Discovery of Potent Poly(ADP-ribose) Polymerase-1 Inhibitors from the Modification of Indeno[1,2-*c*]isoquinolinone

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Abstract: Novel indeno[1,2-*c*]isoquinolinone derivatives were synthesized and evaluated as inhibitors of the nuclear enzyme poly(ADP-ribose) polymerase-1 (PARP-1). These potent non-mutagenic PARP-1 inhibitors possess an additional five-membered ring between the B and C rings of 6(5*H*)-phenanthridinone. The most potent PARP-1 inhibitors were obtained from the substitution of the D ring at the C-9 position, in particular sulfonamide and *N*-acyl analogues (**6** and **11**). The 9-sulfonamide analogues **11a** and **12a** exhibited IC_{50} values of 1 and 10 nM, respectively.

Poly(ADP-ribose) polymerase (PARP) activation plays a role in the pathogenesis of various cardiovascular and inflammatory diseases.[1] At the same time, PARP activation is also relevant for the ability of cells to repair injured DNA. Thus, depending on the circumstances, pharmacological inhibitors of PARP may be able to attenuate ischemic and inflammatory cell and organ injury or may be able to enhance the cytotoxicity of antitumor agents. Both aspects of the "double-edged sword" role of PARP can be exploited for the experimental therapy of many diseases (overviewed in ref 1). Recent articles[2] suggest a new mechanism-based approach for the treatment of patients with BRCA2-associated cancers with PARP inhibitors.

Extensive investigations have been conducted in the identification of novel PARP-1 inhibitors.[2] Various approaches to design the scaffolds for PARP inhibitors based on the prototypical PARP inhibitor 3-aminobenzamide have been developed.[2] Structure−activity relationship studies have provided insight into the structural requirements that are important for the inhibition of PARP. One of the commonly used techniques is to lock the amide group of benzamide in the anti conformation. With this tool, several bi-, tri-, and tetracyclic lactam scaffolds were investigated and modified to achieve potent PARP-1 inhibitory activity.[2,3] Previous reports[4,5] showed that the modifications at the C-2 position of 6(5*H*)-phenanthridinone are well-tolerated (Figure 1). Introduction of appropriate side chains, which carry a terminal amino group, at the C-2 position showed improved PARP-1 inhibition compared to the unsubstituted 6(5*H*)-phenanthridinone. Because of its structural similarity to the prototypical PARP inhibitors[6] 6(5*H*)-phenanthridinone and isoquinolinone, indeno[1,2-*c*]isoquinolinone was chosen as a scaffold for further chemical modifications (Figure 1). In addition and in contrast to the 6(5*H*)-phenanthridinones, the methylene group of the five-membered C ring of indeno[1,2-*c*]isoquinolinone disrupts the planarity of the basic structure so that it is not a DNA intercalator.[7]

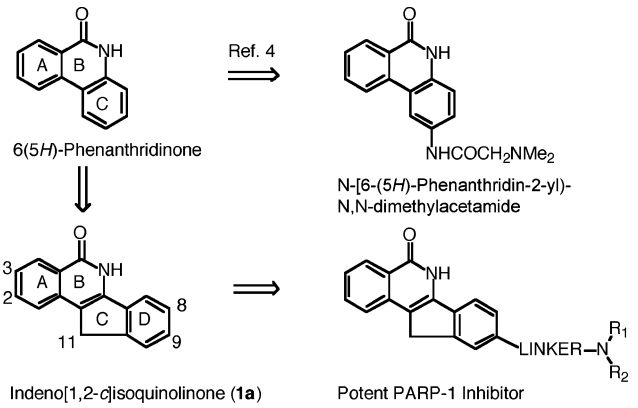
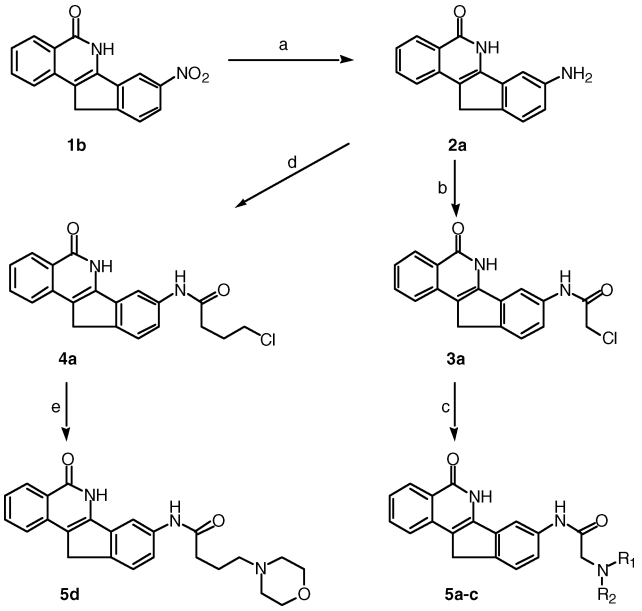


Figure 1. Design of novel PARP-1 inhibitors.

Scheme 1. Synthesis of C-8 Substituted Indeno[1,2-*c*]isoquinolinones[a]



[a] (a) Ammonium formate, Pd−C (10%), DMF, heat, 95%; (b) $ClCOCH_2Cl$, saturated $NaHCO_3$, EtOAc, DMF, 67%; (c) HNR_1R_2, EtOH or DMF, **5a** 75%, **5b** 66%, **5c** 67%; (d) $ClCO(CH_2)_3Cl$, saturated $NaHCO_3$, EtOAc, DMF, 97%; (e) morpholine, DMF, Et_3N, 82%.

Indeno[1,2-*c*]isoquinolinones have been known for topoisomerase-I inhibitory activity.[8] Cushman et al.[7,8] have synthesized a series of 11-keto derivatives of indeno[1,2-*c*]isoquinolinone starting from homophthalic anhydride and benz[*d*]indeno[1,2-*b*]pyran-5,11-dione. The starting compounds **1a**−**e** required in this study were obtained as reported elsewhere.[9] The synthetic routes for the newly synthesized C-8 and C-9 substituted indeno[1,2-*c*]isoquinolinone analogues are outlined in Schemes 1 and 2.

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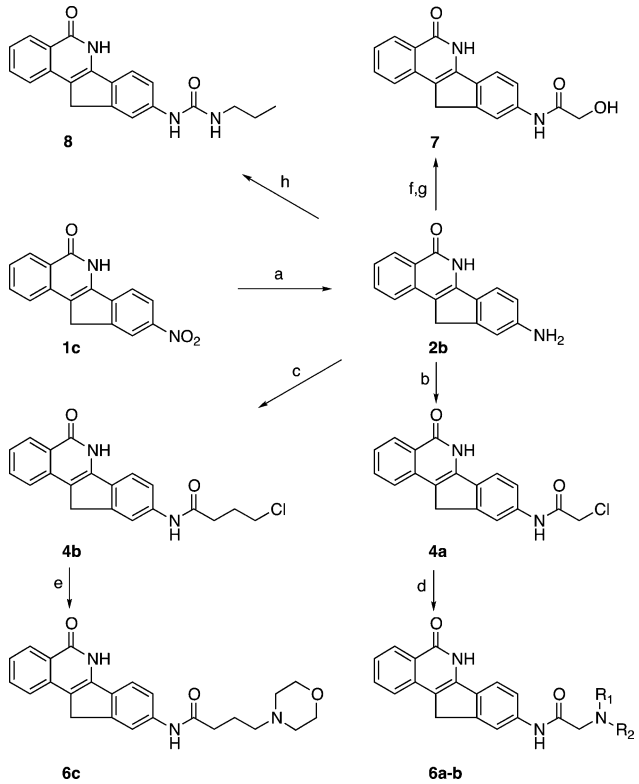
Scheme 2. Synthesis of C-9 Substituted Indeno[1,2-*c*]isoquinolinones[a]

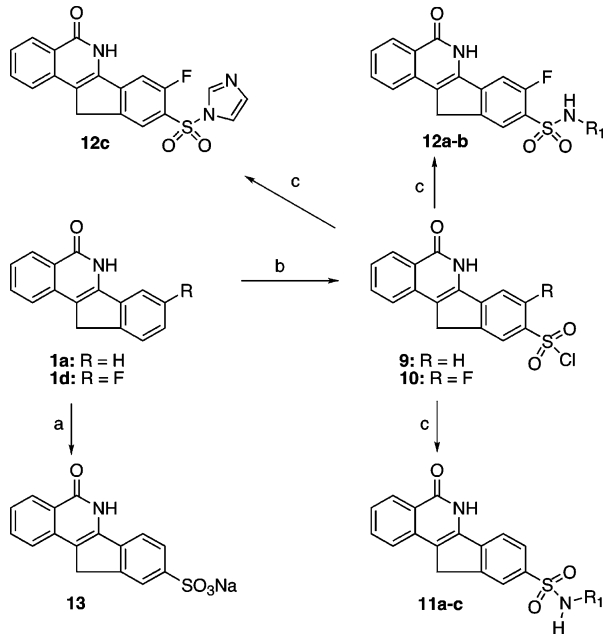


[a] (a) Ammonium formate, Pd−C (10%), DMF, heat, 68%; (b) $ClCOCH_2Cl$, saturated $NaHCO_3$, EtOAc, DMF, 82%; (c) $ClCO(CH_2)_3Cl$, saturated $NaHCO_3$, EtOAc, DMF; (d) HNR_1R_2, EtOH or DMF, **6a** 92%; (e) morpholine, DMF, Et_3N, **6c** 85%; (f) $ClCOCH_2OAc$, saturated $NaHCO_3$, EtOAc, DMF, 72%; (g) $NH_2NH_2 \cdot H_2O$, EtOH, reflux, **7** 82%; (h) *n*-propylisocyanate, DMF, 100 °C, 6 h, **8** 42%.

Our initial attempts to make **1b** or **1c** from the nitration of **1a** were not successful, resulting in mixed nitration products. The nitro derivatives (**1b** and **1c**) obtained from the alternative synthesis[9] were reduced to 8- and 9-aminoindeno[1,2-*c*]isoquinolinones **2a** and **2b** using Pd−C (10%) and ammonium formate in DMF. Schotten−Baumann acylation reaction of **2a** and **2b** with chloroacetyl chloride in ethyl acetate and in the presence of saturated $NaHCO_3$ provided chloroacetyl derivatives **3a** and **4a**, respectively. Following similar reaction conditions, **3b** and **4b** were prepared from **2a** and **2b** using 3-chlorobutyl chloride. Amination of **3a** with dimethylamine, morpholine, and 4-methylpiperazine provided the amino-substituted compounds **5a**, **5b** and **5c** respectively. Under similar conditions, **3b** and morpholine produced **5d**. Similarly, the 8-chloroacetyl derivative **4a** was treated with dimethylamine and 1-(4-fluorophenyl)piperazine in ethanol to produce the amino derivatives **6a** and **6b**, respectively. Compound **6c** was prepared from chlorobutyryl derivative **4b** and morpholine using triethylamine. The analogues **7** and **8** were prepared from **2b** as depicted in Scheme 2.

The synthesis of 9-sulfonamide derivatives is outlined in Scheme 3. Selective C-9 chlorosulfonation of the indeno[1,2-*c*]isoquinolinones **1a** and **1d** was achieved using chlorosulfonic acid. The resulting cholorosulfonyl compounds **9** and **10** were contaminated with corresponding sulfonic acid derivatives. The sulfonamides **11a**−**c** were then obtained by reacting **9** with the

Scheme 3. Synthesis of C-9 Sulfonamide Derivatives of Indeno[1,2-*c*]isoquinolinones[a]



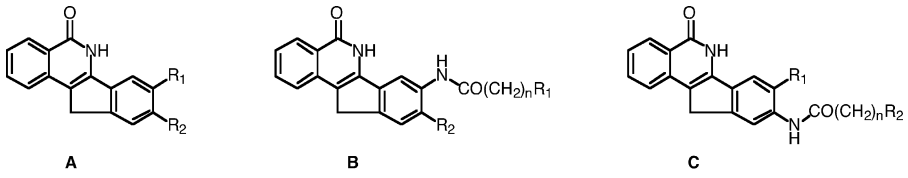
[a] (a) Concentrated H_2SO_4, 0 °C to room temperature, then NaOH; (b) $ClSO_3H$, 0 °C to room temperature, **9** 92%; (c) $H_2N(CH_2)_nNR_1R_2$ or $H_2N(CH_2)_nPh$, 4-aminomorpholine or imidazole, CH_2Cl_2, Et_3N or $^iPr_2EtN$, room temp, **11a** 44%.

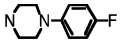
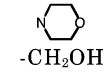
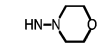
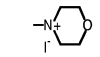
appropriate amines in the presence of triethylamine. Similarly, the **12a**, **12b**, and **12c** were prepared by treating **10** with 4-(3-aminopropyl)morpholine, 4-aminomorpholine, and imidazole, respectively. Compound **13** was obtained from the reaction of **1a** with concentrated H_2SO_4 followed by treatment with NaOH. Quaternary ammonium salt **14** was prepared from **11a** and MeI.

The inhibition of purified PARP-1 enzyme by selected compounds was subsequently determined. The assay was performed in 96-well ELISA plates according to instructions provided with a commercially available PARP inhibition assay kit (Trevigen, Gaithersburg, MD). The inhibitory effect of the compounds on PARP activity is summarized in Table 1. An IC_{50} value of the prototypical PARP-1 inhibitor, 3-aminobenzamide (3-AB), is included in Table 1 as a reference. The unsubstituted indeno[1,2-*c*]isoquinolinone core molecule **1a** showed poor PARP-1 inhibitory activity. The C-8 and C-9 nitro and fluoro derivatives (**1b**−**e**) exhibited moderate activity. The 9-amino compound **2b** showed a 3-fold increase in potency compared to the 8-amino analogue **2a**.

The C-8 N-substituted amide compounds (**5b** and **5c**), which carry morpholine and piperazine groups on the side chain, also showed weak PARP-1 inhibition. However, the dimethylamino derivative **5a**, which contains a one methylene unit linker, and the morpholine derivative **5d** with a three methylene linker exhibited IC_{50} values of 0.8 and 0.4 μM, respectively. The corresponding C-9 substituted derivatives **6a** and **6c** (both camphor sulfonic acid salts) exhibited EC_{50} values of 0.028 and 0.009 μM, respectively. The C-9 analogues with the amine separated from the carbonyl by one or three methylene groups showed increased PARP-1 inhibitory potency. The nature of the linker and the size of the

Table 1. PARP-1 Activity Data of Indeno[1,2-*c*]isoquinolinones



No.	Structure	n	R_1	R_2	IC_{50} μM[a]	EC_{50} μM[b]
1a	A		H	H	>3.0	>3.0
1b	A		NO_2	H	0.5	nd
1c	A		H	NO_2	0.3	nd
1d	A		F	H	0.5	0.225
1e	A		H	F	0.650	0.180
2a	A		NH_2	H	0.3 ± 0.021[c]	nd
2b	A		H	NH_2	0.098 ± 0.005[c]	0.095 ± 0.004[c]
5a	B	1	-NMe_2	H	0.8	0.650
5b	B	1		H	>3.0	nd
5c	B	1		H	>3.0	nd
5d	B	1		H	0.4	0.4
6a	C	1	H	-NMe_2	0.107 ± 0.006[c]	0.028 ± 0.004[c]
6b	C	1	H		0.080	0.070 ± 0.007[c]
6c	C	3	H		0.105	0.009 ± 0.002[c]
7	D		H	-CH_2OH	0.110	0.065
8	D		H	-NHPr	0.100	0.080 ± 0.004[c]
11a	E	3	H		0.001	0.010
11b	E	3	H		0.394 ± 0.021[c]	0.300 ± 0.027[c]
11c	E	3	H		>3.0	nd
12a	E	3	F		0.010	0.009
12b	E	3	F		0.450 ± 0.036[c]	0.3
12c	F		F		0.5	0.4
13	F		H	-SO_3Na	0.7	0.625
14	E	3	H		0.8	nd
3-AB					30[d]	

[a] Assay performed using the commercially available PARP inhibition assay kit (Trevigen, Gaithersburg, MD). [b] EC_{50} values determined using the cell protection assay (see Supporting Information for the assay procedures). nd: not determined. [c] An average of EC_{50} and IC_{50} values measured from three cellular or cell-free assays. [d] Reference 10.

amino group appear to play a major role in the PARP-1 activity in C-8 and C-9 substituted indeno[1,2-*c*]-isoquinolinones.

The 9-sulfonic acid sodium salt (**13**) showed an IC_{50} of 0.7 μM. However, the 9-sulfonamide derivative **11a** mesylate salt exhibited excellent PARP-1 inhibition in both assays (IC_{50} and EC_{50} of 1 and 10 nM, respectively). Replacement of the morpholino group of **11a** with a phenyl ring (**11c**) resulted in deleterious effects on the activity. Compound **14** with the *N*-methylmorpholinium group showed a large decrease in activity. As exemplified above, the C-8 derivatives generally showed weaker PARP-1 inhibition than the corresponding C-9 substituted compounds. However, the C-8 and C-9 disubstituted **12a** was 25-fold more potent than the 9-fluoroindeno[1,2-*c*]isoquinolinone (**1d**). An increase in potency of the disubstituted compound **12a** could be explained by the presence of the sulfonamide linker of **11a** at the C-8 position.

In conclusion, we have synthesized a novel series of potent PARP-1 inhibitors from the modification of indeno[1,2-*c*]isoquinolinones. It is of interest to note that

C-9 substituted and C-8 and C-9 disubstituted derivatives demonstrated considerably higher activity than C-8 derivatives, suggesting that C-9 is the important position for modifications. Additional pharmacokinetics and clinical data will be published in due course.

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Supporting Information Available: Experimental procedures and spectral data for the new and known products and detailed PARP-1 assay procedures. This material is available free of charge via the Internet at http://pubs.acs.org.

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