as an accidental type of cell death but rather as a more severe form of cell demise compared with apoptosis [51]. The most distinctive event of necrosis is the disintegration of the plasma membrane, in contrast with the compaction of apoptotic cells [52]. Furthermore, apoptotic cells are rapidly cleared from the tissues by macrophages, whereas in necrosis the pouring of cell content from necrotic cells into the neighbouring tissue may contribute to organ injury [53]. Nevertheless, despite the morphological and biochemical differences between apoptosis and necrosis, it has been proposed that both pathways are at two ends of a continuum of possible modes of cell death in which apoptosis and necrosis are switched by mild and by severe genotoxic stimuli, respectively [54, 55]. In addition, ATP appears to play a central role in the mode of cell death since severe ATP depletion, causative of necrosis, is brought about by the fall in mitochondrial permeability transition (MPT) induced by a cytotoxic stimulus. This effect is especially important in oxidatively injured cells or in cells treated with DNA-binding antineoplastic drugs such as cisplatin, doxorubicin and etoposide [51, 55-58]. Mitochondrial oxidative phosphorylation that generates ATP is inhibited by MPT failure, which results in ATP depletion that blocks caspase cleavage of PARP-1. Because overactivation of PARP-1 diminishes NAD<sup>+</sup> and ATP storages [59], it is likely that PARP may act as a molecular switch between apoptosis and necrosis.

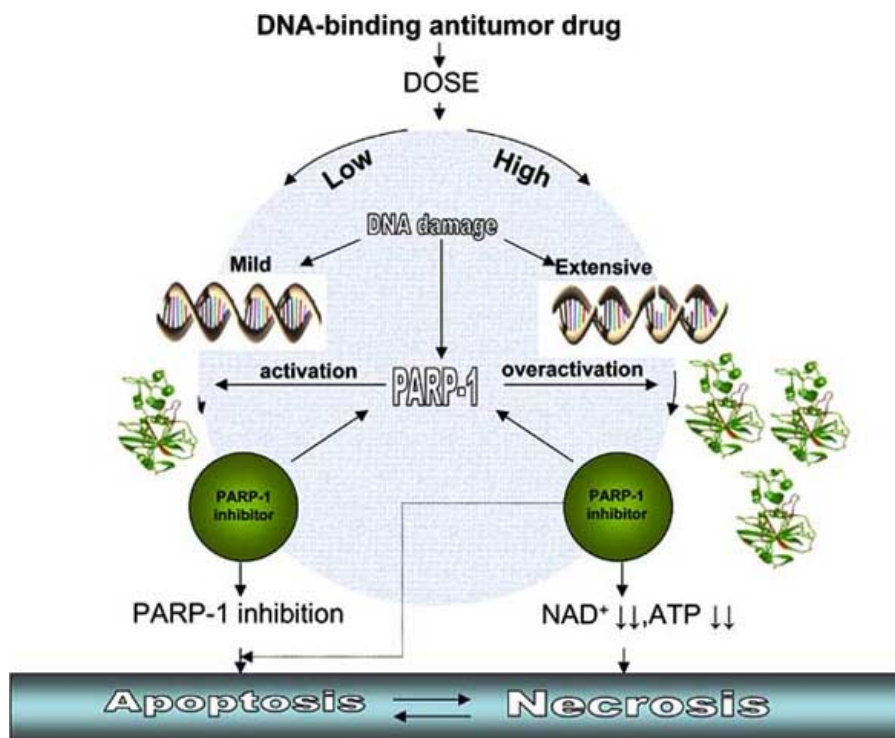
Several research groups have reported that inhibition of PARP-1 activity induces protection against necrotic cell death. However, PARP-1 inhibition does not protect cells from apoptosis [60-62]. Overactivation of PARP-1 has been implicated in the pathogenesis of several diseases, including stroke, myocardial infarction, diabetes, shock, neurodegenerative disorder, allergy, and several other inflammatory processes [10]. A role of PARP-1 overactivation in necrosis is consistent with the fact that the inhibition or absence of PARP-1 provides the most remarkable protection in disease models such as stroke, myocardial infarction, or mesenteric ischemia-reperfusion injury, which are characterized

predominantly by necrotic-type cell death [63]. When PARP-1 is moderately activated, cellular  $\text{NAD}^+$  content may decrease slightly causing cell dysfunction instead of cell death. So, an interesting working hypothesis, which may have therapeutic applications, is that pharmacological inhibition of PARP-1 may rescue dysfunctional cells and thereby can restore cell function by improving the energetic state of the cell.

## 6. PARP-1 INHIBITORS IN CANCER CHEMOTHERAPY

Drug resistance in tumor cells is a common drawback of cancer chemotherapy. Resistance is often a multifactorial process including decreased drug accumulation, intracellular drug detoxification, enhanced DNA repair/tolerance and failure of apoptotic pathways [57]. In addition, highly resistant tumor cells may bear genetic alterations of caspases or they may contain endogenous caspase inhibitors that avoid apoptotic cell death pathways [64]. On the other hand, cancer cells with low cellular ATP levels may be highly resistant to programmed cell death due to the high energetic requirements of apoptosis. So, in these particular tumor cell lines antitumor drugs could only induce cell demise by necrosis. PARP-1 activation in response to DNA breaks and its involvement in cell death, led to investigate the consequences of PARP inhibition on DNA-binding anticancer therapies [4]. These investigations revealed that PARP-1 inactivation may constitute a suitable way to enhance the activity of DNA-binding antitumor compounds,

particularly as PARP-1 function is not essential for cell survival in absence of extensive cell damage [65]. Recent observations indicate that cells may be protected from necrosis by inhibition or inactivation of PARPs (e.g., PARP-1 and PARP-5b) so that the necrotic process is amenable to pharmacological intervention [14]. As shown in Fig. (4), inhibition of PARP-1 in cells exposed to DNA-damaging drugs would decrease DNA repair and would induce apoptotic cell death, decreasing necrotic cell death and preventing the pathological side effects of necrosis. It is interesting to note that PARP inhibitors might be more effective against tumour cells than against normal cells. For instance, in low-grade malignant non-Hodgkin lymphoma cells and hepatocellular carcinomas, increased PARP-1 activity has been reported as compared with healthy lymphocytes or hepatocytes [66]. So, PARP-1 inhibition may increase the sensitivity of tumor cells to DNA-damaging antitumor drugs. In fact, it has been reported that PARP-1 inhibitors 3-aminobenzamide (3-AB, see below) (2) or NU 1025 (see below) (14) increase apoptosis and reduce necrosis induced by the DNA minor groove binder  $\text{MeOSO}_2(\text{CH}_2)_2$ -lexitropsin (Me-Lex) [67]. Moreover, some studies have reported that PARP inhibitors such as 6-(5H)-phenanthridinones (see below) (14) [68] or 4-iodo-3-nitrobenzamide (see below) (20) [69] may have a direct cytotoxic effect on tumor cells by themselves [70, 71]. Nevertheless, most studies have focused on the synergistic cytotoxic effect of PARP-1 inhibitors in combination with gamma-radiation or DNA-binding drugs including alkylating



**Fig. (4).** Pharmacological inhibition of PARP-1 activity in cancer chemotherapy. A low or moderate dose of DNA-binding drug may induce mild DNA damage and subsequent activation of PARP-1. In this case, the use of PARP-1 inhibitors in combination with DNA-binding antitumor drugs may result in efficient block of DNA repair and subsequent apoptotic cell death. A high dose of drug may induce very intense DNA damage and subsequent overactivation of PARP-1 so that depletion of  $\text{NAD}^+$  and ATP stores to lethal-inducing levels may lead to necrotic tumour cell death. In this scenario, the use of PARP-1 inhibitors in combination with DNA-binding drugs may block PARP-1 overactivation and, then, tumor cell death is switched from necrosis to apoptosis.

agents and topoisomerase inhibitors [72, 73]. Thus, taking into account all the above-mentioned considerations, it comes out that the use of PARP-1 inhibitors in combination with DNA-damaging drugs may result in the enhancement of the apoptotic activity of these drugs against resistant cancer cells.

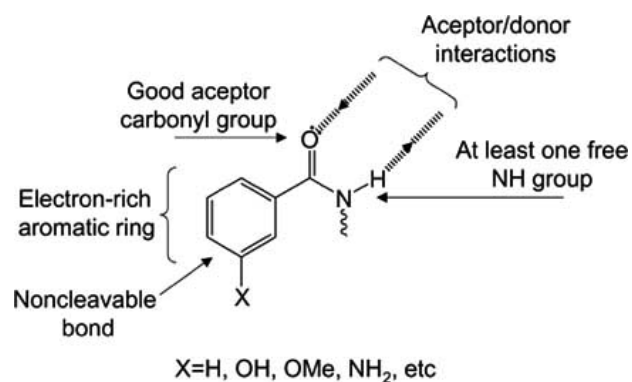
Inhibitors of PARP-1 are very useful tools to study the biological functions of the PARP-1 enzyme and, moreover, they may have applications as therapeutic agents against diseases different from cancer [11]. PARP-1 inhibitors are not cytotoxic by themselves at concentrations necessary to achieve PARP-1 inhibition. However, at higher concentrations, 3-AB (see below) (2) has been shown to inhibit “*de novo*” synthesis of purines, and to stop cellular division in cultures [16, 74]. Most PARP-1 inhibitors have been designed to imitate the substrate-enzyme interactions of NAD<sup>+</sup> with PARP-1, mainly by the knowledge of the crystal structure of chicken catPARP [20, 75]. Because of their structural resemblance to the substrate, these compounds are able to act as competitive inhibitors, blocking NAD<sup>+</sup> binding to the catalytic domain of the enzyme. A common structural characteristic of PARP-1 inhibitors is either the presence of a carboxamide or an imide group built in a polyaromatic heterocyclic skeleton or a carbamoyl group attached to an aromatic ring [14]. The oxygen atom from the carbonyl group seems to function as a hydrogen bond acceptor, and the hydrogen atom from the amide or imide groups acts as a hydrogen bond donor in the hydrogen-bond interaction with PARP-1 [14]. Analyses of the attachment of some inhibitors to the active site of PARP-1 through X-ray crystallographic studies and establishment of structure-activity relationships (SAR), have shown that the potency of PARP-1 inhibition depends on the orientation of the carboxamide oxygen to form three hydrogen bonds with Ser-904 and Gly-863 in the PARP-1 binding site [4]. One of the studied inhibitors is the NAD<sup>+</sup> analogue carbanicotinamide adenine dinucleotide (carba-NAD), in which a methylene group replaces the ring oxygen of the nicotinamide riboside of NAD<sup>+</sup>. This structural difference makes the analogue resistant to nicotinamide ribotide cleavage and consequently, PARP-1 activity is inhibited [76]. Another inhibitor, the nicotinamide analogue PD 128763 (5-methyl dihydroisoquinolinone) (see below) (8) could also be cocrystallized with chicken PARP-CF fragment, and structurally analyzed. It is generally assumed that PARP-1 inhibitors bind to PARP-CF in the same way as the nicotinamide moiety of NAD<sup>+</sup>. However, PD 128763 inhibitor attaches to PARP-CF much better than free nicotinamide. It is thought that the fixed carbamoyl function of PD 128763 accounts for this difference. Consequently, the orientation of the carbamoyl group within the molecule appears to be an important parameter for the design of PARP inhibitors [5].

Second-generation nicotinamide based molecules were designed with the aim of fastening the carboxamide group in its desired *cis*-conformation for the interaction with the NAD<sup>+</sup> binding site of PARP-1. A noteworthy progress was the synthesis of the dihydroisoquinolinones, where the carboxamide group is constrained within a ring. Other constricted carboxamide-like examples include imides, quinazolinones such as the quinazolin-4-[3H]-one NU 1025 (see below) (14), and the benzimidazole-4-carboxamides

NU1064 and NU 1085 (see below) (9). These compounds were identified as more effective PARP-1 inhibitors than their related predecessors, since they were capable of adopting the required *cis*- conformation. Particularly, NU 1025 and NU 1085 were successful enhancers of the cytotoxicity of antitumoral agents in tumor cells *in vitro* [4, 75].

Further improvement of PARP-1 inhibitors was achieved through a rational drug design approach based on crystallographic data. A number of benzimidazoles (see below) (9) and tricyclic lactam indoles (see below) (10) were developed, in which the carboxamide group was held in the favourable orientation by an intramolecular hydrogen bond, as in benzimidazole inhibitors, or through its incorporation into a seven-membered ring, as in tricyclic lactam indole inhibitors (see below) (10). The proper orientation of the carboxamide group of the inhibitor allows the formation of hydrogen bonds between its lactam ring and Gly863 and Ser904 of catPARP.

From all the studies mentioned above, it can be concluded that there are at least three structural features see Fig. (5) that must be taken into account for the design of novel potent PARP-1 inhibitors:



**Fig. (5).** Structural requirements for potent PARP-1 inhibitors. There are at least three structural features for the design of novel potent PARP-1 inhibitors: (i) an electron-rich aromatic ring system, including a carboxamide group with at least one hydrogen on the amide nitrogen, (ii) a noncleavable bond in the 3 position relative to the carboxamide group, and (iii) the carboxamide moiety, which is normally free to rotate, should be restricted to adopt the *cis*- (or *anti*-) configuration required for hydrogen bonding with critical residues in the NAD<sup>+</sup>-binding site.

- (i) More efficient inhibition is achieved by compounds that have an electron-rich aromatic ring system, including a carboxamide group with at least one hydrogen on the amide nitrogen.
- (ii) The compound should also have a noncleavable bond in the 3 position relative to the carboxamide group [11, 78].
- (iii) The carboxamide moiety, which is normally free to rotate, should be restricted to adopt the *cis*- (or *anti*-) configuration required for hydrogen bonding with

critical residues in NAD<sup>+</sup>-binding site. This assumption was confirmed by molecular orbital calculations on the nicotinamide moiety of NAD<sup>+</sup>, which revealed that the conformation with the carbonyl positioned *anti*- to the 2, 3 bond is favoured over the *syn*- conformation.

## 6.1. Chemical Classes of PARP Inhibitors

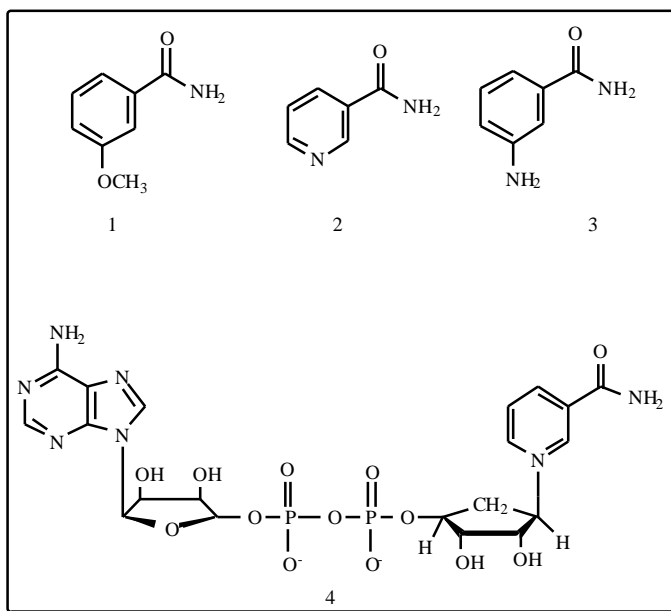
### 6.1.1. Nicotinamide and 3-AB

Nicotinamide (**1**) and the structurally related 3-AB (**2**) were identified as competitive PARP inhibitors in the 1970's. Other substituted benzamides such as 3-methoxybenzamide (**3**) and carba-NAD<sup>+</sup> (**4**) also produced effective inhibition of PARP activity, mainly by interfering with the binding of the natural substrate (NAD<sup>+</sup>) to the enzyme (see below the structures of these PARP-1 inhibitors). The analysis of the mechanism of action of these compounds showed that the carboxamide group was critical in their inhibitory activity. Furthermore, it was reported that benzamides were able to bind to DNA nicks, avoiding their recognition by PARP-1, and therefore preventing the activation of the enzyme [79].

Nicotinamide is a weak PARP-1 inhibitor (inhibitory concentration for 50% reduction of PARP-1 activity, IC<sub>50</sub> = 210 μM) and, moreover, it is not always an inhibitor but often an enhancer of ADP-ribosylation when administered to cells. At millimolar concentrations, nicotinamide interferes with NAD<sup>+</sup> synthesis and becomes a substrate of NAD<sup>+</sup> metabolic enzymes. It may also perturb *S*-adenosylmethionine metabolism at certain doses [15].

Benzamide and its derivatives are more effective than nicotinamide, particularly 3-methoxy and 3-AB (IC<sub>50</sub> = 22 μM for benzamide). However, they have limited solubility in water due to their hydrophobic structure so that the introduction of an additional nitrogen ring improved their solubility. Nevertheless, as it was discussed above, benzamide and its derivatives have several drawbacks as drug candidates. They lack the potency and specificity

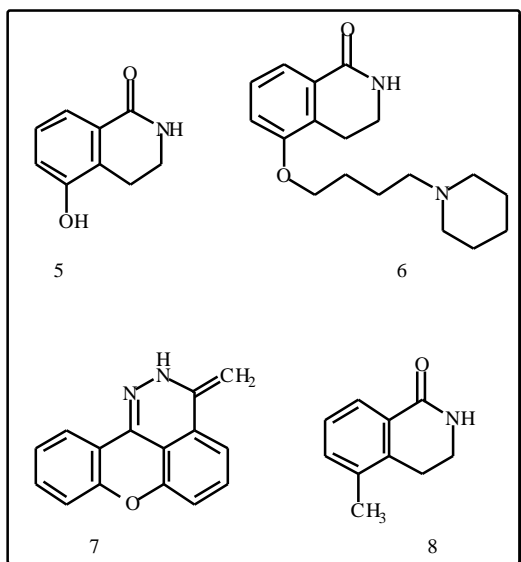
required to make them therapeutically useful, have limited intracellular accumulation and exert some non-specific actions, such as inhibition of mono and poly(ADP-ribosylation) reactions. Benzamide and 3-AB were reported to inhibit glucose metabolism and synthesis of DNA when administered up to 5 mM to lymphoid cells [80]. In addition, the doses of the compounds required for reaching an effective PARP-1 inhibition *in vivo* (up to 3-10 mM or several hundreds of mg/kg/injection), are too high for safe human administration, because at doses above 3 g/day, nicotinamide begins to exert diverse toxic effects [11]. Nevertheless, nicotinamide and 3-AB were used to study the physiological consequences of PARP-1 inhibition *in vivo*. These studies resulted in an improvement of structure-activity knowledge of PARP-1 inhibitors and stimulated further research about the specificity of benzamide inhibitors. The inhibitory activity of benzamide derivatives was quantified against PARP-1 *in vitro*, and the results indicated selective inhibition of PARP-1 by benzamides at μM concentrations. Particularly, benzamide showed 3-4 fold more inhibitory potency against PARP-1 than against other related enzymes [81]. Nicotinamide and benzamide were reported to protect against reperfusion injury in several organs including the brain, and to provide protection in experimental models of shock and inflammation, including arthritis [11]. Furthermore, in early clinical trials, nicotinamide seemed to be able to defend patients against the progression of diabetes Type I, although its efficacy was only slight in the decisive Phase III clinical trials. So, it was not clear that the employed dose was capable of providing sufficient inhibition of PARP-1. Possibly, higher doses of nicotinamide, up to 3 g/day, should be required to reach proper PARP-1 inhibition, which, in turn, would cause several toxic effects in patients. Therefore, nicotinamide and the benzamide derivatives show numerous pitfalls that make them inadequate for therapeutic use so that they are only used as experimental tools to investigate the biological role of PARP-1 activation in cellular processes.



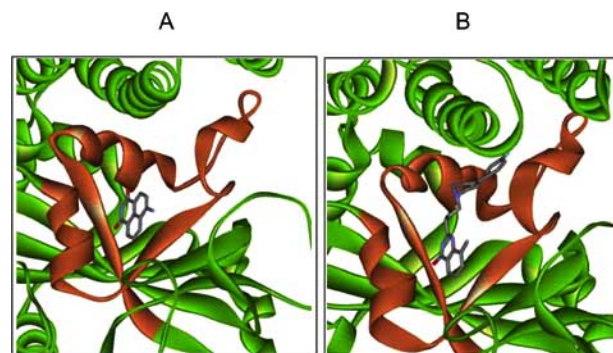
### 6.1.2. Dihydroisoquinolinones and Isoquinolinones

The promising results obtained in PARP-1 inhibition with nicotinamide and benzamides and the rising knowledge about the involvement of PARP-1 in several pathologies and key cellular processes, led to the development of some structurally improved compounds which might avoid the numerous drawbacks of first generation inhibitors.

These new inhibitors were based on the structure of 3-aminobenzamide, but included the carboxamide group attached within a ring structure known as a lactam. Thus, Suto *et al.* synthesized a series of rigid analogues of 3-AB, the dihydroisoquinolinones (see below) (**5-8**) [82]. Experimental data showed noteworthy increases in PARP-1 inhibition by dihydroisoquinolinones and isoquinolinones compared to the effect of aminobenzamide. For instance, 5-methyl-dihydroisoquinolinone PD 128763 (**8**) (see its interaction with catPARP in Fig. (6A) was able to reduce DNA repair and enhanced cell death when used with various anticancer compounds [11, 82].



Dihydroisoquinolinones and isoquinolinones share the structural feature of the lactam within fused cyclic systems, which fastens the carboxamide group in the desired configuration. It is noteworthy that the basic structure of dihydroisoquinolinones and isoquinolinones has been adopted for subsequent development of more potent polycyclic inhibitors. Thus, Guilford have synthesized a series of powerful tetracyclic heterocyclic compounds with  $IC_{50}$  values between 0.046 to 5  $\mu$ M. GPI-6150 (see above) (**7**) [83] is the prototype of the series, and was described as a more potent inhibitor than DPQ (**6**) [84], which shows an  $IC_{50}$  value of 0.15  $\mu$ M. However, its inhibitory effect was unspecific because it was not able to discern between PARP-1 and PARP-2. The isoquinolinone core has also been evaluated in some tricyclic compounds such as 1,8-naphthalimide, and 2,3-dihydrobenzo[de]isoquinolin-1-one [85]. Thus, 1,8-naphthalimide, has been reported to protect cells against oxidative stress *in vitro*.

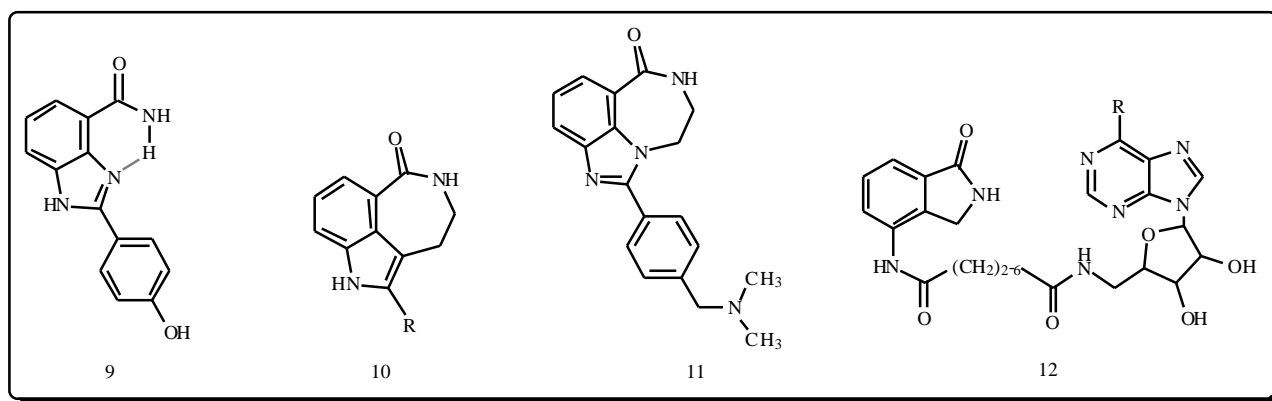


**Fig. (6).** Binding to the catalytic site of PARP-1 (ribbon diagram) of (A) 5-methyldihydroisoquinolinone (PD 128763 inhibitor, stick representation) and (B) 2-[3-[4-[4-fluorophenyl]-3,6-dihydro-1(2H)-pyridinyl]propyl]-8-methyl-4(3H)quinazolinone (FR 257517 inhibitor, sticks representation).

### 6.1.3. Benzimidazoles and Indoles

Based on the favourable results obtained with the improved dihydroisoquinolinones and isoquinolinones, several benzimidazole-4-carboxamides (see below) [86] and benzoxazole-4-carboxamides were designed at the University of Newcastle upon Tyne (**9, 10, 11, and 12**) (see below) [4].

Taking into account the structural requirements for potent PARP-1 inhibition, the carboxamide group was held in the favourable *cis*- orientation by an intramolecular hydrogen bond between the amide proton and the nitrogen within the cycle. Two amide protons are involved in the inhibitory activity of these compounds, one proton establishes the intramolecular hydrogen bond and the other acts as a hydrogen-bonding donor in the critical interaction with the enzyme. Simple benzimidazole-4-carboxamides resulted considerably more active than the classical inhibitor 3-AB. Furthermore, the inhibitory effect was significantly potentiated by the insertion of a phenyl substituent at the 2 position. X-ray diffraction data obtained from co-crystallisation of a 2-aryl-substituted benzimidazole-4-carboxamide with catPARP domain showed that the 2-aryl ring was accommodated into a large cavity of the  $NAD^+$  binding site. Hence, the inhibitor would tolerate the introduction of substituents within the 2-aryl ring without losing activity. Subsequently, various 2-aryl-1*H*-benzimidazole-4-carboxamides [87] containing structural modifications in the neighbourhood of the benzimidazol-4-carboxamide core were synthesised and assayed against recombinant human PARP-1 to investigate structure-activity relationships. These experiments indicated that the new 2-aryl-1*H*-benzimidazole-4-carboxamide derivatives which hold a variety of substituents at either 3- or 4- position of the phenyl ring or at both positions enhanced the potency of 2-phenylbenzimidazole-4-carboxamide [75]. For instance, 2-(4-hydroxyphenyl)-1*H*-benzimidazole-4-carboxamide (NU 1085) (see below) (**9**) was reported to potentiate by 2- or 3-fold the cytotoxicity of the monofunctional alkylating agent temozolomide (TMZ) and of the topoisomerase I inhibitor topotecan in ovarian carcinoma cells [75]. Thus, because of its potency, easy synthesis and reasonably good solubility in



water, NU 1085 has been adopted as a standard PARP-1 inhibitor. Generally, the benzimidazol derivatives resulted more potent than their benzoxazol analogues. Some other examples of 2-substituted benzimidazol-4-carboxamides have been reported. They include alkyl amines as side chains to increase their water solubility, or substituted heterocycles as thiazole, oxazole, oxadiazole or thiophene [11].

A better knowledge of the catalytic domain and crystal structure of PARP-1, has led to the design and synthesis of a series of tricyclic lactam compounds based on indole and benzimidazole structures [4]. In these PARP-1 inhibitors, the carboxamide group has been incorporated in a tricyclic system in an effort to combine the desired qualities of amide-restricted analogues such as AG-14361 (see above) (**11**) with the potency of the benzimidazol-4-carboxamides (see above) (**9**). In this series, lactam functionality is required for potent PARP-1 inhibition, which is consistent with the specific hydrogen bonding interactions in the X-ray crystal structure. X-ray crystallographic analysis also showed that tricyclic derivatives bound to catPARP in the same way as nontricyclic analogues, with equal disposition of the benzamide core and the 2-aryl ring. Interestingly, these compounds turned out to be good chemopotentiators of TMZ and topotecan in cancer cells *in vitro* [8]. In addition, as in their related predecessors, substituted phenyl groups at position 2 also enhanced their potency. In general, tricycles with 2-aryl substituents show good PARP-1 inhibition, with 4-substituted analogues being the most potent. These new PARP-1 inhibitors enhance the cytotoxic effects of topoisomerase I inhibitors, methylating agents and ionizing radiation. Recently, it has been reported that the introduction of amine substituents at the 2-aryl position of 2-aryl-benzimidazole-4-carboxamides also enhances their inhibitory activity [88]. Several 2-aryl-benzimidazole-4-carboxamides [87] containing different amine groups were evaluated against purified human PARP-1. The results indicated that amine substituents at 3- or 4- position of the 2-aryl ring improved the inhibitory effect approximately 3-fold, compared with the unsubstituted compound and 3-AB. It has been hypothesized that this increment in the inhibitory activity may be due to an additional binding interaction between the flexible amine group and the large cavity within the NAD<sup>+</sup> binding domain. Two compounds of this group (*N,N*-dimethylaminomethyl and pyrrolidinomethyl derivatives) have been reported to potentiate the cytotoxicity of TMZ by 4-5 fold in human colorectal cancer cells [88].

New bis-benzamide derivatives have been synthesised for the treatment of cardiovascular, neurodegenerative and cancerous diseases. As an example, 1,5-di[(3-carbamoylphenyl)aminocarbonyloxy]pentane has been reported to inhibit PARP-1 with an IC<sub>50</sub> value of 0.59 μM [15].

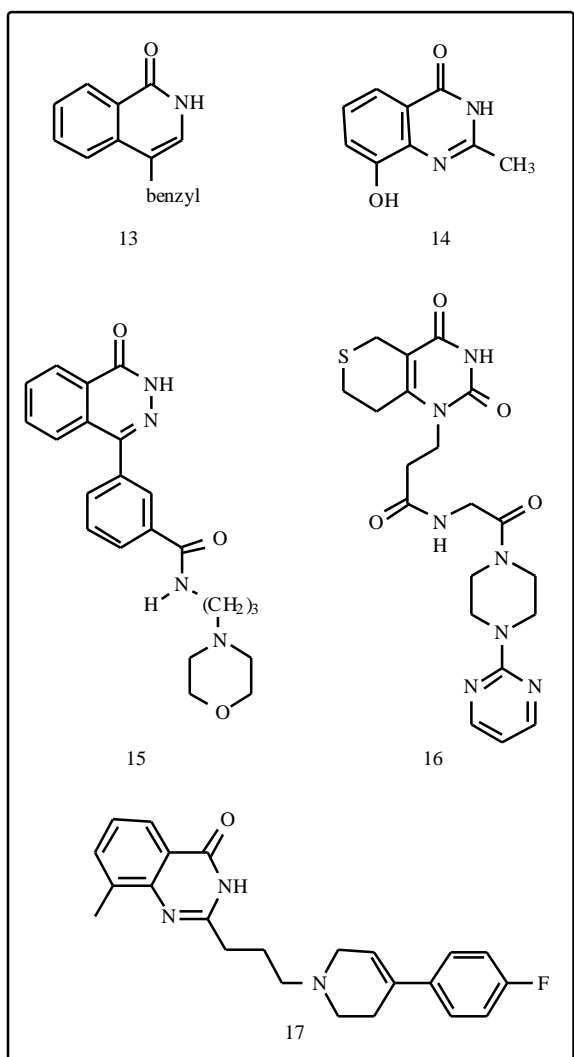
#### 6.1.4. Isoindolinones

Isoindolinones are another example of constricted carboxamide inhibitors. Within these compounds, the carboxamide group is held in the favourable orientation through its incorporation in a five membered ring. The best results are achieved when the isoindolinone is fused to adenosine with an alkyl chain of variable length (see above) (**12**). This kind of inhibitor has shown protection against reperfusion injury and inflammation *in vivo*, and exerts cytoprotective effects in oxidatively damaged cells. However, the unsubstituted isoindolinone core has weak potency by itself [11]. The powerful effect of the adenosine derivative (**12**) in contrast with the poor potency of the isoindolinone core, may be due to the specific recognition of the adenosine in the active site of PARP-1. It has been postulated that the adenosine moiety of NAD<sup>+</sup> establishes two hydrogen bonds with its binding site, through the amine group and the neighbouring ring nitrogen (N1) of adenosine [11]. Also, some polycycles incorporating the isoindolinone group have been reported [11].

#### 6.1.5. Phthalazinones and Quinazolinones

Structures of phthalazinones and quinazolinones (**13-17**) are depicted below. Phthalazin-1(*2H*)-one is a PARP-1 inhibitor with an IC<sub>50</sub> of 12 μM [89]. The compound is not a strong inhibitor by itself, but its potency is increased when methyl, ethyl or benzyl substituents are introduced at position 4 within the ring (see below) (**13**) [11].

KuDOS pharmaceuticals have been optimizing the known 4-aryl-phthalazinones to improve their inhibitory activity and metabolic stability [90]. Through the known crystal structure of chicken PARP-1 and early SAR (structure-activity relationship) studies, additional structural changes over 4-benzyl phthalazinones have been made. Substituents at 3 position of the benzyl moiety enhanced inhibitory potency, especially substituents containing carbonyl groups at the 3-position. KuDOS pharmaceuticals have recently reported the synthesis and preliminary biological evaluation of a new series of 3-substituted 4-benzyl-2*H*-phthalazin-1-ones as potent PARP-1 inhibitors



with low nanomolar inhibitory activity and promising metabolic stability *in vitro* ( $IC_{50}$  values between 30 and 100 nM) [91]. Several phthalazinones with diverse biaryl substituents at the 3 benzylic position also showed high PARP-1 inhibitory effect, particularly amide and imide derivatives. Moreover, the introduction of a fluoro-substituent in the 2 position relative to the imide ring led to a significant enhancement of potency. This compound showed a similar level of inhibition for PARP-1 and -2, whereas for Tankyrase and Vault-PARP inhibitory potency was >100-fold less. Further evaluation of these compounds is still going on [92].

Pfizer developed 5-methoxy-4-methyl-1(2) phthalazinones, while ONO Pharmaceuticals designed 4-substituted phthalazinones with phenyl groups bearing side chains with amines to increase their water solubility. An example is ONO-1924H, whose chemical name is *N*-[3-(4-oxo-3,4-dihydro-phthalazin-1-yl)phenyl]-4-(morpholin-4-yl) butanamide methanesulfonate monohydrate (see above) (**15**). This compound was assayed *in vitro* in a cytotoxicity test induced by hydrogen peroxide, and *in vivo* in cerebral damage induced by middle cerebral artery thrombus occlusion in rat.

It was observed that ONO-1924H (**15**) reduced cell death in a concentration-dependent manner *in vitro*, and decreased cerebral damage in the treated rat group [92]. Other reported variations on phthalazinones include tetracyclic benzopyranyl derivatives, indenyl derivatives, substituents bearing cyclic amines, which improve their water solubility, sulphonamides and heterocycles [11].

Quinazolin-4-ones were derived from phthalazinones through the union of the second ring nitrogen. Quinazolin-4-one and quinazolin-2,4-dione showed moderate PARP-1 inhibition, with  $IC_{50}$  values of 9.5 and 8  $\mu$ M, respectively [88]. Most potent derivatives were the 8-methyl-quinazolinones, with a 2-phenyl group substituted by a 4-nitro, 4-cyano or 4-methoxy group. NU 1025, (8-hydroxy-2-methylquinazolin-4(3H)-one) (see above) (**14**) was found to be 10-fold more effective than 3-aminobenzamide, and to enhance the action of alkylating agents ( $IC_{50}$  value of 0.4  $\mu$ M) [93]. Although Banasik identified uracil derivatives as weak inhibitors of PARP-1, it was found later that if those compounds contained a substituted uracil-derived fused ring gave an optimum inhibitory potency. Particularly, some BAYER-optimized derivatives containing a piperazine or phenyl substituted Ala-Gly side chain exhibited  $IC_{50}$  values below 100 nM against recombinant PARP (see above) (**16**) [94].

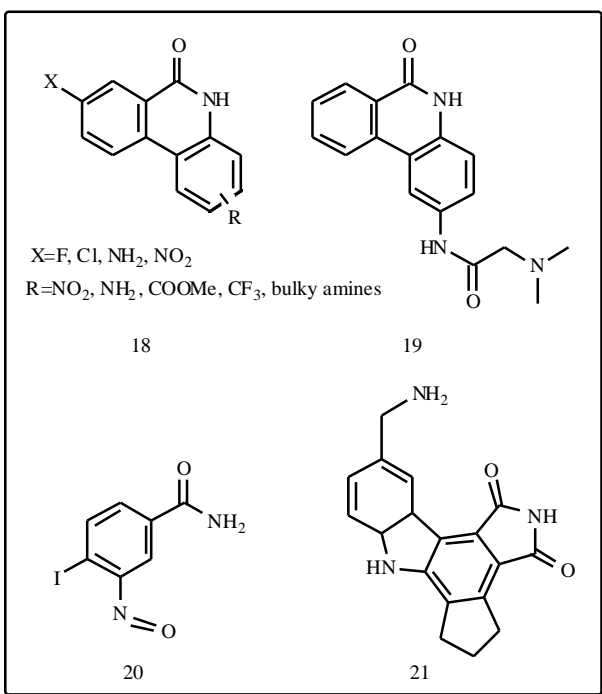
#### 6.1.6. Phenanthridinones

Phenanthridinone was reported by Banasik as a potent PARP-1 inhibitor [89]. Since then, several substituted 6-(5*H*)phenanthridin-6-ones (**18**) (see below) [68] have been synthesized, showing stronger PARP-1 inhibitory effects than the unsubstituted phenanthridinone. Specifically, bulky aliphatic substituents at 2 and 3 positions, usually connected to the phenanthridinone core *via* an amide nitrogen, appear to potentiate the inhibitory effect. Moreover, the introduction of amine and carboxylic acid containing a side chain at 2 or 3 position increases both water solubility and potency of the compound [11]. Cyclopenta(imn)phenanthridine-5(4*H*)-one derivatives were developed from phenanthridine-5-one and showed increased inhibitory potency over the parental compound. Other inhibitors that have also been described include substituents linked *via* sulphonamide or amide groups [11]. INOTEK has reported some novel substituted phenanthridinones that show potent PARP-1 inhibition, with  $IC_{50}$  values from 0.3  $\mu$ M to 1  $\mu$ M. An example is *N*-(6-oxo-5,6-dihydrophenanthridin-2-yl)-2-(*N,N*-dimethylamino)acetamide (PJ 34) (see below) (**19**) [95], which has been shown to protect neurons against oxygen and glucose deprivation *in vitro*. PJ 34 is also useful against various diseases including inflammatory processes, transplanted organ function and allergic encephalomyelitis [96].

#### 6.1.7. Zinc-Fingers PARP-1 Inhibitors

As discussed previously, the asymmetric functionality of F1 and F2 zinc finger motifs within the DBD domain of catPARP has inspired the design of a class of PARP-1 inhibitors. In fact, the two zinc fingers are not required for the recognition of DNA breaks in the same way. Thus, the enzyme results completely inactivated independently of the type of DNA breaks when it is damaged on F1 finger, whereas the destruction of F2 finger avoids PARP-1

activation in response to single strand DNA breaks only (see Fig. (2B)). This property of the zinc fingers has led to the development of several PARP-1 inhibitors that act selectively at the F1 finger. These inhibitors are produced by oxidation of compounds which contain amino groups, to produce the C-nitroso-derivatives. So, the C-nitroso compound only oxidize the first zinc-finger resulting in zinc ion ejection, which leads to the inactivation of PARP-1 activity without halting the binding of the enzyme to DNA. Two compounds of this group (3-nitrosobenzamide and 6-nitroso-1,2-benzopyrone) have been reported to induce apoptosis in tumor cells and to inhibit infection of HIV-1 in human lymphocytes without induction of metabolic changes at doses about 50  $\mu$ M. Other compound, 4-iodo-3-nitrosobenzamide (INO2BA) (see below) (20) [69], has been recently investigated for the treatment of cancer and AIDS. When used against leukaemic cell lines 855-2 and HL-60, INO2BA completely prevented cell proliferation. It has been administered to monkeys in animal safety studies, and doses of 15 mg/kg were well tolerated without noticeable side effects [15].



Important patents on PARP-1 inhibitors are marked with an asterisk in the references and patents section.

## 7. CURRENT AND FUTURE DEVELOPMENTS

Over the last two decades, potent PARP-1 inhibitors have been developed using SAR and crystal structure analysis. These approaches have identified crucial features for potent inhibitor-enzyme interactions, which have rendered large series of compounds based on the existence of the critical carboxamide group for the interaction with the catalytic site of PARP-1 [97]. Hence, noteworthy progress has been achieved in inhibitory potency since the resulting PARP-1

inhibitors are up to 1,000 times more potent than the classical benzamides. These new powerful inhibitors enhance the *in vitro* cytotoxicity of DNA monofunctional alkylating agents (e.g., TMZ and topoisomerase I poisons) and ionizing radiation.

However, it is important to consider the feasible drawbacks associated to the putative use of potent PARP-1 inhibitors in the treatment of human chronic pathologies such as cancer. Thus, a wide variety of safety issues such as pharmacokinetic, pharmacodynamic, toxicity, oral bioavailability, long-term safety for human use and metabolic processes, as well as long-term effects of PARP-1 inhibition need to be solved before human testing for chronic indications can begin. The potential side effects associated with long-term PARP-1 inhibition might be reduced through the combination of proper low-dose of PARP-1 inhibitors with the standard series of drugs that are used for the treatment of a particular disease, paying special attention to the potential interactions of PARP-1 inhibitors with these other therapeutics [98]. Future clinical trials will determine the real potential of PARP-1 inhibitors in cancer chemotherapy as well as the toxicity parameters of affected tissues and organs.

As a general rule, PARP-1 inhibitors bind to the NI site, partially bind to the acceptor site and do not bind to the PH site, or AD site of catPARP. An interesting approach to increase the selectivity of PARP-1 inhibitors has emerged from the observation that FR 257517 (17) inhibitor (see its interaction with catPARP in Fig. (6B)), which has a quinazolinone part as a nicotinamide mimic moiety and a fluorophenyl part, tightly binds to the NI and AD sites but does not bind to the acceptor site nor the PH site [19]. It is known that AD is at the bottom of the active site and surrounds the adenine moiety at the terminus of the  $\text{NAD}^+$  substrate. The result of the unusual binding of FR 257517 (17) to catPARP is the induction of a conformational change of the AD site through the breaking of the bottom of the active site wall. Surprisingly, the movement of a single amino acid in the bottom of AD induces a structural change, which leads to the exposure to the water solvent of the hydrophobic corn-shaped subsite. This observation offers a new concept in PARP-1 inhibitors design using the emerged hydrophobic site, which is situated on the opposite side of the active site, and has been unexploited in inhibitor design yet. Consequently, pharmacological testing of compounds derived from FR 257517 will open new ways to determine whether it is possible to obtain more specific and potent PARP-1 inhibitors.

## 8. ACKNOWLEDGMENTS

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