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Synthesis of Substituted 5[*H*]Phenanthridin-6-ones as Potent Poly(ADP-ribose)polymerase-1 (PARP1) Inhibitors

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Abstract—1-, 2-, 3-, 4-, 8-, or 10-Substituted 5(*H*)phenanthridin-6-ones were synthesized and found to be potent PARP1 inhibitors. Among the 28 compounds prepared, some showed not only low IC_{50} values (compound **1b**, 10 nM) but also desirable water solubility characteristics. These properties, which are superior to the common PARP1 inhibitors such as benzamides and isoquinolin-1-ones, are essential for potential therapeutic usage. The variety of compounds allows SAR analysis of favored substituents and substituted positions on 5(*H*)phenanthridin-6-one ring.

Introduction

Poly(ADP-ribose) polymerase1 (PARP1, EC 2.4.2.30), also known as poly(ADP-ribose) synthetase (PARS), is an abundant nuclear protein in most of the eukaryotic tissues. The discovery of PARP1 involvement in ischemia/reperfusion injuries rekindled interest for possible clinical use of PARP inhibitors.[1] In several studies, we demonstrated that a prototype PARP1 inhibitor, GPI 6150 (1,11b-dihydrobenzopyrano[4,3,2-*de*]isoquinolin-3-one), was efficacious in reducing tissue damage in rodent models of focal cerebral ischemia and regional myocardial ischemia.[2] A common structural feature for the classical PARP1 inhibitors is a carboxamide attached to an aromatic ring or a fused aromatic lactam or imide.[3] Most of these inhibitors are structurally planar and show limited solubility in both organic and aqueous solvents. (5*H*)Phenanthridin-6-one (**1a** in Table 1) was initially identified as a moderate PARP inhibitor.[4] In vivo testing of this compound is hindered by its poor solubility in biocompatible vehicles. It was also found to cross inhibit heterophil arginine ADP-ribose transferase ($IC_{50} = 47$ μM).[4] We report here the improvement of this class of inhibitors in potency and physicochemical properties by installation of appropriate substituent(s) onto 5[*H*]phenanthridin-6-one.

Chemistry

In order to incorporate specific substituents on the ring system of **1a**, several distinct synthetic pathways were designed. Among these approaches, the most efficient method was the one-step Smith or Schmidt reaction using an assortment of commercially available substituted fluoren-9-ones as starting materials and sodium azide as an insertion agent under acidic conditions[5] as illustrated in method I of Scheme 1. When a 2,7-disubstituted fluoren-9-one was used as a starting material, 3,8-disubstituted 5[*H*]phenanthridin-6-ones **1b–f** were obtained.

If the two substituents at the 2,7-position on the fluoren-9-one are not identical, the reaction leads to a mixture of regioisomers. Compound **1b** was obtained by crystallization from a regioisomeric mixture of 3-[8-fluoro-6(5*H*)phenanthridinone] sulfonic acid and of 8-[3-fluoro-6(5*H*)phenanthridinone]sulfonic acid, which were prepared by mixing 7-(2-fluoro-fluoren-9-one)sulfonic acid and sodium azide in concentrated sulfuric acid at 25 °C for 10 h followed by ice-cold water workup. The pure isomer 3-[8-fluoro-6(5*H*)phenanthridinone] sulfuric acid **1b** appeared as a precipitate upon adjusting pH of the solution to 8 and standing at 0 °C for 24 h. The structure of **1b** was distinguished from its regioisomer by observation of NOE between H-4 (δ 7.68, s) and the NH (δ 11.9, br. s) protons in ^{1}H NMR experiments.

In order to enable specific substituent variation at different positions of **1a**, cross-coupling routes were developed. Derivatives of 3-carboxyl-5[*H*]phenanthridin-

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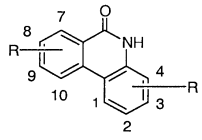
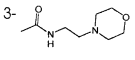
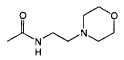
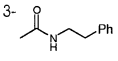
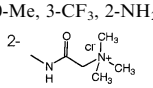
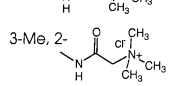
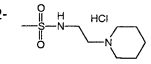
Table 1.

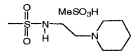
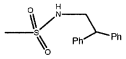


1a: R = H, IC_{50} = 0.52 μM

No.	Substitution	Method	IC_{50} (μM)	Formula[a]	^1H NMR[b] δ	mp (°C)[c]
1b	3-SO_3H, 8-F	I	0.01	$C_{13}H_8FNO_4S$	11.9 (bs, 1H), 8.59 (dd, J=9.0, 5.1 Hz, 1H), 8.33 (d, J=8.4 Hz, 1H), 7.96 (dd, J=9.2, 2.9 Hz, 1H), 7.74 (ddd, J=9.0, 8.8, 2.9 Hz, 1H), 7.68 (s, 1H), 7.47 (d, J=8.4 Hz, 1H)	300+
1c	3-Cl, 8-Cl	I	0.24	$C_{13}H_7Cl_2NO$	11.94 (bs, 1H), 8.55 (d, 1H, J=8.8 Hz), 8.43 (d, J=8.7 Hz, 1H), 8.24 (d, J=2.3 Hz, 1H), 7.92 (dd, J=8.8, 2.3 Hz, 1H), 7.40 (d, J=2.1 Hz, 1H), 7.32 (dd, J=8.7, 2.1 Hz, 1H)	300+
1d	3-NH_2, 8-NH_2	I	3.26	$C_{13}H_{11}N_3O$·0.2AcOH	11.05 (bs, 1H), 7.88 (d, J=8.4 Hz, 1H), 7.76 (d, J=8.4 Hz, 1H), 7.34 (s, 1H), 7.00 (d, J=8.4 Hz, 1H), 6.46 (d, J=8.4 Hz, 1H), 6.42 (s, 1H), 5.35 (s, 2H), 5.32 (s, 2H)	300–301
1e	3-NO_2, 8-NO_2	I	35	$C_{13}H_7N_3O_5$	12.40 (bs, 1H), 9.00 (s, 1H), 8.89 (d, J=8.4 Hz, 1H), 8.77 (d, J=8.2 Hz, 1H), 8.65 (d, J=8.4 Hz, 1H), 8.23 (s, 1H), 8.10 (d, J=8.2 Hz, 1H)	300+
1f	3-SO_3Na, 8-SO_3Na	I	>100	$C_{13}H_7NNa_2O_7S_2$·1.3H_2O	11.69 (bs, 1H), 8.54 (s, 1H), 8.44 (d, J=8.4 Hz, 1H), 8.31 (d, 1H, J=8.3 Hz, 1H), 7.99 (d, J=8.4 Hz, 1H), 7.67 (s, 1H), 7.45 (d, J=8.3 Hz, 1H)	300+
5a1	3-COOMe	II	0.184	$C_{15}H_{11}NO_3$	11.87 (br s, 1H), 8.56 (d, J=8 Hz, 1H), 8.52 (d, J=8 Hz, 1H), 8.36 (d, J=8 Hz, 1H), 8.00 (s, 1H), 7.91 (t, J=8 Hz, 1H), 7.78 (d, J=8 Hz, 1H), 7.73 (t, J=8 Hz, 1H), 3.91 (s, 3H)	276–279
5a2	8-F, 3-COOMe	II	0.037	$C_{15}H_{10}FNO_3$	11.97 (bs, 1H), 8.63 (m, 1H), 8.47 (d, J=8 Hz, 1H), 7.97 (m, 2H), 7.77 (m, 2H), 3.90 (s, 3H)	280–300
5b1	3-COONa	II	0.057	$C_{14}H_9NO_3$·1Na	7.66 (d, J=8.0 Hz, 1H), 7.54 (d, J=8.0 Hz, 1H), 7.45 (d, J=8.0 Hz, 1H), 7.40 (t, J=8.0 Hz, 1H), 7.26 (m, 2H), 7.01 (s, 1H)	300+
5b2	8-F, 3-COONa	II	0.021	$C_{14}H_8FNO_3$·1Na	7.38 (m, 1H), 7.20 (m, 2H), 7.11 (d, J=8.0 Hz, 1H), 7.02 (t, J=8.0 Hz, 1H), 6.95 (s, 1H)	300+
5c1	3- 	II	0.035	$C_{20}H_{21}N_3O_3$	11.82 (bs, 1H), 8.58 (d, J=8.0 Hz, 1H), 8.54 (t, J=8.0 Hz, 1H), 8.49 (d, J=8.0 Hz, 1H), 8.34 (d, J=8.0 Hz, 1H), 7.89 (t, J=8.0 Hz, 1H), 7.83 (s, 1H), 7.70 (m, 2H), 3.58 (m, 5H), 3.40 (m, 2H), 3.31 (m, 5H)	264–267[d]
5c2	8-F, 3- 	II	0.014	$C_{20}H_{20}FN_3O_3$	11.98 (bs, 1H), 8.67 (m, 1H), 8.55 (t, J=8.0 Hz, 1H), 8.47 (d, J=12 Hz, 1H), 7.99 (d, J=8.0Hz, 1H), 7.84 (s, 1H), 7.79 (t, J=8.0 Hz, 1H), 7.70 (d, J=8.0 Hz, 1H), 3.59 (m, 5H), 3.41 (m, 2H), 2.45 (m, 5H)	285–290
5c3	8-F, 3- 	II	0.15	$C_{22}H_{17}FN_2O_2$	11.97 (bs, 1H), 8.70 (m, 2H), 8.47 (d, J=8.0 Hz, 1H), 8.00 (d, J=8.0 Hz, 1H), 7.84 (s, 1H), 7.75 (t, J=8.0 Hz, 1H), 7.68 (d, J=8.0 Hz, 1H), 7.30 (m, 5H), 3.53 (t, J=8.0 Hz, 2H), 2.88 (t, J=8.0 Hz, 2H)	293–298
9a	1-COOH	III	2.01	$C_{14}H_9NO_3$	11.85 (bs, 1H), 8.39 (s, J=7.9 Hz, 1H), 8.37 (s, J=8.1 Hz, 1H), 7.83 (t, J=7.3 Hz, 1H), 7.69 (t, J=7.9 Hz, 1H), 7.54 (t, J=8.1 Hz, 1H), 7.46 (d, J=8.1 Hz, 1H), 7.27 (d, J=7.3 Hz, 1H)	300+
9b	4-NH_2	III	0.312	$C_{13}H_{10}N_2O$	10.69 (bs, 1H), 8.42 (d, J=7.8, 1H), 8.29 (d, J=8.2 Hz, 1H), 7.77 (m, 1H), 7.57 (m, 2H), 7.04 (t, J=7.8 Hz, 1H), 6.86 (d, J=7.4 Hz, 1H), 5.09 (s, 2H)	263–268
9c	4-NO_2	III	6.1	$C_{13}H_8N_2O_3$ 0.4·9H_2O	11.32 (bs, 1H), 8.50 (d, J=8.0 Hz, 1H), 8.46 (d, J=8.0 Hz, 1H), 8.40 (d, J=8.3 Hz, 1H), 8.22 (d, J=8.3 Hz, 1H), 7.80 (t, J=7.7 Hz, 1H), 7.63 (t, J=7.7 Hz, 1H), 7.32 (t, J=8.1 Hz, 1H)	263–264
9d	10-Me, 3-F	III	0.092	$C_{14}H_{10}FNO$	11.81 (bs, 1H), 8.49 (m, 1H), 8.29 (d, J=7.8 Hz, 1H), 7.73 (d, J=6.7 Hz, 1H), 7.54 (t, J=7.6 Hz, 1H), 7.17 (d, J=7.1 Hz, 1H), 7.11 (t, J=8.5 Hz, 1H), 2.91 (s, 3H)	300+
9e	10-COOH	III	>60	$C_{14}H_9NO_3$·1.1H_2O	11.50 (bs, 1H), 8.52 (d, J=8.0 Hz, 1H), 8.12 (d, J=8.2 Hz, 1H), 7.91 (d, J=7.2 Hz, 1H), 7.84 (d, J=8.3 Hz, 1H), 7.34 (t, J=7.7 Hz, 1H), 7.67 (t, 1H), 7.33 (t, J=7.7 Hz, 1H)	272–275[e]
10a1	10-Me, 3-CF_3, 2-NO_2	III	0.136	$C_{15}H_9F_3N_2O_3$	12.37 (bs, 1H), 9.22 (s, 1H), 8.32 (d, J=8.0 Hz, 1H), 7.90 (s, 1H), 7.84 (d, J=8.0 Hz, 1H), 7.70 (t, J=8.0 Hz, 1H), 2.98 (s, 3H)	300+
10a2	2-NO_2	III	0.156	$C_{13}H_8N_2O_3$	12.2 (bs, 1H), 9.21 (s, 1H), 8.69 (d, J=8.0 Hz, 1H), 8.37–8.34 (m, 2H), 7.94 (t, J=8.0 Hz, 1H), 7.75 (t, J=8.0 Hz, 1H), 7.52 (d, J=9.0 Hz, 1H)	381–382
10b1	2-NH_2	III	0.18	$C_{13}H_{10}N_2O$	11.34 (bs, 1H), 8.30 (d, J=7.6 Hz, 1H), 8.22 (d, J=8.0 Hz, 1H), 7.82 (t, J=7.2 Hz, 1H), 7.60 (t, J=7.2 Hz, 1H), 7.48 (s, 1H), 7.11 (d, J=8.4 Hz, 1H), 6.82 (d, J=8.0 Hz, 1H), 5.02 (s, 2H)	284–285
10b2	3-NH_2, 2-NH_2	III	0.18	$C_{13}H_{11}N_3O$·0.4H_2O	11.15 (bs, 1H), 8.18 (d, J=7.6 Hz, 1H), 7.99 (d, J=8.2 Hz, 1H), 7.69 (t, J=7.0 Hz, 1H), 7.38–7.35 (m, 2H), 6.48 (s, 1H), 5.19 (s, 2H), 4.50 (s, 2H)	300+
10b3	10-Me, 3-CF_3, 2-NH_2	III	0.044	$C_{15}H_{11}F_3N_2O$	11.46 (bs, 1H), 8.30 (d, J=8.0 Hz, 1H), 8.00 (s, 1H), 7.71 (d, J=8.0 Hz, 1H), 7.57 (t, J=8.0 Hz, 1H), 7.46 (s, 1H), 5.44 (s, 2H), 2.92 (s, 3H)	300+
10c1	2- 	III	0.03	$C_{18}H_{20}ClN_3O_2$·0.7H_2O	11.76 (bs, 1H), 11.03 (bs, 1H), 8.68 (s, 1H), 8.34 (d, J=8.0 Hz, 1H), 8.27 (d, J=8.0 Hz, 1H), 7.91 (t, J=8.0 Hz, 1H), 7.65 (m, 2H), 7.37 (d, J=8.0 Hz, 1H), 4.41 (s, 2H), 3.34 (s, 9H)	269–272
10c2	3-Me, 2- 	III	0.195	$C_{19}H_{22}ClN_3O_2$·1.5H_2O	11.72 (bs, 1H), 10.30 (bs, 1H), 8.34 (m, 3H), 7.86 (t, J=8.0 Hz, 1H), 7.65 (t, J=8.0 Hz, 1H), 7.23 (s, 1H), 4.43 (s, 2H), 3.32 (s, 9H), 2.31 (s, 3H)	300+
10c3	10-Me, 3-CF_3, 2- 	III	0.033	$C_{17}H_{12}ClF_3N_2O_2$	11.98 (bs, 1H), 10.02 (s, 1H), 8.54 (s, 1H), 8.34 (d, J=8.0 Hz, 1H), 7.70 (m, 2H), 7.66 (t, J=8.0 Hz, 1H), 4.38 (s, 2H)	300+
11a1	2- 	III	0.03	$C_{20}H_{24}ClN_3O_3S$·0.4H_2O	12.1 (bs,1H), 9.65 (bs, 1H), 8.79 (s, 1H), 8.56 (d, J=8.1 Hz, 1H), 8.36 (d, J=7.2 Hz, 1H), 7.93 (m, 2H), 7.75 (t, J=7.5 Hz, 1H), 7.54 (d, J=8.7 Hz, 1H), 3.41 (d, J=12.2 Hz, 2H), 3.14 (m, 4H), 2.89 (t, 2H), 1.71 (m, 6H)	262–267

(continued)

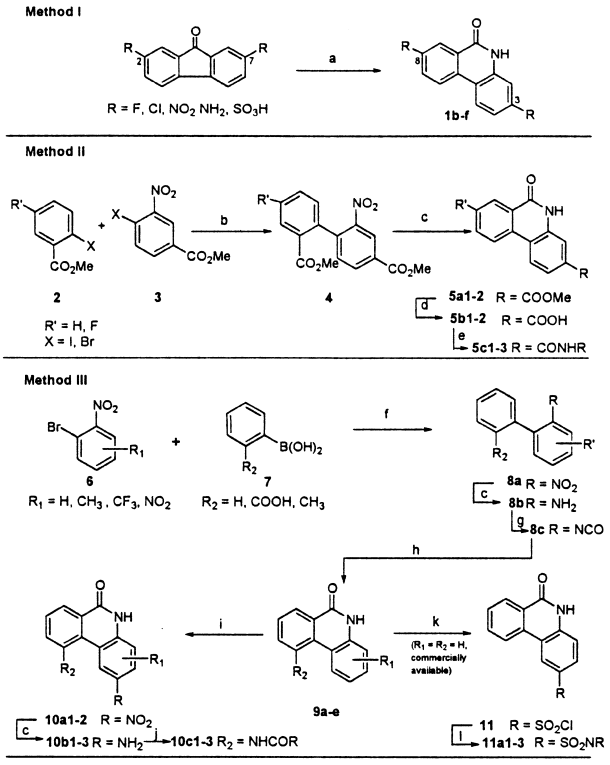
Table 1 (*continued*)

No.	Substitution	Method	IC_{50} (μM)	Formula[a]	^1H NMR[b] δ	mp (°C)[c]
11a2	2- 	III	0.036	$C_{21}H_{27}N_3O_6S2{\cdot}0.5H_2O$	12.10 (bs, 1H), 9.30 (bs, 1H), 8.75 (s, 1H), 8.54 (d, J=8.0 Hz, 1H), 8.36 (d, J=8.0 Hz, 1H), 7.94 (m, 2H), 7.75 (t, J=8.0 Hz, 1H), 7.55 (d, J=8.0 Hz, 1H), 3.43 (d, J=12.0 Hz, 2H), 3.14 (s, 4H), 2.91 (m, 2H), 2.30 (s, 3H), 1.7 (m, 3H)	275–279
11a3	2- 	III	14	$C_{27}H_{22}N_2O_3S{\cdot}0.6\ H_2O$	12.02 (bs, 1H), 8.69 (bs, 1H), 8.45 (d, J=8.0 Hz, 1H), 8.34 (d, J=8.0 Hz, 1H), 7.93 (t, J=7.6 Hz, 1H), 7.84 (d, J=8.6 Hz, 1H), 7.73 (m, 2H), 7.46 (d, J=8.6 Hz, 1H), 7.24 (m, 8H), 7.13 (m, 2H), 5.76 (s, 1H), 4.11 (t, J=7.7 Hz, 1H), 3.41 (d, J=7.7 Hz, 2H)	265–268

[a]Based on elemental analyses experiments.
[b]Measured on a Bruker Avance-400 MHz NMR spectrometer using trimethylsilane as an internal standard and DMSO-d_6 as solvent.
[c]Measured on a Thomas-Hoover melting point apparatus, uncorrected.
[d]Literature:[10a] 227–228 °C.
[e]Literature:[10b] 352.5–353 °C.

6-ones **5** were synthesized as outlined in method II of Scheme 1. Commercially available methyl-2-iodo (or bromo[6]) benzoate **2** reacted with methyl-3-nitro-4-iodo (or bromo[7]) benzoate **3** in an Ullmann coupling to afford biphenyl diester derivatives **4** in 42–50% yield. Reduction of the biphenyl derivative **4** using hydrazine and Raney nickel efficiently produced 3-methyl 5[*H*]phenanthridin-6-one carboxylate **5a**, which was subsequently hydrolyzed with base to afford 5[*H*]phenanthridin-6-one-3-carboxylic acid **5b**. Amidation of this acid using standard EDC coupling conditions with an appropriate amino compound yielded carboxamides **5c**.

Substituted 5[*H*]phenanthridin-6-ones at 1-, 2-, 3-, 4-, or 10-position were synthesized through Suzuki cross-coupling followed by intramolecular Friedel–Crafts acylation using a biphenylisocyanate as an intermediate as described in method III of Scheme 1. The cross-coupling of 5-substituted 2-nitro bromobenzene derivative **6** and phenylboric acid compounds **7** provided the biphenyl product **8** in reasonable yield of 40–60%. The nitro group was easily reduced to the amino functionality **8b** by either treatment with hydrazine and Raney nickel or typical hydrogenation methods. The desired compound **9** was formed in good yield through intramolecular Friedel–Crafts acylation of isocyano intermediate **8c**, generated in situ by condensing the amino group with phosgene.[8]



Scheme 1. Reagents and conditions: (a) NaN_3, H_2SO_4, 25 °C, 3 h; (b) Cu, neat, 200 °C, 8 h; (c) NH_2NH_2, Raney nickel, MeOH; (d) 1 N NaOH; (e) R-NH_2, EDC, DMAP in DCM; (f) Na_2CO_3, $Pd(PPh_3)_4$, Et_3N in DME, reflux, 20 h; (g) phosgene in toluene, reflux; (h) Et_2OBF_3, 0 °C; (i) HNO_3, HOAc, reflux; (j) R-COCl, Et_3N; (k) HOS-O_2Cl neat, 0 °C; (l) R-NH_2/Et_3N in DCM.

Nitration of the resulting 3- or 10-substituted 5[*H*]phenanthridin-6-ones **9** at the 2-position followed by hydrogenation provided 2-amino-5[*H*]phenanthridin-6-ones **10b**. Using the 2-amino group as a linker, desired products of carboxamides **10c** were prepared readily by reaction of **10b** with carboxylic acid halide derivatives in high yields. Monosulfonation of commercially available 6(5*H*)-phenanthridinone **1a** using neat chlorosulfonic acid gave sulfonyl chloride compound **11** in good yield. Amidation of compound **11** using primary amines afforded target sulfonamide compounds **11a1**–**3**. Thus, about 30 compounds were made using the methods described in Scheme 1. Some of these containing a basic amine or carboxylic acid group were converted to either hydrochloride or methansufonic acid salts or sodium salt, respectively. All of the salts showed good solubility in water. For example, both hydrochloride or methansufonic acid salts (entry **11a1** and **11a2**, in Table 1) prepared from the piperidine derivative have solubility in water greater than 10 mg/mL.

Biological Evaluation and Discussion

In the absence of any detailed structure–activity relationship (SAR) information about substituted 5[*H*]phenanthridin-6-ones, optimization of the tricyclic ring was accomplished by systematically varying the substituents at 1-, 2-, 3-, 4-, 8-, and 10-positions. All compounds were fully characterized by ^1H NMR, elemental analysis, and melting point. The IC_{50}'s were determined from full dose–response curves.[9] The structures, inhibition values and physicochemical properties of 5[*H*]phenanthridin-6-ones are given in Table 1. The SAR of these

compounds shows that most substituted 5[*H*]phenanthridin-6-ones are more potent inhibitors than the 'parent' **1a**. Only about a quarter of the substituted compounds have a decreased potency due to unfavorable substitution position or structural character.

Substitution at 2- and 3-positions

A brief examination of the top 10 potent inhibitors confirmed that the 2- or 3-positions were preferred attachment points for substitution. Substitutions at these positions generally resulted in submicromolar inhibitors, with many of the potent ones at low nanomolar range, suggesting beneficial interactions between the substituent and the enzyme. The electronic nature of 2- or 3-substituents appears insignificant to affect the inhibition. This was exemplified by the electron-withdrawing 2-nitro compound **10a2** and electron-donating 2-amino compounds **10b1** and 2,3-diamino **10b2**. All of these three compounds showed values of IC_{50}'s at ca. 0.17 μM and about 3-fold better than the 'parent' **1a**. Compounds with large aliphatic substituents (**5c1**, **5c3**, **11a1**) show very high potency, indicating that the volume tolerance at the direction is sufficiently large to accommodate most substituents. However, there is a limit defined by the pocket, as in the case of the 3-substituted compound (**11a3**, IC_{50} 14 μM), which has a *gem*-diphenyl group at the end of carbon chain and is ca. 30-fold less active than the core **1a**.

Substitution at 8-position

Looking at 3,8-disubstituted compounds (**1b–f**), 8-fluoro is clearly the best among those whereas the sulfonyl worst. The potency increase appears to follow the trend of $F > Cl > H > NH_2 > NO_2 > SO_3Na$, with the 8-sulfonic acid compound **1f** being completely deactivated. Because 3-substitution generally enhances the potency as discussed above, 8-substitution may play a dominant role in the series. In general, bulky 8-substitution (i.e., compare **1f** to **1b**) dramatically diminished the inhibition of PARP1. In contrast, a smaller halogen, such as fluorine, potentiates the in vitro efficacy.

Substitution at 1, 4, or 10 positions

Limited numbers of compounds were prepared in each of these series. But with an IC_{50} value of 2 μM and 4-fold worse than the core structure, 1-carboxylic acid **9a** apparently obstructed the interaction with PARP. Inhibition trend of 4-substituted compounds appeared 4-amino **9b** > **1a** > 4-nitro **9c**. In the 10-substitution series, the methyl group was beneficial (**9d**, 0.092 μM) whereas 10-carboxylic acid showed little activity (**9e**, >60 μM), suggesting that unlike 3-substituted benzamides, the 10-position (corresponding to 3-position of benzamide) is quite sensitive to substitutional modification in PARP1 inhibition.

In summary, introduction of either an acidic or amino-containing side chain at the 2- and/or 3-position not only enhanced potency by more than 20-fold as compared to the core **1a**, but also increases aqueous solubility dramatically in the acid or salt form. For example, three of the most potent inhibitors (**1b**, **10c1**, and **11a2**) exhibited excellent water solubility properties with IC_{50} values as low as 30 nM.

Conclusion

Using several synthetic approaches, we have prepared 1-, 2-, 3-, 4-, 8-, or 10-substituted 5[*H*]phenanthridin-6-ones. The attachment of two optimal substituents onto the core skeleton resulted in the water-soluble compound, 3(10-fluoro-5[*H*]phenanthridin-6-one) sulfonyl acid **1b**, which exhibits an IC_{50} value of 10 nM and 52-fold greater inhibition activity as compared to the core structure of 5[*H*]phenanthridin-6-one **1a**. Thus, by incorporation of substitution with a suitable acidic or basic residue onto the ring system, we have observed improvements of potency in vitro, along with obvious effects on physicochemical characteristics such as water solubility. In vivo data of some of these compounds will be disclosed in future publication.

References and Notes

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9. PARP1 inhibition assay: Purified recombinant human PARP from Trevigan (Gaithersburg, MD, USA) was used to determine the IC_{50} value of a PARP inhibitor. The PARP enzyme assay is set up on ice in a volume of 100 μL consisting of 50 mM Tris–HCl (pH 8.0), 2 mM $MgCl_2$, 30 μg/mL of DNase I activated herring sperm DNA (Sigma, MO, USA), 30 μM [^{3}H]nicotinamide adenine dinucleotide (67 mCi/mmol), 75 μg/mL PARP enzyme, and various concentrations of the compounds to be tested. The reaction is initiated by incubating the mixture at 25 °C. After 15 min of incubation, the reaction is terminated by adding 500 μL of ice cold 20% (w/v) trichloroacetic acid. The precipitate formed is transferred onto a glass fiber filter (Packard Unifilter-GF/B) and washed three times with ethanol. After the filter is dried, the radioactivity is determined by scintillation counting.
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