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Novel poly(ADP-ribose) polymerase-1 inhibitors

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Abstract—Synthesis and activity of a series of 4-thiazol-yl substituted analogs of novel pyrrolocarbazole **1** as poly(ADP-ribose) polymerase-1 (PARP-1) inhibitors have been disclosed.

Poly(ADP-ribose) polymerase-1 (PARP-1) is a nuclear enzyme that catalyzes the synthesis of poly(ADP-ribose) chains from nicotinamide adenine dinucleotide (NAD^+) in relation to single-strand DNA breaks as part of the DNA repair process. Upon activation of PARP-1 in response to DNA damage, synthesis and degradation of this polymer consumes enormous amounts of NAD^+ leading to depletion of energy stores (ATP) and ultimately cell death.[1] A role of PARP-1 has been proposed both in neurodegenerative diseases, for example, stroke, myocardial ischemia, and traumatic brain injury and cancer biology.[2] Thus, development of potent inhibitors of PARP-1 has been the focus of a number of laboratories.[3,4] Recently, our laboratories reported the identification of the novel pyrrolocarbazole compound **1** (Fig. 1), as a potent PARP-1 inhibitor (IC_{50} = 36 nM).[5,6] Compound **2** (Fig. 1), the 3-aminomethyl analog of the parent compound, was also disclosed as a chemopotentiating agent with a variety of clinically effective chemotherapeutic reagents.[7] Additionally, our laboratories reported the synthesis and structure–activity relationships (SAR) of various truncated analogs of compound **1** as well as analogs containing oxygen and sulfur, respectively, in place of indole–NH (position 11) of the parent scaffold.[6] As a part of our continuing search for novel, potent, and cell-permeable inhibitors of this enzyme, additional analogs of compound **1** were prepared. In this report, we disclose the synthesis and enzyme inhibitory activity of a series of heterocycle substituted, especially 4-thiazolyl, analogs of compound **1** (Scheme 1).

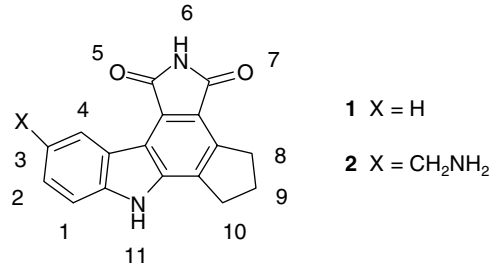

Figure 1.

During our synthetic program, it was discovered that compound **1** underwent facile Friedel–Crafts reaction with acetyl chloride to generate compound **3** in a regioselective fashion.[8] Extension of this synthetic methodology with bromoacetylbromide led to versatile intermediate **4** that was further utilized in subsequent transformations (Scheme 1). Thus, treatment of compound **4** with various thioamides, in parallel fashion, generated a series of thiazoles, followed by additional synthetic manipulations to generate compounds of interest.

Target compounds were evaluated against recombinant human poly(ADP-ribose) polymerase-1 in an in vitro enzyme assay as described previously.[6] They were also tested in a cell-based assay that evaluated their ability to attenuate the depletion of NAD^+ levels following hydrogen peroxide insult in PC12 cells.[9] The results were normalized to the maximal effect of a reference compound (5*H*-phenanthridin-6-one[10]) at 30 μM. The NAD^+ levels are reported as % recovery as compared to untreated cells. The cell % NAD^+ recovery at 1 μM was used as an index of potency, while the maximal

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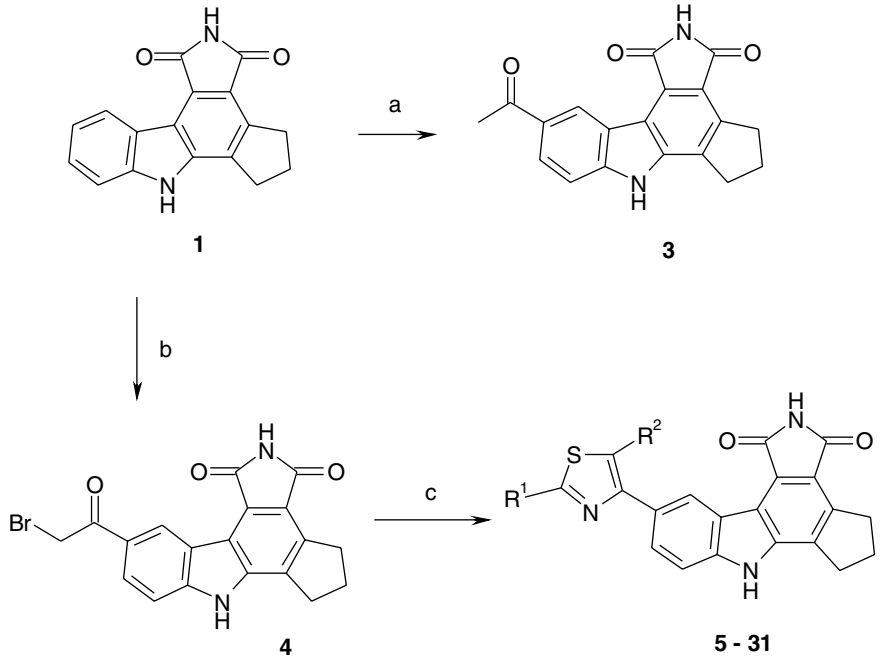


Scheme 1. Reagents and conditions: (a) acetyl chloride, $AlCl_3$, 1,2-dichloroethane, 0 °C to room temperature, 80%; (b) bromoacetyl bromide, $AlCl_3$, 1,2-dichloroethane, 0 °C to room temperature, 84%; (c) various thioamides, absolute ethanol, 80 °C, 41–83% and subsequent manipulations (if needed).

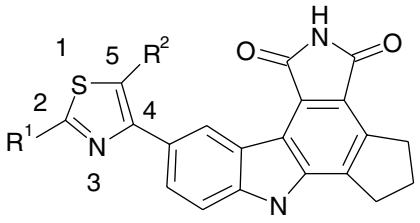
recovery at 30 μM reflects the efficacy. Table 1 describes the biological activities of various target compounds.

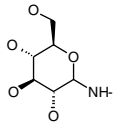
As shown, both carbonyl derivatives **3** and **4** were approximately 2- to 3-fold more potent than the parent compound **1**; however, their cell permeabilities were moderate. In the thiazole series, the 2-methyl analog maintained the potency of the parent molecule (cf. **5** vs **1**). Introduction of another group (halogen) at 5-position of compound **5** resulted in ca. 2-fold drop in potency (cf. **6** vs **5**) indicating that there might be a limitation in steric bulk in that region of the thiazole nucleus. Substitution of one of the hydrogens of the 2-methyl group by a polar amino group maintains the activity (cf. **7** vs **5**). Converting the above-mentioned amine group to a series of amide analogs (**8**–**15**), carbamate (**16**) sulfonamide (**17**), and urea (**18**), respectively, was not detrimental to the enzyme inhibitory activity. However, the inhibitors displayed poor to moderate cell permeability, most probably due to presence of multiple H-bond donor–acceptor polar functionalities. Replacing the methyl group with an amino group at the 2-position of the thiazole nucleus generated compound **19** that was equipotent to parent compound **1**. Interestingly, this compound displayed favorable cell permeability. Introduction of an additional methyl group at the 5-position of the thiazole nucleus in compound **19** generated compound **20** that was about 2-fold less active, reminiscent of compound **6**; compound **20** was also less effective in the cell-based assay. Functionalization of the amino group in compound **19** to an alkyl amine (**21**), guanidine (**22**) or acetyl (**23**), respectively, was detrimental to cell permeability, even though their enzyme activities were acceptable. Introduction of protected and free amino acid moieties (**24**–**25**), sulfonyl derivatives (**26**–**27**), and ester derivatives (**28**–**29**) at the 2-position of the thiazole nucleus displayed various degrees of potency and permeability. Appendage of an additional heterocycle (isoxazole, compound **30**) to the 2-position of the thiazole moiety reduced the potency of the parent molecule as well as cell permeability. On the other hand, introduction of an amino sugar moiety (compound **31**) to improve aqueous solubility was beneficial for potency, but dramatically reduced cell permeability.

Thus, it appears that the position-3 of the parent molecule **1** can accommodate a variety of substituted thiazole moieties in maintaining enzyme inhibitory activity in this class of molecules. From a molecular modeling docking study of parent compound **1** to the catalytic domain of chicken PARP-1, it was subsequently shown that the positions 3 and 4, taken together, offer a pocket that could accommodate various groups to explore the SAR surrounding the parent scaffold.[6,11] Additional work is continuing to exploit this observation. From the current study, compound **19** containing a 2-aminothiazole nucleus emerged as a potent and cell permeable inhibitor of PARP-1.

In this report, we describe a series of potent PARP-1 inhibitors based on our initial lead molecule **1**. The data presented indicate that the enzyme can tolerate a substituted thiazole nucleus at the 3-position of the parent molecule. One such novel analog, compound **19**, displayed potent enzyme activity as well as superior cell permeability. Research is continuing and additional data will be communicated in due course.

Table 1. Biological data of thiazole-derived analogs



Compound	R^1	R^2	IC_{50} (nM) ($n = 2$)	NAD^+ Recovery (PC12 Cells)	
				% Recovery at 1 μM	Maximum % recovery at 30 μM
1	—	—	36	30	Not tested
2			18	84	100
3	—	—	17	35	57
4	—	—	13	32	49
5	CH_3	H	40	19	63
6	CH_3	Br	84	16	57
7	CH_2NH_2 HBr salt	H	42	20	41at 10 μM
8	$CH_2NHCOMe$	H	20	20	51
9	$CH_2NHCOEt$	H	56	16	20
10	CH_2NHCO-*n*-propyl	H	42	19	29
11	CH_2NHCO-*i*-propyl	H	32	10	33
12	CH_2NHCO-*n*-butyl	H	56	15	34
13	CH_2NHCO-*i*-butyl	H	56	13	30
14	CH_2NHCO-cyclopropyl	H	49	11	16
15	CH_2NHCO-cyclopentyl	H	52	21	29
16	CH_2NHCbz	H	20	17	46
17	CH_2NHSO_2Me	H	18	22	28
18	$CH_2NHCONHEt$	H	20	15	22 at 10 μM
19	NH_2	H	25	48	100
20	NH_2	CH_3	50	37	38 at 3 μM
21	$NHCH_3$	H	54	22	56
22	$NHCH(=NH)NH_2$	H	19	19	53
23	$NHCOCH_3$	H	54	10	34
24	$(CH_2)_3CH(NH$ *t*-Boc$)CO_2$*t*-Boc	H	46	17	27 at 10 μM
25	$(CH_2)_3CH(NH_2)CO_2H$ TFA salt	H	22	7	47
26	CH_2SO_2Me	H	38	16	62
27	CH_2SO_2-*t*-butyl	H	21	33	51
28	CH_2CO_2-*t*-butyl	H	29	20	49
29	CH_2OCO-*t*-butyl	H	60	14	27
30	5-Isoxazolyl	H	66	11	27
31		H	17	0	0

Acknowledgments

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8. For parent compound **1**, all the hydrogens were fully characterized via homonuclear decoupling and NOE in 1H NMR (400 MHz) experiments. H-3 displayed a dd-splitting pattern, consistent with its coupling with neighboring H-2 (ortho coupling 7 Hz) and H-4 (ortho coupling, 7 Hz). Applying the same techniques to derivative **3**, it was demonstrated that substitution took place at C-3 position especially from interpretation of NOE interaction data.
9. 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, 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*, 1–13.
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