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Phthalazinones. Part 1: The design and synthesis of a novel series of potent inhibitors of poly(ADP-ribose)polymerase

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Abstract—Screening of the Maybridge compound collection identified 4-arylphthalazinones as micromolar inhibitors of PARP-1 catalytic activity. Subsequent optimisation of both inhibitory activity and metabolic stability led to a novel series of *meta*-substituted 4-benzyl-2*H*-phthalazin-1-ones with low nanomolar, cellular activity as PARP-1 inhibitors and promising metabolic stability in vitro.

The mammalian enzyme poly(ADP-ribose) polymerase-1 (PARP-1) has been implicated in the repair and signalling of DNA damage through its ability to recognise and rapidly bind to DNA single or double strand breaks.[1] Once associated with DNA, activated PARP-1 utilises nicotinamide adenine dinucleotide (NAD^+) to synthesise poly(ADP-ribose) homopolymers onto a variety of nuclear target proteins, most notably itself.[2] It has been postulated that the formation of these negatively charged polymers causes electrostatic repulsion of modified PARP-1, preventing DNA recombination. It is further hypothesised that modified PARP-1 facilitates recruitment of the base excision repair complex to the site of the DNA damage.[3]

PARP-1 activation and subsequent poly(ADP ribosyl)-ation are immediate cellular responses to chemical or radiation-induced DNA damage.[4] Studies have shown that inhibition of PARP-1 activity enhances the effects of radiation by suppressing the repair of potentially lethal damage in cancerous cells.[5] Inhibition of PARP-1 in cells treated with alkylating agents similarly causes enhanced DNA damage and cell killing.[6] PARP-1 activation has also been shown to play a pivotal role in the development of septic shock, ischaemic injury and in neurotoxicity.[7,8] Inhibitors of PARP-1 catalytic activity may therefore have wide-ranging therapeutic potential.

Previous investigators have designed inhibitors of PARP-1 to mimic the substrate–protein interactions of NAD^+ with the enzyme. Mechanistically, these compounds inhibit PARP-1 by blocking the binding of the substrate, particularly the nicotinamide moiety, to the active site of the enzyme. Early weak inhibitors such as 3-aminobenzamide **1**[9] have been developed into more potent PARP-1 inhibitors derived from a range of related pharmacophoric templates, for example the 5-substituted dihydroisoquinolin-1-ones **2**,[10] 2,8-disubstituted quinazolin-4-ones **3**[11] and benzoxazole-4-carboxamides

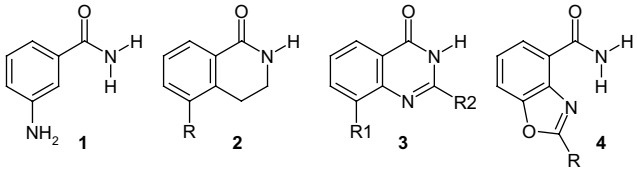

Figure 1. Previously reported PARP-1 Inhibitors.

Keywords: Phthalazinone; PARP; Poly(ADP)polymerase.
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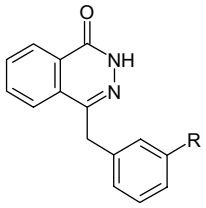
4.[11] In this paper, we describe the synthesis and preliminary biological evaluation of novel 4-benzylphthalazinones as potent inhibitors of PARP-1 (Fig. 1).

High throughput screening of the Maybridge Screening Collection in a FlashPlate scintillation proximity assay[12] identified several 4-aryl-2*H*-phthalazinones as low micromolar PARP-1 inhibitors. These appeared a promising start point, since phthalazinone itself is a known PARP-1 inhibitor.[13] Initial hit exploratory chemistry[14] led to 4-benzyl-2*H*-phthalazin-1-one **5** with a potency against human PARP-1 of IC_{50} 0.77 μM (Table 1). Early SAR studies, backed by structure-based design utilising a homology model derived from the known crystal structure of chicken PARP-1,[15] suggested that further structural elaboration around the *meta* position of the benzyl moiety in **5** could be potency enhancing. In particular, carbonyl-containing substituents at the 3-position led to potent PARP-1 inhibitors exemplified by **6** and **7**. These observations encouraged the synthesis of focused analogue libraries using parallel synthesis methodologies. Preliminary SAR development showed that chain extension to the propionanilide **8** significantly increased PARP-1 inhibitory potency (IC_{50} 20 nM). While further chain extension (**9**) or branching (**10**) tended to reduce potency, constraining the branch into a cyclopropane ring (**11**) reversed this trend. A number of phthalazinones bearing different biaryl substituents at the *meta* benzylic position (**12**–**15**) were also shown to be potent PARP-1 inhibitors. Propionanilide **8** exhibited good PARP-1 inhibitory activity at 1 μM in a cell-based assay (data not shown) making it a suitable candidate for continued optimisation chemistry. However, preliminary pharmacokinetic studies raised concerns that **8** would be rapidly cleared since in vitro it exhibited poor metabolic stability (mouse hepatic microsomes: Cl_i 12 mL min^{-1} g^{-1} liver). It was postulated that the pendant anilide was the most likely point of metabolic instability. Therefore, several structural modifications were undertaken in an attempt to stabilise this group. Trifluoro-substitution α to the carbonyl to give **16** retained potency against isolated PARP-1 enzyme (IC_{50} 13 nM), but reduced in vitro metabolic stability (mouse hepatic microsomes: Cl_i > 50 mL min^{-1} g^{-1} liver). Several bioisosteric amide replacements, such as carbamate **17**, urea **18**, sulfonamide **19** and 1,2,4-oxadiazole **20**, were detrimental to PARP-1 inhibitory potency. However, reversal of the anilide topology in **8** to give amide **21** retained PARP-1 inhibitory potency and considerably enhanced metabolic stability in vitro (mouse hepatic microsomes: Cl_i < 1 mL min^{-1} g^{-1} liver). This compound was selected as a primary candidate for the development of a further novel series of potent PARP-1 inhibitors, which will be reported in detail in due course.

An alternative approach to stabilising the anilide moiety in **8** was to constrain the amide into a ring, as in lactam **22**. This resulted in a moderate increase in metabolic stability (mouse hepatic microsomes: Cl_i 4.9 mL min^{-1} g^{-1} liver), but a reduction in potency. The introduction of a second carbonyl group into the lactam ring resulted in imide **23** (Table 2), which exhibited a 10-fold increase

Table 1. Initial structure–activity relationships of the *meta*-substituted 4-benzyl-2*H*-phthalazin-1-ones



	R	IC_{50} (nM)
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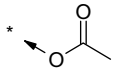
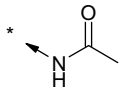
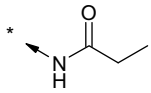
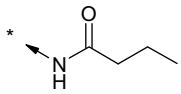
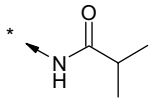
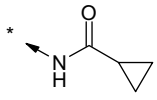
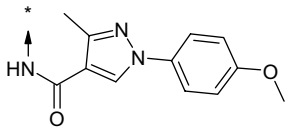
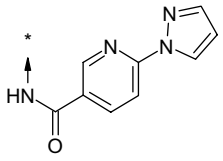
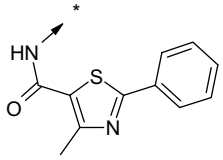
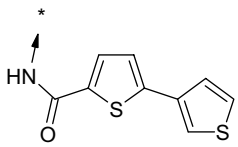
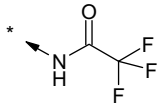
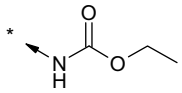
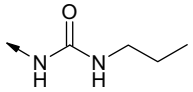
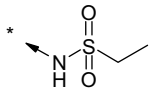
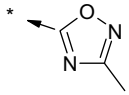
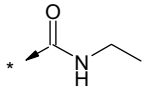
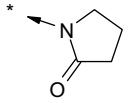
6		36
7		90
8		20
9		90
10		370
11		55
12		10
13		47
14		27
15		56
16		13

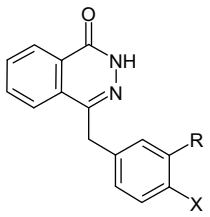
Table 1 (*continued*)

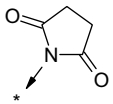
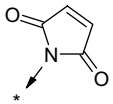
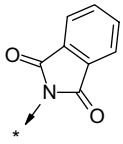
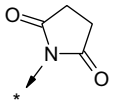
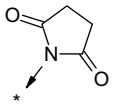
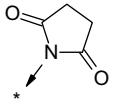
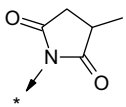
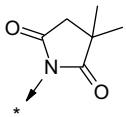
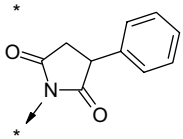
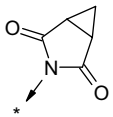
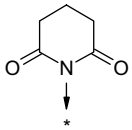
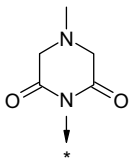
	R	IC_{50} (nM)
17		116
18		540
19		290
20		189
21		50
22		120

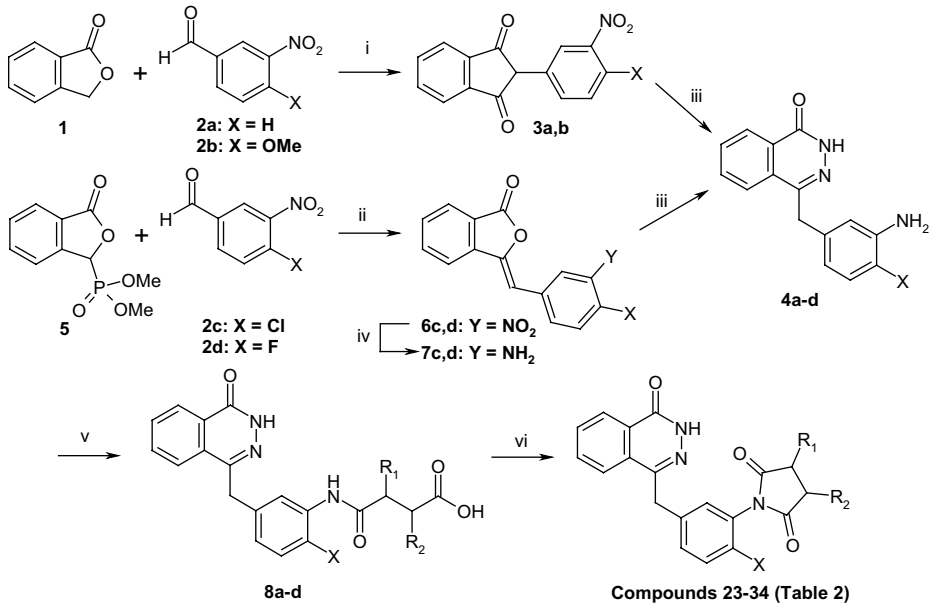
in PARP-1 inhibitory potency. In addition, in vitro stability studies showed that **23** (human hepatic microsomes: $Cl_i < 0.05$ mL min^{-1} g^{-1} liver) has significantly increased metabolic stability over **8**. However, the initial imide lead **23** exhibited only moderate activity in a cell-based assay (PF_{50} 1.74, Table 2).[16] Therefore, further optimisation of **23** was required to improve cellular efficacy whilst maintaining in vitro potency and stability.

Introduction of a double bond into the imide ring resulted in compound **24**, which retained potency against the isolated enzyme but abolished activity in the cell-based assay, as indicated by the low PF_{50} value. Fusion of **23** with a benzene ring (**25**) reduced PARP-1 inhibitory activity overall. Substitution *ortho* to the imide moiety with either a chloro or a methoxy group (**26** and **27**, respectively), did not increase PARP-1 inhibitory potency. However, a significant enhancement of potency in both enzyme and cell-based assays was observed when a fluoro-substituent was introduced *ortho* to the imide ring to give **28**. Indeed, compound **28** retains activity in the cellular assay at concentrations below 10 nM (data not shown). This compound also retained good metabolic stability in vitro (mouse hepatic microsomes: $Cl_i < 1$ mL min^{-1} g^{-1} liver). Testing of compound **28** against other PARP family members indicated a similar level of potency for PARP-2, while for Tankyrase and VaultPARP potency was >100-fold less. Solubility for this compound was limited (0.25 mg mL^{-1}) but in a mouse pharmacokinetic study good bioavailability (50%) was obtained (data not shown). The enhancement of potency via the fluoro substituent appeared quite general for this series as exemplified by compounds **29**–**34**. Furthermore, addition of a methyl

Table 2. Structure–activity relationships of the imide series



	R	X	IC_{50} (nM)	PF_{50}
23		H	12	1.74
24		H	13	0.94
25		H	180	—
26		Cl	19	1.5
27		OMe	33	1.5
28		F	5.0	5.62
29		F	3.8	18.2
30		F	9.8	2.9
31		F	6.8	5.4
32		F	5.0	14.4
33		F	4.1	3.6
34		F	9.5	3.1



Scheme 1. Synthesis route for phthalazinone imides. Reagents: (i) NaOMe, MeOH, reflux; (ii) NEt_3, THF; (iii) $NH_2NH_2{\cdot}H_2O$, reflux; (iv) Fe powder, NH_4Cl; (v) Cyclic anhydride, MeCN, reflux; (vi) HBTU, DMF.

group α to the carbonyl of the imide **28** to give **29** led to a further increase in cellular activity (PF_{50} 18.2). This could be due to enhanced cell penetration as a result of increased lipophilicity. However, geminal-dialkylation α to the carbonyl to give **30** resulted in a slight decrease in potency in both isolated enzyme and cell-based assays. Larger aromatic substituents α to the carbonyl of imide **28** were tolerated, as exemplified by **31**, which retained good PARP-1 inhibitory potency. A high potency PARP-1 inhibitor, **32**, was also obtained by fusion of a cyclopropane ring. Ring expansion of the imide **28** to a piperidinedione such as **33**, or inclusion of a further heteroatom to give a piperazinedione **34** also retained good activity on the free enzyme and in cells.

The synthetic route for the phthalazinone imide analogues is outlined in Scheme 1. For the non-halogenated analogues (X = H and OMe) simple nucleophilic addition of phthalide **1** to the aldehydes **2a**,**b** in the presence of sodium methoxide, followed by simultaneous cyclisation/nitro reduction of the indanediones **3a**,**b** with hydrazine hydrate gave the key amine intermediates **4a**,**b**. For the halogenated analogues (X = Cl and F), in which the halogen *ortho* to the nitro group is labile to nucleophilic displacement, the alternative, milder methodology starting from the phthalide phosphonate **5** was required. Formation of the cyclic imides (**23**–**34**) was problematic under standard conditions. However, a two-step process involving reaction with a cyclic anhydride to give **8** and subsequent cyclisation with a peptide-coupling reagent proved generally applicable.

In conclusion, the synthesis and preliminary biological evaluation of a novel class of phthalazinones as potent PARP-1 inhibitors have been described. Further evaluation of these compounds is ongoing and results will be reported in due course.

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