Bioorganic & Medicinal Chemistry Letters 20 (2010) 448–452

Contents lists available at ScienceDirect

Bioorganic & Medicinal Chemistry Letters

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

Discovery and SAR of novel, potent and selective hexahydrobenzonaphthyridinone inhibitors of poly(ADP-ribose)polymerase-1 (PARP-1)

Caterina Torrisi *, Monica Bisbocci, Raffaele Ingenito, Jesus M. Ontoria, Michael Rowley, Carsten Schultz-Fademrecht, Carlo Toniatti, Philip Jones

IRBM—Merck Research Laboratories Rome, Via Pontina km 30,600, Pomezia, 00040 Rome, Italy

ARTICLE INFO

Article history:
Received 9 November 2009
Revised 1 December 2009
Accepted 1 December 2009
Available online 4 December 2009

Keywords:
PARP
Cancer
Hexahydrobenzonaphthyridinone

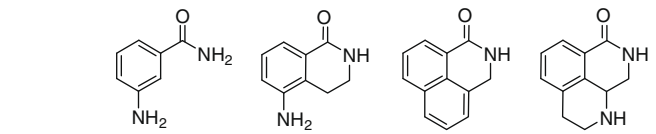
ABSTRACT

A novel hexahydrobenzonaphthyridinone PARP-1 pharmacophore is reported, subsequent SAR exploration around this scaffold led to selective PARP-1 inhibitors with low nanomolar enzyme potency, displaying good cellular activity and promising rat PK properties.

Poly(ADP-ribose) polymerases (PARPs) constitute a super family of 18 nuclear and cytoplasmic enzymes which use NAD^+ as the substrate to catalyze the formation of long and branched ADP-ribose polymers on a number of proteins, including: itself, histones, topoisomerases and p53. This structural modification alters the function of the targeted proteins, and poly(ADP-ribosyl)ation (PARylation) is implicated in the regulation of several fundamental biological processes, including DNA repair, cell death and genomic stability.[1]

PARP-1, the founding member of PARP family, accounts for about 95% of poly(ADP-ribosyl)ation activity in cells and acts as a DNA damage sensor and signaling protein.[2] Through the DNA-binding domain, PARP-1 can detect and bind to DNA single strand breaks. Upon binding to DNA nicks, PARP-1 catalytic activity is promptly stimulated, leading to the formation of poly(ADP-ribose) (PAR) polymers. These PAR chains act as a beacon and recruit the enzymes required for DNA repair to the site of damage, and the lesion is thus repaired.[3] Similarly, PARP-2 elicits DNA-damage-dependent catalytic activity, being thus responsible for the residual PARylation activity observed in PARP-1 deficient mouse fibroblasts.[4] Since DNA repair represents a major mechanism of tumor cell resistance towards the common DNA-damaging cancer therapies, PARP inhibitors (PARPi) are useful as chemosensitizers for use in combination with radiotherapy and/or cytotoxic agents (e.g., temozolomide, topotecan and cisplatin).[5] In addition, PARPi can also be used as context–specific monotherapy of tumors with specific defects in the DNA repair. BRCA-1 and BRCA-2 are key proteins involved in DNA double strand break repair by the homologous recombination pathway, and mutations in these genes have been associated with hereditary cancers, notably breast and ovarian. It has been described that inhibition of PARP activity in BRCA-1 or BRCA-2 deficient tumor cells becomes lethal, whereas PARP inhibition is generally non-toxic to normal cells.[6] A number of inhibitors are currently being explored in the clinic as both mono- and combination therapies.[7]

The vast majority of PARPi known to date are substrate competitive inhibitors and interact with the NAD^+ binding domain of the enzyme through three hydrogen bonds, with Ser904 and Gly863.[8] The byproduct of the PARylation reaction, nicotinamide, was discovered to be a weak PARP inhibitor and structural analogues of nicotinamide such as benzamide **1** and derivatives



	1	**2**	**3**	**4**
PARP-1 IC_{50} (nM)	3300	410	540	140

Figure 1. Known PARP-1 inhibitors (**1**–**3**) with published IC_{50} values,[8] and novel hexahydrobenzophthyridinone inhibitor **4**.

* Corresponding author at present address: Addex Pharma SA, Chemin des Aulx 12, 1228 Plan-les-Ouates, Switzerland. Tel.: +41 228841555; fax: +41 228841556.
E-mail address: caterina.torrisi@addexpharma.com (C. Torrisi).

0960-894X/$ - see front matter © 2009 Elsevier Ltd. All rights reserved.
doi:10.1016/j.bmcl.2009.12.002

(Fig. 1) were among the first compounds to be investigated as PARP inhibitors. However, these acyclic molecules like **1** had only weak inhibitory activity. Restriction of the carboxamide moiety in the bioactive *anti* conformation by intramolecular hydrogen bonding or via a ring connection (e.g., incorporation into a lactam ring) has become a common strategy for PARPi development leading to much more potent compounds such as substituted dihydroisoquinoline **2** and fused tricyclic dihydroisoquinoline **3**.

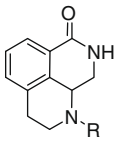
With the aim of finding a new class of potent PARP inhibitors suitable for clinical development, we designed 1,2,3,8,9,9a-hexahydro-7*H*-benzo[*de*]-1,7-naphthyridin-7-one **4** as a new pharmacophore capable of interacting with the NAD^+ binding site. This structure was designed bearing a functional handle to allow the introduction of substituents pointing at the adenine–ribose binding pocket.

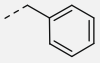
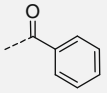
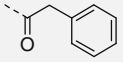
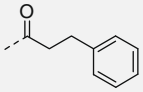
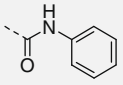
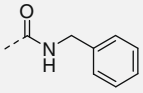
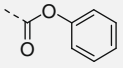
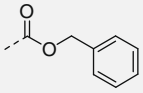
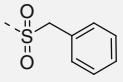
Compound **4** was prepared using a known two-step procedure (Scheme 1).[9] Commercially available 1,2,3,4-tetrahydroisoquinoline-1-acetic acid was subjected to acid-catalyzed cyclization to give **5**, which afforded the desired racemic tricycle **4** as the minor product in 18% yield, together with the corresponding isomer **6** as dominant product (61%), via a Schmidt rearrangement.

We were pleased to find that compound **4** exhibited good levels of inhibition of the activity of both PARP-1 (IC_{50} = 140 nM) and PARP-2 (IC_{50} = 60 nM) with good selectivity over Tankyrase (11% inhibition at 1 μM). The isomer **6** was not active on PARPs 1 and 2 at 1 μM, confirming the importance of the amide bond orientation for PARP activity. Based upon this promising finding a SAR exploration was started to improve the potency of these compounds. Alkyl, acyl, carbamate, sulfonamide and urea derivatives **7**–**49** were prepared in parallel from **4**, applying standard or polymer-supported solution phase synthesis conditions.[10]

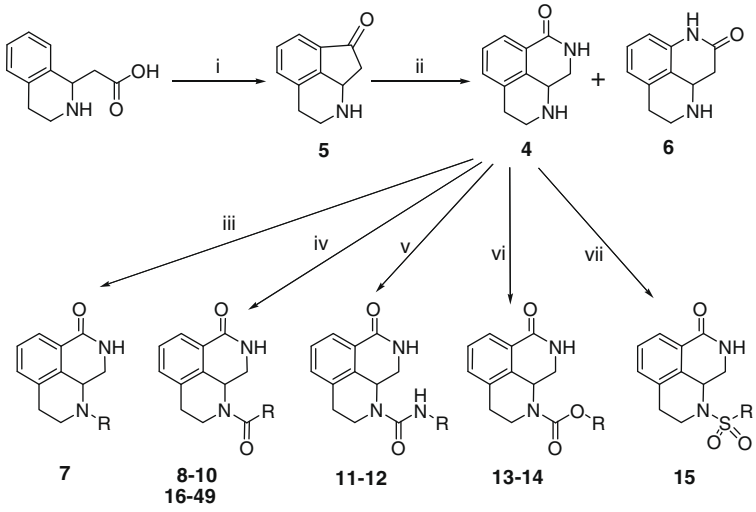
Initial SAR focused on determining the optimal nitrogen substituent, both in terms of functional group and chain length. The compounds were screened as racemates for both inhibition of human PARP-1, and inhibition of PARylation in HeLa cells upon stimulation of DNA damage by treatment with hydrogen peroxide (Table 1).[11] While introduction of a benzyl group to give the tertiary amine **7** gave no improvement in activity with respect to **4**, elimination of the basic center using an amide linker (**8**) led to a boost in activity. In fact, amide analogue **8** displayed inhibitory activity of PARP-1 enzyme in the low nanomolar range with more than 30-fold improvement in potency over the lead **4**. In terms of activity on PARP-1, essentially little or no influence of the length and the nature of the linker was observed (**9**–**14**). Urea and carbamate groups were

Table 1
Enzyme and cellular activities for compounds **4** and **7**–**15**



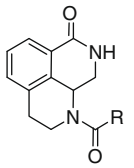
Compds	R	hPARP-1 IC_{50}[a] (nM)	PARylation EC_{50}[a] (nM)
4	H	140	ND[b]
7		190	5200
8		4	840
9		8	230
10		4	350
11		11	1100
12		12	690
13		9	1400
14		14	130
15		83	2700

[a] Values are means of two or more experiments.
[b] Not determined.



Scheme 1. General synthesis of hexahydrobenzophthyridinone compounds. Reagents and conditions: (i) PPA, 150 °C (85%); (ii) NaN_3, H_2SO_4, 0 °C to rt (**4**, 18%; **6**, 61%); (iii) PhCHO, $NaBH_3CN$, MeOH; (iv) RCOOH, HBTU, DIPEA, DMF or RCOOH, PS-DCC, DIPEA, DMF; (v) RNCO, PS-DIPEA, DCM; (vi) ROCOCl, PS-DIPEA, DCM; (vii) RSO_2Cl, PS-DIPEA, DCM.

Table 2
PARP-1 binding assay and PARylation assay results for compounds **16–38**



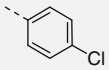
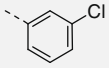
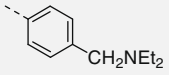
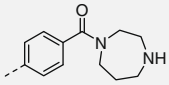
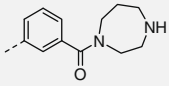
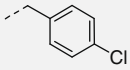
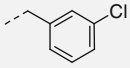
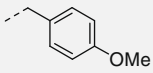
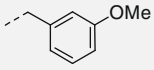
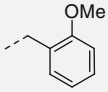
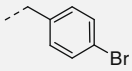
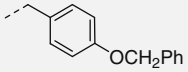
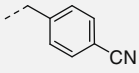
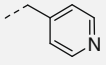
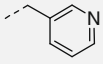
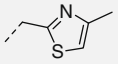
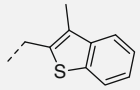
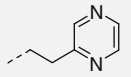
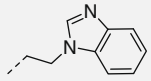
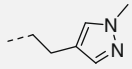
Compds	R	hPARP-1 IC_{50}[a] (nM)	PARylation EC_{50}[a] (nM)
16		7	260
17		37	ND[b]
18		35	ND[b]
19		13	310
20		130	ND[b]
21		50	ND[b]
22		5	650
23		5	1100
24		16	900
25		6	160
26		5	1200
27		27	1700
28		7	200
29		16	580
30		9	200
31		13	230
32		10	4600
33		1	360
34		6	2900

Table 2 (*continued*)

Compds	R	hPARP-1 IC_{50}[a] (nM)	PARylation EC_{50}[a] (nM)
35		13	1300
36		6	330
37		1	1600
38		14	420

[a] Values are means of two or more experiments.
[b] Not determined.

also well tolerated, whereas the sulfonamide **15** was detrimental. However, despite the similarities in enzymatic activity, the amides were generally more active than the corresponding carbamates and ureas in cells, the former typically displaying sub-micromolar inhibition of PARylation. Based on this result, the SAR on this new series of PARPi was extensively explored further focusing on the synthesis of diverse libraries of aromatic, heteroaromatic and aliphatic amides.

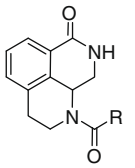
Initially, substituted benzamides were explored (Table 2), and the effect of a simple chlorine substituent was investigated. *Meta* and *ortho* substitution (**17–18**) were detrimental for activity, while introduction of a chlorine in the *para* position (**16**) gave a single digit nanomolar PARP inhibitor similar to **8**, but with a three-fold boost in the cellular activity (EC_{50} = 260 nM). Also a basic amine in the *para* position (**19**) gave improved cellular activity despite slightly weaker enzyme activity. Larger groups such as homopiperazine amides **20**–**21** resulted in a loss of potency. Homologation of **8** into the corresponding phenylacetamide **9** maintained enzyme activity but further improved cellular activity, with **9** displaying EC_{50} = 230 nM. However, preliminary DMPK studies indicated that **9** was highly turned over in rat liver microsomes (Cl_{int} = 240 μl/min/mg P), thus further modifications to these phenylacetamides were aimed at stabilizing the compound toward hepatic metabolism, as well as at improving activity. Again, the *para* position was confirmed to be overall the preferred site for the introduction of substituents (**22–27**), especially for cellular activity, although the regioselectivity was more modest. A variety of substituents were tolerated in position *para*, including methoxy (**25**), bromine (**28**) and cyano groups (**30**). All three showed modestly improved activity in cells compared to **9**. However, a streric limitation was determined as the *para*-benzyloxy group (**29**) resulted in a three-fold loss in enzyme activity compared to **25**.

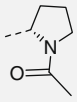
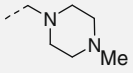
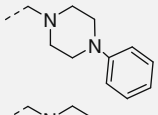
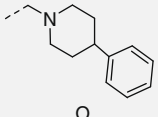
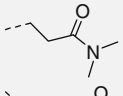
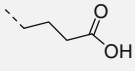
Related heterocyclic replacements were also investigated. Introduction of a heteroatom was better tolerated in the 3-position (**31**–**33**), with **33** being a very potent enzyme inhibitor with good cellular activity. The 3-pyridyl group also gave excellent metabolic stability in vitro (Rat Cl_{int} = 3 μl/min/mg P). Other heterocycles (**34**–**35**) displayed inferior cellular activity.

In the phenylpropamide series, replacement of the phenyl ring of **10** with pyrazine (**36**) did not affect significantly activity compared to **9**. However, **36** showed much better rat liver microsome stability (Cl_{int} = 24 μl/min/mg P). Benzimidazole **37** was a very potent PARP inhibitor (IC_{50} = 1 nM) but in contrast had only micromolar cellular activity. On the contrary pyrazole **38**, despite a decreased potency on the enzyme, showed an acceptable level of cellular potency.

In parallel to the (hetero)aromatic derivatives, a library of aliphatic amides was evaluated (Table 3). The simple cyclopentyl derivative **39** displayed PARP-1 IC_{50} = 11 nM and EC_{50} = 550 nM in cells. To try to pick up additional hydrogen-bond interactions, an amine moiety was incorporated resulting in the L-prolyl derivative **40** which improved both enzyme and cell activity about threefold. The nitrogen needs to be basic to maintain activity as demonstrated by the acetyl analogue **41** which displayed only weak cellular activity (EC_{50} = 2.8 μM). Moving the nitrogen to give the pyrrolidin-3-yl derivative **42** and reducing the size of the ring to give azetidine **43** were both changes detrimental to cellular activity. Other cyclic amines such as (piperidin-1-yl) acetyl **44** were also active cell permeable PARPi, with **44** displaying IC_{50} = 6 nM and EC_{50} = 280 nM, and a remarkable improvement in microsome stability (Cl_{int} = 18 μl/min/mg P). Phenyl piperidines have been claimed as potent PARPi,[12] and while *N*-methyl piperazine **45** was only weakly active, possibly due to the presence of the second amine group, the introduction of a phenyl substituent in the 4-position was confirmed to give a 10-fold boost in activity on the enzyme in both the piperazine and corresponding piperidine compounds (**46**–**47**), with **47** being a sub-nanomolar PARP inhibitor. Other functionalities like an amide (**48**) or a carboxylic acid (**49**) were evaluated but despite giving potent enzyme inhibitors, these modifications were not tolerated in cells.

Table 3
Enzyme and cellular activities for compounds **39–49**



Compds	R	hPARP-1 IC_{50}[a] (nM)	PARylation EC_{50}[a] (nM)
39		11	550
40[b]		4	180
41[b]		14	2800
42		1	3200
43		1	660
44		6	280
45		97	3500
46		11	440
47		0.5	410
48		9	2800
49		15	5000

[a] Values are means of two or more experiments.
[b] Mixture of two diastereoisomers.

Table 4
Activity against various PARP isoforms for compounds **33** and **36**[a]

Compds	PARP-1 IC_{50} (nM)	PARP-2 IC_{50} (nM)	PARP-3 IC_{50} (nM)	v-PARP IC_{50} (nM)	TNKS1 IC_{50} (nM)
33	1	1	50	440	3500
36	6	1	70	660	2000

[a] Values are means of two or more experiments.

Table 5
Rat PK summary for compounds **33** and **36**[a]

Compds	$t_{1/2}$ (h)	Vd (L/kg)	CL (ml/min/kg)	%*F*	po AUC (μM h)
33	1.7	3	38	51	2.3
36	1.2	2	31	ND[b]	ND[b]

[a] Compounds were administered iv and po at 3 mg/kg.
[b] Not determined.

Representative compounds such as **33** and **36**, which were identified as potent PARP-1 inhibitors endowed with sub-micromolar cellular activity and good metabolic stability, were screened against a panel of other PARP isoforms (Table 4). Both compounds resulted in very potent inhibitors of PARPs 1 and 2, with good selectivity towards PARP-3, and excellent selectivity over vPARP and Tankyrase-1.

Given the interest in **33** and **36**, these compounds were evaluated in rat PK studies and results are summarized in Table 5. Both compounds displayed acceptable clearance in vivo, with Cl = 38 and 31 mL/min/kg respectively, which reflects the good microsome stability (Cl_{int} = 3, and 24 μl/min/mg P, respectively). The pyridyl derivative **33** also showed good oral bioavailability (F = 51%). Furthermore, **33** and **36** did not show any CYP inhibition or binding to IKr at 10 μM.

In conclusion, we have described the discovery, SAR and preliminary biological evaluation of a novel series of hexahydrobenzonaphthyridinone carboxamides. These compounds represent a novel class of potent PARP inhibitors, capable of inhibiting the PARylation reaction in whole cells at sub-micromolar concentrations. Furthermore, selective PARP-1 and -2 inhibitors like **33** and **36** having good metabolic stability and promising rat PK profiles were identified.

Acknowledgments

We are grateful to Massimiliano Fonsi and Maria Vittoria Orsale for DMPK support and to Fabrizio Fiore for PK studies.

References and notes

1. (a) Nguewa, P. A.; Fuertes, M. A.; Valladares, B.; Alonso, C.; Perez, J. M. *Prog. Biophys. Mol. Biol* **2005**, *88*, 143; (b) Amé, J. C.; Spenlehauer, C.; de Murcia, G. *BioEssays* **2004**, *26*, 882.
2. Szabó, C.; Jagtap, P. *Nat. Rev. Drug Disc.* **2005**, *4*, 421.
3. Schreiber, V.; Dantzer, F.; Ame, J. C.; de Murcia, G. *Nat. Rev. Mol. Cell. Biol.* **2006**, *7*, 517.
4. (a) Eliasson, M. J.; Sampei, K.; Mandir, A. S.; Hurn, P. D.; Traystman, R. J.; Bao, J.; Pieper, A.; Wang, Z. Q.; Dawson, T. M.; Snyder, S. H.; Dawson, V. L. *Nat. Med.* **1997**, *3*, 1089; (b) Shieh, W. M.; Ame, J. C.; Wilson, M. V.; Wang, Z. Q.; Koh, D. W.; Jacobson, E. L. *J. Biol. Chem.* **1998**, *273*, 30069.
5. Tentori, L.; Graziani, G. *Pharmacol. Res.* **2005**, *52*, 25.
6. (a) 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; (b) Farmer, H.; McCabe, N.; Lord, C. J.; Tutt, A. N. J.; Johnson, D. A.; Richardson, T. B.; Santarosa, M.; Dillon, K. J.; Hickson, I.; Knights, C.; Martin, N. M. B.; Jackson, S. P.; Smith, G. C. M.; Ashworth, A. *Nature* **2005**, *434*, 917.
7. Rodon, J.; Iniesta, M. D.; Papadopoulos, K. *Expert Opin. Investig. Drugs* **2009**, *18*, 31.
8. Peukert, S.; Schwahn, U. *Exp.Opin. Ther. Patents* **2004**, *14*, 1531.
9. Pelletier, J. C.; Cava, M. P. *J. Org. Chem.* **1987**, *52*, 616.

10. Ingenito, R.; Jones, P.; Ontoria, J. M., Schultz-Fademrecht, C.; Torrisi, C. PCT Patent Application WO2008/001134 A1, 2008. This patent describes the general procedures for the synthesis of alkyl, acyl, carbamate, sulfonamide and urea derivatives from compound 4. Characterization data for representative compounds of each class are given.
11. (a) Cheung, A.; Zhang, J. *Anal. Biochem.* **2000**, *282*, 24; (b) Alvarez-Gonzalez, R.; Spring, H.; Muller, M.; Burkle, A. *J. Biol. Chem.* **1999**, *274*, 32122.
12. Ishida, J.; Hattori, K.; Yamamoto, H.; Iwashita, A.; Mihara, K.; Matsuoka, N. *Bioorg. Med. Chem. Lett.* **2005**, *15*, 4221.