Tricyclic Benzimidazoles as Potent Poly(ADP-ribose) Polymerase-1 Inhibitors

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Abstract: Novel tricyclic benzimidazole carboxamide poly(ADP-ribose) polymerase-1 (PARP-1) inhibitors have been synthesized. Several compounds were found to be powerful chemopotentiators of temozolomide and topotecan in both A549 and LoVo cell lines. In vitro inhibition of PARP-1 was confirmed by direct measurement of NAD^+ depletion and ADP-ribose polymer formation caused by chemically induced DNA damage.

Introduction. Poly(ADP-ribose) polymerase-1 (PARP-1; EC 2.4.2.30) is a constitutively expressed nuclear protein that is a critical component of the cellular response to DNA damage.[1] The enzymatic activity of PARP-1 is significantly increased upon binding to DNA single-strand breaks. Once activated, PARP-1 rapidly synthesizes long, branched polymers of ADP-ribose from nicotinamide adenine dinucleotide (NAD^+), which are covalently linked to protein acceptors through surface-accessible glutamate residues. The primary protein acceptor is PARP-1 itself, although other nuclear proteins are also modified. Automodification of PARP-1 results in a decrease in affinity for damaged DNA, facilitating dissociation and allowing repair enzymes access to the strand break.

Repair of DNA damage induced by genotoxic agents during cancer therapy is an important mechanism of resistance. There is considerable evidence suggesting that PARP-1 plays a crucial role in base excision repair and that inhibitors of PARP-1 can act as resistance-modifying agents for radiation and certain chemotherapies.[2] PARP-1 knockout mice,[3] while a viable phenotype, have been shown to be hypersensitive to monofunctional alkylating agents. It is proposed that inhibition of PARP-1 will interfere with automodification, preventing the electrostatic repulsion of PARP-1 from the DNA and thus ultimately interfering with DNA repair.

Early PARP-1 inhibitors were analogues of 3-aminobenzamide[4] (**1**, Figure 1). Unfortunately these compounds lacked specificity and potency. Subsequent inhibitor designs sought to lock the carboxamide in the favored conformation through intramolecular hydrogen bonds (**4**, NU1085) or by incorporation into a ring system (**2**, PD128763;[5] **3**, NU1025). While inhibitors **3** and **4** displayed significant in vitro affinity for PARP-1, they still required concentrations of 10−100 μM for chemopotentiation. Nevertheless, both compounds increased the cytotoxicity of DNA damaging agents in a range of cell lines.[6]

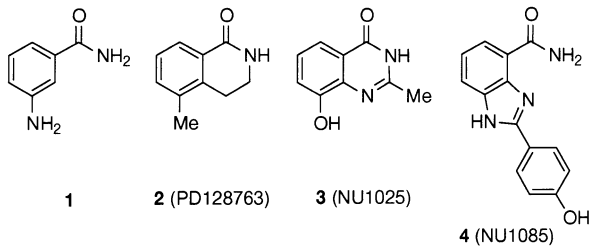

Figure 1. Published PARP-1 inhibitors.

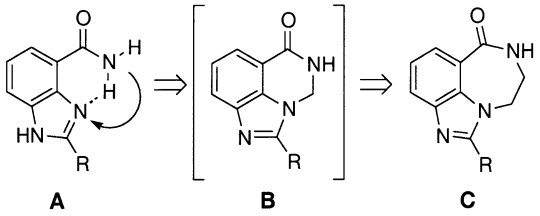

Figure 2. Conversion of benzimidazole carboxamide derivatives to tricyclic analogues.

Utilizing structure-based design, we attempted to combine the desired traits of amide-restricted analogues **2** and **3** with the potency of the 1*H*-benzimidazole-4-carboxamides (i.e., **4**). Figure 2 conceptualizes our design proposal. We designed related cyclic analogues starting from the previously solved cocrystal X-ray structure of 2-(3-methoxyphenyl)-1*H*-benzimidazole-4-carboxamide (**A**; R = 3-MeOPh− [NU1098])[7] bound to the active site of chicken PARP-1. The hypothetical cyclic compound **B** was first considered. It was rejected in favor of tricycle **C** because of possible synthetic difficulties and potential problems with stability and physical properties. Molecular modeling and docking studies suggested the 5,6,7 ring system of **C** would both fit in the active site and form the necessary hydrogen bond network. Furthermore, the tricyclic ring system of **C** was thought to be a stable and synthetically accessible target.

Chemistry. Bicyclic nitroanthranilamide **9** was the first intermediate needed to prepare the desired tricyclic benzimidazoles. Our initial procedure to prepare this compound is outlined in Scheme 1. Acrylonitrile was added to 2-nitroaniline (**5**), via Michael addition, to give propionitrile derivative **6** in 79% yield.[8] Base-catalyzed hydrolysis of the cyano group and subsequent cyclization of the carboxylic acid, using Eaton's reagent, provided intermediate **8**[9] in 90% yield over two steps. Introduction of the requisite amide functionality was achieved by means of a Schmidt reaction. This furnished compound **9** in 86% yield. Although this sequence is quite

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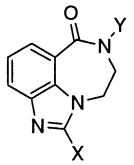
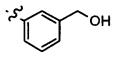
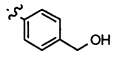
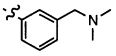
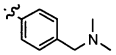
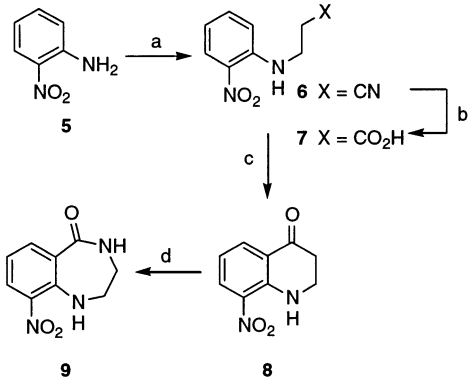
Table 1. Synthesis, Enzymatic and Cellular Activity of Tricyclic Benzimidazole PARP-1 Inhibitors



Compound	X =	Y =	Method[a]	Yield[b] (%)	K_i (nM)[c]	A549 PF_{50} (TM)[d]	A549 PF_{50} (TP)[d]
12	Me	H	B	67	45	1.1	1.2
13	Ph	H	B	72	4.1	ND[e]	1.5
14	4-Cl-Ph	H	A	47	5.7	1.5	2.0
15	2-Cl-Ph	H	A	50	7.7	ND[e]	1.3
16	3-CF_3-Ph	H	A	53	11.2	ND[e]	1.7
17	1-naphthyl	H	A	53	4.9	ND[e]	1.4
18	1-naphthyl	Me	N/A[f]	95[f]	40% Inh[g]	*1.1*[h]	ND[e]
19		H	B	52[i]	8.8	*1.2*[h]	ND[e]
20		H	B	77[j]	4.2	*1.5*[h]	ND[e]
21		H	B	N/A	6.3	1.4	1.8
22		H	B	N/A	5.8	2.2	2.0

[a] See Scheme 3 and text. Details in Supporting Information. [b] Yield of isolated material from cyclization reaction. [c] Assay against human full-length PARP-1. Details in Supporting Information. [d] PF_{50} is defined as the GI_{50} of TM (or TP) alone ÷ GI_{50} of TM (or TP) + 0.4 μM PARP inhibitor. Data are the average from two independent experiments. GI_{50} is defined as the concentration of cytotoxic compound that gives 50% growth inhibition. See ref 7 and Supporting Information. [e] ND = not determined. [f] Compound **18** was prepared by methylation of **17**. Yield of isolated product. Details in Supporting Information. [g] Percent inhibition at 100 μM. [h] Values in italics are measured as EF_{200}, a single point variation of PF_{50}. EF_{200} is defined as the percent growth inhibition at 200 μM TM alone ÷ the percent growth inhibition at 200 μM TM + 0.4 μM PARP inhibitor. [i] Isolated yield of cyclization product from reaction of diamine **11** (Scheme 2) and 3-[1,3]dioxolan-2-ylbenzaldehyde. Details in Supporting Information. [j] Isolated yield of cyclization product **24** (Scheme 4).

Scheme 1[a]

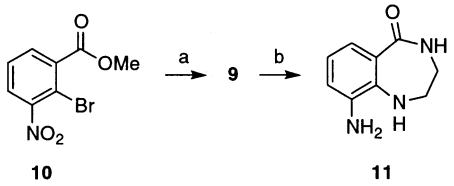


[a] Reagents: (a) CH_2=CHCN, Triton B, 1,4-dioxane; (b) NaOH(aq), MeOH, reflux; (c) P_2O_5, $MeSO_3H$, 70−80 °C; (d) NaN_3, $MeSO_3H$.

efficient, there are problems controlling the regiochemistry in the final step. In addition to the desired product, the regioisomeric amide was also formed in ~10% yield (structure not shown). This unavoidable side product necessitated tedious column chromatography and thus was not amenable to scale-up.

Starting from the known methyl 2-bromo-3-nitrobenzoate (**10**),[10] an alternative shorter route was developed

Scheme 2[a]

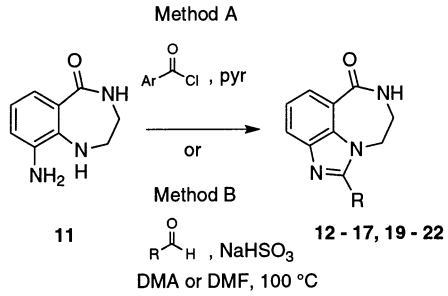


[a] Reagents: (a) ethylenediamine, DMA, 100 °C; (b) H_2, 10% Pd/C, EtOAc/AcOH.

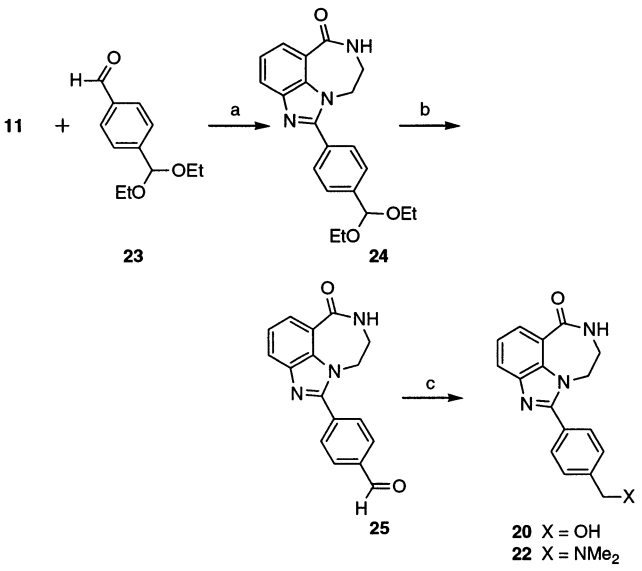
(Scheme 2). Reaction of the aryl bromide with ethylenediamine gave **9** in a single step with no regioisomeric issues (89% yield). The nitro group was efficiently converted to the corresponding dianiline **11** by hydrogenation in 87% yield.

The next step in the synthesis involved formation of the tricycle benzimidazole ring system. This was accomplished by one of the two methods outlined in Scheme 3. Diamine **11** was reacted with either an aryl acid chloride in pyridine (method A)[7,11] or with an aldehyde and sodium bisulfite at elevated temperatures (method B).[12] While both procedures proved to be efficient, method B was more practical and tolerant of a more diverse set of starting materials, including aliphatic aldehydes.

Scheme 3



Scheme 4[a]



[a] Reagents: (a) $NaHSO_3$, DMA, 100 °C; (b) H_2SO_4, EtOH/H_2O, reflux; (c) Me_2NH, $NaBH_3CN$, $ZnCl_2$, HCl, MeOH.

The products of these reactions are outlined in Table 1. For examples **12**−**17**, the desired compounds were isolated directly from the coupling reaction. However, for examples **19**−**22**, further elaboration of the 2-position substituent was necessary. Scheme 4 shows a representative procedure for the preparation of *N*,*N*-dimethylbenzylamine **22**. The diamine **11** and terephthalaldehyde mono(diethyl acetal) (**23**) were coupled, via method B, to give tricyclic intermediate **24** in 77% yield. The acetal was then hydrolyzed to the corresponding aldehyde **25** in 75% yield. Reductive amination with dimethylamine gave the desired compound **22** and the byproduct alcohol **20** in 71% and 19% yield, respectively. In a similar manner, the meta-substituted analogues **19** and **21** were prepared from 3-[1,3]dioxolan-2-ylbenzaldehyde.[13]

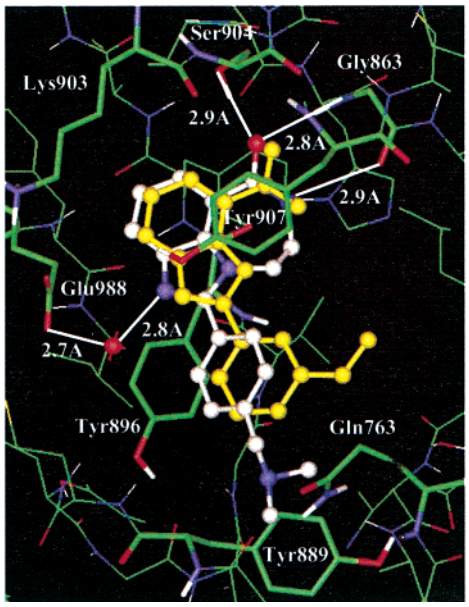


Figure 3. Overlay of compound **22** and NU1098 bound to the PARP-1 active site. Compound **22** is white with heteroatoms being red for oxygen and blue for nitrogen. NU1098 is shown in yellow. The carboxamide hydrogen bond network is indicated with white lines; the heteroatom-to-heteroatom distances are defined.

Results and Discussion. The design concept outlined in Figure 2 was validated by X-ray crystallographic analysis. Figure 3 shows the overlay of compound **22** and NU1098 (vida infra) bound to the PARP-1 active site. Despite the addition of a two-atom tether, compound **22** exhibits the same binding geometry and H-bonding motif as seen in the nontricyclic analogues. The carboxamide makes the same three critical interactions with Ser904 and Gly863. The disposition of the benzimidazole cores and the 2-position aryl rings is essentially identical.

The SAR for the tricyclic benzimidazoles is outlined in Table 1. As seen in a previous study of 1*H*- benzimidazole-4-carboxamides,[7] alkyl substituents at the 2 position have only modest activity against the enzyme (**12**; K_i = 45 nM). Unsubstituted and monosubstituted aryl rings are well tolerated, having inhibition constants ranging from 4.1 to 11.2 nM.

Larger ring systems, such as a 1-naphthyl group, are also easily accommodated (**17**; K_i = 4.9 nM). Elimination of the critical carboxamide hydrogen, as in example **18**, leads to a complete loss of binding affinity.

Good potentiation of cytotoxicity is vital if these compounds are to function as therapeutically useful resistance-modifying agents. Despite potent inhibition of the enzyme in vitro, the PARP-1 inhibitors prepared

Table 2. Effects of PARP-1 Inhibitor **22** on NAD^+ Depletion and ADP-ribose Polymer Formation Following Treatment of A549 Cells with 25 μM *N*-methyl-*N*′-nitro-*N*-nitrosoguanidine (MNNG)

	vehicle control	25 μM MNNG	concentration of **22** with 25 μM MNNG				
			11 μM	0.4 μM	0.15 μM	0.03 μM	0.006 μM
NAD^+ (% remaining versus vehicle control)[a]	100[b]	30 ± 3.5	99 ± 2.8	88 ± 0.7	85 ± 2.8	75 ± 4.2	65 ± 5.7
polymer (% formation with 25 μM MNNG)[c]	3 ± 1.4	100[d]	5.5 ± 0.7	13 ± 4.2	20 ± 6.4	38 ± 12	85 ± 20.5
PF_{50} (TP)				2.3	2.1	1.9	1.6

[a] NAD^+ concentration measured by enzyme recycling method. See ref 15. [b] Normalized amount of NAD^+ for cells without DNA damage. [c] Polymer levels measured by HPLC. See ref 16. [d] Normalized amount of ADP-ribose polymer formation in cells following DNA damage.

Table 3. Summary of Potentiation of Temozolomide (TM) and Topotecan (TP) in LoVo Cell Line by 0.40 μM PARP-1 Inhibitor

	LoVo		
compound	PF_{50} (TM)[a]	PF_{50} (TP)[a]	GI_{50} (μM)[b]
14	2.1	1.5	7.9
21	2.0	1.5	>50
22	5.4	1.7	11.2

[a] See Table 1 and ref 7. [b] GI_{50} of PARP-1 inhibitor alone against LoVo cells.

in this study exhibited a broad array of cellular activity, as measured by the potentiation factor PF_{50}[7,14] (Table 1). Compounds **14** and **22** showed the largest amplification of the cytotoxic effects of temozolomide (TM) and topotecan (TP) against A549 cells (human lung carcinoma). In contrast, the regioisomers of **14** and **22**, **15** and **21**, respectively, contain similar aryl groups and have comparable K_i's yet exhibit lower cellular efficacy.

To confirm inhibition of PARP-1 in intact cells, we measured the NAD^+ [15] and polymer levels[16] within A549 cells. The cultures were initially treated with vehicle, as control, to determine background levels. Subsequently, a set of cells were treated with the monofunctional DNA alkylating agent *N*-methyl-N′-nitro-*N*-nitrosoguanidine (MNNG) at subtoxic concentrations. This gave control levels for NAD^+ depletion and ADP-ribose polymer formation resulting from DNA damage. Finally, cells were treated with MNNG and **22** at various concentrations. Table 2 shows the dose-dependent ability of the inhibitor to suppress depletion of NAD^+ and to inhibit polymer synthesis caused by chromosomal damage. The compound **22** imparts a significant effect even at 30 nM. Concurrent measurement of PF_{50} with TP shows an analogous trend.

PARP inhibitors **14**, **21**, and **22** were also tested against the LoVo cell line (colon cancer). All three showed good levels of activity, with **22** again being the most active (Table 3). These levels of potentiation were obtained at 0.4 μM PARP inhibitor, which is well below the cytotoxic level of the PARP-1 inhibitors alone (see GI_{50}'s in Table 3). By comparison, NU1085 (**4**) required a concentration of 10 μM to achieve similar levels of efficacy.[7] This represents a ~25-fold increase in chemopotentiation efficacy when compared to NU1085.

In summary, we have demonstrated a new and potent class of PARP-1 inhibitors whose mode of action can be directly linked to in vitro enzyme inhibition. Additional preclinical studies will be reported in due course.

Acknowledgment. We thank Suzanne Kyle and Lan-Zhen Wang for their technical assistance in running potentiation assays.

Supporting Information Available: Description of the inhibition, potentiation, NAD^+ depletion, and polymer formation assays, experiment detail and characterization for compounds **6**−**25**, and X-ray data for compound **22**. This material is available free of charge via the Internet at http://pubs.acs.org.

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