Pergamon

SCIENCE DIRECT®

Bioorganic & Medicinal Chemistry Letters 13 (2003) 2513–2518

BIOORGANIC & MEDICINAL CHEMISTRY LETTERS

# Design and Synthesis of Poly(ADP-ribose)polymerase-1 (PARP-1) Inhibitors. Part 3: In Vitro Evaluation of 1,3,4,5-Tetrahydro-benzo[*c*][1,6]- and [*c*][1,7]-naphthyridin-6-ones

Dana Ferraris,* Rica Pargas Ficco, Thomas Pahutski, Susan Lautar, Shirley Huang, Jie Zhang and Vincent Kalish

*Guilford Pharmaceuticals Inc., 6611 Tributary Street, Baltimore, MA 21224, USA*

Received 7 April 2003; revised 25 April 2003; accepted 6 May 2003

**Abstract**—The 1,3,4,5-tetrahydro-benzo[*c*][1,6]- and [*c*][1,7]-napthyridin-6-ones are presented as a potent class of PARP-1 inhibitors. Derivatives of these partially saturated aza-5[*H*]-phenanthridin-6-ones were designed and synthesized with tertiary amines for salt formation, thus enhancing aqueous solubility, iv formulation and their potential use in acute ischemic injuries (i.e., myocardial ischemia and stroke). We found that partial saturation of the C-ring results in derivatives that are several times more potent than the aromatic C-ring derivatives. The general synthetic routes are presented herein as well as thorough in vitro potencies and SAR discussion for selected derivatives.


Poly(ADP-ribose) polymerase-1 (PARP-1, EC 2.4.2.30) is an abundant nuclear enzyme with an important role in the cellular life cycle.[1,2] The PARP-1 enzyme has three structural regions: the DNA binding domain containing two zinc fingers, the automodification domain and the catalytic domain.[3] The catalytic domain is responsible for converting nicotinamide adenine dinucleotide (NAD+) into nicotinamide and adding an ADP-ribose unit to the substrate.[4] When over-activated, PARP-1 can cause the consumption of NAD+ and subsequently the depletion of intracellular ATP. This depletion of ATP results in cell death through a necrotic pathway and eventually results in ischemic tissue damage.[5,6] Recently, studies have demonstrated the utility of aqueous soluble PARP-1 inhibitors in rat models of stroke and heart ischemia.[7] These data indicate that PARP-1 inhibitors could be utilized in a clinical setting to treat ischemia-reperfusion injuries, an area of medicine with unmet therapeutic needs.

Quinazolines (**1a**, Fig. 1) and isoquinolones (**1b**) are examples of two 'first generation' PARP-1 inhibitors whose structure–activity relationships with the enzyme are well established.[8] The closely related tricyclic 5[*H*]-phenanthridin-6-ones (**2**) and aza-5[*H*]-phenanthridin-6-ones (**3a–c**) are also well-known classes of inhibitors whose structure–activity relationships against PARP-1 have recently been established.[7,9] Preliminary findings established that **4a**[10] ($IC_{50}$ = 167 nM) was slightly more potent against PARP-1 than 5[*H*]-phenanthridin-6-one **2**, an unexpected result due to the planar nature of most potent PARP-1 inhibitors (e.g., **1a–b**, **2**, and **3a–c**). Compound **4a** also demonstrated increased intrinsic solubility compared to **2** in common organic solvents. This result coupled with previous findings indicating enhanced in vitro and in vivo efficacy of 1-aza-5[*H*]-phenanthridin-6-one **3a** over 5[*H*]-phenanthridin-6-one **2**[7] prompted the design and synthesis of partially saturated derivatives of the aza-5[*H*]-phenanthridin-6-ones (**4b–d**). In this manuscript, we present the synthesis and in vitro activities of 1,3,4,5-tetrahydro-benzo[*c*][1,6]- and [*c*][1,7]-napthyridin-6-ones as PARP-1 inhibitors (**4b–d**, Fig. 1) whose core potencies are several times better than the related unsaturated derivatives **3b–c**.

The initial synthesis of these partially saturated aza-5[*H*]-phenanthridin-6-ones is outlined in Scheme 1. The first two steps are analogous to a literature synthesis of

*Corresponding author. Tel.: +1-410-631-8126; fax: +1-410-631-6797; e-mail: ferrarisd@guilfordpharm.com

0960-894X/03/$ - see front matter © 2003 Elsevier Ltd. All rights reserved.
doi:10.1016/S0960-894X(03)00465-7

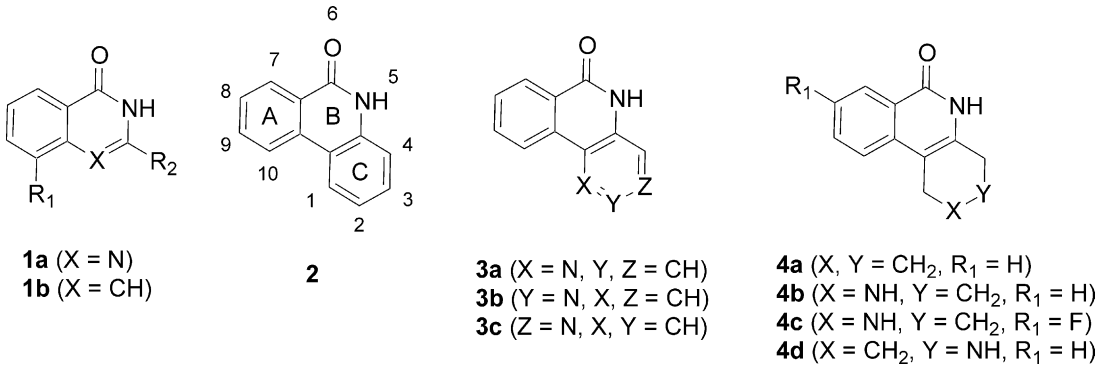
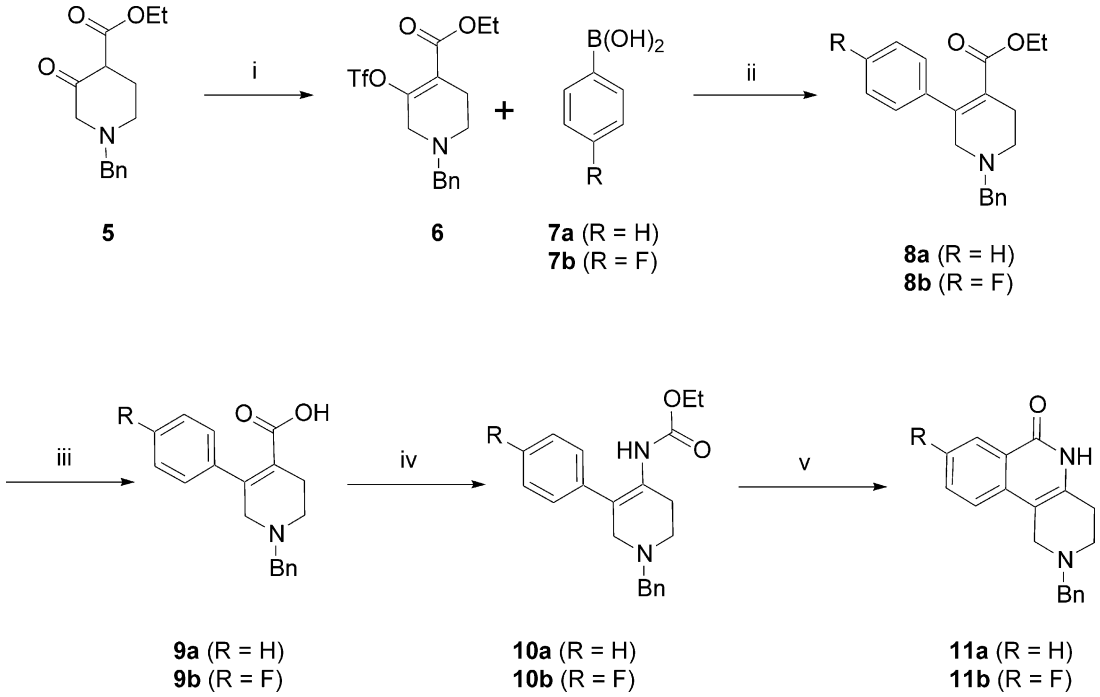



**Figure 1.** Structures of 5[*H*]-phenanthridin-6-ones and related PARP-1 inhibitors.




**Scheme 1.** (i) NaH/$Tf_2O$/$Et_2O$ 95%; (ii) $Pd(PPh_3)_4$/$K_2CO_3$/DME, **8a** = 89%, **8b** = 97%; (iii) LiOH/THF/MeOH, **9a** = 83%, **9b** = 80%; (iv) (1) DPPA/Toluene/$Et_3N$, 85 °C; (2) EtOH, **10a** = 66%, **10b** = 50%; (v) Eaton's Reagent, 100 °C, **11a** = 66%, **11b** = 33%.

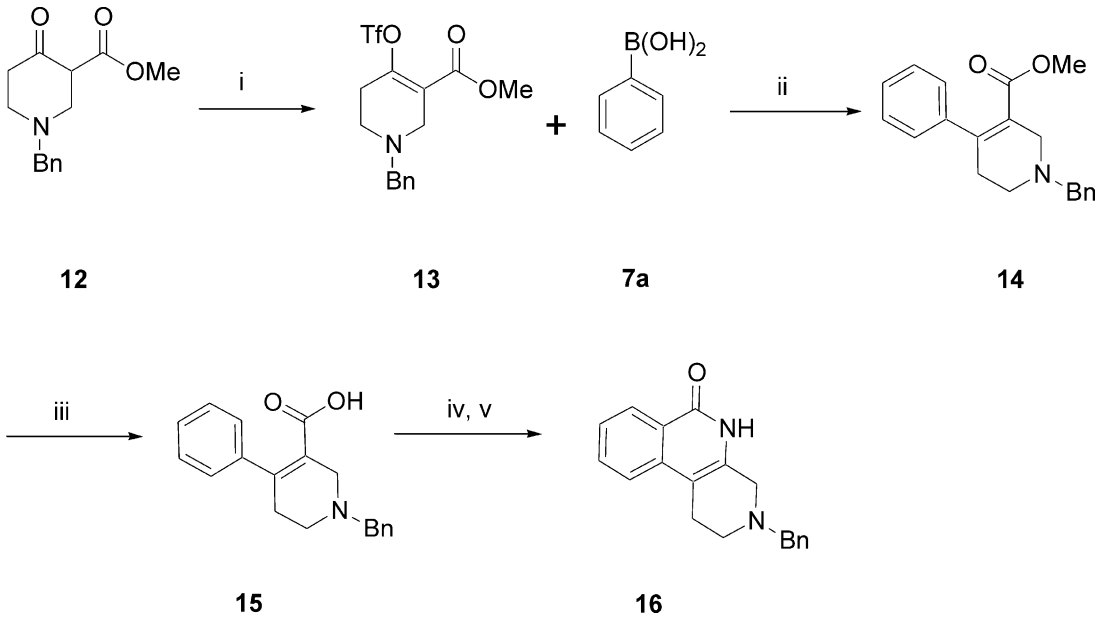
α-1A adrenoreceptor agonists.[11] Commercially available ester **5** was converted to the triflate **6** using sodium hydride and trifluoromethanesulfonic anhydride in 95% yield. Suzuki coupling of **6** with either phenyl boronic acid **7a** or 4-fluorophenyl boronic acid **7b** led to the esters **8a** and **8b** in 89% and 97% yield, respectively. The coupled products were hydrolyzed under basic conditions to afford the carboxylic acids **9a** (83%) and **9b** (80%). The carboxylic acids were subsequently converted into the ethyl carbamates **10a–b** via a Curtius rearrangement and quenching with ethanol. Cyclization of the carbamates **10a–b** was accomplished in moderate yield (33–68%) by refluxing in Eaton's reagent. The final benzyl-protected 1,3,4,5-tetrahydro-benzo[*c*][1,6]-napthyridin-6-ones **11a–b** were formed in 10–39% overall yield.

The isomeric 1,3,4,5-tetrahydro-benzo[*c*][1,7]-napthyridin-6-ones were prepared in a similar manner to **11a–b** as outlined in Scheme 2. Triflation of the starting ester **12** was accomplished in 75% yield to afford compound **13**. The Suzuki coupling of **13** with phenyl boronic acid was achieved in excellent yield to afford ester **14** in 87% yield. The ester was subsequently hydrolyzed in high yield (83%) to form carboxylic acid **15**. The only deviation from Scheme 1 occurs in the cyclization step. The carboxylic acid **15** was converted to the isocyanate in an analogous manner to acid **9a**, but the labile isocyanate was directly converted to the desired *N*-benzyl-1,3,4,5-tetrahydro-benzo[*c*][1,7]-napthyridin-6-one **16** in 25% yield by treatment with aluminum trichloride.

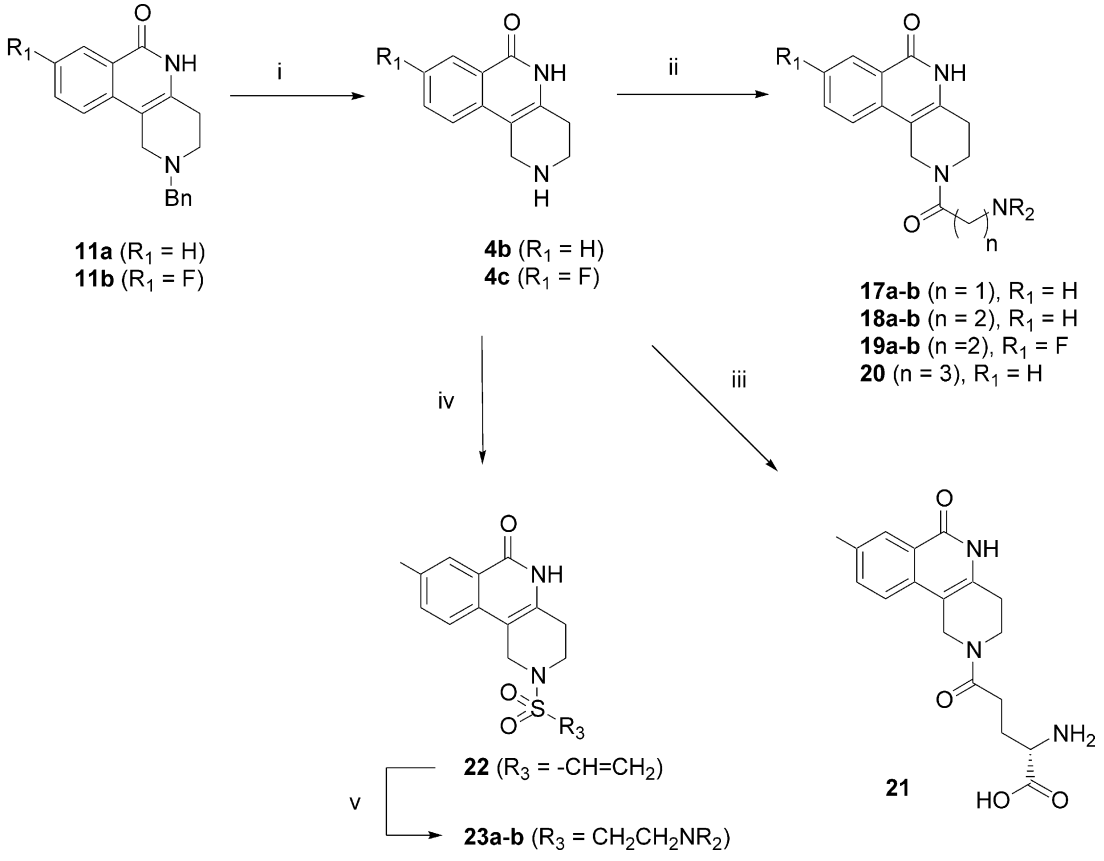
Subsequent derivatization of **11a–b** was carried out as indicated in Scheme 3. Deprotection of benzyl amine **11a** and **11b** led to the secondary amines **4b** and **4c** after workup in 93% yield. Acylation of the latent secondary amine **4b** and **4c** with chloroacetyl-, propionyl- and butyrylchlorides followed by amination led to the tertiary amines **17a–b**, **18a–b**, **19a–b** and **20**.

The glutamate derivative of **4b** was also synthesized by standard EDC coupling with Boc-Glu-OtBu to yield amino acid **21** after deprotection. Sulfonamide derivatives resulted from the addition of chloroethanesulfonyl chloride to **4b** in dichloromethane to afford the sulfonyl olefin **22**. Michael addition of piperidine and 4-pyrrolylpiperidine to **22** led to the tertiary amines **23a–b**.

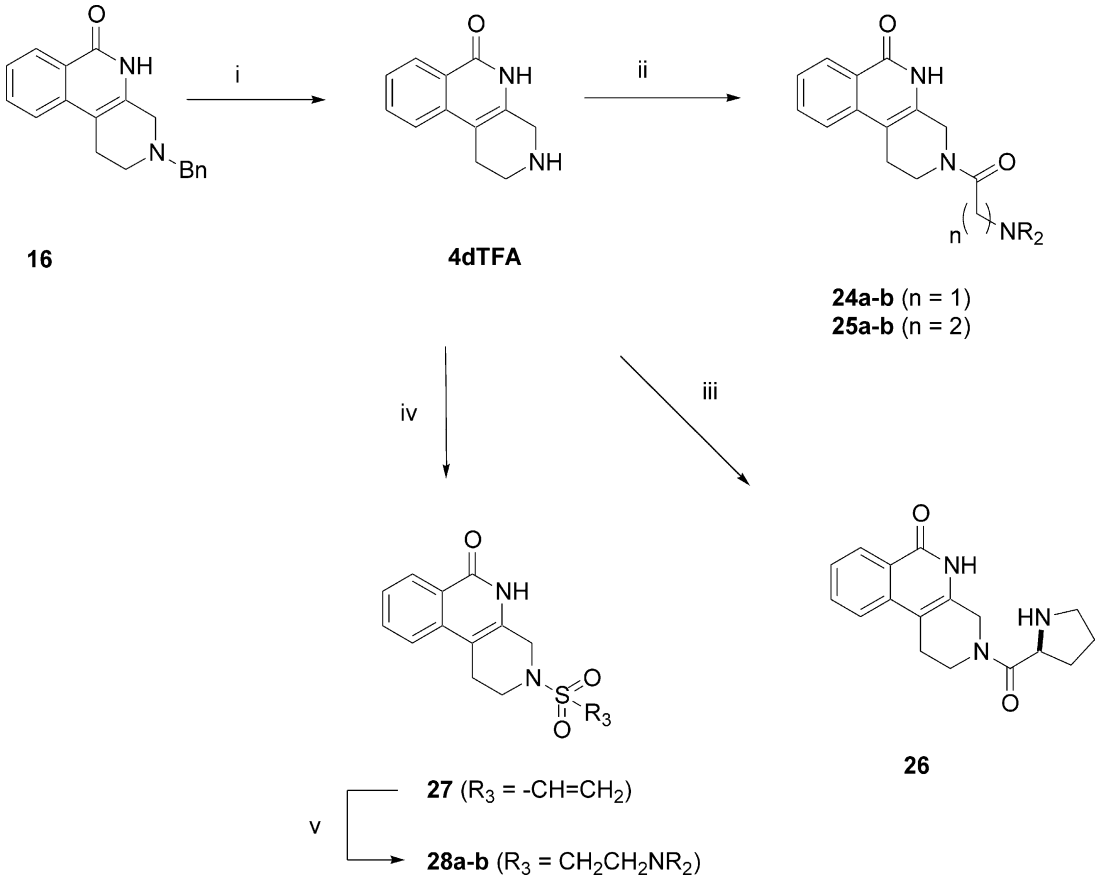
Similarly, deprotection of isomeric benzyl amine **16** in MeOH/TFA led to **4d·TFA** as outlined in Scheme 4.




**Scheme 2.** (i) NaH/$Tf_2O$/$Et_2O$, 75%; (ii) $Pd(PPh_3)_4$/$K_2CO_3$/DME, 87%; (iii) LiOH/THF/MeOH, 83%; (iv) (1) DPPA/Toluene/$Et_3N$, 85 °C; (2) $AlCl_3$, 25%.

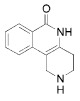
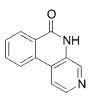



**Scheme 3.** (i) (1) $H_2$/Pd/C/MeOH/TFA; (2) 10% $K_2CO_3$, **4b** = 93%, **4c** = 90%; (ii) (1) $ClCO(CH_2)_nCl$/$Et_3N$, 71–85%; (2) $R_2NH$ (xs), **17a** = 55%, **17b** = 57%, **18a** = 59%, **18b** = 56%, **19a** = 44%, **19b** = 58%, **20** = 59%; (iii) (1) EDC/DMAP/DME, Boc-Glu-O*t*Bu; (2) 10% TFA/DCM, 46% (2 steps); (iv) $ClSO_2CH_2CH_2Cl$, $Et_3N$, 48%; (v) $R_2NH$, xs, **23a** = 58%, **23b** = 69%.




**Scheme 4.** (i) $H_2$/Pd/C/MeOH/TFA, 78%; (ii) $ClCO(CH_2)_nCl/Et_3N$, 51–67%; (2) $R_2NH$ (xs), **24a** = 47%, **24b** = 57%, **25a** = 62%, **25b** = 57%; (iii) (1) EDC/DMAP/DCM/$Et_3N$, Boc-Pro; (2) 10%TFA/DCM, 51% (2 steps) (iv) $ClSO_2CH_2CH_2Cl$, $Et_3N$, 53%; (v) $R_2NH$, xs, **28a** = 59%, **28b** = 76%.

**Table 1.** PARP-1 Inhibition of several tricyclic amides

| Compd | Compound name | Structure | $IC_{50}$ nM[a] |
|---|---|---|---|
| **2** | 5[*H*]-Phenanthridin-6-one |  | 350 |
| **4a** | 1,3,4,5-Tetrahydro-2H-phenanthridin-6-one[b] |  | 167 |
| | 1,3,4,4a,5,10b-Hexahydro-2H-phenanthridin-6-one[c] |  | 7500 |
| **3b** | 2-aza-5[*H*]-phenanthridin-6-one |  | 128 |
| **4b** | 1,3,4,5-Tetrahydro-2H-benzo[*c*][1,6]naphthyridin-6-one |  | 35 |
| **3c** | 3-aza-5[*H*]-phenanthridin-6-one |  | 169 |
| **4d·TFA** | 1,3,4,5-Tetrahydro-2H-benzo[*c*][1,7]naphthyridin-6-one |  | 40 |

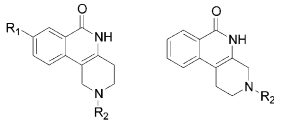
[a]See ref 12 for details.
[b]See ref 10 for synthesis.
[c]See ref 13 for synthesis.

The secondary amine **4d·TFA** was acylated and aminated in a similar manner to **4b** and **4c** leading to **24a–b**, and **25a–b**. Standard EDC coupling of **4d·TFA** with Boc-proline, followed by deprotection of the Boc group led to amine **26** in 53% yield. Sulfonamides **28a–b** were also synthesized from **4d·TFA** in a similar manner to **23a–b**.

The PARP-1 $IC_{50}$'s of saturated and unsaturated 5[*H*]-phenanthridin-6-ones and aza-5[*H*]-phenanthridin-6-ones are outlined in Table 1.[12] The parent tricyclic 5[*H*]-phenanthridin-6-one **2** has an $IC_{50}$ value of 350 nM against PARP-1. By saturating two double bonds in the C ring of 5[*H*]-phenanthridin-6-one, the potency increases by 2-fold as indicated by compound **4a** (X, Y = $CH_2$, Fig. 1).[10] Complete saturation of the C ring as shown by 1,3,4,4a,5,10b-Hexahydro-2H-phenanthridin-6-one,[13] however, leads to a PARP-1 inhibitor that is over an order of magnitude less potent than 5[*H*]-phenanthridin-6-one.

These data indicate that the A and B rings of these tricyclic amides maintain potency when planar, a common characteristic among several classes of PARP-1 inhibitors.[14] Substitution of a nitrogen for a carbon in the C ring leads to the 2- and 3-aza-5[*H*]-phenanthridin-6-ones (**3b** and **3c**, Fig. 1), both of which exhibit activities slightly better than the parent 5[*H*]-phenanthridin-6-one. Likewise, saturation of the C ring of **3b** and **3c**

**Table 2.** PARP-1 Inhibition of several *N*-substituted 1,3,4,5-tetrahydro-*2H*-benzo[*c*][1,6] and [*c*][1,7]napthyridin-6-ones



| Compd | $R_1$ | $R_2$ | *n* | $IC_{50}$ nM[a] | $EC_{50}$ nM[b] |
|---|---|---|---|---|---|
| **4b** | H | H | | 35 | 40 |
| **4c** | F | H | | 20 | 40 |
| **4dTFA** | | H | | 30 | 45 |
| **17a** | H | | 1 | 59 | 60 |
| **18a** | H | | 2 | 42 | 58 |
| **19a** | F | | 2 | 25 | 30 |
| **20** | H | | 3 | 457 | |
| **24a** | | | 1 | 162 | 260 |
| **25a** | | | 2 | 240 | |
| **17b** | H | | 1 | 185 | 850 |
| **18b** | H | | 2 | 99 | |
| **19b** | F | | 2 | 32 | 610 |
| **24b** | | | 1 | 116 | 690 |
| **25b** | | | 2 | 243 | |
| **23a** | H | | | 210 | |
| **28a** | | | | 376 | 2790 |
| **23b** | H | | | 357 | |
| **28b** | | | | 349 | 1780 |
| **21a** | H | | | 89 | 30,000 |
| **27a** | | | | 31 | 100 |

[a]See ref 12 for details.
[b]See ref 15 for details.

leads to compounds **4b** and **4d·TFA** (Fig. 1) whose potencies are over three times better than their unsaturated analogues. As a general trend, compounds with partially saturated C rings are more potent than their unsaturated cores in each case.

Several derivatives of compounds **4b** are outlined in Table 2. The parent compound, **4b**, is among the most potent members of this series ($IC_{50} = 35$ nM). The tertiary amines **17a**, **18a** and **19a** all displayed excellent potencies against PARP-1 with the ethyl linker being preferred (**18a**, **19a**). A similar trend was noted for the pyrrolylpiperazine moiety, that is, compounds **18b** and **19b** are slightly more potent than the one carbon analogue **17b**. Extension of the carbon chain to 3 methylene units as in compound **20**, however, decreased the activity several fold compared to the analogues with a shorter linker. Changing from an amide to a sulfonamide linker also decreased the potency 3–4 times as illustrated by compounds **23a–b**. Glutamate derivative **21a**, despite being several fold less potent than the core **4b**, illustrates that hydrophobic substituents are not necessary to maintain potency.

Previous publications indicate that A-ring substituents of 5[*H*]-phenanthridin-6-ones decrease inhibitory potency,[12] with the exception of a fluorine in the 8 position. As expected, the fluoro-substituted analogues **4c** and **19a–b** maintained or increased the potency in every instance as outlined in Table 2.

The in vitro activities of *N*-substituted derivatives of **4d** are also outlined in Table 2. The parent compound **4d·TFA** is the most active member of the series ($IC_{50} = 30$ nM) followed by the proline derivative **27a**. The amides **24a**, **25a**, have similar potencies to the pyrrolyl piperidine derivatives **24b** and **25b**. Slight loss in potency (i.e., 2–5 times) was observed when changing the amide bond to the sulfonamide linkage as illustrated by compounds **28a–b**.

In general, all of the 1,3,4,5-tetrahydro-benzo[*c*][1,6]-[*c*][1,7]-naphthyridine analogues maintained a good level of inhibition (< 500 nM) regardless of the size or hydrophobicity of the group. These results are indicative of a large pocket adjacent to the nicotinamide binding region of PARP-1, a result also noted in previous publications.[7,9]

The in vitro cellular peroxide toxicity assay provided the $EC_{50}$ data outlined in Table 2.[15] In general, the $EC_{50}$ of any given inhibitor was 1.5–3-fold less potent than the corresponding $IC_{50}$. The only exceptions were the amino acid **21a** and the sulfonamides **28a–b**. These results indicate that most of the 1,3,4,5-tetrahydro-benzo[*c*][1,6]- and [*c*][1,7]-napthyridin-6-ones permeate the cells in high enough concentration to inhibit PARP-1.

In conclusion, several potent PARP-1 inhibitors from the 1,3,4,5-tetrahydro-benzo[*c*][1,6]- and [*c*][1,7]-napthyridin-6-one class of compounds are presented in this text. We have demonstrated the improvement in potency upon partial saturation of the 5[*H*]-phenanthridin-6-one and aza-5[*H*]-phenanthridin-6-one C rings. Finally, we have designed an efficient synthetic route to several, different *N*-substituted 1,3,4,5-tetrahydro-benzo[*c*][1,6]- and [*c*][1,7]-napthyridin-6-ones as a means to further improve the pharmacological properties of these inhibitors for potential use in acute, ischemic injuries.

## References and Notes

1. Decker, P.; Isenberg, D.; Muller, S. *J. Biol. Chem.* **2000**, *275*, 9043.
2. Yu, S.-W.; Wang, H.; Poitras, M. F.; Coombs, C.; Bowers, W. J.; Federoff, H. J.; Poirier, G. G.; Dawson, T. M.; Dawson, V. L. *Science* **2002**, *297*, 259.
3. Rolli, V.; O'Farrell, M.; Menissier-de Murcia, J.; de Murcia, G. *Biochemistry* **1997**, *36*, 12147.
4. Jeggo, P. A. *Curr. Biol.* **1998**, *8*, R49–51.
5. Takahashi, K.; Pieper, A. A.; Croul, S. E.; Zhang, J.; Snyder, S. H.; Greenberg, J. H. *Brain Res.* **1999**, *829*, 46.
6. Nagayama, T.; Simon, R. P.; Chen, D.; Henshall, D. C.; Pei, W.; Stetler, R. A.; Chen, J. *J. Neurochem.* **2000**, *74*, 1636.
7. Ferraris, D.; Ko, Y.; Pahutski, T.; Ficco, R.-P.; Serdyuk, L.; Alemu, C.; Bradford, C.; Chiou, T.; Hoover, R.; Huang, S.; Lautar, S.; Liang, S.; Lin, Q.; Lu, M.-X.-C.; Mooney, M.; Morgan, L.; Qian, Y.; Tran, S.; Williams, L.; Wu, Q.-Y.; Zhang, J.; Zou, Y.; Kalish, V. *J. Med. Chem.* **2003** accepted for publication.

8. Watson, C. Y.; Whish, W. J. D.; Threadgill, M. D. *Bioorg. Med. Chem.* **1998**, *6*, 721.
9. Li, J. H.; Serdyuk, L.; Ferraris, D.; Xiao, G.; Tays, K.; Kletzly, P. W.; Li, W.; Lautar, S.; Zhang, J.; Kalish, V. *Bioorg. Med. Chem. Lett.* **2001**, *11*, 1687.
10. Rigby, J. H.; Holsworth, D. D.; James, K. *J. Org. Chem.* **1989**, *54*, 4019.
11. Patane, M. A.; DiPardo, R. M.; Price, R. P.; Chang, R. S. L.; Ransom, R. W.; O'Malley, S. S.; DiSalvo, J.; Bock, M. G. *Bioorg. Med. Chem. Lett.* **1998**, *8*, 2495.
12. **PARP-1 inhibition assay**: Purified recombinant human PARP from Trevigan (Gaithersburg, MD, USA) was used to determine the $IC_{50}$ values 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 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 was terminated by adding 500 mL 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.
13. Lopes, R. S. C.; Lopes, C. L.; Heathcock, C. H. *Tetrahedron Lett.* **1992**, *33*, 6775.
14. Canan Koch, S. S.; Thorsen, L. H.; Tikhe, J. G.; Maegley, K. A.; Almassy, R. J.; Li, J.; Yu, X.-H.; Zook, S. E.; Kumpf, R. A.; Zhang, C.; Boritzki, T. J.; Mansour, R. N.; Zhang, K. E.; Ekker, A.; Calabrese, C. R.; Crutin, N. J.; Kyle, S.; Thomas, H. D.; Wang, L.-Z.; Calvert, A. H.; Golding, B. T.; Griffin, R. J.; Newell, D. R.; Webber, S. E.; Hostomsky, Z. *J. Med. Chem.* **2002**, *45*, 4961.
15. Zhang, J.; Lautar, S.; Huang, S.; Ramsey, C.; Cheung, A.; Li, J.-H. *Biochem. Biophys. Res. Comm.* **2000**, *278*, 590.