

Poly(ADP-Ribose) Polymerase Inhibitors

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Abstract: Poly(ADP-ribose) polymerase-1 (PARP-1) is the principal member of the PARP enzyme family consisting of PARP-1 and several recently identified novel poly(ADP-ribosyl)ating enzymes. PARP-1 functions as a DNA damage sensor and signalling molecule. Upon binding to DNA breaks, activated PARP cleaves NAD⁺ into nicotinamide and ADP-ribose and polymerizes the latter onto nuclear acceptor proteins including histones, transcription factors and PARP itself. This Poly(ADP-ribosyl)ation contributes to inflammatory signal transduction processes. In addition, oxidative stress-induced overactivation of PARP consumes NAD⁺ and consequently ATP, culminating in cell dysfunction or necrosis. Activation of PARP has been implicated in the pathogenesis of stroke, myocardial ischemia, diabetes, diabetes-associated cardiovascular dysfunction, shock, traumatic central nervous system injury, arthritis, colitis, allergic encephalomyelitis and various other forms of inflammation. Therefore, inhibition of PARP by pharmacological agents may prove useful for the therapy of these diseases, as has been shown in preclinical animal models. Moreover, PARP inhibitors may have additional, potential utility as anticancer agents, radiosensitizers and antiviral agents. In the present article we overview the structures and pharmacological actions of various pharmacological classes of compounds which inhibit the catalytic activity of PARP.

Key words: Poly(ADP-ribose) polymerase, DNA repair, necrosis, apoptosis, peroxynitrite, nitric oxide, reperfusion, stroke, myocardial ischemia, diabetes, inflammation, arthritis, colitis, uveitis, aminobenzamide, nicotinamide

PARP: STRUCTURE AND FUNCTION

Poly(ADP-ribose) polymerase-1 (PARP-1, EC 2.4.2.30) [also known as poly(ADP-ribose) synthetase (PARS) and poly(ADP-ribose) transferase (PADPRT)] is a nuclear enzyme present in eukaryotes. It is a 116 kDa protein consisting of three main domains: the N-terminal DNA-binding domain containing two zinc fingers, the automodification domain, and the C-terminal catalytic domain. PARP-1 functions as a DNA damage sensor and signalling molecule, binding to both single- and double strand breaks in DNA. Upon binding to damaged DNA, mainly through the second zinc finger domain, PARP-1 forms homodimers and catalyzes the cleavage of NAD⁺ into nicotinamide and ADP-ribose. The latter is used to synthesize branched nucleic acid-like polymers with poly(ADP-ribose) units covalently attached to nuclear acceptor proteins (Fig. 1). PARP-1 is the principal member of a growing family of nuclear and cytosolic enzymes that can catalyze poly(ADP-ribosyl)ation reactions. (Unless specified otherwise, the word PARP will refer to PARP-1 in the subsequent sections.) Poly(ADP-ribosyl)ation is a dynamic process as indicated by the short half life of the polymer. Two enzymes - poly(ADP-ribose) glycohydrolase (PARG) and ADP-ribosyl protein lyase - are involved in the

catabolism of poly(ADP-ribose). The biochemistry and molecular biology of PARP and its isoforms have been overviewed in detail elsewhere [1-9].

REASONS FOR INTEREST IN THE DEVELOPMENT OF POTENT PHARMACOLOGICAL INHIBITORS OF PARP

PARP has been implicated in multiple cellular functions, including DNA repair and maintenance of genomic integrity, cell replication and differentiation, as a signal for protein degradation in oxidatively injured cells and in the regulation of cytoskeletal organization (overviewed in 9). Interruption of the above listed processes by pharmacological inhibition of PARP would not be considered beneficial, but may be side effects of inhibitors of PARP (see also below). In certain conditions some of these actions may be considered beneficial. For example, inhibition of DNA repair by pharmacological inhibition of PARP would limit the ability of the tumor cells to repair their damaged DNA and, thereby, enhance the effects of cancer radiotherapy.

There is a highly controversial area of research which implies an active role for the enzymatic cleavage of PARP-1 by caspases in the process of apoptosis. As overviewed elsewhere [9], this process may only have limited importance in most experimental systems, and PARP cleavage serves as a marker, rather than executioner of the apoptotic process. In this process, PARP serves as a substrate of other enzymes (such as caspases), and is not

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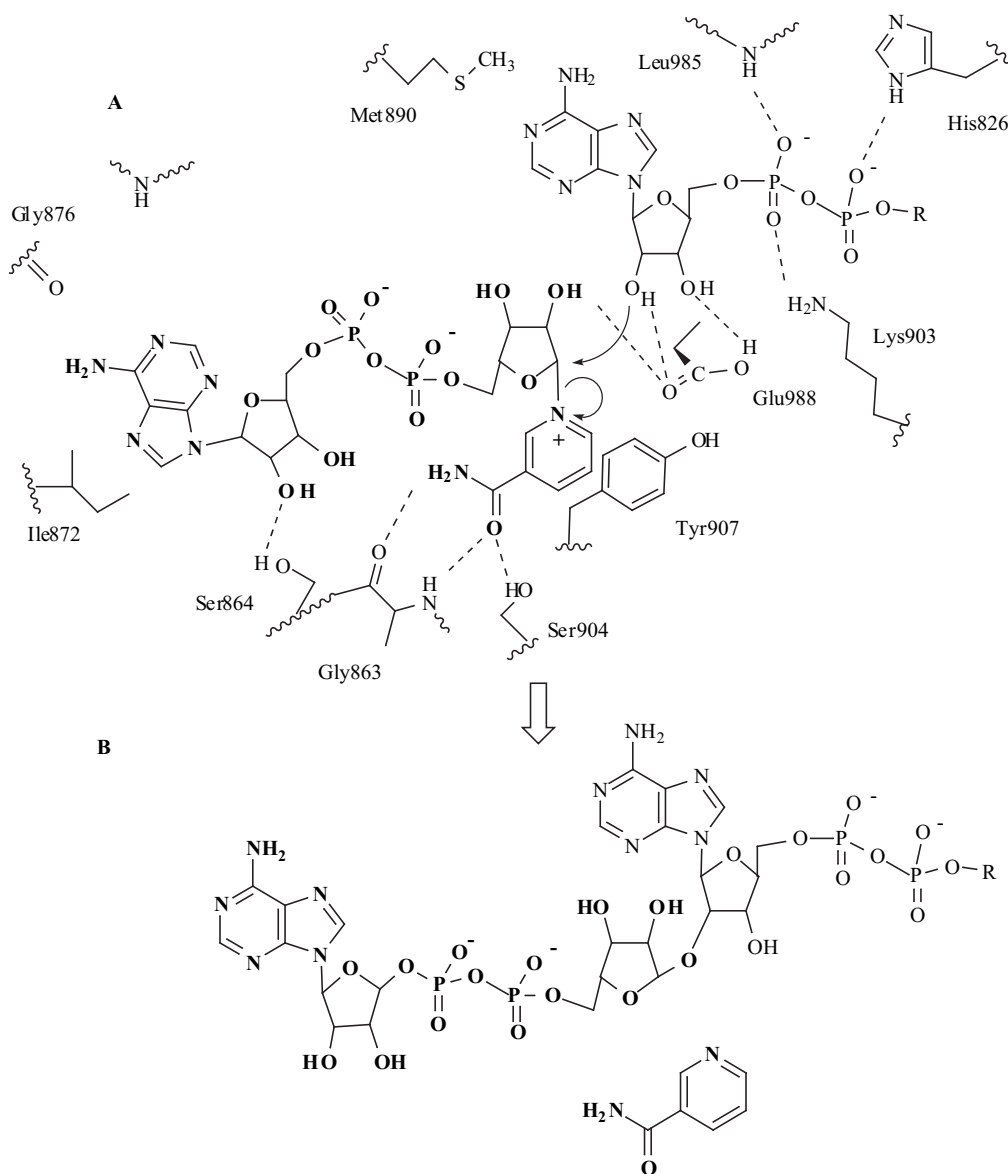


Fig. (1). A representation of the binding of NAD^+ to PARP and the catalysis of the polymerisation elongation reaction. A. NAD^+ (bold atoms) is bound in a cleft in the PARP protein. Some key amino acid residues involved in the interaction with NAD^+ are indicated. Dotted lines represent hydrogen bonding interactions. The curved arrows represent the movement of electrons during nucleophilic attack by the 2' oxygen of the adenosine ribose of the acceptor polymer on the NAD^+ molecule. B. Nicotinamide is displaced from the NAD^+ , the rest of which (ADP-ribose) becomes bonded to the acceptor polymer resulting in elongation of the polymer chain.

catalytically involved. Consequently, inhibition of PARP would not be expected to interfere with this process.

The rationale for the therapeutic benefit for the pharmacological inhibition of PARP in inflammatory and reperfusion injury comes from two lines of observation. Firstly, PARP activation has been found to contribute to an energy-consuming cellular process, which leads to cellular NAD and ATP depletion, mitochondrial dysfunction and an overall cellular dysfunction. This process can eventually culminate in cell death, which occurs via the necrotic pathway [10-14]. Secondly, PARP has been implicated in the process of inflammatory signal transduction [recently overviewed in 8,9]. PARP-1 regulates the expression of various proteins at the transcriptional level. Of special

importance is its regulation of the production of inflammatory mediators such as the inducible nitric oxide synthase, intercellular adhesion molecule 1, tumor necrosis factor alpha and major histocompatibility complex class II [15-20]. NF- κ B is a key transcription factor in the regulation of this set of proteins and PARP has been shown to act as a co-activator in AP-1 and NF- κ B-mediated transcription. There is currently no consensus in the literature as to whether the modulation of signal transcription by PARP is dependent on the catalytic activity of the enzyme, or, alternatively, on its physical presence (or both). Poly(ADP-ribosylation) of histones may also contribute to the transcription-promoting effect of PARP-1 as poly(ADP-ribose) confers negative charge to histones leading to electrostatic repulsion between histones and DNA. Thus,

poly(ADP-ribosyl)ation can loosen up chromatin structure and can thereby make genes more accessible for the transcriptional machinery, thus acting as a “generic” regulator of signal transduction processes. Cellular expression of mRNA under basal conditions or in response to inflammatory mediators is markedly altered by PARP

deficiency, as demonstrated by mRNA microchip analysis [19,20]. PARP deficiency suppresses the expression of a variety of gene products including tumor necrosis factor alpha, interferon gamma, inducible nitric oxide synthase, intercellular adhesion molecule-1 and P-selectin [8,9,16,17].

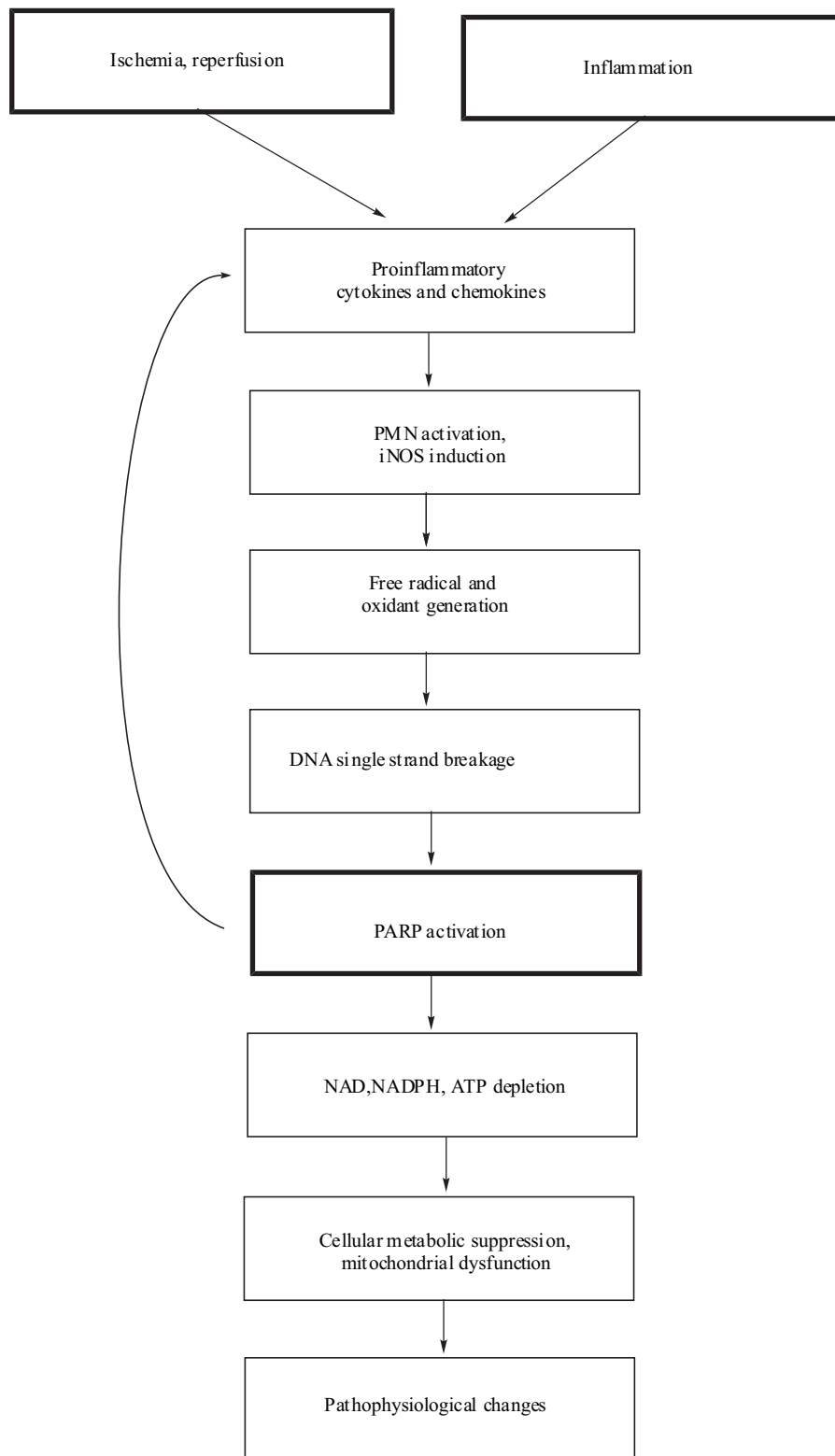


Fig. (2). The involvement of PARP in the chain of events leading to pathophysiological changes in response to various insults.

The participation of PARP activation in both the promotion of oxidant-induced cell dysfunction and the promotion of pro-inflammatory signal transduction processes (Fig. 2) is responsible for resistance of PARP-1^{-/-} cells (10-14) or PARP-1^{-/-} animals [21-28] to the development of injury in various disease models, when compared to their wild-type (PARP-1^{+/+}) counterparts. These diseases include stroke [21,22], neurotrauma [23] and neurodegeneration [24]; myocardial ischemia and infarction [16, 25-27]; diabetes mellitus [28-31] and its vascular complications [32]; inflammatory bowel disease [33], endotoxic, septic and hemorrhagic shock [17,34,35] and acute respiratory distress syndrome [36]. The fact that PARP deficient mice show marked resistance in the above disease models (as well as the confirmation of this protection by using potent pharmacological inhibitors of PARP, see below) makes PARP an attractive target for drug development. An additional indication for the development of pharmacological inhibitors of PARP is the sensitisation of tumor cells to anticancer therapies such as radiation (as mentioned above) and cytotoxic agents. Finally, PARP inhibitors may interfere with the integration of HIV into the genome and may act as anti-HIV agents. The mechanistic aspects of the participation of PARP in these disease processes have been reviewed [8,9], and the known actions of the various classes of pharmacological PARP inhibitors are listed in the subsequent chapters.

CHEMICAL CLASSES OF PARP INHIBITORS

Nicotinamide and 3-Aminobenzamide

Nicotinamide [Fig. (3), Structure 3.1] and its 5-methyl derivative were shown to be competitive inhibitors of PARP thirty years ago [37]. Being a natural compound, nicotinamide also acts as a substrate for other NAD-metabolizing enzymes. Structurally similar compounds such as benzamide, pyrazinamide and substituted benzamides, in particular 3-aminobenzamide [3-AB, Structure 3.2a] and 3-methoxybenzamide [3.2b], also demonstrated inhibition of PARP [38, 39]. Studies with other derivatives of benzamide indicated the importance of the carboxamide group, as N-alkylation [40] or replacement with a thioamide [41] greatly

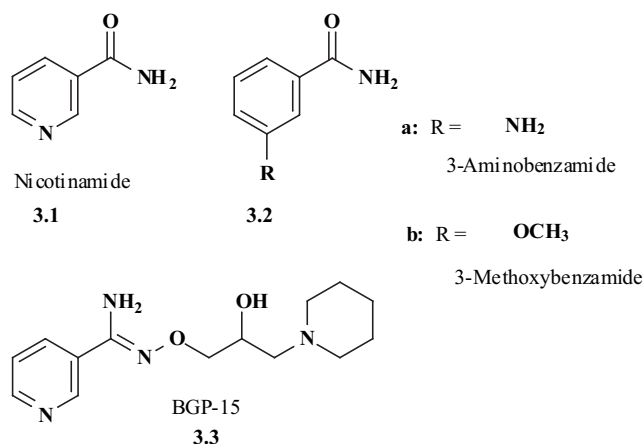


Fig. (3). Structures of nicotinamide, 3-aminobenzamide and related compounds.

reduced activity. It is generally assumed that benzamides inhibit PARP by interfering with the binding of NAD to the enzyme's active site. However, an additional action of benzamides may be related to their binding to DNA and thereby preventing the recognition of DNA breaks by PARP and thus prevention of the activation of PARP [42].

A large body of early and more recent work utilized 3-AB to investigate the *in vivo* effects of PARP inhibition, due to its ready commercial availability and apparent lack of acute toxicity. The IC₅₀s of 3-aminobenzamide and nicotinamide in the isolated PARP-1 enzyme are in the vicinity of 30 μM. Both 3-AB and nicotinamide (at high millimolar concentrations) are effective in inhibiting the death of various cell types exposed to various forms of oxidative or nitrosative stress *in vitro*. These compounds were also able to block the pro-inflammatory upregulation of various signal transduction pathways and mediators *in vitro* [as overviewed in 2, 8 and 9]. As far as *in vivo* studies are concerned, 3-AB has been shown to protect against ischemia-reperfusion injury of the brain [22,42-45] and heart [46-50]. The compound was also found to be effective in protecting against reperfusion injury in various other organs, including the gut [51,52], kidney [53, 54], skeletal muscle [48], liver [55], ear [56], retina [57] and skeletal muscle [47], as well as providing protection in experimental models of shock [58-63] and inflammation [33,44,64-68]. Similarly, nicotinamide has been shown to be protective in a variety of disease models ranging from various forms of stroke [69-73] and ischemia-reperfusion injury [52,54, 74, 75] to various forms of shock [61,76] and inflammation, including arthritis [77-79].

Both 3-aminobenzamide and nicotinamide have been shown to protect against oxidant-mediated islet cell death *in vitro* [80,81] and against the development of diabetes *in vivo* [82-87]. It is noteworthy that human clinical trials with nicotinamide in Type I diabetic patients [88-90] and a pilot trial in osteoarthritic patients [79] represent the only example to date where a PARP inhibitor (nicotinamide) has been administered to human beings. This is possible as nicotinamide is a vitamin, with an established safety profile in humans. Although it appears that, after several promising small Phase II trials, the ultimate Phase III trials in diabetes do not show efficacy, these disappointing results may be related to the choice and the dose of the compound used [overviewed in 88-90]. In the diabetes trials, no surrogate markers (e.g. poly(ADP-ribosyl)ation in peripheral blood leukocytes) were measured, and so it is unclear whether or to what extent the dosage regimen was able to block the activity of the PARP enzyme. By extrapolating from the animal studies (where nicotinamide needs to be given at doses of several hundred mg/kg/day), one would expect that much higher doses of nicotinamide would be required to provide sufficient inhibition of PARP in humans. It is unlikely that doses of nicotinamide that are sufficient to block PARP can be reached in humans, as at doses above 3 g/day in humans, nicotinamide begins to exert various toxic effects [91].

Overall, many aspects of the early work using 3-AB and nicotinamide [92,93] have been confirmed using more potent inhibitors (see below) and from studies with PARP-1^{-/-} mice

[8-27]. Nevertheless, there are many concerns regarding the specificity of these compounds due to the relatively high concentrations and doses required for PARP inhibition *in vitro* and *in vivo* (up to 3-10 mM or several hundreds of mg/kg, respectively). Thus, one must be aware of the fact that nicotinamide and 3-AB have additional, antioxidant effects and, therefore, some of the protection seen with these compounds *in vitro* or *in vivo* may be a consequence of the compounds acting upstream from the catalytic activity of PARP, i.e. at the level of oxidative stress [15]. Direct measurements have actually demonstrated that 3-AB, when given at several hundred mg/kg doses *in vivo*, only exerts a partial inhibition of poly(ADP-ribose) accumulation in the heart and other organs [50]. Nicotinamide has been shown to reduce the markers of oxidative and nitrosative stress in various disease models, which may be consistent with a direct antioxidant action. In addition, the finding that nicotinamide reduces both necrosis and apoptosis in a stroke model [72] may be consistent with pharmacological actions of the compound other than PARP inhibition.

A variety of benzamide derivatives have been described in the patent and scientific literature [overviewed in 94]. In many cases it is difficult to determine from the available data the relationship between the pharmacological actions of the compounds, their PARP inhibitory properties (if any) and their additional non-PARP related pharmacological actions. For example, in the case of N-substituted benzamide antiemetics compounds, their reversal of multidrug resistance, induction of cell differentiation and their antitumor properties may or may not be related to inhibition of PARP. Declopramide (3-chloro procainamide), and other N-substituted analogs are distinguished from their unsubstituted analogues because they (i) are susceptible to radiolysis, (ii) induce cytotoxicity by apoptosis but not necrosis, (iii) inhibit cell proliferation, (iv) *activate* PARP, and (v) have reduced effects on microregional tumor blood perfusion [95]. Thus, it appears the above mentioned analogs exert their effects independently of the inhibition of PARP.

Overall, we feel that aminobenzamide or its derivatives (even the ones that do have some PARP inhibitory activity) have many disadvantages as drug candidates due to a combination of factors including low potency, short cellular residence time, a variety of non-specific actions including inhibition of mono- as well as poly(ADP-ribosyl)ation reactions [1,92,93] and non-proprietary status.

Nicotinamide, as a vitamin, can be administered to humans, but it is unlikely that at the safe doses of the compound effective inhibition of PARP can be reached in humans. A non-carboxamide derivative termed BGP15 (i.e. O-(2-hydroxy-3-piperidino-propyl)-3-carboxylic acid amidoxime, **3.3**) is also a weak inhibitor of PARP [96-99], but exerts many pharmacological actions which are consistent with PARP inhibition as a mode of action, including inhibition of oxidant-induced cell damage in the heart [97], skin [98] and kidney [99]. Nevertheless, the doses required to achieve protection are rather high, in the range of several hundreds of μM *in vitro* [97], 100-200 mg/kg when administered systemically [99] and 5-10% topically [98]. The compound also exerts antioxidant effects that are more

consistent with direct oxidant scavenging than with catalytic inhibition of PARP [96-99].

In our estimation, benzamide analogue PARP inhibitors and nicotinamide remain primarily experimental tools for investigating the role of PARP activation in various cellular processes. However, due to the non-specific actions of these compounds it is advisable to confirm the effects using more potent PARP inhibitors (see below) and/or PARP deficient cells or animals.

Isoquinolinones and Dihydroisoquinolinones

Various structures where the carboxamide group is incorporated in an anti-(or cis-) configuration within a ring structure (lactam) were reported as considerably more effective at inhibiting PARP than 3-AB [93, 100]. Of these, the substituted 3,4-dihydroisoquinolin-1(2H)-ones [Fig. (4), **4.1**] and isoquinolin-1(2H)-ones [**4.2**], were investigated as potential enhancers of radiotherapy or chemotherapy agents and were claimed as such [101, 102], together with the less potent 1,6-naphthyridine-5(6H)-ones [e.g. **4.3**] [103]. While the substituted dihydroisoquinolinones and isoquinolinones, with IC_{50} s in the 100s of nM range, have been widely used as research tools and are discussed later, the 1,6-naphthyridine-5(6H)-ones are generally less potent [103], with the lowest IC_{50} reported as 500 nM for the methyl derivative shown [**4.3**].

The Banasik article mentioned above [93] compared a large number of compounds from a variety of structural classes for their ability to inhibit PARP and mono(ADP-ribosyl)transferase. The most potent compounds against PARP all contained the lactam structure in fused cyclic systems and these formed the basis for many of the polycyclic PARP inhibitors subsequently developed (see below, Figs. **4.6,7,9**). These compounds were 4-amino-1,8-naphthalimide (**4.4**, EC_{50} =180 nM), 5[H]phenanthridin-6-one (**9.1**, X, R = H, IC_{50} =300 nM), isoquinolinone (**4.2**, R=H, 7 μM), 4-hydroxyquinazoline (**7.1**, X, R=H, 9.5 μM), 1(2H) phthalazinone (**6.1**, R=H, 12 μM) and substituted derivatives thereof. These classes of compounds, and ones that can be considered as derived from them, are described in subsequent sections.

The initial impetus for the earlier work and patent filings was the concept that inhibitors of PARP would enhance the cytotoxic effects of chemotherapy or radiotherapy agents used in the treatment of cancer. In this respect, many compounds were, and are, tested for potentiation of the cytotoxic effects of chemotherapeutic agents and ionizing radiation *in vivo*. While not universal, potentiation is often seen to correlate with dose and, for different drugs, with the ability of the drugs to inhibit PARP. This area was overviewed recently [104]. Generally, it seems that high doses of the drugs are required for these effects, suggesting that a near-complete inhibition of PARP-1 is necessary. Thus, the development of potent PARP inhibitors for this therapeutic area remains a valid concept. It remains to be seen whether the same PARP inhibitors which are being developed for cancer sensitisation purposes will also be applicable for protective purposes in inflammation or

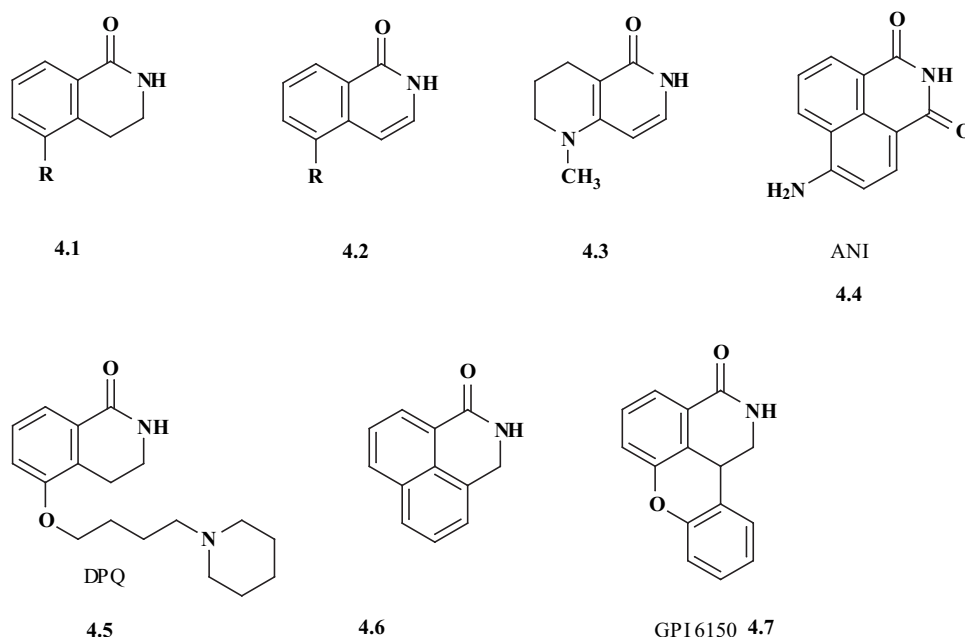


Fig. (4). Structures of isoquinolinones, dihydroisoquinolinones and related PARP inhibitors.

reperfusion injury, or whether the latter indications will require the development of separate, distinct classes of compounds.

From the above referenced studies, it became apparent that the following features are essential or desirable for the competitive inhibition of PARP at the catalytic site by this broad range of compounds: 1) an aromatic ring system bearing a 2) carboxamide group, which bears 3) at least one proton on the amide nitrogen. 4) The carboxamide group must be able to adopt the anti- (or cis-) configuration, and is preferably held in this conformation. 5) There is a non-cleavable bond in the 3 position relative to the carboxamide group.

In the last five or so years, the growing realisation that PARP may be involved in the pathogenesis of many diseases (see above) led to the development of numerous types of novel PARP inhibitors. Several of the classes of PARP inhibitors originally described in Banasik's article [93] have undergone elaboration in order to enhance potency, improve pharmacokinetic characteristics, increase solubility in water, and to allow the filing of new patent applications.

We must note in advance that the differences in the PARP assay conditions used in the cited literature below may cause considerable variations to be found in the inhibition constants found by different groups of investigators. Therefore, comparisons should be made with caution. In the following discussion, the use of K_i and IC_{50} imply that isolated PARP preparations were used and EC_{50} implies a cell-based assay. Generally, for a given compound, the K_i will tend to be lower than the IC_{50} , and the EC_{50} higher. Whenever available, we will also include potency values for reference compounds obtained under the same conditions.

The dihydroisoquinolinones [4.1], and isoquinolinones [4.2], as described by Suto and Banasik [100, 93], are

considerably more potent than aminobenzamide [3.2a] ($IC_{50}=33 \mu M$). The parent compounds [4.1, 4.2, R=H] had IC_{50} s of 1.5 μM and 6.2 μM , respectively. The potency was enhanced by substituents in the 5 position, the hydroxyl group proving the best with IC_{50} s of 100 and 140 nM for the 5-hydroxy dihydroisoquinolinone [DHIQ; 4.1, R=OH] and 5-hydroxyisoquinolinone [4.2, R=OH], respectively [93]. 5-methyl dihydroisoquinolinone [PD 128763; 4.1, R=CH₃; $IC_{50}=140$ nM] was reported to sensitise cells to ionising radiation and to reduce DNA repair [100-102, 105, 106] as well as to potentiate the cell death induced by various anticancer drugs [107]. The same compound also exerts protective effects through PARP inhibition against oxidative injury in cultured cells *in vitro* [11,108]. 5-Hydroxyisoquinolinone [4.2, R=OH] was found to exert PARP inhibitory and cardioprotective effects in an ischemic-reperfused heart preparations *in vitro* [47, 109]. The 5-amino derivative (5-AIQ; 4.2, R=NH₂) proved useful in protecting against organ damage in rat models of hemorrhagic shock, acute respiratory distress syndrome and myocardial infarction [61, 110,111]. The concentration at which this compound provides protection against oxidative injury (100-300 μM) in cultured cells does not appear to be consistent with the unusually high efficacy of the compound *in vivo* (effective protection at as low as 30 $\mu g/kg$ i.v. dosing in rodents) [61]. It is possible that pharmacological actions other than PARP inhibition also contribute to the protection seen with this particular compound. 3,4-dihydro-5-[4-(1-piperidinyl)butoxy]-1(2H)-isoquinolinone (DPQ; 4.5) is another example of the same class, which has been shown to exert neuroprotective effects in cultured cells *in vitro* and in stroke models *in vivo* [21, 112-115]. It is worthwhile to note also that some isoquinolinones (such as 3,4-dihydro-5-[¹¹C]methoxy-1(2H)-isoquinolinone) have been proposed as potential positron emission tomography tracers [116].

The isoquinolinone core is also present in various tricyclic compounds: Banasik reported the IC_{50} s of 1,8 naphthalimide ($IC_{50}=1400$ nM) and of the 4-amino

derivative (**4.4**, 180 nM) [93]. Based on these, Guilford described 2,3-dihydrobenzo[de]isoquinolin-1-one (**4.6**, IC_{50} = 540 nM) and analogues that incorporate heteroatoms in the additional ring [117]. Tang and colleagues reported that 1,8-naphthalimide exerts protective effects in oxidatively stressed endothelial cells *in vitro* [118]. The 4-amino substituted derivative (**4.4**) has been found to reduce ischemia-reperfusion injury in the heart, liver and skeletal muscle [47, 55]. The same compound also exerts radio- and chemosensitizing effects *in vitro* [119,120]. SmithKline Beecham [121] have prepared a limited number of thieno[2,3-c]isoquinolines [**9.4**]. These are discussed later under phenanthridinones.

The benzopyrano[4,3,2-de]isoquinolinones [**4.7**] [122] are a related class of tetracyclic lactams of which GPI-6150 (K_i = 60 nM) has been studied most intensely. GPI-6150 (**4.7**) *in vitro* exerts protection against the death of oxidatively stressed cells in the concentration range of 1-30 μ M, with half-maximal protection seen at approximately 3 μ M [123]. The compound also blocked the cytokine induced expression of mucosal addressin cell adhesion molecule-1, intracellular adhesion molecule 1 and E-selectin *in vitro* [124,125], effects also seen with genetic PARP-1 deficiency. In the dosing range of 20-40 mg/kg/day, GPI-6150 exerts significant protective effects in experimental models of stroke, traumatic brain injury, neurodegeneration, circulatory shock, diabetes mellitus and various inflammatory conditions including colitis and gouty arthritis [126-130].

Benzimidazoles, Indoles and Related Compounds

On the assumption that restriction of the carboxamide into the favorable conformation should result in increased inhibitory potency, researchers at the University of Newcastle-upon-Tyne designed a series of benzoxazole-4-carboxamides [Fig. **5.1**, X = O] [131] and benzimidazole-4-carboxamides compounds [**5.1**, X = NH] [132,133] that favored the active conformation by intramolecular hydrogen bonding between the amide proton and the cyclic nitrogen [**5.1**] [reviewed in detail by the inventors in 104]. These compounds exhibit potencies superior to those usually shown by monocyclic carboxamides. The variously substituted 2-phenyl derivatives [**5.1**, R = Phenyl] are the most potent examples and the benzimidazole analogues [**5.1**, X = NH] are a few times more potent than the corresponding benzoxazoles [X = O]: benzimidazoles substituted in the 2-position [**5.1**, X = NH, R] with phenyl and 2-(3-methoxyphenyl) had EC_{50} s of 950 and 60 nM respectively, while the corresponding benzoxazoles (**5.1**, X = O) had values of 2100 and 1100 nM in a cell based assay of PARP [131,132]. The same benzimidazole compounds were tested against expressed human PARP [134] where the said 3-methoxyphenyl derivative had a K_i of 6 nM (3AB was 3100 nM on the same assay). 3- and 4- (mono and di-) substituted phenyl groups were well tolerated in the 2-position [R in **5.1**] with a dozen examples with single digit nM K_i . The above-mentioned patents claim the compounds as enhancers of chemotherapy or radiotherapy agents and the example tested in the article, 2-(4-hydroxyphenyl)benzimidazole-4-carboxamide ("NU 1085"; **5.1**, X = NH, R = 3-hydroxyphenyl) potentiated the cytotoxic effects of

tempozomide (a topoisomerase I inhibitor) and topotecan (an alkylating agent) [133-134].

A series of patents by BASF also claimed 2-substituted benzimidazoles-4-carboxamides [135-137], including 2-phenyl derivatives with side chains containing alkyl amines to increase their solubility water [137]. Such compounds apparently maintain their PARP inhibitory activity and are claimed for the treatment of neurological, cardiovascular, and proliferative diseases [135-137].

Variations on this theme from Yamanouchi Pharmaceuticals [138] and BASF [136] include benzimidazoles with heterocycles at the 2-position. A later Yamanouchi patent describes further examples [139] where the heterocycle is further, more elaborately substituted. Examples prepared include R groups where two or three cyclic groups are attached sequentially, either directly or via linkers such as alkyl chains. The cyclic groups incorporated include thiophene, thiazole, oxadiazole, oxazole or other heterocycles and substituted phenyls. Hundreds of examples are disclosed [139], but no efficacy data are presented.

More recent variants from BASF are the indoles [**5.2**], with one less nitrogen [140] and compounds of type **5.3** where either of the Xs may be nitrogen (and the other CH) [141]. In the latter case, claims are aimed to cover a 2-substituents of mono-, di-, and tricycles, although scant biological data is given to indicate activities. The isomer with the carbamide on the opposite carbon, adjacent to the nitrogen atom, are also included. As is the case with many structure of matter patents, biological data are at a minimum, if present at all. In some instances the same irrelevant data are disclosed in multiple patents on compounds not actually claimed in the patent.

A natural extension of the above is to replace the hydrogen bond that encourages the molecule to adopt the active conformation with a covalent bond that anchors it there. This approach was tried by several groups: Agouron Pharmaceuticals [142,143], BASF [144] and Guilford Pharmaceuticals [145, 146].

Agouron tested several hundred tricyclic compounds based on the indole [142] [**5.4**, **5.5**], and benzimidazole [143] [**5.6**] structures. They found that, as above, substituted phenyl groups at C2 enhanced the potency over the parent (unsubstituted) structures. For example, the parent 1,3,4,5-tetrahydro-azepino[5,4,3-cd]indol-6-one [**5.4**, R = H] has a K_i of 38 nM, whereas many 2-phenyl substituted examples [**5.4 a,b,c**] have K_i of less than 10 nM: the respective K_i for **5.4a** and **5.4b** are 4 nM and 9 nM. Bulkier substituents than these tend towards higher K_i values.

The 4-fluorophenyl derivative [**5.4c**] has a K_i of 4 nM, while the corresponding 4-fluorophenyl derivative of the 1,5-dihydro-[1,2]diazepino[4,5,6-cd]indolin-6-one [**5.5**, R = 4-fluorophenyl] has a K_i of only 1 nM, suggesting a slight advantage in potency for the diazepine variants. This is in accord with similar work by Guilford [145] who used human recombinant PARP: the parent dihydrodiazapinoindolinone [**5.5**, R = H] has a IC_{50} (18 nM) substantially lower than that (366 nM) for the tetrahydroazapinoindolinone [**5.4**, R = H]

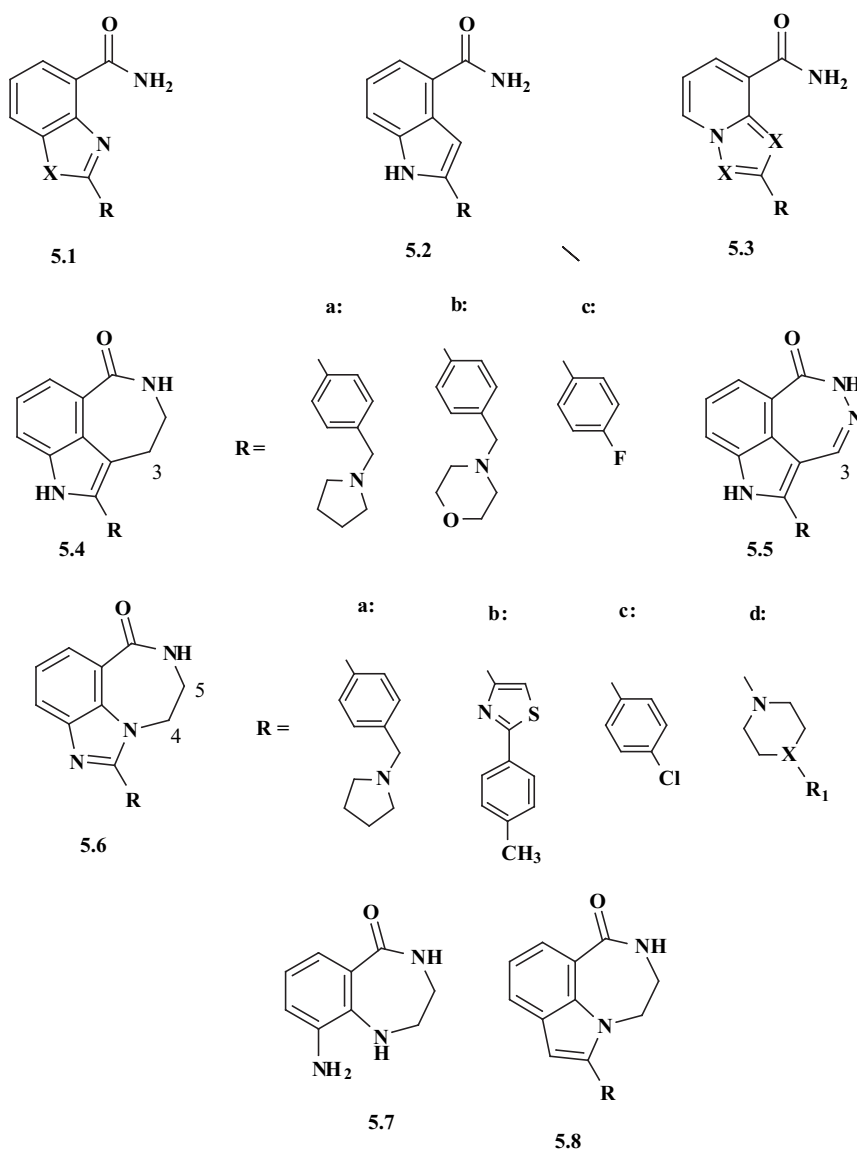


Fig. (5). Structures of benzimidazoles, indoles and related PARP inhibitors.

[145]. However, when the latter has an extra oxo group at C3 on the lactam ring [5.4, =O at C3], the EC_{50} drops by 10 fold to 35 nM. When the indole nitrogen of this dione is substituted with a methyl group, the IC_{50} is about the same (28 nM) [145]. However, a benzyl on the indole N of 5.5 [R=H] gives a $K_i = 13 \mu\text{M}$ [142].

The parent benzimidazole-based 5,6-dihydroimidazo [4,5,1-j,k][1,4]benzodiazopin-7(4H)-one [5.6, R=H] has an IC_{50} of 300 nM [145], similar to that of the indole analogue [5.4, R = H]. Again, many 2-phenyl derivatives show reduced K_i in the low nM range: the respective K_i for compounds 5.6a and b are 5 and 8 nM, indicating that even multicyclic side groups retain the activity of the molecule [143].

Generally those compounds with the substituent on the 7-membered (lactam) ring are not as potent as those with the substitution at the 2-position. However, some 3- substituted dihydroazepinoindolinones (5.5, substituent on the C3) have K_i under 10 nM, e.g. the 3-(4-trifluoromethylphenyl)-

derivative [142]. The orientation of the substituent relative to the plane of the rest of the molecule may have a bearing on this. The substituents at C2 (R in 5.4, 5.5 and 5.6) are connected to a sp^2 carbon that means the C-R bond is in the plane of the ring system. Similarly, the C3 in the dihydroazepinoindolinones [5.5] is also sp^2 . In contrast, substituents attached to the sp^3 carbons of the 7-membered rings of 5.4 and 5.6 will be forced out of the plane of the ring and may interfere with binding within the PARP NAD binding site (see discussion below). This is consistent with the stereoselectivity observed for the azepine ring of 5.6: if a methyl group is introduced at the C5 of structure 5.6c, two optical isomers are possible. The S isomer has a K_i of 5 nM while that of the R-isomer is 113 nM [143]. The degree to which the methyl group projects above the face of the molecule may influence the interactions in the active site. Some of the above Agouron compounds were tested and found to potentiate the cytotoxic effects of the chemotherapy agents topotecan and temozolomide. There was some correlation between K_i and degree of potentiation for some sub-groups of related compounds, but the comparison of

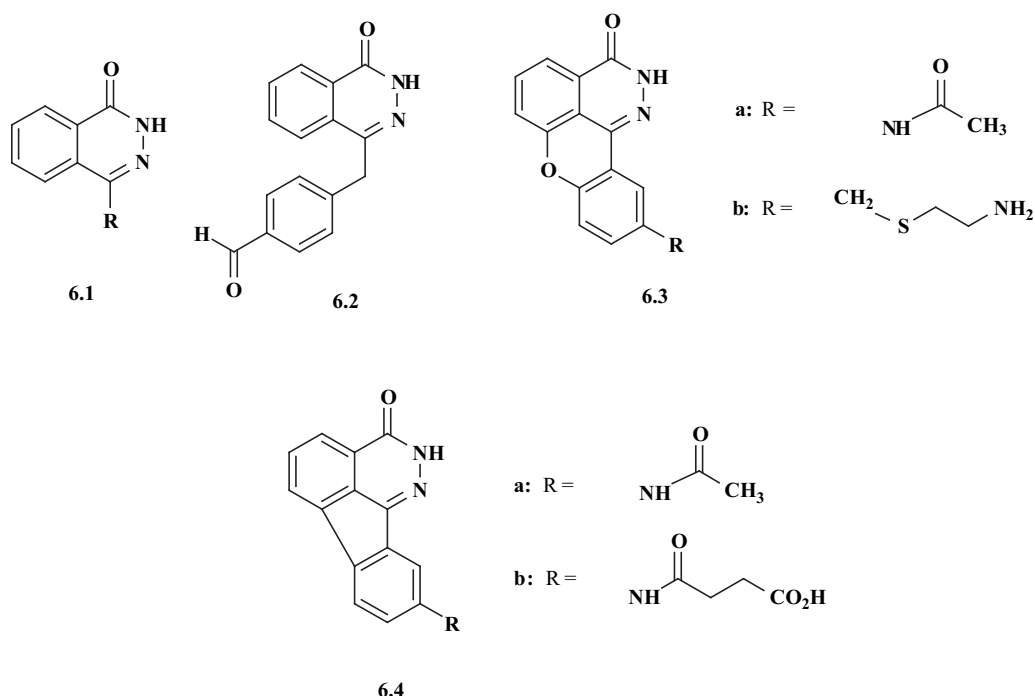


Fig. (6). Structures of phthalazinones and related PARP inhibitors.

enzyme and cell-based assays are prone to complicating factors.

At a recent presentation at the ACS meeting in Boston, Guilford Pharmaceuticals compared the IC_{50} s of a variety of related compounds [146]. They showed that the tricyclic version [5.6, R=H, IC_{50} = 300 nM] was no more potent than the benzimidazole [5.1, X=NH, R=H; IC_{50} = 100 nM], but was 10 fold better than the bicyclic compound without a carbon between the two nitrogens [5.7; IC_{50} = 2.6 μ M]. It was also slightly more potent than the analogue [5.8, R=H,] where a CH replaces the other imidazole nitrogen (IC_{50} =

700nM). If this carbon bears a substituent (e.g. acetyl or carboxamide) the potency is further reduced [143]. Even so, substituted aryl groups in the usual position (R in 5.8) reduce K_i to below 10 nM in some cases [143].

It was found that potency could be further increased for the 2-phenyl substituent (R) of the imidabenzodiazapines [5.6] if it was attached via a, preferably unsubstituted, ethylene bridge (IC_{50} = 26 nM) [146]. This compares to IC_{50} s of 90 and 108 nM if the phenyl is attached directly or via a methylene bridge. The ethylene phenyl compound was shown to be protective in rat models of both transient and

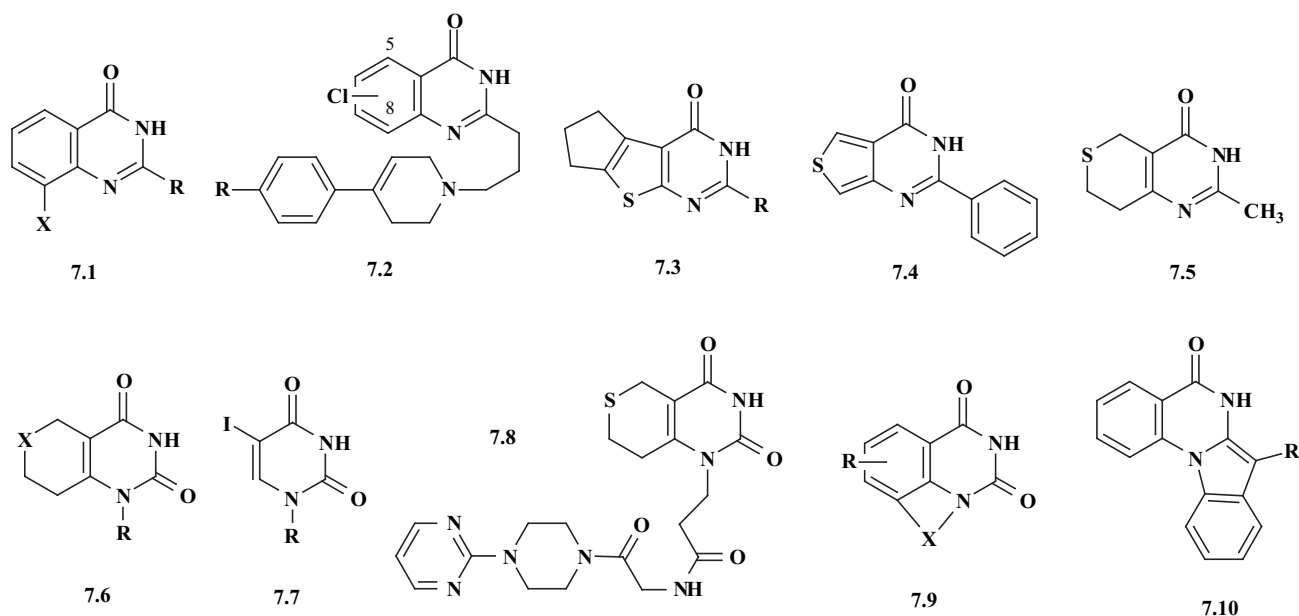


Fig. (7). Structures of quinazolinones and related PARP inhibitors.

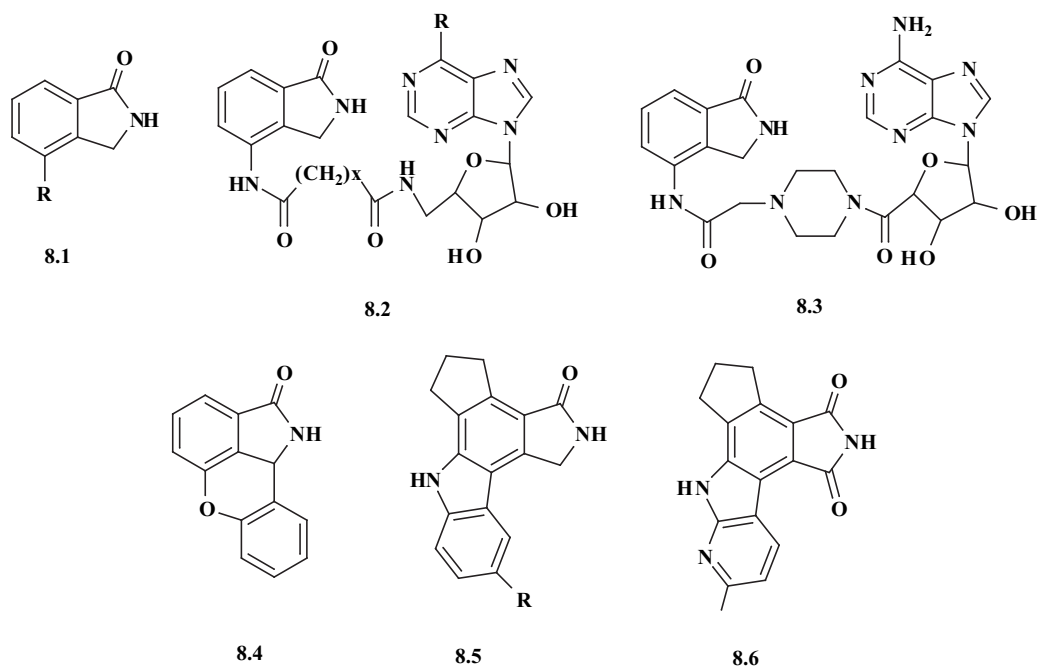


Fig. (8). Structures of isoindolinones and related PARP inhibitors.

permanent ischemia at doses of 40 mg/Kg [146]. These and other, on the whole, less potent examples are found in a Guilford filing [145]. However, amongst these are a naphthyl derivative linked via the naphthyl C2 (alpha-naphthyl, Fig 5.6, R= 2-naphthyl, IC₅₀= 32 nM) and the isomer linked via the naphthyl C1 (Beta-naphthyl, IC₅₀= 382 nM) which is 10 fold less active. Other derivatives where R is a 4-substituted piperazine or piperidine [5.6d, X = N or CH] have also been disclosed by Sanofi-Synthelab [147]. Although compounds where the 4-substituents [R₁] include alkyl, phenyl and heterocycles, are disclosed, the *in vitro* efficacies are reported (for "les composés les plus actifs") as "between 5 and 500 nM" for both PARP-1 and PARP-2.

Several other structural permutations are disclosed in the above patents with additional nitrogens, for example, in the 2-position of structures 5.4 and 5.5, but no data are presented on cellular or *in vivo* activity [145].

Phthalazin-1(2H)-Ones and Quinazolinones

Phthalazin-1(2H)-one [Fig. 6, structure 6.1, R=H] was reported by Banasik to have an IC₅₀ of 12 μM [93]. Iconox Pharmaceuticals [148] reported a EC₅₀ of 100 μM when they used an assay based on the inhibition of growth of yeast cells when expressing a PARP vector. The EC₅₀ improved somewhat to around 40 μM when R [6.1] was methyl or ethyl, but these were no better than phenanthridinone (10 μM).

However, 4-aryl substituted compounds appear to be better; Kudos Pharmaceuticals [149], using mammalian PARP from the nuclear fraction of HeLa cells, tested 4-benzyl analogues [6.1, R = substituted benzyl]. Most (of 52 tested) had IC₅₀s between 300-2000 nM (cf Phthalazinone=7.2 μM in the same assay), but 3,4-difluorobenzyl phthalazin-1(2H)-one (70 nM) and compound

6.2 (40 nM) were exceptions. Those examples tested enhanced the inhibitory effects of the anticancer agent bleomycin on cell growth.

Other groups to work on these compounds include Warner-Lambert (now Pfizer) [101, 102] who disclosed 5-methoxy-4-methyl-1(2) phthalazinone with an IC₅₀ of 80 nM, and ONO Pharmaceuticals [150]. ONO claimed phthalazinones substituted in the 4-position with phenyl groups bearing amine-containing side chains to improve water solubility, while maintaining potency.

Other substituents tested at position C4 [6.1, R] include heterocycles linked directly or via alkyl chains [151] and 4-substituted piperazines [152].

Further variations include a series of tetracyclic benzopyrano[4,3,2-de]phthalazinone [6.3] and indeno[1,2,3-de]phthalazinone [6.4] compounds [153]. Here, the 4-phenyl group is held rigid by linking to the 5- position either via an oxygen [6.3] or directly [6.4]. The basic unsubstituted core molecules [145] have IC₅₀s of 80 nM [6.3, R=H] and 140 nM [6.4, R=H]. The value for phenanthridinone [9.1] was 350 nM in this assay. Modest improvements were made with substituents in the 10 position: 6.3a has an IC₅₀ of 37 nM and 6.4a = 69 nM. In the cases where R incorporated the usual cyclic amines (piperazine, morpholine etc.) good water-solubility was claimed [154], probably the goal of the exercise for such compounds. In the corresponding patent filings [153,155], data for a further 40 compounds are disclosed. These are all substituted at the 10 position (this becomes the 9- position for the indenophthalazines, 6.4) with substituents that include sulphonamides, heterocycles and alkyl chains. Many IC₅₀s were below 100 nM and the lowest 11 nM. The more efficacious substituents include several 2 - 6 atom chains that terminate in an acid or an amine group: 6.3b and 6.4b had IC₅₀s of 28 and 29 nM.

The tricyclic compounds of type **5.5**, discussed above, may also be considered as a variation on the phthalazinone core.

Juxtaposition of the second ring nitrogen of phthalazinones to give the quinazolin-4-one derivatives maintains the potency. As reported by Banasik [93], quinazolin-4-one itself [**7.1**, X=H, R=H] and quinazolin-2,4-dione [**7.1**, X=H, R=OH] are moderate inhibitors of PARP with IC₅₀s of 9.5 and 8 μM respectively. Simple 2-methyl and 2-phenyl compounds (**7.1**, X=H, R= methyl etc.) have IC₅₀s of around 200 nM or above, with some tendency for the phenyl to have reduced activity vs. methyl for given 8-substituent [X, **7.1**] [156,157]. However, electron withdrawing groups in the 4-position of the aryl ring substituent do increase potency against PARP enzyme [158,159]. As with the dihydroisoquinolines (above), 8-hydroxy and 8-methyl derivatives [**7.1**, X = OH, CH₃] exhibited greater activity than the 8-methoxy analogues [X = OCH₃], regardless of 2-substituent. Consequently, the most potent examples reported were the 8-methyl-quinazolinones [**7.1**, X = CH₃] with a 2-phenyl group [R] which was substituted with a 4-nitro, 4-cyano or a 4-methoxy group. All had EC₅₀s below 300 nM. Of this series, 8-hydroxy-2-methylquinazolin-4(3H)-one (NU 1025; **7.1**, X=OH, R= methyl; IC₅₀ 400 nM) was tested and found to be far more effective (>10x) than 3-AB at potentiating the effects of gamma radiation and alkylating agents [158,159]. However, concentrations of 100 μM were required to see a significant effect.

The inherent problem of limited water solubility of these compound was addressed by derivatizing the 8-hydroxy group (and a hydroxy phenyl group if present at C2) to phosphate groups and then forming the water soluble ammonium or sodium salts. These can then be used to give prodrugs for parenteral administration [160,161].

Fujisawa Pharmaceuticals [162] also describe 2-substituted quinazolines, but with more elaborate side chains incorporating heterocycles and fused ring systems as well as chlorine at positions C5 or C8 [**7.2**]. For the 4 examples reported as tested for potency, the EC₅₀s are quoted as "< 0.5 μM". One compound [**7.2**, R=H, Cl at C5] was tested and shown to be effective in preventing the reduction of striatal dopamine (and metabolites) in response to 1-methyl-4-phenyl-1,2,3,6-tetrahydropyridine - an experimental model for Parkinson's disease.

Some examples where the ring attached to the carbamoyl deviates from the commonly employed aryl, include compounds **7.3** and **7.4**. Iconix [148] claimed **7.3** (R=H) as showing selectivity for PARP1 over PARP2, with EC₅₀s of 5.5 and 41 μM respectively. If R= ethyl, the selectivity is lost as the EC₅₀s are the same (70 μM). Of the thieno[3,4-d]pyrimidin-4(3)ones, the most potent example [**7.4**] gives 93% inhibition of PARP (mouse) at a concentration of approx 10 μM [163].

Meiji Seika Kaisha [164] describe pyrimidinones fused to a saturated heterocycle, with EC₅₀s in the 100s of nM range [**7.5**]. So, too, do Bayer [**7.6**], but their compounds have the additional keto group to give compounds derived

from uracil [165,166]. Incidentally, uracil derivatives were found by Banasik [93] to have weak inhibitory effects on PARP: iodouracil (5-iodo-2,4-pyrimidindiol, **7.7**, R =H) had an IC₅₀ of 70 μM and iodouridine (**7.7**, R= ribofuran) was slightly better (43 μM). Due to the additional oxo group in the Bayer compounds, functionalization is at N1 [**7.6**]. It was found that the compounds bearing a ring system connected via a beta-alanine-glycine spacer gave an optimum potency. The attached ring system leading to the most potent compounds were either substituted phenyls or piperazines. Also, the 3,6-dihydro-2-thiopyrane subunit (**7.6**, X=S) resulted in a 3-10 fold increase in potency compared to a cyclohexyl moiety (X=CH₂). For such compounds [e.g. **7.8**] IC₅₀ values were consistently below 100 nM against recombinant human PARP. Corresponding EC₅₀ values in a cell protection assay were in the order of 10 times higher [165,166].

Tricyclic compounds incorporating the uracil ring [**7.9**] are disclosed by Sumitomo pharmaceuticals [167] and Novartis have also come up with their variation with the indoloquinazolines [**7.10**] [168]. Again, data are scant, but the preferred compound 7-(1H-tetrazol-5-yl)-6H-indolo[1,2-a]quinazolin-5-one [**7.10**, R = 5-tetrazole] has an *in vitro* IC₅₀ of 12 nM and reduces infarct size in a rabbit model of myocardial infarction if given 5 minutes before reperfusion in the dose range of 1-22 mg/kg (60% reduction at 22 mg/kg).

Isoindolinones

Isoindolinones incorporate the requisite lactam in a five membered ring. The simple 5-substituted derivatives (**8.1**) have only poor potency (EC₅₀ > 10 μM) when tested on a cell-protection assay, but more elaborate side chains improve the effects [169]. This patent application discloses a series of homologues, where the isoindolinone is linked to adenosine with a alkyl chain of varying length [**8.2**, x=1 to 8, R = NH₂]. The potency is maximal when x = 2 or 6. A phenyl ring incorporated into the linker reduces the apparent potency, but the piperazine analog EB-47 [**8.3**] shows 100% inhibition at 100 nM. This compound exerts cytoprotective effects in oxidatively damaged cells, and shows protection in *in vivo* models of reperfusion injury and inflammation. The striking difference in potency between the weak isoindolinone core, the adenosine derivatives with the C6 alkyl chain (**8.2**, x = 6, R= NH₂; 98% inhibition at 10 μM) and its inosine analogue (**8.2**, R = O, X = 6; 68% inhibition at 100 μM), suggests that the adenosine is recognised by a pocket within the active site, as may be expected to exist for the binding of NAD⁺. Indeed, in reviewing recent crystallographic data, Rolli and colleagues [170] postulated two hydrogen bonding interactions between the adenosine of NAD⁺ (see Fig. 1) and the NAD⁺ binding site of PARP. These are via the primary amino group and the adjacent ring nitrogen (N1) of adenosine. This is consistent with the weak inhibition of PARP *in vitro* by adenosine itself and, to a lesser extent, by hypoxanthine and inosine, which do not possess the 6-amino function [171]. This work illustrates the statistical synergism of having two discreet binding functions on the same molecule.

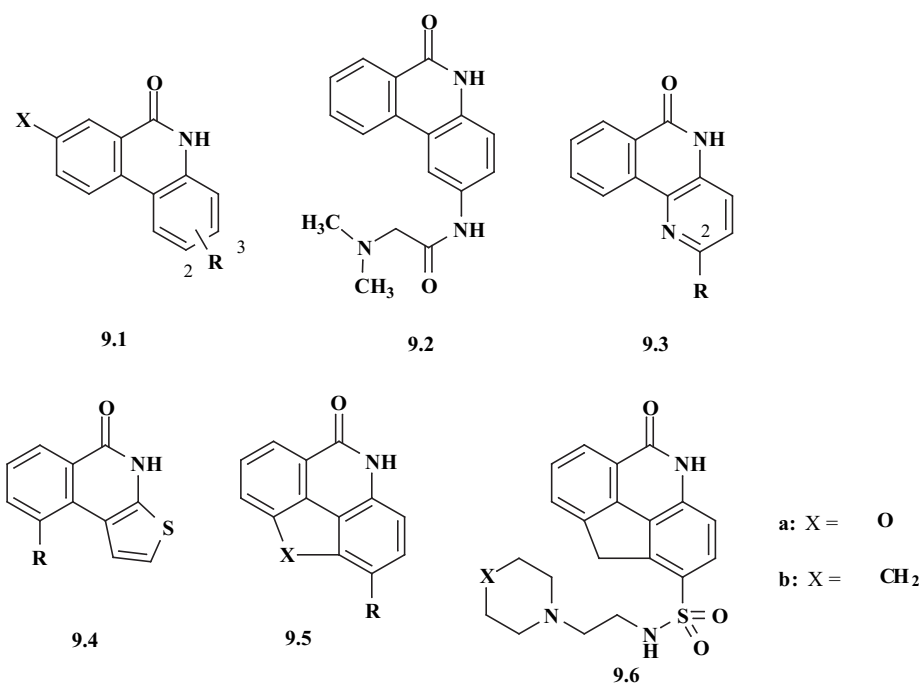


Fig. (9). Structures of phenanthridinones and related PARP inhibitors.

Guildford have claimed the tetracyclic isoindolinone [8.4] [172] for use in peripheral neuropathy caused by stroke, trauma, spinal cord damage, ischemia, reperfusion injury, neurodegeneration etc. Specific data for this compound are not disclosed (even though it is the only compound claimed), but the IC_{50} against human PARP is between “a few nM to 20 M”

Polycycles incorporating the isoindolinone group (8.5) have since been published [173]. IC_{50} data are given for over 200 compounds, the vast majority of which have IC_{50} s below 100 nM. Despite this, there still seems to be some sensitivity to the substituent R: when R goes from methyl to cyano, the reported EC_{50} drops from 800 to 18 nM. When an additional keto group is adjacent to the ring nitrogen to give the imide, the potency is enhanced. The example given in [8.6] has a reported IC_{50} of 2 nM.

Phenanthridinones

A variety of substituted 5[H]phenanthridin-6-ones have been reported as PARP inhibitors [174-178] with activities up to 100-fold greater than the unsubstituted parent phenanthridinone [Fig. 9, 9.1, X=R=H] as reported by Banasik (IC_{50} = 300 nM) [93].

The 2- and 3- positions [9.1, R] appear to be the preferred points of attachment for substituents, with their electronic nature having no clear influence on potency. Additionally, large aliphatic substituents (alkylmorpholine, piperidine) at these positions can result in quite potent compounds (~30 nM) suggesting that the enzyme is quite tolerant in this area and that the hydrophobic surface area enhances binding. The reverse is true on the other end of the molecule where bulky 8-substituents [9.1, X] diminish the inhibition of PARP1, and only the small fluoro [X=F] actually enhances potency at that position [174,178]. The introduction of an acid or amino containing side chain at the 2 or 3 position has the dual benefit of increasing water solubility as well as increasing potency over the parent molecule.

Inotek Pharmaceuticals Corporation has published extensively on one such compound, PJ-34 [9.2] [178]. PJ-34, with an EC_{50} of approximately 40 nM, restores the viability of isolated murine thymocytes exposed to cytotoxic concentrations of oxidants [178]. The compound also induces a dose-dependent suppression of pro-inflammatory cytokine and chemokine production in immunostimulated macrophages, although the required concentrations of PJ-34 in this system are somewhat higher (5-10 μ M) [178]. As far as *in vivo* models are concerned, PJ-34, in the dose range of

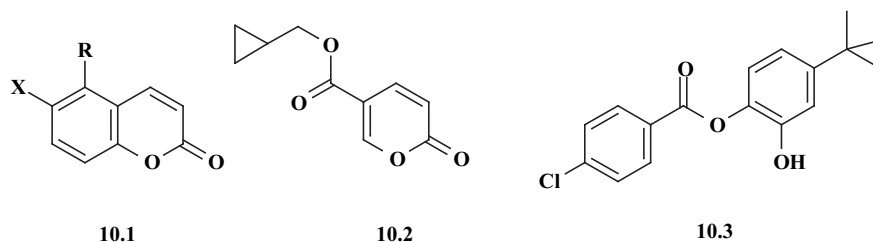


Fig. (10). Structures of miscellaneous PARP inhibitors.

3-30 mg/kg/day, has been shown to reduce cell necrosis and improve functional outcome in stroke [179], myocardial infarction and reoxygenation injury [180,181], circulatory shock [178,182], diabetes and its vascular complications [183-185], colitis [185], arthritis [185] and uveitis [185]. PJ34 also protects in various forms of chronic heart failure and cardiomyopathy resulting from cytotoxic drugs [186], ischemia [187] and diabetes [188]. PARP inhibition with PJ34 also improves endothelium-dependent vascular relaxant function in various models of vascular injury, including aging, hypertension, diabetes and chronic ischemic heart failure [187-190].

Many of the examples, including PJ-34, link the substituent group to the phenanthridine core via an amide nitrogen. In a recent patent filing [175], Guilford claim sulphonamides (**9.1**, R = -SO₂NH-R₁) and the sulfonic acid derivative (R = -SO₃H) which has an IC₅₀ of 10 nM, with or without an 8-fluoro substituent (X = F or H).

Replacement of C1 with a nitrogen yields a series [**9.3**] of which only a few examples have IC₅₀s comparable to the phenanthridinones discussed above [145]. When R = H, hydroxy or piperazine, IC₅₀s are 120, 114 and 45 nM respectively. Other examples where R is a saturated heterocycle (or two) are disclosed with IC₅₀s below 100 nM. The isomer with the second nitrogen at C2 is more potent (IC₅₀ = 42nM) than the one illustrated (**9.3**, R = H, IC₅₀ = 120nM).

SmithKline Beecham [121] have prepared a limited number of thieno[2,3-c]isoquinolines [**9.4**]. The most potent against PARP (purified from fetal calf thymus) is the 9-amino- (R = NH₂) with an IC₅₀ of 50 nM. The hydroxy and methoxy derivatives (R = OH and OCH₃) had IC₅₀s of 100 and 300 nM respectively (the value for phenanthridinone in the same assay was 12 μM). The unsubstituted compound (R = H, IC₅₀ = 300 nM) was, apparently, very effective at reducing post-ischemic neuronal death in primary cultures and also reduced necrotic brain in a permanent middle cerebral artery occlusion model of stroke in rats at 3-10 mg/kg IP [121].

Cyclopenta[imn]phenanthridine-5(4H)-one derivatives [**9.5**] have also been described [191,192] and show improved inhibitory capacity over phenanthridine-5-one. From the small sample of variants of X tested (**9.5**, R = H), X can be CH₂ (IC₅₀ = 100 nM), C=O (70 nM), CHOH (120 nM) or NH while retaining good activity. If X is CHOC(O)CH₃ activity is diminished eight-fold. Various substituents (R), including cyclic compounds, linked via sulphonamide or amide functions, reduce IC₅₀s to between 40 – 70 nM (**9.5**, X = CH₂). Compounds **9.6a** and **b** have IC₅₀s of 70 and 50 nM.

Miscellaneous Classes of Compounds

A recently published patent filing from Guilford [193] filed in 1998, claims a broad range of compounds, including some of the bicyclic, tricyclic and tetracyclic compounds discussed above. With very few specific examples disclosed, the patent claims their use in treating stroke,

neurodegenerative disease, cardiovascular, renal disorders, AIDS and renal failure.

While much development centered around the lactam/carboxamide pharmacophore, there are several groups of compounds which do not possess this functionality. Most of these are inhibitors of only moderate potency compared to those discussed above, but less effort has been directed towards their optimisation and elucidation of mode of action. That said, 1,2-benzopyrone [**10.1**, X = R = H] was reported as a non-competitive inhibitor of PARP [194]. It was subsequently proposed that the 6-nitroso benzopyrone [**10.1**, X = NO, R = H] inhibits PARP by interacting with the DNA binding zinc finger in the N-terminal domain of PARP [195]. The compound 5-iodo-6-aminobenzopyrone (INHBP; **10.1**, X = NH₂, R = I; IC₅₀ = 10 μM) has been shown to protect oxidatively damaged cells *in vitro* [15, 196] and exerts beneficial effects in stroke [197], circulatory shock [196] and autoimmune diabetes [34]. Some of the above referenced benzopyrone derivatives also exert anti-HIV effects *in vitro*, although the relationship between PARP inhibitory and anti-HIV actions has not yet been clarified [198,199]. Some coumalic acid derivatives, also containing the lactone function, have potencies in the same region. One of the better ones with an IC₅₀ under 10 μM is illustrated in [**10.2**] (unpublished observations).

As mentioned previously, early work showed that thio analogues of carboxamide compounds were very weak inhibitors of PARP. Guilford have claimed thio analogous to the isoquinolines and tetrahydroisoquinolinones. Although the thio compounds were claimed in a US patent application, the only biological data disclosed relate to the parent lactams [200].

Several patents from N-Gen Kutato KFT disclose hydroxamic acids and amidoximes [201,202] as inhibitors of PARP. Biological data on the amidoxime BGP-15 have been discussed in a preceding section of the current review.

Guilford have reported some ortho diphenyl compounds containing a 4-t-butylbenzene-1,2-diol fragment that is, apparently, required for activity [203]. This is linked to an aryl carboxylic acids as shown in [**10.3**]. This example has an IC₅₀ of 2.2 μM.

CRYSTAL STRUCTURE STUDIES

The empirical SAR findings listed above have been rationalized by X-ray crystallographic studies that have studied the binding of the substrate, NAD⁺, and inhibitors to the active site of PARP in the crystalline state. The studies confirm that those inhibitors possessing the carbamoyl pharmacophore do, indeed, compete for binding in the same pocket as the nicotinamide portion of NAD⁺ and indicate the likely interactions of the molecules with the pertinent residues.

Ruf *et al.* [204-206] expressed the C-terminal fragment of chicken PARP, which bears a close homology to the human PARP, and studied the binding of inhibitors to the NAD⁺ binding site. Although attempted, crystallization

with NAD⁺ was not possible, but the investigators surmised from modelling experiments and from analogy to other closely related enzymes that there is a well defined subsite within the NAD⁺ binding site that accommodates the nicotinamide portion of NAD⁺. This is the subsite to which the competitive PARP inhibitors bind. Certain conserved interactions were observed between the nicotinamide binding cleft and inhibitors such as the 5-methyl isoquinolinone [4, R= CH₃] and 3-methoxybenzamide [206]. All showed H-bonding interactions between the amide or lactam function of the inhibitor and the nitrogen and oxygen of GLY 863 and SER 904 on the protein (Fig. 1). In addition, all showed non-polar interactions with the aromatic ring of TYR 907 and, to some degree, TYR896, which lines the other face of the pocket. 3-Methoxy-benzamide did not exhibit as extensive an interaction with these tyrosine residues as do the bi- and tri-cyclic inhibitors tested. In addition, NU 1025 [7.1, X=OH, R = CH₃] and 4-amino-naphthalimide [4.4] also have additional H-bonding [204]. Crystallographic data for the binding of the 2-(3-methoxyphenyl)-1(H)-benzimidazole-4-carboxamide [5.1 R=3-methoxyphenyl, X=NH] compound [134] show similar interactions of the benzimidazole portion within the deep binding pocket, while the phenyl side group is largely exposed to solvent (on both faces). The methoxy group at the 3 position of the phenyl is accommodated by space, whereas 4 and 5 substituents would be less well accommodated.

After extensive modelling, involving docking experiments and QSAR studies of 42 inhibitors found in the literature, Constantino and colleagues [207] concluded that, beyond the requirement for the carboxamide/lactam group, "potency seems to be given by the extension of the contact surfaces between inhibitor and enzyme, although the presence of H-bonding forming groups can increase the affinity within a homologous series of compounds." This seems to be consistent for the majority of inhibitors studied.

Ferraris and colleagues also found, in their modelling, increased potency for an extended planar structures even those extending into a hydrophobic pocket adjacent to the nicotinamide sub-pocket [146]. The ASP766 and GLN763 amino acid residues appear to form part of this hydrophobic pocket [146].

Although there is little difference in structure between the ligated and non-ligated protein, there is some general increase in order upon ligand binding among those sub-structures that contribute to the binding site.

GENERAL DISCUSSION

The work of the last few years has seen the emergence of an array of compounds based around the carbamoyl function that appears to be so critical for potent inhibition of PARP. Ten- to hundred-fold increases in potency have been achieved over the compounds described by Banasik ten years ago [93]. It is noteworthy that increased potency can be considered a good starting point for the pharmaceutical development of PARP inhibitors, but a variety of other important factors (selectivity, toxicity, bioavailability,

metabolism, *in vivo* efficacy, etc.) must also be considered. While information on potency can usually be found in many of the publications and in some patent filings, information on these additional factors is scarce. For example, there is practically no information available on the toxicity of the various novel PARP inhibitor compounds, and detailed efficacy data are only available for a handful of compounds. It is important to note that the increase in potency is associated with the emergence of various inherent practical problems. The extended planar cyclic systems that enhance binding of inhibitors to the enzyme - as rationalized by crystallography and modelling experiments - also reduces their solubility in water. Planar cyclic systems are also prone to DNA intercalation and are associated with increased potential risk of genotoxic effects. Many of the novel PARP inhibitor structures can tolerate a variety of side chains and substituents that can be chosen to enhance solubility, alter pharmacokinetic and toxicological characteristics and allow continued patenting and therefore development by pharmaceutical companies.

Despite extensive work in the area of PARP over the last decade, and the multitude of indications for the pharmaceutical inhibition of PARP (including many severe, life-threatening indications), there are no drugs on the market, and - judged from the publicly available information - not even in clinical testing. This slow progress is in contrast to many other areas of drug development, where identification of a novel target was followed by the emergence of approved and marketed drugs within 10 years or so (see the example of the selective COX-2 inhibitors). Based on the available data on the progress with the preclinical development of various PARP inhibitors at pharmaceutical companies, we predict that PARP inhibitors will be tested in clinical trials starting in 2003 or 2004.

The relatively slow progress may be related to the inherent difficulty in targeting the PARP enzyme, and/or the suboptimal safety profile of the classes of compounds that entered early clinical development. As mentioned above, many planar structures that are inhibitors of PARP can have DNA-binding and DNA-intercalating properties. This may represent an example of a non-mechanism based side effect that may be relevant for some classes of compounds (but not others). PARP inhibitors, in theory, may also interfere with the binding of NAD to other enzymes that require NAD for their function. Such interference may result in various untoward effects. One must also keep in mind that multiple studies indicate that PARP has important protective functions in terms of DNA repair. Interfering with such a function may be acceptable for the therapy of acute, life-threatening indications (such as stroke, myocardial infarction, circulatory shock, chronic heart failure or cancer), where the therapeutic alternatives are limited or only marginally effective. On the other hand, for long-term inhibition of PARP, the risk/benefit ratio of PARP inhibition must be carefully considered, especially for indications that have a smaller incidence of fatal outcome (arthritis, diabetes, diabetic complications), and where alternative therapies are available or expected to emerge soon.

How strong, then, is the evidence for the function of PARP as the "guardian angel" of the genome? This issue has

been discussed by multiple authors over recent years [e.g. 1,3,4,8,9]. PARP deficient mice were found to be more sensitive than wild-type animals to ionizing radiation or monofunctional alkylating agents [208]. Furthermore, embryonic fibroblasts derived from PARP^{-/-} exhibited a reduced rate of proliferation after exposure to various genotoxic compounds than did PARP^{+/+} mice [208, 209]. PARP deficient cells also exhibited genomic instability as evidenced by an increased number of micronuclei (chromatin fragments indicating chromosomal damage) in response to genotoxic agents. In addition, PARP^{-/-} fibroblasts exhibit slower rejoining of DNA breaks. It was also found that lysates from PARP-1 deficient fibroblasts had no long patch repair system activity and their short patch repair system activity was also reduced by about 50%, as compared to PARP-1 proficient cell lysates [210]. Based on these data the conclusion can be drawn that PARP-1 contributes to the maintenance of genomic integrity and also enhances base excision repair in irradiated or alkylating agent-treated cells. The involvement of PARP-1 in genomic surveillance is also indicated by the interaction of PARP-1 with other nick sensors such as DNA ligase III, adaptor factors such as XRCC1, and DNA repair effectors (e.g. DNA polymerase β and DNA ligase III) [211]. Regulation of the activity of these proteins by PARP-1 is carried out both via physical interaction and poly(ADP-ribosylation). The results obtained from studies using PARP deficient experimental systems usually do not distinguish between findings related to the physical absence of the enzyme – i.e. “scaffolding” functions, versus the lack of PARP’s catalytic activity – i.e. the “enzymatic” function. Because of the reasons mentioned above, it is not surprising that the findings obtained with PARP deficient cell lines do not always or necessarily mirror the findings seen with chronic treatment with PARP inhibitors. For example, it has been shown that PARP-deficient cells have disturbed cell cycle progression and contain a tetraploid population, a finding that could not be reproduced in wild type cells with pharmacological PARP inhibition using GPI-6150 [212,213].

As mentioned above, the marked beneficial effects of PARP inhibitors in many animal models of various diseases suggest that PARP inhibitors can be exploited to treat human diseases. However, before potent PARP inhibitors should be used to treat chronic diseases in humans, several crucial safety issues must be addressed. As PARP has been implicated in DNA repair and maintenance of genomic integrity, one possible risk associated with chronic PARP inhibition is an increased mutation rate and cancer rate. It is important to point out in this respect that PARP-deficient mice have not been reported to have increased occurrence of spontaneous tumors. However, an increased number of chemically-induced tumors has been observed in PARP-deficient mice as compared to wild type ones [214]. The crossing of the PARP deficient mice with the p53 deficient mice was recently conducted by two independent groups and yielded conflicting results [215,216]. Carcinogenesis studies have not yet been published in mice chronically treated with PARP inhibitors. It is possible that enhanced environmental carcinogenesis is due to the lack of PARP protein, but not to decreased poly(ADP-ribosylation), as several examples showed dissociation of these two functions (see above). If poly(ADP-ribosylation) is required to prevent

carcinogenesis, appropriate dosage may provide a level of PARP inhibition sufficient to improve disease signs with sufficient residual PARP activity to facilitate DNA repair. When considering the risk/benefit ratios associated with the development of PARP inhibitors for therapeutic purposes, clear distinctions must be made between acute versus chronic treatments, as well as between life-threatening diseases versus other disease indications.

The discovery of new poly(ADP-ribosyl)ating enzymes (also termed “minor PARP isoforms”) presents new challenges and opportunities to the field. Based on their intracellular localization, dependence or non-dependence on DNA damage for activation, the novel PARP enzymes are likely to have distinct biological functions. It is not known at present, how PARP inhibition therapy affects the function of these minor isoforms and whether or not inhibition of these isoforms contributes to the well-established therapeutic effects (or possibly to the side effects?) of PARP inhibitors. Bearing in mind that results of most pharmacological studies could be reproduced by using PARP-deficient animals and cells, we can conclude that PARP-1 is the major target of PARP inhibitors in disease. Whether the development of *PARP-1 selective* PARP inhibitors is necessary, given the small amount of PARP isoforms other than PARP-1 in the cell, and/or feasible, considering the highly conserved structure of the NAD⁺ binding site across the various PARP isoforms, remains to be determined by future studies.

NOTES ADDED IN PROOF

1. A recent publication from Pfizer/Agouron [217] describes selected examples of compounds [5.4] originally disclosed in the Agouron patent [142].
2. A recent patent [218] describes compounds very similar to those Fig. 8.5 as inhibitors of inflammation, but without specifically mentioning PARP.

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