Bioorganic & Medicinal Chemistry Letters 18 (2008) 5126–5129

Contents lists available at ScienceDirect

Bioorganic & Medicinal Chemistry Letters

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

Identification of ring-fused pyrazolo pyridin-2-ones as novel poly(ADP-ribose)polymerase-1 inhibitors

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ARTICLE INFO

Article history:
Received 27 May 2008
Revised 23 July 2008
Accepted 24 July 2008
Available online 27 July 2008

Keywords:
PARP-1 inhibitor
Cancer
DNA repair

ABSTRACT

A novel class of PARP-1 inhibitors was identified containing a non-aromatic heterocycle or carbocycle fused to a pyrazolo pyridin-2-one. Compounds displayed low nanomolar binding activity in the PARP-1 binding assay and submicromolar activity in a cell based chemosensitization assay.

Poly(ADP-ribose)polymerase-1 (PARP-1) is a highly abundant nuclear enzyme that is activated by single or double DNA strand breaks.[1] Activated PARP-1 uses nicotinamide adenine dinucleotide as a substrate to catalyze the addition of long branched chains of poly ADP ribose onto itself and other target proteins involved in DNA replication, transcription, and repair.[2]

The involvement of PARP-1 in the regulation of DNA integrity has made it an attractive target for cancer therapy. PARP-1 has been demonstrated to be important for repair of damage induced by radiation as well as by different classes of chemotherapy agents, including the topoisomerase I inhibitors and the mono-functional DNA alkylating agents. Disruption of PARP-1 expression or function results in increased sensitivity of tumor cells to these cancer agents.[3] Indeed, PARP-1 inhibitors have been in development as sensitizers in radiation and chemotherapy treatment of cancer for many years.[4] In addition, PARP-1 inhibitors are currently being studied as protective agents against tissue damage occurring from ischemia/reperfusion injuries[5] and various forms of inflammation.[6]

The vast majority of PARP-1 inhibitors described to date are competitive with NAD^+, are analogs of nicotinamide, and have a carboxamide attached to an aromatic ring or contain a fused aromatic lactam (Fig. 1).[7]

In this letter, we describe the discovery, synthesis, and SAR of a novel series of tricyclic PARP-1 inhibitors that contains a non-aromatic heterocycle or carbocycle fused to a pyrazolo pyridin-2-one.[8]

To initiate this program, a large collection of commercial samples from different vendors was screened in a PARP-1 binding assay. From this screening effort, compound **5** was identified as a potent PARP-1 inhibitor with an IC_{50} of 93 nM (Fig. 2). However, after resynthesis of this compound and some close analogs, no significant binding affinity for PARP-1 was observed. NMR analysis of the commercial sample **5**[9] showed strong NOE enhancements between methyl protons and methylene protons suggesting that the correct structure was in fact compound **6**, containing a pyridin-2-one (Fig. 2). This pharmacophore with a s-trans conformation of an amide functionality is known to be a favorable binding conformation for PARP-1 inhibitors as described in the literature.[10,11] Close analogs of structure **6** were synthesized, varying both the substituents off the pyrazole unit and the non-aromatic heterocycle fused to the pyridin-2-one.

Two synthetic routes were employed for the synthesis of analogs around compounds **5** and **6**:

Method A: Cyclic β-ketoesters (**7**) were reacted with 5-aminopyrazoles (**8**) in PPA at ~130 °C to generate pyridin-4-ones predominantly[12] (**9**) (Scheme 1). If reaction times surpassed 15 min

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doi:10.1016/j.bmcl.2008.07.091

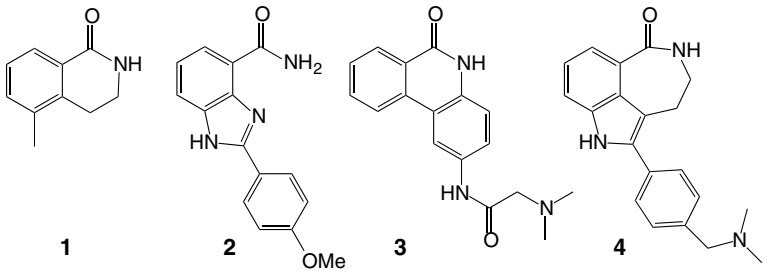


Figure 1. PARP-1 inhibitors **1, 2, 3**, and **4** with PARP-1 IC_{50} values of 160, 60, 20, and 5 nM, respectively.

for the tetrahydrothiophene subseries, significant aromatization to the thiophene pyridine-4-one occurred. When cyclic β-ketoesters (**7**) were reacted with 5-aminopyrazoles (**8**) under basic conditions[13] amide intermediates (**10**) were formed which were purified by HPLC chromatography. Subsequent cyclization under acidic conditions or at high temperature in vacuo gave the pyrazolo pyridin-2-ones (**11**). In early reports, it is described that the enamine condensation product from 5-amino-1,3-dimethylpyrazole and ethyl acetoacetate does not cyclize in acetic acid to yield the expected 4-pyridinone but rather the 2-pyridinone due to interconversion of the enamine intermediate to the amide condensation product (**10**).[14] This possibly led to the disparity in assignment of the commercial HTS lead (**5**).

Method B by Winters et al. [15]: Cyclic ketones (**12**) were reacted with 5-aminopyrazoles (**8**) to give dienamines (**13**), converted to ureas and subjected to thermal cyclization to give pyrazolo pyridin-2-ones (**14**) (Scheme 2).[16] When tetrahydrothiophen-3-one was used in this sequence, the major product isolated upon condensation with 5-aminopyrazole (**8**) was a fully aromatized thiophene aminopyrazole (**13**). Therefore this method was only employed with the carbocyclic ketones. All compounds were isolated by either filtration from crude reaction mixtures or reverse phase HPLC/MS purification using mass triggered fraction collection.[17]

Compounds were evaluated in a PARP-1 binding assay,[18] and the results are summarized in Tables 1–4. Representatives of the pyridin-4-one series (Table 1), including **5**, the reported structure of the high-throughput screening hit, did not display the inhibitory activity for PARP-1 as one would have expected based on the results (IC_{50} = 93 nM) for the commercial sample. None of the other compounds in this series, in which the pyrazolo substituents and the non-aromatic heterocycle attached to the pyridin-4-one core were varied, showed PARP-1 inhibition with IC_{50} values <5 μM (data not shown).

In contrast, the pyridin-2-one series resulted in potent PARP-1 inhibitors (Tables 2–4). In the dihydrothiophene (I), cyclopentene (II) and cyclohexene (III) subseries, the most active compounds contained methyl substituents at both R^1 and R^2 positions (**6, 21, 27**). Replacement of the methyl at R^2 by phenyl or 2-thienyl typically resulted in a small decrease (up to 5-fold) in inhibition (**17, 18, 22, 23, 28, 29**). A more dramatic decrease in binding affinity was observed with bulky substituents at R^1 such as t-butyl or phenyl in the presence of methyl at R^2 (**19, 20, 24, 25, 30**). Phenyl substituents at both R^1 and R^2 (**26, 31**) caused a complete loss of inhibitory activity.

Preliminary docking experiments using the co-crystal structure of chicken PARP-1 (1PAX) and the Parke-Davis/Pfizer inhibitor[11a] (**1**) indicated that the pyrazolo pyridin-2-ones have a similar binding mode[19] and provided a rationalization for the observed PARP-1 data. In Figure 3, a high scoring pose of compound **22** is shown, making two hydrogen bonds between the amide bond and Gly-863 and Ser 904. A fairly large binding pocket can accommodate the phenyl group or a variety of other substituents at R^2 in subseries II. The volume tolerance in the direction of R^1 in subseries II was substantially less. Figure 3 displays a high scoring pose of inactive compound **25**. In this orientation the amide can only make one of the two key donor/acceptor interactions because of the orientation required to accommodate the R^1 phenyl group (subseries II) in the active site.

Comparison of dihydrothiophene (I), cyclopentene (II) and cyclohexene (III) sub-series, indicates a preference for binding affinity according to cyclohexene III > cyclopentene II > dihydrothiophene I. These results are consistent with the predicted outcome from modeling. Docking shows subseries III to be the most favored as the additional methylene from the cyclohexyl provides additional favorable hydrophobic contact with PARP-1. In a comparison of **6, 21**, and **27**, the hydrophobic surface area is 7% larger for **27** than for **6** and **21** and the ligand/protein contact in this area of the binding site is predominantly aromatic/hydrophobic. A similar

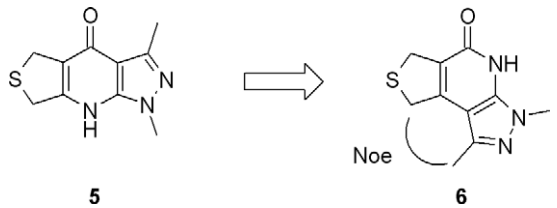
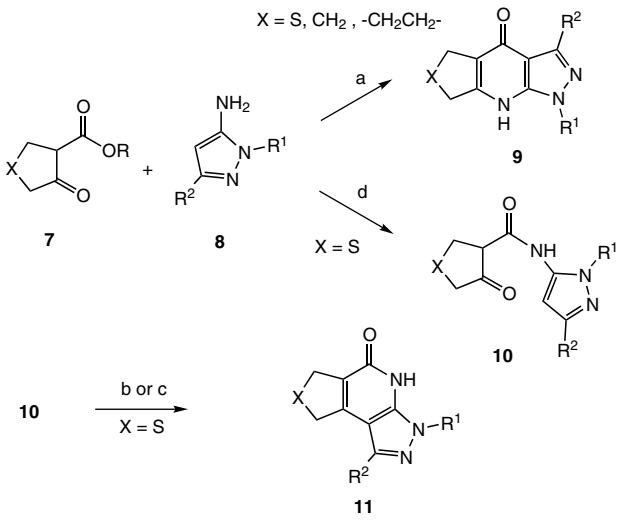
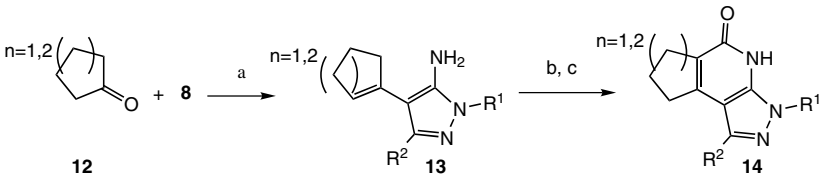


Figure 2. Reported structure (**5**) and correct structure (**6**) of high-throughput screening hit.

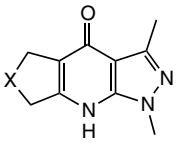


Scheme 1. Reagents and conditions: (a) PPA, 130 °C, 2 h (X = CH_2 and CH_2CH_2), 15 min (X = S); (b) HOAc, reflux, 1 h; (c) ~220 °C, vacuo, 15 min; (d) Pyr, o-Xylene, 110 °C, 2–6 h.



Scheme 2. Reagents and conditions: (a) HOAc, rt, 45 h; (b) PhN=C=O, toluene, 3 h, rt then 15 min–3 h at 85 °C (c) ~200 °C, 5–15 min.

Table 1
PARP-1 inhibition for pyrazolo pyridin-4-ones



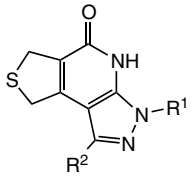
Compound	X	PARP-1 Inhibition IC_{50}, μM[a]
5	–S–	>10
15	$–CH_2–$	7
16	$–CH_2CH_2–$	>10

[a] Values for single experiments.

increase in binding potency was observed in a bicyclic uracil series when a cyclopentyl group was replaced by a cyclohexyl moiety.[20]

Potent compounds were subsequently tested for their ability to augment cytotoxicity of the alkylating agent streptozotocin with the human colon carcinoma HCT 116 cell line[21] (Table 5). Compounds from the cyclohexene subseries III, the most potent in the enzyme binding assay (**27**–**29**), were also most active in the

Table 2
PARP-1 inhibition for dihydrothiophene subseries I

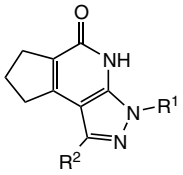


I

Compound	R^1	R^2	PARP-1 Inhibition IC_{50}[a], nM
6	Me	Me	92.7
17	Me	Ph	110.3
18	Me	2-Thienyl	428.1
19	*t*-Bu	Me	1841.5
20	Ph	Me	>10,000

Single determinations when IC_{50} > 1000 nM.
[a] Values of duplicate determinations are within twofold of each other.

Table 3
PARP-1 inhibition for cyclopentene subseries II

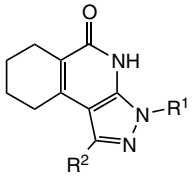


II

Compound	R^1	R^2	PARP-1 Inhibition IC_{50}[a], nM
21	Me	Me	29.8
22	Me	Ph	106.0
23	Me	2-Thienyl	148.6
24	*t*-Bu	Me	519.2
25	Ph	Me	>10,000
26	Ph	Ph	>10,000

Single determinations when IC_{50} > 1000 nM.
[a] Values of duplicate determinations are within twofold of each other.

Table 4
PARP-1 inhibition for cyclohexene subseries III



III

Compound	R^1	R^2	PARP-1 Inhibition IC_{50}[a], nM
27	Me	Me	2.2
28	Me	Ph	7.3
29	Me	2-Thienyl	6.9
30	Ph	Me	9720
31	Ph	Ph	>10,000

Single determinations when IC_{50} > 1000 nM.
[a] Values of duplicate determinations are within twofold of each other.

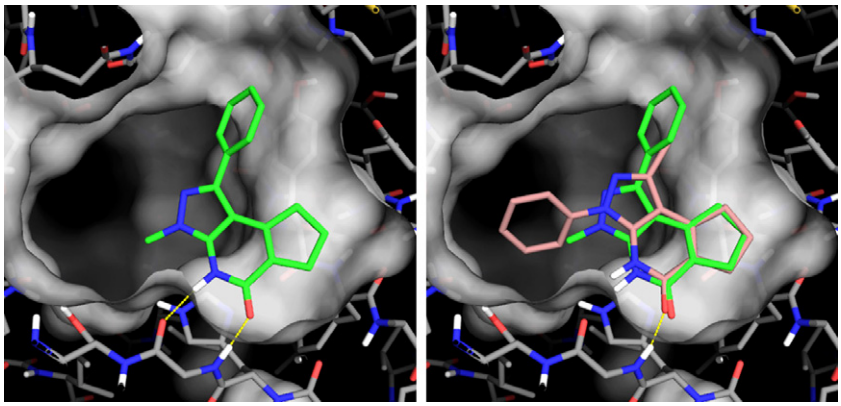


Figure 3. Dock of **22** (left) and **25** (right) using the co-crystal structure of chicken PARP-1 (1PAX) and the Parke-Davis/Pfizer inhibitor (**1**).

Table 5
Chemosensitization, cell toxicity, and therapeutic index for compounds **6** and **17, 21–23**, and **27–29**

Compound	EC_{sens}, μM[a]	LD_{40}, μM	TI[c]
6	28.6	>50	>1.8
17	15.4	21.2	1.4
21	19.3	27.2	1.4
22	26.8	42.5	1.6
23	3.1 (1.3)	11.2	3.6
27	1.9 (1.2)	>12.5[b]	>6.6
28	0.38 (0.15)	7.5[b]	19.7
29	0.38 (0.21)	>12.5[b]	>32.9

[a] Values for quadruplicate experiments. Single determinations if EC_{sens} values were >10 uM.
[b] Values of duplicate determinations are within twofold of each other.
[c] In vitro therapeutic index (LD_{40}/EC_{sens}).

chemosensitization assay and displayed the most favorable therapeutic index. Addition of an aromatic group at R2 in compounds **28** and **29** appeared to accentuate chemosensitizer potency relative to the dimethyl analog **27**. Since analogs **27-29** exhibited similar enzyme potency in vitro, this result implies that other factors are contributing to the enhanced potency of the aryl-substituted analogs in the cell-based assay. Possible factors would include solubility and cellular permeability, which is consistent with the observed lack of activity for compound **21** in cyclopentene subseries II.

In conclusion, tricyclic pyrazolo pyridin-2-ones containing a non-aromatic carbocyle or heterocycle fused to the pyridin-2-one were identified as a novel series of potent low nanomolar inhibitors of PARP-1. Structure activity was further developed in the pyrazolo moiety resulting in the identification of analogs with submicromolar activity in a chemosensitization assay.

The compounds described herein could be useful for further studies on the role of PARP-1 inhibitors as adjunct agents in cancer therapy.

Acknowledgments

The authors thank Dr. Mark J. Suto, Dr. Peter L. Myers, Dr. Kerry W. Fowler and Dr. Michael Gallatin for support of this program, Dr. Catherine Jolivet for NMR analysis, and Mr. Tao Wang for analytical assistance.

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16. Synthesis of compound **28**: 4-Cyclohex-1-enyl-2-methyl-5-phenyl-2H-pyrazol-3-ylamine (**13**, n = 2, R^1 = Me, R^2 = Ph), prepared as described by Winters et al.[15a] (50 mg, 0.20 mmol), was suspended in 800 μl toluene and treated with phenylisocyanate (26 μl, 0.24 mmol).[15b] After stirring at rt for 3 h and heating at 85 °C for 2.5 h, the mixture was concentrated in vacuo to give a yellow oil. The crude product was heated at 200 °C for 5 min, cooled down to rt and triturated with EtOAc (400 μL). The precipitate was filtered and washed with EtOAc to yield 41 mg of **28** (72%). MS: *m/z* 280 $[M+H]^+$, expected 280 $[M+H]^+$. ^{1}H NMR (d^6-DMSO) δ 1.46–1.56 (2H, m, CH_2), 1.62–1.70 (2H, m, CH_2), 2.36–2.47 (4H, m, CH_2), 3.85 (3H, s, CH_3N), 7.40–7.57 (5H, m, Ph), 8.63 (1H, bs, NH).
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21. The ability of PARP inhibitors to augment the cytotoxicity of the alkylating agent streptozotocin (ICN Pharmaceuticals) was tested in the human colon carcinoma HCT116 cell line (ATCC). PARP inhibitors and streptozotocin were diluted in culture media and added to 96-well plates. HCT116 cells were trypsinized and seeded into the plates at a final concentration of 1000 cells/well. Plates were incubated in a 37 °C, 5% CO_2 incubator for four days. Cells were then labeled with [methyl-^{3}H]-thymidine (NEN) at a concentration of 1 μCi/well. The plates were incubated for another 24 h at 37 °C, 5% CO_2. The plates were harvested and then counted using a Matrix 96 Direct Beta Counter (Packard). To compare the potency of PARP inhibitors, the concentration of PARP inhibitor was calculated that reduced by half the amount of chemotherapy agent required for 90% inhibition of cell growth (EC_{sens}). The concentration of PARP inhibitor that is significantly toxic as a single agent (LD_{40}) was also determined.