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Potentiation of cytotoxic drug activity in human tumour cell lines, by amine-substituted 2-arylbenzimidazole-4-carboxamide PARP-1 inhibitors

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Abstract—The synthesis and biological evaluation of a new series of amine-substituted 2-arylbenzimidazole-4-carboxamide inhibitors of the DNA-repair enzyme poly(ADP-ribose) polymerase-1 (PARP-1) is reported. The introduction of an amine substituent at the 2-aryl position is not detrimental to activity, with most inhibitors exhibiting K_i values for PARP-1 inhibition in the low nanomolar range. Two compounds in this series were found to potentiate the cytotoxicity of the DNA-methylating agent temozolomide by 4–5-fold in a human colorectal cancer cell line.

1. Introduction

The abundant eukaryotic nuclear enzyme poly(ADP-ribose) polymerase-1 (PARP-1), is the founding member of a burgeoning family of poly(ADP-ribosyl)ating enzymes of diverse and complex function.[1] A major role of PARP-1 is in the base excision repair of DNA, mediated by the ability of the enzyme to detect DNA strand breaks.[2–4] Utilising intracellular NAD^+ as a substrate, PARP-1 catalyses the formation of long linear and branched poly(ADP-ribose) polymers that modify a variety of nuclear proteins, including histones (hetero-modification), and the enzyme itself (automodification). This is part of the immediate cellular response to various forms of genotoxic stress, including DNA damage caused by ionising radiation and DNA alkylating agents. Poly(ADP-ribose) polymers have a short half life, estimated to be less than 1 min, due to rapid hydrolysis by poly(ADP-ribose) glycohydrolase.[5] Depletion of NAD^+, as a consequence of inappropriate PARP-1 activity, results in critically low intracellular ATP concentrations leading to extensive cell death. The enzyme is thus implicated in the pathology of several disease states, including diabetes, arthritis, and cardiovascular disease.[6,7]

PARP-1 mediated DNA repair in response to DNA damaging agents represents a mechanism of tumour resistance, and inhibition of this enzyme has been shown to enhance the activity of ionising radiation and several cytotoxic antitumour agents, including temozolomide and topotecan.[8–10] PARP-1 is thus a potentially important therapeutic target for enhancing DNA-damaging cancer therapies. As part of a programme to develop clinically useful inhibitors, we have employed a crystal structure-based inhibitor design approach, utilising an understanding of structure–activity relationships for NAD^+-competitive PARP-1 inhibition. These studies have resulted in the identification of potent inhibitors, including the benzimidazole-4-carboxamides (**1**),[11] and inhibitors that incorporate the essential carboxamide motif within a tricyclic system (**2** and **3**).[12,13]

Keywords: PARP-1; Inhibitors; Benzimidazole-4-carboxamides; Chemopotentiation.
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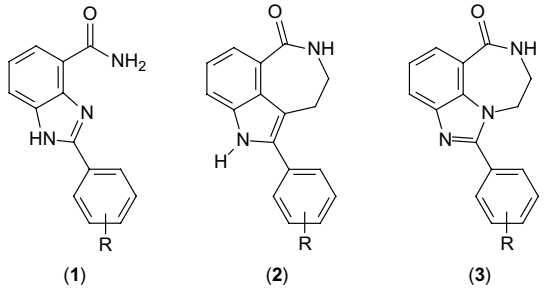
Co-crystallisation of a 2-aryl-substituted benzimidazole-4-carboxamide (**1**; R = 3-OMe) with the C-terminal catalytic domain of chicken PARP-1, revealed that the 2-aryl ring of the inhibitor occupied a large cavity within the NAD^+ binding site.[11] Importantly, this observation suggested that substituents on the 2-aryl ring would be tolerated without detriment to inhibitory activity. In this communication we report the synthesis and biological evaluation of a series of benzimidazole-4-carboxamides bearing a range of amine groups on the 2-aryl ring. These compounds were designed to probe the NAD^+ binding pocket, with a view to enhancing potency and modulating the pharmacokinetic and physicochemical properties of this class of PARP-1 inhibitor.

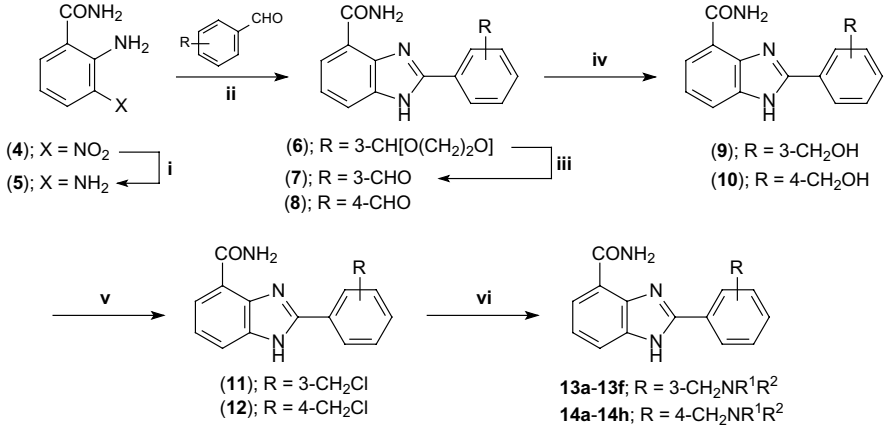


2. Chemical synthesis

Although we have previously reported the synthesis of a wide range of benzimidazole-4-carboxamides, including the key alcohols **9** and **10**,[11] an alternative synthetic route was required to enable the preparation of these intermediates on a multi-gram scale (Scheme 1). Catalytic hydrogenation of 2-amino-3-nitrobenzamide (**4**), prepared as described previously,[11] afforded the unstable anthranilamide **5** in quantitative yield, and this was used directly in the next reaction step. The required benzimidazole-4-carboxamides were prepared from **5** using a modification of a literature procedure.[14] Thus, condensation of **5** with 2-(3-formylphenyl)-1,3-dioxolane[15] in DMA at 140 °C, in the presence of sodium hydrogen sulfite, afforded the acetal derivative **6**, which gave the corresponding aldehyde **7** on acid hydrolysis.[16] The analogous reaction between **5** and terephthalaldehyde mono(diethylacetal) gave aldehyde **8** directly, and reduction of **7** and **8** with sodium borohydride furnished the required alcohols **9** and **10**, respectively. The target amine-substituted 2-arylbenzimidazole-4-carboxamides (**13a**–**13f** and **14a**–**14h**) were prepared in variable yields from **9** and **10**, by conversion into the respective chlorides **11** and **12** under Vilsmeier-like conditions ($SOCl_2$-DMF), followed by treatment with the appropriate amine.[17]

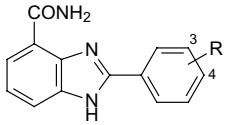
3. Results and discussion

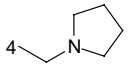
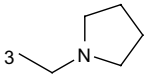
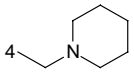
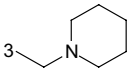
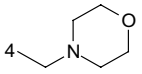
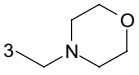
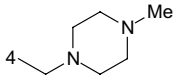
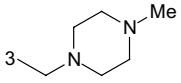
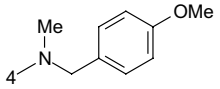
All new compounds were assayed for inhibition of purified human PARP-1 as described previously,[11] and the results are presented in Table 1. The benchmark PARP-1 inhibitor 3-aminobenzamide (3-AB), and the unsubstituted 2-phenylbenzimidazole-4-carboxamide NU1070,[11,18] are included for comparison. In general, amine substitution at the 3- or 4-position of the 2-aryl ring resulted in an enhancement of PARP-1 inhibitory activity compared with NU1070, this effect being most pronounced at the 4-position. The *N*-methylaminomethyl (**14a**) and *N*,*N*-dimethylaminomethyl (**13a** and **14b**) derivatives proved approximately 3-fold more potent than NU1070, and while this activity was retained with the 4-*N*,*N*-di-(2-hydroxyethyl)aminomethyl analogue (**14c**), a dramatic reduction in potency was observed when this group was introduced at the 3-position (**13b**). The reason for this loss of activity remains uncertain, but the high potency of the corresponding piperidine and *N*-methylpiperazine derivatives (**13d** and **13f**) mitigates against unfavourable steric interactions within the NAD^+ binding domain. Interestingly, a morpholine function is also detrimental to PARP-1 inhibitory activity when introduced at the 3-position, but not the 4-position (compare **13e** and **14f**).



Scheme 1. Synthesis of benzimidazole-4-carboxamides. Reagents and yields: (i) 10% Pd/C, H_2, MeOH, (100%); (ii) $NaHSO_3$, DMA, 140 °C, (70–80%); (iii) 0.5 M HCl, THF, (92%); (iv) $NaBH_4$, H_2O, EtOH, (94%); (v) $SOCl_2$, DMF (cat.), MeCN, (93%); (vi) R^1R^2NH, $(Et)_3N$, MeCN, (8–89%).

Table 1. Inhibition of PARP-1 by amine-substituted 2-arylbenzimidazole-4-carboxamides



Compound no.	R	K_i (nM)[a]	Compound no.	R	K_i (nM)
3-AB[b]	—	3100	**14a**	4-CH_2NHMe	3.2
NU1070	H	15	**14b**	4-CH_2NMe_2	4.5
13a	3-CH_2NMe_2	5.6	**14c**	4-$CH_2N(CH_2CH_2OH)_2$	4.5
13b	3-$CH_2N(CH_2CH_2OH)_2$	272	**14d**		5.9
13c		10.2	**14e**		7.2
13d		11.7	**14f**		12
13e		31	**14g**		8.7
13f		9.8	**14h**		5.9

[a] Assayed against human PARP-1 full length protein; for further details see Ref. 11. Data are averages of at least two independent experiments, standard deviation $\leqslant 10\%$.
[b] 3-Aminobenzamide.

Although cycloalkylamino substitution at the 3- or 4-position did not increase potency to the same degree as did the introduction of alkyl- or dialkyl-amines, the differences are modest, and bulky amine substituents appear to be well tolerated (compare **14a** with **14h**).

These observations indicate that the increased potency of this compound series compared with NU1070 may arise through an additional binding interaction between the pendant amine function and the large cavity within the NAD^+ binding domain. Our previous studies with tricyclic PARP-1 inhibitors bearing analogous amine groups have demonstrated that unique changes in the protein conformation occur on inhibitor binding.[13] Notably, the co-crystal structure of the tricyclic indole lactam (**2**, R = 4-CH_2NMe_2) with the PARP-1 catalytic domain, revealed that disruption of a key hydrogen bond between residues Tyr-889 and Asp-766 is necessary for the 4-dimethylaminomethyl group to be accommodated in the large cavity, resulting in relocation of an entire loop between Gly-888 and Gly-892. Given that the benzimidazole-4-carboxamide (**1**) and tricyclic lactam (**2** and **3**) pharmacophores exhibit near-identical binding geometries and hydrogen bonding interactions, it is likely that similar conformational changes to the protein are elicited by the amine-substituted 2-arylbenzimidazole-4-carboxamides. Although not observed in complexes of the tricyclic inhibitors **2** and **3** (R = 4-CH_2NMe_2) with the chicken PARP-1 catalytic domain,[12,13] an ionic interaction between Glu-763 (Gln-763 in chicken PARP) and the protonated amine substituent of inhibitors in the 2-arylbenzimidazole-4-carboxamide series is also possible, and may account for the increase in potency observed (Fig. 1).

The activity of selected PARP-1 inhibitors as potentiators of DNA-damaging antitumour agent cytotoxicity, was assessed in vitro by treating human lung (A549)

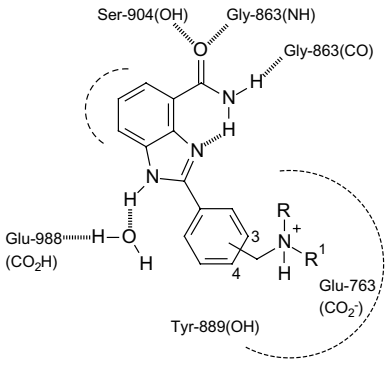


Figure 1. Interactions of 2-arylbenzimidazole-4-carboxamides with the PARP-1 NAD^+ binding domain, indicating the likely positioning of an amine substituent within the cavity.[12,13]

Table 2. Potentiation of temozolomide (TM) and topotecan (TP) activity by selected PARP-1 inhibitors in A549 and LoVo human tumour cell lines[a]

Compound no.[b]	PF_{50} (A549)[c]		PF_{50} (LoVo)[d]	
	TP	TM	TP	TM
13a	ND	ND	1.4 ± 0.3	1.7 ± 0.1
13b	1.1	1.1	ND	ND
13c	ND	ND	1.1 ± 0.1	1.7 ± 0.2
14b	1.9	1.6	1.7 ± 0.4	4.0 ± 1.0
14c	1.3	ND	ND	ND
14d	1.8	1.5	1.5 ± 0.3	5.2 ± 1.9
14e	1.9	ND	ND	ND

[a] Cell growth was estimated by the SRB assay.[20]
[b] 0.4 μM inhibitor concentration.
[c] Data are mean of two independent experiments.
[d] Data are mean ± standard deviation of at least three independent experiments.

and/or colorectal (LoVo) cancer cell lines with increasing concentrations of two antitumour drugs, the DNA methylating agent temozolomide (TM) and the topoisomerase I inhibitor topotecan (TP), in the presence of a fixed concentration of PARP-1 inhibitor (Table 2). The cytotoxic potentiation factor (PF_{50}) of these agents was expressed as the ratio of the GI_{50} for cells treated with cytotoxic agent and PARP-1 inhibitor, against the GI_{50} of cells exposed to the cytotoxic drug alone (where GI_{50} is the concentration of drug required to inhibit growth by 50%). Thus a PF_{50} value of 1.0 indicates no effect.[9,19]

In general, the PARP-1 inhibitors exhibited a modest potentiation of the activity of TM and TP in the A549 tumour cell line, with compounds **14b**, **14d**, and **14e** giving similar activity consistent with their PARP-1 inhibitory activity. Compound **13b**, a much weaker PARP-1 inhibitor, exhibited negligible potentiation, and the lower PF_{50} value observed for **14c** in combination with TP, perhaps reflects the lower cell permeability arising from the polar diethanolamino group. Although a similar modest degree of potentiation of TP activity was seen in the LoVo tumour cell line, in the presence of PARP-1 inhibitors, a significant effect was observed with TM in these cells. Thus, the cytotoxicity of TM was increased 4-fold and 5-fold, respectively, by the *N*,*N*-dimethylaminomethyl (**14b**) and pyrrolidinomethyl (**14d**) derivatives, both of which are potent PARP-1 inhibitors. The results for compound **14b** are also represented graphically in Figure 2.

In conclusion, we report the synthesis and biological evaluation of a series of potent PARP-1 inhibitors based on the 2-arylbenzimidazole-4-carboxamide pharmacophore, and have demonstrated that the introduction of amine substituents on the 2-aryl ring is generally tolerated without loss of inhibitory activity compared with the parent inhibitor NU1070. Importantly, two compounds in this series (**14b** and **14d**) show excellent activity as potentiators of the cytotoxicity of the antitumour agent temozolomide in a human colorectal cancer cell line. Preliminary data (not shown) also indicate that this compound series combines good metabolic stability in human liver microsome preparations, with improved water solubility compared with NU1070, when formulated as amine salts.

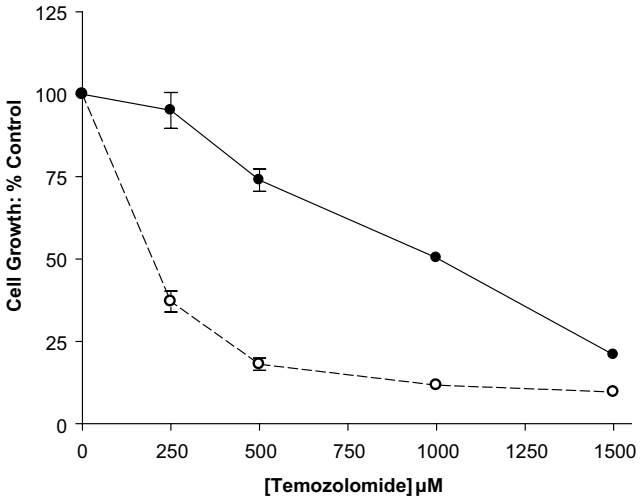


Figure 2. Inhibition of LoVo cell growth by temozolomide (TM); potentiation by **14b**. Cells were exposed to increasing concentrations of temozolomide alone (solid circles and line) or in the presence of 0.4 μM **14b** (open circles, broken line) for five days. Data (normalised to no drug or 0.4 μM **14b** alone) are from a single representative experiment in which the IC_{50} for temozolomide alone was 1006 μM, but 199 μM in the presence of **14b**, giving a PF_{50} of 5.1 in this experiment.

Acknowledgements

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16. A general procedure for the synthesis of benzimidazole-4-carboxamides **7** and **8** is as follows: To a suspension of 2,3-diaminobenzamide (0.5 g) and sodium hydrogen sulfite (1.5 mol equivalents) in *N*,*N*-dimethylacetamide (10 mL/g), was added the appropriate aryl aldehyde (1 mol equivalent) dropwise over 15 min. After heating the reaction mixture for 5 h at 140 °C with vigorous stirring, water (200 mL) was added, and the mixture was stirred for a further 2 h. The product was collected by filtration, and washed thoroughly with water.
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