

## Synthesis and Evaluation of Tricyclic Derivatives Containing a Non-Aromatic Amide as Poly(ADP-ribose)polymerase-1 (PARP-1) Inhibitors

Chun-Ho Park,<sup>†,‡</sup> Kwangwoo Chun,<sup>†</sup> Jong-Hee Choi,<sup>†</sup> Wan-Keun Ji,<sup>†</sup> Hyun Young Kim,<sup>‡</sup>  
Seung Hyun Kim,<sup>‡</sup> Gyoonee Han,<sup>§</sup> and Myung-Hwa Kim<sup>†,\*</sup>

<sup>†</sup>Drug Discovery Laboratory, R&D Center, Jeil Pharmaceutical Co., Kyunggi-Do 449-861, Korea  
<sup>\*</sup>E-mail: mhkim@jeilpharm.co.kr

<sup>‡</sup>Department of Neurology, Hanyang University College of Medicine, Seoul 133-791, Korea

<sup>§</sup>Department of Biotechnology, Yonsei University, Seoul 120-740, Korea

<sup>#</sup>Graduate Program in Biomaterials Science and Engineering, Yonsei University, Seoul 120-740, Korea

Received February 24, 2011, Accepted March 31, 2011

A series of potent tricyclic derivatives with a non-aromatic amide as potent PARP-1 inhibitors were successfully synthesized and their PARP-1 inhibitory activity was evaluated. Among the derivatives, 2-(1-propylpiperidin-4-yloxy)-7,8,9,10-tetrahydrophenanthridin-6(5H)-one **23c** displayed potent activity in a PARP-1 enzymatic assay and cell-based assay ( $IC_{50} = 0.142 \mu\text{M}$ ,  $ED_{50} = 0.90 \mu\text{M}$ ) with good water solubility. Further, molecular modeling studies confirmed the obtained biological results.

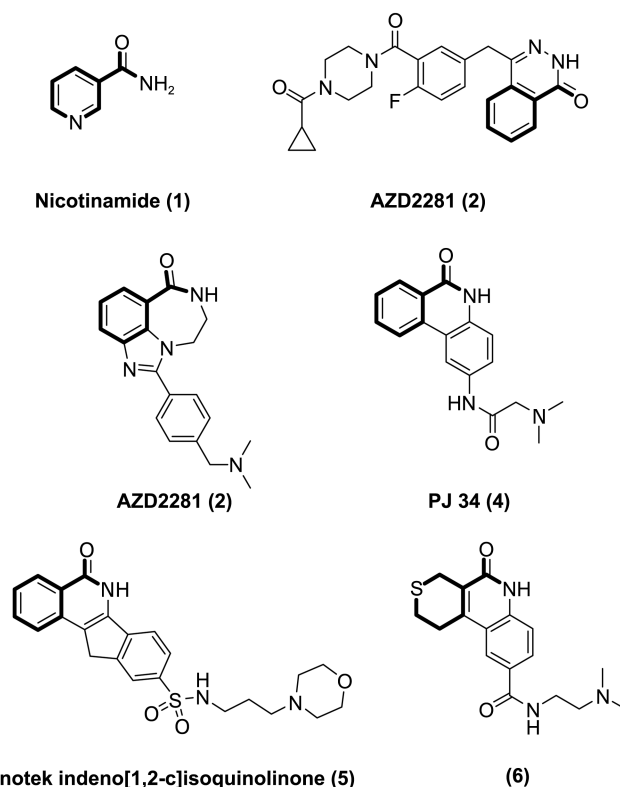
**Key Words** : Poly(ADP-ribose)polymerase, PARP-1 inhibitor, Phenanthridines

### Introduction

Poly(ADP-ribose) polymerase-1 (PARP-1, EC 2.4.2.30) is an abundant chromatin-bound nuclear enzyme in eukaryotes as a key DNA damage sensor that facilitates the repair of DNA.<sup>1,2</sup> The role of PARP-1 is important in a number of cellular process related to ischemia-reperfusion injury and cancer. When triggered by DNA damage, PARP-1 catalyzes the transfer of ADP-ribose units from intracellular nicotinamide adenine dinucleotide ( $\text{NAD}^+$ ) to synthesize a polymer of ADP-ribose on either the PARP protein itself or various other protein acceptors. Subsequently, DNA repair, recombination, cell proliferation, or cell death occurs based upon genomic stability.<sup>2</sup> Furthermore, PARP-1 has also been implicated in a caspase-independent apoptosis pathway mediated by apoptosis-inducing factor.<sup>3</sup> PARP-1 inhibitors provided remarkable protection from tissue damage in various forms of reperfusion injury, inflammation, and neurodegenerative disorders in animal models.<sup>4</sup> Indeed, inhibition of PARP-1 by pharmacological agents could be useful in the treatment of stroke, traumatic brain injury, Parkinson's disease, cancer, and several other diseases involved in PARP-1 activation.<sup>5-7</sup>

Over the last two decades, a large number of PARP-1 inhibitors such as AZD2281 (**2**), AG14361 (**3**), and PJ34 (**4**) have been developed, many of which are currently in clinical trials.<sup>6,8-15</sup> Most PARP-1 inhibitors are competitive with  $\text{NAD}^+$  and these structures are typically nicotinamide or benzamide analogs (Fig. 1). The aromatic amide group of these compounds has been shown to form hydrogen bonds with the Gly-863 and Ser-904 amino acid residues of the PARP enzyme and also binds effectively to the aryl residues, Tyr907 and Tyr896, through a sandwiched hydrophobic  $\pi$ - $\pi$  interaction.<sup>8,10,13</sup>

We have recently disclosed tricyclic PARP-1 inhibitors, substituted thiopyrano[3,4-*c*]quinoline-9-carboxamide derivatives.<sup>16</sup> These compounds include a non-aromatic A-ring and fit well to the active site even though their conformations are not flat. In addition, these derivatives displayed good enzyme inhibitory activity and a few compounds



**Figure 1.** Published PARP-1 inhibitors and thiopyrano[3,4-*c*]quinoline (**6**).

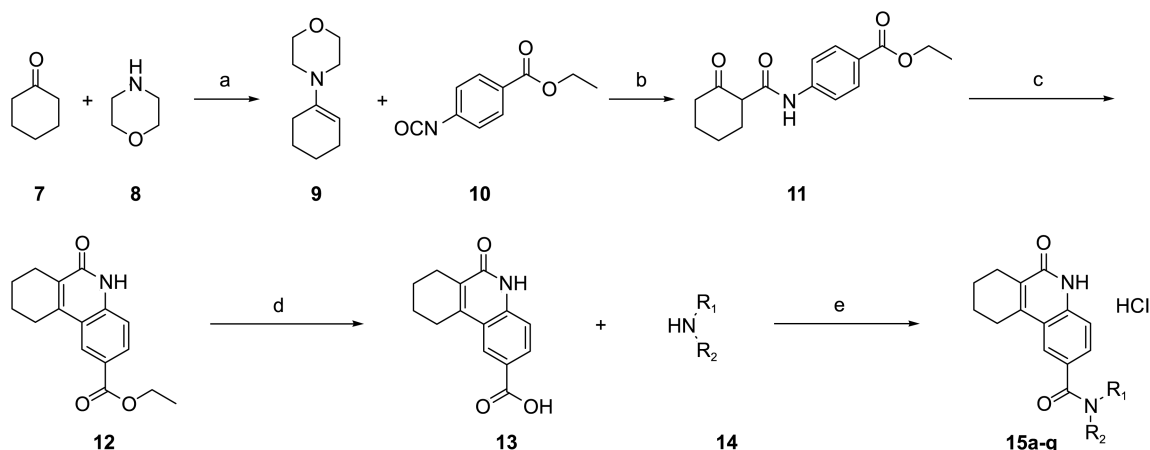
displayed significant cellular activity. A fair number of compounds such as inhibitor **6**, however, suffered from poor cellular potency. In effort to further optimize thiopyrano[3,4-*c*]quinoline-9-carboxamides in order to improve the the potency on the cellular level, substituted 6-oxo-5,6,7,8,9,10-hexahydrophenanthridin-2-carboxamides and 7,8,9,10-tetrahydrophenanthridin-6(5*H*)-ones were designed and synthesized. Crystallographic structure of the human PARP-1 catalytic domain (PDB code: 1UK0) was obtained from the Protein Data Bank (PDB) database,<sup>10</sup> and docking studies of these compounds were performed to approach the development of more potent inhibitors. Additionally, their PARP-1 inhibitory activities were evaluated.

## Results and Discussion

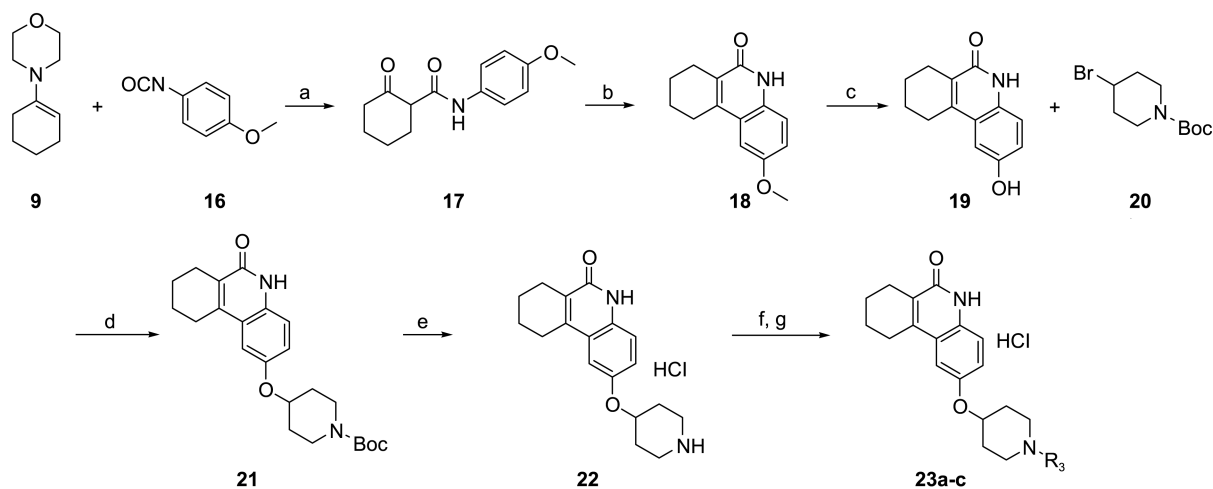
**Chemistry.** The synthetic routes for the newly synthesized substituted 6-oxo-5,6,7,8,9,10-hexahydrophenanthridin-2-carboxamide derivatives are outlined in Schemes 1 and 2. Cyclohexanone **7** reacted with morpholine **8** in the presence of catalytic *p*-toluenesulfonic acid monohydrate

and subsequently treated without further purification.<sup>17,18</sup> The ketamide **11**, prepared by reaction of the enamine **9** with ethyl 4-isocyanobenzoate **10**, was cyclized in 70% sulfuric acid to give the ethyl 6-oxo-5,6,7,8,9,10-hexahydrophenanthridin-2-carboxylate **12**.<sup>19</sup> Hydrolysis of the carboxylate **12** with 1*N* NaOH solution gave its corresponding carboxylic acid **13**, which was optimized by conversion of acid group to amide. 6-oxo-5,6,7,8,9,10-hexahydrophenanthridin-2-carboxamides **15a-g** were synthesized by amide coupling with aliphatic amine or Boc-protected diamine using 1-ethyl-3-(3-dimethylaminopropyl) carbodiimide hydrochloride (EDCI). The analogues **23a-c** were prepared from **9** as depicted in Scheme 2.

2-Methoxy-7,8,9,10-tetrahydrophenanthridin-6(5*H*)-one **18** was obtained from the enamine **9** according to the method described for the preparation of the carboxylate **12**. Demethylation of compound **18** with boron tribromide in dichloromethane gave alcohol **19**, which was alkylated with the bromide **20** in the presence of base to afford the Boc-protected amine **21**. After deprotection of **21** under acidic condition, alkyl amine **23** was synthesized by reductive



**Scheme 1.** Reagents and conditions: (a) *p*-TsOH·H<sub>2</sub>O, toluene, reflux, 12 hr (b) i) toluene, rt, 1 day ii) 2 *N* HCl, rt, overnight (c) 70% H<sub>2</sub>SO<sub>4</sub>, rt, 14 hr (d) 1 *N* NaOH, MeOH, 50 °C, 12 hr (e) i) EDC, HOBt, DMF, rt, 10-15 hr ii) 3.7 *M* HCl in 1,4-dioxane, rt, 1-10 hr.



**Scheme 2.** Reagents and conditions: (a) i) toluene, rt, 1 day ii) 2 *N* HCl, rt, overnight; (b) 70% H<sub>2</sub>SO<sub>4</sub>, rt, 14 hr; (c) BBr<sub>3</sub>, CH<sub>2</sub>Cl<sub>2</sub>, rt, 2 hr; (d) KOH, *i*-PrOH, reflux, 2 day; (e) 3.7 *M* HCl, 1,4-dioxane, rt, 12 hr; (f) aldehyde, NaB(OAc)<sub>3</sub>H, AcOH, MeOH/dichloromethane, rt, 3-5 hr; (g) 3.7 *M* HCl, 1,4-dioxane, rt, 10-12 hr.

amination using an appropriate aldehyde. Finally, the HCl salt of *N*-substituted amine **23** was prepared by 3.7*N* HCl in 1,4-dioxane to enhance water solubility. The structure of the synthesized phenanthridine analogues was established on the basis of <sup>1</sup>H NMR and mass spectral analysis.

**Biology.** The PARP-1 inhibitory activity of synthesized phenanthridine derivatives **15a-g** was evaluated *in vitro* PARP-1 enzymatic assay and cell-based assay, which is summarized in Table 1 and 2. An IC<sub>50</sub> and ED<sub>50</sub> value of the indeno[1,2-*c*]isoquinolinone (**5**) is included in Table 1 as a reference. A series of indeno[1,2-*c*]isoquinolinone (**5**) recently described by the Inotek group showed very potent PARP-1 inhibitory activity.<sup>12</sup> Generally, synthesized phenanthridine compounds showed a profile of reasonable enzymatic potencies. Some compounds (**15a**, **15c** and **15d**), however, displayed poor cellular activity. The polar group at the end of the derivative (**15c** and **15d**) chain seems to prevent cell permeability. The piperidin derivative **15b** showed

**Table 1.** Enzyme and cellular activity of the synthesized compounds **15a-g**

Comps	NR <sub>1</sub> R <sub>2</sub>	IC <sub>50</sub> (μM) <sup>a</sup>	ED <sub>50</sub> (μM) <sup>b</sup>
<b>5</b> <sup>c</sup>		0.057	1.01
<b>15a</b>		0.085	25.31
<b>15b</b>		0.050	7.63
<b>15c</b>		0.076	> 30
<b>15d</b>		0.071	26.73
<b>15e</b>		0.065	2.11
<b>15f</b>		0.098	> 30
<b>15g</b>		0.288	2.36

<sup>a</sup>Enzymatic assays followed a commercially available protocol (Trevigen kit, 4671-096-K) in 384 well plates. Values are the mean of triplicate experiments. <sup>b</sup>The CHO-K1 (Chinese hamster ovary) cell line was used for cell based assay. Values are the mean of quadruplicate experiments. <sup>c</sup>Indeno[1,2-*c*]isoquinolinone (**5**, Ref 12). The data was taken from our assay protocol.

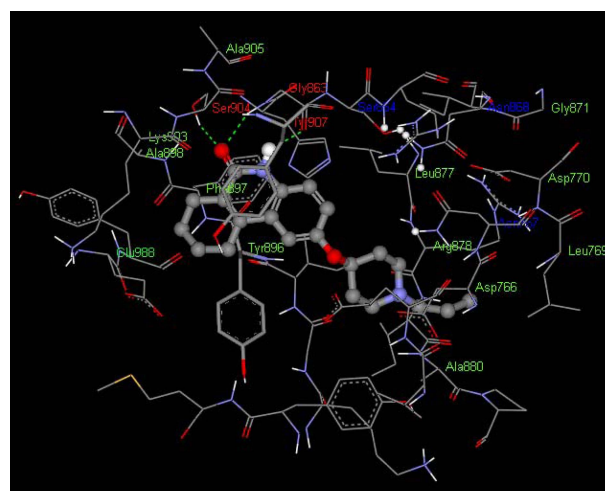
moderate PARP-1 cellular activity. The *N*-alkyl piperazine derivative **15e** and *N*-cycloalkyl piperazine derivatives **15g** displayed significant activities in enzyme assays and slightly potent activities in cellular assays. As mentioned above, the compound **15f** with the polar group at the end of the chain decreased cellular activity significantly.

The activities of substituted 7,8,9,10-tetrahydrophenanthridin-6(5*H*)-one derivatives **23a-c** are given in Table 2. When piperidine analogs were fused to the C-2 hydroxy position, hydrophobic interactions with the adenine-ribose binding site (AD site) and water solubility were increased significantly. The *N*-methyl, ethyl, and propyl piperidine derivatives (**23a-23c**) demonstrated a dramatic increase in cellular potency relative to parent **22**, while maintaining good enzyme potency. Moreover, **23a** and **23c** showed good

**Table 2.** Enzyme and cellular activity of the synthesized compounds **22** and **23a-c**

Comps	R <sub>3</sub>	IC <sub>50</sub> (μM) <sup>a</sup>	ED <sub>50</sub> (μM) <sup>b</sup>
<b>22</b>		0.089	2.51
<b>23a</b>		0.158	0.96
<b>23b</b>		0.091	1.41
<b>23c</b>		0.142	0.90

<sup>a</sup>Enzymatic assays followed a commercially available protocol (Trevigen kit, 4671-096-K) in 384 well plates. Values are the mean of triplicate experiments. <sup>b</sup>The CHO-K1 (Chinese hamster ovary) cell line was used for cell based assay. Values are the mean of quadruplicate experiments.



**Figure 2.** Docking of compound **23c** in the catalytic domain of human PARP-1 (PDB code: 1UK0). (Kinoshita, Nakanishi, Warizaya, Iwashita, Kido, Hattori and Fujii 2004).

potencies similar to **5** in the cell-based assay. In an attempt to comprehend the binding conformation of piperidine analogs fused to the C-2 hydroxy position in the active site of the enzyme, propyl piperidine derivative **23c** was selected for due to its overall profile. An X-ray co-crystal structure of PARP-1 with **23c** is shown in Figure 2. The carboxamido group of the compound (**23c**) formed key hydrogen bond interactions to Ser904 and Gly863 of the enzyme. The amine of the piperidine group was posted near backbone of Ala880 and side chain of Asp766 for polar interaction. Furthermore, the propyl group of the compound (**23c**) formed hydrophobic interaction with hydrophobic pocket (Asp766, Leu769, Arg878, Ile879, and Ala880).

### Conclusion

In conclusion, we have described a straightforward synthesis, docking study and biological evaluation of tricyclic derivatives with a non-aromatic amide as potent PARP-1 inhibitors. Compound **23c** was found to be highly potent in enzyme and cell-based assays ( $IC_{50} = 0.142 \mu\text{M}$ ,  $ED_{50} = 0.90 \mu\text{M}$ ) with good water solubility. These findings suggest that a potent PARP-1 inhibitor **23c** could be a useful therapeutic candidate for cancers and ischemic stroke. Further evaluation of these derivatives such as pharmacokinetic (PKs), CNS penetration is ongoing and will be reported in the near future.

### Experimental Section

**General.** Melting points were determined on a Stuart SMP3 melting point apparatus and are uncorrected.  $^1\text{H}$  NMR spectra were recorded at 400 MHz on a Varian 400 Mercury plus spectrometer; chemical shifts are reported in  $\delta$  (ppm) units relative to the internal reference tetramethylsilane (TMS). Mass spectra were performed on an Applied Biosystems API 2000 mass spectrometer and Agilent 1200 series LC system. All compounds were routinely checked by TLC and  $^1\text{H}$  NMR. TLC was performed on glass-backed silica gel plates (Merck, DC Kieselgel 60 F<sub>254</sub>). Flash chromatography was carried out on E. Merck Kieselgel 60 silica gel (230-400 mesh). All chemicals were obtained commercially. All solvents were reagent-grade and were used without further purification, unless otherwise stated.

**Ethyl 4-(2-oxocyclohexancarboxamido)benzoate (11).** To a stirred solution of 4-cyclohexanone **7** (3.0 g, 30.56 mmol) in toluene (60 mL) were added morpholine **8** (3.2 mL, 36.67 mmol) and catalytic *p*-TsOH·H<sub>2</sub>O (290 mg, 1.53 mmol). The resulting mixture was refluxed for 12 hr with a Dean-Stark trap. After completion, the mixture was cooled to room temperature and evaporated under reduced pressure. The residue was concentrated *in vacuo* to afford the unstable enamine **9**, which was used without further purification.

Ethyl 4-isocyanatobenzoate **10** (8.9 g, 46.55 mmol) in toluene (20 mL) was added dropwise to a stirred solution of unstable enamine **9** in toluene (60 mL) at room temperature, and the stirring was continued at room temperature for 1 day.

Then 2*N* HCl (12.0 mL, 24.00 mmol) was added, and the resulting mixture was stirred at room temperature overnight. The mixture was cautiously neutralized with 2*N* NaOH, and the precipitate was filtered. The filtrate was taken up with water and extracted with dichloromethane. The organic layer was washed with brine, dried over Na<sub>2</sub>SO<sub>4</sub>, filtered and concentrated. The residue was then purified by flash column chromatography (hexane:ethyl acetate = 1:5) to afford the title compound **11** as a yellow solid (2.5 g, 21%, 2 step).

$^1\text{H}$  NMR (400 MHz, DMSO-*d*<sub>6</sub>):  $\delta$  9.69 (s, 1H), 8.02 (d, *J* = 8.4 Hz, 2H), 7.64 (d, *J* = 8.6 Hz, 2H), 7.59 (d, *J* = 8.6 Hz, 2H), 4.35 (q, *J* = 7.2 Hz, 2H), 2.33-2.29 (m, 4H), 1.80-1.74 (m, 4H), 1.60 (s, 1H), 1.38 (t, *J* = 6.8 Hz, 3H).

**Ethyl 6-oxo-5,6,7,8,9,10-hexahydrophenanthridin-2-carboxylate (12).** Ketamide **11** (2.0 g, 6.91 mmol) was suspended in 70 mL of 70% H<sub>2</sub>SO<sub>4</sub> and the resulting mixture was stirred at room temperature for 14 hr. The reaction mixture was poured into ice water and stirred for 0.5 hr. The precipitate was filtered, washed with water, and dried *in vacuo* to afford the title compound **12** as a brown solid (1.0 g, 53%).

$^1\text{H}$  NMR (400 MHz, DMSO-*d*<sub>6</sub>):  $\delta$  13.11 (br, 1H), 8.47 (s, 1H), 8.13 (d, *J* = 8.8 Hz, 1H), 7.57 (d, *J* = 8.8 Hz, 1H), 4.38 (q, *J* = 7.2 Hz, 2H), 3.02 (s, 2H), 2.66 (d, *J* = 6.0 Hz, 2H), 1.90 (d, *J* = 6.0 Hz, 2H), 1.83 (d, *J* = 6.0 Hz, 2H), 1.38 (t, *J* = 6.8 Hz, 3H).

**6-Oxo-5,6,7,8,9,10-hexahydrophenanthridin-2-carboxylic acid (13).** To a stirred solution of carboxylate **12** (1.0 g, 3.68 mmol) in MeOH (12 mL) was added aqueous 1*N* NaOH solution (18.4 mL). The mixture was then heated to 50 °C and stirred for 12 hr. After completion, the cooled mixture was poured on water and acidified to pH 3 with 2*N* HCl. The resulting precipitate was filtered and washed with water, yielding the title compound **13** as a white solid (710 mg, 79%).

$^1\text{H}$  NMR (400 MHz, DMSO-*d*<sub>6</sub>):  $\delta$  12.86 (br, 1H), 11.92 (s, 1H), 8.23 (s, 1H), 7.96 (d, *J* = 8.4 Hz, 1H), 7.32 (d, *J* = 8.8 Hz, 1H), 2.84 (s, 2H), 1.78 (d, *J* = 4.4 Hz, 2H), 1.70 (d, *J* = 4.8 Hz, 2H).

**General Procedure for the Synthesis of 6-Oxo-5,6,7,8,9,10-hexahydrophenanthridin-2-carboxamide Derivatives (15a-g).** To a stirred solution of 6-oxo-5,6,7,8,9,10-hexahydrophenanthridin-2-carboxylic acid **13** (40 mg, 0.16 mmol) in DMF (3 mL) were added *N*-(3-dimethylamino-propyl)-*N'*-ethylcarbodiimide hydrochloride (EDCI, 35 mg, 0.18 mmol), hydroxybenzotriazole (HOBt, 25 mg, 0.18 mmol), and an appropriate amine compound (0.18 mmol). The resulting mixture was stirred at room temperature for 10-15 hr and poured into saturated aqueous NaHCO<sub>3</sub> solution. The mixture was extracted with ethyl acetate, washed with brine, dried over Na<sub>2</sub>SO<sub>4</sub>, and concentrated to dryness. The residue was then purified by flash column chromatography (hexane:ethyl acetate = 1:1 or chloroform:methanol = 1:10) to afford 6-oxo-5,6,7,8,9,10-hexahydrophenanthridin-2-carboxamide as a free base.

3.7*M* HCl in 1,4-dioxane (30 equiv.) was added to a stirred solution of 6-oxo-5,6,7,8,9,10-hexahydrophenanthri-

din-2-carboxamide in 1,4-dioxane (3-5 mL) at 0 °C. After stirring for 1-10 hr at room temperature, the reaction mixture was concentrated *in vacuo* to give the title compound **15**.

The physical and analytical data of the synthesized title compounds are given, as follows:

**N-[2-(Dimethylamino)ethyl]-6-oxo-5,6,7,8,9,10-hexahydrophenanthridin-2-carboxamide (15a)**. White solid; 70%; <sup>1</sup>H NMR (400 MHz, DMSO-*d*<sub>6</sub>): δ 11.88 (s, 1H), 10.63 (br, 1H), 8.95-8.93 (m, 1H), 8.27 (s, 1H), 7.96 (d, *J* = 8.4 Hz, 1H), 7.31 (d, *J* = 8.8 Hz, 1H), 3.65-3.64 (m, 2H), 3.27-3.26 (m, 2H), 2.90-2.88 (m, 2H), 2.84 (s, 3H), 2.82 (br, 2H), 1.81-1.73 (m, 4H).

**6-Oxo-N-[2-(piperidin-1-yl)ethyl]-5,6,7,8,9,10-hexahydrophenanthridin-2-carboxamide (15b)**. White solid; 54%; <sup>1</sup>H NMR (400 MHz, DMSO-*d*<sub>6</sub>): δ 11.88 (s, 1H), 9.84 (br, 1H), 8.98 (br, 1H), 8.27 (s, 1H), 7.96 (d, *J* = 8.6 Hz, 1H), 7.31 (d, *J* = 8.4 Hz, 1H), 3.68-3.67 (m, 2H), 2.56-3.52 (m, 2H), 3.24-3.22 (m, 2H), 2.97-2.85 (m, 4H), 2.32 (br, 2H), 1.90-1.72 (m, 8H), 1.43-1.30 (m, 2H).

**N-(2-Aminoethyl)-6-oxo-5,6,7,8,9,10-hexahydrophenanthridin-2-carboxamide (15c)**. White solid; 56%; <sup>1</sup>H NMR (400 MHz, DMSO-*d*<sub>6</sub>): δ 11.86 (s, 1H), 8.95 (br, 1H), 8.28 (s, 1H), 8.16 (m, 3H), 7.98 (d, *J* = 8.8 Hz, 1H), 7.31 (d, *J* = 8.4 Hz, 1H), 3.56-3.52 (m, 2H), 3.02-2.98 (m, 2H), 2.90 (m, 2H), 2.45 (m, 2H), 1.80-1.79 (m, 2H), 1.73-1.71 (m, 2H).

**2-(Piperazine-1-carbonyl)-7,8,9,10-tetrahydrophenanthridin-6(5H)-one (15d)**. Pale yellow solid; 69%; <sup>1</sup>H NMR (400 MHz, DMSO-*d*<sub>6</sub>): δ 11.82 (s, 1H), 9.58 (s, 1H), 7.74 (s, 1H), 7.51 (d, *J* = 8.0 Hz, 1H), 7.32 (d, *J* = 8.0 Hz, 1H), 3.71 (br, 1H), 3.12 (br, 4H), 2.82 (s, 2H), 2.44 (br, 2H), 1.77 (d, *J* = 5.2 Hz, 2H), 1.70 (d, *J* = 5.2 Hz, 2H).

**2-(4-{2-[4-(4-Fluorophenyl)-5,6-dihydropyridin-1(2H)-yl]ethyl}piperazine-1-carbonyl)-7,8,9,10-tetrahydrophenanthridin-6(5H)-one (15e)**. White solid; 41%; <sup>1</sup>H NMR (400 MHz, DMSO-*d*<sub>6</sub>): δ 11.87 (s, 1H), 11.61 (br, 1H), 11.10 (br, 1H), 7.77 (s, 1H), 7.57-7.53 (m, 3H), 7.36 (d, *J* = 8.0 Hz, 1H), 7.23 (t, *J* = 8.8 Hz, 2H), 6.21 (s, 1H), 4.15 (m, 2H), 3.72-3.24 (m, 14H), 2.84 (m, 4H), 2.46 (m, 2H), 1.79-1.78 (m, 2H), 1.72-1.71 (m, 2H).

**2-[4-(Pyridin-4-yl)piperazine-1-carbonyl]-7,8,9,10-tetrahydrophenanthridin-6(5H)-one (15f)**. Yellow solid; 49%; <sup>1</sup>H NMR (400 MHz, DMSO-*d*<sub>6</sub>): δ 14.06 (br, 1H), 11.87 (s, 1H), 8.29 (t, *J* = 5.6 Hz, 2H), 7.77 (s, 1H), 7.55 (d, *J* = 8.4 Hz, 1H), 7.37 (d, *J* = 8.4 Hz, 1H), 7.17 (d, *J* = 5.6 Hz, 2H), 3.78-3.60 (m, 8H), 2.82 (m, 2H), 2.46 (m, 2H), 1.78-1.77 (m, 2H), 1.72-1.70 (m, 2H).

**2-(4-Cyclopentylpiperazine-1-carbonyl)-7,8,9,10-tetrahydrophenanthridin-6(5H)-one (15g)**. White solid; 41%; <sup>1</sup>H NMR (400 MHz, DMSO-*d*<sub>6</sub>): δ 11.81 (s, 1H), 11.39 (br, 1H), 7.74 (s, 1H), 7.52 (d, *J* = 8.4 Hz, 1H), 7.32 (d, *J* = 8.4 Hz, 1H), 3.54-3.36 (m, 6H), 3.10-3.02 (m, 2H), 2.82 (m, 2H), 2.45 (m, 2H), 1.97 (m, 2H), 1.78-1.70 (m, 7H), 1.54-1.51 (m, 4H).

**N-(4-Methoxyphenyl)-2-oxocyclohexanecarboxamide (17)**. 4-Methoxyphenyl isocyanate **16** (9 g, 60.34 mmol) in toluene (30 mL) was added dropwise to a stirred solution of unstable enamine **9** (6.8 g, 40.75 mmol) in toluene (90 mL)

at room temperature, and the stirring was continued at room temperature for 1 day. Then 2*N* HCl (12.0 mL, 24.00 mmol) was added, and the resulting mixture was stirred at room temperature overnight. The mixture was taken up with water and extracted with ethyl acetate. The organic layer was washed with brine, dried over Na<sub>2</sub>SO<sub>4</sub>, filtered and concentrated. The residue was then purified by flash column chromatography (hexane:ethyl acetate = 1:6) to afford the title compound **17** as a white solid (2.5 g, 25%, 2 step).

<sup>1</sup>H NMR (400 MHz, DMSO-*d*<sub>6</sub>): δ 9.20 (s, 1H), 7.48 (d, *J* = 8.4 Hz, 2H), 6.88 (d, *J* = 8.4 Hz, 2H), 3.79 (s, 3H), 3.31 (q, *J* = 6.0 Hz, 1H), 2.58-2.39 (m, 3H), 2.11-1.94 (m, 3H), 1.87-1.71 (m, 2H).

**2-Methoxy-7,8,9,10-tetrahydrophenanthridin-6(5H)-one (18)**. Ketamide **17** (2.0 g, 8.09 mmol) was suspended in 50 mL of 70% H<sub>2</sub>SO<sub>4</sub> and the resulting mixture was stirred at room temperature for 14 hr. The reaction mixture was poured into ice water and stirred for 30 min. The precipitate was filtered, washed with water, and dried *in vacuo* to afford the title compound **18** as a brown solid (1.7 g, 91%).

<sup>1</sup>H NMR (400 MHz, DMSO-*d*<sub>6</sub>): δ 11.52 (s, 1H), 7.22 (d, *J* = 8.0 Hz, 2H), 7.10 (s, 1H), 7.09 (d, *J* = 8.0 Hz, 2H), 3.79 (s, 3H), 2.80 (s, 2H), 2.46 (d, *J* = 6.8 Hz, 2H), 1.78 (d, *J* = 5.6 Hz, 2H), 1.71 (d, *J* = 5.6 Hz, 2H).

**2-Hydroxy-7,8,9,10-tetrahydrophenanthridin-6(5H)-one (19)**. To a stirred solution of 2-methoxy-7,8,9,10-tetrahydrophenanthridin-6(5H)-one **18** (1.0 g, 4.36 mmol) in dichloromethane (1.5 mL) was added dropwise 1M BBr<sub>3</sub> in dichloromethane (20 mL). The resulting mixture was stirred at room temperature for 2 hr. After completion, the mixture was poured into ice water and stirred for 0.5 hr. The precipitate was filtered, washed with water, and dried *in vacuo* to afford the title compound **19** as a yellow solid (800 mg, 85%).

<sup>1</sup>H NMR (400 MHz, DMSO-*d*<sub>6</sub>): δ 11.42 (s, 1H), 9.33 (br, 1H), 7.13 (d, *J* = 8.0 Hz, 1H), 6.97 (s, 1H), 6.92 (d, *J* = 8.0 Hz, 1H), 2.71 (s, 2H), 2.44 (d, *J* = 5.6 Hz, 2H), 1.76 (d, *J* = 4.8 Hz, 2H), 1.69 (d, *J* = 4.8 Hz, 2H).

**tert-Butyl 4-(6-oxo-5,6,7,8,9,10-hexahydrophenanthridin-2-yloxy)piperidine-1-carboxylate (21)**. To a stirred solution of the alcohol **19** (100 mg, 0.46 mmol) in isopropyl alcohol (3 mL) was added KOH (117 mg, 2.09 mmol), and the mixture was refluxed for 30 min. Then, *tert*-butyl 4-bromopiperidine-1-carboxylate **20** (430 mg, 1.63 mmol) in isopropyl alcohol (3 mL) was added dropwise and the stirring was continued at reflux temperature for 2 day. The mixture was cooled to room temperature and evaporated under reduced pressure. The residue was then purified by flash column chromatography (hexane:ethyl acetate = 1:1) to afford the title compound **21** as a yellow solid (48 mg, 26%).

<sup>1</sup>H NMR (400 MHz, DMSO-*d*<sub>6</sub>): δ 10.61-10.38 (br, 1H), 7.20 (d, *J* = 4.2 Hz, 1H), 7.15 (d, *J* = 4.4 Hz, 1H), 4.46 (m, 1H), 3.75-3.69 (m, 2H), 3.37-3.31 (m, 2H), 2.83-2.80 (m, 2H), 2.70-2.67 (m, 2H), 1.95-1.72 (m, 8H), 1.47 (s, 9H).

**2-(Piperidin-4-yloxy)-7,8,9,10-tetrahydrophenanthridin-6(5H)-one Hydrochloride (22)**. To a stirred solution of

Boc-(6-oxo-5,6,7,8,9,10-hexahydrophenanthridin-2-yloxy)-piperidine **21** (48 mg, 0.12 mmol) in 1,4-dioxane (3 mL) was added 3.7M HCl in 1,4-dioxane (1 mL) at 0 °C, and the mixture was stirred for 12 hr at room temperature. The reaction mixture was concentrated *in vacuo* to give the title compound **22** as a yellow solid (37 mg, 92%).

<sup>1</sup>H NMR (400 MHz, DMSO-*d*<sub>6</sub>): δ 11.56 (s, 1H), 9.16 (br, 2H), 7.25-7.15 (m, 3H), 4.70-4.67 (m, 1H), 3.27-3.18 (m, 2H), 3.07-3.02 (m, 2H), 2.82-2.77 (m, 2H), 2.46-2.41 (m, 2H), 2.12-2.07 (m, 2H), 1.88-1.69 (m, 6H).

**General Procedure for the Synthesis of 2-(1-substitutedpiperidin-4-yloxy)-7,8,9,10-tetrahydrophenanthridin-6(5H)-one Derivatives (23a-c).** To a stirred solution of 2-(piperidin-4-yloxy)-7,8,9,10-tetrahydrophenanthridin-6(5H)-one hydrochloride **22** (50 mg, 0.15 mmol) in MeOH/dichloromethane (3 mL/3 mL) were added sequentially an appropriate aldehyde (2 equiv.), AcOH (10 μL, 0.18 mmol), and NaB(OAc)<sub>3</sub>H (79 mg, 0.37 mmol). The resulting mixture was stirred at room temperature for 3-5 hr and extracted with chloroform. The organic layer was washed with brine, dried over Na<sub>2</sub>SO<sub>4</sub>, and concentrated to dryness. The residue was then purified by flash column chromatography (chloroform:methanol = 10:1) to afford 2-(1-alkylpiperidin-4-yloxy)-7,8,9,10-tetrahydrophenanthridin-6(5H)-one as a free base.

To a stirred solution of 2-(1-alkylpiperidin-4-yloxy)-7,8,9,10-tetrahydrophenanthridin-6(5H)-one in 1,4-dioxane (3-5 mL) was added 3.7M HCl in 1,4-dioxane (30 equiv.) at 0 °C. After stirring for 10-12 hr at room temperature, the reaction mixture was concentrated *in vacuo* to give the title compound **23**.

The physical and analytical data of the synthesized title compounds are given, as follows:

**2-(1-Methylpiperidin-4-yloxy)-7,8,9,10-tetrahydrophenanthridin-6(5H)-one (23a).** White solid; 73%; <sup>1</sup>H NMR (400 MHz, DMSO-*d*<sub>6</sub>): δ 11.56 (s, 1H), 10.81 (br, 1H), 7.26-7.13 (m, 3H), 4.55 (m, 1H), 3.45-3.37 (m, 2H), 3.27-3.04 (m, 4H), 2.78-2.72 (m, 4H), 2.44 (s, 3H), 2.22-1.87 (m, 4H), 1.76-1.69 (m, 2H); MS (ESI+) *m/z*: [M+H]<sup>+</sup> 313.2.

**2-(1-Ethylpiperidin-4-yloxy)-7,8,9,10-tetrahydrophenanthridin-6(5H)-one (23b).** Pink solid; 87%; <sup>1</sup>H NMR (400 MHz, DMSO-*d*<sub>6</sub>): δ 11.53 (s, 1H), 10.62 (br, 1H), 7.24-7.11 (m, 3H), 4.76-4.54 (m, 1H), 3.49-3.46 (m, 1H), 3.32-3.30 (m, 1H), 3.12-2.94 (m, 4H), 2.77 (m, 2H), 2.48 (m, 2H), 2.21-2.09 (m, 2H), 2.02-1.86 (m, 2H), 1.76-1.68 (m, 4H), 1.25 (t, *J* = 7.2 Hz, 3H); MS (ESI+) *m/z*: [M+H]<sup>+</sup> 327.2.

**2-(1-Propylpiperidin-4-yloxy)-7,8,9,10-tetrahydrophenanthridin-6(5H)-one (23c).** White solid; 66%; <sup>1</sup>H NMR (400 MHz, DMSO-*d*<sub>6</sub>): δ 11.56 (s, 1H), 10.82 (br, 1H), 7.26-7.14 (m, 3H), 4.79-4.56 (m, 1H), 3.52-3.49 (m, 1H), 3.34-3.31 (m, 1H), 3.16-2.93 (m, 4H), 2.79 (m, 2H), 2.45 (m, 2H), 2.22-2.16 (m, 2H), 2.03-1.93 (m, 2H), 1.77-1.70 (m, 6H), 0.91 (t, *J* = 7.2 Hz, 3H); MS (ESI+) *m/z*: [M+H]<sup>+</sup> 341.2.

**Docking Study.** All the molecular docking studies were performed using the docking software LigandFit module of Discovery Studio 2.5 (Accelrys, San Diego, CA). In this

study, the docking of PARP-1 inhibitors into the active site of human PARP-1 was performed. The crystal structure of human PARP-1 (pdb code: 1UK0) obtained from the Protein Data Bank was renet to remove water molecules and to add hydrogen atoms to the whole enzyme at a pH of 7.0. The ligands were optimized using a forcefield function, CHARMM. Ten poses were docked for each ligand. Among the docked conformations, the over-fitted conformation was eliminated.

**PARP-1 Enzyme Inhibitory Activity.** The IC<sub>50</sub> of PARP inhibitor compounds was determined using Trevigen PARP inhibition kit (Gaithersburg, USA). The assay was performed in 384-well, small volume microplates following a modified previously reported method,<sup>20</sup> as follows:

The PARP-1 enzyme assay was set up in a volume of 12 μL. Wells were coated with diluted histones and incubated at 25 °C for 2 hr. After the plates were washed four times with PBS, all the liquid was removed following each wash by tapping the plate onto paper towels. To block the nonspecific signal, the wells were blocked by adding the Strep-diluent and incubated at 25 °C for 1 hr. The plates were washed four times with PBS, and serial dilutions of compound were added. The diluted PARP-1 enzyme was then added to each well (0.12 unit/well), except for the negative control wells, and combined with a 2 x PARP cocktail. The reaction was allowed to proceed for 30 min at 25 °C. After washing four times with PBS, streptavidin-linked peroxidase was added to detect the extent of ribosylation, and the plate was incubated at 37 °C for 30 min. The plates were then washed 4 times with PBS, then the TACS-Sapphire substrate was added and the color allowed to develop for 10 min. Finally, the reaction was stopped by adding 0.2N HCl, and the optical densities were read at 450 nm using a Wallac EnVision™ plate reader (PerkinElmer Oy, Turku, Finland). All the data points were determined in triplicate and the data were analyzed using SigmaPlot 10 (Systat Software Inc., USA).

**Intracellular PARP Inhibitory Activity.** This protocol describes a real-time assay to assess an imbalance of DNA single-strand break repair by indirectly measuring PARP activation through the depletion of intracellular NAD(P)H.<sup>21</sup> Chinese hamster ovary cells (CHO-K1) were cultured in RPMI media supplemented with 10% fetal bovine serum (FBS). The cultured CHO-K1 cells were seeded at a density of 2.9 × 10<sup>3</sup> cells/well in 96-well plate and cultured at 37 °C and 5% CO<sub>2</sub> for 16 hr. The cells were then treated with various concentrations of the synthesized compounds and incubated at 37 °C for 2 hr. DNA damage was induced using 1.5 mM MMS (methyl methanesulfonate), and the cells were simultaneously treated with a CCK-8 (Cell Count Kit-8) solution (DOJINDO, CK01-13) for colorimetric assay. At 4 hr after the treatment with MMS, the amount of NAD(P)H secreted into the culture media was quantified using a Wallac EnVision™ plate reader at 450 nm. The results obtained according to various concentrations of the compounds are the average values obtained from four wells, and the results were calculated by regression analysis.

**Acknowledgments.** This study was supported by a grant

from the Korea Healthcare Technology R&D Project, Ministry for Health, Welfare & Family Affairs, Republic of Korea (A100453-1001-0000100).

### References

1. Malanga, M.; Althaus, F. R. *Biochemistry and Cell Biology* **2005**, *83*, 354.
2. Amé, J.-C.; Spenlehauer, C.; de Murcia, G. *BioEssays* **2004**, *26*, 882.
3. Yu, S. W.; Wang, H.; Poitras, M. F.; Coombs, C.; Bowers, W. J.; Federoff, H. J.; Poirier, G. G.; Dawson, T. M.; Dawson, V. L. *Science* **2002**, *297*, 259.
4. Szabo, C.; Dawson, V. L. *Trends Pharmacol Sci.* **1998**, *19*, 287.
5. Zaremba, T.; Curtin, N. J. *Anticancer Agents Med. Chem.* **2007**, *7*, 515.
6. Calabrese, C. R.; Almassy, R.; Barton, S.; Batey, M. A.; Calvert, A. H.; Canan-Koch, S.; Durkacz, B. W.; Hostomsky, Z.; Kumpf, R. A.; Kyle, S.; Li, J.; Maegley, K.; Newell, D. R.; Notarianni, E.; Stratford, I. J.; Skalitzky, D.; Thomas, H. D.; Wang, L. Z.; Webber, S. E.; Williams, K. J.; Curtin, N. J. *J. Natl. Cancer Inst.* **2004**, *96*, 56.
7. Graziani, G.; Battaini, F.; Zhang, J. *Pharmacol Res.* **2005**, *52*, 1.
8. Ishida, J.; Yamamoto, H.; Kido, Y.; Kamijo, K.; Murano, K.; Miyake, H.; Ohkubo, M.; Kinoshita, T.; Warizaya, M.; Iwashita, A.; Mihara, K.; Matsuoka, N.; Hattori, K. *Bioorg. Med. Chem.* **2006**, *14*, 1378.
9. Ferraris, D.; Ko, Y. S.; Pahutski, T.; Ficco, R. P.; Serdyuk, L.; Alemu, C.; Bradford, C.; Chiou, T.; Hoover, R.; Huang, S.; Lautar, S.; Liang, S.; Lin, Q.; Lu, M. X.; Mooney, M.; Morgan, L.; Qian, Y.; Tran, S.; Williams, L. R.; Wu, Q. Y.; Zhang, J.; Zou, Y.; Kalish, V. *J. Med. Chem.* **2003**, *46*, 3138.
10. Kinoshita, T.; Nakanishi, I.; Warizaya, M.; Iwashita, A.; Kido, Y.; Hattori, K.; Fujii, T. *FEBS Lett* **2004**, *556*, 43.
11. Menear, K. A.; Adcock, C.; Boulter, R.; Cockcroft, X. L.; Copsey, L.; Cranston, A.; Dillon, K. J.; Drzewiecki, J.; Garman, S.; Gomez, S.; Javaid, H.; Kerrigan, F.; Knights, C.; Lau, A.; Loh, V. M., Jr.; Matthews, I. T.; Moore, S.; O'Connor, M. J.; Smith, G. C.; Martin, N. M. *J. Med. Chem.* **2008**, *51*, 6581.
12. Jagtap, P. G.; Baloglu, E.; Southan, G. J.; Mabley, J. G.; Li, H.; Zhou, J.; van Duzer, J.; Salzman, A. L.; Szabo, C. *J. Med. Chem.* **2005**, *48*, 5100.
13. Matsumoto, K.; Kondo, K.; Ota, T.; Kawashima, A.; Kitamura, K.; Ishida, T. *Biochim. Biophys. Acta* **2006**, *1764*, 913.
14. Lord, A. M.; Mahon, M. F.; Lloyd, M. D.; Threadgill, M. D. *J. Med. Chem.* **2009**, *52*, 868.
15. Penning, T. D.; Zhu, G. D.; Gandhi, V. B.; Gong, J.; Liu, X.; Shi, Y.; Klinghofer, V.; Johnson, E. F.; Donawho, C. K.; Frost, D. J.; Bontcheva-Diaz, V.; Bouska, J. J.; Osterling, D. J.; Olson, A. M.; Marsh, K. C.; Luo, Y.; Giranda, V. L. *J. Med. Chem.* **2009**, *52*, 514.
16. Kim, M. H.; Park, C. H.; Joe, B. Y.; Chun, K. W. *Patent; KR 10-2009-0046431*.
17. Khuthier, A.-H.; Al-Mallah, K. Y.; Hanna, S. Y.; Abdulla, N.-A. I. *J. Org. Chem.* **1987**, *52*, 1710.
18. Stork, G.; Brizzolara, A.; Landesman, H.; Szmuszkovicz, J.; Terrell, R. *J. Am. Chem. Soc.* **1963**, *85*, 207.
19. Park, C. H.; Chun, K.; Joe, B. Y.; Park, J. S.; Kim, Y. C.; Choi, J. S.; Ryu, D. K.; Koh, S. H.; Cho, G. W.; Kim, S. H.; Kim, M. H. *Bioorganic & Medicinal Chemistry Letters* **2010**, *20*, 2250.
20. Lee, S.; Koo, H. N.; Lee, B. H. *Methods Find Exp. Clin. Pharmacol* **2005**, *27*, 617.
21. Nakamura, J.; Asakura, S.; Hester, S. D.; de Murcia, G.; Caldecott, K. W.; Swenberg, J. A. *Nucleic. Acids Res.* **2003**, *31*, e104.