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Communication

Synthesis and biological evaluation of piperidyl benzimidazole carboxamide derivatives as potent PARP-1 inhibitors and antitumor agents

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ABSTRACT

We have synthesized a series of compounds based on a piperidyl benzimidazole carboxamide structure, and tested their PARP-1 inhibitory activity, as well as cellular inhibitory activity. Some of them show great potency as PARP-1 inhibitors and antitumor activity, which are valuable for further research. In addition, the predicted ADME properties and proposed binding mode with PARP-1 of the compounds were obtained *via* computational simulation.

During the growing progress of cells, DNA is unpreventably damaged due to many exo- or endogenous factors. Therefore, a bunch of mechanisms have been established naturally in living organisms in order to recognize and repair DNA damages, including DNA single-strand break (SSB) and double-strand break (DSB), to maintain the normal physiologic functions of cells [1]. Poly(ADP-ribose) polymerases (PARPs) are a series of enzymes that play important roles in repairing DNA damages, which closely participate in base excision repair (BER) of DNA single strand breaks [2,3]. Among all 18 members of the PARP family, PARP-1 is the most abundant one in eukaryotic cells, and therefore, the most deeply studied one [3]. In the 1970s, Clark *et al.* found that nicotinamide had a weak inhibitory effect on PARP activity (IC_{50} = 210 μmol/L) [4]. Since then, a number of benzamides with PARP inhibitory activity have been found [5,6].

In the 1990s, scientists used *meta*-substituted benzamide PARP inhibitors as templates, immobilized amide conformation by introducing cyclic lactam structure, which greatly improved their PARP inhibitory activity [7]. Based on those structures, the first PARP-1 inhibitory antitumor drug, olaparib, has been developed (Fig. 1) [8,9] and approved by FDA in 2014.

In the mid-1990s, the group lead by Golding and Griffin reported a series of compounds with a basic structure skeleton of benzimidazole-4-carboxamide [10], which performed significant PARP-1 inhibitory activity, and were developed by the subsequent researches during the following decades [11–15]. Till now, numbers of PARP-1 inhibitors based on benzimidazole carboxamide structures have already entered the stage of clinical research [16,17]. Among them, veliparib (Fig. 1) is considered as one of the most competitive members to obtain approval in the future [18]. In 2001, Abbott Labs synthesized hundreds of 2-alkylamine substitutes and screened out compounds with both PARP-1 and cellular IC_{50} under 10 nmol/L [12,18]. After further optimization, the final structure of veliparib was achieved. In 2006, Veliparib entered clinical research stage. Currently, more than 100 clinical

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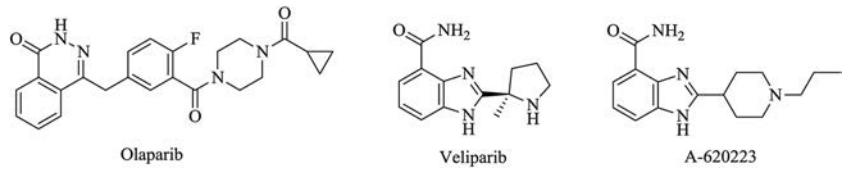


Fig. 1. Structures of olaparib, veliparib and A-620223.

studies are conducting on veliparib along or combination with radiation therapy or chemotherapy drugs, such as carboplatin and paclitaxel, and six of them are in phase III [19–25].

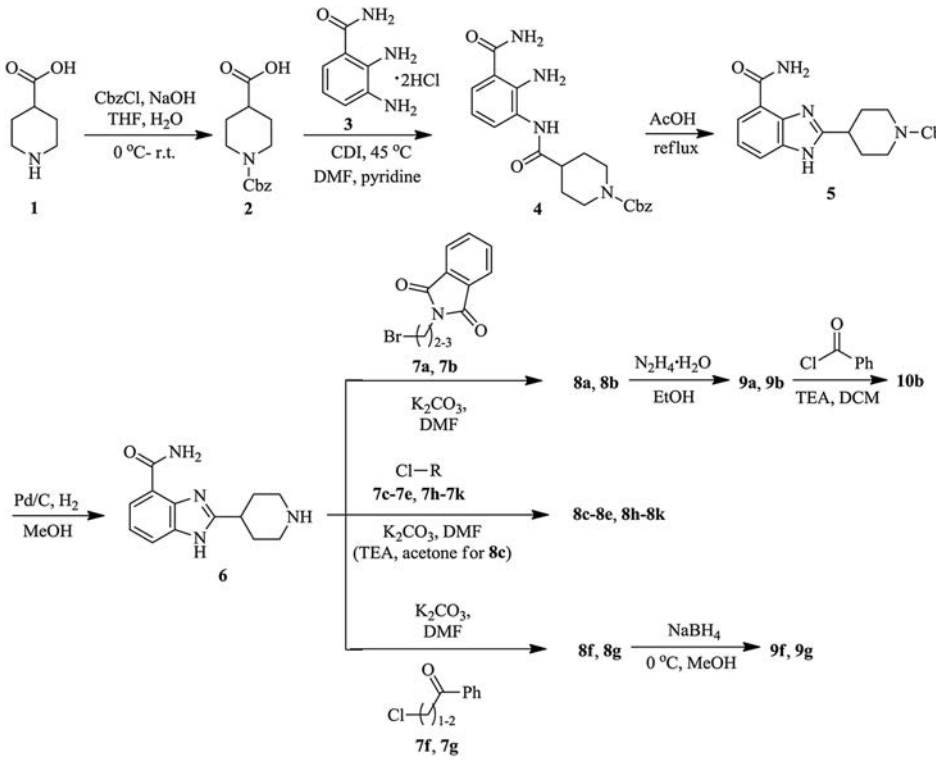
In the process of structural optimization to veliparib, A-620223 (Fig. 1), a piperidyl-containing benzimidazole carboxamide compound was found with good PARP-1 enzyme potency, as well as cellular potency [12]. Although A-620223 was abandoned afterwards since the pyrrolidine substitution structure with a quaternary carbon was eventually proved to be better, the results of previous biological experiments have aroused our great interest in this kind of skeleton. In this work, we have developed a novel series of piperidyl-containing benzimidazole carboxamide derivatives based on the structure-activity relationship of benzimidazole PARP-1 inhibitors [12] and the above optimized precursor of veliparib (A-620223).

The substrate NAD^+ binds in the catalytic domain of PARP-1 and the binding region was usually characterized as two sub-pockets. One is the nicotinamide-ribose binding site (NI site), and the other is the adenine-ribose binding site (AD site) which contained a large hydrophobic pocket [26]. Therefore, a large hydrophobic group at the end of the liner could possibly improve the activity of PARP-1 inhibitors. Based on this, we introduced a phenyl ring, expecting to enhance the binding to the AD site. In consideration of water solubility, hydroxyl, carbonyl groups and/or nitrogen atom were added onto the linker. Furthermore, the length of the linker varied from 2 atoms to 5 atoms, in order to find the optimal length of the side chain. The phenyl ring on the side chain was also functionalized with various groups to test the tolerance of PARP-1 to substituents. In addition, compounds without phenyl ring on the side chain were designed to explore the importance of the hydrophobic group in PARP-1 inhibition.

According to these thoughts, 16 target compounds were obtained. The derivatives displayed very good potency against both PARP-1 and PARP-2, along with moderate cytotoxic activity.

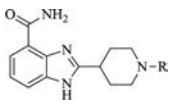
The piperidyl-containing benzimidazole carboxamide derivatives were prepared according to the synthetic route as outlined in Scheme 1 and their chemical structures are presented in Table 1. Piperidine-4-carboxylic acid (**1**) was protected with a CBZ-group, to give protected piperidine-4-carboxylic acid (**2**). The carboxylic acid was coupled to 2,3-diaminobenzamide dihydrochloride (**3**) under CDI condition, to provide the amide (**4**). Side chains (**7a-7k**) were connected to the piperidine ring by heating with K_2CO_3 or triethylamine to give 11 compounds (**8a-8k**). Hydrazinolysis of substituted phthalimides (**8a**, **8b**) gave amines (**9a**, **9b**), and acylation of **9b** with benzoyl chloride provided benzamide (**10b**). Reduction of the ketone **8f** and **8g** gave alcohols (**9f**, **9g**). We changed the length of the linker, as well as the number and position of N atoms in the linker, in order to study the structure-activity relationship. Detailed procedures and spectral data of the target compounds were listed in Supporting information.

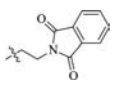
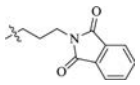
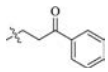
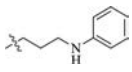
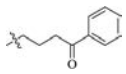
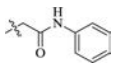
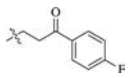
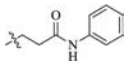
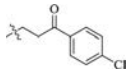
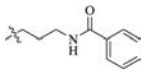
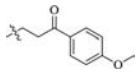
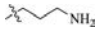
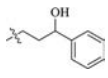
All the target compounds were evaluated *in vitro* for their PARP-1 enzyme inhibition activity at the concentration of 10 nmol/L, and



Scheme 1. Synthesis of designed compounds.

Table 1
Structures of designed compounds and their inhibition rate and IC_{50} against PARP-1 and PARP-2.



Compd.	-R	%Inhibition rate at 10 nmol/L		IC_{50} (nmol/L)		Compd.	-R	%Inhibition rate at 10 nmol/L		IC_{50} (nmol/L)	
		PARP-1	PARP-2	PARP-1	PARP-2			PARP-1	PARP-1	PARP-1	PARP-2
8a		8.6	21.8	/	/	**8f**		32.6	77.7	/	/
8b		25.2	70.1	/	/	**8g**		70.2	54.0	4.4	2.6
8c		51.4	91.9	5.6	1.6	**8h**		54.2	87.4	6.9	2.4
8d		11.6	40.7	/	/	**8i**		58.6	71.4	4.6	2.1
8e		-[a]	4.5	/	/	**8j**		76.9	86.6	2.4	1.5
10b		-[a]	1.4	/	/	**8k**		70.1	81.0	3.1	1.7
9a		15.3	30.4	/	/	**9f**		46.4	76.3	/	/
9b		14.3	31.2	/	/	**9g**		43.0	78.1	/	/
Veliparib		54.3	78.3	5.3	1.6						

[a] Undetectable.

compounds with PARP-1 inhibitory ratio >50% were selected to determine the IC_{50} values. Veliparib was used as the reference molecule. The results were presented in Table 1.

Every target compound was constituted by a piperidyl-containing benzimidazole carboxamide, a linker and a variable substitution. Among these compounds, **8d**, **8e** and **10b** which containing an amide group on the linker showed little inhibitory activity. Compounds bearing a primary amine (**9a** and **9b**) and compounds containing a phthalimide substitution (**8a** and **8b**) were also failed to demonstrate significant inhibitory effect. While the aniline propyl derivative **8c** exhibited good potency with an IC_{50} value of 5.6 nmol/L. We also synthesized phenyl ketone alkyl derivatives with a linker of different length. Compound **8f** showed stronger inhibitory activity than most of the derivatives described above, which indicated that phenyl ketone was an effective substituent group for PARP-1 enzyme inhibitory activity. Compound **8g** with a longer linker exhibited more potent inhibitory activity than **8f** with IC_{50} value of 4.4 nmol/L. While increasing the length of linker of **8g** to give **8h** led to a slight decrease in potency against PARP-1.

Encouraged by the above results, further modification of the phenyl at the para position of **8g** with fluoro (**8i**), chloro (**8j**) and methoxyl (**8k**) was conducted. The results showed that all the three subsitutions were tolerated and produced comparable activity with the non-substituted counterpart with IC_{50} value of 4.6, 2.4 and 3.1 nmol/L. Reduction of the ketone on the linker to a hydroxyl group (**9f** and **9g**) resulted in a moderate decrease in enzyme potency, thus confirming the crucial role of the ketone group. All the phenyl ketone alkyl derivatives exhibited more potent than the positive control veliparib.

In addition to PARP-1, PARP-2 is another enzyme that has been characterized to be involved in DNA repair [27]. PARP-2 is the closest homolog of PARP-1 in the PARP superfamily and its catalytic domain shares 69% similarity to that of PARP-1 [4,28], therefore,

Table 2
Results of compound screening by cell proliferation assay.

Compd.	IC_{50} (μmol/L)	
	MDA-MB-436	CAPAN-1
8a	>100	>100
8b	>100	>100
8c	>100	74.7
8d	>100	>100
8e	61.5	>100
8f	74.8	66.1
8g	15.8	28.8
8h	77.6	88.0
8i	29.0	19.6
8j	18.9	11.3
8k	34.9	17.2
9a	>100	>100
9b	>100	>100
9f	>100	>100
9g	>100	>100
10b	>100	>100
Olaparib	30.2	>100
Veliparib	>100	>100

most of the initially known PARP-1 inhibitors also bound PARP-2 with comparable binding affinity [28]. In this study, all target compounds were evaluated for their PARP-2 inhibitory activity at the concentration of 10 nmol/L. As shown in Table 1, all tested compounds produced marked inhibition activities against PARP-2 and processed stronger potency than those against PARP-1, which were consistent with most of the PARP inhibitors reported previously. The IC_{50} values of **8c** and **8g**-**8k** against PARP-2 were also determined, which were 1.6, 2.6, 2.4, 2.1, 1.5 and 1.7 nmol/L respectively.

Given the profile of PARP-1 and PARP-2 inhibition, we further evaluated all the target compounds *in vitro* for their antitumor activities. The growth inhibitory activity was determined against MDA-MB-436, a BRCA1-deficient triple negative breast cancer cell line and CAPAN-1 pancreatic cancer cells line carrying BRCA2 gene mutation. Veliparib and olaparib were both used as the positive controls and the results were summarized in Table 2. The steps of the cell proliferation assay can be found in Supporting information.

As showed in Table 2, it was obvious that the trend of antitumor activities of most compounds was basically the same as that of inhibition to PARP-1 and PARP-2. In both cell lines, the antiproliferative effect of **8g**, **8i**, **8j** and **8k**, which were the most potent PARP-1 inhibitors in this work, was ranging from 15 μmol/L to 35 μmol/L. These compounds were more potent than veliparib and olaparib. **8f** and **8h** exhibited relative lower inhibition activities in both cell lines, which was in agreement with the result of the PARP inhibition experiment. This indicated that perhaps the PARP inhibitory potency of the tested derivatives contributes to its growth inhibitory. However, **8c**, **9f** and **9g** performed moderate to good PARP inhibitory activity but poor cellular potency, perhaps due to the lack of appropriate membrane permeability, since the polar amido/hydroxyl groups on the linker possibly reduced the lipophilicity of the compounds. The weak cytotoxicity in MDA-MB-436 of compound **8e** which has no effect on PARP-1 and PARP-2 suggests off-target effects beside PARP-1 and PARP-2. The result also indicated that compounds **8a**, **8b**, **8d**, **9a**, **9b** and **10b** were inactive in both cell lines, as no inhibition of cell growth was observed up to 100 μmol/L drug concentration.

To further study the pharmaceutical potential of these compounds, we chose **8g**, **8i**, **8j** and **8k**, which showed the best activities in both PARP inhibition experiments and cell proliferation assay, to predict their ADME properties using ACD/Percepta. A brief introduction of the operation process of ADME prediction on ACD/Percepta was provided in Supporting information.

Table 3
ADME profiling of **8g, 8i, 8j** and **8k**.

Compd.	Caco-2[a] (Pe, μm/s)	PPB[b] (%)	CNS[c] (Score)
8g	0.17	84	−3.60
8i	0.22	78	−3.35
8j	0.49	87	−3.24
8k	0.22	78	−3.45

[a] Caco-2 permeability.
[b] Plasma protein binding rate.
[c] Central nervous system distribution.

As listed in Table 3, the Pe values in Caco-2 monolayer model of the four compounds were all above 0.1 μm/s, indicating that these substitutions are supposed to be highly permeable in the process of intestinal transport *in vivo*, as Caco-2 cells are similar with differentiated human intestinal epithelial cells in structure and functions. As for PPB (plasma protein binding), the binding rates of all of the four compounds were predicted to be above 75%, presenting that they would be strongly bound to plasma protein *in vivo*. In addition, CNS (central nervous system) distribution scores under −3 showed that these compounds were not able to penetrate blood-brain barrier, suggesting the CNS would not directly suffer from any potential side-effect caused by these compounds.

As the most potent inhibitor, compound **8j** was docked into the active site of PARP-1 (PDB ID code: 2RCW [13]) on SYBYL-X 1.3, in order to explore the binding mode (Fig. 2). As we expected, the benzimidazole scaffold was nicely situated in the NI site and formed two key hydrogen bonds with the backbone of Gly-863. Another hydrogen bond was characterized between the hydrogen atom on the imidazole ring and Glu-988, which was not found in other benzimidazole inhibitors. It also formed strong π-π stacking with Tyr-907, which was consistent with previous reports [12]. Furthermore, the phenyl ketone moiety extended into the AD site, which formed hydrophobic contacts with the hydrophobic pocket. We also carried out docking simulation of compound **8f**, which was similar to **8j** in structure but did not perform an equal level of activity in biological experiments. As shown in Fig. 2, the benzimidazole carboxamide part of **8f** was in a different direction, which led to the reduction in number of hydrogen bonds. In addition, the phenyl ring at the end of the side chain failed to enter the hydrophobic pocket of PARP-1 in the case of **8f**. These results indicated that the binding mode of piperidyl benzimidazole carboxamide derivatives with PARP-1 was supposed to be significantly affected by the length of the linker. Details of the docking simulation were given in Supporting information.

In summary, we have described a series of potent PARP-1 inhibitors based on piperidyl benzimidazole carboxamide structure. According to the result of the PARP inhibition experiments and cell proliferation assay, the phenyl ketone alkyl derivatives (**8g**, **8i**, **8j** and **8k**) showed impressive PARP-1 inhibitory activity. **8f** and **8h** performed moderate inhibitory activity as the length of the linker changed. **8c**, **9g** and **9h** showed fine activity in enzyme inhibition assay but poor cellular potency, possibly due to weak membrane permeability. In addition, the result of ADME prediction indicated that the compounds have proper pharmaceutical properties. Besides, the binding mode between the designed inhibitors and PARP-1 was confirmed *via* docking simulation of **8f** and **8j** to PARP-1.

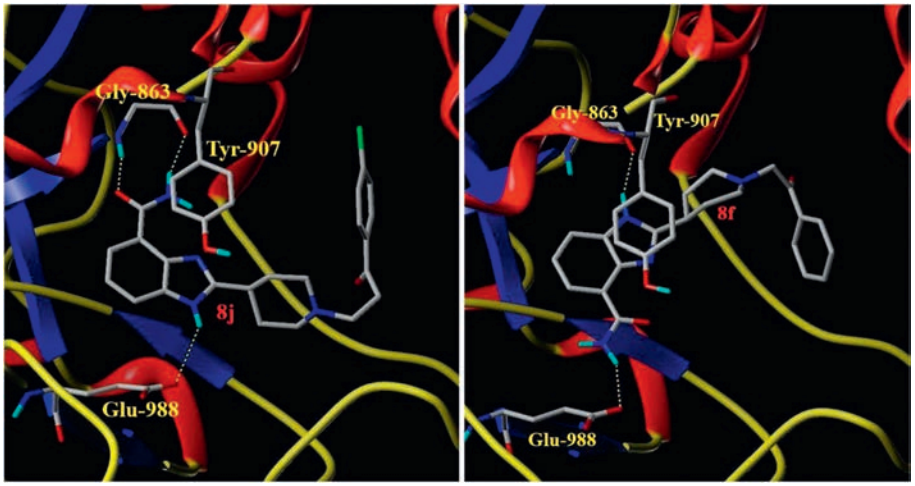


Fig. 2. Proposed binding modes of **8j** and **8f** with PARP-1.

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Appendix A. Supplementary data

Supplementary material related to this article can be found, in the online version, at doi:https://doi.org/10.1016/j.cclet.2019.04.045.

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