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Novel alkoxybenzamide inhibitors of poly(ADP-ribose) polymerase

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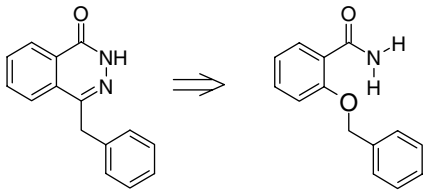
ABSTRACT

We have previously described poly(ADP-ribose) polymerase-1 (PARP-1) inhibitors based on a substituted benzyl-phthalazinone scaffold. As an alternative chemical template, a novel series of alkoxybenzamides were developed with restricted conformation through intramolecular hydrogen bond formation; the compounds exhibit low nM enzyme and cellular activity as PARP-1 inhibitors.

The role of PARP-1 (poly(ADP-ribose) polymerase-1) has been recognized as a key component in a number of cellular processes including necrosis and apoptosis, transcriptional regulation and DNA repair.[1] Due to this, PARP-1 has been implicated in several important disease states including cerebro-vascular disease, reperfusion injury and cancer resistance.[2,3] PARP-1 is an abundant nuclear enzyme that binds to predominantly single stranded damaged DNA, from where it becomes activated and leads to the repair of the DNA via the base-excision repair (BER) pathway.[1] Utilizing nicotinamide adenine dinucleotide (NAD^+) as substrate, PARP-1 catalyzes the formation of ADP-ribose polymers through either automodification of itself or ribosylation of other protein acceptors that aid in the process of repair.

PARP-1 inhibition is known to potentiate the effects of radiation as well as a variety of chemotherapeutic agents in tumour models.[4] Moreover, recent studies have also indicated that PARP-1 inhibitors may be used as potential stand-alone therapies where the tumours have become deficient in certain DNA repair pathways.[5,6] Tumours that have lost their BRCA1 and BRCA2 components of the homologous recombination DNA repair pathways, for example, show high sensitivity to the effects of PARP inhibition. This concept of synthetic lethality along with the combination of PARP inhibitors and radio/chemotherapies is now under investigation preclinically and in the clinic.

We have previously reported a series of substituted benzyl-phthalazinones originating from compound **1** (Fig. 1) as potent, cellularly active inhibitors of PARP-1.[7,8] Typical examples of optimized compounds from the phthalazinone series are shown in Table 1. Here, the 3-piperazine-1-carbonyl substituent **2** and acylated derivatives **3** and **4** (tested as the racemate) demonstrate excellent activity with PARP-1 IC_{50} values in the low nM range and cellular potency, as measured by the potentiation factor PF_{50} to the methylating agent methyl methanesulfonate (MMS), greater than 2. For compound **4**, a near maximal sensitization is seen (PF_{50} 29.7) in the absence of intrinsic cellular toxicity, which may indicate high cellular permeability or differing kinetics of PARP inhibition.



1 PARP-1 IC_{50} 0.77μM

Figure 1. Compound **1** PARP-1 IC_{50} 0.77 μM.

In an effort to explore alternative chemical templates, we took the expedient of dissecting the phthalazinone B ring and introducing an oxygen linker into the system (Fig. 1). The hypothesis for this approach was that the subsequent intramolecular hydrogen bond between the aryl C-2 oxygen and benzamide NH may counter a loss in entropy in the acyclic system. Additionally this arrangement will conformationally lock the system in the required *syn* configuration necessary for bidentate hydrogen bonding to the

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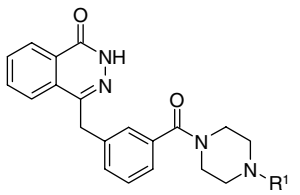
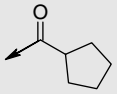
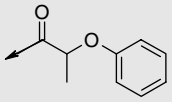
Table 1



Compound	R^1	PARP IC_{50} (μM)	PF_{50}[a]
2	H	0.002	2.7
3		0.008	4.8
4		0.005	29.7

[a] The PF_{50} value is the potentiation factor, calculated as the ratio of the GI_{50} growth curve for the alkylating agent, methyl methane sulfonate (MMS) divided by the GI_{50} of the curve of MMS + PARP inhibitor. The cells used were HeLa B and the test compounds were used at a fixed concentration of 200 nM. PARP-1 IC_{50} and PF_{50} values are means from 2 to 3 independent dose–response curves.

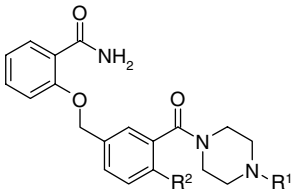
enzyme (carbonyl of Ser904 and NH of Gly863) characteristic of this class of inhibitor.[9] A similar tactic has been described previously for benzamidazole carboxamides; however, the approach was detrimental to potency in comparison to equivalent bonded ring systems.[10,11]

A further consideration for developing the alkoxybenzamide template was ready chemical access to explore SAR, which might otherwise have proven difficult with the phthalazinone class. The assumption, however, was that SAR between the phthalazinone series and the putative alkoxybenzamide scaffold would be transferable. To establish this, matched pairs of compounds with the equivalent substituted phthalazinones **2**, **3** and **4** were initially proposed for synthesis and testing.

Gratifyingly, the benchmark 2-[3-(piperazine-1-carbonyl)benzyloxy]benzamide **5** (Table 2) had excellent inhibitory potency against PARP-1 with an IC_{50} of 18 nM. This initial compound therefore proved the equivalence of the two chemical templates towards PARP-1 inhibition. Likewise the cyclopentanecarbonyl **7** and the phenoxypropionylcarbonyl **9** compounds were made, but in these cases, although still potent, a loss of relative activity was observed. All three compounds were tested in cellular assays to establish their effect on the potentiation of cytotoxicity to MMS. For compounds **5** and **7**, little effect was seen; however, **9** gave good cellular activity with a PF_{50} of 2.0. We have previously observed that for the phthalazinone series cellular activity can be enhanced by 4-fluoro substitution on the benzyl linker.[7,8] This trend is also noted for the alkoxybenzamides with the corresponding 4-fluoro analogues **6**, **8** and **10** all showing improved PF_{50} values over the des-fluoro derivatives (**5**, **7** and **9**). Indeed, the phenoxypropionyl **10** proved to be the most cellularly active compound from the series with a PF_{50} of 3.7.

As a further exploration of the SAR around the pendant ether linker, a series of phenethyloxybenzamides were synthesized, again maintaining the three benchmark side chains for comparison (Table 3). Homologation of the linker by one carbon atom to the simple 3-piperazine carbonyl **11** results in a dramatic reduction in PARP-1 potency (over 50×). The decline in potency was also observed for **15** (vs **9**) but not, interestingly, for **13** which is equipotent to **7**. Again the tactic of introducing a 4-fluoro-benzyl substituent in order to improve enzyme and cell potency was attempted and gave varying degrees of success. For the 3-piperazine

Table 2
Structure–activity relationships of substituted 2-benzyloxybenzamides



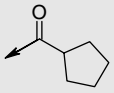
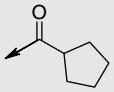
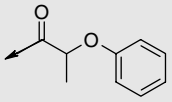
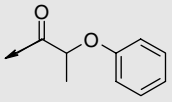
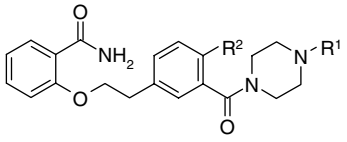
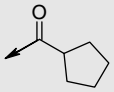
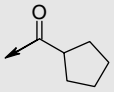
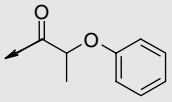
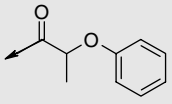
Compound	R^1	R^2	PARP-1 IC_{50} (μM)	PF_{50}
5	H	H	0.018	1.3
6	H	F	0.061	1.4
7		H	0.188	1.1
8		F	0.024	1.96
9		H	0.036	2.0
10		F	0.050	3.7

Table 3
Structure–activity relationships of substituted 2-phenethyloxybenzamides

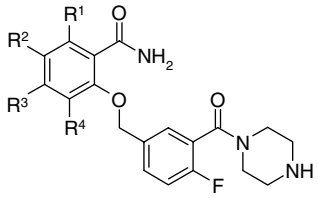


Compound	R^1	R^2	PARP-1 IC_{50} (μM)	PF_{50}
11	H	H	0.97	1.06
12	H	F	>10	ND
13		H	0.2	ND
14		F	0.103	ND
15		H	0.2	ND
16		F	0.019	1.5

carbonyl **12** derivative activity was markedly reduced, whereas for **14**, and to a greater extent for **16**, activity was enhanced. Curiously for **16**, the potency against PARP-1 was improved over the corresponding benzyl-linked compound **10** (IC_{50} 19 nM vs 50 nM, respectively). The reason for this paradox is uncertain but the SAR suggests alternative PARP-1 binding modes for the differing sub-series of compounds.

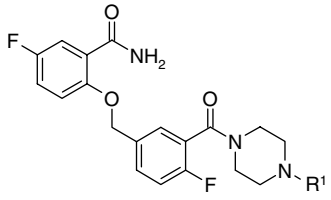
Our attention next turned to an exploration of substituents on the benzamide ring. An examination of the homology model of

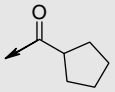
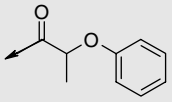
Table 4
Structure–activity relationships of substituted 2-benzyloxyfluorobenzamides



Compound	R^1	R^2	R^3	R^4	PARP-1 IC_{50} (μM)	PF_{50}
17	H	F	H	H	0.029	1.6
18	H	F	F	H	0.24	1.08
19	H	H	F	H	0.25	1.2
20	H	F	H	F	1.5	0.9
21	F	H	H	F	>10	ND

Table 5
Structure–activity relationships of difluoro-substituted 2-benzyloxybenzamides



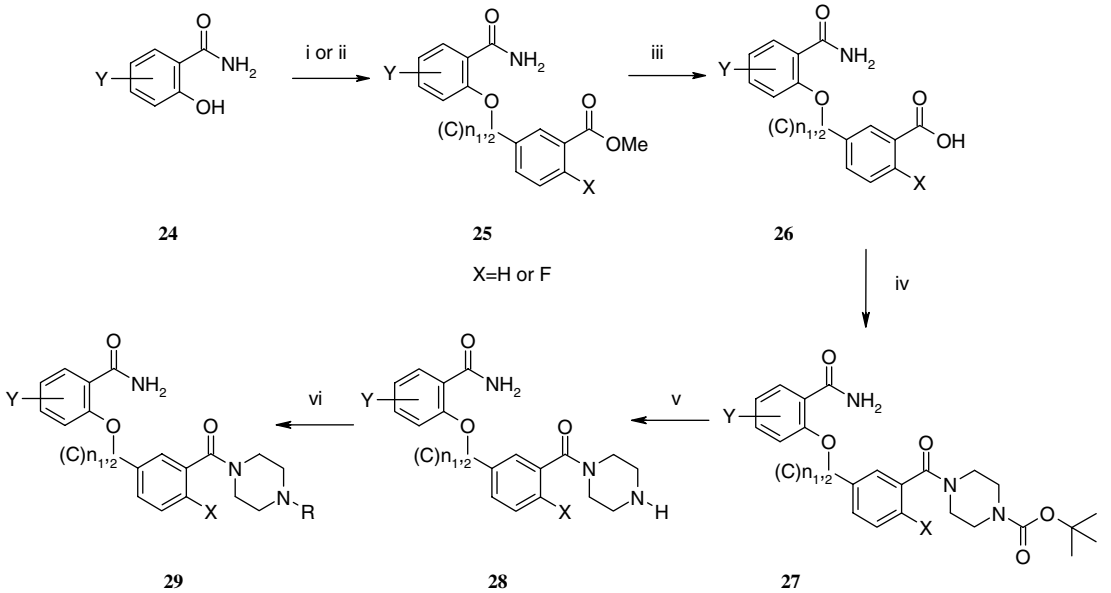
Compound	R^1	PARP-1 IC_{50} (μM)	PF_{50}
22		0.012	1.5
23		0.014	1.12

PARP-1 (previously described) and co-crystallized inhibitors from other workers[11] makes it clear that the binding pose for inhibitors in this class has limited room for substitution especially at C4–6 (R1–3 in Table 4).

With this in consideration and also from our experience showing that an aryl fluorine substitution at C-4 of the benzyloxy linker gives enhanced potency, the chemistry was restricted to variously decorated fluorobenzamides (Table 4). To this end, compounds **17–21** were synthesized. Of greatest value was the observation that the 5-fluoro-2-alkoxy benzamide **17** had good potency against the enzyme in excess (by over threefold) of the C-5 des-fluoro derivative **6**. Table 4 shows that a significant reduction in potency results from the other exemplified patterns of fluoro substitution with the difluoro analogue **21** in particular being significantly less active with an $IC_{50} > 10$ μM.

In an attempt to determine whether the C-5 fluoro-benzamide in **17** would function additively with the capped piperazine side chains, the adducts **22** and **23** were synthesized (Table 5). In both cases potency was improved in comparison to the C-5 des-fluoro analogues **8** and **10**. On going from **8** to **22** IC_{50} enhanced by 2×, on going from **10** to **23** IC_{50} enhanced by 4×. Disappointingly, the potency advantage at the level of the enzyme was not reflected in an increase in cellular activity where only modest values were noted.

For the analogues described above, synthetic access was readily achieved through ether formation of an appropriately substituted hydroxylbenzamide **24** (Scheme 1). Two standard methods were employed to achieve this result; for the benzyl-substituted compounds, classical Williamson ether formation with the requisite benzyl bromide methyl ester (either X = F or H) gives intermediate **25**. Alternatively, Mitsunobu reaction with either an appropriately substituted benzyl alcohol or substituted 2-phenylethanol using diisopropyl azodicarboxylate (DIAD) gave **25** where C_n, n = 1 or 2, respectively. For the extended linker (C_n, n = 2, compounds **11–16**) this was the method of choice. Simple base hydrolysis of the methyl ester **25** to the carboxylic acid **26**, using methanolic sodium hydroxide, followed. Coupling of **26** with BOC-piperazine under standard conditions furnished **27**, which underwent deprotection to the free amine **28** under acidic conditions. Where necessary, capping of the piperazine side chain was conducted under basic conditions with either cyclopentane carbonyl chloride or 2-phenoxy propionyl chloride to give intermediates **29**.



Scheme 1. Reagents and conditions: (i) substituted benzyl bromide, acetone, K_2CO_3, rt; (ii) substituted benzyl alcohol or substituted 2-phenylethanol, acetone, Ph_3P, DIAD, rt; (iii) NaOH, MeOH, rt; (iv) DMF, HBTU, DIPEA, *tert*-butyl piperazine-1-carboxylate; (v) concd HCl, EtOH, rt; (vi) DCM, DIPEA, ROCl.

In conclusion, we have developed a novel class of potent PARP-1 inhibitors based on an understanding of the established SAR of a series of benzyl-phthalazinones. Further evaluation of this class of compounds is ongoing and will be reported in due course.

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