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Synthesis and SAR of novel, potent and orally bioavailable benzimidazole inhibitors of poly(ADP-ribose) polymerase (PARP) with a quaternary methylene-amino substituent

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ABSTRACT

Poly(ADP-ribose) polymerases (PARPs) play significant roles in various cellular functions including DNA repair and control of RNA transcription. PARP inhibitors have been demonstrated to potentiate the effect of cytotoxic agents or radiation in a number of animal tumor models. Utilizing a benzimidazole carboxamide scaffold in which the amide forms a key intramolecular hydrogen bond for optimal interaction with the enzyme, we have identified a novel series of PARP inhibitors containing a quaternary methylene-amino substituent at the C-2 position of the benzimidazole. Geminal dimethyl analogs at the methylene-amino substituent were typically more potent than mono-methyl derivatives in both intrinsic and cellular assays. Smaller cycloalkanes such as cyclopropyl or cyclobutyl were tolerated at the quaternary carbon while larger rings were detrimental to potency. In vivo efficacy data in a B16F10 murine flank melanoma model in combination with temozolomide (TMZ) are described for two optimized analogs.

Poly(ADP-ribose) polymerases (PARPs) are a family of DNA-binding proteins found in nearly all eukaryotic cells.[1] When activated by nicks in DNA occurring during inflammation, ischemia, neurodegeneration, or cancer therapy, PARPs catalyze the transfer of ADP-ribose units from nicotinamide adenine dinucleotide (NAD^+) to the acceptor proteins with NAD^+ as substrate, leading to formation of protein-bound ADP-ribose polymers and dramatic cellular ATP depletion.[2] This cellular ADP-ribose transfer process is pivotal for DNA repair machinery and maintenance of genomic stability.[1,2] PARP-1, the most abundant member of the PARP family, is therefore regarded as a promising target for treating diseases related to inflammation and ischemia-reperfusion injury. This DNA repair mechanism also contributes to the drug resistance that often develops after cancer therapy.[3] In addition, enhanced PARP expression and/or activation has been observed in a number of hematological and solid tumors as compared to normal cells, suggesting a potential selectivity of PARP inhibitors against tumor cells. Inhibition of PARP-1 would retard the intracellular DNA repair and therefore sensitize tumor cells to cytotoxic agents or ionizing radiation.[4–8]

There have been a plethora of PARP inhibitors developed in the past two decades.[9] The majority of the known inhibitors bind to the nicotinamide binding site and structurally mimic the binding mode of nicotinamide, where the amide functional group forms multiple hydrogen bonds with Gly-863 and Ser-904.[9] One structural class of inhibitors, namely benzimidazole carboxamide, was first developed by researchers at the University of Newcastle.[10] This series of compounds displayed relatively high intrinsic potency against PARP-1, but suffered from poor activity in a whole cell assay. Herein, utilizing the same benzimidazole carboxamide scaffold, we incorporated a basic amino functionality to the C-2 position of the pharmacophore, and discovered a series of PARP inhibitors with greater cellular activity. Two optimized analogs, **9b** and **9k**, with a quaternary methylene-amino substituent, displayed excellent intrinsic and cellular potency, adequate pharmaceutical properties, and potentiated the efficacy of cytotoxic agent temozolomide (TMZ) in a B16F10 flank melanoma model.

Outlined in Scheme 1 are general syntheses of the PARP inhibitors with a representative structure **6** or **7**. 2,3-Diaminobenzamide dihydrochloride **1**[11] was first coupled to an appropriately protected amino acid **2** to provide mono-acylated **3**. The 1,1′-carbonyldiimidazole (CDI)-mediated amidation proceeded predominately at the 3-amino group. A 1:1 mixture of anhydrous DMF and pyridine was typically used as solvent in which pyridine freed up the

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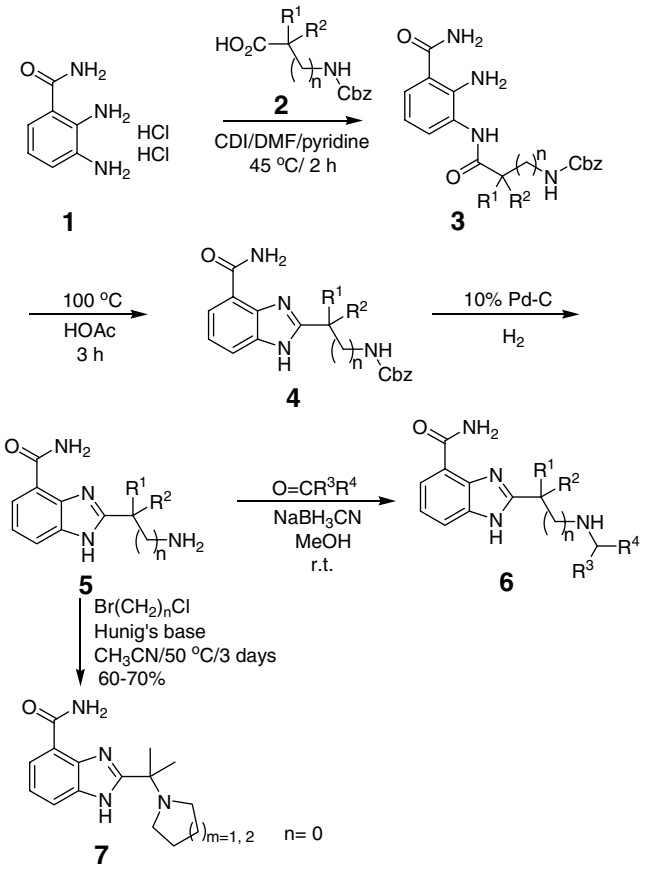


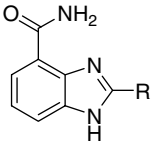
Scheme 1.

diamine from its hydrochloric salt form. The amides **3** were not purified at this stage. Solvents were removed, and the residue was directly heated in acetic acid, leading to benzimidazole product **4** in good to excellent yields. Deprotection of compound **4** under hydrogenation conditions provided the free amines **5** that were converted to the corresponding alkyl analogs **6** through reductive amination (Scheme 1). Alkylation of the free amines **5** with 1-bromo-3-chloropropane or 1-bromo-4-chlorobutane in the presence of Hunig's base afforded cyclic amines **7**.

As summarized in Table 1, benzimidazole carboxamide **8a** is a relatively potent scaffold with a K_i of 240 nM against PARP-1. Installation of a methyl group at the C-2 position of the benzimidazole as exemplified by **8b** improved its binding affinity by 2-fold. An additional amino group (**8c**) is beneficial for both activity and increased aqueous solubility. Attachment of an isopropyl group onto the amino functionality (**8e**) improved the potency against PARP-1 by 4-fold, whereas other alkylations (**8d**, **8f**-**j**) appeared to be less beneficial. Extension of the amino group with an ethylene spacer displayed no significant impact (**8k** and **8l**). Dimethylaminosulfonylation on the amine was found to be detrimental to potency.

In an attempt to make a bulkier C-2 substituent where the amino functionality attaches to a quaternary carbon, an additional methyl group was installed, and resulted in **8n** with improved intrinsic potency. Ring-formation at the quaternary carbon (**8o**–**8s**) retained moderate activity. Smaller cycloalkanes such as cyclopropyl or cyclobutyl were tolerated at the quaternary carbon while some other ring systems such as in **8q** and **8s** showed a small drop in potency. Tolerance of a bulky group at the C-2 position of the benzimidazole scaffold is consistent with the large binding pocket of PARP in this area as indicated in a previous report.[10]

Table 1
SAR of C-2 substituted benzimidazole carboxamide



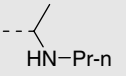
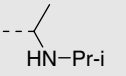
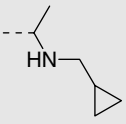
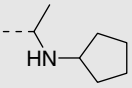
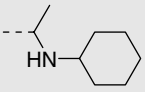
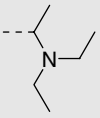
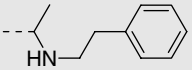
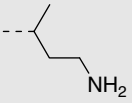
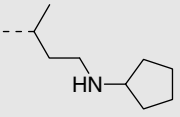
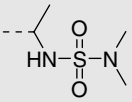
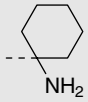
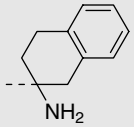
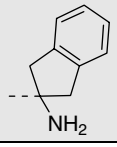
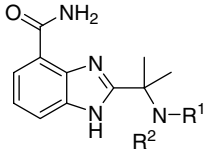
Compound	R	PARP-1[a] (K_i, nM)
a	H	240 nM
b	CH_3	99
c		50
d		41
e		12
f		24
g		33
h		53
i		61
j		35
k		32
l		45
m		140
n		19
o		25

Table 1 (*continued*)

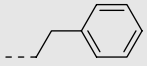
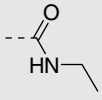
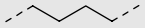
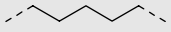
Compound	R	PARP-1[a] (K_i, nM)
p		25
q		50
r		18
s		58

[a] Assays follow the same protocols as previously described.[5] Values are means of two or more experiments, all assays generated data within 2-fold of mean.

Table 2
SAR of C-2 substituted benzimidazole carboxamide



9

Compound	R^1	R^2	PARP-1[a] (K_i, nM)	Cellular[a] (EC_{50}, nM)
a	*n*-Pr	H	10	3
b	*i*-Pr	H	7	2
c		H	7	2
d		H	9	4
e		H	37	9
f		H	870	ND[b]
g	Me	H	4	1.3
h	Me	Me	9	1.8
i	Me		19	ND[b]
j	Me		17	ND[b]
k			3	0.9
l			15	3.1

[a] Assays follow the same protocols as previously described.[5] Values are means of two or more experiments, all assays generated data within 2-fold of mean.
[b] Not determined.

Compound **8n** was then chosen as a lead for further optimization. As shown in Table 2, installation of an isopropyl or cyclopropylmethyl group on the amino functionality, led to compounds **9b** or **9c**, with superior activity in both intrinsic and cellular assays. Attachment of a straight chain alkyl group (**9a**) or a larger cyclic substituent (**9d**) was slightly less beneficial. As indicated by compound **9e**, an additional phenyl group seems to be detrimental as well. A replacement of the basic amino group with urea (**9f**) resulted in significant loss in potency against PARP-1.

Compounds **9g**–**9j** are methyl analogs of amine **8n** with or without an extra alkyl substituent, among which the less substituted **9g** showed superior activity in both enzyme and cellular assays. Tethering of the two alkyl groups in the bis-alkylated amine derivatives results in **9k** and **9l** with a cyclic structure as **7**. Both compounds displayed excellent potency against PARP, with the 5-membered ring **9k** more active.

Listed in Table 3 are the pharmacokinetic properties of select PARP inhibitors. All four compounds screened in the PK studies displayed a relatively short intravenous half-life, but demonstrated respectable oral drug exposures with modest to excellent bioavailability. With the excellent potency and acceptable PK property in hand, we evaluated the efficacy of **9b** and **9k** in a B16F10 murine flank melanoma model.[12] Temozolomide, a cytotoxic alkylating agent that is currently used to treat CNS malignancies and melanoma, was chosen as the DNA damaging agent of choice. The PARP inhibitor was administered orally twice a day on days 6–10 at doses of 3, 10, 30 mg/kg, while temozolomide was dosed daily at 50 mg/kg, which mimics the exposure in human at the clinically relevant dose. As illustrated in Figure 1 and 2, both **9b** and **9k** induced profound potentiation of the efficacy of temozolomide at 30 mg/kg as reflected by tumor volumes (bottom green curves), whereas TMZ monotherapy had only modest tumor growth inhibition (bottom red curves). At lower doses (3 mg/kg for **9b** and 10 mg/kg for **9k**), less significant potentiation of the TMZ activity was observed. The combination of PARP inhibitor with temozolo-

Table 3
Mouse PK summary for selected PARP inhibitors[a]

Compound	$t_{1/2}$ (h)	V_d (L/kg)	CL (L/h/kg)	%*F*	Oral AUC (μM h)
9b	0.6	2.1	4.0	82	5.78
9c	0.8	13.3	11.5	100	3.38
9g	0.6	3.5	4.2	85	8.64
9k	0.4	1.8	3.0	52	6.33

[a] Compounds were administered intravenously at 5 mg/kg or orally at 10 mg/kg.

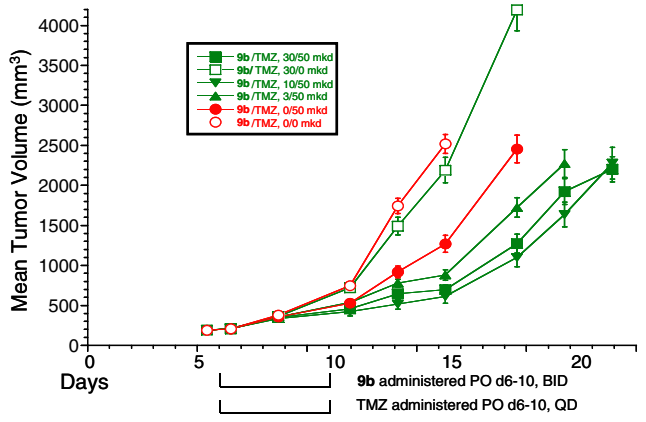


Figure 1. Efficacy of compound **9b** in combination with temozolomide in a B16F10 murine melanoma model.[12]

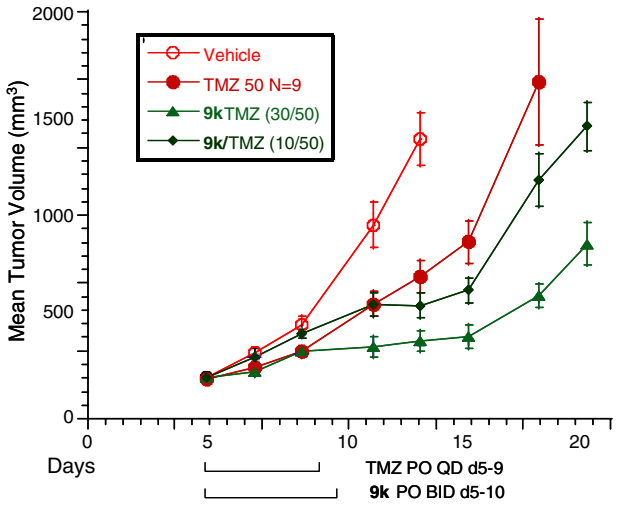


Figure 2. Efficacy of compound **9k** in combination with temozolomide in a B16F10 murine melanoma model.[12]

Table 4
PK summary of compound **9b** in different animal species[a]

Animal	$t_{1/2}$ (h)	V_d (L/kg)	CL (L/h/kg)	%*F*	Oral AUC (μM h)
Mouse	0.6	2.1	4.0	82	5.78
Rat	1.0	3.7	5.1	26	0.74
Dog	2.8	2.9	0.85	70.5	6.07
Monkey	0.6	1.1	1.4	12.6	0.65

[a] Intravenous and oral doses for the above pharmacokinetic studies are as follows: 3 and 10 mg/kg for mouse, 5 and 5 mg/kg for rat, 2.5 and 2.5 mg/kg for dog, and 2.5 and 2.5 mg/kg for monkey.

mide was well tolerated, with minimal loss of body weight at highest doses.

As shown in Table 4, **9b** is orally bioavailable across all animals species we tested, with significantly better bioavailability in mouse and dog (82% and 70%, respectively), and modest bioavailability in rat and monkey (26% and 12%, respectively). Largely due to a slow rate of clearance, the IV half-life of this compound in dog is relatively longer than that in other species. In addition, compound **9b** displayed an excellent aqueous solubility (>5 mg/mL), showed modest human plasma binding (60–70%), and demonstrated minimal inhibition of several cytochrome p450s (<10% at 10 μM).

In summary, starting from a benzimidazole carboxamide scaffold, we developed a novel series of PARP inhibitors containing a quaternary methylene-amino substituent at the C-2 position of the benzimidazole. Two optimized inhibitors in this series, **9b** and **9k**, displayed an excellent intrinsic potency against PARP-1, and showed good pharmaceutical properties, including aqueous solubility and pharmacokinetics across multiple species. In a murine flank melanoma model in combination with temozolomide, both compounds significantly potentiated the efficacy of temozolomide, without an observable increase in toxicity.

Acknowledgment

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12. For B16F10 syngeneic studies, 6×10^4 cells were mixed with 50% matrigel (BD Biosciences, Bedford, MA) and inoculated by sc injection into the flank of 6–8-week old female C57BL/6 mice, 20 g (Charles River Laboratories, Wilmington, MA), on day 1. Mice were injection-order allocated to treatment groups and PARP inhibitor therapy was initiated on days 6 (for **9b**) and 5 (for **9k**) following inoculation, with temozolomide treatment starting on days 6 and 5, respectively.