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The discovery and synthesis of novel adenosine substituted 2,3-dihydro-1*H*-isoindol-1-ones: potent inhibitors of poly(ADP-ribose) polymerase-1 (PARP-1)

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Abstract—A series of novel 4-(*N*-acyl)-2,3-dihydro-1*H*-isoindol-1-ones have been prepared from methyl-3-nitro-2-methylbenzoate and linked through various spacers to the adenosine derivatives **11** and **12**. We found that potent inhibition of poly(ADP-ribose)-polymerase-1 (PARP-1) was achieved when isoindolinone was linked to adenosine by a spacer group of a specific length. Introduction of piperazine and succinyl linkers between the isoindolinone and adenosine core structures resulted in highly potent compounds **8a** and **10b**, which showed IC_{50} values of 45 and 100 nM, respectively.

1. Introduction

Poly(ADP-ribose) polymerase-1 (PARP-1, EC 2.4.2.30) also known as poly(ADP-ribose) synthetase (PARS) and poly(ADP-ribose) transferase (PADPRT) is a nuclear enzyme present in eukaryotes. PARP-1 is the principal member of a family of poly(ADP-ribosyl)ating enzymes and functions both as a DNA damage sensor and as a signalling molecule. Upon binding to damaged DNA, PARP-1 forms homodimers and catalyzes the cleavage of NAD^+ into nicotinamide and ADP-ribose. The latter is used to synthesize branched nucleic acid-like polymers with poly(ADP-ribose) units covalently attached to nuclear acceptor proteins including histones, transcription factors and PARP. The poly(ADP-ribosyl)-ation of these proteins contributes to inflammatory signal transduction processes. In addition, oxidative stress-induced over-activation of PARP consumes NAD^+ and, consequently, ATP culminating in cell dysfunction or necrosis. Activation of PARP has been implicated in the pathogenesis of stroke, myocardial ischemia, diabetes, diabetes-associated cardiovascular dysfunction, shock, traumatic central nervous system injury, arthritis, colitis, allergic encephalomyelitis and various other forms of inflammation.[1] Therefore, inhibition of PARP by pharmacological agents may prove useful for the treatment of these diseases.

Various compounds that incorporate a carboxamide group in an *anti*- (or *cis*-) configuration, within a ring structure (i.e., lactams) were reported[2] as considerably more effective at inhibiting PARP than a prototypical inhibitor, 3-aminobenzamide (3-AB). Of these, the substituted 3,4-dihydroisoquinolin-1(2*H*)-ones and isoquinolin-1(2*H*)-ones, were investigated as potential enhancers of radiotherapeutic or chemotherapeutic agents.[2b,3] The initial impetus for the earlier work was the concept that inhibitors of PARP would enhance the cytotoxic effects in the treatment of cancer.[4] It was shown that high doses of the compounds were required for these effects, suggesting that a near-complete inhibition of PARP-1 was necessary. Thus, the development of potent PARP inhibitors for this therapeutic area remains a valid concept.

An original study by Banasik[2a] compared a large number of compounds from a variety of structural classes for their ability to inhibit PARP. The most potent compounds contain a lactam ring as a part of their cyclic systems. These findings formed the basis for many of the polycyclic PARP inhibitors subsequently developed. Recently,[5] the growing realization that PARP may be involved in the pathogenesis of many diseases led to the development of numerous types of novel PARP inhibitors.[5a] Several classes of PARP inhibitors originally described by Banasik[2a] were later optimized in order to enhance potency, improve pharmacokinetic characteristics and increase solubility in water. Recently, Costan-

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tino et al.[5b] reported a quantitative structure–activity relationship (QSAR) analysis of several PARP inhibitors.

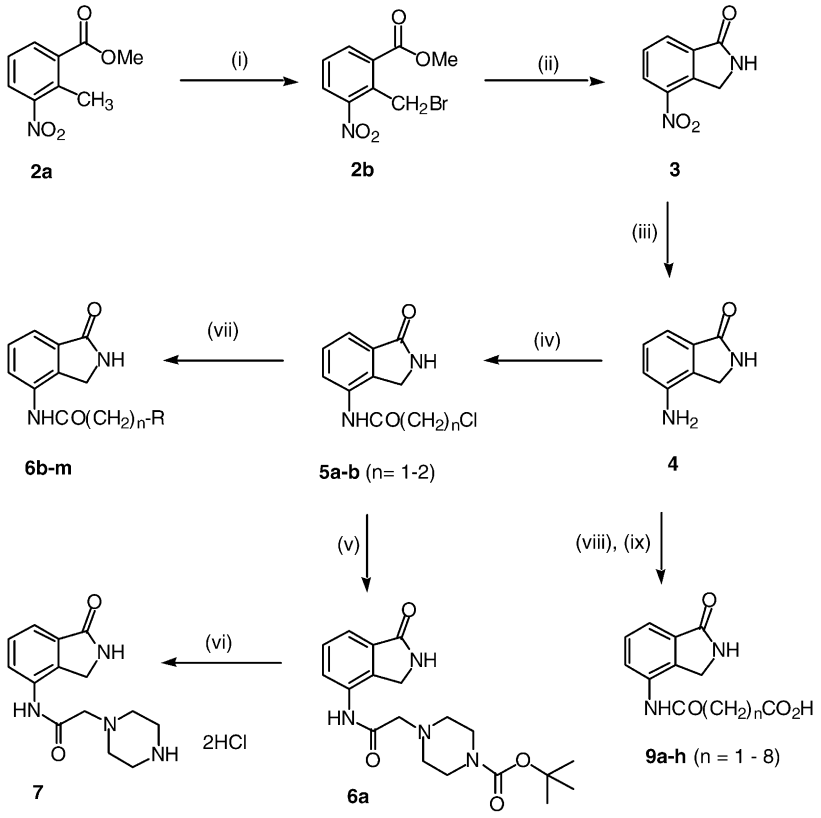
While much emphasis has been placed on extending the ring systems to include tri-, tetra- and penta-cyclic systems with all the inherent problems or concerned associated with this,[6] we tried to improve the binding characteristics of modest inhibitors by coupling them to adenosine, a structural element of NAD, which is recognized by the NAD binding site of PARP. Adenosine by itself is only a weak inhibitor of PARP activity, but we postulated that appropriately substituted adenosine derivatives might show good PARP inhibitory activity. To expand on this hypothesis, we prepared a series of 4-substituted isoindolinones and linked them through various spacers to adenosine. We report herein, that potent inhibition of PARP was achieved with the selection of appropriate linkers comprising cyclic or alkyl chains.

2. Results and discussion

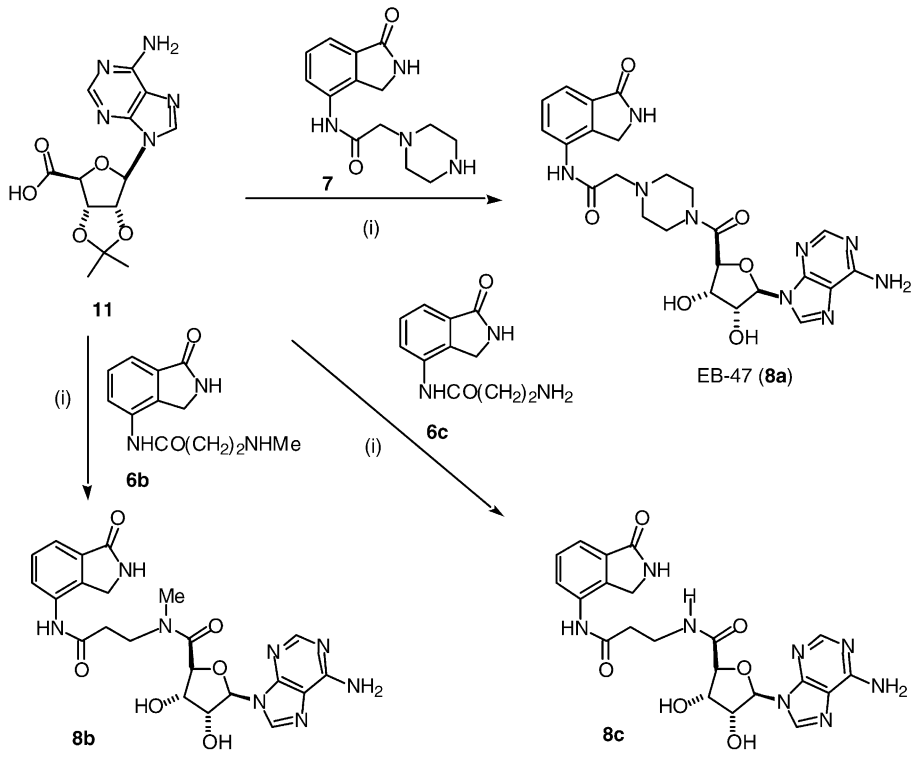
The synthetic routes to prepare the adenosine derivatives **8a–c** and **10a–h**, with various combinations of the side chains at the isoindolinone C-4 position, were designed to use acid and base coupling reactions (Schemes 1–3). These couplings were accomplished by introducing acid or amine functional groups at the 5′-position of 2′,3′-isopropylidene protected adenosine derivatives (**11** and **12**)[7] and coupling them with 4-substituted-isoindolinones carrying the appropriate linkers bearing an amine or acid group.

A refluxing solution of commercially available ester **2a** and *N*-bromosuccinimide (NBS) in CH_2Cl_2 was treated with 2,2′-azobisisobutyronitrile (AIBN) to give bromo ester **2b** in excellent yield, which upon treatment with a solution of ammonia in methanol at room temperature furnished 4 - nitro - 2,3 - dihydro - 1*H* - isoindolinone **3** (Scheme 1). The nitro compound **3** was reduced to the intermediate amino compound **4** using ammonium formate and palladium on carbon (10%). Reaction of **4** with various acid chlorides in an aqueous solution of sodium bicarbonate and ethyl acetate (Schotten Baumann reaction) provided **5a–b.** Compounds **9a–h** were prepared from isoindolinone **4** using a similar acylation reaction approach, followed by hydrolysis of the corresponding esters. Reaction of **5a** with 4-′BOC-piperazine in methanol at 50 °C provided **6a** in 59% yield. Isoindolinone **7** was then prepared by treating **6a** with HCl (4.0 M solution in dioxane) at 0 °C to room temperature in 98% yield. Similarly, reaction of **5a–b** with various amines in methanol provided compounds **6b–m** in good yields.

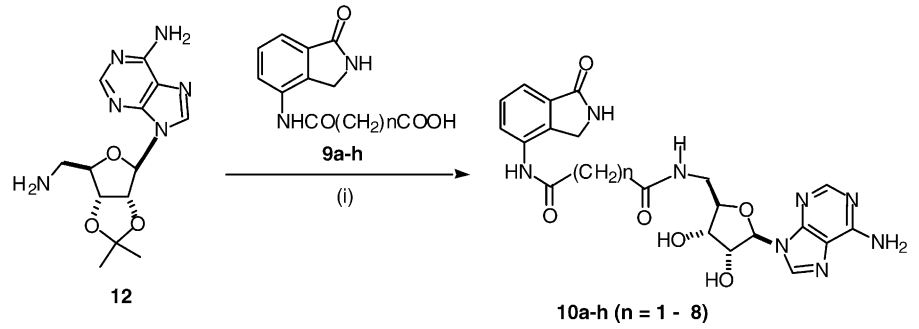
The amines **6b–c** and **7** were subsequently treated with 2′,3′-isopropylideneadenosine-5′-carboxylic acid **11**, as shown in Scheme 2. This was followed by deprotection of the adenosine-coupled product to give compounds **8a–c**.



Scheme 1. Reagents and conditions: (i) NBS, AIBN, methylene chloride, reflux, 16 h, 98%; (ii) ammonia–MeOH (7 N), rt, 2 h, 81%; (iii) ammonium formate, Pd/C (10%), DMF, 100 °C, 30 min, 83%; (iv) $ClCO(CH_2)_nCl$, $NaHCO_3$, EtOAc, rt, 30 min, 80–85%; (v) 4-*N*-′BOC-piperazine, MeOH, 50 °C, 48 h, 59%; (vi) HCl–dioxane, MeOH, 0 °C to rt, 16 h, 98%; (vii) amine, MeOH, rt, 2–24 h; (viii) $ClCO(CH_2)_nCOOMe$, $NaHCO_3$, EtOAc, rt, 30 min; (ix) NaOH, MeOH, rt.



Scheme 2. Reagents and conditions: (i) DMF–methylene chloride, diisopropylethylamine, EDAC, rt, 24 h; then acid, water, rt, 1–2 h, 49–55%.



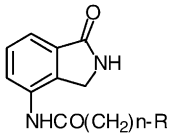
Scheme 3. Reagents and conditions: (i) DMF–methylene chloride, diisopropylethylamine, EDAC, rt, 24 h; then acid, water, rt, 1–2 h.

The synthetic routes for preparing compounds **10a–h** were similar except that 2′,3′-isopropylideneadenosine-5′-methyleneamine (**12**) was used for the coupling reaction (Scheme 3). The products obtained after this reaction were treated with TFA and water as described above to yield **10a–h**, which contain aliphatic chains ranging from 1 to 8 methylene units between the adenosine and the isoindolinone.

2,3-Dihydro-1*H*-isoindol-1-ones incorporate the requisite lactam in a five-membered ring. Polycycles containing the isoindolinone structure have been reported to be potent PARP inhibitors.[8] However, isoindolinone **1** showed only 33% inhibition at 10 μM in a cell-based assay.[9] The simple 4-substituted isoindolinones also had poor potency (IC_{50} >10 μM) in this assay, but more elaborate side chains showed improved potency. The 4-*N*-substituted isoindolinones have weak to moderate potency against the isolated enzyme and offered only moderate protection against cell injury in the peroxynitrite toxicity assay. The various substituents at the 4-position had only minor effects activity. However, significant increases in potency were achieved when benzimidazole (**6l**) and N^6-dimethyladenine (**6m**) were introduced at the 4-position as shown in Tables 1 and 2, respectively. These compounds (**6l** and **6m**) showed a 10-fold increase in activity.

When adenosine is incorporated into the 4-position of isoindolinone using various spacers, the nature of the linker between the isoindolinone core and the adenosine had a large impact on efficacy. As shown in Schemes 2 and 3, adenosine derivatives **8b–c** and **10a–h** with various chain length [$(CH_2)_n$] (n = 1–8) were prepared. The piperazine and phenyl containing adenosine derivatives **8a** and **13** (Fig. 1) represent derivative with cyclic linkers. The potency was best for the succinyl and propanoyl linked compounds **10b** and **8b** (IC_{50} = 0.1 and 0.25 μM, respectively). Compound **8c**, which contains a secondary amide was less active than the *N*-methyl amide

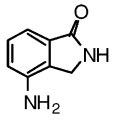
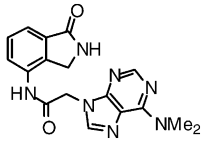
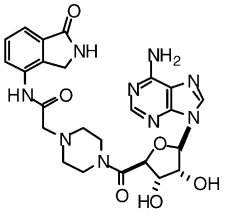
Table 1. PARP-1 Inhibition data of 4-substituted-2,3-dihydro-1*H*-isoindol-1-ones



Compd	*n*	R	IC_{50}, μM[a]
6d	1	–NHMe	> 30
6e	1	–NHEt	18
6f	7	–NHMe	30
6g	1	$-NEt_2$	30
6h	1	–4-*N*-Me-piperazine	> 30
6i	2	–4-*N*-Me-piperazine	> 30
6j	1	–Morpholine	> 30
6k	1	–1,2,3,4-Tetrahydroisoquinoline	30
6l	1	–Benzimidazole	3
7	1	–Piperazine·2HCl	18
8b	2	NMe-5′-CO-adenosine	0.25
8c	2	NH-5′-CO-adenosine	0.7
10a	1	CO-5′-NH-CH_2-adenosine	1.9
10b	2	CO-5′-NH-CH_2-adenosine	0.1
10c	3	CO-5′-NH-CH_2-adenosine	0.45
10d	4	CO-5′-NH-CH_2-adenosine	0.9
10e	5	CO-5′-NH-CH_2-adenosine	3
10f	6	CO-5′-NH-CH_2-adenosine	0.7
10g	7	CO-5′-NH-CH_2-adenosine	2
10h	8	CO-5′-NH-CH_2-adenosine	3

[a] IC_{50} values have been determined by using a commercially available in vitro PARP-1 inhibition assay kit (Trevigen, Gaithersburg, MD, USA) following manufacturer's recommendations.

Table 2. PARP-1 Inhibition data of isoindolinones **4**, **6m** and **8a**

Compd	R	IC_{50}, μM[a]
4		30
6m		3
EB-47 (**8a**)		0.045

[a] See Table 1.

derivative **8b**. Compounds **10e–h**, showed deleterious effects on activity. Compound **13**, with a styrene group incorporated into the linker, had reduced potency. However, the piperazine analogue EB-47 (**8a**) showed 100% inhibition at 200 nM and exhibited the lowest IC_{50} (0.045 μM). The inosine-linked compound **14** (Fig. 1) was 10-fold less potent than the adenosine analogue **10f**.

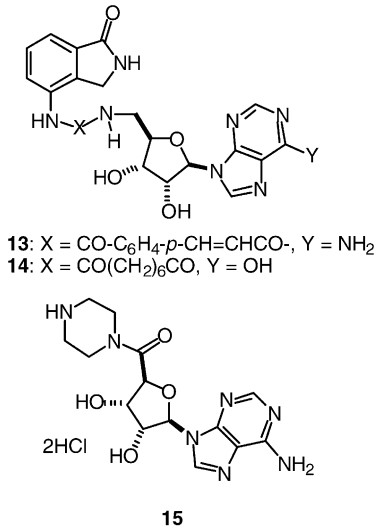
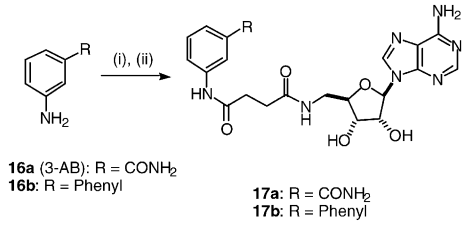


Figure 1.



Scheme 4. Reagents and conditions: (i) succinic anhydride, chloroform, reflux, 2.5 h; (ii) DMF–CH_2Cl_2, diisopropylethylamine, EDAC, **12**, rt, 24 h; then TFA, water, rt, 1–2 h.

Table 3. PARP-1 Inhibition data of compounds **1** and **13–18**

Compd	% PARP-1 inhibition[9] at 10 μM	IC_{50}, μM[a]
1	33	> 30
13	51	10
14	68	30
15	n.d. at 10 μM	> 300
16a	28	n.d.
17a	52	75
17b	5	> 300

[a] See Table 1. n.d., not determined.

3-Aminobenzamide (**16a**) is a commercially available PARP inhibitor that has an IC_{50} in the range of 30 μM in the isolated PARP-1 enzyme assay.[10] To see the effect of an adenosine linked 3-aminobenzamide analogue on PARP-1 inhibition, a succinyl linker was coupled to 3-AB through the 3-position to adenosine as shown in Scheme 4. In the cell based assay,[9] 3-AB (**16a**) showed 28% inhibition of PARP-1 at 10 μM, whereas adenosine linked 3-AB derivative **17a** showed 52% inhibition at 10 μM (Table 3). In the same assays, the 5′-piperazine amide **15**, adenosine, inosine and the biphenyl derivative **17b** had only weak effects on PARP activity.

The difference in potency between the weakly active compounds **1** and **6d–m**, and the potent compounds **10f** (98% inhibition at 1 μM) and its inosine analogue (**14**, Y = OH, *n* = 6; 68% inhibition at 10 μM), suggests that the adenosine is recognized by a pocket within the active

site, as may be expected to exist for the binding of NAD^+. Rolli and colleagues[11] postulated that two hydrogen-bonding interactions between the adenosine of NAD^+ and the NAD^+ binding site of PARP in their recent report. These are via the primary amino group and the adjacent ring nitrogen (N^1) of adenosine. This is consistent with the weak PARP activity of adenosine and the weak activity of the other nucleosides hypoxanthine and inosine, which lack the 6-amino functionality.[12]

3. Conclusion

A series of novel adenosine-linked analogues of isoindolinone have been synthesized and evaluated in an in vitro PARP-1 inhibition assays. The cyclic derivative of 3-aminobenzamide **4**, and many smaller 4-substituted isoindolinones (**6d–k**) demonstrated weak PARP-1 inhibition. Introducing benzimidazole and N^6-dimethyladenine to the isoindolinone core resulted in a 10-fold increase in potency. However, adenosine linked via succinyl and propanyl spacers (**10b** and **8b**) yielded highly potent compounds. In addition, the piperazine linked adenosine analogue **8a** exhibited the greatest potency with an IC_{50} value of 45 nM, which is 650-fold more potent than the parent isoindolinone core.[13] The current work illustrates the synergism of having two discreet binding functions on the same molecule.

Acknowledgements

This work was supported by grants from the National Institutes of Health (R44DK054099 and R44GM058986).

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6. (a) An inherent problem for the plainer heteroaromatic compounds is poor solubility in water and other routinely used organic solvents. Another concern for plainer compounds is the potential for mutagenic and/or carcinogenic activity as seen for 2-acetamidocarbazole, 2-acylaminofluorine, 5-fluoroquinoline and dibenz[*a*,*j*] acridine. Warshawsky, D.; talaska, G.; Xue, W.; Schneider, J. *Crit. Rev. Toxicol.* **1996**, *26*, 213. (b) Heflich, R. H.; Neft, R. E. *Mutat. Res.* **1994**, *318*, 73.
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9. Cell protection assay: Raw murine macrophages were treated with test compounds for 15 min prior to the addition of peroxynitrite (750 μM) for a further 15 min. For the measurement of PARP activity, the media was removed and replaced with 0.5 mL HEPES (pH 7.5) containing 0.01% digitonin and ^{3}H-NAD (0.5 μCi mL^{-1}, final concentration of NAD^+ in buffer is 20 nM/L) for 20 min. The cells were then scraped from the wells and placed in Eppendorf tubes containing 200 μL of 50% (w/v) ice-cold trichloroacetic acid (TCA). The tubes were then placed at 4 °C. After 4 h, the tubes were centrifuged at 1800*g* for 10 min and the supernatant removed. The pellets were washed twice with 500 μL ice-cold 5% TCA. The pellets were solubilized in 250 μL NaOH (0.1 M) containing 2% SDS overnight at 37 °C and the PARP activity was then determined by measuring the radioactivity incorporated using a Wallac scintillation counter. The solubilized protein (250 μL) was mixed with 5 mL of scintillant (ScintiSafe Plus, Fisher Scientific) before being counted for 10 min. The compounds described as inhibitors of PARP-1 are capable of permeating whole cells, a property necessary for therapeutic efficacy.
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13. Compound **8a** (EB-47) was used for in vivo experimentation. This piperazine linked adenosine derivative exerts cytoprotective effects in oxidatively damaged cells, and shows protection in in vivo models of reperfusion injury and inflammation. The in vivo experimental results will be published elsewhere.