Bioorganic & Medicinal Chemistry Letters 20 (2010) 1023–1026

Contents lists available at ScienceDirect

Bioorganic & Medicinal Chemistry Letters

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

# Discovery and SAR of substituted 3-oxoisoindoline-4-carboxamides as potent inhibitors of poly(ADP-ribose) polymerase (PARP) for the treatment of cancer

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ARTICLE INFO

*Article history:*
Received 20 October 2009
Revised 5 December 2009
Accepted 10 December 2009
Available online 14 December 2009

*Keywords:*
PARP inhibitor
Inhibitor of poly(ADP-ribose) polymerase
3-Oxoisoindoline-4-carboxamide

ABSTRACT

Through conformational restriction of a benzamide by formation of a seven-membered hydrogen-bond with an oxindole carbonyl group, a series of PARP inhibitors was designed for appropriate orientation for binding to the PARP surface. This series of compounds with a 3-oxoisoindoline-4-carboxamide core structure, displayed modest to good activity against PARP-1 in both intrinsic and cellular assays. SAR studies at the lactam nitrogen of the pharmacophore have suggested that a secondary or tertiary amine is important for cellular potency. An X-ray structure of compound **1e** bound to the protein confirmed the formation of a seven-membered intramolecular hydrogen bond. Though revealed previously in peptides, this type of seven-membered intramolecular hydrogen bond is rarely observed in small molecules. Largely due to the formation of the intramolecular hydrogen bond, the 3-oxoisoindoline-4-carboxamide core structure appears to be planar in the X-ray structure. An additional hydrogen bond interaction of the piperidine nitrogen to Gly-888 also contributes to the binding affinity of **1e** to PARP-1.



Poly(ADP-ribose) polymerases (PARPs) constitute a family of cell signaling enzymes abundant in all eukaryotes.[1] Poly(ADP-ribose) polymerase-1 and 2 (PARP-1 and 2), key members of the PARP family, play a significant role in preservation of genomic integrity through DNA repair and control of RNA transcription.[2] In the event of a stimuli caused during pathophysiological processes such as inflammation, stroke, myocardial infarction, neurodegenerative disorder or radiation therapy, PARP is activated to catalyze the synthesis of poly(ADP-ribose) chains from nicotinamide adenine dinucleotide ($NAD^+$) through automodification or ribosylation to aid the process of cell repair.[3] Thus PARPs are critical regulatory components and act as gatekeepers in maintaining cell fidelity.[4] The formation of ADP-ribose polymers is crucial in the repair of damaged DNA caused by radiation therapy or chemotherapeutic agents. Consequently, PARP-1 and PARP-2 interfere with the aforementioned therapies and diminish its effectiveness in treating cancer. Moreover, it has been observed that PARP expression is enhanced in a number of hematological and solid tumors as compared to normal cells. The dual role played by the enzyme therefore suggests that pharmacological modulation of its activity could increase the efficacy of DNA-damaging antitumor drugs.[5–12] Additionally, tumors with deficiencies in DNA-repair genes such as BRCA-1 or BRCA-2 have displayed acute sensitivity towards inhibition of PARP-1, suggesting a potential utilization of PARP inhibitors as single agents in treating certain mutations of breast cancer.[13] PARP-1 is therefore regarded as a valuable target in the exploration of new cancer treatment regimens.

Figure 1 is a schematic presentation of the X-ray structure of $NAD^+$ binding to PARP-1, and formation of multiple hydrogen bond

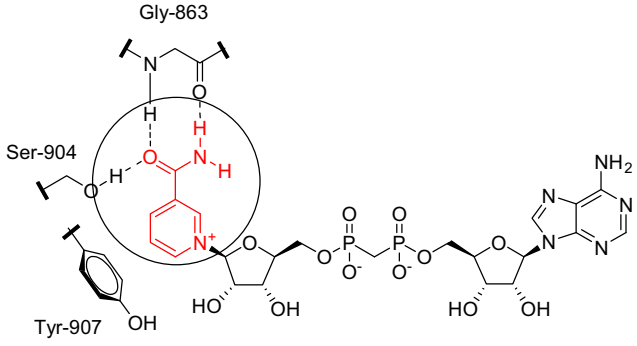


**Figure 1.** A schematic presentation of the X-ray structure of $NAD^+$ binding to PARP-1. The cycled part of structure (red) showed critical hydrogen bond interactions of $NAD^+$ with PARP-1.

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doi:10.1016/j.bmcl.2009.12.042

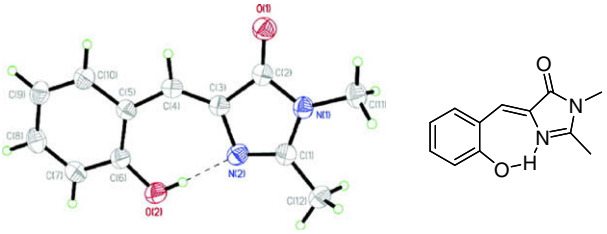


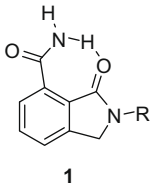
**Figure 2.** The molecular structure of *o*-HBDI. (Picture adopted from Journal of the American Chemical Society, 2007, 129, 4534, and copyright granted from American Chemical society (License number 2312091023118).)

interactions between the carboxamide moiety and two key amino acid residues in the PARP active site, Gly-863 and Ser-904.[5–7] Most known PARP inhibitors mimic the $NAD^+$ binding mode, by conformational restriction of a primary amide or by incorporation of an amide into a lactam.[5–12] Formation of a more planar structure as shown in the circle of Figure 1 is more likely optimal for the binding.

Restricted rotation of a functional group has been frequently achieved through formation of five- or six-membered ring intramolecular hydrogen bond.[5–7] An intramolecular hydrogen bond incorporating in a larger ring has been rarely observed, primarily in peptide type of structures. Recently, a seven-membered-ring hydrogen-bonding system 4-(2-hydroxybenzylidene)-1,2-dimethyl-1*H*-imidazol-5(4*H*)-one (*o*-HBDI) has been reported,[14] and formation of the intramolecular hydrogen bond is believed to be critical to an excited-state intramolecular proton transfer. The structure of the seven-membered ring hydrogen bond has been confirmed by an X-ray structure and the ring structure incorporating the hydrogen bond appears to be planar (Fig. 2).

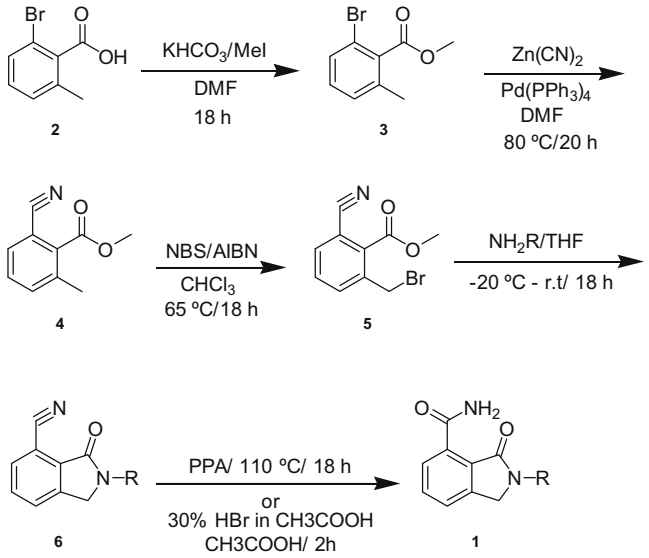
To our surprise, no significant difference on the binding energy was observed in peptides among 7–10-membered-ring intramolecular hydrogen bond, ranging from 5 to 10 kcal/mol.[15,16] This binding energy is also in the same range as compared to five- or six-membered intramolecular hydrogen bonds. Since a seven-membered ring intramolecular hydrogen bond contributes to the same level of orientation restriction as compared to that of smaller rings, we have designed 3-oxoisoindoline-4-carboxamide **1** in which the carboxamide is restricted into the shown orientation as in Figure 3, through formation of seven-membered ring hydrogen bond.

Scheme 1 outlines the general procedure used in the synthesis of our PARP inhibitors. Commercially available 2-bromo-6-methylbenzoic acid **2** was treated with methyl iodide and potassium bicarbonate in DMF to provide the methyl ester **3** in nearly quantitative yields. Benzonitrile **4** was obtained in excellent yield through a palladium-catalyzed cyanation of aryl bromide **3** and zinc cyanide as the cyanide source. Free radical bromination of **4** with AIBN and NBS yielded **5** in good yield. The penultimate transformation to provide the cyclized oxoisoindoline core **6** was achieved by treating **5** with selected alkyl and aryl primary amines in THF. Carboxamides **1a**–**1q** were obtained through hydrolysis of nitrile **6** in PPA. Alternatively, the nitrile to amide transformation can be accomplished by heating in 30% HBr in acetic acid.



**1**

**Figure 3.** Structure of designed scaffold 1.

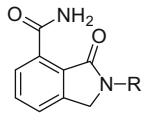



**Scheme 1.**

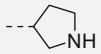
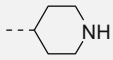
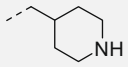
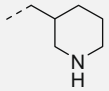
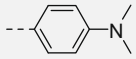
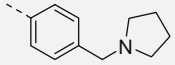
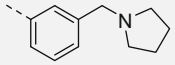
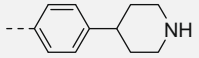
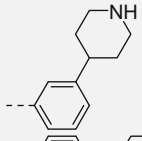
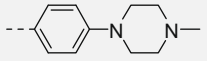
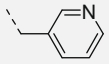
Table 1 outlines a variety of PARP analogs synthesized employing the procedure as described above in Scheme 1. Alkyl analogs **1a** and **1b** showed modest intrinsic potencies but poor cellular activity. Introduction of a cyclic amine, in particular, **1d** and **1e** resulted in significant boost in PARP activity, with enzymatic activity of 59 nM and 33 nM and cellular activity of 160 nM and 51 nM, respectively. Based on the initial observation it was evident that a basic amino functionality was beneficial for improved cellular activity. To this end, compounds **1f**–**1i,** containing 3-piperidine, 2-pyrrolidine, 3- and 4-methyl piperidines were synthesized. However, this modification resulted in ca. fivefold to 10-fold drop in potency as compared to piperidine **1e** demonstrating that the presence and position of the basic amino functionality had significant impact on the PARP activity, both in an enzyme and cellular assays. In an attempt to understand the binding mode of these compounds in the active site and explore other potential interactions, substituted phenyl and aryl analogs **1j**–**1q** were synthesized. Generally, these analogs showed a similar enzyme and cellular potency profile compared to aforementioned cycloalkyl analogs. However, 4-substituted phenyl analogs were significantly more potent compared to the 3-substituted analogs. For instance, compound **1l** with a *para* substitution has enzymatic potency of 53 nM, while **1m** is significantly less potent at 391 nM. Similarly, analog **1o**, *para*-substituted piperidine, is 10-fold more potent in comparison with the *meta*-substituted piperidine analog **1n**. A comparison of analogs **1n** and **1p** indicated that addition of second nitrogen in the ring results in a slight drop in enzymatic potency while **1p** gained more than fivefold boost in cellular potency. Finally, incorporation of a pyridine-3-methyl functionality **1q** led to loss of PARP activity. Selected compounds (e.g., **1e**) were tested against the closely-related PARP-2 enzyme, and displayed equal potency as PARP-1.

A versatile characteristic of our novel 3-oxoisoindoline-4-carboxamide class of PARP inhibitors is the favorable orientation of the carboxamide moiety relative to the 3-oxo substitution. As shown in Figure 3, the nitrogen of the carboxamide moiety and the 3-oxo substitution are involved in a seven-membered intramolecular hydrogen bonding interaction thereby restricting the free rotation of the amide functionality. The locked conformation of the molecule is presumably responsible for its optimal interaction with the PARP enzyme and its inhibition. The favorable orientation of the amide enables the 3-oxoisoindoline-4-carboxamide moiety

**Table 1**
SAR of 2-substituted 3-oxoisoindoline-4-carboxamide



**1**

| Compound | R | PARP-1[a] ($K_i$, nM) | Cellular[a] ($EC_{50}$, nM) |
|---|---|---|---|
| **a** | $CH_3$ | 123 | ND |
| **b** | *i*-Bu | 112 | >1000 |
| **c** |  | 172 | >1000 |
| **d** |  | 59 | 160 |
| **e** |  | 33 | 51 |
| **f** |  | 140 | ND |
| **g** |  | 377 | 374 |
| **h** |  | 384 | ND |
| **i** |  | 164 | ND |
| **j** |  | 261 | ND |
| **k** |  | 70 | 372 |
| **l** |  | 53 | 53 |
| **m** |  | 391 | ND |
| **n** |  | 32 | 120 |
| **o** |  | 314 | ND |
| **p** |  | 48 | 22 |
| **q** |  | >9500 | ND |

ND = not determined.
[a] Assays follow the same protocol as previously described.[5] Values are means of two or more experiments, all assays generated data within twofold of mean.

to form critical hydrogen-bond interactions with the serine and glycine amino acid residues of the enzyme.

An X-ray co-crystal structure of PARP-1 with **1e** is depicted in Figure 4. The carboxamide group of **1e** is involved in key hydrogen-bond interactions with both Gly-863 and Ser-904, in accordance with previous literature reports.[17] In addition, the NH of the piperidine ring is involved in a hydrogen-bond interaction with Gly-888 and lastly the π-stacking interaction between the oxoisoindoline ring and Tyr-907 is also observed. This Gly-888 interaction likely explains why significant differences in potency are observed with changes in the orientation of the basic amino group.

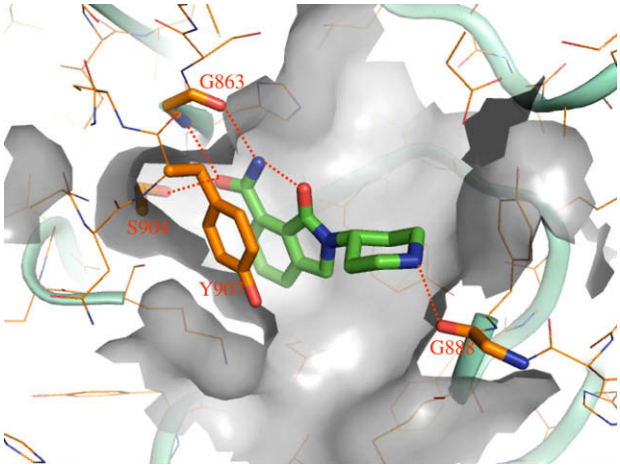



**Figure 4.** X-ray co-crystal structure of PARP-1 and **1e**.[18]

In conclusion, through rational design of a seven-membered ring intramolecular hydrogen bond utilizing a carbonyl group and a carboxamide functionality, we have identified 3-oxoisoindoline-4-carboxamide as a lead inhibitor of poly(ADP-ribose) polymerase-1 (PARP-1). Preliminary SAR study of this series of PARP inhibitors indicates that the presence and position of an amine functionality has significant impact both on enzymatic and cellular potency. An X-ray co-crystal structure of PARP-1 with **1e** confirmed the critical multiple hydrogen bonding interactions of the 4-carboxamide with two PARP residues, as well as intramolecularly with the 3-carbonyl to lock the amide in an optimal planar conformation. Additional hydrogen bond interaction to Gly-888 was also achieved through an appropriately-oriented piperidine nitrogen.

## Acknowledgment

The authors are grateful to the Abbott analytical department for acquisition of $^1$H NMR and MS.

## References and notes

1. Virág, L.; Szabó, C. *Pharmacol. Rev.* **2002**, *54*, 375.
2. (a) Bellocchi, D.; Macchiarulo, A.; Costantino, G.; Pellicciari, R. *Bioorg. Med. Chem.* **2005**, *13*, 1151; (b) Jagtap, P.; Szabó, C. *Nat. Rev. Drug Disc.* **2005**, *4*, 421.
3. (a) Dunn, D.; Husten, J.; Ator, M. A.; Chatterjee, S. *Bioorg. Med. Chem. Lett.* **2007**, *17*, 542; (b) Menear, K. A.; Adcock, C.; Cuenca Alonso, F.; Blackburn, K.; Copsey, L.; Drzewiecki, J.; Fundo, A.; Le Gall, A.; Gomez, S.; Javaid, H.; Fenandez Lence, C.; Martin, N. M. B.; Mydlowski, C.; Smith, G. C. M. *Bioorg. Med. Chem. Lett.* **2008**, *18*, 3942.
4. Cepeda, V.; Fuertes, M.; Castilla, J.; Alonso, C.; Quevedo, C.; Soto, M.; Perez, J. M. *Recent Patents on Anticancer Drug Discovery* **2006**, *1*, 39.
5. (a) Penning, T. D.; Zhu, G.-D.; Gandhi, V. B.; Gong, J.; Thomas, S.; Lubisch, W.; Grandel, R.; Wernet, W.; Park, C. H.; Fry, E. H.; Liu, X.; Shi, Y.; Klinghofer, V.; Johnson, E. F.; Donawho, C. K.; Frost, D. J.; Bontcheva-Diaz, V.; Bouska, J. J.; Olson, A. M.; Marsh, K. C.; Luo, Y.; Rosenberg, S. H.; Giranda, V. L. *Bioorg. Med. Chem.* **2008**, *16*, 6965; (b) Griffin, R. J.; Srinivasan, S.; White, A. W.; Bowman, K.; Calvert, A. H.; Curtin, N. J.; Newell, D. R.; Golding, B. T. *Pharm. Sci.* **1996**, *2*, 43; (c) 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.
6. Zhu, G.-D.; Gandhi, V. B.; Gong, J.; Thomas, S.; Luo, Y.; Liu, X.; Shi, Y.; Klinghofer, V.; Johnson, E. F.; Frost, D.; Donawho, C.; Jarvis, K.; Bouska, J.; Marsh, K. C.; Rosenberg, S. H.; Giranda, V. L.; Penning, T. D. *Bioorg. Med. Chem. Lett.* **2008**, *18*, 3955.
7. Penning, T. D.; Zhu, G.-D.; Gandhi, V.; Gong, J.; Liu, X.; Shi, Y.; Klinghofer, V.; Johnson, E. F.; Donawho, C.; Frost, D.; Bontcheva-Diaz, K.; Bouska, J.; Osterling, D.; Olson, A.; Marsh, K.; Luo, Y.; Giranda, V. L. *J. Med. Chem.* **2009**, *52*, 514.
8. (a) Plummer, E. R. *Curr. Opin. Pharmacol.* **2006**, *6*, 364; (b) Horváth, E. M.; Szabó, C. *Drug News Perspect.* **2007**, *20*, 171; (c) Ratnam, K.; Low, J. A. *Clin. Cancer Res.* **2007**, *13*, 1383.
9. Donawho, C. K.; Luo, Y.; Penning, T. D.; Bauch, J. L.; Bouska, J. J.; Bontcheva-Diaz, V. D.; Cox, B. F.; DeWeese, T. L.; Dillehay, L. E.; Ferguson, D. C.;

Ghoreishi-Haack, N. S.; Grimm, D. R.; Guan, R.; Han, E. K.; Holley-Shanks, R.; Hristov, B.; Idler, K. B.; Jarvis, K.; Johnson, E. F.; Kleinberg, L. E.; Klinghofer, V.; Lasko, L. M.; Liu, X.; Marsh, K. C.; McGonigal, T. P.; Meulbroek, J. A.; Olson, A. M.; Palma, J. P.; Rodriguez, L. E.; Shi, Y.; Stavropoulos, J. A.; Tsurutani, A. C.; Zhu, G.-D.; Rosenberg, S. H.; Giranda, V. L.; Frost, D. J. *Clin. Cancer Res.* **2007**, *13*, 2728.

10. Lapidus, R. G.; Tentori, L.; Graziani, G.; Leonetti, C.; Scarsella, M.; Vergati, M.; Muzi, A.; Zhang, J. *J. Clin. Oncol* **2005**, *23*, 3136.
11. Calabrese, C. R.; Almassy, R.; Barton, S.; Batey, M. A.; Calvert, A. H.; CananKoch, S.; Durkacz, B. W.; Hostomsky, Z.; Kumpf, R. A.; Kyle, S.; Li, J.; Maegley, K.; Newell, D. R.; Notarianni, E.; Stratford, I. J.; Skalitsky, D.; Thomas, H. D.; Wang, L.-Z.; Webber, S. E.; Williams, K. J.; Curtin, N. J. *J. Natl. Cancer Inst.* **2004**, *95*, 56.
12. Tentori, L.; Leonetti, C.; Scarsella, M.; d'Amati, G.; Vergati, M.; Portarena, I.; Xu, W.; Kalish, V.; Zupi, G.; Zhang, J.; Graziani, G. *Clin. Cancer Res.* **2003**, *9*, 5370.
13. (a) Ashworth, A. *J. Clin. Oncol.* **2008**, *26*, 3785; (b) Fong, P. C.; Boss, D. S.; Yap, T. A.; Tutt, A.; Wu, P.; Mergui-Roelvink, M.; Mortimer, P.; Swaisland, H.; Lau, A.; O'Connor, M. J.; Ashworth, A.; Carmichael, J.; Kaye, S. B.; Schellens, J. H. M.; de Bono, J. S. *N. Eng. J. Med.* **2009**, *361*, 123.
14. Chen, K.-Y.; Cheng, Y.-M.; Lai, C.-H.; Hsu, C.-C.; Ho, M. L.; Lee, G.-H.; Chou, P. T. *J. Am. Chem. Soc.* **2007**, *129*, 4534.
15. Wang, C.-S.; Zhang, Y.; Gao, K.; Yang, Z.-Z. *J. Chem. Phys.* **2005**, *123*, 1.
16. Zhang, Y.; Wang, C.-S.; Yang, Z.-Z. *J. Theor. Comput. Chem.* **2009**, *8*, 279.
17. (a) Ferraris, D.; Ficco, R. P.; Dain, D.; Ginski, M.; Lautar, S.; Lee-Wisdom, K.; Linag, 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; (b) Canan-Koch, S. S.; Thoresen, L. H.; Tikhe, J. G.; Maegley, K. A.; Yu, X.-H.; 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.-Z.; Calvert, A. H.; Golding, B. T.; Griffin, R. J.; Newell, D. R.; Webber, S. E.; Hostomsky, Z. *J. Med. Chem.* **2002**, *45*, 4961; (c) Costatino, G.; Macchiarulo, A.; Camaioni, E.; Pellicciari, R. *J. Med. Chem.* **2001**, *44*, 3786.
18. Crystallographic data for compound **1e** has been deposited with PDB (ID:3L3L).