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4-Phenyl-1,2,3,6-tetrahydropyridine, an excellent fragment to improve the potency of PARP-1 inhibitors

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Abstract—We have shown that a 4-phenyl-1,2,3,6-tetrahydropyridine fragment plays an important role in improving inhibitory potency against poly(ADP-ribose) polymerase-1 (PARP-1). Various benzamide analogues linked with this fragment via alkyl spacers have been prepared and evaluated. As a result, some of them have been found to be highly potent PARP-1 inhibitors.
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1. Introduction

Poly(ADP-ribose) polymerase-1 (PARP-1: EC 2.4.2.30) is a chromatin-bound nuclear enzyme that plays an important role in the process of genomic repair.[1] PARP-1 is activated by DNA strand breaks and catalyzes the transfer of ADP-ribose units from its substrate nicotinamide adenine dinucleotide (NAD^+) to nuclear acceptor proteins, such as histones, transcription factors, and PARP itself. However, overactivation of PARP caused by DNA-damaging stimuli, such as exposure to reactive oxygen species could lead to depletion of NAD^+ and ATP. This depletion of ATP culminates in cell death via a necrotic pathway.[2] Recent studies, including the availability of knockout mice, have demonstrated that a lack of PARP-1 offers protection in animal models of stroke, traumatic brain injury, and Parkinson's disease.[3] These data indicate that inhibition of PARP-1 may prove useful for the treatment of these diseases and consequently, a variety of PARP-1 inhibitors have been reported.[4] We have recently found FR247304 (**1**) as a potent lead inhibitor of PARP-1 using a structure-based drug design (Fig. 1).[5] This structure is characterized by two main fragments, quinazolin-4(3H)-one and 4-phenyl-1,2,3,6-tetrahydropyridine. Quinazolin-4(3H)-one contains a benzamido moiety that is essential for binding to the catalytic domain of PARP-1. Meanwhile, 4-phenyl-1,2,3,6-tetrahydropyridine plays an important role in increasing PARP-1 inhibitory potency by binding the AD site of the PARP enzyme.[6] We therefore expected utilization of 4-phenyl-1,2,3,6-tetrahydropyridine by application to other published benzamide analogues, such as 3,5,7,8-tetrahydro-thiino[4,3-d]pyrimidine-4-one (**3**),[7] phthalazin-1(2H)-one (**4**),[8] and phenanthridin-6(5H)-one (**5**),[9] to lead to potent PARP-1 inhibitors (Fig. 2). Although PARP-1 inhibitors containing these scaffolds have been published, there still remain problems with regard to potencies, pharmacokinetics, and physical properties. We report herein the synthesis and in vitro activity of related derivatives linked with a 4-phenyl-1,2,3,6-tetrahydropyridine moiety via alkyl spacers.

2. Chemistry

The bicyclic pyrimidin-4(3H)-one derivatives were synthesized, as outlined in Scheme 1. Alkylation of commercially available 4-phenyl-1,2,3,6-tetrahydropyridine hydrochloride (**6**) with 4-bromobutyronitrile (**7**) in the presence of triethylamine in DMF afforded 4-(4-phenyl-3,6-dihyro-1(2H)-pyridinyl)butanenitrile (**8**). This intermediate was converted to the corresponding amidine (**9**) by treatment with methylchloroaluminum amide.[10] Coupling of this amidine with various cyclic β-ketoesters in the presence of potassium carbonate afforded the target compounds (**10**).[11]

Keywords: PARP-1; Poly(ADP-ribose) polymerase; 4-phenyl-1,2,3,6-tetrahydropyridine.
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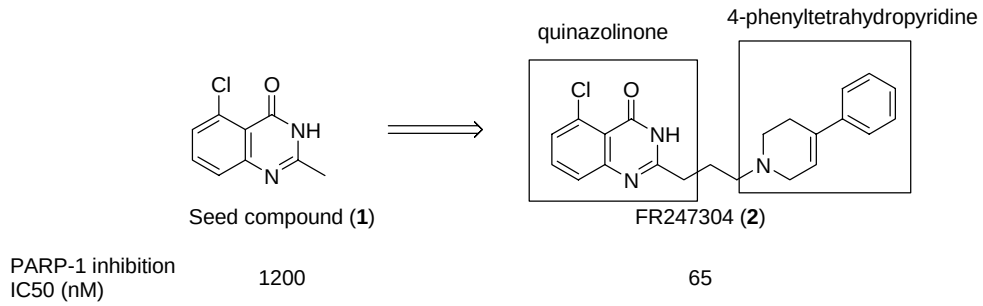


Figure 1. Dramatic improvement of PARP-1 inhibitory potency by introducing 4-phenyltetrahydropyridine.

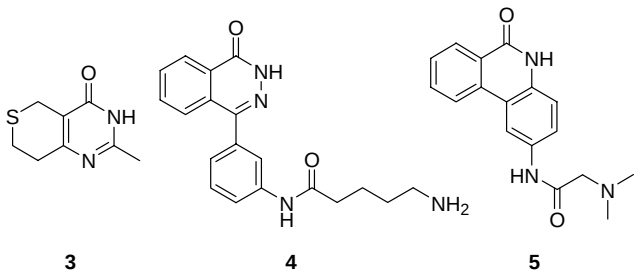


Figure 2. Three examples of published PARP-1 inhibitors containing 3,5,7,8-tetrahydro-thiino[4,3-d]pyrimidine-4-one (**3**), phthalazin-1(2H)-one (**4**), and phenanthridin-6(5H)-one (**5**) scaffolds.

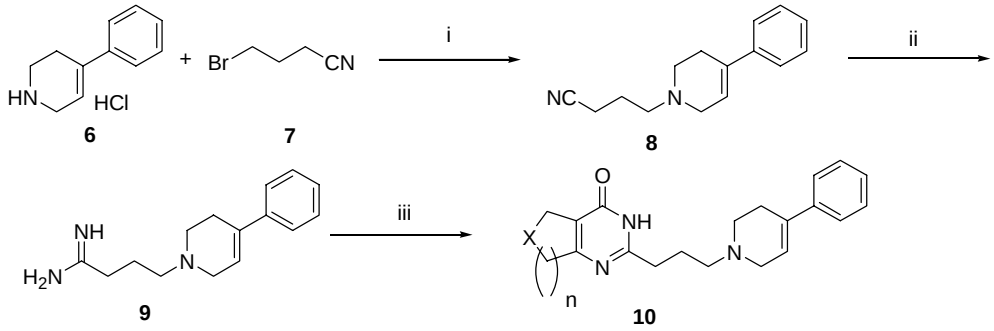
The synthesis of the requisite 4-substituted phthalazin-1(2H)-one is shown in Scheme 2. The phthalazin-1(2H)-one core was prepared, as described in the literature by Napoletano et al.[12] Wittig olefination of the phosphonium bromide (**11**) with benzyloxy alkanal (**12**, $n = 1$, 2), followed by coupling with hydrazine hydrate in EtOH, afforded the corresponding 4-benzyloxyalkylphthalazin-1(2H)-one (**13**). Direct bromination of the benzyl ethers (**13**) with BBr_3 in CH_2Cl_2 gave the bromide (**14**),[13] which upon amination with an appropriate amine in the presence of Et_3N gave the target compounds (**15**). We also attempted to prepare propyl analogue ($n = 0$) through the same procedure, but bromination of **13** failed owing to decomposition of the phthalazin-1(2H)-one nucleus.

2-Substituted phenanthridin-6(5H)-ones were synthesized, as shown in Scheme 3. Monobromination at the para position of the carbamoyl group on the phenyl rings of **16** with bromine and sodium acetate in AcOH afforded **17**, which was converted to the corresponding alkyl bromide (**18**) by treatment with PBr_3 in ethyl acetate. Suzuki coupling of the bromobenzene derivatives (**18**) with phenyl boronic acid gave the biphenyl product (**19**). Construction of the phenanthridin-6(5H)-one core was effected by treatment with P_2O_5 and $POCl_3$ to provide **20**.[14] The target compound (**21**) was obtained by amination of the 2-bromoalkyl phenanthridin-6(5H)-one (**20**) with 4-phenyl-1,2,3,6-tetrahydropyridine hydrochloride (**6**) in the presence of triethylamine.

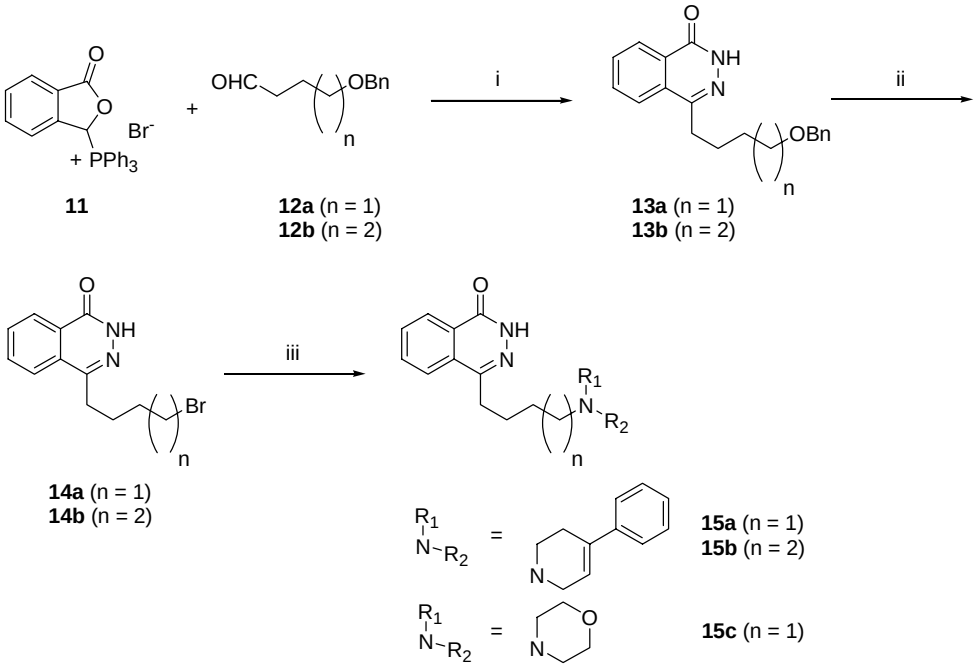
The synthesis of 3-substituted phenanthridin-6(5H)-ones is outlined in Scheme 4. The intermediate 3-carboxylic acid (**22**) was prepared, according to the literature.[15] Reduction of **22** using a mixed anhydride method gave the 3-hydroxymethyl derivative (**23**). Selective chlorination of the hydroxylmethyl group of **23** using $SOCl_2$ failed on account of the extremely low solubility of **23** in organic solvents. Dichlorination of both the hydroxymethyl and amide functionalities of **23** in refluxing $POCl_3$ was carried out, followed by amination with an appropriate amine and hydrolysis of the chloropyridine moiety under acidic conditions to give the desired compound (**24**).

3. Results and discussion

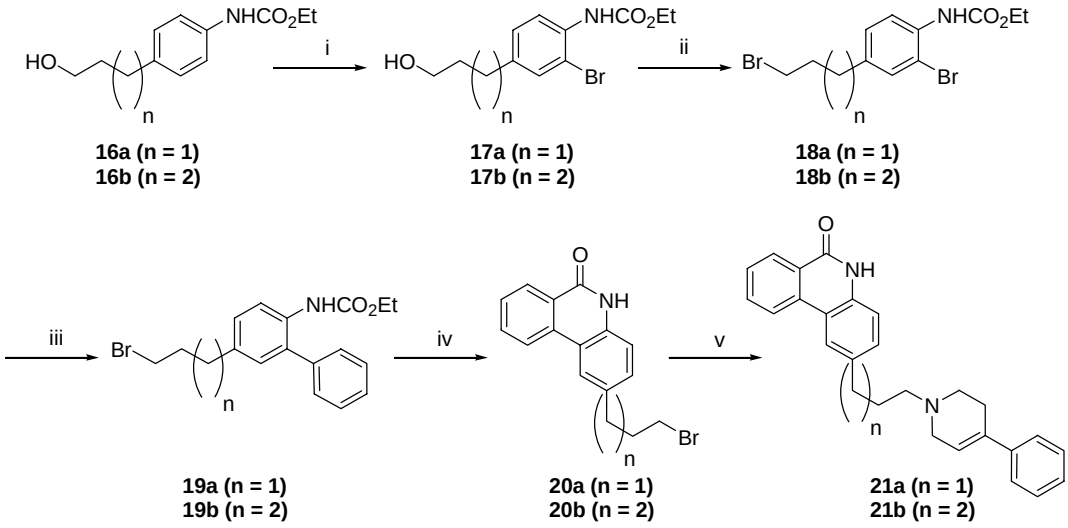
The PARP-1 IC_{50}'s for the new bicyclic pyrimidinone derivatives prepared in this work are given in Table 1.[16] Although the thia-containing core (**10d**) was expected to have the highest potency amongst these derivatives according to literature precedent, we optimized this aliphatic ring to generate information on the SAR of analogues bearing a 4-phenyl-1,2,3,6-tetrahydropyridine



Scheme 1. Reagents: (i) Diisopropylethylamine, DMF, 78%; (ii) $MeAl(Cl)NH_2$, toluene, 76%; and (iii) β-ketoester, K_2CO_3, EtOH 28–57%.



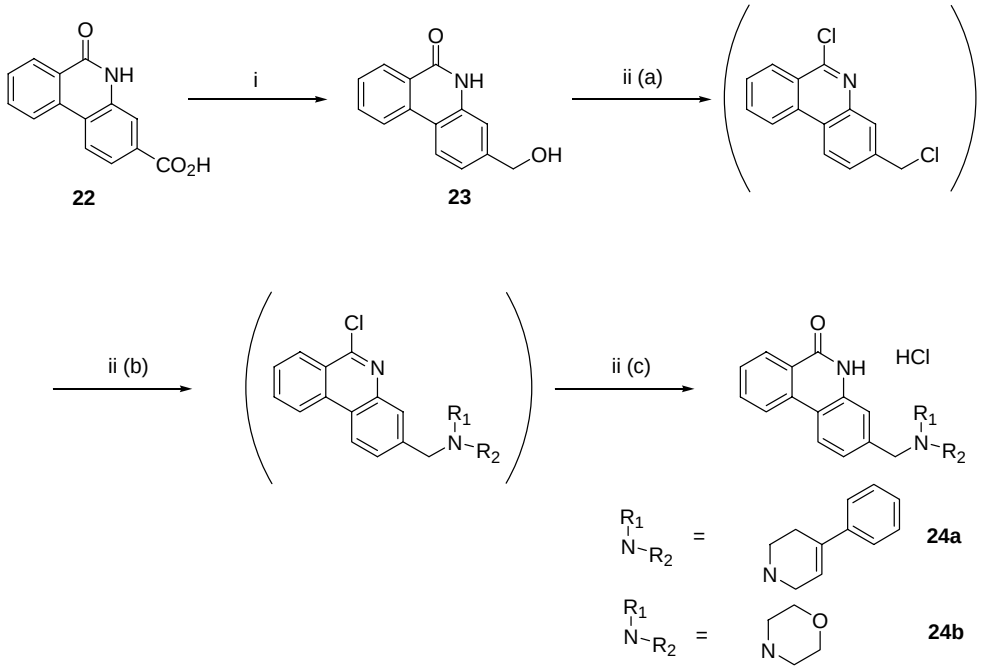
Scheme 2. Reagents: (i) (a) Et_3N, THF, (b) $NH_2NH_2{\cdot}H_2O$, EtOH, **13a** = 51%, **13b** = 40%; (ii) BBr_3, CH_2Cl_2, **14a** = 58%, **14b** = 75%; and (iii) **6** or morpholine, Et_3N, DMF, **15a** = 48%, **15b** = 27%, **15c** = 25%.



Scheme 3. Reagents: (i) Br_2, NaOAc, AcOH, **17a** = 92%, **17b** = 100%; (ii) PBr_3, AcOEt, **18a** = 66%, **18b** = 62%; (iii) phenylboronic acid, $Pd(PPh_3)_4$, DME, 2 M Na_2CO_3 (aq), **19a** = 100%, **19b** = 87%; (iv) (a) P_2O_5, $POCl_3$, **20a** = 74%, **20b** = 79%; and (v) **6**, Et_3N, DMF, **21a** = 45%, **21b** = 29%.

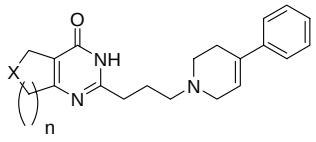
moiety. Optimization of the ring size fused with the pyrimidinone showed that a six-membered ring (**10b**) resulted in having the highest potency among the three different sizes of aliphatic rings prepared (20 nM). As expected, the thia analogue (**10d**) had the highest potency among these derivatives (8.9 nM). According to our assay protocol, known compound (**3**), which has no 4-phenyl-1,2,3,6-tetrahyropyridine, had an IC_{50} value of only 498 nM therefore, we could demonstrate that introduction of a 4-phenyl-1,2,3,6-tetrahyropyridine moiety potentiates PARP-1 inhibition drastically. It is noteworthy that replacement of the carbon atom at the 6 position of the tetrahydroquinazolinone (**10b**) by a sulfur atom (**10d**) led to an approximately 2 times increase in potency, whilst a similar substitution with nitrogen or oxygen (**10e**, **10f**) reduced activity 1 or 2 orders of magnitude. The decrease in potency of **10e** and **10f** may be attributed to the hydrophilic nitrogen or oxygen interacting electrostatically with hydrophobic residues of the PARP enzyme around this region. The addition of methyl groups to the cyclohexene moiety resulted in a dramatic loss of potency (**10g**, **10h**), presumably on account the planar pocket formed by two parallel tyrosine residues (Tyr 896 and Tyr 907) could not accommodate a cyclohexene moiety having bulky methyl groups efficiently.

The activity of 4-substituted phthalazin-1(2H)-one derivatives is given in Table 2. Compound **15a** linked



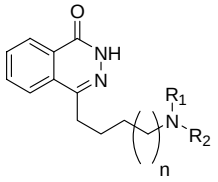
Scheme 4. Reagents: (i) (a) *i*-BuOCOCl, Et_3N, THF, (b) $NaBH_4$, THF, H_2O, 60%; (ii) (a) $POCl_3$, (b) **6** or morpholine, Et_3N, DMF, (c) 4 N HCl (aq), EtOH, **24a** = 68%, **24b** = 87%.

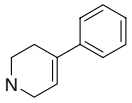
Table 1. PARP-1 inhibition of pyrimidin-4(3H)-one derivatives



Compound	*n*	X	IC_{50} (nM)
10a	1	CH_2	249
10b	2	CH_2	20
10c	3	CH_2	495
10d	2	S	8.9
10e	2	NH	8600
10f	2	O	200
10g	2	NMe	6100
10h	2	(Me)CH	274
10i	2	$(Me)_2C$	>10,000
3			467

Table 2. PARP-1 inhibition of phthalazin-1(2H)-one derivatives

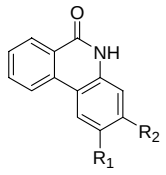


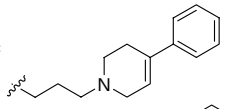
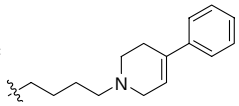
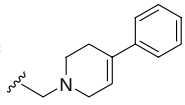
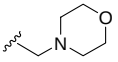
Compound	*n*	R_1 R_2	IC_{50} (nM)
15a	1		64
15b	2		119
15c	1		950

with phenyltetrahydropyridine via four methylene units, having an IC_{50} value of 64 nM, was twofold more potent than the five carbon analogue **15b** (119 nM). To validate the effect of a 4-phenyl-1,2,3,6-tetrahyropyridine, the morpholine analogue (**15c**) was estimated, revealing that absence of a phenyl ring led to a severe loss of PARP-1 inhibitory potency (950 nM).

The activity of phenanthridin-6(5H)-one derivatives is given in Table 3. In the case of 2-substituted derivatives, the compound bearing a propyl linker (**21a**) had more potency (46 nM) than the butyl analogue (**21b**, 119 nM). The 3-substituted derivative (**24**), however, had the highest activity (12 nM) against PARP-1 within this series, revealing that substitution at the 3-position is the most favorable for improving PARP-1 inhibitory potency. On the other hand, known compound **5** had an IC_{50} value of 110 nM , which was relatively potent, regardless of the absence of a 4-phenyl-1,2,3,6-tetrahydropyridine. This result implied that the SAR of phenanthridin-6(5H)-one derivatives was different from the previous two cases therefore; the morpholine analogue (**24c**) was prepared to verify the role of a 4-phenyl-1,2,3,6-tetrahydropyridine. Unexpectedly, **24c** had very high potency against PARP-1 (23 nM). This is presumably because conformation around the nitrogen atom was fixed effectively to interact with the residue of the PARP-1 enzyme in the case of **24c**. In other words, in the case of phenanthridin-6(5H)-one derivatives, the fused benzene ring connected with an alkylamino chain may play a crucial role in fixing the nitrogen atom to have high affinity. Meanwhile, in the case of bicyclic pyri-

Table 3. PARP-1 inhibition of phenanthridin-6(5H)-one derivatives



Compound			IC_{50} (nM)
21a	R_1 = 	R_2 = H	46
21b	R_1 = 	R_2 = H	119
24a	R_1 = H	R_2 = 	16
24b	R_1 = H	R_2 = 	23
5			110

midinone and phthalazin-1(2H)-one derivatives, the phenyl ring of the 4-phenyl-1,2,3,6-tetrahydropyridine moiety may permit this vital fixation of the nitrogen atom by efficiently binding the AD site.

In conclusion, we have demonstrated that 4-phenyl-1,2,3,6-tetrahydropyridine is an excellent fragment to improve PARP-1 inhibitory potency by connecting it via alkyl spacers to published scaffolds, such as pyrimidine-4-one, phenanthridin-6(5H)-one, and phthalazin-1(2H)-one. We have obtained highly potent PARP-1 inhibitors **10b**, **10d**, **15a**, **21a**, and **24a**. This utilization of 4-phenyl-tetrahydropyridine could be extended to other published PARP-1 inhibitors containing scaffolds that mimic benzamide. Detailed SAR, pharmacokinetic, and in vivo data for these derivatives will be reported in due course.

Acknowledgment

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References and notes

1. (a) For recent reviews on PARP see: Nguewa, P. A.; Fuertes, M. A.; Alonso, C.; Petéz, J. M. *Mol. Pharmacol.* **2003**, *64*, 1007; (b) Chiarugi, I. *TIPS* 2002, *23*, 122; Szabó, C.; Virag, L. *Pharmacol. Rev.* **2002**, 54, 375; (c) de Murcia, G., Shall, S. Eds; *From DNA Damage and Stress Signaling to Cell Death: Poly ADP-ribosylation Reactions*; Oxford University Press: Oxford, 2000; (d) Szabó, C., Ed.; *Cell Death: The Role of PARP*; CRC Press: Boca Raton, FL, 2000 and references therein.
2. (a) Nagayama, T.; Simon, R. P.; Henshall, D. C.; Pei, W.; Stetler, R. A.; Chen, J. *J. Neurochem.* **2000**, *74*, 1636; (b) Takahashi, K.; Pieper, A. A.; Croul, S. E.; Zhang, J.; Snyder, S. H.; Greenberg, J. H. *Brain Res.* **1999**, *829*, 46.
3. (a) Laplaca, M. C.; Zhang, J.; Raghupathi, R.; Li, J. H.; Smith, F.; Bareyre, F. M.; Snyder, S. H.; Graham, D. I.; Mclntosh, T. K. *J. Neurotrauma* **2001**, *18*(4), 369; (b) Adbelkarim, G. E.; Harms, C.; Katchanov, J.; Dirnagl, U.; Szabó, C.; Endres, M. *Int. J. Mol. Med.* **2001**, *7*, 255; (c) Cosi, C.; Colpaert, F.; Koek, W.; Degryse, A.; Marien, M. *Brain Res.* **1996**, *729*, 264; (d) Mandir, A. S.; Przedborski, S.; Jackson-Lewis, V.; Wang, Z.; Simbulan-Rosenthal, C. M.; Smulson, M. E.; Hoffman, B. E.; Guastella, D. B.; Dawson, V. L.; Dawson, T. M. *Proc. Natl. Acad. Aci. USA* **1999**, *96*, 5774.
4. (a) For recent reviews: Southan, G. J.; Szabó, C. *Curr. Med. Chem.* **2003**, *10*, 321; (b) Cosi, C. *Expert Opinion Ther. Patents*, **2002**, 12, 1047.
5. Hattori, K.; Kido, Y.; Yamamoto, H.; Ishida, J.; Kamijo, K.; Murano, K.; Ohkubo, M.; Kinoshita, T.; Iwashita, A.; Mihara, K.; Yamazaki, S.; Matsuoka, N.; Teramura, Y.; Miyake, H. *J. Med. Chem.* **2004**, *47*, 4151.
6. Kinoshita, T.; Nakanishi, I.; Warizaya, M.; Iwashita, Y.; Kido, Y.; Hattori, K.; Fujii, T. *FEBS* **2004**, *556*, 43.
7. Meiji: WO 00/42025 2000.
8. Ono: WO 00/44726 2000.
9. Inotek: WO 01/42219 2001.
10. Garigipati, R. S. *Tetrahedron Lett.* **1990**, *31*, 1969.
11. Badawey, E.-S. A. M.; El-Ashmawey, I. M. *Eur. J. Med. Chem.* **1998**, *33*, 349.
12. Napoletano, M.; Norcini, G.; Pellacini, F.; Marcini, F.; Morazzoni, G.; Ferlenga, P.; Pradella, L. *Bioorg. Med. Chem. Lett.* **2001**, *11*, 33.
13. Kuene, M. E.; Pitner, J. B. *J. Org. Chem.* **1989**, *54*, z4553.
14. Wang, X-j.; Tan, J.; Grozinger, K. *Tetrahedron Lett.* **1998**, *39*, 6609.
15. Li, J.-H.; Serdyruk, L.; Ferraris, D. V.; Xiao, G.; Tays, K. L.; Kletzly, P. W.; Li, W.; Lautar, S.; Zhang, J.; Kalish, V. *J. Bioorg. Med. Chem. Lett.* **2001**, *11*, 1687.
16. For PARP-1 inhibition assay protocol see: Iwashita, A.; Yamazaki, S.; Mihara, K.; Hattori, K.; Yamamoto, H.; Ishida, J.; Matsuoka, N.; Mutoh, S. *J. Pharmacol. Exp. Ther.* **2004**, *309*, 1067.