

Design, Synthesis, and Evaluation of 3,4-Dihydro-2H-[1,4]diazepino[6,7,1-*hi*]indol-1-ones as Inhibitors of Poly(ADP-Ribose) Polymerase

Jayashree G. Tikhe,^{*,†} Stephen E. Webber,[‡] Zdenek Hostomsky,[†] Karen A. Maegley,[†] Anne Ekkers,[†] Jianke Li,[†] Xiao-Hong Yu,[†] Robert J. Almassy,[§] Robert A. Kumpf,[†] Theodore J. Boritzki,[†] Cathy Zhang,[†] Chris R. Calabrese,^{||} Nicola J. Curtin,^{||} Suzanne Kyle,^{||} Huw D. Thomas,^{||} Lan-Zhen Wang,^{||} A. Hilary Calvert,^{||} Bernard T. Golding,^{||} Roger J. Griffin,^{||} and David R. Newell^{||}

Pfizer Global R&D—La Jolla Laboratories, 10770 Science Center Drive, San Diego, California 92121, Anadys Pharmaceuticals, 3115 Merryfield Row, San Diego, California 92121, Quorex Pharmaceuticals, 1890 Rutherford Road, #200, Carlsbad, California 92008, and University of Newcastle, Newcastle upon Tyne NE2 4HH, United Kingdom

Received October 10, 2003

The design, synthesis, and biological evaluation of potent inhibitors of poly(ADP-ribose) polymerase-1 (PARP-1) are reported. A novel series of 3,4-dihydro-2H-[1,4]diazepino[6,7,1-*hi*]indol-1-ones were designed using a combination of protein structure-based drug design, molecular modeling, and structure–activity relationships (SAR). These novel submicromolar inhibitors possess a tricyclic ring system conformationally restricting the benzamide in the preferred *cis* orientation. The compounds were designed to optimize space-filling and atomic interactions within the NAD⁺ binding site of PARP-1. Previously described and newly adapted methods were applied to syntheses of these tricyclic inhibitors. Various modifications were made to the diazepinoindolones at the 6- and 7-positions in order to study this region of the active site and optimize noncovalent interactions. The electron density of derivative **28** bound to chicken PARP-1 revealed that the oxime makes a tight hydrogen bond with the catalytic γ -carboxylate of glutamic acid (Glu) 988 in accordance with our original designs and models. Most of the compounds have been evaluated for inhibition of human PARP-1. Selected inhibitors were also tested for the ability to potentiate the cytotoxic effect of the DNA-damaging agent Topotecan.

Introduction

Poly(ADP-ribose) polymerases (PARPs), nuclear enzymes found in almost all eukaryotic cells, catalyze the transfer of ADP-ribose units from nicotinamide adenine dinucleotide (NAD⁺) to nuclear acceptor proteins and are responsible for the formation of protein-bound linear and branched homo-ADP-ribose polymers.^{1–8} The activation of PARP and the formation of poly(ADP-ribose) are the result of DNA strand breaks induced from exposure to certain chemotherapeutics, ionizing radiation, oxygen free radicals, or nitric oxide.^{9,10} The acceptor proteins of poly(ADP-ribose), including histones, topoisomerases, DNA and RNA polymerases, DNA ligases, and metal-dependent endonucleases, are involved in maintaining DNA integrity.^{7,11–14}

The cellular ADP-ribose transfer process may “indirectly” contribute to the resistance that often develops to various types of cancer therapies through its role in repairing DNA strand breaks caused by radiotherapy or chemotherapy. Consequently, inhibition of PARP-1 (EC 2.4.2.30), the most abundant form of the PARPs, may retard intracellular DNA repair and thereby enhance and prolong the antitumor effects of certain anticancer therapies. In vitro and in vivo data show that

many PARP inhibitors potentiate the effects of ionizing radiation or cytotoxic agents and, therefore, may serve as effective adjunct cancer chemotherapeutics.^{15–20}

PARP-1 has also been implicated in several other disease states. In addition to anticancer therapies, inhibitors may have utility against myocardial infarction, neurotoxicity consequent to stroke, head trauma, neurodegenerative diseases, insulin-dependent diabetes mellitus, and arthritis and inflammation.^{10,21–25}

Many PARP inhibitors, designed as nicotinamide mimics, have been reported. Representative examples are shown in Figure 1. 3-Aminobenzamide, **I**, has been widely used in numerous studies; however, this simple compound lacks the potency and efficacy required to make it therapeutically useful.^{26,27} Second-generation nicotinamide-based molecules made use of tetrahydroisoquinolin-2-one to restrict the amide in its *cis* conformation. PD128763, **II**, was shown to be approximately 50-fold more active than 3-aminobenzamide; however, development of this compound was discontinued.^{15,28} Other constricted carboxamide-like examples include imides such as **III**²⁷ and quinazolinones such as **IV**.¹⁷ More recently, a class of potent benzimidazole PARP inhibitors, **V**, was developed at the University of Newcastle.²⁹ The requisite *cis* amide geometry is locked in place through an eloquent design of an intramolecular hydrogen bond between the –NH₂ and the lone pair electrons of N-3.

Our group has recently reported three novel classes of tricyclic PARP-1 inhibitors, shown in Figure 2.^{20,30,31}

* To whom correspondence should be addressed. Tel: 858-622-3172. Fax: 858-678-8156. E-mail: jayashree.tikhe@pfizer.com.

[†] Pfizer Global R&D—La Jolla Laboratories.

[‡] Anadys Pharmaceuticals.

[§] Quorex Pharmaceuticals.

^{||} University of Newcastle.

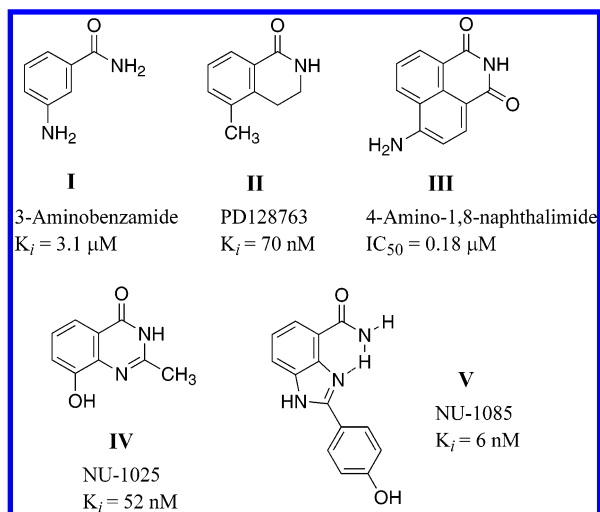


Figure 1.

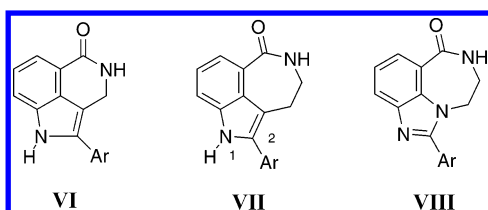


Figure 2.

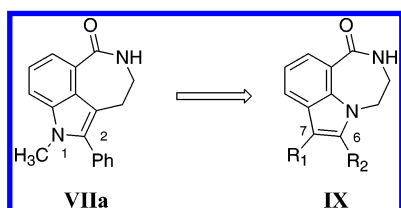


Figure 3.

The conception of these unique tricyclic inhibitors was based on the combination of reported information and protein–ligand structure-based design. As a continuation of our research, we have expanded this series to include 3,4-dihydro-2*H*-[1,4]diazepino[6,7,1-*hi*]indol-1-ones.

Molecular Design

In preceding publications, our group has described the design and syntheses of tricyclic PARP-1 inhibitors.^{30,31} Incorporation of the seven-membered ring in **VII** and **VIII** not only locks the critical amide in the required *cis* geometry but also better disrupts the planarity of the tricyclic core, thereby filling more hydrophobic space while conceivably enhancing the overall physical properties of the molecule. Close examination of cocrystal structures of chicken PARP-1 complexed with inhibitors of type **V** or **VII** revealed a region adjacent to *N*-1 accessible for further modification.^{29,30} It was previously shown that *N*-1 methylation of 2-phenyl-1,3,4,5-tetrahydroazepino[5,4,3-*cd*]indol-6-one (**VIIa**) is well-tolerated and not detrimental to binding.³⁰ We envisaged the tricyclic 3,4-dihydro-2*H*-[1,4]diazepino[6,7,1-*hi*]indol-1-one **IX** (Figure 3) to be an attractive alternative scaffold since synthetic modifications at C-7 would allow us to readily probe this scantily explored region of the active site.

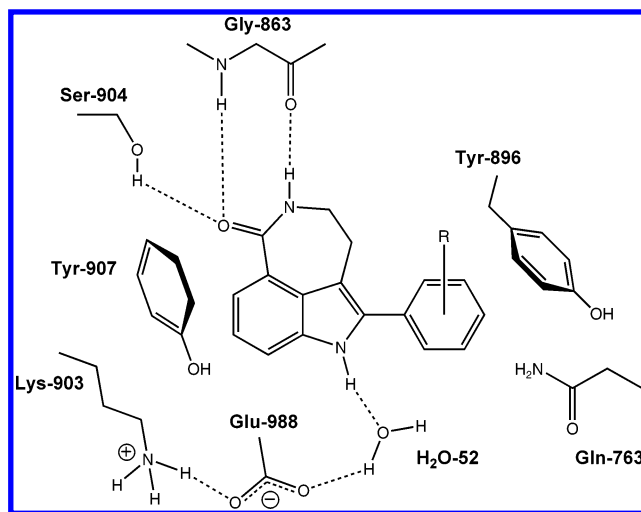


Figure 4.

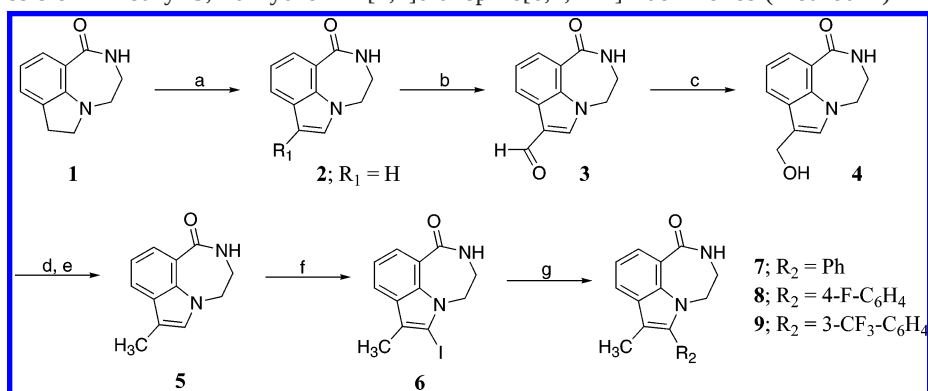
Models of structure **IX** in the PARP-1 active site also show C-7 to be in close proximity to the catalytically relevant γ -carboxylic acid of Glu-988. Glu-988 functions in the polymerization reaction by forming a crucial hydrogen bond with the 2'-hydroxyl of the growing poly-(ADP-ribose) chain, thus activating this hydroxyl for nucleophilic attack of carbon in the nicotinamide–ribose bond of the donor NAD⁺ molecule.^{32–34} In addition to activation, the hydrogen bond to the donor ribose hydroxyl stabilizes an oxocarbenium-like transition state.^{34–36}

From crystallographic data, a water molecule is often observed situated between the nitrogen atom of the inhibitor and the γ -carboxylate of Glu-988, forming hydrogen bonds that “bridge” the ligand to the enzyme.^{30,31} Figure 4 illustrates a schematic depiction of crystallographically observed atomic interactions between inhibitors of type **VII** and the active site residues of chicken PARP-1.³⁰

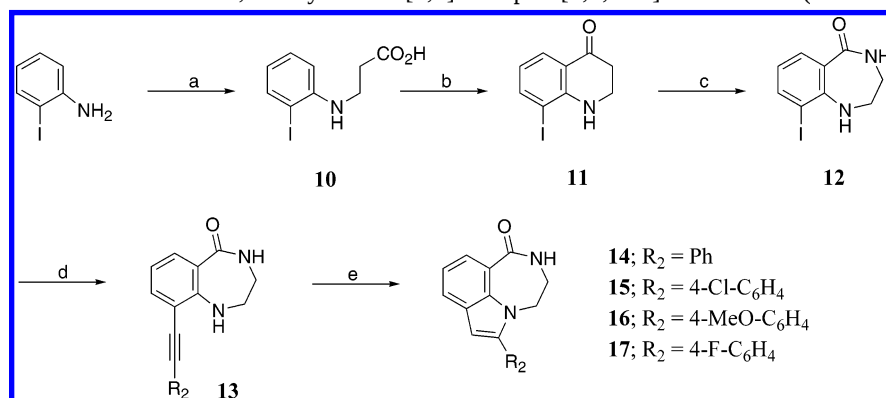
We became very interested in the design of inhibitors that would possibly interact directly with Glu-988. Other research groups have also explored this region of the active site. Through crystallographic studies, Ruf et al. provided evidence that the 4-amino group of **III** forms a hydrogen bond with the γ -carboxylate of Glu-988.³⁷ Watson et al. designed electrophilic benzamide and isoquinolinone derivatives in an attempt to test whether Glu-988 would act as a nucleophile to form a covalent bond in a mechanism-based fashion.³⁸

Our initial molecular modeling and design indicated that H₂O-52 could be displaced by other hydrogen bond donors, in particular the NOH of an *E*-carboxaldehyde oxime. A number of C-7-substituted diazepinoindolones were designed and prepared in order to determine whether direct interactions to Glu-988 would enhance inhibition of PARP-1 and to test the limitations of H₂O-52 displacement or reorientation.

From our previous studies, we also learned that aryl groups at C-2 of tricyclic compounds **VII** and **VIII** were advantageous to binding.^{30,31} Aryl rings occupy hydrophobic space in this region of the active site, predominantly interacting with the side chains of Tyr-889 and Tyr-896. Capitalizing on the structure–activity relationship (SAR) information learned from the published series, several 6-substituted diazepinoindolones were

Scheme 1. Synthesis of 7-Methyl-3,4-dihydro-2H-[1,4]diazepino[6,7,1-*hi*]indol-1-ones (Method A)^a

^a Conditions and reagents: (a) DDQ, Ph-H. (b) ex POCl₃, DMF. (c) NaBH₄, EtOH. (d) AcOAc, pyr, DMAP. (e) 10% Pd/C, H₂, AcOH, MeOH. (f) I₂, (CF₃CO₂)₂C₆H₅, CH₂Cl₂. (g) R₂-B(OH)₂, catalytic (Ph₃P)₄Pd, LiCl, Na₂CO₃, DMF, H₂O.

Scheme 2. Synthesis of 6-Substituted 3,4-Dihydro-2H-[1,4]diazepino[6,7,1-*hi*]indol-1-ones (Method B)^a

^a Conditions and reagents: (a) β-Propiolactone, CH₃CN, reflux. (b) P₂O₅, CH₃SO₃H, room temperature–65 °C. (c) NaN₃, CH₃SO₃H. (d) R₂–≡–H, catalytic (Ph₃P)₄Pd, CuI, Et₂NH, DMF. (e) Catalytic PdCl₂, CH₃CN, 60–70 °C.

therefore prepared and tested. The *meta*- and *para*-substituted *N*-alkylaminomethyl and *N,N*-dialkylaminomethyl phenyl analogues were designed with the primary intent to improve the aqueous solubility of the compounds. These substituents were previously exploited in tricyclic series **VI**–**VIII**.^{30,31} The aminomethylene groups are generally well-accommodated in the enzyme active site. Molecular modeling of these functional groups and crystallographic data for a related inhibitor³¹ suggest that the positively charged amino group participates in favorable electrostatic interactions to the side chains of Gln-763,³⁹ Asp-766, and Tyr-896.

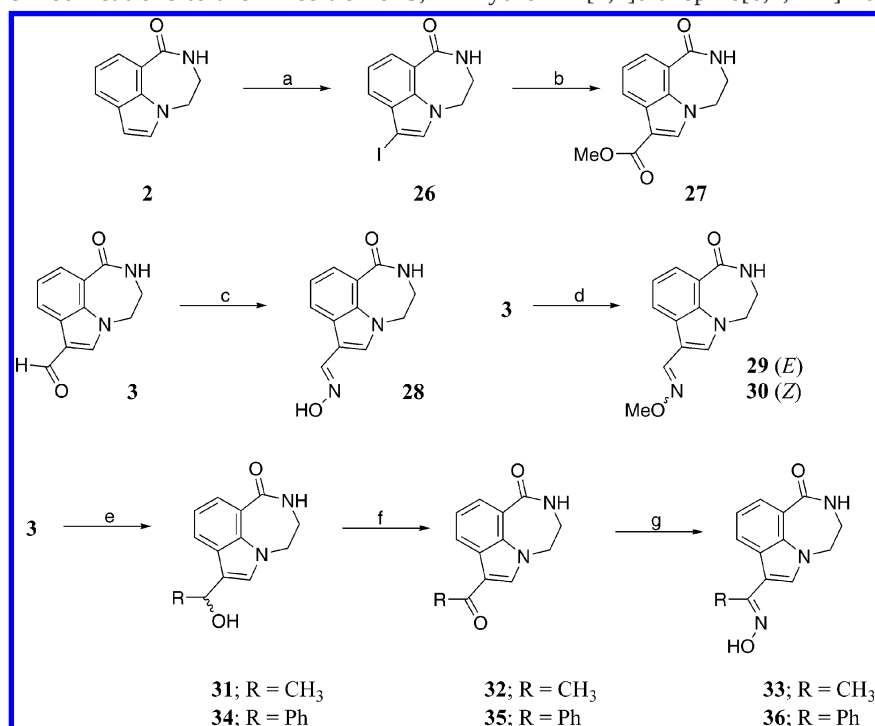
Finally, we were curious to learn whether 6,7-disubstituted derivatives would have additive effects on PARP inhibition. The 3,4-dihydro-2H-[1,4]diazepino[6,7,1-*hi*]indol-1-one tricycle is an ideal ring system that readily allowed us to concurrently access both active site regions of interest via disubstitutions at the 6- and 7-positions.

Chemistry

We initially considered the unsubstituted 3,4-dihydro-2H-[1,4]diazepino[6,7,1-*hi*]indol-1-one **2** to be a versatile intermediate. It was synthesized from **1** using reported procedures.⁴⁰ Formylation or iodination via electrophilic aromatic substitution of **2** at C-7 allowed for the preparation of several derivatives as shown in Schemes 1 and 3. Scheme 1 (method A) depicts the syntheses of key intermediates **2** and **3** and further describes the

preparation of 7-methyl-3,4-dihydro-2H-[1,4]diazepino[6,7,1-*hi*]indol-1-one **5** by the catalytic deoxygenation of the acetate of **4**. Iodide **6**, generated in moderate yield from **5**, was used as a common intermediate in Suzuki type⁴¹ palladium-catalyzed coupling of arylboronic acids to prepare the 6-aryl-7-methyl analogues **7**–**9** listed in Table 3.

Achieving monosubstitution at C-6 and disubstitution at C-6 and C-7 with greater diversity turned out not to be very convenient using **2** as a starting material. Because of synthetic limitations, an alternative synthesis of 3,4-dihydro-2H-[1,4]diazepino[6,7,1-*hi*]indol-1-ones was investigated as outlined in Scheme 2 (method B). Our new strategy relied on indole ring formation in the final step of the construction of the tricyclic core. Starting from 2-iodoaniline, compound **10** was prepared following a literature procedure.⁴² In the same report, however, the authors were unsuccessful in their attempts to convert **10** to 8-iodo-tetrahydro-quinolin-4-one **11** using polyphosphoric acid and only observed position scrambling of iodine and dehalogenation during cyclization.⁴² We were able to circumvent this problem and thus transform **10** to **11** in good yields when Eaton's reagent (P₂O₅/CH₃SO₃H)⁴³ was used. Ring expansion under Schmidt⁴⁴ reaction conditions gave iodo-diazepinone **12** in very good yield. Intermediate **12** was then subjected to tandem palladium-catalyzed acetylene coupling and indole cyclization to afford the tricyclic lactams **14**–**19**.^{45,46} This new route also proved to be a more efficient method to prepare compound **2**. The 6-

Scheme 3. Synthetic Modifications to the 7-Position of 3,4-Dihydro-2*H*-[1,4]diazepino[6,7,1-*hi*]indol-1-ones^a

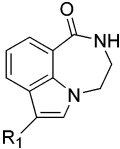
^a Conditions and reagents: (a) I₂, DMF, KOH. (b) CO, Et₃N, catalytic (Ph₃P)₂PdCl₂, DMF, MeOH. (c) NH₂OH·HCl, H₂O, NaOH, EtOH. (d) NH₂OMe·HCl, pyr., EtOH. (e) RLi, THF. (f) *o*-Iodoxybenzoic acid, DMSO. (g) NH₂OH·HCl, pyr.

substituted 3,4-dihydro-2*H*-[1,4]diazepino[6,7,1-*hi*]indol-1-ones prepared are listed in Table 2.

The syntheses of 7-substituted derivatives via the highly versatile 7-formyl or 7-iodo intermediates **3** and **26**, respectively, are described in Scheme 3. Intermediate **3** was directly converted to oximes **28**–**30**, while a three-step sequence was used to synthesize keto-oximes **33** and **36**. Table 1 lists all 7-substituted diazepinoindolones synthesized.

Next, we focused our efforts on the synthesis of 6,7-disubstituted 3,4-dihydro-2*H*-[1,4]diazepino[6,7,1-*hi*]indol-1-ones. Schemes 4 and 5 describe the synthesis of 6,7-disubstituted compounds with functional groups at the C-7 other than methyl and capable of displacing or hydrogen bonding to H₂O-52. As shown in Scheme 4, oximes **38** and **40** were readily prepared from aldehydes **37** and **39**, respectively. Oxime **40** was dehydrated with thiocarbonyldiimidazole to give nitrile **41**, a common intermediate used to prepare amide **42** and thioamide **43**. The methyl thiuronium salt **44** was readily prepared from **43** and further converted to *N*-hydroxyamidine **45** with hydroxylamine or *N*-aminoamidine **46** with hydrazine. Scheme 5 describes the synthesis of 7-acetyl-diazepinoindolone **48** from the corresponding iodide **47**. Acetyl **48** is used to prepare keto-oxime **49** and pyrrazole **51**. The data for the 6,7-disubstituted 3,4-dihydro-2*H*-[1,4]diazepino[6,7,1-*hi*]indol-1-ones prepared are listed in Table 3.

The last sets of compounds, *meta*- and *para*-arylamino-methylene derivatives **20**–**25**, were derived under reductive amination conditions of the appropriate aldehyde. As exemplified in Scheme 6, the *meta*-dimethylaminomethylene analogue **20** was prepared using method C. This route was developed and chosen over method B since it provided a more convenient and higher yielding synthesis of the penultimate aldehydes from common

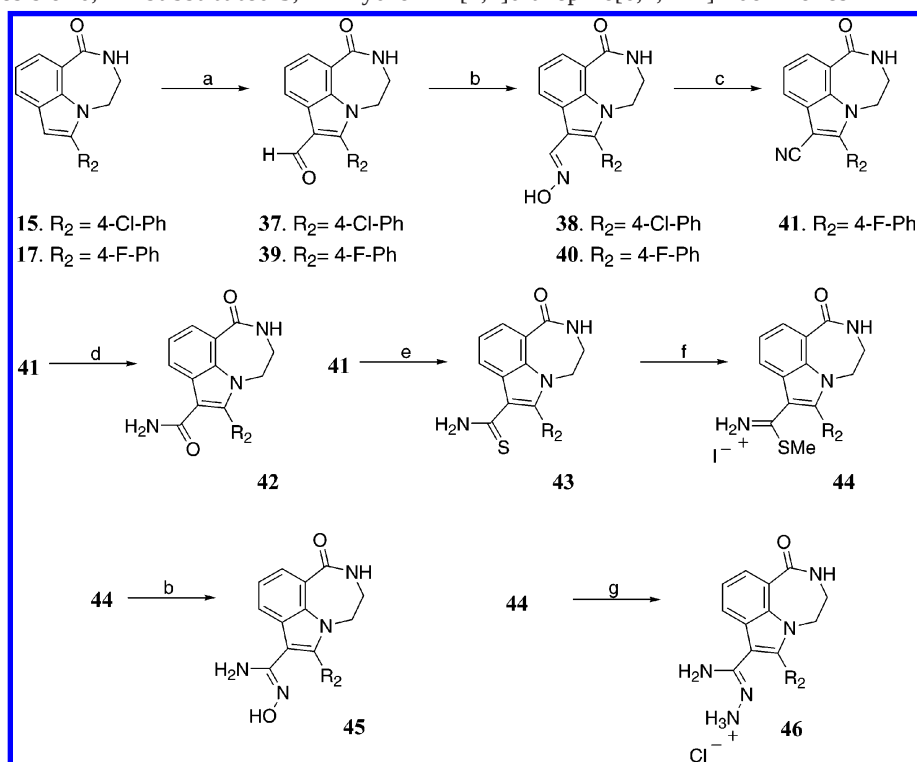
Table 1. 7-Substituted 3,4-Dihydro-2*H*-[1,4]diazepino[6,7,1-*hi*]indol-1-ones


no.	R ₁	mp (°C)	formula ^a	K _i (nM) ^{b,c}
2	H	165–167	C ₁₁ H ₁₀ N ₂ O·0.05H ₂ O	105
3	CHO	238–240	C ₁₂ H ₁₀ N ₂ O ₂ ·0.1H ₂ O	176
4	CH ₂ OH	180–182	C ₁₂ H ₁₂ N ₂ O ₂ ·0.20H ₂ O	79
5	CH ₃	135–137	C ₁₂ H ₁₂ N ₂ O	122
26	I	188–190	C ₁₁ H ₉ N ₂ OI ^a	304
27	COOCH ₃	259–261	C ₁₃ H ₁₂ N ₂ O ₃ ·0.25H ₂ O	900
28	CH=NOH	262–264	C ₁₂ H ₁₁ N ₃ O ₂	9.4
29	(<i>E</i>)-CH=NOMe	173–175	C ₁₃ H ₁₃ N ₃ O ₂ ·0.25H ₂ O	809
30	(<i>Z</i>)-CH=NOMe	210–212	C ₁₃ H ₁₃ N ₃ O ₂ ·0.1H ₂ O ^a	121
31	CH(OH)CH ₃	295–297	C ₁₃ H ₁₄ N ₂ O ₂	692
32	COCH ₃	285–287	C ₁₃ H ₁₂ N ₂ O ₂	606
33	C(CH ₃)=NOH	238–240	C ₁₃ H ₁₃ N ₃ O ₂	39
34	C(OH)C ₆ H ₅	178–180	C ₁₈ H ₁₆ N ₂ O ₂ ·0.25H ₂ O	380
35	COC ₆ H ₅	229–230	C ₁₈ H ₁₄ N ₂ O ₂	337
36	(<i>E</i>) and (<i>Z</i>)-C(C ₆ H ₅)=NOH	263–265	C ₁₈ H ₁₅ N ₃ O ₂ ·0.1H ₂ O	38

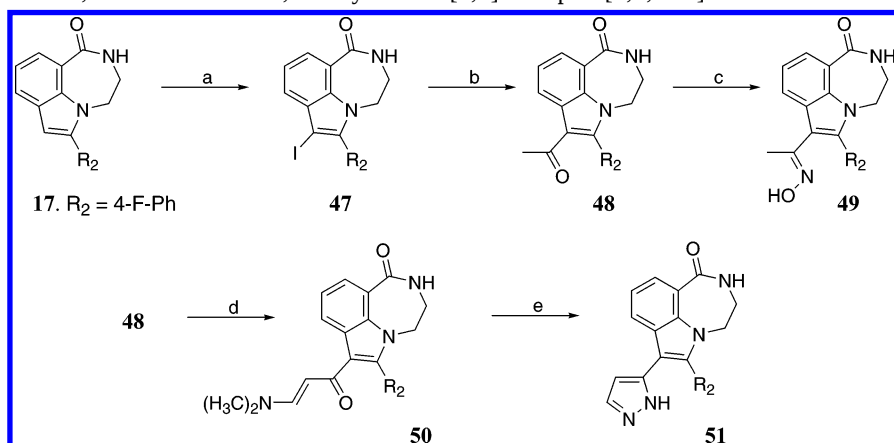
^a Compounds were analyzed for C, H, and N; the results are in agreement to within ±0.4% of the theoretical values except for **26**: C, calcd, 42.33; found, 42.81. Compound **30**: analysis passed only with 0.1EtOAc. ^b Human PARP-1 enzyme inhibition; values are an average of at least two independent experiments; % error ≤ ±20%. ^c See Experimental Section for conditions.

intermediate **53** and commercially available iodo-benzaldehydes.

Enzyme Inhibition and Crystallographic Analysis. Data for the synthesized diazepinoindolinones are reported in Tables 1–3. Enzyme inhibition constants were determined using a previously reported method as described in the Experimental Section.^{30,31,33} Oxime

Scheme 4. Synthesis of 6,7-Disubstituted 3,4-Dihydro-2H-[1,4]diazepino[6,7,1-hi]indol-1-ones^a

^a Conditions and reagents: (a) ex POCl₃, DMF. (b) NH₂OH·HCl, pyr. (c) Thiocarbonyldiimidazole, THF. (d) 85% H₃PO₄, 90–100 °C. (e) H₂S, pyr., Et₃N, 0 °C. (f) CH₃I, THF. (g) NH₂NH₂, CH₃CN, then HCl/MeOH.

Scheme 5. Synthesis of 6,7-Disubstituted 3,4-Dihydro-2H-[1,4]diazepino[6,7,1-hi]indol-1-ones^a

^a Conditions and reagents: (a) I₂, DMF, KOH. (b) H₂C=C(OEt)SnBu₃, (Ph₃P)₄Pd, 2,6-di-*t*-Bu-4-Me-phenol, 1,4-dioxane, DMF. (c) NH₂OH·HCl, pyr. (d) (CH₃)₂NCH(OCH₃)₂, DMF, 110–120 °C. (e) NH₂NH₂·H₂O, THF.

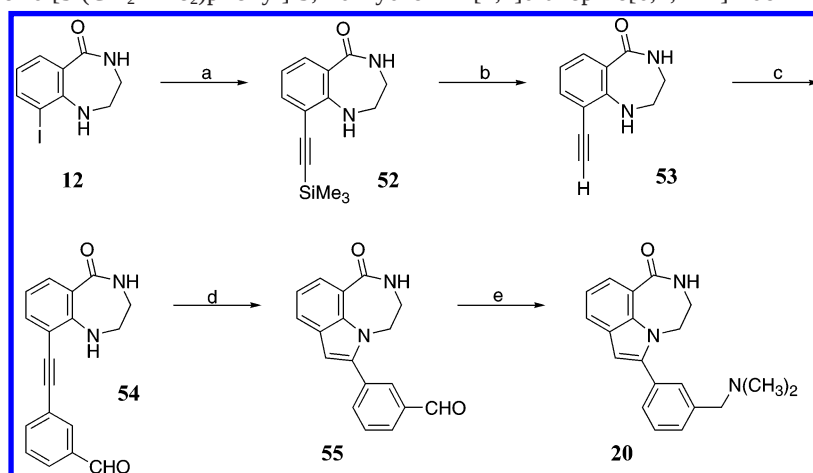
derivatives were the best enzyme inhibitors of all of the C-7 monosubstituted diazapinoindolinones (Table 1). The simplest *trans*-oxime, compound **28**, a 9.8 nM inhibitor of PARP-1, was the most potent derivative in this series with a *K_i* value 11 times lower than the parent compound **2**.

Compound **28** was cocrystallized with the C-terminal catalytic domain of chicken PARP-1 protein, and a 2.8 Å structure was solved (Figures 5 and 6). As anticipated from previous studies, the tricyclic core is wedged between the side chains of Tyr-896 and Tyr-907, while three key hydrogen bonds between the ligand amide and the protein are made.^{30,31} The C-1 lactam carbonyl forms two hydrogen bonds, one to the side chain hydroxyl of Ser-904 and the other to the backbone amide of Gly-863. The N-2 lactam NH hydrogen bonds to the back-

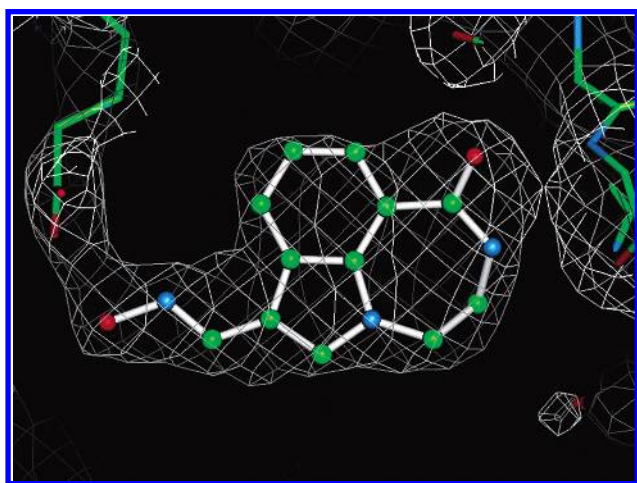
bone carbonyl of Gly-863. The three-dimensional structure also confirmed our modeling. The *E*-oxime displaced a water molecule, and the –OH formed a very tight H-bond to the catalytic γ -carboxylic acid of Glu-988.

The important role this interaction has on binding was confirmed by the loss of affinity upon *O*-methylation of the oxime group. The corresponding *trans*-*O*-methyl analogue **29** has a *K_i* value 86 times greater than **2**, whereas the *cis*-*O*-methyl oxime **30** has a *K_i* about 13 times higher. Models of either **29** or **30** into the crystallographic complex of **28** and PARP-1 suggest that neither compound can be situated in the active site as well as **28**, especially since the *O*-methyl group prevents a direct interaction to Glu-988.

Oximes **33** and **36**, derived from methyl ketone **32** and phenyl ketone **35**, respectively, have *K_i* values four times

Scheme 6. Synthesis of 6-[3-(CH₂NMe₂)phenyl]-3,4-dihydro-2H-[1,4]diazepino[6,7,1-*hi*]indol-1-ones (Method C)^a

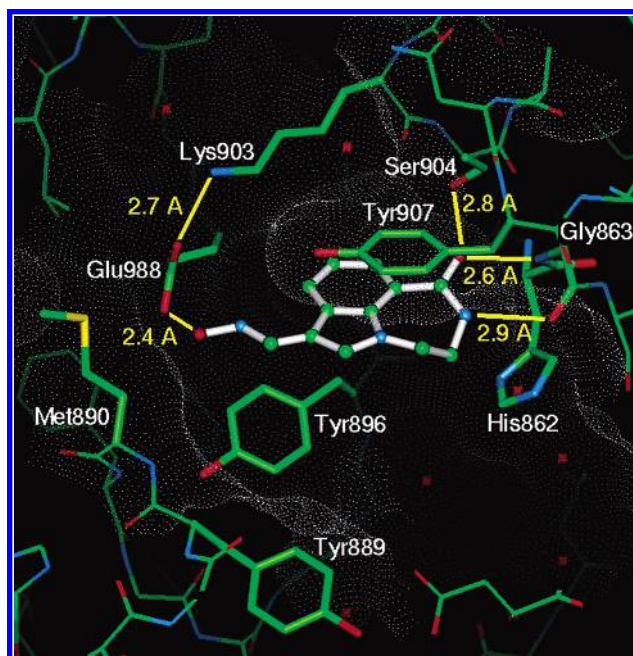
^a Conditions and reagents: (a) Trimethylsilylacetylene, catalytic (Ph₃P)₄Pd, CuI, Et₂NH, DMF. (b) Catalytic K₂CO₃, MeOH. (c) 4-Iodobenzaldehyde, catalytic (Ph₃P)₄Pd, CuI, Et₂NH, DMF. (d) PdCl₂, CH₃CN, 70–80 °C. (e) HN(CH₃)₂, MeOH, NaBH₃CN, HCl, H₂O.

**Figure 5.** Electron density for compound **28** bound to chicken PARP-1. The initial 2.8 Å 2Fo-Fc electron density contoured at 1σ and phased with the PARP-1 protein and solvent model from a previous cocrystal structure.²⁹

greater than **28**. Models of both compounds into the cocrystal structure suggest that there is inadequate space in the active site to accommodate either the methyl or the phenyl substituents, forcing the tricyclic ring to shift, thereby perturbing the optimal binding orientation observed with **28**. Hydroxymethyl derivative **4** was the only compound other than the oximes with a *K_i* value less than parent compound **2**.

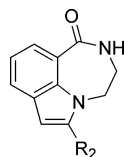
Next, we focused our attention on C-6 monosubstituted diazepinoindolinones. As expected, the addition of various aryl groups at C-6 markedly enhances PARP-1 inhibition. Previous studies also established that potency could be enhanced with *meta*- or *para*-aminomethylene groups while improving aqueous solubility.^{30,31} In general, *para*-aminomethylene derivatives **23**–**25** were indeed three of the most potent enzyme inhibitors prepared. The inhibition data for the C-6 monosubstituted diazepinoindolinones are listed in Table 2.

Examples of 6,7-disubstituted diazepinoindolinones are shown in Table 3. Compound **7**, 7-methyl-6-phenyl-3,4-dihydro-2H-[1,4]diazepino[6,7,1-*hi*]indol-1-one, is closely related structurally to *N*-1-methyl-2-phenyl-1,3,4,5-tetrahydroazepino[5,4,3-*cd*]indol-6-one (**VIIa**), a 7 nM in-

**Figure 6.** X-ray structure complex of **28** and chicken PARP-1 highlighting key protein residues and hydrogen bonds. Ordered water molecules are represented as red crosses, and the solvent-exposed surface of the PARP-1 active site is drawn with white dots.

hibitor of PARP-1 reported by Canan Koch et al.³⁰ However, when comparing their inhibitory activities against PARP-1, **7** has a *K_i* value of 41 nM, approximately six times higher than **VIIa**. Modeling results imply that the reduction in potency may be due, at least in part, to the loss of a weak hydrogen bond to Tyr-907 (CH₃N- -H-O) and a less favorable electrostatic interaction with the aromatic ring of Tyr-907.

We postulated that a 6,7-disubstituted diazepinoindolinone combining the carboxaldehyde of **28** and the 4-fluorophenyl of **17** might provide a significant boost in activity against PARP-1. Analogue **40** is a 6.0 nM inhibitor of PARP-1, only marginally better than either **28** or **17**. Of interest is the 6,7-disubstituted diazepinoindolinone analogue **49**, a combination of the methyl ketoxime of **33** and the 4-fluorophenyl of **17**. Compound **49**, a 9.1 nM enzyme inhibitor, has a *K_i* about four times less than **33**, but when compared to **17**, **28**, or **40**, the

Table 2. 6-Substituted 3,4-Dihydro-2H-[1,4]diazepino[6,7,1-hi]indol-1-ones

	R ₂	mp (°C)	formula ^a	K _i (nM) ^{b,c}
14	C ₆ H ₅	165–167	C ₁₇ H ₁₄ N ₂ O·0.1H ₂ O	14.3
15	4-Cl-C ₆ H ₄	238–240	C ₁₇ H ₁₃ N ₂ OCl	11
16	4-MeO-C ₆ H ₄	188–190	C ₁₈ H ₁₆ N ₂ O ₂ ·0.1H ₂ O	8.2
17	4-F-C ₆ H ₄	198–200	C ₁₇ H ₁₃ N ₂ OF	11
18	(CH ₂) ₂ C ₆ H ₄	188–190	C ₁₉ H ₁₈ N ₂ O	27
19	2-pyridyl	185–187	C ₁₆ H ₁₃ N ₃ O·0.8H ₂ O	68
20	3-[CH ₂ N(CH ₃) ₂]-C ₆ H ₄	98–100	C ₂₀ H ₂₁ N ₃ O·0.25H ₂ O	9.1
21	3-[CH ₂ NHCH ₃]-C ₆ H ₄	128–130	C ₁₉ H ₁₉ N ₃ O·0.6H ₂ O	6.9
22	3-[CH ₂ N-pyrrolyl]-C ₆ H ₄	158–160	C ₂₂ H ₂₃ N ₃ O·0.4H ₂ O	12.5
23	4-[CH ₂ N(CH ₃) ₂]-C ₆ H ₄	140–142	C ₂₀ H ₂₁ N ₃ O·0.3H ₂ O	6.4
24	4-[CH ₂ NHCH ₃]-C ₆ H ₄	178–180	C ₁₉ H ₁₉ N ₃ O·0.1H ₂ O	3.8
25	4-[CH ₂ N-pyrrolyl]-C ₆ H ₄	146–148	C ₂₂ H ₂₃ N ₃ O·0.25H ₂ O	6.8
55	3-CHO-C ₆ H ₄	192–194	C ₁₈ H ₁₄ N ₂ O ₂ ·0.25H ₂ O	ND

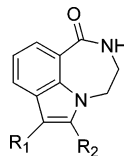
^a Compounds were analyzed for C, H, and N; the results are in agreement to within ±0.4% of the theoretical values. ^b Human PARP-1 enzyme inhibition; values are an average of at least two independent experiments; % error ≤ ±20%. ^c See Experimental Section for conditions.

K_i values are relatively equal. Other 6-aryl-diazepinoindolones with various functional groups at the 7-position capable of forming H-bonds directly to Glu-988, or via the mobile bridging water molecule, were investigated. None of these, including amide **42**, *N*-hydroxy-amidine **45**, and *N*-aminoamidine **46**, showed enhanced binding to PARP-1 when compared to oximes **38**, **40**, and **49** or to the simpler 6-aryl- or 7-carboxaldehyde monosubstituted diazepinoindolones.

In Vitro Cellular Growth Inhibition; Enhancement of Topotecan (TP) Cytotoxicity. To evaluate

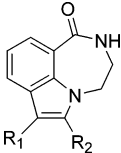
their possible use as resistance-modifying agents, selected 3,4-dihydro-2H-[1,4]diazepino[6,7,1-hi]indol-1-one PARP-1 inhibitors were evaluated for potentiation of topoisomerase I inhibitor TP cytotoxicity against human lung carcinoma A549 cells. Alone, diazepinoindolone PARP-1 inhibitors did not inhibit A549 cell growth at 400 nM, the standard concentration selected to investigate the potentiation of TP. This concentration is well below the IC₅₀ values of those compounds tested, except for compound **40**, where the single agent IC₅₀ was less than twice the standard concentration used in the potentiation assay. The potentiation factor is reported as a PF₅₀. PARP-1 inhibitors with a PF₅₀ value >1 increase the growth inhibitory effects of TP to that degree, while inhibitors with values = 1 have no effect.^{30,31} As learned from previous studies, a correlation between the K_i and the PF₅₀ value does not exist; however, low nanomolar inhibition of PARP-1 is undoubtedly required for potentiation. Of the inhibitors tested, compound **23** provided the best enhancement, reducing the IC₅₀ of TP by a factor of 1.9. The data are listed in Table 4.

In summary, 3,4-dihydro-2H-[1,4]diazepino[6,7,1-hi]indol-1-ones (**IX**), a third generation of novel 5,6,7-tricyclic PARP-1 inhibitors with structural homology to the 3,4,5,6-tetrahydro-1*H*-azepino[5,4,3-*cd*]indol-6-ones (**VII**) and 8,9-dihydro-7*H*-2,7,9a-triaza-benzo[*cd*]azulen-6-ones (**VIII**), were designed with the aid of protein structure-based drug design, molecular modeling, and SAR. An efficient synthesis was developed to prepare various C-7-substituted molecules that allowed us to study additional intermolecular interactions within the catalytic site, particularly with Glu-988. The C-7 carboxaldehyde derivatives are some of the most potent PARP-1 inhibitors reported to date. The cocrystal structure of compound **28** bound to the PARP-1 validated our design process. The combination of an oxime

Table 3. 6,7-Disubstituted 3,4-Dihydro-2H-[1,4]diazepino[6,7,1-hi]indol-1-ones

no.	R ₁	R ₂	mp (°C)	formula ^a	K _i (nM) ^{b,c}
7	CH ₃	C ₆ H ₅	278–280	C ₁₈ H ₁₆ N ₂ O·0.4H ₂ O	41
8	CH ₃	4-F-C ₆ H ₄	270–272	C ₁₈ H ₁₅ N ₂ OF·0.1H ₂ O	22
9	CH ₃	3-CF ₃ -C ₆ H ₄	258–260	C ₁₉ H ₁₅ N ₂ OF ₃ ^d	79
37	CHO	4-Cl-C ₆ H ₄	278–279	C ₁₈ H ₁₃ N ₂ O ₂ Cl·0.25H ₂ O	43
38	CH=NOH	4-Cl-C ₆ H ₄	249–251	C ₁₈ H ₁₄ N ₂ O ₂ Cl ^a	7.5
39	CHO	4-F-C ₆ H ₄	268–270	C ₁₈ H ₁₃ N ₂ O ₂ F ^e	ND
40	CH=NOH	4-F-C ₆ H ₄	277–279	C ₁₈ H ₁₄ N ₂ O ₂ F·0.3H ₂ O ^a	6
41	CN	4-F-C ₆ H ₄	248–250	C ₁₈ H ₁₂ N ₃ OF·0.1H ₂ O	54
42	CONH ₂	4-F-C ₆ H ₄	287–289	C ₁₈ H ₁₄ N ₃ O ₂ F·0.5H ₂ O	200
43	CSNH ₂	4-F-C ₆ H ₄	238–240	C ₁₈ H ₁₄ N ₃ OSF·0.4H ₂ O	219
45	CH=NOHNH ₂	4-F-C ₆ H ₄	257–259	C ₁₈ H ₁₅ N ₄ O ₂ F·0.5H ₂ O	87
46	CH=NHNNH ₂	4-F-C ₆ H ₄	272–274	C ₁₈ H ₁₆ N ₅ OF·1H ₂ O ^a	57
47	I	4-F-C ₆ H ₄	283–285	C ₁₇ H ₁₂ NOIF	ND
48	COCH ₃	4-F-C ₆ H ₄	275–276	C ₁₉ H ₁₅ N ₂ O ₂ F ^f	306
49	C(CH ₃)=NOH	4-F-C ₆ H ₄	248–250	C ₁₉ H ₁₆ N ₃ O ₂ F·0.1H ₂ O	9.1
51	2-pyrazolyl	4-F-C ₆ H ₄	173–175	C ₂₀ H ₁₅ N ₄ OF ^a	249

^a Compounds were analyzed for C, H, and N; the results are in agreement to within ±0.4% of the theoretical values except for **38**: analysis passed only with 0.75CH₂Cl₂. Compound **40**: N, calcd, 12.78; found, 12.32. Compound **46**: analysis passed only with 4.5HCl. Compound **51**: analysis passed only with 1.0MeOH. ^b Human PARP-1 enzyme inhibition; values are an average of at least two independent experiments; % error ≤ ±20%. ^c See Experimental Section for method. ^d 90% pure by HPLC. ^e 99% pure by HPLC. ^f 98% pure by HPLC.

Table 4. Potentiation of TP-Induced Growth Inhibition by 6,7-Disubstituted 3,4-Dihydro-2H-[1,4]diazepino[6,7,1-*hi*]indol-1-ones in A549 Cells


no.	R ₁	R ₂	K _i (nM) ^a	IC ₅₀ (alone) (nM) ^b	IC ₅₀ (+TP) (nM) ^c	PF ₅₀ ^d
8	CH ₃	4-F-C ₆ H ₄	22	6000	21.8	1.1
15	H	4-Cl-C ₆ H ₄	11	3000	13.3	1.8
17	H	4-F-C ₆ H ₄	11	8000	13.3	1.8
20	H	3-[CH ₂ N(CH ₃) ₂]C ₆ H ₄	9.1	11000	15.0	1.6
23	H	4-[CH ₂ N(CH ₃) ₂]C ₆ H ₄	6.4	7000	12.6	1.9
28	CH=NOH	H	9.4	8000	18.5	1.3
40	CH=NOH	4-F-C ₆ H ₅	6	<800	20.0	1.2
TP				24		

^a Human PARP-1 enzyme inhibition; values are an average of at least two independent experiments; % error $\leq \pm 20\%$. ^b Cytotoxicity of single agent. ^c Cytotoxicity of TP plus 0.4 μM corresponding PARP-1 inhibitor. ^d $\text{PF}_{50} = (\text{IC}_{50} \text{ of TP})/(\text{IC}_{50} \text{ of TP} + 0.4 \mu\text{M PARP-1 inhibitor})$. ^{a-d} See Experimental Section for methods.

at C-6 and an aryl group at C-7 did not produce a substantial increase in PARP-1 binding potency or in vitro potentiation of TP cytotoxicity. Further crystallographic studies may help lead to the design of an improved class of 3,4-dihydro-2H-[1,4]diazepino[6,7,1-*hi*]indol-1-one PARP-1 inhibitors with better cytotoxic potentiation.

Experimental Section

Proton magnetic resonance spectra (NMR) were determined using a Bruker Avance 300DPX or 500DRX spectrometer operating at a field strength of 300 and 500 MHz, respectively. Chemical shifts are reported in parts per million (δ) with references set such that in CDCl_3 the CHCl_3 is at 7.26 ppm, in acetone- d_6 the acetone is at 2.02 ppm, and in $\text{DMSO}-d_6$ the DMSO is at 2.49 ppm. Standard and peak multiplicities are designated as follows: s, singlet; doublet; dd, doublet of doublets; t, triplet; q, quartet; br s, broad singlet; and m, multiplet. Mass spectra were determined at the University of California, Riverside, or Scripps Research Institute, San Diego, CA, Mass Spectrometry Facilities. Infrared absorption (IR) spectra were taken on a MIDAC Corp. FTIR or a Perkin-Elmer 1600 series FTIR spectrometer. Elemental microanalyses were performed by Atlantic Microlab Inc. (Norcross, GA) and gave results for the elements stated with $\pm 0.4\%$ of the theoretical values. Analytical HPLC was performed using a Hewlett-Packard (HP) Series 1100 Quaternary system, equipped with a HP 1100 variable wavelength detector set at 254 nm; sensitivity, 0.02–50 AUFS. A Phenomenex Prodigy 5 ODS (3) column (250 mm $\times$ 4.6 mm; 5 μm) was used. Typically, a gradient mobile phase starting with 90% H_2O with 0.1% TFA, 10% CH_3CN with 0.1% TFA up to 20 min (min), then 35% H_2O with 0.1% TFA, 65% CH_3CN with 0.1% TFA up to 25 min, then 10% H_2O with 0.1% TFA, 90% CH_3CN with 0.1% TFA thereafter was used. Flow rate = 1 mL/min. Preparative HPLC was performed using Gilson model 806 Manometric module, equipped with a Gilson 811c dynamic mixer, two Gilson model 306 pumps, a Gilson 215 liquid handler, and a Gilson model 119 UV/visible detector set at 2145 or 220 and 254 nm; sensitivity, 0.02–50 AUFS. A Metasil AQ C18 column (250 mm $\times$ 212 mm; 10 μm) was used. Typically, a gradient mobile phase starting with 90% H_2O with 0.1% TFA, 10% CH_3CN with 0.1% TFA up to 2 min, then reaching 35% H_2O with 0.1% TFA, 65% CH_3CN with 0.1% TFA after 22 min or 90% 0.1M

NH_4OAc , 10% CH_3CN up to 2 min, then reaching 100% CH_3CN after 22 min, was used. Flow rate = 25 mL/min. Flash column chromatography was performed using silica gel 60 (Merck Art 9385). Thin layer chromatographs were performed on pre-coated sheets of silica 60 F₂₅₄ (Merck Art 5719). Melting points were determined on a Mel-Temp apparatus and are uncorrected. All commercial solvents were reagent grade or better and used as supplied. All reactions were performed in septum-sealed flasks under a slight positive pressure of argon, unless otherwise noted.

PARP Enzyme Inhibition Assay. The PARP enzyme-inhibiting activities of the compounds were assayed as described by Simonin et al.⁴⁷ and Marsischky et al.³³ with minor modifications as follows. Samples (50 μL) containing 20 nM purified PARP protein, 10 $\mu\text{g/mL}$ DNase I-activated calf thymus DNA (sigma), 500 μM NAD^+ , 0.5 μCi [^{32}P]NAD⁺, 2% DMSO, and various concentrations of test compounds were incubated in sample buffer [50 mM Tris, pH 8.0, 10 mM MgCl_2 , and 1 mM tris(carboxyethyl)phosphine HCl] at 25 $^\circ\text{C}$ for 4 min. Under these conditions, the reaction rate was linear up to 10 min. The reaction was stopped by the addition of an equal volume of ice-cold 40% trichloroacetic acid. The samples were then incubated on ice for 15 min, transferred to a Bio-Dot microfiltration apparatus (Biorad), and filtered through Whatman GF/C glass-fiber filter paper. Filters were washed three times with 150 μL of wash buffer (5% trichloroacetic acid and 1% inorganic pyrophosphate) and dried. [^{32}P]ADP-ribose incorporation into the acid insoluble material was quantified using a PhosphorImager (Molecular Dynamics) and Image-Quant software. Inhibition constants (K_i) were calculated by nonlinear regression analyses using the velocity equation for competitive inhibition.⁴⁸ In the case of tight-binding inhibitors, 5 nM enzyme was used and the reaction was incubated at 25 $^\circ\text{C}$ for 15 min. K_i values for tight-binding inhibitors were calculated using the equation described by Sculley et al.⁴⁹

Potentiation of TP Growth Inhibition Assay. A549 cells (ATCC, Rockville, MD) were seeded into 96 well cell culture plates (Falcon brand, Fisher Scientific, Pittsburgh, PA) 16–24 h before experimental manipulation. The cells were then treated with a test compound (or a combination of test compounds where indicated) each at a concentration of 0.4 μM for either 3 or 5 days. At the end of treatments, the relative cell number was determined by either MTT assay or SRB assay. For the MTT assay, 0.2 $\mu\text{g}/\mu\text{L}$ of MTT [3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide] (Sigma Chemical Co., St. Louis, MO) was added to each well of a plate, and the plate was incubated in a cell culture incubator for 4 h. Metabolized MTT in each well was solubilized in 159 μL of DMSO (Sigma Chemical Co.) with shaking and quantified with a Wallac 1420 Victor plate reader (EG&G Wallad, Gaithersburg, MD) at 540 nm. For the SRB assay, the cells were fixed with 10% trichloroacetic acid (Sigma Chemical Co.) for an hour at 4 $^\circ\text{C}$. After they were extensively washed, fixed cells were stained for 30 min with 0.4% sulforhodamine B (SRB, Sigma Chemical Co.) in 1% acetic acid (Sigma Chemical Co.). Unbound SRB was washed away with 1% acetic acid. Then, the cultures were air-dried, and bound dye was solubilized with 10 mM unbuffered Tris base (Sigma Chemical Co.) with shaking. The bound dye was measured photometrically with the Wallac Victor plate reader at 515 nm. The ratio of the OD (optical density) value of a compound-treated culture to the OD value of a mock-treated culture, expressed in percentage, was used to quantify the cytotoxicity of a compound. The concentration at which a compound causes 50% cytotoxicity is referred to as IC_{50} . To quantify the potentiation of the cytotoxicity of TP by test compounds, a dimensionless parameter PF_{50} is used and defined as the ratio of the IC_{50} of TP alone to the IC_{50} of TP in combination with a test compound.

Crystallographic Methods. Purified protein and cocrystals for the C-terminal catalytic domain of chicken PARP-1 were obtained using procedures similar to those previously described. In this study, 100 mM Tris, pH 8.5, 16–32% PEG-600, and 8% isopropyl alcohol were used as a precipitant. X-ray diffraction data were collected with a MAR345 image plate to

2.8 Å resolution. The space group is $P2_12_12_1$, with $a = 59.31$ Å, $b = 63.80$ Å, and $c = 96.93$ Å. The 19100 observations were scaled and merged into 8942 unique reflections using DENZO and SCALEPACK. The overall R -merge was 6.0%, the ratio $I/\sigma(I)$ was 14.2, and the data were 94% complete. Corresponding values for the high-resolution data shell (2.9–2.8 Å) were 16.4, 4.7, and 91.3%, respectively. The structure of **28** bound to chicken PARP-1 was solved and refined using these data, the program CNX,⁵⁰ and the 2.2 Å protein and solvent model.²⁹ The conventional and free R -factors for the refined model are 16.5 and 24%. The rms deviations between model and ideal bond distances, bond angles, dihedral angles, and improper angles are 0.007 Å, 1.3°, 22.7°, and 0.8°.

Computational Methods. Molecular modeling of inhibitors with a chimeric human PARP-1 model was performed with MacroModel version 5.5.⁵¹ The model was created from the X-ray structure of compound **28** complexed with chicken PARP-1; however, residue 763 was changed from a glutamine to a glutamate. A Monte Carlo conformational search of the Glu-763 side chain was performed using the Amber* force field.

Method A. 3,4,6,7-Tetrahydro-2H-[1,4]diazepino[6,7,1-hi]indol-1-one (1).⁴⁰ ¹H NMR (DMSO- d_6): δ 2.92 (t, 2H, $J = 7.5$ Hz), 3.29–3.31 (m, 4H), 3.47 (t, 2H, $J = 7.5$ Hz), 6.49 (t, 1H, $J = 7.5$ Hz), 7.04 (d, 1H, $J = 7.5$ Hz), 7.49 (d, 1H, $J = 7.5$ Hz), 7.86 (br s, 1H). HRMS calcd for $C_{11}H_{12}N_2O$ (M^+), 188.0950; found (M^+), 188.0957. Anal. ($C_{11}H_{12}N_2O$) C, H, N.

3,4-Dihydro-2H-[1,4]diazepino[6,7,1-hi]indol-1-one (2).⁴⁰ mp 165–167 °C. ¹H NMR (DMSO- d_6): δ 3.52–3.56 (m, 2H), 4.31–4.36 (m, 2H), 6.53 (d, 1H, $J = 3.0$ Hz), 7.11 (t, 1H, $J = 6.0$ Hz), 7.38 (d, 1H, $J = 3.0$ Hz), 7.70 (d, 1H, $J = 6.0$ Hz), 7.80 (d, 1H, $J = 6.0$ Hz), 8.30 (br s, 1H). LRMS (M^+) 186. Anal. ($C_{11}H_{10}N_2O \cdot 0.05H_2O$) C, H, N.

1-Oxo-1,2,3,4-tetrahydro[1,4]diazepino[6,7,1-hi]indole-7-carbaldehyde (3). POCl₃ (16.37 g, 106.76 mmol) was slowly added to DMF (225 mL) at 0 °C. The mixture was stirred for 15 min and then was treated with a solution of **2** (1.46 g, 7.85 mmol) in DMF (10 mL). The reaction mixture was warmed to room temperature and stirred for 17 h. After all of the solvent was removed, the residue was taken up in H₂O, made basic (pH 12) using 20% aqueous NaOH, and extracted with EtOAc several times. The organic layer was dried over anhydrous MgSO₄ and concentrated to give a pale yellow solid, which was used without further purification; mp 238–240 °C. ¹H NMR (DMSO- d_6): δ 3.58–3.61 (m, 2H), 4.48 (br s, 2H), 7.37 (t, 1H, $J = 7.5$ Hz), 7.97 (d, 1H, $J = 7.5$ Hz), 8.33–8.35 (m, 2H), 8.43–8.45 (m, 1H), 9.95 (s, 1H). HRMS calcd for $C_{12}H_{10}N_2O_2$ (M^+), 214.0742; found (M^+), 214.0737. Anal. ($C_{12}H_{10}N_2O_2 \cdot 0.1H_2O$) C, H, N.

7-Hydroxymethyl-3,4-dihydro-2H-[1,4]diazepino[6,7,1-hi]indol-1-one (4). Sodium borohydride (0.018 g, 0.466 mmol) was added to a suspension of **3** (0.050 g, 0.233 mmol) in 15 mL EtOH. The reaction mixture was refluxed for 1.5 h and cooled, and the solvent was evaporated. The residue was partitioned between 1% aqueous NaOH and EtOAc. The organic extract was dried over anhydrous MgSO₄ and evaporated to give a pale yellow solid (88%); mp 180–182 °C. ¹H NMR (DMSO- d_6): δ 3.52–3.55 (m, 2H), 4.31 (br s, 2H), 4.63 (d, 2H, $J = 5$ Hz), 4.84 (t, 1H, $J = 5$ Hz), 7.12 (t, 1H, $J = 7.5$ Hz), 7.29 (s, 1H), 7.80–7.83 (m, 2H), 8.24–8.26 (m, 1H). HRMS calcd for $C_{12}H_{12}N_2O_2$ (M^+), 216.0899; found (M^+), 216.0908; Anal. ($C_{12}H_{12}N_2O_2 \cdot 0.2H_2O$) C, H, N.

Acetic Acid 1-Oxo-1,2,3,4-tetrahydro[1,4]diazepino[6,7,1-hi]indol-7-ylmethyl Ester (4a). 4-(Dimethylamino)pyridine (0.057 g, 0.466 mmol) was added to a solution of the alcohol **4** (1.007 g, 0.466 mmol) in a mixture of acetic anhydride (1.1 mL, 11.65 mmol) and pyridine (25 mL). The mixture was stirred for 15 h at room temperature, and then, the solvent was evaporated. The residue was purified by flash silica gel chromatography eluting with a gradient of 0–3% MeOH in CHCl₃ to give 0.925 g (77%) of the acetate. ¹H NMR (DMSO- d_6): δ 2.0 (s, 3H), 3.42–3.44 (br s, 2H), 4.23–4.25 (br s, 2H), 5.30 (s, 2H), 9.10 (t, 1H, $J = 7.5$ Hz), 7.50 (s, 1H), 7.75 (d, 1H, $J = 7.5$ Hz), 7.85 (d, 1H, $J = 7.5$ Hz), 8.30 (m, 1H).

7-Methyl-3,4-dihydro-2H-[1,4]diazepino[6,7,1-hi]indol-1-one (5). Acetate **4a** (0.508 g, 1.97 mmol) was dissolved in MeOH (70 mL) and glacial acetic acid (30 mL). Pd/C (10%) (0.076 g) was added, and the suspension was stirred under an atmosphere of H₂ for 4.5 h at room temperature. The black suspension was filtered, and the filtrate was concentrated to give a white solid, which was purified by flash silica gel chromatography eluting with a gradient of 0–1% MeOH in CHCl₃ to give 0.296 g (75%) of the product; mp 135–137 °C. ¹H NMR (DMSO- d_6): δ 2.52 (s, 3H), 3.51–3.54 (m, 2H), 4.27–4.28 (m, 2H), 7.11 (t, 1H, $J = 7.5$ Hz), 7.15 (s, 1H), 7.69 (d, 1H, $J = 7.5$ Hz), 7.81 (d, 1H, $J = 7.5$ Hz), 8.22–8.24 (m, 1H). HRMS calcd for $C_{12}H_{12}N_2O$ (M^+), 200.0950; found (M^+), 200.0955. Anal. ($C_{12}H_{12}N_2O$) C, H, N.

6-Iodo-7-methyl-3,4-dihydro-2H-[1,4]diazepino[6,7,1-hi]indol-1-one (6). To a solution of **5** (0.030 g, 0.150 mmol) in CH₂Cl₂ (5 mL) was added iodine (0.038 g, 0.150 mmol) and bis(trifluoroacetoxy)iodobenzene (0.077 g, 0.180 mmol). The reaction mixture was stirred at room temperature for 5 min, diluted with CH₂Cl₂, and washed with 10% Na₂S₂O₃. The organic layer was dried over anhydrous MgSO₄ and concentrated. The residue was purified by flash silica gel chromatography eluting with a gradient of 0–1% MeOH in CHCl₃ to give 0.026 g (53%) of the desired product. ¹H NMR (DMSO- d_6): δ 2.20 (s, 3H), 3.33–3.35 (br s, 2H), 4.32–4.35 (br s, 2H), 7.10 (t, 1H, $J = 7.5$ Hz), 7.60 (d, 1H, $J = 7.5$ Hz), 7.80 (d, 1H, $J = 7.5$ Hz), 8.30 (br s, 1H).

7-Methyl-6-phenyl-3,4-dihydro-2H-[1,4]diazepino[6,7,1-hi]indol-1-one (7). To a solution of the iodide **6** (0.024 g, 0.736 mmol) in DMF (3 mL) was added phenylboronic acid (0.010 g, 0.0810 mmol), Na₂CO₃ (0.020 g, 0.184 mmol) dissolved in minimum H₂O, LiCl (0.010 g, 0.221 mmol), and tetrakis(triphenylphosphine) palladium (5.0 mg, 0.0037 mmol) at room temperature. The reaction mixture was stirred at 80–90 °C for 19 h, and then, the solvent was evaporated. The residue was taken up in H₂O and extracted with EtOAc several times. The combined organic layers were dried over anhydrous MgSO₄ and concentrated to give a brown solid. The crude mixture was purified by flash silica gel chromatography eluting with a gradient of 0–1% MeOH in CHCl₃ to give 0.014 g (70%) of the product; mp 278–280 °C. ¹H NMR (DMSO- d_6): δ 2.23 (s, 3H), 3.46 (br s, 2H), 4.13 (br s, 2H), 7.17 (t, 1H, $J = 7.5$ Hz), 7.45–7.56 (m, 5H), 7.76 (d, 1H, $J = 7.5$ Hz), 7.84 (d, 1H, $J = 7.5$ Hz), 8.29–8.31 (m, 1H). LRMS (M^+) 276. Anal. ($C_{18}H_{16}N_2O \cdot 0.4H_2O$) C, H, N.

Compounds **8** and **9** were prepared from **6** using method A.

6-(4-Fluorophenyl)-7-methyl-3,4-dihydro-2H-[1,4]diazepino[6,7,1-hi]indol-1-one (8). White solid (73%); mp 270–272 °C. ¹H NMR (DMSO- d_6): δ 2.77 (s, 3H), 3.74 (br s, 2H), 3.39–4.37 (m, 2H), 7.45 (t, 1H, $J = 7.5$ Hz), 7.63–7.67 (m, 2H), 7.81–7.83 (m, 2H), 8.04 (d, 1H, $J = 7.5$ Hz), 8.12 (d, 1H, $J = 7.5$ Hz), 8.57–8.59 (m, 1H). HRMS calcd for $C_{18}H_{15}N_2OF$ (M^+), 294.1168; found (M^+), 294.1175. Anal. ($C_{18}H_{15}N_2OF \cdot 0.1H_2O$) C, H, N.

7-Methyl-6-(3-trifluoromethyl-phenyl)-3,4-dihydro-2H-[1,4]diazepino[6,7,1-hi]indol-1-one (9). White solid (81%); mp 258–260 °C. ¹H NMR (DMSO- d_6): δ 2.25 (s, 3H), 3.44–3.48 (m, 2H), 4.13–4.16 (m, 2H), 7.19 (t, 1H, $J = 7.5$ Hz), 7.77–7.88 (m, 6H), 8.32–8.36 (m, 1H). HRMS calcd for $C_{19}H_{15}N_2OF_3$ (M^+), 344.136; found (M^+), 344.136. Anal. HPLC $R_t = 14.9$ min. Purification done using preparative HPLC. A gradient mobile phase, starting with 90% 0.1 M NH₄OAc, 10% CH₃CN up to 2 min, then reaching 100% CH₃CN after 22 min, was used. $R_t = 17.59$ min.

Method B. 8-Iodo-2,3-dihydro-1H-quinolin-4-one (11). A mixture of the acid 3-(2-iodo-phenylamino)propionaldehyde carboxylic acid **10**⁴² (0.103 g, 0.354 mmol) in 7% P₂O₅:CH₃SO₃H (Eaton's reagent)⁴³ (2 mL) was heated at 60–70 °C for 3 h. Ice-cold water was added to the reaction mixture, made basic (pH 12) with 50 wt % NaOH, and extracted with EtOAc several times. The combined organic extracts were dried over anhydrous MgSO₄ and concentrated to give 0.070 g (72%) of the product as orange oil. The product was used without further purification. ¹H NMR (CDCl₃): δ 2.71 (t, 2H, $J = 6.0$ Hz), 3.65

(t, 2H, $J = 6.0$ Hz), 4.86 (br s, 1H), 6.50 (t, 1H, $J = 9.0$ Hz), 7.79 (d, 1H, $J = 9.0$ Hz), 7.85 (d, 1H, $J = 9.0$ Hz). LRMS (M^+) 272.

9-Iodo-1,2,3,4-tetrahydro-benzo[e][1,4]diazepin-5-one (12). To a solution of **11** (3.47 g, 0.0127 mmol) in $\text{CH}_3\text{SO}_3\text{H}$ (50 mL) was added NaN_3 (1.074 g, 0.0165 mmol) slowly at room temperature. The reaction mixture was stirred at room temperature for 0.5 h. Upon consumption of starting material by TLC, ice-cold water was added to the reaction mixture and made basic (pH 13) using 50 wt % NaOH whereupon the product (3.05 g, 83%) precipitates. The solids were filtered, washed with water, and dried; mp 182–184 °C. ^1H NMR ($\text{DMSO}-d_6$): δ 3.25–3.27 (m, 2H), 3.48 (br s, 2H), 5.43 (br s, 1H), 6.41 (t, 1H, $J = 6.0$ Hz), 7.73 (d, 1H, $J = 6.0$ Hz), 7.80 (d, 1H, $J = 6.0$ Hz), 8.15 (br s, 1H). LRMS (M^+) 288.

9-Phenylethynyl-1,2,3,4-tetrahydro-benzo[e][1,4]diazepin-5-one (13a). A mixture of **12** (0.144 g, 0.5 mmol), phenylacetylene (0.055 mL, 0.5 mmol), tetrakis(triphenylphosphine) palladium chloride (6 mg, 0.005 mmol), CuI (2 mg, 0.01 mmol) in diethylamine (4 mL), and DMF (2 mL) was stirred at room temperature for 2 h. The solvent was evaporated to dryness, and the residue was taken in water and extracted with EtOAc. The organic extract was dried over anhydrous MgSO_4 and concentrated. The crude mixture was purified by flash silica gel chromatography eluting with a gradient of 0–3% MeOH in CHCl_3 to give 0.102 g (78%) of the desired product. ^1H NMR ($\text{DMSO}-d_6$): δ 3.27–3.29 (m, 2H), 3.53–3.56 (m, 2H), 6.26 (t, 1H, $J = 6.0$ Hz), 6.61 (t, 1H, $J = 6.0$ Hz), 7.40–7.47 (m, 4H), 7.62–7.65 (m, 2H), 7.80 (d, 1H, $J = 6.0$ Hz), 8.13 (t, 1H, $J = 6.0$ Hz). LRMS (M^+) 262. IR (KBr) 3400, 3190, 3051, 1641, 1589, 1518, 1446, 1250, 756, 690 cm^{-1} .

6-Phenyl-3,4-dihydro-2H-[1,4]diazepino[6,7,1-*hi*]indol-1-one (14). To a clear solution of **13a** (0.08 g, 0.305 mmol) in CH_3CN (10 mL) was added PdCl_2 (3.0 mg, 0.0153 mmol) at room temperature. The reaction mixture was heated at 70–80 °C for 3.5 h. Upon consumption of starting material (TLC), the solvent was evaporated and the crude residue was purified by flash silica gel chromatography eluting with a gradient of 0–3% MeOH in CHCl_3 to give 0.058 g (73%) of the desired product; mp 165–167 °C. ^1H NMR ($\text{DMSO}-d_6$): δ 3.46–3.51 (m, 2H), 4.31–4.33 (m, 2H), 6.71 (s, 1H), 7.17 (t, 1H, $J = 9.0$ Hz), 7.42–7.55 (m, 3H), 7.60–7.63 (m, 2H), 7.78 (d, 1H, $J = 9.0$ Hz), 7.82 (d, 1H, $J = 9.0$ Hz), 8.38 (t, 1H, $J = 6.0$ Hz). HRMS calcd for $\text{C}_{17}\text{H}_{14}\text{N}_2\text{O}$ (M^+), 262.1106; found (M^+), 262.1109. Anal. ($\text{C}_{17}\text{H}_{14}\text{N}_2\text{O} \cdot 0.1\text{H}_2\text{O}$) C, H, N.

Compounds **15–18** were made using method B.

6-(4-Chloro-phenyl)-3,4-dihydro-2H-[1,4]diazepino[6,7,1-*hi*]indol-1-one (15). Pale yellow solid (69%); mp 238–240 °C. ^1H NMR ($\text{DMSO}-d_6$): δ 3.47–3.50 (m, 2H), 4.29–4.32 (m, 2H), 6.74 (s, 1H), 7.18 (t, 1H, $J = 9.0$ Hz), 7.58 (d, 2H, $J = 9.0$ Hz), 7.65 (d, 2H, $J = 9.0$ Hz), 7.80 (d, 2H, $J = 9.0$ Hz), 7.83 (d, 2H, $J = 9.0$ Hz), 8.39 (t, 1H, $J = 4.5$ Hz). HRMS calcd for $\text{C}_{17}\text{H}_{13}\text{N}_2\text{OCl}$ (M^+), 296.0716; found (M^+), 296.0715. Anal. ($\text{C}_{17}\text{H}_{13}\text{N}_2\text{OCl}$) C, H, N.

6-(4-Methoxy-phenyl)-3,4-dihydro-2H-[1,4]diazepino[6,7,1-*hi*]indol-1-one (16). Pale yellow solid (84%); mp 188–190 °C. ^1H NMR ($\text{DMSO}-d_6$): δ 3.48–3.50 (m, 2H), 4.27–4.30 (m, 2H), 6.60 (s, 1H), 7.07 (d, 2H, $J = 9.0$ Hz), 7.15 (t, 1H, $J = 6.0$ Hz), 7.54 (d, 2H, $J = 9.0$ Hz), 7.75 (d, 1H, $J = 6.0$ Hz), 7.79 (d, 1H, $J = 6.0$ Hz), 8.36 (t, 1H, $J = 6.0$ Hz). HRMS calcd for $\text{C}_{18}\text{H}_{16}\text{N}_2\text{O}_2$ (M^+), 292.1212; found (M^+), 292.1218. Anal. ($\text{C}_{18}\text{H}_{16}\text{N}_2\text{O}_2 \cdot 0.1\text{H}_2\text{O}$) C, H, N.

6-(4-Fluoro-phenyl)-3,4-dihydro-2H-[1,4]diazepino[6,7,1-*hi*]indol-1-one (17). Pale yellow solid (79%); mp 198–200 °C. ^1H NMR ($\text{DMSO}-d_6$): δ 3.48–3.50 (m, 2H), 4.28–4.30 (m, 2H), 6.70 (s, 1H), 7.15 (t, 1H, $J = 6.0$ Hz), 7.33–7.39 (m, 2H), 7.65 (d, 1H, $J = 6.0$ Hz), 7.68 (d, 1H, $J = 6.0$ Hz), 7.78 (d, 1H, $J = 6.0$ Hz), 7.82 (d, 1H, $J = 6.0$ Hz), 8.38 (t, 1H, $J = 6.0$ Hz). HRMS calcd for $\text{C}_{17}\text{H}_{13}\text{N}_2\text{OF}$ (M^+), 280.1012; found (M^+), 280.1002. Anal. ($\text{C}_{17}\text{H}_{13}\text{N}_2\text{OF}$) C, H, N.

6-Phenethyl-3,4-dihydro-2H-[1,4]diazepino[6,7,1-*hi*]indol-1-one (18). Pale yellow solid (70%); mp 188–190 °C. ^1H NMR ($\text{DMSO}-d_6$): δ 2.96–3.06 (m, 4H), 3.49–3.50 (m, 2H), 4.21 (br s, 2H), 6.37 (s, 1H), 7.07 (t, 1H, $J = 6.0$ Hz), 7.18–

7.29 (m, 5H), 7.65 (d, 1H, $J = 6.0$ Hz), 7.74 (d, 1H, $J = 6.0$ Hz), 8.26 (t, 1H, $J = 6.0$ Hz). HRMS calcd for $\text{C}_{19}\text{H}_{18}\text{N}_2\text{O}$ (M^+), 290.1419; found (M^+), 290.1421. Anal. ($\text{C}_{19}\text{H}_{18}\text{N}_2\text{O}$) C, H, N.

6-Pyridin-2-yl-3,4-dihydro-2H-[1,4]diazepino[6,7,1-*hi*]indol-1-one (19). To a solution of 9-pyridin-2-ylethynyl-1,2,3,4-tetrahydro-benzo[e][1,4]diazepin-5-one (0.050 g, 0.190 mmol) in DMF (6 mL) were added CuI (0.003 g, 0.012 mmol) and PdCl_2 (5.0 mg, 0.029 mmol) at room temperature. The reaction mixture was heated at 80–85 °C for 4 h. Upon completion, the solvent was evaporated to dryness. The crude residue was purified by flash silica gel chromatography eluting with a gradient of 0–3% MeOH in CHCl_3 to give 0.010 g (20%) of the product. ^1H NMR ($\text{DMSO}-d_6$): δ 3.38–3.55 (m, 2H), 4.64 (br s, 2H), 7.06 (s, 1H), 7.19 (t, 1H, $J = 9.0$ Hz), 7.37–7.41 (m, 1H), 7.82–7.96 (m, 4H), 8.38 (t, 1H, $J = 6.0$ Hz), 8.70 (d, 1H, $J = 3.0$ Hz). HRMS calcd for $\text{C}_{16}\text{H}_{13}\text{N}_3\text{O}$ (M^+), 263.1059; found (M^+), 263.1062. Anal. ($\text{C}_{16}\text{H}_{13}\text{N}_3\text{O} \cdot 0.8\text{H}_2\text{O}$) C, H, N.

Method C. 9-Trimethylsilanylethynyl-1,2,3,4-tetrahydro-benzo[e][1,4]diazepin-5-one (52). A mixture of **12** (1.0 g, 3.47 mmol), TMS acetylene (5.0 mL, 34.70 mmol), tetrakis(triphenylphosphine) palladium chloride (0.040 g, 0.0347 mmol), CuI (0.013 g, 0.0694 mmol) in diethylamine (10 mL), and DMF (10 mL) was stirred at room temperature for 5 h. The solvent was evaporated, and the residue was taken up in H_2O and extracted with EtOAc several times. The combined organic layers were dried over anhydrous MgSO_4 and concentrated. The crude mixture was purified by flash silica gel chromatography eluting with a gradient of 0–5% MeOH in CHCl_3 to give 0.733 g (82%) of a brown solid; mp 180–182 °C. ^1H NMR ($\text{DMSO}-d_6$): δ 0.25 (s, 9H), 3.25–3.33 (m, 2H), 3.51–3.55 (m, 2H), 5.90 (br s, 1H), 6.57 (t, 1H, $J = 6.0$ Hz), 7.35 (d, 1H, $J = 6.0$ Hz), 7.78 (d, 1H, $J = 6.0$ Hz), 8.13 (t, 1H, $J = 6.0$ Hz). LRMS (M^+) 258.

9-Ethynyl-1,2,3,4-tetrahydro-benzo[e][1,4]diazepin-5-one (53). A mixture of **52** (0.712 g, 2.76 mmol) and K_2CO_3 (0.038 g, 0.276 mmol) in MeOH (35 mL) was stirred at room temperature for 2.5 h. The solvent was evaporated, and the residue taken up in H_2O and extracted with EtOAc several times. The combined organic extracts were dried over anhydrous MgSO_4 and concentrated to give 0.504 g (98%) of a brown solid. The solid was used without further purification; mp 146–148 °C. ^1H NMR ($\text{DMSO}-d_6$): δ 3.15–3.23 (m, 2H), 3.48–3.52 (m, 2H), 4.50 (s, 1H), 6.13 (br s, 1H), 6.57 (t, 1H, $J = 9.0$ Hz), 7.37 (d, 1H, $J = 9.0$ Hz), 7.79 (d, 1H, $J = 9.0$ Hz), 8.10 (t, 1H, $J = 6.0$ Hz). LRMS (M^+) 186.

3-(5-Oxo-2,3,4,5-tetrahydro-1H-benzo[e][1,4]diazepin-9-ylethynyl)benzaldehyde (54). Using the procedure described for the preparation of **13**, 3-iodobenzaldehyde and **53** were used to synthesize **54** in 62% yield as a yellow solid; mp 176–178 °C. ^1H NMR ($\text{DMSO}-d_6$): δ 3.30–3.33 (m, 2H), 3.54–3.57 (m, 2H), 6.40 (br s, 1H), 6.63 (t, 1H, $J = 6.0$ Hz), 7.49 (d, 1H, $J = 6.0$ Hz), 7.66 (t, 1H, $J = 9.0$ Hz), 7.83 (d, 1H, $J = 6.0$ Hz), 7.90–7.97 (m, 2H), 8.15 (br s, 1H), 8.31 (s, 1H), 10.03 (s, 1H). LRMS (M^+) 291.

3-(1-Oxo-1,2,3,4-tetrahydro[1,4]diazepino[6,7,1-*hi*]indol-6-yl)benzaldehyde (55). Using the procedure described for the preparation of **14**, compound **54** was converted to **55** in 66% yield as a pale yellow solid; mp 192–194 °C. ^1H NMR ($\text{DMSO}-d_6$): δ 3.49–3.51 (m, 2H), 4.33–4.36 (m, 2H), 6.83 (s, 1H), 7.19 (t, 1H, $J = 6.0$ Hz), 7.75 (t, 1H, $J = 9.0$ Hz), 7.80–7.86 (m, 2H), 7.96 (d, 2H, $J = 6.0$ Hz), 8.15 (s, 1H), 8.41 (t, 1H, $J = 6.0$ Hz), 10.11 (s, 1H). LRMS (M^+) 290.

6-(3-Dimethylaminomethyl-phenyl)-3,4-dihydro-2H-[1,4]diazepino[6,7,1-*hi*]indol-1-one (20). Dimethylamine (2 M) in methanol (16.34 mmol, 8.2 mL) was added to a suspension of **55** (1.90 mmol, 0.55 g) in MeOH (110 mL) at room temperature. The reaction mixture was heated to reflux until the suspended solids went into solution. The reaction mixture was cooled to room temperature, and a solution of NaCNBH_3 (2.09 mmol, 0.131 g) and ZnCl_2 (1.05 mmol, 0.143 g) in MeOH (55 mL) was slowly added. The pH of the reaction was adjusted to 3–4 using 2 M methanolic HCl. The reaction mixture was stirred at room temperature for 2.5 h. Upon completion (TLC), concentrated HCl was added (pH 1) and the solvent was

removed in vacuo. The residue was diluted with H₂O, made basic (pH 12–14) with 50% aqueous NaOH, and extracted with EtOAc several times. The combined organic layers were dried over anhydrous MgSO₄ and concentrated. The crude mixture was purified by flash silica gel chromatography eluting with a gradient of 0–7% MeOH in CHCl₃ followed by 3–7% MeOH/NH₃ in CHCl₃ to give 0.527 g (87%) of a white solid; mp 98–100 °C. ¹H NMR (DMSO-*d*₆): δ 2.18 (s, 6H), 3.47 (br s, 4H), 4.30–4.32 (m, 2H), 6.70 (s, 1H), 7.17 (t, 1H, *J* = 6.0 Hz), 7.35–7.37 (m, 1H), 7.43–7.50 (m, 3H), 7.78 (d, 1H, *J* = 6.0 Hz), 7.81 (d, 1H, *J* = 6.0 Hz), 8.38 (t, 1H, *J* = 6.0 Hz). HRMS calcd for C₂₀H₂₁N₃O (M⁺), 319.1685; found (M⁺), 319.1682; Anal. (C₂₀H₂₁N₃O · 0.25 H₂O) C, H, N.

Compounds **21–25** were made using method C.

6-(3-Methylaminomethyl-phenyl)-3,4-dihydro-2H-[1,4]-diazepino[6,7,1-hi]indol-1-one (21). Pale yellow solid (94%); mp 128–130 °C. ¹H NMR (DMSO-*d*₆): δ 2.29 (s, 3H), 3.48 (br s, 2H), 3.71 (s, 2H), 4.30–4.33 (m, 2H), 6.69 (s, 1H), 7.17 (t, 1H, *J* = 9.0 Hz), 7.38–7.39 (m, 1H), 7.44–7.46 (m, 2H), 7.54 (s, 1H), 7.80 (t, 2H, *J* = 9.0 Hz), 8.39 (t, 1H, *J* = 6.0 Hz). HRMS calcd for C₁₉H₁₉N₃O (M⁺), 305.3828; found (M⁺), 305.3820. Anal. (C₁₉H₁₉N₃O · 0.6 H₂O) C, H, N.

6-(3-Pyrrolidin-1-ylmethyl-phenyl)-3,4-dihydro-2H-[1,4]-diazepino[6,7,1-hi]indol-1-one (22). Pale yellow solid (92%); mp 158–160 °C. ¹H NMR (DMSO-*d*₆): δ 1.71 (br s, 4H), 2.49 (br s, 4H), 3.49 (br s, 2H), 3.68 (br s, 2H), 4.30–4.33 (m, 2H), 6.70 (s, 1H), 7.17 (t, 1H, *J* = 9.0 Hz), 7.38–7.52 (m, 4H), 7.79 (d, 1H, *J* = 9.0 Hz), 7.82 (d, 1H, *J* = 9.0 Hz), 8.38 (t, 1H, *J* = 6.0 Hz). HRMS calcd for C₂₂H₂₃N₃O (M⁺), 345.1841; found (M⁺), 345.1848. Anal. (C₂₂H₂₃N₃O · 0.4 H₂O) C, H, N.

6-(4-Dimethylaminomethyl-phenyl)-3,4-dihydro-2H-[1,4]-diazepino[6,7,1-hi]indol-1-one (23). Pale yellow solid (86%); mp 140–142 °C. ¹H NMR (DMSO-*d*₆): δ 2.18 (s, 6H), 3.45 (s, 2H), 3.47–3.50 (m, 2H), 4.32 (m, 2H), 6.69 (s, 1H), 7.16 (t, 1H, *J* = 10.0 Hz), 7.42 (d, 2H, *J* = 10 Hz), 7.56 (d, 2H, *J* = 10 Hz), 7.77 (d, 1H, *J* = 10 Hz), 7.81 (d, 1H, *J* = 10.0 Hz), 8.36 (t, 1H, *J* = 5.0 Hz). HRMS calcd for C₂₀H₂₁N₃O (M⁺), 319.1685; found (M⁺), 319.1678. Anal. (C₂₀H₂₁N₃O · 0.3 H₂O) C, H, N.

6-(4-Methylaminomethyl-phenyl)-3,4-dihydro-2H-[1,4]-diazepino[6,7,1-hi]indol-1-one (24). Pale yellow solid (71%); mp 178–180 °C. ¹H NMR (DMSO-*d*₆): δ 2.29 (s, 3H), 3.48 (br s, 2H), 3.70 (s, 2H), 4.30–4.33 (m, 2H), 6.68 (s, 1H), 7.16 (t, 1H, *J* = 9.0 Hz), 7.45 (d, 2H, *J* = 9.0 Hz), 7.55 (d, 2H, *J* = 9.0 Hz), 7.77 (d, 1H, *J* = 9.0 Hz), 7.80 (d, 1H, *J* = 9.0 Hz), 8.38 (t, 1H, *J* = 6.0 Hz). HRMS calcd for C₁₉H₁₉N₃O (M⁺), 305.3828; found (M⁺), 305.1536. Anal. (C₁₉H₁₉N₃O · 0.1 H₂O) C, H, N.

6-(4-Pyrrolidin-1-ylmethyl-phenyl)-3,4-dihydro-2H-[1,4]-diazepino[6,7,1-hi]indol-1-one (25). Pale yellow solid (76%); mp 146–148 °C. ¹H NMR (DMSO-*d*₆): δ 1.71 (br s, 4H), 2.49 (br s, 4H), 3.48 (br s, 2H), 3.64 (br s, 2H), 4.30–4.33 (m, 2H), 6.69 (s, 1H), 7.16 (t, 1H, *J* = 9.0 Hz), 7.43 (d, 2H, *J* = 9.0 Hz), 7.55 (d, 2H, *J* = 9.0 Hz), 7.77 (d, 1H, *J* = 9.0 Hz), 7.80 (d, 1H, *J* = 9.0 Hz), 8.38 (t, 1H, *J* = 6.0 Hz). HRMS calcd for C₂₂H₂₃N₃O (M⁺), 345.1841; found (M⁺), 345.1835. Anal. (C₂₂H₂₃N₃O · 0.25 H₂O) C, H, N.

7-Iodo-3,4-dihydro-2H-[1,4]diazepino[6,7,1-hi]indol-1-one (26). To a pale yellow solution **2** (0.051 g, 0.274 mmol) in 5 mL of DMF were added KOH (0.058 g, 1.03 mmol) and iodine (0.139 g, 0.548 mmol) at room temperature. The reaction mixture was stirred at room temperature overnight. The solvent was removed in vacuo, and the residue was taken up in EtOAc and washed with 0.1% sodium bisulfite, H₂O, and brine. The organic layer was dried over anhydrous MgSO₄ and concentrated to give 0.078 g (92%) of a pale yellow solid, which was used without further purification; mp 188–190 °C. ¹H NMR (DMSO-*d*₆): δ 3.56–3.59 (m, 2H), 4.40 (m, 2H), 7.26 (t, 1H, *J* = 7.5 Hz), 7.52 (d, 1H, *J* = 7.5 Hz), 7.67 (s, 1H), 7.93 (d, 1H, *J* = 7.5 Hz), 8.37 (t, 1H, *J* = 5.3 Hz). HRMS calcd for C₁₁H₉N₂OI (M⁺), 311.9761; found (M⁺), 311.9776. Anal. calcd for C₁₁H₉N₂O₂I: % C, 42.33; % H, 2.91; % N, 8.98. Found: % C, 42.81; % H, 2.98; % N, 8.85.

1-Oxo-1,2,3,4-tetrahydro[1,4]diazepino[6,7,1-hi]indole-7-carboxylic Acid Methyl Ester (27). Triethylamine (0.11

mL, 0.747 mmol) was added to a mixture of **26** (0.074 g, 0.37 mmol) and bistrisphenylphosphine palladium chloride (8.4 mg, 0.012 mmol) in 8 mL of MeOH and 3 mL of DMF at room temperature. The reaction mixture was heated at 50–55 °C for 18 h under a CO atmosphere (balloon). The solvent was removed under vacuo, and the residue was taken up in EtOAc and washed with water. The organic layer was dried over anhydrous MgSO₄ and concentrated to give a yellow solid, which was purified by flash silica gel chromatography eluting with a gradient of 0–3% MeOH in CHCl₃ to give 0.025 g of the methyl ester; mp 259–261 °C. ¹H NMR (DMSO-*d*₆): δ 3.34–3.60 (m, 2H), 3.83 (s, 3H), 4.46 (br s, 2H), 7.36 (t, 1H, *J* = 7.5 Hz), 7.95 (d, 1H, *J* = 7.5 Hz), 8.23 (s, 1H), 8.27 (d, 1H, *J* = 7.5 Hz), 8.40–8.50 (m, 1H). HRMS calcd for C₁₃H₁₂N₂O₃ (M⁺), 244.0848; found (M⁺), 244.0850. Anal. (C₁₃H₁₂N₂O₃ · 0.25 H₂O) C, H, N.

1-Oxo-1,2,3,4-tetrahydro[1,4]diazepino[6,7,1-hi]indole-7-carbaldehyde Oxime (28). To a mixture of **3** (0.050 g, 0.233 mmol) in EtOH (5 mL) and H₂O (0.5 mL) were added NH₂OH · HCl (0.041 g, 0.583 mmol) and NaOH (0.024 g, 0.583 mmol) at room temperature. The reaction mixture was heated at 80–85 °C for 2 days. The resulting suspension was filtered, and the white solid (0.047 g) was washed with water and dried; mp 262–264 °C. ¹H NMR (DMSO-*d*₆): δ 3.56 (br s, 2H), 4.36 (br s, 2H), 7.23 (t, 1H, *J* = 7.5 Hz), 7.68 (s, 1H), 7.90 (d, 1H, *J* = 7.5 Hz), 8.21 (d, 1H, *J* = 7.5 Hz), 8.26 (s, 1H), 8.33–8.35 (m, 1H), 10.66 (s, 1H). HRMS calcd for C₁₂H₁₁N₃O₂ (M⁺), 229.0851; found (M⁺), 229.0843. Anal. (C₁₂H₁₁N₃O₂) C, H, N.

1-Oxo-1,2,3,4-tetrahydro[1,4]diazepino[6,7,1-hi]indole-7-carbaldehyde O-Methyl-oxime (29 and 30). A solution **3** (0.050 g, 0.234 mmol) and MeONH₂ · HCl (0.020 g, 0.242 mmol) in EtOH (5 mL) and pyridine (5 mL) was refluxed for 20 h. The reaction mixture was then evaporated to dryness. The residue was taken up in H₂O and extracted with EtOAc several times. The combined organic layers were dried over anhydrous MgSO₄ and concentrated. The crude was purified by flash silica gel chromatography eluting with a gradient of 0–1% MeOH in CHCl₃ to give 0.036 g of the (*E*) and 0.013 g of the (*Z*) isomer.

Compound 29. (*E*) isomer: mp 173–175 °C. ¹H NMR (DMSO-*d*₆): δ 3.55 (br s, 2H), 3.87 (s, 3H), 4.37 (br s, 2H), 7.27 (t, 1H, *J* = 7.5 Hz), 7.75 (s, 1H), 7.91 (d, 1H, *J* = 7.5 Hz), 8.24 (d, 1H, *J* = 7.5 Hz), 8.34–8.38 (m, 2H). HRMS calcd for C₁₃H₁₃N₃O₂ (M⁺), 243.1008; found (M⁺), 243.1016. Anal. (C₁₃H₁₃N₃O₂ · 0.25 H₂O) C, H, N.

Compound 30. (*Z*) isomer: mp 210–212 °C. ¹H NMR (DMSO-*d*₆): δ 3.54–3.58 (m, 2H), 3.96 (s, 3H), 4.43 (br s, 2H), 7.27 (t, 1H, *J* = 9.0 Hz), 7.89–7.92 (m, 2H), 8.14 (d, 1H, *J* = 9.0 Hz), 8.21 (s, 1H), 8.35–8.39 (m, 1H). HRMS calcd for C₁₃H₁₃N₃O₂ (M⁺), 243.1008; found (M⁺), 243.1020. Anal. (C₁₃H₁₃N₃O₂ · 0.1 H₂O · 0.1 EtOAc) C, H, N.

7-(1-Hydroxy-ethyl)-3,4-dihydro-2H-[1,4]diazepino[6,7,1-hi]indol-1-one (31). MeLi (1.5 M) (3.744 mmol, 2.5 mL) was added to a solution of **3** (1.17 mmol, 0.250 g) in 100 mL of THF at –78 °C. The reaction mixture was warmed to room temperature and stirred for 5–10 min. Upon consumption of **3** as indicated by TLC, the reaction was quenched with H₂O and then extracted with EtOAc several times. The combined organic extracts were dried over anhydrous MgSO₄ and concentrated to give a yellow solid. The solid was purified by flash silica gel chromatography eluting with a gradient of 0–5% MeOH in CHCl₃ to give 0.222 g (69%) of a pale yellow solid; mp 295–297 °C. ¹H NMR (DMSO-*d*₆): δ 1.47 (d, 3H, *J* = 6.0 Hz), 3.50–3.55 (m, 2H), 4.29–4.31 (m, 2H), 4.95 (d, 1H, *J* = 6.0 Hz), 4.97–5.03 (m, 1H), 7.10 (t, 1H, *J* = 6.0 Hz), 7.25 (s, 1H), 7.81 (d, 1H, *J* = 6.0 Hz), 7.86 (d, 1H, *J* = 6.0 Hz), 8.25 (t, 1H, *J* = 6.0 Hz). HRMS calcd for C₁₃H₁₄N₂O₂ (M⁺), 231.1134; found (M⁺), 231.1143. Anal. (C₁₃H₁₄N₂O₂) C, H, N.

7-Acetyl-3,4-dihydro-2H-[1,4]diazepino[6,7,1-hi]indol-1-one (32). *o*-Iodoxybenzoic acid (2.217 mmol, 0.621 g) was added to a solution of **31** (0.739 mmol, 0.170 g) in DMSO (8 mL) at room temperature, and the mixture was stirred at for 2.5 h. The solvent was removed in vacuo, and the residue was taken up in EtOAc and washed with 5% Na₂S₂O₃/5% NaHCO₃,

H₂O, and brine. The organic layer was dried over anhydrous MgSO₄ and concentrated to give an orange solid. The solid was purified by flash silica gel chromatography eluting with a gradient of 0–5% MeOH in CHCl₃ to give 0.094 g (75%) of a pale pink solid; mp 285–287 °C. ¹H NMR (DMSO-*d*₆): δ 2.42 (s, 3H), 3.56–3.61 (m, 2H), 4.44 (br s, 2H), 7.32 (t, 1H, *J* = 6.0 Hz), 7.92 (d, 1H, *J* = 6.0 Hz), 8.40–8.44 (m, 3H). HRMS calcd for C₁₃H₁₂N₂O₂ (M⁺), 228.0899; found (M⁺), 228.0890. Anal. (C₁₃H₁₂N₂O₂) C, H, N.

7-(1-Hydroxyimino-ethyl)-3,4-dihydro-2H-[1,4]diazepino[6,7,1-*hi*]indol-1-one (33). NH₂OH·HCl (1.928 mmol, 0.134 g) was added to a solution of **32** (0.241 mmol, 0.055 g) in pyridine (6 mL) at room temperature. The reaction mixture was heated at 70–80 °C for 5 h. The solvent was removed, and ice-cold H₂O was added to the residue, whereupon a precipitate formed, which was then filtered, washed with H₂O, and dried to give 0.040 g (68%) of a pale yellow solid; mp 238–240 °C. ¹H NMR (DMSO-*d*₆): δ 2.16 (s, 3H), 3.56 (br s, 2H), 4.36 (br s, 2H), 7.19 (t, 1H, *J* = 6.0 Hz), 7.77 (s, 1H), 7.87 (d, 1H, *J* = 6.0 Hz), 8.33 (t, 1H, *J* = 6.0 Hz), 8.38 (d, 1H, *J* = 6.0 Hz), 10.67 (s, 1H). HRMS calcd for C₁₃H₁₃N₃O₂ (M⁺), 243.1008; found (M⁺), 243.0997. Anal. (C₁₃H₁₃N₃O₂) C, H, N.

7-(1-Hydroxy-1-phenyl-methyl)-3,4-dihydro-2H-[1,4]diazepino[6,7,1-*hi*]indol-1-one (34). Using the procedure described for the preparation of **31**, **3** was exposed to phenyllithium yielding 74% of **34** as a yellow solid; mp 178–180 °C. ¹H NMR (DMSO-*d*₆): δ 3.51–3.52 (m, 2H), 4.28–4.29 (m, 2H), 5.70 (d, 1H, *J* = 6.0 Hz), 5.96 (d, 1H, *J* = 6.0 Hz), 7.05 (t, 1H, *J* = 6.0 Hz), 7.14 (s, 1H), 7.17–7.22 (m, 1H), 7.30 (t, 2H, *J* = 6.0 Hz), 7.45 (d, 2H, *J* = 6.0 Hz), 7.72 (d, 1H, *J* = 6.0 Hz), 7.79 (d, 1H, *J* = 6.0 Hz), 8.24 (t, 1H, *J* = 6.0 Hz). HRMS calcd for C₁₈H₁₆N₂O₂ (M⁺), 292.1212; found (M⁺), 292.1202. Anal. (C₁₈H₁₆N₂O₂·0.25 H₂O) C, H, N.

7-(1-Phenyl-methanoyl)-3,4-dihydro-2H-[1,4]diazepino[6,7,1-*hi*]indol-1-one (35). Using the procedure described for the preparation of **32**, *o*-iodooxybenzoic acid and **34** were used to synthesize **35** in 80% yield as a pale yellow solid; mp 229–230 °C. ¹H NMR (DMSO-*d*₆): δ 3.58–3.61 (m, 2H), 4.47 (br s, 2H), 7.40 (t, 1H, *J* = 6.0 Hz), 7.52–7.65 (m, 3H), 7.79–7.82 (m, 2H), 7.98 (d, 1H, *J* = 6.0 Hz), 8.08 (s, 1H), 8.44 (t, 1H, *J* = 6.0 Hz), 8.51 (d, 1H, *J* = 6.0 Hz). HRMS calcd for C₁₈H₁₄N₂O₂ (M⁺), 290.1055; found (M⁺), 290.1042. Anal. (C₁₈H₁₄N₂O₂) C, H, N.

7-(1-Hydroxyimino-1-phenyl-methyl)-3,4-dihydro-2H-[1,4]diazepino[6,7,1-*hi*]indol-1-one (36). Using the procedure described for the preparation of **33**, NH₂OH·HCl and **35** were used to synthesize **36** in 76% yield as a pale yellow solid; mp 263–265 °C (mixture of syn and anti oximes). ¹H NMR (DMSO-*d*₆): δ 3.51 (br s, 2H), 3.60 (br s, 2H), 4.29 (br s, 2H), 4.45 (br s, 2H), 6.97–7.04 (m, 3H), 7.24 (t, 1H, *J* = 6.0 Hz), 7.34–7.46 (m, 10H), 7.82 (d, 1H, *J* = 6.0 Hz), 7.89–7.93 (m, 2H), 8.31–8.36 (m, 3H), 10.74 (s, 1H), 11.37 (s, 1H). HRMS calcd for C₁₈H₁₅N₃O₂ (M⁺), 305.1164; found (M⁺), 305.1177. Anal. (C₁₈H₁₅N₃O₂·0.1 H₂O) C, H, N.

6-(4-Chloro-phenyl)-1-oxo-1,2,3,4-tetrahydro[1,4]diazepino[6,7,1-*hi*]indole-7-carbaldehyde (37). POCl₃ (0.3 mL, 3.19 mmol) was slowly added to DMF (3 mL) at 0 °C. The reaction mixture was stirred for 15 min and then was treated with a solution of **15** (0.070 g, 0.236 mmol) in DMF (2 mL) and warmed to room temperature for 4 h. After all solvent was removed, the residue was taken up in H₂O and made basic (pH 12–14) using 50% aqueous NaOH whereupon solids formed. The product was filtered, washed with water several times, and dried (0.077 g); mp 278–279 °C. ¹H NMR (DMSO-*d*₆): δ 3.41–3.52 (m, 2H), 4.20–4.22 (m, 2H), 7.43 (t, 1H, *J* = 9.0 Hz), 7.68 (d, 2H, *J* = 9.0 Hz), 7.74 (d, 2H, *J* = 9.0 Hz), 8.00 (d, 1H, *J* = 6.0 Hz), 8.47 (d, 1H, *J* = 6.0 Hz), 8.51 (t, 1H, *J* = 6.0 Hz), 9.65 (s, 1H). HRMS calcd for C₁₈H₁₃N₂O₂Cl (M⁺), 324.0665; found (M⁺), 324.0668. Anal. (C₁₈H₁₃N₂O₂Cl·0.25H₂O) C, H, N.

6-(4-Chloro-phenyl)-1-oxo-1,2,3,4-tetrahydro[1,4]diazepino[6,7,1-*hi*]indole-7-carbaldehyde oxime (38). NH₂OH·HCl (0.027 g, 0.385 mmol) and NaOH (0.016 g, 0.385 mmol) were added to a suspension of **37** (0.050 g, 0.154 mmol) in

EtOH (5 mL) and H₂O (0.5 mL). After the reaction mixture was heated at 80–85 °C for 3 h, it was evaporated to dryness. The residue was taken up in ice-cold H₂O when a pale yellow solid formed. The solid was filtered, washed with H₂O, and then purified by flash silica gel chromatography eluting with a gradient of 0–5% MeOH in CHCl₃ to give 0.035 g (67%) of the oxime; mp 249–251 °C. ¹H NMR (DMSO-*d*₆): δ 3.40 (br s, 2H), 4.0–4.1 (m, 2H), 7.30 (t, 1H, *J* = 9.0 Hz), 7.55 (d, 2H, 9.0 Hz), 7.64 (d, 2H, *J* = 9.0 Hz), 7.90 (s, 1H), 7.94 (d, 1H, *J* = 9.0 Hz), 8.34 (d, 1H, *J* = 9.0 Hz), 8.41 (t, 1H, *J* = 6.0 Hz), 10.83 (s, 1H). HRMS calcd for C₁₈H₁₄N₃O₂Cl (M⁺ + H), 340.0853; found (M⁺ + H), 340.0862. Anal. (C₁₈H₁₄N₃O₂Cl·0.75CH₂Cl₂) C, H, N.

6-(4-Fluoro-phenyl)-1-oxo-1,2,3,4-tetrahydro[1,4]diazepino[6,7,1-*hi*]indole-7-carbaldehyde (39). Using the procedure described for the preparation of **37**, **17** was transformed to **39** in 94% yield as a white solid; mp 268–270 °C. ¹H NMR (DMSO-*d*₆): δ 3.52–3.54 (m, 2H), 4.19–4.22 (m, 2H), 7.40–7.50 (m, 3H), 7.75 (d, 1H, *J* = 6.0 Hz), 7.78 (d, 1H, *J* = 6.0 Hz), 8.46 (d, 1H, *J* = 6.0 Hz), 8.52 (t, 1H, *J* = 6.0 Hz), 9.64 (s, 1H). LRMS (M⁺ + H) 309. HPLC *R*_t = 3.522 min.

6-(4-Fluoro-phenyl)-1-oxo-1,2,3,4-tetrahydro[1,4]diazepino[6,7,1-*hi*]indole-7-carbaldehyde Oxime (40). NH₂OH·HCl (0.325 mmol, 0.100 g) was added to a solution of **39** (0.813 mmol, 0.056 g) in pyridine (10 mL) at room temperature. The reaction mixture was stirred at room temperature for 20 h. Upon completion of the reaction, the solvent was removed in vacuo. The residue was taken up in 2 N HCl and extracted with EtOAc several times. The combined organic extracts were dried over anhydrous MgSO₄ and concentrated to give 0.097 g (92%) of a pale yellow solid, which was used without further purification; mp 277–279 °C. ¹H NMR (DMSO-*d*₆): δ 3.50 (br s, 2H), 4.12–4.14 (m, 2H), 7.30 (t, 1H, *J* = 6.0 Hz), 7.43 (t, 2H, *J* = 9.0 Hz), 7.57–7.62 (m, 2H), 7.89 (s, 1H), 7.94 (d, 1H, *J* = 9.0 Hz), 8.33 (d, 1H, *J* = 6.0 Hz), 8.41 (t, 1H, *J* = 6.0 Hz), 10.80 (s, 1H). HRMS calcd for C₁₈H₁₄N₃O₂F (M⁺), 323.1070; found (M⁺), 323.1066. Anal. calcd for C₁₈H₁₄N₃O₂F·0.3H₂O: % C, 65.76; % H, 4.48; % N, 12.78. Found: % C, 66.06; % H, 4.51; % N, 12.32.

6-(4-Fluoro-phenyl)-1-oxo-1,2,3,4-tetrahydro[1,4]diazepino[6,7,1-*hi*]indole-7-carbonitrile (41). Thiocarbonyldiimidazole (2.33 mmol, 0.415 g) was added to a solution of **40** (0.932 mmol, 0.301 g) in THF (70 mL) at room temperature. The reaction mixture was stirred at room temperature for 4 h. Upon consumption of **40**, the solvent was removed in vacuo. The residue was dissolved in EtOAc and washed with 10% HCl and then with saturated NaHCO₃. The organic layer was dried over anhydrous MgSO₄ and concentrated to give a yellow oil, which was purified by flash silica gel chromatography eluting with a gradient of 0–3% MeOH in CHCl₃ to give 0.268 g (94%) of a pale yellow solid; mp 248–250 °C. ¹H NMR (DMSO-*d*₆): δ 3.52 (br s, 2H), 4.29–4.31 (m, 2H), 7.41–7.53 (m, 3H), 7.77 (d, 1H, *J* = 6.0 Hz), 7.80 (d, 1H, *J* = 6.0 Hz), 7.90 (d, 1H, *J* = 6.0 Hz), 8.01 (d, 1H, *J* = 6.0 Hz), 8.55 (t, 1H, *J* = 6.0 Hz). HRMS calcd for C₁₈H₁₂N₃OF (M⁺), 305.0964; found (M⁺), 305.0951. Anal. (C₁₈H₁₂N₃OF·0.1H₂O) C, H, N.

6-(4-Fluoro-phenyl)-1-oxo-1,2,3,4-tetrahydro[1,4]diazepino[6,7,1-*hi*]indole-7-carboxylic Acid Amide (42). A suspension of **41** (0.164 mmol, 0.050 g) in 85% H₃PO₄ (7 mL) was heated at 90–100 °C for 22 h. Upon completion (as indicated by TLC), the reaction mixture was poured into cold H₂O and extracted with EtOAc several times. The combined organic extracts were dried over anhydrous MgSO₄ and concentrated to give a pink oil, which was purified by flash silica gel chromatography eluting with a gradient of 0–5% MeOH in CHCl₃ to give 0.042 g (79%) of a pale yellow solid; mp 287–289 °C. ¹H NMR (DMSO-*d*₆): δ 3.47 (br s, 2H), 3.98–4.06 (m, 2H), 6.46 (br s, 1H), 7.09 (br s, 1H), 7.28 (t, 1H, *J* = 6.0 Hz), 7.38 (t, 2H, *J* = 9.0 Hz), 7.56 (d, 1H, *J* = 6.0 Hz), 7.60 (d, 1H, *J* = 6.0 Hz), 7.90 (d, 1H, *J* = 6.0 Hz), 8.15 (d, 1H, *J* = 6.0 Hz), 8.40 (t, 1H, *J* = 6.0 Hz). HRMS calcd for C₁₈H₁₄N₃O₂F (M⁺), 323.1070; found (M⁺), 323.1063. Anal. (C₁₈H₁₄N₃O₂F·0.5H₂O) C, H, N.

6-(4-Fluoro-phenyl)-1-oxo-1,2,3,4-tetrahydro[1,4]diazepino[6,7,1-hi]indole-7-carbothioic Acid Amide (43). In an opened sealed tube apparatus, H₂S gas was bubbled into a solution of **41** (0.5 mmol, 0.153 g) in Et₃N (1 mL) and pyridine (2.4 mL) at 0 °C for 1 h until saturated. The tube was then carefully sealed, allowed to warm to room temperature, and stirred at room temperature for 4 days. The tube was cooled to 0 °C and carefully vented, and the remaining H₂S was purged with argon. The dark green solution was diluted with EtOAc and washed with 2 N HCl and then with H₂O. The organic layer was dried over anhydrous MgSO₄ and concentrated to give a yellow solid, which was purified by flash silica gel chromatography eluting with a gradient of 0–3% MeOH in CHCl₃ to give 0.107 g (63%) of a yellow solid; mp 238–240 °C. ¹H NMR (DMSO-*d*₆): δ 3.47 (br s, 2H), 4.01–4.11 (m, 2H), 7.27 (t, 1H, *J* = 9.0 Hz), 7.37 (t, 2H, *J* = 9.0 Hz), 7.54–7.58 (m, 2H), 7.88 (d, 1H, *J* = 9.0 Hz), 8.19 (d, 1H, *J* = 9.0 Hz), 8.42 (t, 1H, *J* = 6.0 Hz), 8.63 (br s, 1H), 9.50 (br s, 1H). HRMS calcd for C₁₈H₁₄N₃OSF (M⁺), 339.084162; found (M⁺), 339.0833. Anal. (C₁₈H₁₄N₃OSF·0.3H₂O·0.3 MeOH) C, H, N.

6-(4-Fluoro-phenyl)-1-oxo-1,2,3,4-tetrahydro[1,4]diazepino[6,7,1-hi]indole-7-carboximidothioic Acid Methyl Ester (44). Iodomethane (3.218 mmol, 0.2 mL) was added to a solution of the **43** (0.354 mmol, 0.120 g) in 50 mL of THF at room temperature and stirred for 18 h. The solvent was removed to give a yellow solid (0.130 g), which was used without further purification. ¹H NMR (DMSO-*d*₆): δ 2.63 (s, 3H), 3.51 (br s, 2H), 4.01–4.05 (m, 2H), 7.42–7.53 (m, 3H), 7.62 (d, 1H, *J* = 6.0 Hz), 7.65 (d, 1H, *J* = 6.0 Hz), 8.02 (d, 1H, *J* = 3.0 Hz), 8.05 (d, 1H, *J* = 3.0 Hz), 8.57 (t, 1H, *J* = 6.0 Hz).

6-(4-Fluoro-phenyl)-N-hydroxy-1-oxo-1,2,3,4-tetrahydro[1,4]diazepino[6,7,1-hi]indole-7-carboxamide (45). NH₂OH·HCl (0.852 mmol, 0.059 g) was added to a solution of the **44** (0.142 mmol, 0.05 g) in 5 mL of pyridine at room temperature and the mixture was stirred for 15 min. The solvent was removed to give an oil, which was purified by flash silica gel chromatography eluting with a gradient of 0–5% MeOH in CHCl₃ initially, followed by 2–10% MeOH/NH₃ in CHCl₃ to give 0.025 g (52%) of a pale yellow solid; mp 257–259 °C. ¹H NMR (DMSO-*d*₆): δ 3.45–3.47 (m, 2H), 4.10–4.12 (m, 2H), 5.41 (br s, 2H), 7.23 (t, 1H, *J* = 6.0 Hz), 7.34 (t, 2H, *J* = 9.0 Hz), 7.57 (d, 1H, *J* = 6.0 Hz), 7.59 (d, 1H, *J* = 6.0 Hz), 7.88 (d, 1H, *J* = 9.0 Hz), 7.93 (d, 1H, *J* = 9.0 Hz), 8.39 (br s, 1H), 9.33 (br s, 1H). HRMS calcd for C₁₈H₁₅N₄O₂F (M⁺), 338.1179; found (M⁺), 338.1182. Anal. (C₁₈H₁₅N₄O₂F·0.5 H₂O) C, H, N.

6-(4-Fluoro-phenyl)-N-amino-1-oxo-1,2,3,4-tetrahydro[1,4]diazepino[6,7,1-hi]indole-7-carboxamide Hydrochloride (46). Anhydrous hydrazine (2.92 mmol, 0.092 mL) was added to a solution of the **44** (0.139 mmol, 0.049 g) in 25 mL of acetonitrile at room temperature. The reaction mixture was stirred for 48 h, and the solvent was removed to give an oil, which was purified by flash silica gel chromatography eluting with a gradient of 0–10% MeOH in CHCl₃ initially, followed by 2–10% MeOH/NH₃ in CHCl₃ yielding 0.028 g (64%) of a white crystalline solid. This solid was dissolved in MeOH saturated with HCl gas and stirred at room temperature for 30 min. Diethyl ether was added to the solution, and the solvent was then evaporated to give an orange solid (9 mg); mp 272–274 °C. ¹H NMR (DMSO-*d*₆): δ 3.58 (br s, 2H), 4.22–4.23 (m, 2H), 5.18 (br s, 2H), 7.37–7.46 (m, 3H), 7.54–7.58 (m, 2H), 7.82 (d, 1H, *J* = 6.0 Hz), 8.00 (d, 1H, *J* = 6.0 Hz), 8.55 (t, 1H, *J* = 6.0 Hz), 8.79 (br s, 1H), 9.08 (br s, 1H), 10.60 (br s, 1H). HRMS calcd for C₁₈H₁₆N₅OF (M⁺), 337.1339; found (M⁺), 337.1326. Anal. (C₁₈H₁₆N₅OF·1.0H₂O·4.5HCl) C, H, N.

6-(4-Fluoro-phenyl)-7-iodo-3,4-dihydro-2H-[1,4]diazepino[6,7,1-hi]indol-1-one (47). Using the procedure described for the preparation of **26**, **17** was converted with I₂ and KOH to **47** in 78% yield as a pale yellow solid; mp 283–285 °C. ¹H NMR (DMSO-*d*₆): δ 3.48 (br s, 2H), 4.15–4.18 (m, 2H), 7.29 (t, 1H, *J* = 6.0 Hz), 7.41 (t, 2H, *J* = 9.0 Hz), 7.58–7.64 (m, 3H), 7.94 (d, 1H, *J* = 6.0 Hz), 8.41 (t, 1H, *J* = 6.0 Hz). LRMS (M⁺ + H) 407.

7-Acetyl-6-(4-fluoro-phenyl)-3,4-dihydro-2H-[1,4]diazepino[6,7,1-hi]indol-1-one (48). A solution of the **47** (0.246 mmol, 0.100 g), ethoxyvinyl tributyltin (0.320 mmol, 0.11 mL), tetrakis(triphenyl phosphine) palladium (0.0123 mmol, 0.014 g), and ~1.0 mg of 2,6-di-*tert*-butyl-4-methyl phenol in 1,4-dioxane (20 mL) and DMF (1 mL) was heated at 90–95 °C for 20 h. After it was cooled to ambient temperature, the solvent was evaporated to dryness and the residue was taken up in 1 N HCl and extracted with EtOAc several times. The combined organic layers were dried over anhydrous MgSO₄ and concentrated to give a yellow oil, which was purified by flash silica gel chromatography eluting with a gradient of 0–3% MeOH in CHCl₃ to yield 0.049 g (64% crude) of a yellow solid. Further purification by preparative HPLC yielded 26% of 98% pure product using gradient solvent system of:

time (min)	H ₂ O w/ 0.1% TFA	CH ₃ CN w/ 0.1% TFA
0	90	10
2	90	10
22	35	65

mp 275–276 °C. ¹H NMR (DMSO-*d*₆): δ 1.86 (s, 3H), 3.45–3.52 (m, 2H), 3.96–3.98 (m, 2H), 7.37 (t, 1H, *J* = 6.0 Hz), 7.45 (t, 2H, *J* = 9.0 Hz), 7.64 (d, 1H, *J* = 6.0 Hz), 7.67 (d, 1H, *J* = 6.0 Hz), 7.96 (d, 1H, *J* = 6.0 Hz), 8.42 (t, 1H, *J* = 6.0 Hz), 8.55 (d, 1H, *J* = 6.0 Hz). HRMS calcd for C₁₉H₁₅N₂O₂F (M⁺), 322.1117; found (M⁺), 322.1131. HPLC *R*_t = 10.608 min.

6-(4-Fluoro-phenyl)-7-((E)-2-hydroxy-1-methyl-vinyl)-3,4-dihydro-2H-[1,4]diazepino[6,7,1-hi]indol-1-one (49). NH₂OH·HCl (0.93 mmol, 0.066 g) was added to a solution of the **48** (0.124 mmol, 0.040 g) in 4 mL of pyridine at room temperature. The reaction mixture was stirred for 24 h whereupon the reaction mixture was evaporated to dryness. The residue was taken up in 2 N HCl and extracted with EtOAc several times. The combined organic layers were dried over anhydrous MgSO₄ and concentrated to give a pale yellow solid, which was purified by flash silica gel chromatography eluting with a gradient of 0–3% MeOH in CHCl₃ to give 0.025 g (60%) of a white solid; mp 248–250 °C. ¹H NMR (DMSO-*d*₆): δ 1.70 (s, 3H), 3.49 (br s, 2H), 4.07–4.09 (m, 2H), 7.21 (t, 1H, *J* = 6.0 Hz), 7.37 (t, 2H, *J* = 9.0 Hz), 7.51 (d, 1H, *J* = 6.0 Hz), 7.55 (d, 1H, *J* = 6.0 Hz), 7.89 (d, 1H, *J* = 6.0 Hz), 8.09 (d, 1H, *J* = 6.0 Hz), 8.37 (t, 1H, *J* = 6.0 Hz), 10.93 (s, 1H). HRMS calcd for C₁₉H₁₆N₃O₂F (M⁺), 337.1226; found (M⁺), 337.1230. Anal. for (C₁₉H₁₆N₃O₂F·0.1H₂O) C, H, N.

7-((E)-3-Dimethylamino-acryloyl)-6-(4-fluoro-phenyl)-3,4-dihydro-2H-[1,4]diazepino[6,7,1-hi]indol-1-one (50). N,N'-Dimethylformamide dimethyl acetal (13.88 mmol, 2 mL) was added to a solution of **48** (0.217 mmol, 0.070 g) in DMF (1 mL) at room temperature. The reaction mixture was stirred at 110–120 °C for 18 h. Upon completion of the reaction as indicated by TLC, the solvent was removed in vacuo to give 0.101 g (quantitative yield) of an orange solid, which was used without further purification. ¹H NMR (DMSO-*d*₆): δ 3.30 (s, 6H), 3.50 (br s, 2H), 3.98–4.05 (m, 2H), 4.61 (d, 1H, *J* = 12.0 Hz), 7.26 (t, 1H, *J* = 6.0 Hz), 7.35–7.43 (m, 3H), 7.54–7.58 (m, 2H), 7.89 (d, 1H, *J* = 6.0 Hz), 8.37–8.43 (m, 2H). LC mass (M⁺ + H) 378.

6-(4-Fluoro-phenyl)-7-(2H-pyrazol-3-yl)-3,4-dihydro-2H-[1,4]diazepino[6,7,1-hi]indol-1-one (51). Hydrazine monohydrate (5.14 mmol, 0.26 mL) was added to a solution of **50** (0.257 mmol, 0.097 g) in 10 mL of THF at room temperature. The reaction mixture was stirred at room temperature for 42 h and then evaporated to dryness. The residue was taken up in 2 N HCl and extracted with EtOAc several times. The combined organic layers were dried over anhydrous MgSO₄ and concentrated to give a yellow oil, which was purified by flash silica gel chromatography eluting with a gradient of 0–3% MeOH in CHCl₃ to give 0.020 g (23%) of a yellow solid; mp 173–175 °C. ¹H NMR (DMSO-*d*₆): δ 3.45–3.52 (m, 2H), 4.03–4.08 (m, 2H), 5.64 (br s, 1H), 7.23 (t, 1H, *J* = 6.0 Hz), 7.32 (t, 2H, *J* = 9.0 Hz), 7.38–7.55 (m, 3H), 7.88 (d, 1H, *J* =

6.0 Hz), 8.36–8.43 (m, 2H), 12.67 (br s, 1H). HRMS calcd for $C_{20}H_{15}N_4O_4F$ (M^+), 346.1221; found (M^+), 346.1225. Anal. for ($C_{20}H_{15}N_4O_4F \cdot 1.0MeOH$) C, H, N.

References

- (1) *From DNA Damage and Stress Signaling to Cell Death: Poly ADP-Ribosylation Reactions*; de Murcia, G., Shall, S., Eds; Oxford University Press: Oxford, United Kingdom, 2000; and references therein.
- (2) *Cell Death: The Role of PARP*; Szabo, C., Ed.; CRC Press: Boca Raton, FL, 2000; and references therein.
- (3) Ame, J. C.; Rolli, V.; Schreiber, V.; Niedergang, C.; Apiou, F.; Decker, P.; Muller, S.; Hoger, T.; Ménissier de Murcia, J.; de Murcia, G. PARP-2, a novel mammalian DNA damage-dependent poly (ADP-ribose) polymerase. *J. Biol. Chem.* **1999**, *274*, 17860–17868.
- (4) Johansson, M. A human poly (ADP-ribose) polymerase gene family (ADPRTL): cDNA cloning of two novel poly (ADP-ribose) polymerase homologues. *Genomics* **1999**, *57*, 442–445.
- (5) Kickhoefer, V. A.; Siva, A. C.; Kedersha, N. L.; Inman, E. M.; Ruland, C.; Streuli, M.; Rome, L. H. The 193-kD vault protein, VPAP, is a novel poly (ADP-ribose) polymerase. *J. Cell Biol.* **1999**, *146*, 917–928.
- (6) Smith, S.; Girat, I.; Schmitt, A.; De Lange, T. Tankyrase, a poly (ADP-ribose) polymerase at human telomeres. *Science* **1998**, *282*, 1484–1487.
- (7) de Murcia, G.; Ménissier de Murcia, J. Poly (ADP-ribose) polymerase: A molecular nick-sensor. *TIBS* **1994**, *19*, 172–176.
- (8) Benjamin, R. C.; Gill, D. M. Poly (ADP-ribose) synthesis in vitro programmed by damaged DNA. *J. Biol. Chem.* **1981**, *255*, 10502–10508.
- (9) Satoh, M.; Lindahl, T. Role of poly (ADP-ribose) formation in DNA repair. *Nature* **1992**, *356*, 356–358.
- (10) Pieper, A. A.; Verma, A.; Zhang, J.; Snyder, S. H. Poly (ADP-ribose) polymerase, nitric oxide and cell death. *TIPS* **1999**, *20*, 171–181.
- (11) D'Amours, D.; Desnoyers, S.; D'Silva, I.; Poirier, G. G. Poly (ADP-ribose)ylation reactions in the regulation of nuclear functions. *Biochem. J.* **1999**, *342*, 249.
- (12) Lautier, D.; Lagueux, J.; Thibodeau, J.; Menard, L.; Poirier, G. G. Molecular and biochemical features of poly (ADP-ribose) metabolism. *Mol. Cell. Biochem.* **1993**, *122*, 171–193.
- (13) Lindahl, T.; Satoh, M. S.; Poirier, G. G.; Klungland, A. Post-translational modification of poly (ADP-ribose) polymerase induced by DNA strand breaks. *TIBS* **1995**, *20*, 405–411.
- (14) Dantzer, F.; Schreiber, V.; Niedergang, C.; Trucco, C.; Flatter, E.; De La Rubia, G.; Pliver, J.; Rolli, V.; Ménissier de Murcia, J.; de Murcia, G. Involvement of poly (ADP-ribose) polymerase in base excision repair. *Biochimie* **1999**, *81*, 69–75.
- (15) Arundel-Suto, C. A.; Scavone, S. V.; Turner, W. R.; Suto, M. J.; Sebolt-Leopold, J. S. Effects of PD 128763, a new potent inhibitor of poly (ADP-ribose) polymerase, on X-ray-induced cellular recovery processes in chinese hamster v79 cells. *Radiat. Res.* **1991**, *126*, 367–371.
- (16) Sheng, H.; Olive, P. L. Reduction of tumor hypoxia and inhibition of DNA repair by nicotinamide after irradiation of SCCVII murine tumor and normal tissue. *Cancer Res.* **1996**, *56*, 2801–2808.
- (17) Griffin, R. J.; Srinivasan, S.; Bowman, K.; Calvert, A. H.; Curtin, N. J.; Newell, D. R.; Pemberton, L. C.; Golding, B. T. Resistance-modifying agents. 5. Synthesis and biological properties of quinazoline inhibitors of the DNA repair enzyme poly(ADP-ribose) polymerase (PARP). *J. Med. Chem.* **1998**, *41*, 5247–5256.
- (18) Delaney, C. A.; Wang, L. Z.; Kyle, S.; White, A. W.; Calvert, A. H.; Curtin, N. J.; Durkacz, B. W.; Hostomsky, Z.; Newell, D. R. Potentiation of temozolomide and topotecan growth inhibition and cytotoxicity by novel poly(adenosine diphosphoribose) polymerase inhibitors in a panel of human tumor cell lines. *Clin. Cancer Res.* **2000**, *6*, 2860–2867.
- (19) Bowman, K. J.; Newell, D. R.; Calvert, A. H.; Curtin, N. J. Differential effects of the poly(ADP-ribose) polymerase (PARP) inhibitor NU1025 on topoisomerase I and II inhibitor cytotoxicity. *Br. J. Cancer* **2001**, *84*, 106–112.
- (20) Calabrese, C. R.; Almasy, R.; Barton, S.; Batey, M. A.; Boritzki, T.; Calvert, A. H.; Canan-Koch, S.; Durkacz, B. W.; Hostomsky, Z.; Kumpf, R. A.; Kyle, S.; Li, J.; Maegley, K.; Newell, D. R.; North, M.; Notarianni, E.; Stratford, I. J.; Skaltitzky, D.; Thomas, H. D.; Wang, L.-Z.; Webber, S. E.; Williams, K. J.; Curtin, N. J. Anticancer chemo- and radio-sensitization in vitro and in vivo by a potent novel poly(ADP-ribose) polymerase inhibitor, AG14361. *J. Natl. Can. Inst.* **2004**, *96*, 56–67.
- (21) Tentori, L.; Portarena, I.; Graziani, G. Potential clinical applications of poly(ADP-ribose) polymerase (PARP) inhibitors. *Pharmacol. Res.* **2002**, *45*, 73–85.
- (22) Zhang, J. PARP inhibition: A novel approach to treat ischemia/reperfusion and inflammation-related injuries. *Emerging Drugs* **1999**, *4*, 209–221 and references therein.
- (23) Szabó, C.; Dawson, V. L. Role of poly(ADP-ribose) synthetase in inflammation and ischemia-reperfusion. *TIPS* **1998**, *19*, 287–298.
- (24) Cusi, C.; Suzuki, H.; Skaper, S. D.; Milani, D.; Facci, L.; Menegazzi, M.; Vantini, G.; Kanai, Y.; Degryse, A.; Colpaert, F.; Koek, W.; Marien, M. R. Poly(ADP-ribose) polymerase (PARP) revisited. A new role for an old enzyme: PARP involvement in neurodegeneration and PARP inhibitors as possible neuroprotective agents. *Ann. N. Y. Acad. Sci.* **1997**, *825* (Neuroprotective Agents), 366–379.
- (25) Szabó, C.; Virág, L.; Cuzzocrea, S.; Scott, G. S.; Hake, P.; O'Connor, M. P.; Zingarelli, B.; Salzman, A.; Kun, E. Protection against peroxy-nitrite-induced fibroblast injury and arthritis development by inhibition of poly(ADP-ribose) synthetase. *Proc. Natl. Acad. Sci. U.S.A.* **1998**, *95*, 3867–3872.
- (26) Purnell, M. R.; Whish, W. J. D. Novel inhibitors of poly(ADP-ribose) synthetase. *Biochem. J.* **1980**, *185*, 775–777.
- (27) Banasik, M.; Komura, H.; Shimoyama, M.; Ueda, K. Specific inhibitors of poly(ADP-ribose) synthetase and mono(ADP-ribosyl)transferase. *J. Biol. Chem.* **1992**, *267*, 1569–1575.
- (28) Suto, M. J.; Turner, W. R.; Arundel-Suto, C. M.; Werbel, L. M.; Sebolt-Leopold, J. S. Dihydroisoquinolinones: The design and synthesis of a new series of potent inhibitors of poly(ADP-ribose) polymerase. *Anti-Cancer Drug Des.* **1991**, *7*, 107–117.
- (29) White, A. W.; Almasy, R.; Calvert, A. H.; Curtin, N. J.; Griffin, R. J.; Hostomsky, Z.; Maegley, K.; Newell, D. R.; Srinivasan, S.; Golding, B. T. Resistance-modifying agents. 9. Synthesis and biological properties of benzimidazole inhibitors of the DNA repair enzyme poly(ADP-ribose) polymerase. *J. Med. Chem.* **2000**, *43*, 4084–4097.
- (30) Canan Koch, S. S.; Thoresen, L. H.; Tikhe, J. G.; Maegley, K. A.; Almasy, 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.; Curtin, 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. Novel tricyclic poly(ADP-ribose) polymerase-1 inhibitors with potent anticancer chemoprotecting activity: Design, synthesis, and X-ray cocrystal structure. *J. Med. Chem.* **2002**, *45*, 4961–4974.
- (31) Skaltitzky, D. J.; Marakovits, J. T.; Maegley, K. A.; Ekker, A.; Yu, X.-H.; Hostomsky, Z.; Webber, S. E.; Eastman, B. W.; Almasy, R. J.; Li, J.; Curtin, N. J.; Newell, D. R.; Calvert, A. H.; Griffin, R. J.; Golding, B. T. Tricyclic benzimidazoles as potent PARP-1 inhibitors. *J. Med. Chem.* **2003**, *46*, 210–213.
- (32) Ruf, A.; Rolli, V.; de Murcia, G.; Schulz, G. E. The mechanism of the elongation and branching reaction of poly(ADP-ribose) polymerase as derived from crystal structures and mutagenesis. *J. Mol. Biol.* **1998**, *278*, 57–65.
- (33) Marsischky, G. T.; Wilson, B. A.; Collier, R. J. Role of glutamic acid 988 of human poly(ADP-ribose) polymerase in polymer formation. *J. Biol. Chem.* **1995**, *270*, 3247–3254.
- (34) Ruf, A.; de Murcia, J. M.; de Murcia, G. M.; Schulz, G. E. Structure of the catalytic fragment of poly(ADP-ribose) polymerase from chicken. *Proc. Natl. Acad. Sci. U.S.A.* **1996**, *93*, 7481–7485.
- (35) Chen, X.-Y.; Berti, P. J.; Schramm, V. L. Ricin A-chain: Kinetic isotope effect and transition state structure with stem-loop RNA. *J. Am. Chem. Soc.* **2000**, *122*, 1609–1617.
- (36) Han, S.; Craig, J. A.; Putnam, C. D.; Carozzi, N. B.; Tainer, J. A. Evolution and mechanism from structures of an ADP-ribosylating toxin and NAD complex. *Nat. Struct. Biol.* **1999**, *6*, 932–936.
- (37) Ruf, A.; de Murcia, G.; Schulz, G. E. Inhibitor and NAD⁺ binding to poly(ADP-ribose) polymerase as derived from crystal structures and homology modeling. *Biochemistry* **1998**, *37*, 3893–3900.
- (38) Watson, C. Y.; Whish, W. J. D.; Threadgill, M. D. Synthesis of 3-substituted benzamides and 5-substituted isoquinolin-1-(2H)-ones and preliminary evaluation as inhibitors of poly(ADP-ribose) polymerase (PARP). *Bioorg. Med. Chem.* **1998**, *6*, 721–734.
- (39) In the human PARP-1 enzyme, amino acid 763 is a glutamic acid. See Computational Methods in the Experimental Section for details.
- (40) Hester, J. B.; Rudzik, A. D.; Veldkamp, W. Pyrrolo[3,2,1-jk][1,4]benzodiazepines which have central nervous system activity. *J. Med. Chem.* **1970**, *13*, 827–835.
- (41) Miyauchi, N.; Suzuki, A. Palladium-catalyzed cross-coupling reactions of organoboron compounds. *Chem. Rev.* **1995**, *95*, 2457–2483.
- (42) Bradley, G.; Clark, J. 2,3-Dihydroquinolin-4(1H)-ones. Part I. Halogen-substituted 2,3-dihydroquinolin-4(1H)-ones and their 1-(2-acylethyl) derivatives. *J. Chem. Soc., Perkins 1* **1972**, 2019–2023.

- (43) Eaton, P. E.; Carlson, G. R.; Lee, J. T. Phosphorous pentoxide-methanesulfonic acid. A convenient alternative to polyphosphoric acid. *J. Org. Chem.* **1973**, *38*, 4071–4073.
- (44) For a review of the Schmidt reaction, see Wolff, H. *Organic Reactions*; 1946; Vol. 3, Chapter 8, pp 307–336.
- (45) Taylor, E. C.; Katz, A. H.; Salgado-Zamora, H.; McKillop, A. Thallium in organic synthesis. 68. A convenient synthesis of 2-phenylindoles from anilides. *Tetrahedron Lett.* **1985**, *26*, 5963–5966.
- (46) Sakamoto, T.; Kondo, Y.; Yamanaka, H. Synthesis of condensed heteroaromatic compounds using palladium-catalyzed reaction, *Heterocycles* **1988**, *27*, 2225–2249.
- (47) Simonin, F.; Poch, O.; Delarue, M.; de Murcia, G. Identification of potential active-site residues in the human poly(ADP-ribose) polymerase. *J. Biol. Chem.* **1993**, *268*, 8529–8535.
- (48) Segel, I. H. *Enzyme Kinetics: Behavior and Analysis of Rapid Equilibrium and Steady-State Enzyme Systems*; John Wiley & Sons: New York, 1975; pp 100–125.
- (49) Sculley, M. J.; Morrison, J. F. The determination of kinetic constants governing the slow, tight-binding inhibition of enzyme-catalyzed reactions. *Biochim. Biophys. Acta* **1986**, *874*, 44–53.
- (50) Brunger, A. T.; Adams, P. D.; Clore, G. M.; DeLano, W. L.; Gros, P.; Grosse-Kunstleve, R. W.; Jiang, J. S.; Kuszewski, J.; Nilges, M.; Pannu, N. S.; Read, R. J.; Rice, L. M.; Simonson, T.; Warre, G. L. Crystallography and NMR system: A new software suite for macromolecular structure determination. *Acta Crystallogr. D: Biol. Crystallogr.* **1998**, *54*, 905–921.
- (51) Mohamadi, F.; Richards, N. G. J.; Guida, W. C.; Liskamp, R.; Lipton, M.; Caufield, C.; Chang, G.; Hendrickson, T.; Still, W. C. Macromodel V5.5; Macromodel—An integrated software system for modeling organic and bioorganic molecules using mechanics. *J. Comput. Chem.* **1990**, *11*, 440–467.

JM030513R