Available online at www.sciencedirect.com
SCIENCE DIRECT
Bioorganic & Medicinal Chemistry Letters 16 (2006) 938–942
Bioorganic & Medicinal Chemistry Letters

Synthesis and structure–activity relationships of novel poly(ADP-ribose) polymerase-1 inhibitors

Ming Tao,* Chung Ho Park, Ron Bihovsky,† Gregory J. Wells, Jean Husten, Mark A. Ator and Robert L. Hudkins

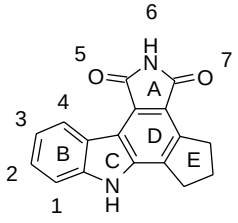
Cephalon, Inc., 145 Brandywine Parkway, West Chester, PA 19380-4245, USA

Received 19 September 2005; revised 26 October 2005; accepted 28 October 2005
Available online 15 November 2005

Abstract—A series of novel pyrrolocarbazoles was synthesized as potential PARP-1 inhibitors. Pyrrolocarbazole **1** was identified as a potent PARP-1 inhibitor (IC_{50} = 36 nM) from our internal database. Synthesis of analogs around this template with the aid of modeling studies led to the identification of the truncated imide **14**. Compound **14** (IC_{50} = 40 nM), with deleted B-ring, was found to be an equipotent PARP-1 inhibitor.

Poly(ADP-ribose) polymerase-1 (PARP-1) is a nuclear enzyme that catalyzes the synthesis of poly(ADP-ribose) chains from NAD^+ in response to single-strand DNA breaks as part of the DNA repair process.[1,2] The PARP-1 enzyme is comprised of three functional regions: an N-terminal DNA binding domain containing two zinc fingers, a linker region, and a C-terminal catalytic domain. Upon activation in response to DNA damage, PARP-1 synthesis and degradation consumes massive amounts of NAD^+, which leads to depletion of ATP energy stores, and ultimately necrotic cell death. PARP-1 has been implicated in many important pathophysiological processes such as stroke, myocardial ischemia, diabetes, shock, and traumatic CNS injury.[3] PARP-1 inhibition in tumor cells may potentiate radiotherapy and cancer chemotherapeutic agents targeting DNA due to its involvement in processes related to DNA repair.[3] Therefore, potent, selective, soluble PARP-1 inhibitors might be therapeutically useful in the treatment of neurodegenerative disorders and cancers.

In the literature, a variety of scaffolds, which mimic and bind to the nicotinamide site of NAD^+, have been reported as inhibitors of PARP-1.[4] The X-ray crystal structures of 3-aminobenzamides and several other classes have been reported.[5] Although a variety of PARP-1 inhibitors have been disclosed in the literature, many suffer from development problems such as toxicity, poor solubility, or poor pharmacokinetic profiles. In search of novel PARP-1 inhibitors, we identified pyrrolocarbazole **1** (IC_{50} = 36 nM) as a potent inhibitor from our internal database.[6] Described here are the synthesis and evaluation of the structure–activity relationships around this novel pyrrolocarbazole PARP-1 template.

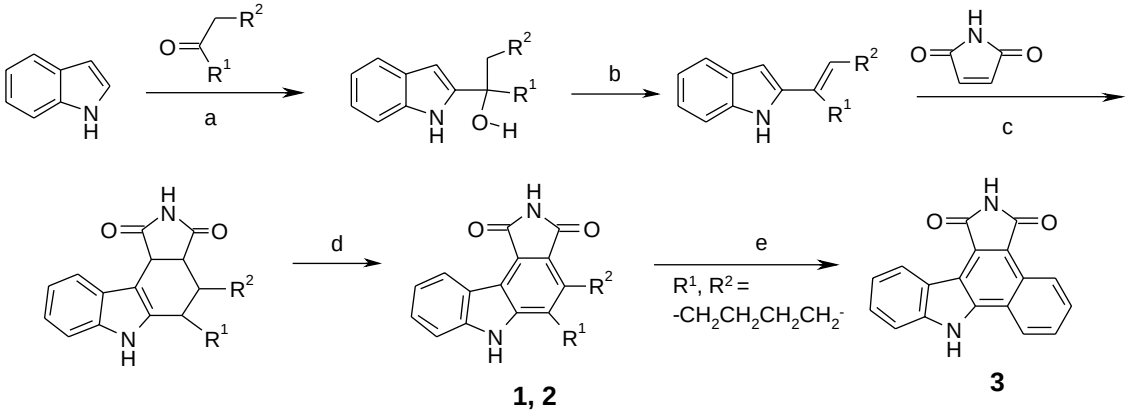


1

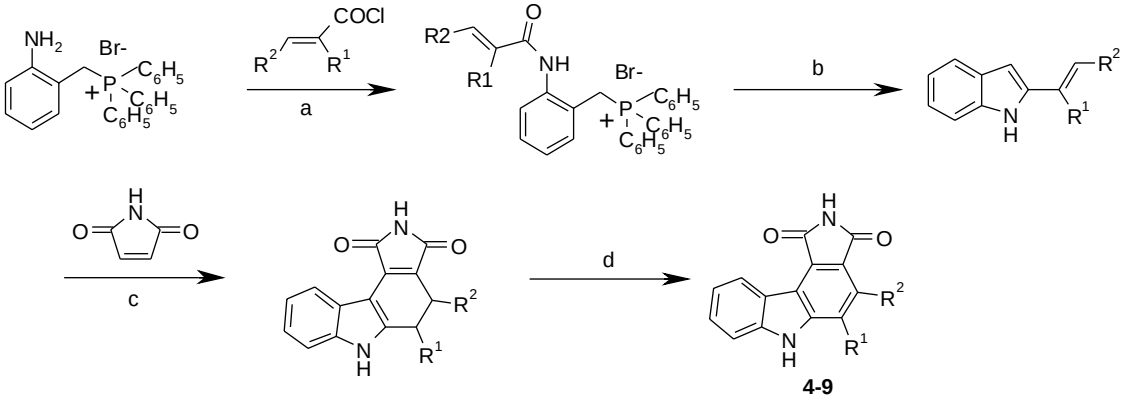
The synthesis of the pyrrolocarbazole imides is illustrated in Schemes 1 and 2 . In Scheme 1, protection of the indole nitrogen with carbon dioxide, followed by 2-lithiation and addition to the cycloketone, provided the 2-substituted indole–alcohol intermediate. Acid catalyzed elimination of the alcohol at room temperature to the diene, Diels–Alder reaction with neat maleimide, followed by DDQ oxidation at 60 °C in toluene gave the pyrrolocarbazole targets **1**–**3**. Alternatively, as shown in Scheme 2, the indole diene was prepared using an intramolecular Wittig reaction.[7] The indole diene reacted with maleimide, followed by DDQ oxidation to form the pyrrolocarbazole imides **4**–**9**.

Keywords: Pyrrolocarbazoles; PARP-1 inhibitors.
* Corresponding author. E-mail: mtao@cephalon.com
† Present address: Key Synthesis LLC, 804 Primrose Lane, Wynnewood, PA 19096, USA.

0960-894X/$ - see front matter © 2005 Elsevier Ltd. All rights reserved.
doi:10.1016/j.bmcl.2005.10.099



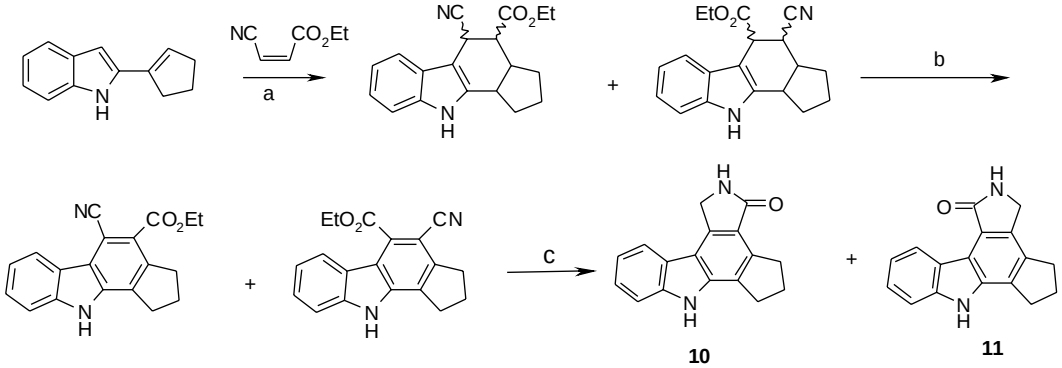
Scheme 1. Reagents and conditions: (a) BuLi/THF, −78 °C, $CO_{2(g)}$, *t*-BuLi/THF, then, the ketone, 60–80%; (b) HCl, rt, 80–90%; (c) 190 °C, neat, 1 h, 50%; (d) DDQ, toluene, 60 °C, 75–85%; (e) Pd/C/hexene, reflux.



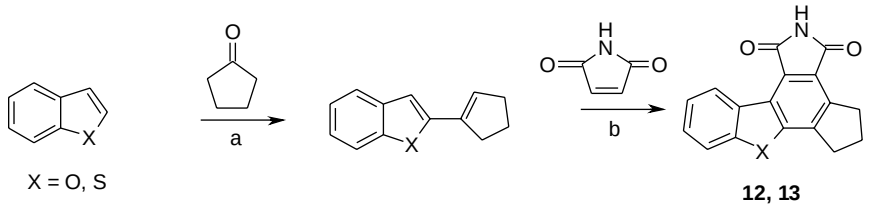
Scheme 2. Reagents and conditions: (a) pyridine, CH_2Cl_2, reflux, 30 min, 76%; (b) potassium *t*-butoxide/toluene, reflux, 30 min, 48%; (c) neat, 190 °C, 61%; (d) DDQ, toluene, 40 °C, 40%.

The pyrrolocarbazole lactam regioisomers were prepared using ethyl *cis*-β-cyanoacrylate as a dienophile in a thermal Diels–Alder reaction with 2-cyclopentenyl indole to form mainly *cis*-tetrahydrocarbazole regioisomers. DDQ oxidation and reductive cyclization of 5- and 7-cyano-esters using Raney® nickel in DMF produced lactams **10** and **11** after fractional recrystallizations from DMF and acetone (Scheme 3). The benzofuran (**12**) and benzothiophene (**13**) analogs were prepared in a similar manner for compounds **1**–**3**, as outlined in Scheme 4.

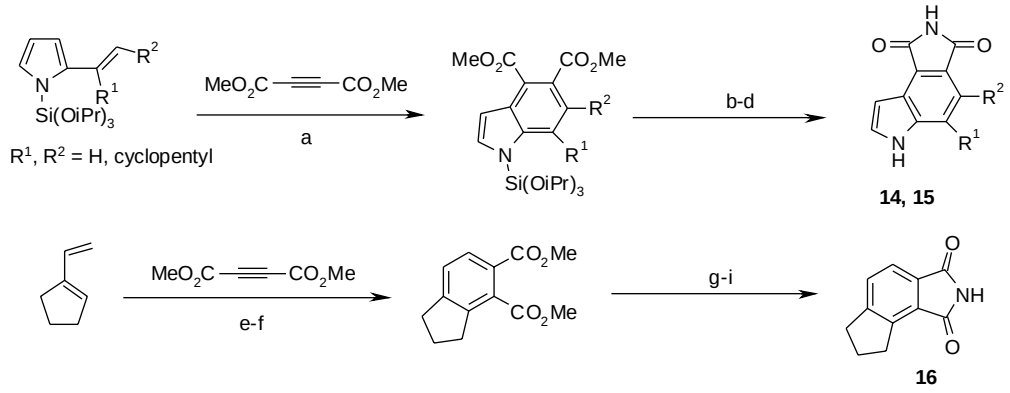
Truncated analogs were prepared as shown in Scheme 5. The triisopropylsilyl-protected 2-cyclopentenyl pyrrole diene was reacted with neat dimethyl acetylenedicarboxylate at 150 °C to form the indole diester.[8] Hydrolysis of the diester to the diacid, anhydride formation, and subsequent transamination with $(TMS)_2NH$ gave **14**



Scheme 3. Reagents and conditions: (a) neat, 200–205 °C, 1.5 h, >95%; (b) DDQ, toluene, 35–40 °C, 20 h, 25%; (c) Raney® Nickel/DMF, 45 psi, 7 days; 28%.



Scheme 4. Reagents and conditions: (a) conditions for benzothiophene: (i)—BuLi/ether, 0 °C; (ii)—TsOH, reflux for 5 min, 47%; conditions for benzofuran: (iii)—BuLi/ether, 0 °C; (iv)—TsOH, 40 °C for 30 min, 36%; (b) tetrachloroquinone, neat, 190 °C, 23–29%.



Scheme 5. Reagents and conditions: (a) neat, 150 °C, 64 h, 21–35%; (b) 10 N NaOH in EtOH, reflux, 3 h, 96%; (c) acetic anhydride, 73 h, 66%; (d) $(TMS)_2NH$/MeOH, DMF, 73 °C, 4 h, 88%; (e) neat, rt, 18 h, 40%; (f) DDQ/toluene, rt, 1 h, 82%; (g) 5 N NaOH/MeOH, rt, 1 h, 66%; (h) Ac_2O, 4 h, 85%; (i) NH_2CONH_2, neat, 150 °C, 30 min, 60%.

and **15**. Compound **16** lacking the indole ring was prepared by the same procedure as **14** and **15** but 1-vinylcyclopentene was utilized as the starting material and transamination was accomplished with urea (Scheme 5).

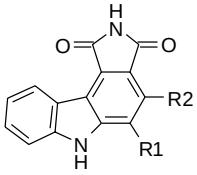
The pyrrolocarbazole analogs were evaluated as inhibitors of recombinant human poly(ADP-ribose) polymerase-1 as shown in Tables 1–4. As mentioned earlier, pyrrolocarbazole **1** inhibits PARP-1 with the IC_{50} value of 36 nM. Modification of the ring size led to a loss of potency. The cyclohexyl analog **2** and fused phenyl analog **3** displayed IC_{50} values >10 μM, indicating that the cyclopentyl ring is critical for activity. The fused furano analog **4** is also a weak PARP-1 inhibitor as shown in Table 1. Deleting the cyclopentyl ring (**5**) or replacement with alkyl and dialkyl groups led to a significant loss in potency. The dimethyl analog **6** displayed modest PARP-1 activity (IC_{50} = 700 nM), while the other alkyl and dialkyl analogs showed weaker activity.

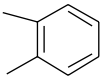
As shown in Table 2, both 5-oxo (**11**) and 7-oxo (**10**) lactam pyrrolocarbazoles were synthesized to explore the role of the imide functionality. The 7-oxo lactam **10** is 2.5-fold less potent (IC_{50} = 90 nM) than the imide **1**, while the 5-oxo analog **11** is essentially inactive (IC_{50} = 10 μM). The 7-oxo lactam isomer **10** is >100-fold more potent than the 5-oxo isomer **11**, indicating that the 7-oxo carbonyl is required to bind to PARP-1. The role of the indole N–H was evaluated by synthesizing the benzofuran and benzothiophene analogs **12** and **13**. As shown by the data in Table 3 both **12** and **13** are inactive for PARP-1, indicating that the indole nitrogen in compound **1** is important for activity.

Carbazole **1** was truncated to further explore the contribution of the different rings. The indole imide **14**, which lacks phenyl ring B, displayed an equally potent PARP-1 activity (IC_{50} = 40 nM), compared to the lead compound **1** (IC_{50} = 36 nM). However, further truncated compound **15**, in which pyrrole ring C is also absent, is a weaker PARP-1 inhibitor. Indole imide **16**, where both phenyl ring B and the cyclopentane ring have been removed, is almost 20-fold less potent (IC_{50} = 750 nM). The activity of **16** compared to, **15** reveals the importance of the indole N–H group and represents the minimum pharmacophore for retaining PARP-1 activity in the series.

A molecular docking study of carbazole **1** to the catalytic domain of chicken PARP-1 was conducted and is illustrated in Figure 1. A binding model for PARP-1 was derived using the coordinates for 4-amino-1,8-naphthalimide bound at the NAD^+ site of the catalytic fragment of chicken PARP-1 (PDB 2PAX).[5a] Compound **1** was docked and minimized in the model. The key interactions identified and supported by the SAR were hydrogen-bond donor–acceptor interactions with the imide/lactam 7-oxo C=O and NH with backbone carbonyl and amino of Gly863. Ser904 is involved in a H-bond with the 7-oxo C=O. The indole N–H shares a hydrogen-bond with the carboxyl side chain of Glu988. The aromatic π-stacking interactions occur between carbazole rings B and D, and the aryl groups

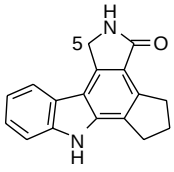
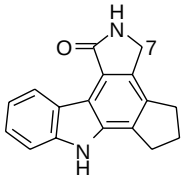
Table 1. PARP-1 inhibition by pyrrolocarbazoles[9]



Compound	R1	R2	PARP-1 IC_{50}[a,b] (nM)
1			36
2			~10,000
3			>10,000
4			~10,000
5	H	H	~10,000
6	CH_3	CH_3	700
7	CH_3	H	5000
8	H	CH_3	~2000
9	Et	*n*Pr	>10,000

[a] 4-Amino-1,8-naphthalimide (IC_{50} = 26 nM).
[b] Values of duplicate determinations were within 2-fold of each other.

Table 2. PARP-1 inhibition by pyrrolocarbazole lactams

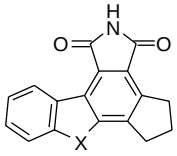
Compound	Structure	PARP-1 IC_{50}[a] (nM)
10		90
11		~10,000

[a] Values of duplicate determinations were within 2-fold of each other.

of Tyr896 and Tyr907. The cyclopentyl fits closely into a fold formed by the Lys861 side chain, Ala898, Trp861, and Asn987 which locks the inhibitor in the pocket.

In summary, we identified a novel pyrrolocarbazole PARP-1 inhibitor (**1**) through high throughput screening of our internal library. Structural modification to the core identified the key pharmacophore elements necessary for PARP-1 inhibition. The cyclopentyl ring

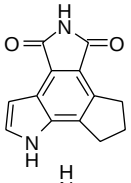
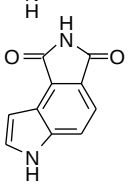
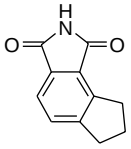
Table 3. Benzofuran and benzothiophene imides



Compound	X	PARP-1 IC_{50}[a] (nM)
12	O	>10,000
13	S	>10,000

[a] Values of duplicate determinations were within 2-fold of each other.

Table 4. PARP-1 inhibition by truncated analogs

Compound	Structure	PARP-1 IC_{50}[a] (nM)
14		40
15		750
16		2220

[a] Values of duplicate determinations were within 2-fold of each other.

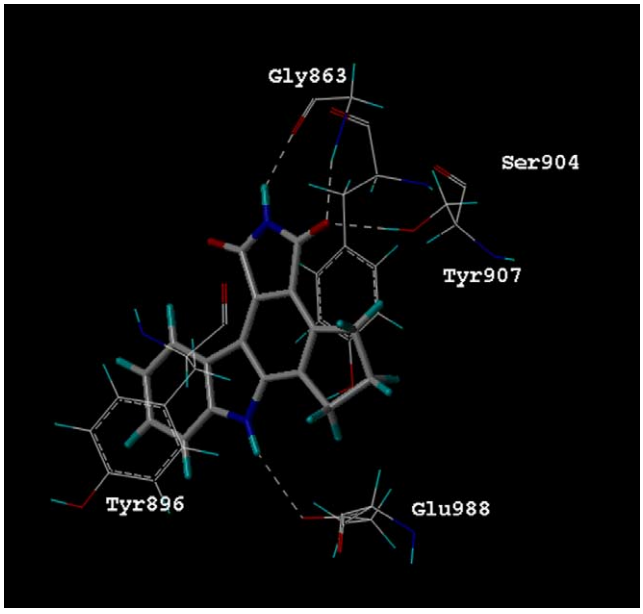


Figure 1. Important interactions of compound **1** with PARP-1.

is required for potency and fits into a steric pocket with the enzyme. Expanding, deleting, or opening the cyclopentyl ring led to weak or inactive inhibitors. The indole NH is required and forms a significant H-bond with PARP-1 as the benzofuran and benzothiophene analogs were inactive. The truncated pyrrole imide **14** was found to be equipotent to **1**, indicating that the B-ring is not required. The des-aryl compound **14**, with a lower molecular weight, would be anticipated to have improved physical chemical properties and represents a novel small molecule PARP-1 scaffold.

Acknowledgments

The authors acknowledge the support of and discussions with Drs. John Mallamo, Ed Bacon, and Jeffry Vaught.

References and notes

1. (a) Alvarez-Gonzalez, R.; Pacheco-Rodriquez, G.; Mandoza-Alvarez, H. *Mol. Cell. Biochem.* **1994**, *138*, 33; (b) Yu, S.-W.; Wang, H.; Poitras, M. F.; Coombs, C.; Browers, W. J.; Federoff, H. J.; Poirier, G. G.; Dawson, T. M.; Dawson, V. L. *Science* **2002**, *297*, 259; (c) Bryant, H. E.; Schultz, N.; Thomas, H. D.; Parker, K. M.; Flower, D.; Lopez, E.; Kyle, S.; Meuth, M.; Curtin, N. J.; Helleday, T. *Nature* **2005**, *434*, 913; (d) Farmer, H.; McCabe, N.; Lord, C. L.; Tutt, A. N. J.; Johnson, D. A.; Richardson, T. B.; Santarosa, M.; Dillon, K. J.; Hickson, I.; Knights, C.; Martin, N. M.; Jackson, S. P.; Smith, G. C. M.; Ashworth, A. *Nature* **2005**, *424*, 917.
2. Ruf, A.; Menissier de Murcia, J.; de Muricia, G. M.; Schulz, G. E. *Proc. Nat. Acad. Sci. U.S.A.* **1996**, *93*, 7481.
3. (a) Virág, L.; Szabó, C. *Pharmacol. Rev.* **2002**, *54*, 375; (b) Skaper, S. D. *Curr. Drug Targets CNS Neurol. Disord.* **2003**, *2*, 279; (c) Endres, M.; Wang, Z. Q.; Namura, S.; Waeber, C.; Moskowitz, M. A. *J. Cereb. Blood Flow Metab.* **1997**, *17*, 1143; (d) Takahashi, K.; Pieper, A. A.; Croul, S. E.; Zhang, J.; Synder, S. H.; Greenberg, J. H. *Brain Res.* **1999**, *829*, 46; (e) Nagayama, T.; Simon, R. P.; Chen, D.; Henshall, D. C.; Pei, W.; Stetler, R. A.; Chen, J. *J. Neurochem.* **2000**, *74*, 1636; (f) Shall, S. *Mol. Cell. Biochem.* **1994**, *138*, 71; (g) Satoh, M. S.; Lindahl, T. *Nature (London)* **1992**, *356*, 356; (h) Miknyoczki, S. J.; Jones-Bolin, S.; Pritchard, S.; Hunter, K.; Zhao, H.; Wan, W.; Ator, M.; Bihovsky, R.; Hudkins, R.; Chatterjee, S.; Klein-Szanto, A.; Dionne, C.; Ruggeri, B. *Mol. Cancer Ther.* **2003**, *2*, 371.
4. (a) Banasik, M.; Komura, H.; Shimiyama, M.; Ueda, K. *J. Biol. Chem.* **1992**, *267*, 1569; (b) Southan, G. J.; Szabó, C. *Curr. Med. Chem.* **2003**, *10*, 321; (c) 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; (d) Jagtap, P. G.; Southan, G. J.; Baloglu, E.; Ram, S.; Mabley, J. G.; Marton, A.; Salzman, A.; Szabo, C. *Bioorg. Med. Chem. Lett.* **2004**, *14*, 81; (e) Ferraris, D.; Ko, Y.; 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.-C.; 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.
5. (a) Ruf, A.; de Murcia, G.; Schulz, G. E. *Biochemistry* **1998**, *37*, 3893; (b) White, A. W.; Almassy, R.; Calvert, A. H.; Curtin, N. J.; Griffin, R. J.; Hostomsky, Z.; Maegley, K.; Newell, D. R.; Srinivasan, S.; Golding, B. T. *J. Med. Chem.* **2000**, *43*, 4084; (c) Costantino, G.; Macchiarulo, A.; Camaioni, E.; Pellicciari, R. *J. Med. Chem.* **2001**, *44*, 3786; (d) Koch, S. S. C.; Thoresen, L. H.; Tikhe, J. G.; Maegley, K. A.; Almassy, R. J.; Li, J.; Yu, X.; Zook, S. E.; Kumpf, R. A.; Zhang, C.; Boritzki, T. J.; Mansour, R. N.; Zhang, K. E.; Ekker, A.; Calabrese, C. R.; Curtin, N. J.; Kyle, S.; Thomas, H. D.; Wang, L.; Calvert, A. H.; Golding, B. T.; Griffin, R. J.; Newell, D. R.; Webber, S. E.; Hostomsky, Z. *J. Med. Chem.* **2002**, *45*, 4961; (e) Ferraris, D.; Ficco, R. P.; Dain, D.; Ginski, M.; Lautar, S.; Lee-Wisdom, K.; Liang, S.; Lin, Q.; Lu, M. X.-C.; Morgan, L.; Thomas, B.; Williams, L. R.; Zhang, J.; Zhou, Y.; Kalish, V. J. *Bioorg. Med. Chem.* **2003**, *11*, 3695.
6. Ator, M. A.; Bihovsky, R.; Chatterjee, S.; Dunn, D.; Hudkins, R. L. W.O. Patent 01/85686 A2, 2001.
7. Eitel, M.; Pindur, U. *Synthesis* **1989**, *364*.
8. Noland, W. E.; Lee, C. K.; Bae, S. K.; Chung, B. Y.; Hahn, C. S.; Kim, K. J. *J. Org. Chem.* **1983**, *48*, 2488.
9. PARP-1 inhibition assay: PC12 cells were plated onto polyornithine/laminin-coated 96-well plates. Prior to addition of inhibitors, growth medium was replaced with low serum medium. Cells were pre-treated for 1 h with PARP inhibitors and then with 0.5 mM hydrogen peroxide for 30 min in the presence of inhibitors. After washing away the peroxide, fresh PARP inhibitors were added. One hour after peroxide treatment the cell surface was washed once with phosphate-buffered saline. NAD^+ was released into phosphate-buffered saline by subjecting the cells to two freeze/thaw cycles. After centrifugation, the supernatant was analyzed for NAD^+ using the method described in Shah, G. M.; Poirier, D.; Duchaine, C.; Brochu, G.; Desnoyers, S.; Lagueux, J.; Verreault, A.; Hoflack, J. C.; Kirkland, J. B.; Poirier, G. G. *Anal. Biochem.* **1995**, *227*, 1.