Contents lists available at ScienceDirect



Bioorganic & Medicinal Chemistry Letters

journal homepage: www.elsevier.com/locate/bmcl



Synthesis and evaluation of tricyclic derivatives containing a non-aromatic amide as inhibitors of poly(ADP-ribose)polymerase-1 (PARP-1)

Chun-Ho Park^a, Kwangwoo Chun^{a,c}, Bo-Young Joe^a, Ji-Seon Park^a, Young-Chul Kim^a, Ji-Soo Choi^a, Dong-Kyu Ryu^a, Seong-Ho Koh^b, Goang Won Cho^b, Seung Hyun Kim^b, Myung-Hwa Kim^{a,*}

^a Drug Discovery Laboratory, R&D Center, Jeil Pharmaceutical Co., Ltd. 117-1, Keungok-Ri, Baekam-Myun, Cheoin-Gu, Yongin-City, Kyunggi-Do 449-861, Republic of Korea ^b Department of Neurology, Hanyang University College of Medicine, Seoul, Republic of Korea ^c Department of Biotechnology, Yonsei University, Seodaemun-Gu, Seoul 120-740, Republic of Korea

ARTICLE INFO

Article history: Received 21 October 2009 Revised 1 February 2010 Accepted 3 February 2010 Available online 8 February 2010

Keywords: Poly(ADP-ribose)polymerase PARP-1 Tricyclic derivatives

ABSTRACT

Highly potent poly(ADP-ribose)polymerase-1 (PARP-1) inhibitors, including 9-hydroxy-1,2-dihydro-4*H*-thiopyrano[3,4-*c*]quinolin-5(6*H*)-one derivatives with a non-aromatic A-ring, were synthesized. Among the derivatives, **12a** showed low nanomolar enzyme and cellular activity ($IC_{50} = 42 \text{ nM}$, $ED_{50} = 220 \text{ nM}$) with good water solubility. Further, **12a** exhibited microsomal stability in vitro and brain permeability in vivo.

© 2010 Elsevier Ltd. All rights reserved.

The poly(ADP-ribose)polymerase (PARP) family is immediately stimulated by DNA damage.¹ When DNA is damaged, PARPs use nicotinamide adenine dinucleotide (NAD⁺) as substrate to synthesize a polymer of ADP-ribose on the PARP protein itself and various other protein acceptors, and then carry out DNA repair, recombination, cell proliferation, or cell death and genomic stability.² PARP

activation has been shown to mediate both ischemic brain injury and cancer by caspase-independent cell death and DNA repair.³ PARP-1 (EC 2.4.2.30) was the first enzyme of this family to be discovered, and is the most abundant member of the PARP enzyme family in eukaryotes.^{2,4} The role of PARP-1 is important in a number of cellular processes, thus it is regarded as a target for treating



Figure 1. Previously reported PARP-1 inhibitors and compound 7.

* Corresponding author. Tel.: +82 31 332 4457; fax: +82 31 333 0337. *E-mail address:* mhkim@jeilpharm.co.kr (M.-H. Kim).

⁰⁹⁶⁰⁻⁸⁹⁴X/\$ - see front matter \odot 2010 Elsevier Ltd. All rights reserved. doi:10.1016/j.bmcl.2010.02.014



Scheme 1. Reagents and conditions: (a) *p*-TsOH?H₂O, toluene, reflux, 12 h; (b) (i) toluene, 1 h; (ii) 2 N HCl, 24 h; (c) 70% H₂SO₄, 14 h; (d) BBr₃, CH₂Cl₂, 2 h; (e) K₂CO₃ or KOH, *i*-PrOH, 24 h; (f) HCl, 1,4-dioxane, 12 h; (g) K₂CO₃, MeCN, 70 °C; (h) HCl, 1,4-dioxane.



Figure 2. Docking of compound **7** (red color, ball and stick) and FR257517 (blue color, stick)¹¹ in the catalytic domain of human PARP-1 (PDB code: 1UK0).

diseases related to ischemia-reperfusion injury and cancer. Indeed, PARP-1 inhibitors have been shown to be effective in animal models of ischemic stroke, traumatic brain injury, Parkinson's disease and cancer.^{5–7}

Recently, a variety of PARP-1 inhibitors have been reported.^{6.8–} ¹⁵ Most of the PARP-1 inhibitors are competitive with NAD⁺ and these structures are typically nicotinamide or benzamide analogs (Fig. 1). The aromatic amide group of these compounds form hydrogen bonds with the Gly-863 and Ser-904 of the PARP enzyme and also bind to Tyr907 and Tyr896 of the nicotinamide-ribose binding site (NI site) by a sandwiched hydrophobic interaction.^{8,11,14} But, some of the PARP-1 inhibitors, fused uracils, had a non-aromatic amide.¹⁶ On the basis of these compounds, we carried out novel class of PARP-1 inhibitors.

In this Letter, we describe the synthesis, the structure–activity relationship (SAR) study and the biological evaluation of tricyclic compounds that contain non-aromatic amides as potential PARP-1 inhibitors. Crystallographic structure of the human PARP-1 catalytic domain (PDB code: 1UK0) was obtained from the Protein Data Bank (PDB) database,¹¹ and a docking study was performed to approach the development of potent inhibitors. The result led us to design analogs containing a non-aromatic A-ring. The first compound **7** which included a non-aromatic A-ring, fit the NI site very well even though its conformation was not flat (Fig. 2).

To verify this docking study, tricyclic 1,2-dihydro-4*H*-thiopyrano[3,4-*c*]quinolin-5(6*H*)-one derivatives were synthesized, as outlined in Scheme 1. The 4-thianone **1**, which was synthesized by method in previous report, reacted with a secondary amine by azeotropic distillation with toluene.^{17,18} The reaction of the enamine **3** with the isocyanate **4** gave the ketoamide **5**, which was cyclized to the thiopyranoquinolinone **6** in 70% sulfuric acid at room temperature.¹⁹ Demethylation of **6** with Boron tribromide in dichloromethane gave alcohol **7**. Alkylation of **7** with the appropriate bromide **8** in the presence of base led to the Boc-protected amine **9**. Alkyl amine **11** was synthesized by deprotection under acidic condition and coupling reaction under basic condition. Final-



Scheme 2. Reagents: (a) Cu(OAc)₂, Et₃N, MeCN; (b) HCl, 1,4-dioxane.

Table 1

Enzyme and cellular activity of the synthesized compounds

Compound	R ¹	IC_{50}^{a} (μM)	$ED_{50}^{b}(\mu M)$
15	_	0.040	1.00
7	Н	0.099	ND ^c
12a		0.042	0.22
12b		0.045	0.58
12c		0.071	0.74
12d	. Слудон	0.029	1.60
12f	~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~	0.040	1.26
12g		0.173	>30
14		0.695	1.86
12h	$\tilde{\Omega}$	0.053	0.80
12i		0.038	0.36
12j		>10	9.44
12k	`́С ^{NH}	0.028	0.89
121		0.039	0.58
12m		0.025	0.28
12n		0.056	0.74
120	<u> </u>	0.080	0.73
12q		0.013	0.35

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

Values are the mean of quadruplicate experiments.

^c ND; not determined.

ly, the N-substituted amine salt **12** was prepared by 3.7 N HCl in 1,4-dioxane to enhance water solubility. In addition to **12**, the synthetic procedure for phenyl amine salt **14** was outlined in Scheme 2. N-Arylation of amine **10a** with phenylboronic acid in the presence of Copper(II) acetate gave phenyl amine **13**, followed by 4.0 N HCl in 1,4-dioxane at room temperature to form **14**.²⁰

Recently, the indeno[1,2-*c*]isoquinolinone **15**, reported by Inoteck, showed 1 nM inhibitory activity (Table 1).¹³ However, according to our assay protocol, indeno[1,2-*c*]isoquinolinone **15** had an IC_{50} value of 40 nM. The activity of various 1,2-dihydro-4*H*-thiopyrano[3,4-*c*]quinolin-5(6*H*)-one derivatives is summarized in

Table 2

Rat PK profile of $12a^a$ (10 mg/kg), n = 3

Compound	12a
Dose (mg/kg)	10
AUC _{0-inf} (h ng/ml)	2908
CL (l/h/kg)	3.4
t _{1/2} (h)	3.45

^a Values were detected by LC/MS/MS after intravenous administration.

Table 3

Brain/plasma	concentrations	of 12a	in the	e rat ^a	(10 mg/kg),	n = 3

Time (h)	Brain (ng/g)	Plasma (ng/ml)	Ratio
0.17	1705	2480	0.69
0.75	391	610	0.64
4.00	13	18	0.72

^a The concentration was detected by LC/MS/MS.

 Table 4

 Human liver microsomal stability of 12a^a

Drug	<i>t</i> _{1/2} (min)
Buspirone	3.8
12a	52.6

^a Microsomal activity was detected at 0, 15, 45, and 80 min by LC/MS/MS. The incubation temperature was 37 $^{\circ}$ C. Values are the mean of triplicate experiments. Buspirone was used as reference.

Table 1. When piperidine analogs were fused to the C-9 hydroxy position, hydrophobic interactions with the adenine-ribose binding site (AD site) and water solubility were increased. The N-propyl and pentyl piperidine derivatives (12a, 12b) showed good enzyme and cellular activity, and water solubility compared to 7. Moreover, 12a was 4.5-fold more active than 15 in the cell-based assay. The *N*-bulky alkyl piperidine derivative **12c** was less active than **12a** due to bumping slightly to the active site. The N-(2-hydroxy) and *N*-(2-methoxy)ethyl piperidine derivatives (**12d**, **12f**) retained moderate activity and the N-(2-piperidinyl)ethyl piperidine derivative 12g was twofold less active than 7 in the enzyme assay. However, **12d**, **12f**, and **12g** displayed weaker activity in the cell-based assay. Additionally, N-aryl alkyl piperidine derivatives (12h, 12i) demonstrated good potency, but rigid or polar bulky derivatives (14, 12j) displayed a loss of potency. Modification of the nitrogen position (12k, 12l, 12m, and 12n) showed good potency, similar to that of 12a. Reduction of ring size (12o, 12q) led to moderate potency and 12q showed a sixfold improved potency compared to 120.

We examined pharmacokinetic (PK) characteristics, brain/plasma concentration, and microsomal stability of **12a**. In the PK studies, **12a** showed a relatively long intravenous half-life and high AUC concentration (Table 2). The concentration of **12a** in plasma and brain was detected by LC/MS/MS after intravenous injection in rats. The brain/plasma ratio was 0.64–0.72 over four hours (Table 3). In the human liver microsomal stability test, **12a** showed good metabolic stability with a 52.6 min half-life (Table 4).²¹

In conclusion, we report the synthesis and biological evaluation of tricyclic derivatives with a non-aromatic amide as potent PARP-1 inhibitors. Compound **12a** was found to be highly potent in enzyme and cell-based assays ($IC_{50} = 42 \text{ nM}$, $ED_{50} = 220 \text{ nM}$) with good water solubility and brain penetration. These findings suggest that the PARP-1 inhibitor **12a** could be a useful therapeutic candidate for ischemic stroke and cancers. Further evaluation of this class of derivatives is ongoing and will be reported in the near future.

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 (A080747-0902-0000200). We thank Professor Gyoonhee Han (Department of Biotechnology, Yonsei University, Republic of Korea) for his assistance with the docking study.

References and notes

- 1. Malanga, M.; Althaus, F. R. Biochem. Cell Biol. 2005, 83, 354.
- 2. Ame, J. C.; Spenlehauer, C.; de Murcia, G. BioEssays 2004, 26, 882.
- 3. Hong, S. J.; Dawson, T. M.; Dawson, V. L. Trends Pharmacol. Sci. 2004, 25, 259.
- 4. Burkle, A. FEBS J. 2005, 272, 4576.
- 5. Zaremba, T.; Curtin, N. J. Anticancer Agents Med. Chem. 2007, 7, 515.
- 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.
 Graziani, G.; Battaini, F.; Zhang, J. Pharmacol. Res. 2005, 52, 1.
- 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.
- 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.

- Hattori, K.; Kido, Y.; Yamamoto, H.; Ishida, J.; Iwashita, A.; Mihara, K. Bioorg. Med. Chem. Lett. 2007, 17, 5577.
- Kinoshita, T.; Nakanishi, I.; Warizaya, M.; Iwashita, A.; Kido, Y.; Hattori, K.; Fujii, T. FEBS Lett. 2004, 556, 43.
- 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.
- 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.
- Matsumoto, K.; Kondo, K.; Ota, T.; Kawashima, A.; Kitamura, K.; Ishida, T. Biochim. Biophys. Acta 2006, 1764, 913.
- Lord, A. M.; Mahon, M. F.; Lloyd, M. D.; Threadgill, M. D. J. Med. Chem. 2009, 52, 868.
- Steinhagen, H.; Gerisch, M.; Mittendorf, J.; Schlemmer, K. H.; Albrecht, B. Bioorg. Med. Chem. Lett. 2002, 12, 3187.
- 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. Castan, F.; Schambel, P.; Enrici, A.; Rolland, F.; Bigg, D. C. H. Med. Chem. Res. 1996, 6, 81.
- 20. Quach, T. D.; Batey, R. A. Org. Lett. 2003, 5, 4397.
- Xu, R.; Nemes, C.; Jenkins, K. M.; Rourick, R. A.; Kassel, D. B. J. Am. Soc. Mass. Spectrom. 2002, 13, 155.