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Identification of 2-substituted pyrrolo[1,2-*b*]pyridazine derivatives as new PARP-1 inhibitors

Hao-Yue Xiang [a,b,e], Jian-Yang Chen [b,e], Xia-Juan Huan [b,e], Yi Chen [c], Zhao-bing Gao [d], Jian Ding [b], Ze-Hong Miao [b,*], Chun-Hao Yang [b,*]

[a] College of Chemistry and Chemical Engineering, Central South University, Changsha, Hunan 410083, PR China
[b] State Key Laboratory of Drug Research, Shanghai Institute of Materia Medica, Chinese Academy of Sciences, Shanghai 201203, PR China
[c] Division of Anti-tumor Pharmacology, Shanghai Institute of Materia Medica, Chinese Academy of Sciences, Shanghai 201203, PR China
[d] Key Laboratory of Receptor Research, Shanghai Institute of Materia Medica, Chinese Academy of Sciences, Shanghai 201203, PR China

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ABSTRACT

A library of new 2-substituted pyrrolo[1,2-*b*]pyridazine derivatives were rapidly assembled and identified as PARP inhibitors. Structure-activity relationship for this class of inhibitor resulted in the discovery of most potent compounds **15a** and **15b** that exhibited about 29- and 5- fold selective activity against PARP-1 over PARP-2 respectively. The antiproliferative activity of the as-prepared compounds were demonstrated by further cellular assay in BRCA2-deficient V-C8 and BRCA1-deficient MDA-MB-436 cell lines, displaying that compound **15b** could robustly reduce the corresponding cell proliferation and growth with CC_{50}s of 340 and 106 nM respectively. The PK property of **15b** was also investigated here.

Poly(ADP-ribose)polymerase (PARP), as a nuclear enzyme, plays critical roles in a variety of physiological functions related to cell survival, proliferation and apoptosis.[1–4] Especially, DNA strand breaks can selectively activate PARP, accompanied by catalyzing the poly-ADP ribosylation of corresponding acceptor proteins with nicotinamide adenine dinucleotide (NAD^+).[5–6] Drug resistance is frequently appeared in cancer patients during the procedure of chemotherapeutics and radiation by repair of DNA damages.[7] Given its implication in processes related to DNA damages in the base excision repair pathway, PARP has emerged to be an efficient target for cancer treatment.[8,9]

There are at least 17 members consisted in PARP family and all of them share a conserved domain. Between these members, DNA-binding domain is only found in PARP-1 and PARP-2, with PARP-1 contributing up to 95% of total activity for this family in response to DNA damage.[10,11] As a result, targeting PARP-1 is regarded as a rationale strategy for cancer therapy and substantial breakthroughs in the realm of PARP-1 inhibitors have been achieved.[12]

Designing structural analogues of NAD^+ to compete with NAD itself at the level of the catalytic domain is the most direct strategy thus to inhibit the activity of PARP.[13] By now, a number of PARP inhibitors mimicking the nicotinamide moiety of NAD^+ have been developed (Fig. 1).[14–24] Encouragingly, the concept that inhibition of PARP-1 as a clinically efficacious cancer-therapeutic strategy have been well documented by the approval of olaparib (AZD2281).[25,26] Afterwards, other three PARP-1 inhibitors including rucaparib, talazoparib and niraparib have also been approved.[8] Currently a set of PARP-1 inhibitors[25,26] such as veliparib[27] or our own mefuparib[28] are active in a variety of clinical trials. Not surprisingly, all these reported inhibitors share similar structural and chemical features, including an aromatic ring and carboxamide moiety, which can interact with the catalytic domain of PARP-1 through hydrogen bonds and π-stacking.[13] Recently, a straightforward methodology to 2-substituted pyrrolo[1,2-*b*]pyridazine derivatives was reported by our lab.[29] After analyzing the structural features of the obtained pyrrolo[1,2-*b*]pyridazine products, we found that the structure of these products possessed the common moiety in PARP-1 inhibitors and thus rationalized that pyrrolo[1,2-*b*]pyridazine could be served as novel scaffold for PARP-1 inhibitors. In this letter, we focused on investigating the influence of the substituents at the 2-position of pyrrolo[1,2-*b*]pyridazine framework on the activity of the newly designed compounds to inhibit PARP1/2 and cell proliferation, culminating in the discovery of potent PARP-1 inhibitor **15b**.

As shown in Scheme 1, the commercially available starting materials

* Corresponding authors.
E-mail addresses: zhmiao@simm.ac.cn (Z.-H. Miao), chyang@simm.ac.cn (C.-H. Yang).
[e] These authors contributed eaqually.

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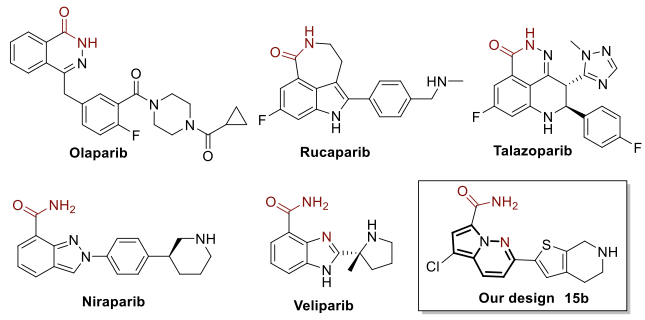
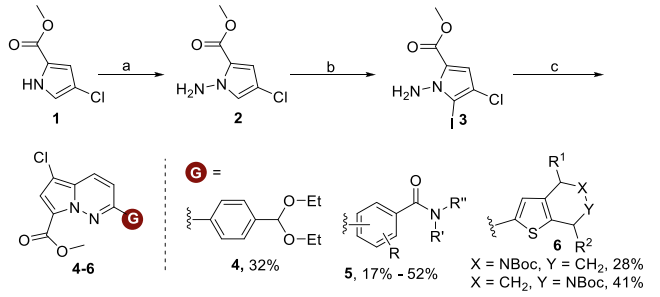
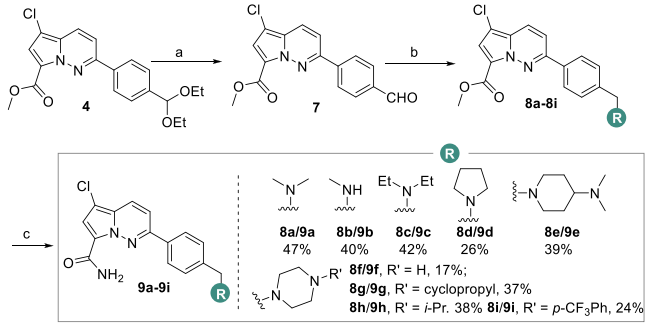


Fig. 1. Representative PARP-1 inhibitors and our design.



Scheme 1. Preparation of the key intermediates **4**–**6**. Reagents and conditions: (a) NaH, NH_2Cl in ether, DMF 0 °C ~ rt, yield 85%; (b) KI, H_2O_2, AcOH, rt, yield 78% ; (c) $Pd(OAc)_2$, CuI, DBU, toluene, MW, 90 °C.

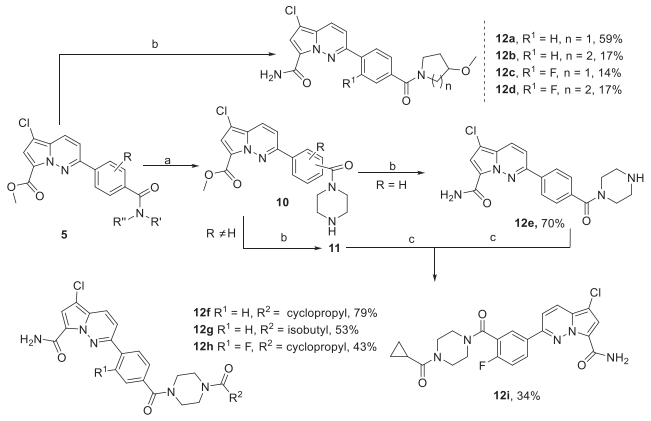


Scheme 2. Route for the synthesis of compounds **9**. Reagents and conditions: (a) 3 M HCl, dioxane, reflux, yield 80% ; (b) amines, $NaBH_3CN$, AcOH, CH_3OH, rt ; (c) NH_3 in CH_3OH, sealed tube, 90 °C, yields for two steps.

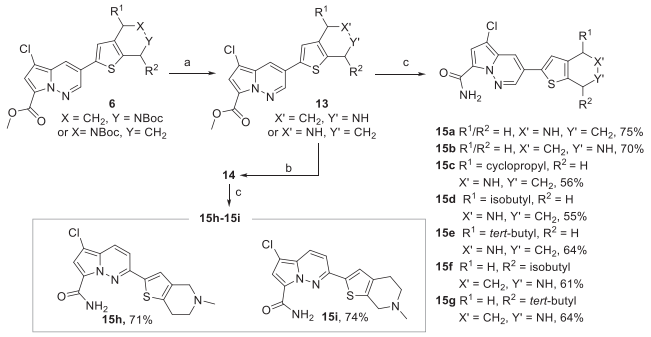
methyl 4-chloro-1*H*-pyrrole-2-carboxylate **1** was able to converted to methyl 1-amino-4-chloro-5-iodo-1*H*-pyrrole-2-carboxylate **3** smoothly by sequential amination and iodization process. Following, the key intermediates **4**–**6** were easily prepared through a domino coupling–isomerization–condensation reaction between compound **3** and corresponding (hetero)aryl propargyl alcohols. Subsequently, three series of designed target compounds were prepared according to the routes displayed in Schemes 2–4 respectively.

Hydrolysis of acetal **4** gave the corresponding aldehyde **7**. Further reductive amination reaction of aldehyde **7** by being treated with a range of diverse amines, provided the desired 2-(4-(aminomethyl) phenyl)-pyrrolo[1,2-*b*]pyridazine analogs **8**. Final aminolysis of compounds **8** delivered the first series of target compounds **9** .

As shown in Scheme 3, the second series of designed 2-substituted pyrrolo[1,2-*b*]pyridazine derivatives **12** could be obtained from compounds **5**. Direct aminolysis of intermediates **5a-5d** yielded the desired final products **12a-12d**. Analogously, the products **11** and **12e** were formed through a two-step process of deprotection and aminolysis from



Scheme 3. Route for the synthesis of compounds **12**. Reagents and conditions: (a) HCl, CH_2Cl_2, reflux to remove Boc group; (b) NH_3 in CH_3OH, tube sealed, 90 °C; (c) RCOCl, NEt_3, CH_2Cl_2, rt.



Scheme 4. Route for the synthesis of compounds **15**. Reagents and conditions: (a) CF_3COOH, CH_2Cl_2, rt; (b) 37% formaldehyde aqueous solution, AcOH, $NaBH_3CN$, CH_3OH; (c) NH_3 in CH_3OH, tube sealed, 90 °C.

the corresponding intermediate **5e** (yield 35%). Alternatively, acylation of the deprotection product of intermediate **10** with appropriate acyl chlorides produced the amide derivatives **12f-12i**. Similarly, the third series of products **15** could be conveniently prepared according to the route outlined in Scheme 4 and the key intermediates **6** were prepared according to our previous report.[31]

With these as-prepared pyrrolo[1,2-*b*]pyridazine derivatives in hands, we next shifted our attention to evaluate their biological activity at both enzymatic and cellular level with AZD2281 as reference compound (Table 1). *BRCA1*-deficient human breast cancer cells MDA-MB-436 and *BRCA2*-deficient Chinese hamster cell mutant V-C8 were employed in cell assays to verify the antiproliferative activity. Most of the tested compounds exhibited potent inhibition against PARP1 with the IC_{50} values in the nanomolar range. Generally, the third series of designed derivatives **15** bearing tetrahydrothienopyridinyl segments showed more potent than the other two series of 2-substituted pyrrolo [1,2-*b*]pyridazine derivatives **9** and **12** at both enzymatic and cellular level. Interestingly, most of these compounds displayed stronger inhibition toward PARP-1 over PARP-2. Specifically, as for the first series, the substitutions on the nitrogen atom in the side chain of compounds **9** had tremendous effect on the inhibition potency and selectivity. The inhibitory activity for PARP1/2 usually decreased with the increase volume of basic side chain of compounds **9**. Simplest methyl group substituted on nitrogen atom led to compound **9b**, resulting in a 47-fold selectivity toward PARP-1 over PARP-2, however its enzymatic and cellular potency against *BRCA1*-deficient MDA-MB-436 cells both reduced compared to **9a**. The introduction of piperazinyl moiety was unable to improve their biological profiles. Obviously, the appendants

Table 1
Enzymatic and cellular assays of compounds **9**, **12** and **15**.[a]

Cpd.	PARP-1 IC_{50} (nM)	PARP-2 IC_{50} (nM)	V-C8 (BRCA2$^{-/-}$) CC_{50} (μM)	MDA-MB-436 (BRCA1$^{-/-}$) CC_{50} (nM)	Selectivity[b] PARP −1 vs −2
9a	93.27	704.62	6.20	686.95	7.5
9b	248.69	1118.31	2.00	1284.32	47.7
9c	433.50	2202.99	3.51	3734.56	5.1
9d	432.39	1118.78	2.15	5398.52	2.6
9e	818.27	1126.00	2.78	5478.26	1.4
9f	626.68	2325.82	n.d.	9668.86	3.7
9g	1082.86	997.49	>10	75231.91	0.92
9h	368.98	1444.72	2.70	3546.08	3.9
9i	3328.75	445.06	>10	8092.39	0.13
12a	361.84	1714.13	n.d.	9644.52	4.74
12b	3512.86	4508.40	4.42	>10	1.28
12c	660.67	1594.00	n.d.	>10	2.4
12d	114.09	1642.75	n.d,	>10	8.9
12e	340.29	1005.03	>10	8092.39	3.0
12f	443.20	1104.91	2.48	9538.09	2.5
12g	501.91	1010.30	5.75	3836.46	2.0
12h	218.51	2324.48	2.31	2953.37	10.7
12i	166.15	1923.75	7.03	>10	11.6
15a	7.24	209.38	1.33	213.99	29.0
15b	14.20	66.81	0.34	106.31	4.7
15c	127.54	1198.40	5.52	2983.54	9.4
15d	184.96	2756.47	6.65	1846.58	14.9
15e	434.19	811.39	n.d.	n.d.	1.9
15f	240.49	501.60	2.68	1363.07	2.1
15g	1033.81	999.67	2.04	1461.94	0.97
15h	156.53	363.35	2.64	3052.52	2.3
15i	140.83	382.13	3.62	2579.66	2.7
AZD 2281[c]	5.22	1.87	0.40	11.38	0.36

[a] IC_{50} values were calculated by Logit method from the results of at least three independent tests with six concentrations each (standard deviations were within 25% of the mean values); Cytotoxic effect (CC_{50}) means the concentration required to reduce cell proliferation and growth by 50%. Values were calculated by Logit method from the results of at least three independent tests with six concentrations each (standard deviations were within 25% of the mean values).[b]fold selectivity of PARP-1 vs PARP-2 (PARP-2 IC_{50}/PARP-1 IC_{50}). [c]AZD2281 is olaparib.

on piperazinyl segment such as cyclopropyl, *i*-Pr and *p*-CF_3Ph groups in compounds **9g-9i** were detrimental for their inhibition activity.

Subsequently, learning from the success of the discovery of the PARP inhibitor olaparib,[30] we introduced a range of benzamide side chains to this scaffold and designed the corresponding compounds **12**. These modification maintained the PARP inhibition potency, but resulted in decreased antiproliferative activity, which probably caused by their low aqueous solubility. In our recent work,[31] we demonstrated that the tetrahydrothienopyridinyl motifs were well tolerated in a series of benzimidazole carboxamides as PARP inhibitors. Therefore, various tetrahydrothienopyridinyl segment was incorporated and evaluated. Pleasingly, significantly increasing of the inhibition potency against both PARP-1/2 and antiproliferative activity against both *BRCA1*-deficient MDA-MB-436 cells and *BRCA2*-deficient V-C8 cells, were observed in analogues **15**. Especially, the compounds **15a** and **15b** exhibited the highest PARP-1 (**15a**, IC_{50} = 7.24 nM; **15b**, IC_{50} = 14.2 nM) and PARP-2 potency (**15a**, IC_{50} = 209.38 nM; **15b**, IC_{50} = 66.81 nM), as well as cellular potency for *BRCA2*-deficient V-C8 cell (**15a**, CC_{50} = 1.33 μM; **15b**, IC_{50} = 340 nM) and *BRCA1*-deficient MDA-MB-436 cell (**15a**, CC_{50} = 213.99 nM; **15b**, IC_{50} = 106.31 nM), among all the investigated compounds. Similar to the analogues **9g-9i**, masking the nitrogen atom in pyridinyl ring, resulted in decreased potency (compounds **15h-15i**). As a consequence, it could be concluded that a free *NH* group seems to be beneficial for securing its high biological activity.

To further elucidate the druggability for this new class of PARP inhibitors, we firstly tested the hERG (human ether-a-go-go-related gene)-inhibitory activity of the most potent compounds **15a** and **15b** to assess their cardiotoxicity.[32] The results showed that compound **15b** (hERG IC_{50} = 11.31 μM) were much less toxic than compound **15a** (hERG IC_{50} = 1.31 μM), and cisapride was used as the positive control (hERG IC_{50} = 0.27 μM). As such, the compound **15b** was further selected to investigate its pharmacokinetic properties (PK).[33] The mouse PK studies were performed on **15b** hydrochloride and the results of the behavior of **15b·HCl** *in vivo* were expressed in Table 2. Low oral exposure with high clearance and short half-life was observed. Comparing to the clinical PARP inhibitor **AZD2281**, the oral availability of **15b·HCl** were acceptable (23%), which support itself for its anti-tumor efficacy *in vivo*. A preliminary *in vivo* antitumor studies were carried out on *BRAC*-1-mutated MAD-MB-436 xenograft model, revealing that **15b·HCl** significantly inhibited the growth of tumor with administrating orally once a day for 21 days in a dose-dependent manner (Table S1) with no obvious weight loss (Table S2).[33] Even so, further efforts to improve the PK and other druggable profile of this series of compounds still need to be devoted.

Table 2
PK properties of **15b·HCl**.

Dose (mg/kg)	$AUC_{0-\infty}$ (ng·h/mL)	Cmax (ng/mL)	MRT (h)	CL (l/h/kg)	$T_{1/2}$ (h)	F (%)
5 (i.v.)	942	–	1.41	5.36	0.92	–
10 (p.o.)	519	93.3	4.17	–	1.5	23.5

Abbreviations: i.v., intravenous injection; p.o., per oral; AUC, area under the concentration–time curve; Cmax, peak plama concentration of a drug after administratration; MRT, mean residence time; CL, plasma clearance; $T_{1/2}$, elimination half-life; F, bioavailability.

In an effort to develop novel PARP inhibitors, a series of 2-substituted pyrrolo[1,2-*b*]pyridazine derivatives were synthesized and evaluated. Noticeably, pyrrolo[1,2-*b*]pyridazine was widely encountered in the realm of kinase inhibitors, but it is the first time to be identified as PARP inhibitors. Most of the newly prepared analogues potently inhibits the target enzyme and the proliferation of *BRCA1*-deficient MDA-MB-436 cells and *BRCA2*-deficient V-C8 cells. In somewhat, the selectivity of inhibition activity toward PARP-1 over PARP-2 was observed in several tested compounds. Intensive exploration of the side chain on the 2-position of pyrrolo[1,2-*b*]pyridazine skeleton delivered compound **15b** with superior inhibitory against PARP1/2 and antiproliferative activity. The results of the PK studies revealed that the oral availability of **15b** was acceptable. Preliminary antitumor efficacy assays reinforced the lead-like performance of **15b**, rendering it eligible for further development or optimization.

Declaration of Competing Interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

Acknowledgments

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Appendix A. Supplementary data

Supplementary data to this article can be found online at https://doi.org/10.1016/j.bmcl.2020.127710.

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33 The protocol for this study was reviewed and approved by the Institutional Animal Care and Use Committee of Shanghai Institute of Materia Medica, Chinese Academy of Sciences. (Shanghai, China).