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Rational design of conformationally restricted quinazolinone inhibitors of poly(ADP-ribose)polymerase

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Abstract—A successful design of conformationally restricted novel quinazolinone derivatives linked via a cyclopentene moiety as potent poly(ADP-ribose)polymerase-1 (PARP-1) inhibitors has been developed. One selected member of the new series, 8-chloro-2-[(3*S*)-3-(4-phenylpiperidin-1-yl)cyclopent-1-en-1-yl]quinazolin-4(3H)-one (*S*-**16d**), was found to be highly potent with IC_{50} = 8.7 nM and good brain penetration.

Poly(ADP-ribose)polymerase-1 (PARP-1: EC 2.4.2.30) is a chromatin-bound nuclear enzyme involved in a variety of physiological functions related to genomic repair, including DNA replication and repair, cellular proliferation and differentiation, and apoptosis.[1] PARP-1 functions as a DNA damage sensor and signaling molecule. Upon binding to DNA breaks, activated PARP cleaves NAD^+ into nicotinamide and ADP-ribose and polymerizes the latter onto nuclear acceptor proteins including histones, transcription factors, and PARP itself. A cellular suicide mechanism of both necrosis and apoptosis by PARP activation has been implicated in the pathogenesis of brain injury and neurodegenerative disorders and PARP inhibitors have been shown to be effective in animal models of stroke, traumatic brain injury, and Parkinson's disease.[2,3] And also, the use of PARP inhibitors as potential adjuvant cancer therapy with alkylating agents and/or radiation has been recently an area of primary interest in this field.[4] Therefore, inhibition of PARP by pharmacological agents may prove useful for the therapy of neurodegenerative disorders and several other diseases involved in PARP activation.

Over the last two decades, extensive investigations have been conducted in the identification of novel PARP-1 inhibitors. Various approaches to design the scaffolds for PARP inhibitors based on the prototypical PARP inhibitor 3-aminobenzamide have been developed. Our laboratory has previously reported the structure-based design and synthesis of the series of quinazolinones shown in Scheme 1.[5] We designed quinazolinone **3**, which is a structural hybrid of **1** and **2** based on molecular modeling of the human active site of PARP-1, and found that quinazolinone **4e** shows strong potency (IC_{50} = 13 nM) and good oral bioavailability with a high brain/plasma concentration ratio of approximately five. Having identified a general template for the design of PARP-1 inhibitors, we set out to further explore this series by examining modification to the ubiquitous propylene side chain present in **4e**. Accordingly, we have designed a new series of analogues, in which the propylene linker unit has been replaced with a conformationally restricted cyclic structure to reduce the number of rotatable bonds. In this paper, we describe the SAR results of this novel series of modified cyclic linker of quinazolinones against PARP-1.

Table 1 shows the SAR result of various cyclic structures in place of linear linker.[6] Cyclopentene ring compound **6a** displayed the same potency as the original compound **4a**, while cyclopentane ring analogues **7a** and **8a** and pyrrolidine ring analogue **14a** were appropriately 20 times less potent than **6a**. Cyclohexene ring **9a** and cyclohexane ring analogues **10a**, **11a**, and **12a** were 10-fold and 100-fold less active, respectively. As these results, we have selected the restricted cyclopentene linker as a new lead compound of quinazolinone analogue.

Keywords: Poly(ADP-ribose)polymerase PARP-1; SBDD; X-ray; Quinazolinone; Conformationally restricted.
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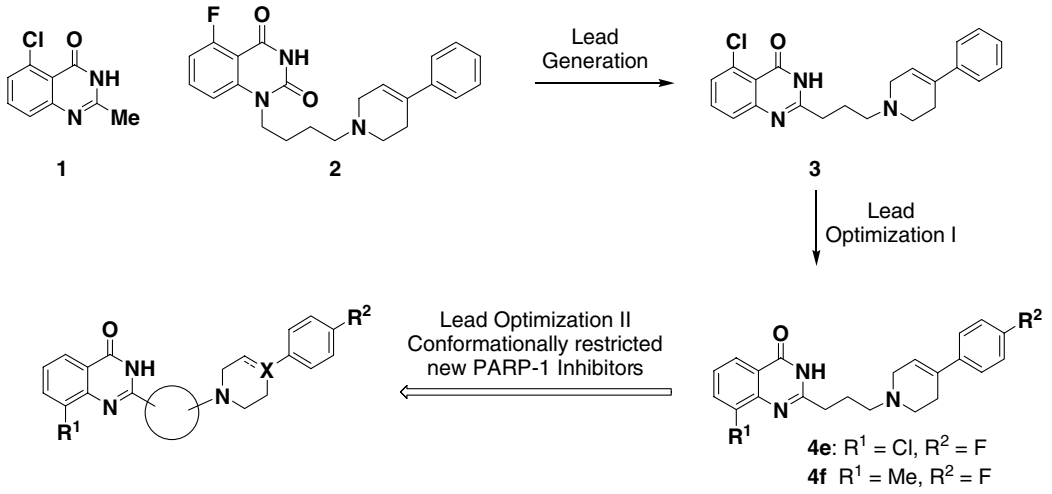
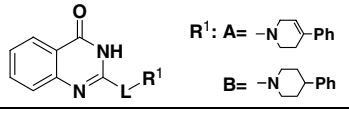


Scheme 1. Design of quinazolinone PARP-1 inhibitors.

Table 1. SAR of cyclic-linker analogues[a]



Compound	L	R^1	PARP-1 IC_{50} (nM)
4a		A	16
5a		B	46
6a[b]		A	16
(*cis* or *trans*)-**7a**[b]		B	435
(*trans* or *cis*)-**8a**[b]		B	555
9a[b]		A	160
10a[b,c]		A	1800
(*cis*)-**11a**		A	1600
(*trans*)-**12a**		A	8400
13a		A	504
14a[b]		A	300
15a		A	131

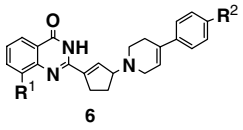
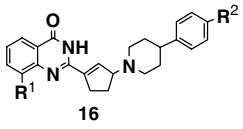
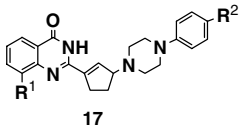
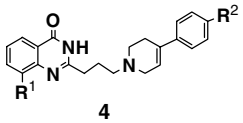
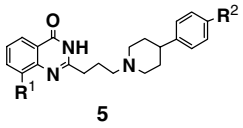
[a] Values are averages from at least two independent dose–response curves, and standard errors for PARP inhibition assay is typically ±5% of the mean or less.
[b] Racemic compounds.
[c] Compound was mixture of *cis* and *trans*.

Table 2 shows the results of quinazolinone analogues linked via a cyclopentene linker. Regarding the chirality of the cyclopentene ring, the (*S*)-enantiomer showed more potent activity than the (*R*)-enantiomer, which agrees with predictions based on the result of molecular modeling of the human active site of PARP-1. It is noteworthy that substitution of a fluoro group on the phenyl ring did not improve activity for the cyclopentene linker in spite of a 2-fold improvement in activity for the linear linker **4** and **5**. The phenylpiperidine (*S*)-**16d** in particular showed 12-fold improved potency relative to the corresponding linear linker compound **5d** and 4-fold more potency than the fluorophenylpiperidine (*S*)-**16e**. Figure 1 displays an overlay of (*S*)-**16e** (green color) and **4f** (pink color). The conformation of **4f** was based on the structure in a X-ray co-crystal structure with the **4f**/PARP-1 complex (accession code of the PDB: 1 UK0).[7] Indeed, docking study suggested that both the quinazolinone core and the terminal phenylpyridine unit of (*S*)-**16e** could be occupied the similar space as an important pharmacophore for PARP inhibitory activity.

Table 3 shows the brain/plasma concentrations after oral administration in mice.[8] In general, the compounds linked with cyclopentene showed improved plasma or brain concentrations compared to the corresponding compounds with a linear linker, which would be explained by metabolic stability in Table 4.[9] We have already confirmed the metabolic pathway of **3** and identified the main metabolites which were produced by oxidation of the linker unit in both rat and human liver microsomes shown in Scheme 3.[10] Accordingly, the cyclopentene linker was more efficacious to block this primary site of metabolism. The tetrahydropyridine type (*S*)-**6d** and **6e**, and the piperazine type **17c**, had decreased brain penetration. The phenylpiperidine type (*S*)-**16d**, however, showed good brain/plasma ratios over two hours, an important prerequisite for treatment of brain-related neurodegenerative disorders.

Compound (*S*)-**16d** was synthesized using the procedures outlined in Scheme 2. Briefly, the racemate compound was synthesized using the same procedure as in our previous publication.[5] Bromination of **18** with *N*-bromosuccinimide gave **19** and its isomer in a 2:1 ratio. After removal of isomer **20** by column chromatography, hydrolysis of the methyl ester group gave the corresponding carboxylic acid. Treatment with $SOCl_2$, followed by condensation with chloroanthranilamide, gave amide derivative **21**. Amination of **21** with

Table 2. SAR of cyclopentene-linker analogues[a]

Compound		R^1	R^2	PARP-1 IC_{50} (nM)
 6	**6a**	H	H	16
	6b	H	F	18
	(*S*)-**6d**	Cl	H	15
	(*R*)-**6d**	Cl	H	286
	6e	Cl	F	36
	6f	Me	F	25
 16	(*S*)-**16d**	Cl	H	8.7
	(*S*)-**16e**	Cl	F	35
	(*R*)-**16e**	Cl	F	257
	16f	Me	F	30
	16g	Me	Cl	46
 17	**17c**	H	Cl	25
	17e	Cl	F	25
	17g	Me	Cl	12
 4	**4a**	H	H	16
	4b	H	F	8.9
	4c	H	Cl	23
	4d	Cl	H	26
	4e	Cl	F	13
	4f	Me	F	8.0
	4g	Me	Cl	16
 5	**5a**	H	H	46
	5d	Cl	H	103
	5e	Cl	F	47

[a] Values are the averages from at least two independent dose–response curves, and standard error for PARP inhibition assay is typically ±5% of the mean or less.

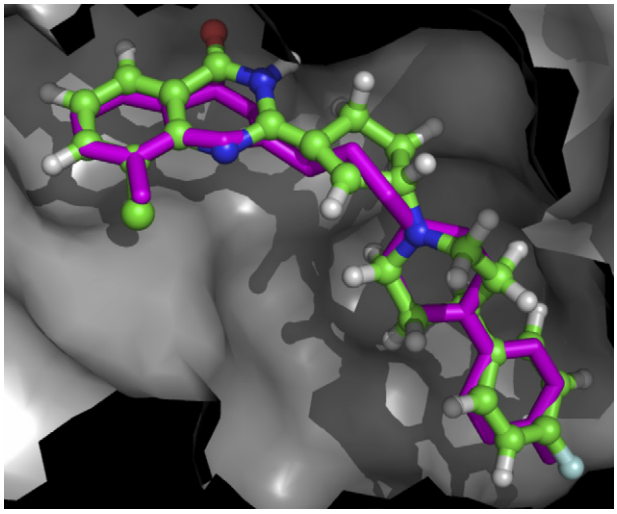


Figure 1. Overlay of (*S*)-**16e** (green color) and **4f** (pink color).

Table 3. Brain/plasma concentration in mice[a]

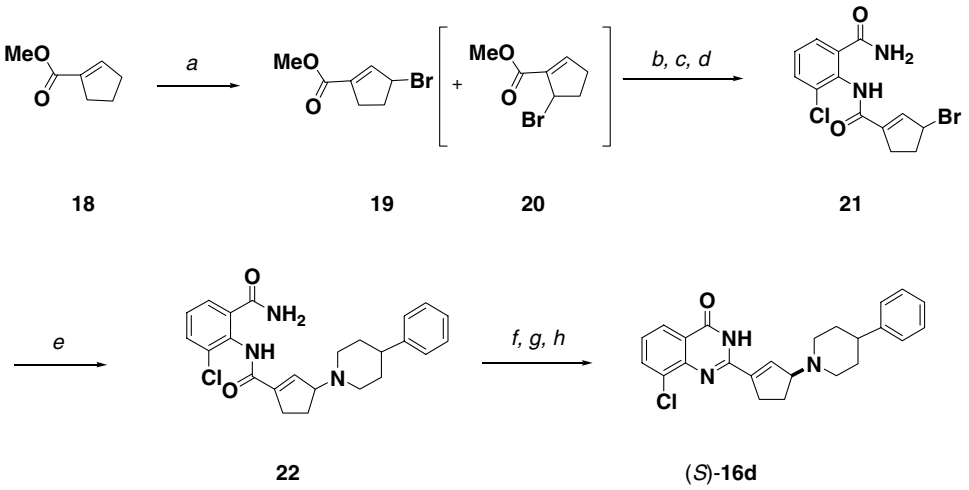
	Brain (μg/g tissue)		Plasma (μg/mL)	
	0.5 h	2.0 h	0.5 h	2.0 h
(*S*)-**6d**	2.0	3.0	17.1	18.1
6e	0.4	0.9	15.2	17.7
(*S*)-**16d**	19.8	18.1	8.7	4.4
(*S*)-**16e**	6.7	12.5	13.6	11.3
17c	1.3	2.2	>20.1	>20.4
4d	8.0	8.4	1.3	1.2
4e	8.4	2.8	1.9	1.2
5d	11.6	3.9	12.5	9.4

[a] The concentration is determined by ex vivo assay after po administration, see Ref. 8.

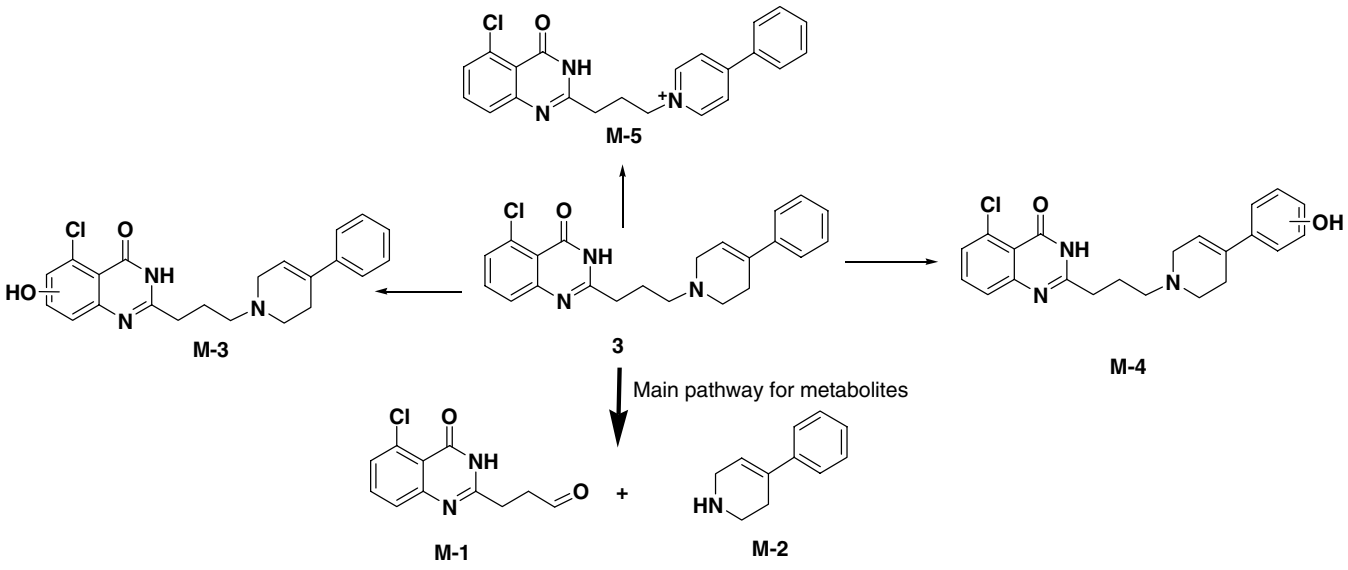
Table 4. Metabolism by liver microsomes[a]

	Clint (mL/min/kg)	
	Mouse	Human
6e	1333	54
(*S*)-**16d**	1164	22
(*S*)-**16e**	686	17
4e	3910	156

[a] Results are average of two experiments, see Ref. 9.



Scheme 2. Reagents and conditions: (a) *N*-bromosuccinimide, CCl_4, reflux; (b) 1 N NaOH, THF–MeOH, rt; (c) $SOCl_2$, CH_2Cl_2, rt; (d) chloroanthranilamide, TEA, CH_2Cl_2, rt; (e) phenylpiperidine, TEA, DMF, rt; (f) 1 N NaOH, dioxane, rt; (g) (L)-di-*p*-toluoyl-tartaric acid, EtOH; (h) 1 N NaOH, rt.



Scheme 3. Metabolites pathway of **3**.

phenylpiperidine in the presence of triethylamine gave **22**, which was treated with 1 N NaOH in dioxane at room temperature to yield **16d** as a racemic mixture. The optical resolution of **16d** by crystallization with (L)-di-*p*-toluoyl-tartaric acid from ethanol (three times) produced the optically pure (*S*)-**16d** with > 98% ee.[11] The preparation of the tetrahydropyridine type **6** and the piperazine type **17** was synthesized using the similar procedure in Scheme 2.

In conclusion, we have refined our previously described PARP-1 inhibitor templates, replacing the propylene linker unit found in **4** with a conformationally restricted cyclopentene linker. This has led to a new series of PARP-1 inhibitors described herein. The phenylpiperidine type (*S*)-**16d** was found to be very potent with good bioavailability and high brain penetration. These findings suggest (*S*)-**16d** could be an attractive therapeutic candidate for neurodegenerative disorders such as Parkinson's disease.

Acknowledgments

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8. Measurements of the concentration of inhibitors in brain and plasma were performed in mice following po administration (32 mg/kg, $n = 2$) by ex vivo assay. See Ref. 5.
9. Compounds were incubated at 37 °C for 60 min with mice and humans in the presence of the MADPH-generating system.
10. M-1 and M-2 were the main metabolites in both mouse and human liver microsomes shown in Scheme 3.
11. The ee ratio was determined by HPLC analysis with a chiral column.