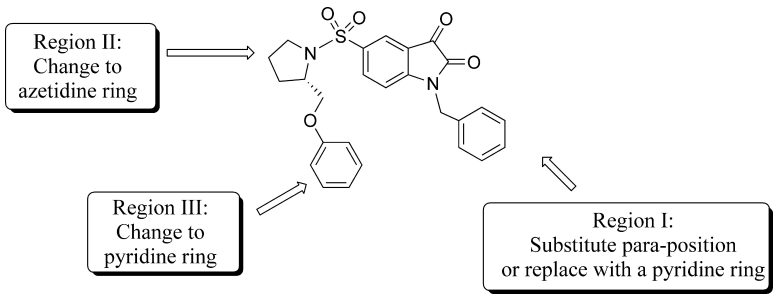
Journal of Medicinal Chemistry

Article

N-Benzylisatin Sulfonamide Analogues as Potent Caspase-3 Inhibitors: Synthesis, in Vitro Activity, and Molecular Modeling Studies

Wenhua Chu, Jun Zhang, Chenbo Zeng, Justin Rothfuss, Zhude Tu, Yunxiang Chu, David E. Reichert, Michael J. Welch, and Robert H. Mach

J. Med. Chem., **2005**, 48 (24), 7637-7647• DOI: 10.1021/jm0506625 • Publication Date (Web): 05 November 2005



More About This Article

Additional resources and features associated with this article are available within the HTML version:

- Supporting Information
- Links to the 4 articles that cite this article, as of the time of this article download
- Access to high resolution figures
- Links to articles and content related to this article
- Copyright permission to reproduce figures and/or text from this article

View the Full Text HTML

N-Benzylisatin Sulfonamide Analogues as Potent Caspase-3 Inhibitors: Synthesis, in Vitro Activity, and Molecular Modeling Studies

Wenhua Chu,† Jun Zhang,† Chenbo Zeng, Justin Rothfuss, Zhude Tu, Yunxiang Chu, David E. Reichert, Michael J. Welch, and Robert H. Mach*

Division of Radiological Sciences, Washington University School of Medicine, 510 South Kingshighway Boulevard, St. Louis, Missouri 63110

Received July 13, 2005

A number of isatin sulfonamide analogues were prepared and their potencies for inhibiting caspase-1, -3, -6, -7, and -8 were evaluated in vitro. Several compounds displaying a nanomolar potency for inhibiting the executioner caspases, caspase-3 and caspase-7, were identified. These compounds were also observed to have a low potency for inhibiting the initiator caspases, caspase-1 and caspase-8, and caspase-6. Molecular modeling studies provided further insight into the interaction of this class of compounds with activated caspase-3. The results of the current study revealed a number of non-peptide-based caspase inhibitors that may be useful in assessing the role of inhibiting the executioner caspases in minimizing tissue damage in disease conditions characterized by unregulated apoptosis.

Introduction

Apoptosis, or programmed cell death, is a conserved process that is mediated by the activation of a series of cysteine aspartyl-specific proteases termed caspases. Apoptosis plays an important role in a wide variety of normal cellular processes including fetal development, tissue homeostasis, and maintenance of the immune system.[1] However, abnormal apoptosis has been observed in large number of pathological conditions, including ischemia-reperfusion injury (stroke and myocardial infarction), cardiomyopathy, neurodegeneration (Alzheimer's Disease, Parkinson's Disease, Huntington's Disease, ALS), sepsis, Type I diabetes, fulminant liver disease, and allograft rejection.[2,3] Therefore, the development of drugs that can halt the process of apoptosis has been an active area of research in the pharmaceutical industry.[2,4]

There are two different classes of caspases involved in apoptosis, the initiator caspases and the executioner caspases.[5] The initiator caspases, which include caspase-6, -8, -9, and -10, are located at the top of the signaling cascade; their primary function is to activate the executioner caspases, caspase-2, -3, and -7. The executioner caspases are responsible for the physiological (e.g., cleavage of the DNA repair enzyme PARP-1, nuclear laminins, and cytoskeleton proteins) and morphological changes (DNA strand breaks, nuclear membrane damage, membrane blebbing) that occur in apoptosis.[2] A third class of caspases, caspases-1, -4, -5, and -13, are involved in cytokine maturation and are not believed to play an active role in apoptosis.

Although it was initially thought that apoptosis could not be stopped once the process was initiated, recent studies suggest that pharmacological intervention can minimize cell death and tissue damage via these pathways. For example, inhibitors of caspase-3 have been shown to reduce the amount of cellular and tissue damage in cell culture and animal models of disease.[6,7] To date, most inhibitors of caspase-3 have been peptide-based compounds that either reversibly or irreversibly inhibit the catalytic activity of this enzyme.[6−13] In vivo studies have shown that the peptide-based inhibitors of caspase-3, M-791 and M-826, reduce apoptosis and tissue damage in animal models of sepsis and neonatal hypoxic-ischemic brain injury.[7,13]

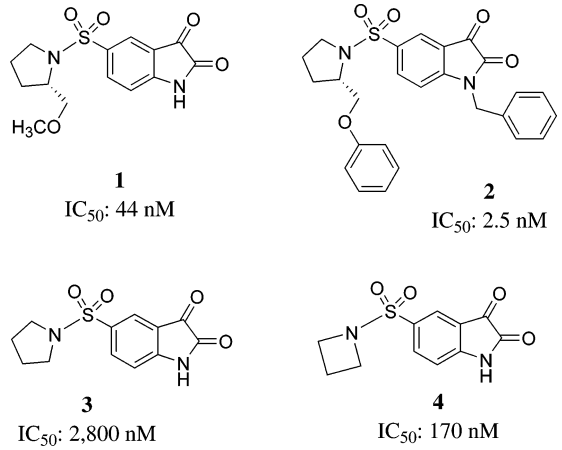


Figure 1. Structure of isatin sulfonamide analogues reported previously.

One of the potential problems of peptide-based caspase inhibitors is their poor metabolic stability and poor cell penetration,[12] which has resulted in a search for non-peptide-based inhibitors of caspase-3 and caspase-7. Recently, a number of isatin-based inhibitors of caspase-3 and caspase-7 have been reported. One compound, (*S*)-(+)-5-[1-(2-methoxymethyl-pyrrolidine)sulfonyl]isatin, **1** (Figure 1), has been shown to reduce tissue damage in an isolated rabbit heart model of ischemic injury.[14,15] Additional structure−activity relationship studies have revealed that replacement of the 2-methoxymethyl group with a phenoxymethyl moiety and the introduction of an alkyl group on the isatin nitrogen group

* To whom correspondence should be addressed. E-mail: rhmach@mir.wustl.edu. Phone: (314) 362-8538. Fax: (314) 362-0039.
† These authors contributed equally to this work.

10.1021/jm0506625 CCC: $30.25
Published on Web 11/05/2005

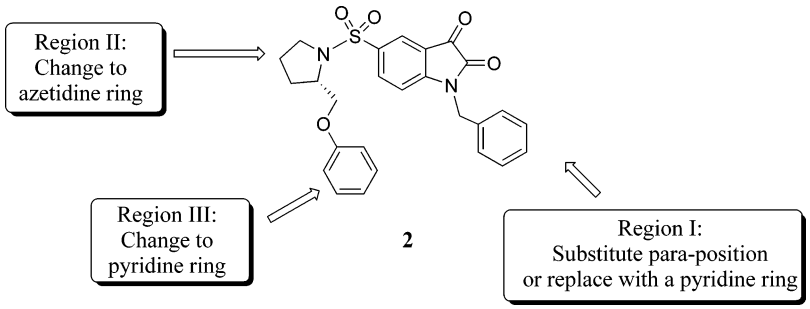


Figure 2. Strategy used in the current structure−activity relationship study.

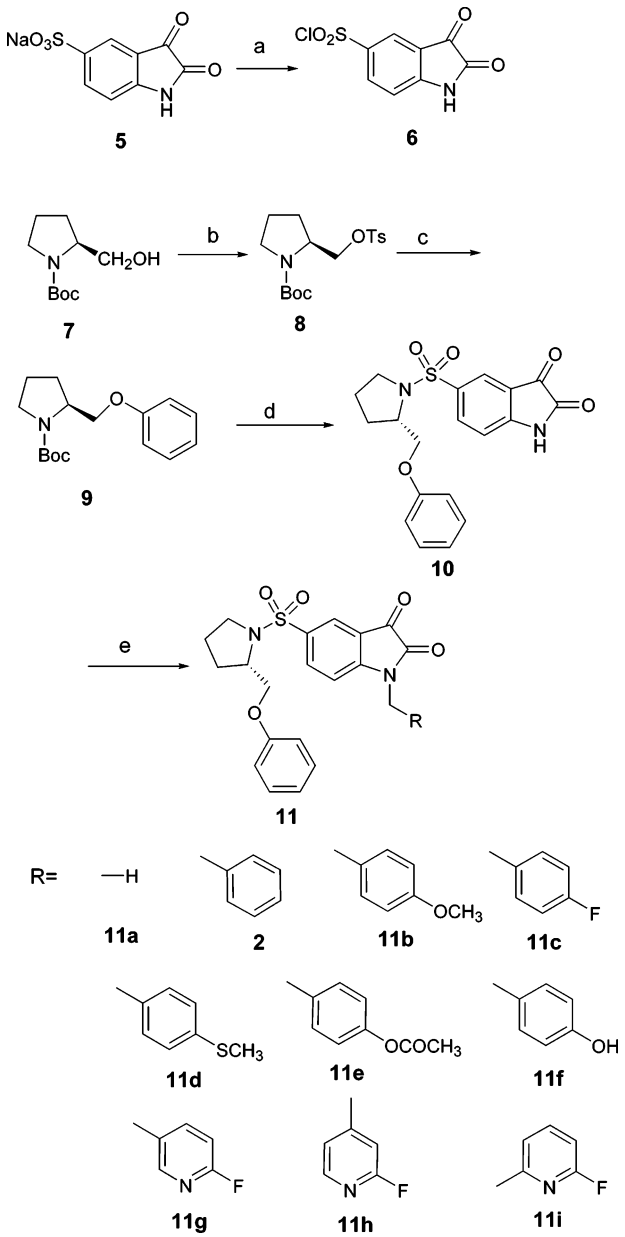
resulted in a dramatic improvement in potency for inhibiting caspase-3 activity (**2**) (Figure 1).[16] An additional improvement in potency was also reported when the pyrrolidine ring of **3** was replaced with an azetidine ring to give compound **4**.[16] The goal of the current study is to extend the structure−activity relationship study of this class of compounds by incorporating the following changes into lead compound **2** (Figure 2): (1) substituting the para position of the N-benzyl group in order to determine if there are any substituent effects with respect to caspase-3 inhibition potency; (2) replacing the pyrrolidine ring with an azetidine ring; and, (3) replacing the benzene ring of the phenoxymethyl moiety with a pyridine ring. Docking studies were performed in order to gain insight into structural features which determine potency of this class of compounds for inhibiting caspase-3 activity.

Results

Chemistry. The synthesis of 5-(2-phenoxymethyl-pyrrolidine-1-sulfonyl)isatin analogues is shown in Scheme 1. The 5-chlorosulfonylisatin **6** was prepared by reaction of 5-isatinsulfonic acid, sodium salt hydrate (**5**) with phosphorus oxychloride in tetramethylene sulfone at 60 °C for 3 h. The hydroxyl group of *N*-Boc-2-pyrrolmethanol (**7**) was first tosylated with *p*-toluenesulfonyl chloride in pyridine to give compound **8**, followed by displacement of the tosylate group by sodium phenoxide in DMF to afford *N*-Boc-2-(phenoxymethyl)pyrrolidine **9**. The *N*-Boc group of **9** was removed with TFA, and the secondary amine was coupled with **6** in THF using triethylamine as an acid scavenger to afford the 5-(2-phenoxymethyl-pyrrolidine-sulfonyl)-1*H*-2,3-dione **10** in 84% yield. The isatin nitrogen was alkylated by treatment of **10** with sodium hydride in DMF at 0 °C followed by addition of various alkyl halides to give compounds **2** and **11a**−**e**,**g**−**i**. Compound **11f** was prepared by hydrolysis of **11e** with sodium hydroxide in aqueous methanol.

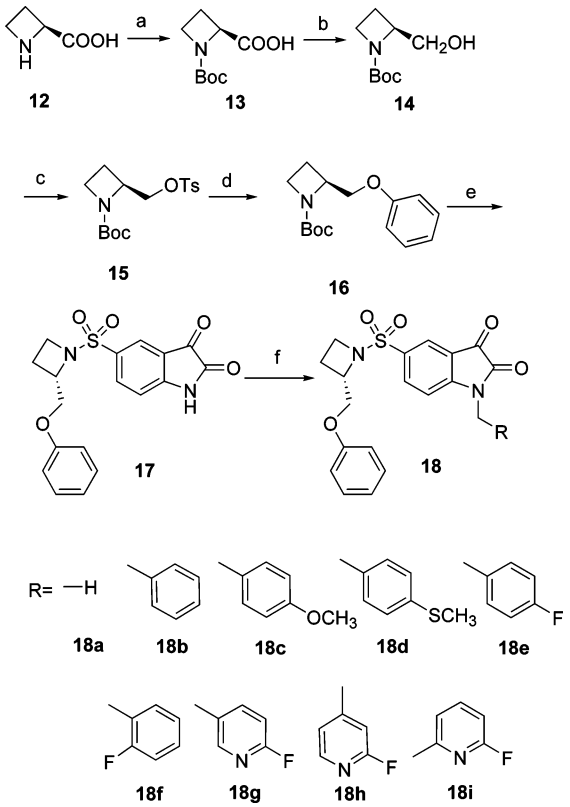
The synthesis of 5-(2-phenoxymethyl-azetidine-1-sulfonyl)isatin analogues is shown in Scheme 2. The intermediate (*S*)-*N*-Boc-2-azetidinemethanol **14** was prepared from (*S*)-2-azetidinecarboxylic acid **12** according to the literature method.[17] The hydroxy group of **14** was tosylated with *p*-toluenesulfonyl chloride in pyridine to afford compound **15**, which was converted to the corresponding phenoxyl group as described above to give **16**. Compound **16** was deprotected with TFA, and the secondary amine was coupled with **6** using triethylamine as the base to afford **17** in 63% yield. The nitrogen of **17** was alkylated by the same procedure as that of **10** to give compounds **18a**−**i**. Similarly, the 5-(2-pyridin-3-yl-oxymethyl)pyrrolidine-1-sulfonyl)isatin analogues were prepared by using the same sequence of reactions described in the synthesis of **11a**−**i** to afford compounds **21a**−**e** (Scheme 3). The synthesis of the 4-pyridyl analogue **23** is also outlined in Scheme 3.

Scheme 1[a]



[a] Reagents: (a) $POCl_3$; (b) *p*-toluenesulfonyl chloride, pyridine; (c) phenol, NaH, THF; (d) (1) TFA, CH_2Cl_2, (2) **6**, triethylamine; (e) NaH, DMF, R-CH_2X (X = Cl, Br, I).

Scheme 2[a]



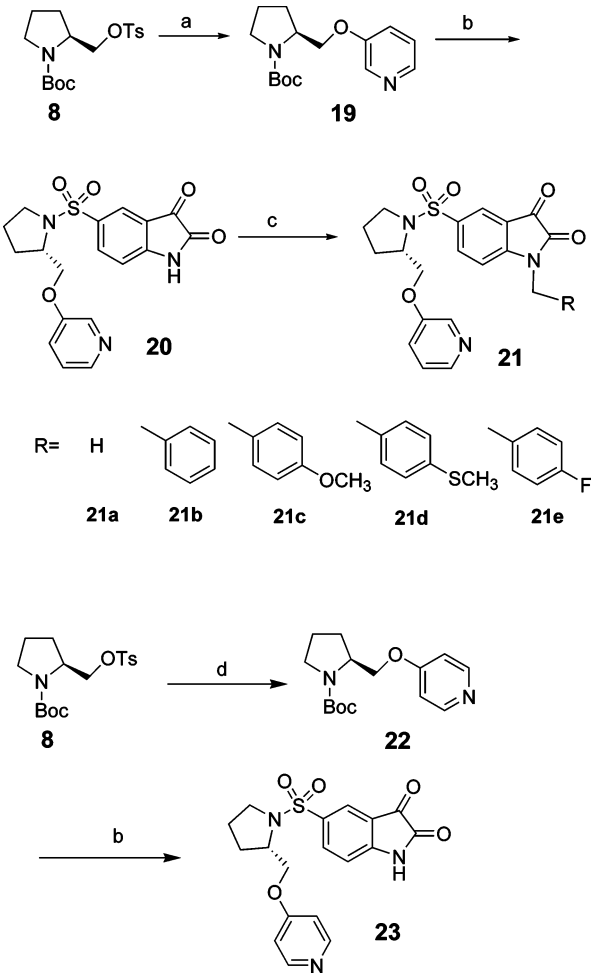
[a] Reagents: (a) Di-*tert*-butyl dicarbonate; (b) BH_3, THF; (c) *p*-toluenesulfonyl chloride, pyridine; (d) phenol, NaH, THF; (e) (1) TFA, (2) **6**, triethylamine; (f) NaH, DMF, R-CH_2X (X = Cl, Br, I).

Enzyme Assays. Inhibition of recombinant human caspase-3 and other caspases by the isatin analogues was assessed using a fluorometric assay by measuring the accumulation of a fluorogenic product, 7-amino-4-methylcoumarin (7-AMC). All of the tested compounds inhibited caspase-3 and caspase-7 in a concentration-dependent manner with similar potency. The IC_{50} values from the enzyme assays are summarized in Tables 1−3. Alkylation of the isatin nitrogen of **10** with a benzyl group (i.e., **2**) or substituted benzyl group (i.e., **11c**−**e**) resulted in a 10 to 20-fold increase in potency for inhibiting caspase-3, and a 9 to 37-fold increase in potency for inhibiting caspase-7. The isatin analogues were also evaluated for their inhibitory activity against a panel of three other caspases (caspases-1, -6, and -8). As shown in Table 1, they demonstrated high selectivity against caspase-3 and -7, with IC_{50} values at least 100-fold higher versus caspases-1, -6, and -8.

The azetidine analogue **17** had a similar potency for inhibiting caspase-3 as that of the corresponding pyrrolidine analogue **10**. However, compound **17** was >2-fold less potent for inhibiting caspase-7 relative to the corresponding pyrrolidine analogue, **10**. Substitution of **17** with either a benzyl (i.e., **18b**), a substituted benzyl (**18c**−**f**), or a pyridylmethyl group (**18g**−**i**) resulted in a 10 to 50-fold increase in potency against caspase-3 and a 10 to 80-fold increase in potency for inhibiting caspase-7 relative to **17** (Table 2). Again, these compounds exhibited at least 100-fold greater selectivity for caspase-3 and -7 versus caspases-1, -6, and -8.

Interestingly, a higher caspase-3 potency was achieved upon replacing the benzene ring of the 2-(phenoxymethyl)pyrrolidine moiety with a pyridine ring (Table 3). All pyridine-containing analogues had a lower IC_{50} value for inhibiting caspase-3 than the corresponding benzene-containing congeners (eg., **11a** vs **21a**, **11d** vs **21d**). Compound **21c** was found to be the most potent inhibitor of caspase-3, with IC_{50} of 3.9 nM. These compounds demonstrated similar potency against caspase-3 and 7, but at least 100 fold less potent versus caspases-1, -6, and -8.

Scheme 3[a]



[a] Reagents: (a) 3-Hydroxypyridine, NaH, THF/DMF; (b) (1) TFA, CH_2Cl_2, (2) **6**, triethylamine; (c) NaH, DMF, R-CH_2X (X = Cl, Br, I); (d) 4-hydroxypyridine, NaH, THF/DMF.

Kinetic studies were also conducted in order to determine the mechanism of inhibition of caspase-3 activity by compound **21c**. The kinetic pattern indicated that **21c** displays competitive inhibition versus Ac-DEVD-AMC with a calculated K_i value of 4.4 nM (Figure 3). These data are consistent with previous studies demonstrating that the isatin analogues bind to the catalytic site of activated caspase-3.[16]

Modeling Studies. Becker et al.[12] analyzed a series of seven caspase-3 inhibitor complexes and found that the tertiary and quaternary structures were remarkably similar regardless of the inhibitors involved. The key interactions found in these crystal structures, particularly the covalent attachment to Cys285, hydrogen bonds to Cys285 and Gly238, and polar interactions with Arg179 and Gln283, were remarkably similar to those found in the isatin-based inhibitor developed by Lee et al.[14] (PDB ID: 1GFW), shown in Figure 4. Lee

Table 1. Inhibitor Selectivity of Pyrrolidine Isatin Analogues for Caspases-1, -3, -6, -7, and -8

	IC_{50} (nM)					
	caspase-1	caspase-3	caspase-6	caspase-7	caspase-8	Log P
10	>10000	240.0 ± 10.0	>5000	540.0 ± 56.6	>50000	2.23
11a	>20000	119.2 ± 17.0	>5000	310.0 ± 14.1	>50000	2.27
2	>10000	12.2 ± 0.3	>5000	28.0 ± 0.7	>50000	4.05
11b	>10000	14.5 ± 1.6	>5000	21.8 ± 3.5	>50000	3.96
11c	>50000	12.1 ± 2.1	>5000	23.0 ± 1.4	>50000	4.1
11d	>50000	12.4 ± 2.1	>5000	41.0 ± 1.4	>50000	4.54
11e	>50000	12.0 ± 1.5	>5000	34.8 ± 0.4	>50000	3.39
11f	>5000	13.5 ± 2.4	>5000	44.0 ± 0.1	>50000	3.31
11g	>50000	10.3 ± 1.5	>5000	14.5 ± 0.9	>50000	2.67
11h	>50000	21.3 ± 3.2	>5000	58.0 ± 2.8	>50000	2.67
11i	>50000	9.1 ± 1.8	>5000	22.2 ± 4.0	>50000	2.67
standard						
Ac-YVAD-CHO	8.1 ± 2.1	-	-	-	-	
Ac-DEVD-CHO	-	3.8 ± 0.8	-	8.5 ± 1.0	-	
Ac-VEID-CHO	-	-	60.5 ± 7.6	-	-	
Ac-IETD-CHO	-	-	-	-	4.7 ± 0.9	

Table 2. Inhibitor Selectivity of the Azetidine Isatin Analogues **17**, **18a**−**i** for Caspases-1, -3, -6, -7, and -8

	IC_{50} (nM)					
	caspase-1	caspase-3	caspase-6	caspase-7	caspase-8	Log P
17	>10000	286.7 ± 24.7	>5000	1350.0 ± 141.4	>50000	1.66
18a	>10000	91.7 ± 7.6	>5000	362.5 ± 3.5	>50000	1.71
18b	>10000	9.7 ± 1.6	>5000	29.5 ± 4.9	>50000	3.49
18c	>50000	8.4 ± 1.2	>5000	23.2 ± 3.0	>50000	3.4
18d	>50000	11.3 ± 1.2	>5000	26.7 ± 7.2	>50000	3.97
18e	>10000	8.8 ± 1.4	>5000	21.0 ± 5.6	>50000	3.54
18f	>10000	9.4 ± 0.3	>5000	26.0 ± 5.2	>50000	3.54
18g	>50000	10.9 ± 1.4	>5000	17.0 ± 3.0	>50000	2.11
18h	>50000	29.2 ± 5.2	>5000	135.0 ± 7.1	>50000	2.11
18i	>10000	5.8 ± 1.0	>5000	22.7 ± 3.1	>50000	2.11

Table 3. Selectivity Profile of the Pyridine Analogues within the Caspase Family

	IC_{50} (nM)					
	caspase-1	caspase-3	caspase-6	caspase-7	caspase-8	Log P
20	>5000	58.3 ± 7.6	>5000	214.9 ± 49.5	>50000	1.17
21a	>10000	23.3 ± 3.1	>5000	94.9 ± 21.6	>50000	1.21
21b	>10000	5.2 ± 1.6	>5000	14.1 ± 3.4	>50000	2.99
21c	>10000	3.9 ± 0.9	>5000	15.1 ± 1.2	>50000	2.91
21d	>50000	4.4 ± 1.4	>5000	23.3 ± 0.7	>50000	3.48
21e	>10000	8.4 ± 2.0	>5000	15.1 ± 0.1	>50000	3.04
23	>5000	20.4 ± 1.7	>5000	142.3 ± 22.6	>50000	1.04

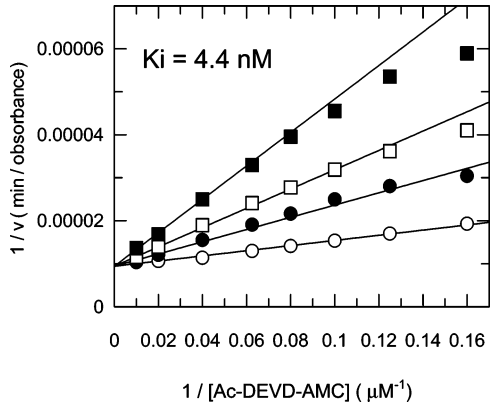


Figure 3. Competitive inhibition of caspase-3 by **21c**. The concentration of **21c** was 0 (○), 5 (●), 10 (□), and 20 nM (■).

et al.[14] found that the isatin-based inhibitors developed their selectivity through hydrophobic contacts between the pyrrolidine ring and the residues of the S_2 pocket.

To understand how the compounds described in this work interact with caspase-3, docking studies were performed using the program Gold. The binding site of caspase-3 for these inhibitors is quite wide and shallow with extensive solvent accessibility which tends to lead to a large number of accessible poses in docking studies. By utilizing covalent constraints, Gold was able to successfully dock all twenty-eight compounds. The compounds all bound in a fashion similar to that of the isatin MSI found in the crystal structure 1GFW. Figure 5 shows the superimposition of the highest scoring poses of the pyrrolidines **2**, **11a**−**i**, and the azetidines **18a**−**i**.

Discussion

It was previously reported that isatin sulfonamides are potent and selective non-peptide-based inhibitors of the executioner caspases, caspase-3 and -7.[16] The key structural properties that resulted in high potency for inhibiting caspase-3 and -7 identified in this initial structure−activity relationship study were the presence of an *N*-benzyl moiety off the isatin nitrogen atom and an (*S*)-2-phenoxymethyl moiety in the 2-position of the pyrrolidine ring (Figure 1). The goal of the current study was to extend the structure−activity relationships of

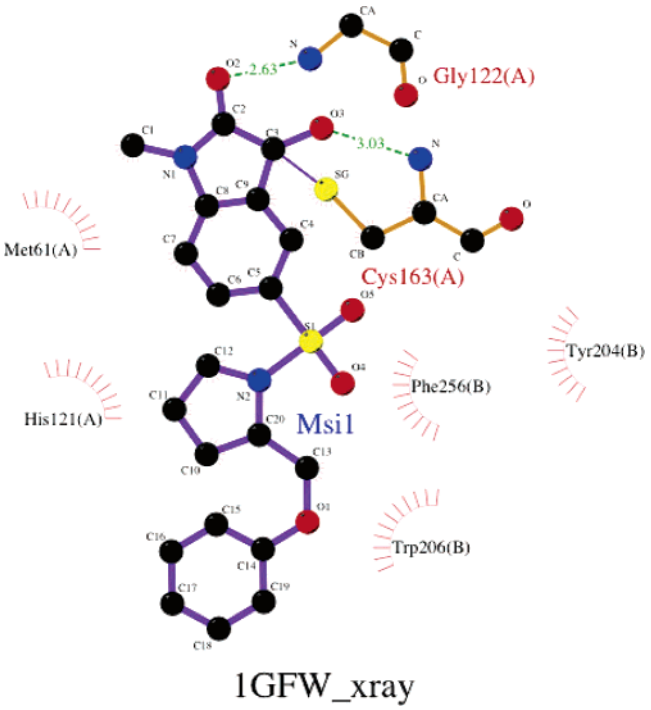


Figure 4. Binding interactions of the isatin-based inhibitor MSI found in the crystal structure 1GFW. Hydrogen bonds are shown as green lines; hydrophobic contacts are shown as radial hemispheres. Binding interactions were calculated with the program LigPlot.[20]

this class of compounds by using the strategy outlined in Figure 2. The first conclusion that can be reached from the current study was the absence of a substituent effect in the aromatic ring of the *N*-benzyl moiety of compound **2**. The results outlined in Table 1 indicate that either substitution of the para position of **2** or replacement of the benzene ring with a pyridine ring results in little change in potency for inhibiting caspase-3 and caspase-7. These results are consistent with the earlier observations regarding the substitution of the isatin nitrogen with hydrophobic substituents.[16]

A second, and somewhat unexpected, observation was the similar potency between the pyrrolidine analogues **11b**−**i** and the azetidine analogues **18b**−**i**, given the difference in potency for inhibiting caspase-3 by compound **3** and compound **4** (Figure 1). However, the results were explained by molecular modeling studies which revealed a high degree of overlap in binding of the azetidine and pyrrolidine analogues to activated caspase-3 (Figure 5). An analysis of the docked poses for **11a**−**i** and **18a**−**i** found little difference between the two classes in terms of the relative orientation of the phenoxymethyl group and the isatin core. For each molecule, two planes were defined: one containing the phenyl carbons of the phenoxymethyl group, and the second containing the isatin heavy atoms. For the pyrrolidines, these two planes intersected with a mean angle of 43.4 ± 1.7°, while the azetidines had a mean intersection angle of 55.6 ± 3.7°. These differences are too small to have a major effect on the binding mode of the molecules.

The predominant noncovalent interactions appear to be a $\pi-\pi$ interaction between the phenyl of the isatin

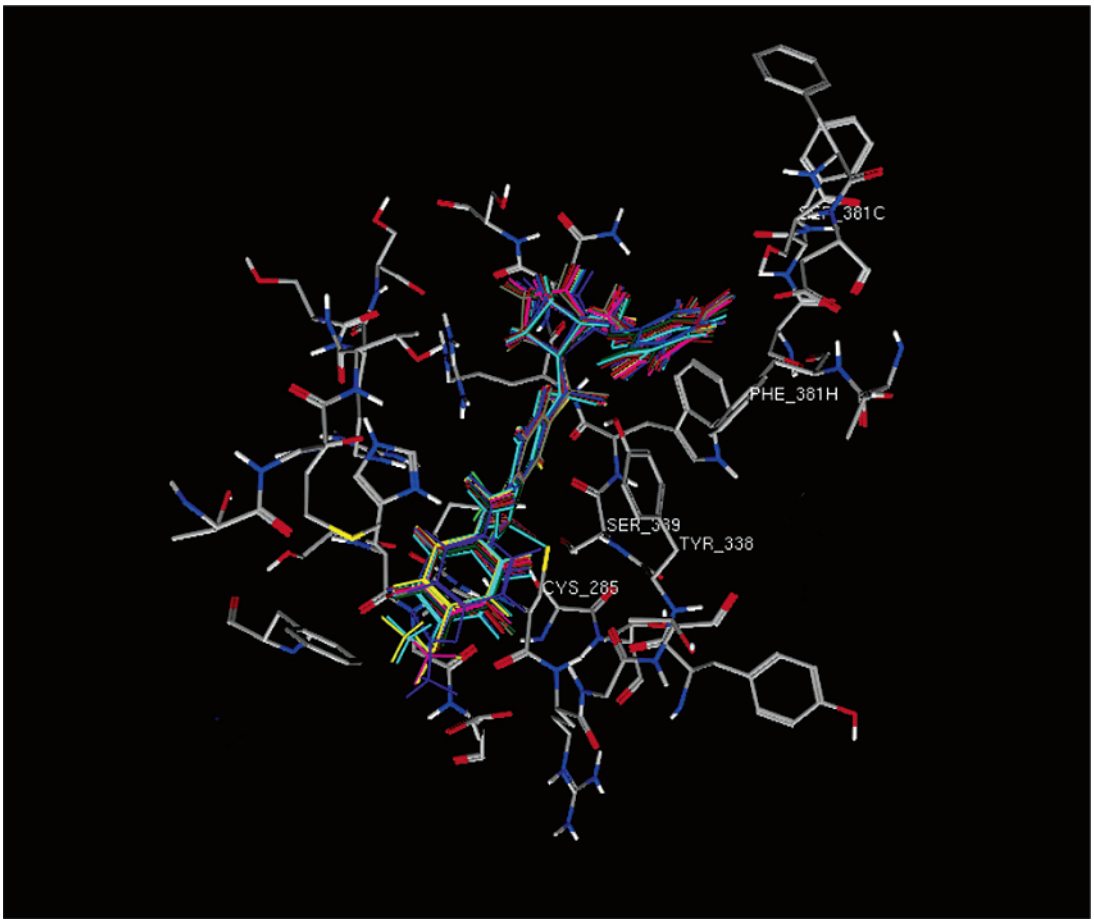


Figure 5. Superimposition of the top poses of pyrrolidines **2**, **11a**−**i** and azetidines **18a**−**i** in the active site of caspase-3.

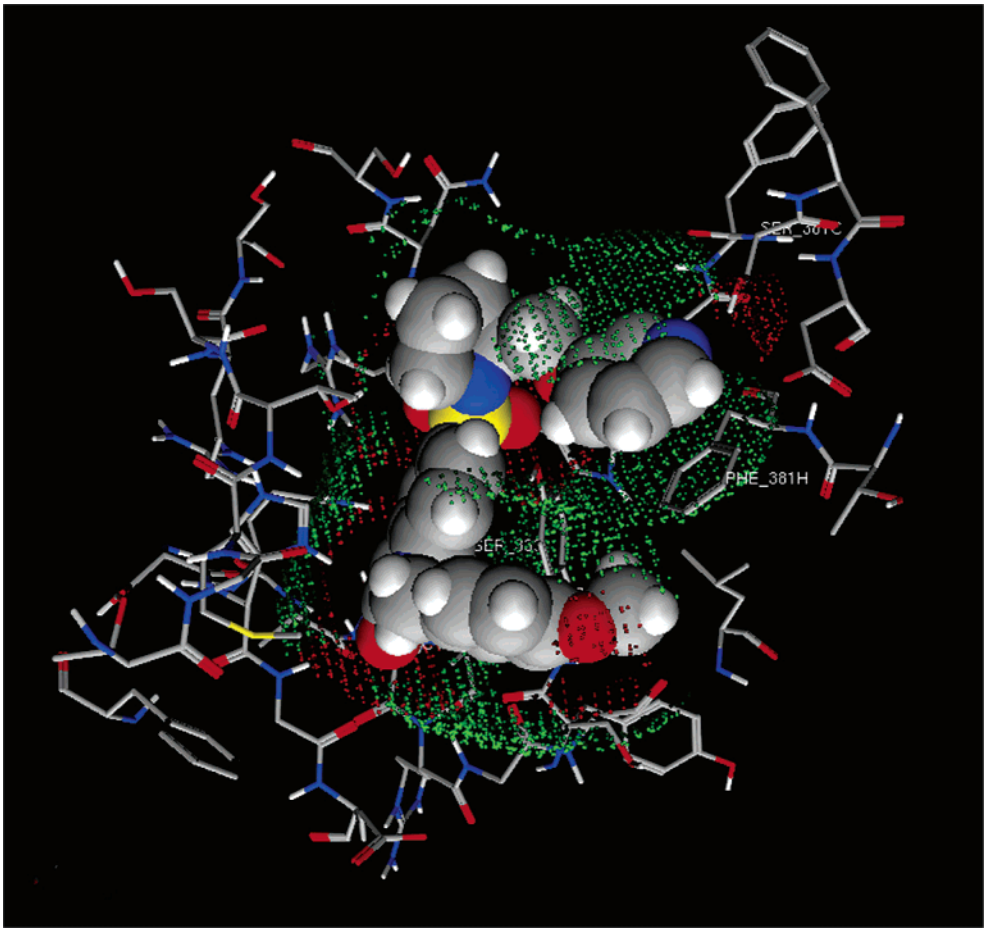


Figure 6. Compound **21c** (space filling) superimposed on the contact statistics surface of the binding site of caspase-3 (1RHJ). Hydrophobic preferences are shown in green, and the hydrophilic preferences are shown in red.

core and Tyr338, and a stabilization of the oxyanion through interactions with the amide hydrogen of Ser339 or the amide hydrogen of Cys285 (also found in the crystal structure 1GFW). A final key interaction appears to be a $\pi-\pi$ interaction between the aromatic group in Region III (Figure 2) and Phe381.

Another unexpected observation was the high potency of the pyridine analogues **21b**−**e** relative to their phenyl congeners, **2** and **11b**−**d**. These data suggest a possible hydrophilic interaction between the phenoxymethyl moiety and the S_3 binding domain of caspase-3 (Figure 6). As already mentioned, all compounds were found to involve a $\pi-\pi$ interaction with Phe381. The pyridine analogues **21b**−**e** also appear perfectly oriented to involve a hydrogen bond between the pyridine nitrogen and the hydroxyl group of Ser381. In compound **21c** this distance is 2.21 Å, although this distance is slightly long it falls within the observed range of this interaction found in the Cambridge database. Comparison of the distance between the same ring position for the phenyl congener **11b** to the Ser381 hydroxyl was 2.92 Å. Compound **23**, with the pyridine attached at the four position instead of at the 3 position, showed a distance of 2.60 Å. While a single hydrogen bond is unlikely to be the sole cause of the differences in inhibitory potencies, the ability to hydrogen bond with Ser381 is consistent with the assay data.

Substitution of the pyridine ring for benzene ring in the phenoxymethyl moiety also resulted in a dramatic reduction in the overall lipophilicity of the isatin analogues.[18,19] For example, compound **2** has a calculated log *P* value of 4.05 whereas the corresponding pyridine analogue, **21b**, has a calculated log *P* value of 2.99. Therefore, the pyridine analogues may have a higher potency for inhibiting activated caspase-3 in which there is an intact cell membrane. Studies evaluating the potency of these compounds in cell culture and animal models of apoptosis are currently ongoing in our laboratory.

In conclusion, we have completed the synthesis and in vitro evaluation of a series of isatin analogues having a high potency for inhibiting the executioner caspases, caspase-3, and caspase-7. The results of this study have extended the structure−activity relationships of this class of compounds and provide further insight into the development of non-peptide-based inhibitors of caspase-3 and caspase-7. The compounds described above may be useful probes for determining the effectiveness of inhibiting caspase-3 and caspase-7 for minimizing tissue damage in pathological conditions characterized by unregulated apoptosis.

Experimental Section

All reactions were carried out under an inert nitrogen atmosphere with dry solvents using anhydrous conditions unless otherwise stated. Reagents and grade solvents were used without further purification. Flash column chromatography was conducted using Scientific Adsorbents, Inc. silica gel, 60a, "40 Micron Flash" (32−63 μm). Melting points were determined using MEL-TEMP 3.0 apparatus and uncorrected. ^{1}H NMR spectra were recorded at 300 MHz on a Varian

Mercury-VX spectrometer. All chemical shift values are reported in ppm (δ). Elemental analyses (C, H, N) were determined by Atlantic Microlab, Inc.

2,3-Dioxo-2,3-dihydro-1*H*-indole-5-sulfonyl Chloride (6).[16] Phosphorus oxychloride (13.2 mL, 141.6 mmol) was added to a solution of 5-isatinsulfonic acid (**5**), sodium salt hydrate (8.0 g, 30.0 mmol) in tetramethylene sulfone (40 mL). The mixture was heated to 60 °C for 3 h, then cooled to 0 °C. The reaction mixture was poured into 150 g of ice. The solid was filtered out and washed with cold water, then the solid was dissolved in ethyl acetate (100 mL), washed with water (50 mL × 2) and saturated NaCl (50 mL), and dried over Na_2SO_4. The ethyl acetate was evaporated in reduced pressure to afford 6.12 g (83%) of **6** as a pale yellow solid, mp 188.2−190.1 °C. ^{1}H NMR (300 MHz, DMSO) δ 11.1 (s, 1H), 7.82 (dd, J = 8.4 Hz, J = 1.8 Hz, 1H), 7.60 (s, 1H), 6.89 (d, J = 8.1 Hz, 1H).

((*S*)-1-(*tert*-Butoxycarbonyl)pyrrolidin-2-yl)methyl 4-Methylbenzesulfonate (8). A solution of **7** (5.03 g, 25.0 mmol) and pyridine (15 mL) in CH_2Cl_2 (50 mL) was reacted with *p*-toluenesulfonyl chloride (5.96 g, 31.2 mmol) at 0 °C. The mixture was stirred overnight at room temperature, then CH_2Cl_2 (50 mL) was added. The solution was washed with water (50 mL × 2), 10% citric acid (50 mL × 2), and saturated NaCl (50 mL) and dried over Na_2SO_4. After evaporation of the CH_2Cl_2, the crude product was purified with hexanes−ethyl ether (1:1) to afford 8.9 g (100%) of **8** as a colorless oil. ^{1}H NMR (300 MHz, DMSO) δ 7.78 (d, J = 8.4 Hz, 2H), 7.49 (d, J = 8.1 Hz, 2H), 4.02 (m, 2H), 3.83 (m, 1H), 3.18 (m, 2H), 2.43 (s, 3H), 1.92 (m, 1H), 1.72 (m, 3H), 1.35 and 1.29 (s, 9H).

(*S*)-*tert*-Butyl 2-(Phenoxymethyl)pyrrolidine-1-carboxylate (9). A solution of phenol (7.37 g, 78.4 mmol) in THF (100 mL) was reacted with 60% NaH (3.14 g, 78.4 mmol) at 0 °C in 20 min. The mixture was warmed to room temperature and stirred 20 min, then a solution of **8** (5.57 g, 15.7 mmol) in THF (25 mL) was added. The mixture was heated to reflux for 24 h. After evaporation of the THF, ether (200 mL) was added, washed with water (40 mL), 1 N NaOH (40 mL × 3), and saturated NaCl (40 mL), and dried over Na_2SO_4. After evaporation of the ether, the crude product was purified with hexanes−ether (2:1) to afford 2.37 g (54%) of **9** as a colorless oil. ^{1}H NMR (300 MHz, DMSO) δ 7.28 (t, J = 8.4 Hz, 2H), 6.95 (m, 3H), 4.04 (m, 2H), 3.87 (m, 1H), 3.27 (m, 2H), 1.93−1.80 (m, 4H), 1.41 (s, 9H).

(*S*)-5-(2-Phenoxymethyl-pyrrolidine-1-sulfonyl)-1*H*-indole-2,3-dione (10). To a solution of **9** (1.46 g, 5.2 mmol) in CH_2Cl_2 (5 mL) was added trifloroacetic acid (5 mL) at 0 °C. The mixture was stirred at 0 °C for 15 min. After evaporation of the solvent in vacuo, CH_2Cl_2 (15 mL) and triethylamine (2 mL) were added, then a solution of **6** (1.44 g, 5.9 mmol) in THF (25 mL) was added at 0 °C. The reaction mixture was stirred overnight at room temperature. The solvent was evaporated in vacuo, then ethyl acetate (150 mL) was added, washed with water (50 mL × 2) and saturated NaCl (50 mL), and dried over Na_2SO_4. After evaporation of the ethyl acetate, the crude product was purified with ether to afford 1.7 g (84%) of **10** as a yellow solid, mp 204.5−205.9 °C. ^{1}H NMR (300 MHz, $CDCl_3$) δ 8.94 (s, 1H), 7.77 (dd, J = 8.25 Hz, J = 2.1 Hz, 1H), 7.67 (s, 1H), 7.02 (t, J = 8.7 Hz, 2H), 6.84 (d, J = 8.1 Hz, 1H), 6.69 (t, J = 7.2 Hz, 1H), 6.63 (d, J = 7.8 Hz, 2H), 3.89 (m, 1H), 3.75−3.66 (m, 2H), 3.23 (m, 1H), 2.96 (m, 1H), 1.72 (m, 2H), 1.54−1.42 (m, 2H). LRMS (FAB) *m/e*: 387.1 (M + H, 100). Anal. Calcd for $C_{19}H_{18}N_2O_5S$: C, 59.06, H, 4.70, N, 7.25. Found: C, 58.99, H, 4.74, N, 7.11.

(*S*)-1-Methyl-5-(2-phenoxymethyl-pyrrolidine-1-sulfonyl)-1*H*-indole-2,3-dione (11a). To a solution of **10** (193 mg, 0.5 mmol) in DMF (3 mL) was added 60% NaH (30 mg, 0.75 mmol) at room temperature. The mixture was stirred 15 min, then iodomethane (0.5 mL) was added. The mixture was stirred overnight at ambient temperature, then ether (75 mL) was added, washed with water (30 mL) and saturated NaCl (30 mL), and dried over Na_2SO_4. After evaporation of the solvent, the crude product was purified with ether to afford 85 mg (43%) of **11a** as a yellow solid, mp 160.1−160.9 °C. ^{1}H NMR (300 MHz, $CDCl_3$) δ 8.07 (dd, J = 8.25 Hz, J = 2.1 Hz, 1H), 8.01 (s, 1H), 7.25 (t, J = 8.4 Hz, 2H), 6.92 (m, 3H), 6.81 (d, J = 7.8 Hz, 2H), 4.15 (dd, J = 9.0 Hz, J = 2.7 Hz, 1H), 4.00 (m, 1H), 3.92 (m, 1H), 3.51 (m, 1H), 3.30 (m, 1H), 3.26 (s, 3H), 2.04 (m, 2H), 1.81 (m, 2H). Anal. Calcd for $C_{20}H_{20}N_2O_5S$: C, 59.99, H, 5.03, N, 7.00. Found: C, 59.80, H, 5.03, N, 6.91.

(*S*)-1-Benzyl-5-(2-phenoxymethyl-pyrrolidine-1-sulfonyl)-1*H*-indole-2,3-dione (2) was prepared according to the same procedure for compound **11a**, except using benzyl bromide, and purified with hexanes−ether (1:2) to afford 152 mg (64%) of **2** as a yellow solid, mp 97.2−99.1 °C. ^{1}H NMR (300 MHz, $CDCl_3$) δ 8.01 (d, J = 1.5 Hz, 1H), 7.94 (dd, J = 8.4 Hz, J = 1.8 Hz, 1H), 7.36 (m, 5H), 7.22 (m, 2H), 6.95−6.79 (m, 4H), 4.92 (s, 2H), 4.15 (dd, J = 8.85 Hz, J = 2.4 Hz, 1H), 3.97−3.87 (m, 2H), 3.49 (m, 1H), 3.23 (m, 1H), 2.01 (m, 2H), 1.78 (m, 2H). Anal. Calcd for $C_{26}H_{24}N_2O_5S$: C, 65.53, H, 5.08, N, 5.88. Found: C, 65.27, H, 5.32, N, 5.58.

(*S*)-1-(4-Methoxybenzyl)-5-(2-phenoxymethyl-pyrrolidine-1-sulfonyl)-1*H*-indole-2,3-dione (11b) was prepared according to the same procedure for compound **11a**, except using 4-methoxybenzyl chloride, and purified with hexanes−ether (1:3) to afford 175 mg (69%) of **11b** as a yellow solid, mp 126.7−128.8 °C. ^{1}H NMR (300 MHz, $CDCl_3$) δ 8.00 (s, 1H), 7.95 (dd, J = 8.25 Hz, J = 2.1 Hz, 1H), 7.28−7.21 (m, 4H), 6.96−6.80 (m, 6H), 4.86 (s, 2H), 4.18−4.11 (m, 1H), 3.97−3.88 (m, 2H), 3.80 (s, 3H), 3.50 (m, 1H), 3.23 (m, 1H), 2.02 (m, 2H), 1.78 (m, 2H). Anal. Calcd for $C_{27}H_{26}N_2O_6S$: C, 64.02, H, 5.17, N, 5.53. Found: C, 64.76, H, 5.24, N, 5.06.

(*S*)-1-(4-Fluorobenzyl)-5-(2-phenoxymethyl-pyrrolidine-1-sulfonyl)-1*H*-indole-2,3-dione (11c) was prepared according to the same procedure for compound **11a**, except using 4-fluorobenzyl bromide, and purified with hexanes−ether (1:2) to afford 196 mg (79%) of **11c** as an orange solid, mp 74.5−75.4 °C. ^{1}H NMR (300 MHz, $CDCl_3$) δ 7.99 (s, 1H), 7.95 (dd, J = 8.25 Hz, J = 2.1 Hz, 1H), 7.34−7.19 (m, 4H), 7.06 (t, J = 8.7 Hz, 2H), 6.92 (t, J = 7.2 Hz, 1H), 6.87−6.79 (m, 3H), 4.89 (s, 2H), 4.13 (m, 1H), 3.93 (m, 2H), 3.47 (m, 1H), 3.23 (m, 1H), 2.01 (m, 2H), 1.78 (m, 2H). Anal. Calcd for $C_{26}H_{23}FN_2O_5S$: C, 63.15, H, 4.69, N, 5.66. Found: C, 63.05, H, 4.69, N, 5.60.

(*S*)-1-(4-Methylthiobenzyl)-5-(2-phenoxymethyl-pyrrolidine-1-sulfonyl)-1*H*-indole-2,3-dione (11d) was prepared according to the same procedure for compound **11a**, except using 4-methylthiobenzyl bromide, and purified with hexanes−ether (1:2) to afford 152 mg (64%) of **11d** as a yellow solid, mp 175.4−176.8 °C. ^{1}H NMR (300 MHz, $CDCl_3$) δ 8.05 (s, 1H), 7.99 (d, J = 10.5 Hz, 1H), 7.27 (m, 6H), 6.96 (t, J = 7.2 Hz, 1H), 6.86 (t, J = 8.1 Hz, 3H), 4.90 (s, 2H), 4.19 (d, J = 8.7 Hz, 1H), 3.96 (m, 2H), 3.53 (m, 1H), 3.25 (m, 1H), 2.50 (s, 3H), 2.05 (m, 2H), 1.82 (m, 2H). Anal. Calcd for $C_{27}H_{26}N_2O_5S_2$: C, 62.05, H, 5.01, N, 5.36. Found: C, 61.81, H, 4.95, N, 5.34.

Acetic Acid (*S*)-4-[2,3-Dioxo-5-(2-phenoxymethyl-pyrrolidine-1-sulfonyl)-2,3-dihydroindol-1-yl-methyl]phenyl Ester (11e). This compound was prepared according to the same procedure for compound **11a**, except using 4-(chloromethyl)phenyl acetate, and purified with hexanes−ether (1:2) to afford 112 mg (42%) of **11e** as a yellow solid, mp 191.5−193.2 °C. ^{1}H NMR (300 MHz, $CDCl_3$) δ 8.03 (d, J = 2.1 Hz, 1H), 7.98 (d, J = 8.4 Hz, 1H), 7.35 (d, J = 8.4 Hz, 2H), 7.23 (t, J = 7.2 Hz, 2H), 7.10 (d, J = 8.7 Hz, 2H), 6.95−6.81 (m, 4H), 4.90 (s, 2H), 4.17 (m, 1H), 3.93 (m, 2H), 3.51 (m, 1H), 3.23 (m, 1H), 2.30 (s, 3H), 2.02 (m 2H), 1.79 (m, 2H). Anal. Calcd for $C_{28}H_{26}N_2O_7S$: C, 62.91, H, 4.90, N, 5.24. Found: C, 62.99, H, 5.02, N, 5.13.

(*S*)-1-(4-Hydroxybenzyl)-5-(2-phenoxymethyl-pyrrolidine-1-sulfonyl)-1*H*-indole-2,3-dione (11f). To a solution of **11e** (53 mg, 0.1 mmol) in methanol (3 mL) and water (1 mL) was added NaOH (4.4 mg, 0.11 mmol) at ambient temperature. The mixture was stirred overnight, then acidified with 1 M HCl to pH of 4 and extracted with ethyl acetate (50 mL). The ethyl acetate was washed with NaCl (30 mL) and dried over Na_2SO_4. After evaporation of the solvent, the crude product was purified with ether to afford 36 mg (73%) of **11f** as a yellow solid, mp 170.5−172.4 °C. ^{1}H NMR (300 MHz, $CDCl_3$) δ 8.01 (s, 1H), 7.97 (dd, J = 8.25 Hz, J = 2.1 Hz, 1H), 7.24−7.19 (m, 4H), 6.96−6.80 (m, 6H), 4.85 (s, 2H), 4.16 (m, 1H), 3.98−3.88

(m, 2H), 3.49 (m, 1H), 3.21 (m, 1H), 2.03 (m, 2H), 1.80 (m, 2H). Anal. Calcd for $C_{26}H_{24}N_2O_6S \cdot 0.25H_2O$: C, 62.83, H, 4.97, N, 5.64. Found: C, 62.87, H, 4.74, N, 5.69.

(*S*)-1-(6-Fluoropyridin-3-yl-methyl)-5-(2-phenoxymethyl-pyrrolidine-1-sulfonyl)-1*H*-indole-2,3-dione (11g) was prepared according to the same procedure for compound **11a**, except using 5-(bromomethyl)-2-fluoropyridine,[21] and purified with ether to afford 94 mg (76%) of **11g** as yellow solid, mp 113.3−114.7 °C. ^{1}H NMR (300 MHz, $CDCl_3$) δ 8.28 (s, 1H), 8.03 (m, 2H), 7.82 (m 1H), 7.27−7.20 (m, 2H), 7.00−6.79 (m, 5H), 4.92 (s, 2H), 4.13 (m, 1H), 3.95 (m, 2H), 3.50 (m, 1H), 3.26 (m, 1H), 2.05 (m, 2H), 1.80 (m, 2H). Anal. Calcd for $C_{25}H_{22}FN_3O_5S$: C, 60.60, H, 4.47, N, 8.48. Found: C, 60.60, H, 4.59, N, 8.33.

(*S*)-1-(2-Fluoro-pyridin-4-yl-methyl)-5-(2-phenoxymethyl-pyrrolidine-1-sulfonyl)-1*H*-indole-2,3-dione (11h) was prepared according to the same procedure for compound **11a**, except using 4-(bromomethyl)-2-fluoropyridine,[21] and purified with ether to afford 41 mg (33%) of **11h** as a yellow solid, mp 180.1−181.9 °C. ^{1}H NMR (300 MHz, $CDCl_3$) δ 8.25 (d, J = 5.4 Hz, 1H), 8.07 (s, 1H), 8.00 (d, J = 8.4 Hz, 1H), 7.23 (m, 2H), 7.12 (d, J = 4.2 Hz, 1H), 6.96−6.73 (m, 5H), 4.94 (s, 2H), 4.13 (m, 1H), 4.00−3.89 (m, 2H), 3.49 (m, 1H), 3.28 (m, 1H), 2.04 (m, 2H), 1.82 (m, 2H). Anal. Calcd for $C_{25}H_{22}FN_3O_5S$: C, 60.60, H, 4.47, N, 8.48. Found: C, 60.32, H, 4.34, N, 8.35.

(*S*)-1-(6-Fluoro-pyridin-2-yl-methyl)-5-(2-phenoxymethyl-pyrrolidine-1-sulfonyl)-1*H*-indole-2,3-dione (11i) was prepared according to the same procedure for compound **11a**, except using 6-(bromomethyl)-2-fluoropyridine,[21] and purified with ether to afford 57 mg (46%) of **11i** as a yellow solid, mp 128.6−129.4 °C. ^{1}H NMR (300 MHz, $CDCl_3$) δ 8.02 (m, 2H), 7.82 (m, 1H), 7.28−7.10 (m, 4H), 6.92 (m, 2H), 6.85 (m, 2H), 4.96 (s, 2H), 4.14 (m, 1H), 3.94 (m, 2H), 3.51 (m, 1H), 3.23 (m, 1H), 2.03 (m, 2H), 1.79 (m, 2H). Anal. Calcd for $C_{25}H_{22}FN_3O_5S \cdot 0.25H_2O$: C, 60.05, H, 4.54, N, 8.40. Found: C, 60.06, H, 4.49, N, 8.24.

(*S*)-1-(*tert*-Butoxycarbonyl)azetidine-2-carboxylic Acid (13). To a solution of (*S*)-2-azetidinecarboxylic acid **12** (1.0 g, 10.0 mmol) and di-*tert*-butyl dicarbonate (2.83 g, 12.5 mmol) in ethanol (20 mL) and water (10 mL) was added NaOH (420 mg, 10.5 mmol) at 0 °C. The mixture was stirred overnight at ambient temperature. After evaporation of the ethanol, water (20 mL) was added, then acidified with diluted HCl to a pH of 3 and extracted with ethyl acetate (50 mL × 3). The combined ethyl acetate was washed with water (30 mL) and saturated NaCl (30 mL), and dried over Na_2SO_4. After evaporation of the ethyl acetate to afford 1.98 g (100%) of **13** as a white solid. ^{1}H NMR (300 MHz, $CDCl_3$) δ 4.79 (m, 1H), 3.93 (m, 2H), 2.46 (m, 2H), 1.48 (s, 9H).

(*S*)-*tert*-Butyl 2-(Hydroxymethyl)azetidine-1-carboxylate (14).[17] To a solution of **13** (0.94 g, 4.7 mmol) in THF (10 mL) was added slowly a 1 M BH_3 in THF (21.0 mL) at 0 °C. The mixture was stirred 2 days at ambient temperature, then cold water (20 mL) was added at 0 °C. After evaporation of the THF in vacuo, an 10% aqueous solution of citric acid (15 mL) was added and extracted with ethyl acetate (50 mL × 2). The combined ethyl acetate was washed with saturated $NaHCO_3$ (30 mL) and NaCl (30 mL), and dried over Na_2SO_4. Evaporation of the ethyl acetate in vacuo afforded 0.86 g (100%) of **14** as a colorless oil. ^{1}H NMR (300 MHz, $CDCl_3$) δ 4.40 (m, 1), 3.85−3.70 (m, 3H), 2.13 (m, 1H), 1.90 (m, 1H), 1.42 (s, 9H).

((*S*)-1-(*tert*-Butoxycarbonyl)azetidine-2-yl)methyl 4-methylbenzenesulfonate (15) was prepared according to the same procedure for compound **8**, except using compound **14**, and purified with hexanes−ether (1:1) to afford 1.34 g (86%) of **15** as a colorless oil. ^{1}H NMR (300 MHz, $CDCl_3$) δ 7.79 (d, J = 8.7 Hz, 2H), 7.34 (d, J = 8.1 Hz, 2H), 4.33−4.24 (m, 2H), 4.10 (m, 1H), 3.78 (m 2H), 2.44 (s, 3H), 2.21 (m, 2H), 1.36 (s, 9H).

(*S*)-*tert*-Butyl 2-(phenoxymethyl)azetidine-1-carboxylate (16) was prepared according to the same procedure for compound **9**, except using compound **15**, and purified with hexanes−ether (2:1) to afford 0.81 g (79%) of **16** as a colorless oil. ^{1}H NMR (300 MHz, $CDCl_3$) δ CDCl3 7.30 (m, 2H), 6.94 (m, 3H), 4.53 (m, 1H), 4.26 (m, 1H), 4.12 (m, 1H), 3.93 (m, 2H), 2.33 (m, 2H), 1.43 (s, 9H).

(*S*)-5-(2-Phenoxymethyl-azetidine-1-sulfonyl)-1*H*-indole-2,3-dione (17) was prepared according to the same procedure for compound **10**, except using compound **16**, and purified with ether to afford 715 mg (63%) of **17** as a yellow solid, mp 173.2−174.5 °C. ^{1}H NMR (300 MHz, DMSO) δ 11.48 (s, 1H), 7.98 (dd, J = 8.25 Hz, J = 2.1 Hz, 1H), 7.77 (s, 1H), 7.27 (m, 2H), 7.10 (d, J = 8.1 Hz, 1H), 6.91 (d, J = 7.8 Hz, 3H), 4.20−4.02 (m, 3H), 3.70 (m, 1H), 3.55 (m, 1H), 2.22 (m, 1H), 2.02 (m, 1H). LRMS (FAB) *m/e*: 373.0 (M + H, 100). Anal. Calcd for $C_{18}H_{16}N_2O_5S \cdot 0.5H_2O$: C, 56.68, H, 4.49, N, 7.34. Found: C, 56.96, H, 4.39, N, 7.30.

(*S*)-1-Methyl-5-(2-phenoxymethyl-azetidine-1-sulfonyl)-1*H*-indole-2,3-dione (18a) was prepared according to the same procedure for compound **11a**, except using compound **17**, and purified with ether to afford 46 mg (48%) of **18a** as an orange solid, mp 173.5−174.9 °C. ^{1}H NMR (300 MHz, $CDCl_3$) δ 8.04 (m, 2H), 7.24 (m, 2H), 6.94 (m, 2H), 6.79 (m, 2H), 4.46 (m, 1H), 4.10 (m, 2H), 3.86 (m, 2H), 3.25 (m, 3H), 2.30 (m, 2H). Anal. Calcd for $C_{19}H_{18}N_2O_5S$: C, 59.06, H, 4.70, N, 7.25. Found: C, 58.98, H, 4.75, N, 7.19.

(*S*)-1-Benzyl-5-(2-phenoxymethyl-azetidine-1-sulfonyl)-1*H*-indole-2,3-dione (18b) was prepared according to the same procedure for compound **11a**, except using compound **17** and benzyl bromide, and purified with hexanes−ether (1:2) to afford 92 mg (80%) of **18b** as an orange solid, mp 157.1−158.9 °C. ^{1}H NMR (300 MHz, $CDCl_3$) δ 8.05 (d, J = 2.1 Hz, 1H), 7.95 (dd, J = 8.25 Hz, J = 2.1 Hz, 1H), 7.34 (m, 5H), 7.22 (m, 2H), 6.94 (m, 1H), 6.87−6.78 (m, 3H), 4.93 (s, 2H), 4.46 (m, 1H), 4.10 (m, 2H), 2.82 (m, 2H), 2.32 (m 2H). Anal. Calcd for $C_{25}H_{22}N_2O_5S$: C, 64.92, H, 4.79, N, 6.06. Found: C, 64.82, H, 4.79, N, 7.97.

(*S*)-1-(4-Methoxybenzyl)-5-(2-phenoxymethyl-azetidine-1-sulfonyl)-1*H*-indole-2,3-dione (18c) was prepared according to the same procedure for compound **11a**, except using compound **17** and 4-methoxybenzyl chloride, and purified with hexanes−ether (1:2) to afford 62 mg (50%) of **18c** as an orange solid, mp 159.8−161.5 °C. ^{1}H NMR (300 MHz, $CDCl_3$) δ 8.04 (s, 1H), 7.96 (dd, J = 8.25 Hz, J = 2.1 Hz, 1H), 7.25 (m, 4H), 6.97−6.87 (m, 4H), 6.80 (d, J = 7.8 Hz, 2H), 4.87 (s, 2H), 4.45 (m, 2H), 4.11 (m, 2H), 3.84 (m, 2H), 3.81 (s, 3H), 2.32 (m, 2H). Anal. Calcd for $C_{26}H_{24}N_2O_6S$: C, 63.40, H, 4.91, N, 5.69. Found: C, 63.65, H, 4.93, N, 5.59.

(*S*)-1-(4-Methylthiobenzyl)-5-(2-phenoxymethyl-azetidine-1-sulfonyl)-1*H*-indole-2,3-dione (18d) was prepared according to the same procedure for compound **11a**, except using compound **17** and 4-methylthiobenzyl bromide, and purified with hexanes−ether (1:2) to afford 57 mg (45%) of **18d** as an orange solid, mp 167.6−169.2 °C. ^{1}H NMR (300 MHz, $CDCl_3$) δ 8.05 (d, J = 1.5 Hz, 1H), 7.96 (dd, J = 8.4 Hz, J = 1.8 Hz, 1H), 7.25 (m, 6H), 6.95 (t, J = 7.2 Hz, 1H), 6.86−6.78 (m, 3H), 4.89 (s, 2H), 4.46 (m, 1H), 4.11 (m, 2H), 3.82 (m, 2H), 2.49 (s, 3H), 2.39−2.25 (m, 2H). Anal. Calcd for $C_{26}H_{24}N_2O_5S_2$: C, 61.40, H, 4.76, N, 5.51. Found: C, 60.99, H, 4.71, N, 5.36.

(*S*)-1-(4-Fluorobenzyl)-5-(2-phenoxymethyl-azetidine-1-sulfonyl)-1*H*-indole-2,3-dione (18e) was prepared according to the same procedure for compound **11a**, except using compound **17** and 4-fluorobenzyl bromide, and purified with hexanes−ether (1:2) to afford 85 mg (71%) of **18e** as an orange solid, mp 164.6−165.7 °C. ^{1}H NMR (300 MHz, $CDCl_3$) δ 8.05 (d, J = 1.8 Hz, 1H), 7.97 (dd, J = 8.4 Hz, J = 2.1 Hz, 1H), 7.34−7.20 (m, 4H), 7.07 (t, J = 8.7 Hz, 2H), 6.94 (t, J = 7.2 Hz, 1H), 6.86−6.77 (m, 3H), 4.90 (s, 2H), 4.47 (m, 1H), 4.10 (m, 2H), 3.85 (m, 2H), 2.36−2.22 (m, 2H). Anal. Calcd for $C_{25}H_{21}FN_2O_5S$: C, 62.49, H, 4.41, N, 5.83. Found: C, 62.27, H, 4.48, N, 5.69.

(*S*)-1-(2-Fluorobenzyl)-5-(2-phenoxymethyl-azetidine-1-sulfonyl)-1*H*-indole-2,3-dione (18f) was prepared according to the same procedure for compound **11a**, except using compound **17** and 2-fluorobenzyl bromide, and purified with

hexanes−ether (1:2) to afford 81 mg (68%) of **18f** as an orange solid, mp 147.1−148.0 °C. ^{1}H NMR (300 MHz, $CDCl_3$) δ 8.05 (d, J = 1.8 Hz, 1H), 8.00 (dd, J = 8.25 Hz, J = 2.1 Hz, 1H), 7.35 (m, 2H), 7.24−7.11 (m, 3H), 7.02−6.78 (m, 5H), 4.98 (s, 2H), 4.47 (m, 1H), 4.11 (m, 2H), 3.85 (m, 2H), 2.35−2.25 (m, 2H). Anal. Calcd for $C_{25}H_{21}FN_2O_5S$: C, 62.49, H, 4.41, N, 5.83. Found: C, 62.25, H, 4.47, N, 5.68.

(*S*)-1-(6-Fluoropyridin-3-ylmethyl)-5-(2-phenoxymethylazetidine-1-sulfonyl)-1*H*-indole-2,3-dione (18g) was prepared according to the same procedure for compound **11a**, except using compound **17** and 5-(bromomethyl)-2-fluoropyridine, and purified with ether to afford 74 mg (62%) of **18g** as an orange solid, mp 176.8−178.3 °C. ^{1}H NMR (300 MHz, $CDCl_3$) δ 8.27 (d, J = 2.4 Hz, 1H), 8.07 (d, J = 2.1 Hz, 1H), 8.01 (dd, J = 8.25 Hz, J = 2.1 Hz, 1H), 7.79 (td, J = 8.1 Hz, J = 2.4 Hz, 1H), 7.22 (m, 2H), 7.00−6.77 (m, 5H), 4.92 (s, 2H), 4.49 (m, 1H), 4.09 (m, 2H), 3.85 (m, 2H), 2.35−2.23 (m, 2H). Anal. Calcd for $C_{24}H_{20}FN_3O_5S$: C, 59.87, H, 4.19, N, 8.73. Found: C, 59.81, H, 4.16, N, 8.62.

(*S*)-1-(2-Fluoropyridin-4-yl-methyl)-5-(2-phenoxymethylazetidine-1-sulfonyl)-1*H*-indole-2,3-dione (18h) was prepared according to the same procedure for compound **11a**, except using compound **17** and 4-(bromomethyl)-2-fluoropyridine, and purified with ether to afford 36 mg (30%) of **18h** as an orange solid, mp 159.0−159.9 °C. ^{1}H NMR (300 MHz, $CDCl_3$) δ 8.23 (d, J = 5.1 Hz, 1H), 8.08 (s, 1H), 7.98 (dd, J = 8.7 Hz, J = 2.1 Hz, 1H), 7.22 (m, 2H), 7.10 (d, J = 5.4 Hz, 1H), 6.93 (t, J = 7.5 Hz, 1H), 6.84−6.72 (m, 4H), 4.93 (s, 2H), 4.49 (m, 1H), 4.07 (m, 2H), 3.91−3.81 (m, 2H), 2.35−2.22 (m, 2H). Anal. Calcd for $C_{24}H_{20}FN_3O_5S{\cdot}0.5H_2O$: C, 58.77, H, 4.32, N, 8.57. Found: C, 58.69, H, 4.45, N, 8.26.

(*S*)-1-(6-Fluoropyridin-2-yl-methyl)-5-(2-phenoxymethylazetidine-1-sulfonyl)-1*H*-indole-2,3-dione (18i) was prepared according to the same procedure for compound **11a**, except using compound **17** and 6-(bromomethyl)-2-fluoropyridine, and purified with ether to afford 62 mg (52%) of **18i** as an orange solid, mp 144.7−146.1 °C. ^{1}H NMR (300 MHz, $CDCl_3$) δ 8.03 (s, 1H), 8.00 (dd, J = 8.25 Hz, J = 2.1 Hz, 1H), 7.82 (m, 1H), 7.27−7.11 (m, 4H), 6.92 (m, 2H), 6.79 (m, 2H). Anal. Calcd for $C_{24}H_{20}FN_3O_5S$: C, 59.87, H, 4.19, N, 8.73. Found: C, 59.59, H, 4.27, N, 8.48.

2-(Pyridin-3-yl-oxymethyl)-pyrrolidine-1-carboxylic acid *tert*-butyl ester (**19**) was prepared according to the same procedure for compound **9**, except using 3-hydroxypyridine. The crude product was purified with ether to afford 1.70 g (61%) of **19** as a colorless oil. ^{1}H NMR (300 MHz, $CDCl_3$) δ 8.32 (s, 1H), 8.21 (s, 1H), 7.21 (m, 2H), 4.16 (m, 2H), 3.99−3.86 (m, 1H), 3.38 (m, 2H), 2.05−1.84 (m, 4H), 1.47 (s, 9H).

5-(2-(Pyridin-3-yl-oxymethyl)-pyrrolidine-1-sulfonyl)-1*H*-indole-2,3-dione (**20**) was prepared according to the same procedure for compound **10**, except using compound **19**, and the crude product was recrystallized from ethyl acetate to afford 1.75 g (82%) of **20** as a yellow solid, mp 215.9−217.8 °C. ^{1}H NMR (300 MHz, DMSO) δ 11.42(s, 1H), 8.26 (d, J = 3.0 Hz, 1H), 8.16 (d, J = 4.5 Hz, 1H), 8.02 (d, J = 8.4 Hz, 1H), 7.78 (s, 1H), 7.38 (m, 1H), 7.33 (m, 1H), 7.05 (d, J = 8.4 Hz, 1H), 4.15−4.02 (m, 2H), 3.90 (m, 1H), 3.34 (m, 1H), 3.12 (m, 1H), 1.87 (m, 2H), 1.67−1.54 (m, 2H). LCMS *m/e*: 387.8 (M + H). Anal. Calcd for $C_{18}H_{17}N_3O_5S{\cdot}0.5H_2O$: C, 54.54, H, 4.58, N, 10.60. Found: C, 54.56, H, 4.70, N, 10.04.

1-Methyl-5-(2-(pyridin-3-yl-oxymethyl)-pyrrolidine-1-sulfonyl)-1*H*-indole-2,3-dione (**21a**) was prepared according to the same procedure for compound **11a**, except using compound **20**, and the crude product was purified with ethyl acetate to afford 55 mg (55%) of **21a** as a yellow solid, mp 142.1−143.4 °C. ^{1}H NMR (300 MHz, $CDCl_3$) δ 8.24 (d, J = 2.7, 1H), 8.22 (dd, J = 3.9 Hz, J = 2.1 Hz, 1H), 8.08 (dd, J = 8.4, J = 2.1 Hz, 1H), 7.26 (s, 1H), 7.21 (m, 2H), 7.00 (d, J = 8.4 Hz, 1H), 4.22 (m, 1H), 3.98 (m, 2H), 3.53 (m, 1H), 3.30 (s, 3H), 3.22 (m, 2H), 2.03 (m, 2H), 1.80 (m, 2H). LCMS *m/e*: 401.84 (M + H). Anal. Calcd for $C_{19}H_{19}N_3O_5S$: C, 56.85, H, 4.77, N, 10.47. Found: C, 56.48, H, 4.87, N, 10.19.

1-Benzyl-5-(2-(pyridin-3-yl-oxymethyl)-pyrrolidine-1-sulfonyl)-1*H*-indole-2,3-dione (**21b**) was prepared according to the same procedure for compound **11a**, except using compound **20** and benzyl bromide, and the crude product was purified with ether to afford 61 mg (51%) of **21b** as a yellow solid, mp 79.6−80.7 °C. ^{1}H NMR (300 MHz, $CDCl_3$) δ 8.25 (s, 1H), 8.22 (t, J = 2.7 Hz, 1H), 8.04 (d, J = 2.1 Hz, 1H), 7.98 (dd, J = 8.4 Hz, J = 2.1 Hz, 1H), 7.36 (m, 5H), 7.22 (m, 2H), 6.91 (d, J = 8.8 Hz, 1H), 4.97 (s, 2H), 4.25 (m, 1H), 3.99−3.94 (m, 2H), 3.55−3.48 (m, 1H), 3.21−3.15 (m, 1H), 2.10−1.97 (m, 2H), 1.84−1.75 (m, 2H). LRMS (FAB) *m/e*: 484.1 (M + Li, 100); HRMS (FAB) *m/e* calcd for $C_{25}H_{23}N_3O_5SLi$ (M + Li) 484.1518, found 484.1539.

1-(4-Methoxybenzyl)-5-(2-(pyridin-3-yl-oxymethyl)-pyrrolidine-1-sulfonyl)-1*H*-indole-2,3-dione (**21c**) was prepared according to the same procedure for compound **11a**, except using **20** and 4-methoxybenzyl chloride. The crude product was purified with ether to afford 45 mg (36%) of **21c** as a yellow solid, mp 156.7−158.4 °C. ^{1}H NMR (300 MHz, $CDCl_3$) δ 8.26 (s, 1H), 8.23 (t, J = 2.7 Hz, 1H), 8.03 (d, J = 1.5 Hz, 1H), 7.98 (dd, J = 8.25 Hz, J = 2.1 Hz, 1H), 7.28 (d, J = 8.7 Hz, 2H), 7.22 (t, J = 2.1 Hz, 2H), 6.94 (d, J = 8.1 Hz, 1H), 6.90 (d, J = 8.7 Hz, 2H), 4.90 (s, 2H), 4.24 (m, 1H), 4.01−3.90 (m, 2H), 3.81 (s, 3H), 3.55−3.49 (m, 1H), 3.20−3.15 (m, 1H), 2.05 (m, 2H), 1.78 (m, 2H). LCMS *m/e*:507.9 (M + H). Anal. Calcd for $C_{26}H_{25}N_3O_6S$: C, 61.53, H, 4.96, N, 8.28. Found: C, 61.27, H, 4.95, N, 8.17.

1-(4-Methylthiobenzyl)-5-(2-(pyridin-3-yl-oxymethyl)-pyrrolidine-1-sulfonyl)-1*H*-indole-2,3-dione (**21d**) was prepared according to the same procedure for compound **11a**, except using **20** and 4-methylsulfanylbenzyl bromide. The crude product was purified with ether to afford 57 mg (44%) of **21d** as a yellow solid, mp 81.5−83.1 °C. ^{1}H NMR (300 MHz, $CDCl_3$) δ 8.25−8.21 (m, 2H), 8.03 (d, J = 1.8 Hz, 1H), 7.24 (s, 3H), 7.21 (m, 1H), 6.89 (d, J = 8.4 Hz, 1H), 4.91 (s, 2H), 4.23 (m, 1H), 4.00−3.89 (m, 2H), 3.51 (m, 1H), 3.14 (m, 1H), 2.47 (s, 3H), 2.02 (m, 2H), 1.78 (m, 2H). LRMS (FAB) *m/e*: 530.1 (M + Li, 100); HRMS (FAB) *m/e* calcd for $C_{26}H_{25}N_3O_5S_2Li$ (M + Li) 530.1396, found 530.1397.

1-(4-Fluorobenzyl)-5-(2-(pyridin-3-yl-oxymethyl)-pyrrolidine-1-sulfonyl)-1*H*-indole-2,3-dione (**21e**) was prepared according to the same procedure for compound **11a**, except using **20** and 4-fluorobenzyl bromide. The crude product was purified with ether to afford 35 mg (28%) of **21e** as a yellow solid, mp 77.1−78.3 °C. ^{1}H NMR (300 MHz, $CDCl_3$) δ 8.25 (m, 2H), 8.05 (s, 1H), 8.03−7.99 (m, 1H), 7.36−7.32 (m, 2H), 7.23 (m, 2H), 7.09 (t, J = 8.7 Hz, 2H), 6.90 (d, J = 8.7 Hz, 1H), 4.94 (s, 2H), 4.25 (d, J = 6.0 Hz, 1H), 3.98 (m, 2H), 3.52 (m, 1H), 3.19 (m, 1H), 2.05 (m, 2H), 1.80 (m, 2H). LRMS (FAB) *m/e*: 502.1 (M + Li, 100); HRMS (FAB) *m/e* calcd for $C_{25}H_{22}FN_3O_5SLi$ (M + Li) 502.1424, found 502.1420.

2-(Pyridin-4-yl-oxymethyl)-pyrrolidine-1-carboxylic acid *tert*-butyl ester (**22**) was prepared according to the same procedure for compound **9**, except using 4-hydroxypyridine. The crude product was purified with ethyl acetate to afford 1.31 g (47%) of **22** as a colorless oil. ^{1}H NMR (300 MHz, CDCl3) δ 8.42 (m, 2H), 6.87 (m, 2H), 4.15 (m, 3H), 3.43 (m, 2H), 1.98 (m, 4H), 1.50 (s, 9H).

5-(2-(Pyridin-4-yl-oxymethyl)-pyrrolidine-1-sulfonyl)-1*H*-indole-2,3-dione (**23**) was prepared according to the same procedure for compound **10**, except using compound **22**, purified with ethyl acetate to afford 1.17 g (55%) of **23** as a yellow solid, mp 204.2−205.3 °C. ^{1}H NMR (300 MHz, $CDCl_3$) δ 11.44 (s, 1H), 8.37 (d, J = 5.7 Hz, 2H), 8.03 (dd, J = 8.4 Hz, J = 2.1 Hz, 1H), 7.79 (s, 1H), 7.06 (d, J = 8.4 Hz, 1H), 6.96 (d, J = 6.0 Hz, 2H), 4.17−4.05 (m, 2H), 3.90 (m, 1H), 3.32 (m, 1H), 3.10 (m, 1H), 1.85 (m, 2H), 1.60 (m, 2H). LCMS *m/e*: 387.9 (M + H). Anal. Calcd for $C_{18}H_{17}N_3O_5S{\cdot}0.75H_2O$: C, 53.92, H, 4.65, N, 10.48. Found: C, 54.14, H, 4.39, N, 10.35.

Enzyme Inhibition Assays. Recombinant human caspases (3, 6, 7, and 8) and their peptide-specific substrates (Ac-DEVD-AMC, Ac-VEID-AMC, Ac-DEVD-AMC, and Ac-IETD-AMC, respectively) were purchased from Sigma-Aldrich (St. Louis, MO) with the, exception of caspase 1 and its substrate (Ac-YVAD-AMC) which were obtained from BIOMOL Research Laboratories (Plymouth Meeting, PA). The enzymatic activity

of caspases was determined by measuring the accumulation of the fluorogenic product 7-amino-4-methylcoumarin (AMC). All assays were prepared in 96-well format at a volume of 210 μL per well and consisted of 100 mM Na^+ HEPES (pH 7.4), 10% sucrose, 100 mM NaCl, 0.1% CHAPS, 5 mM 2-mercaptoethanol, 2 mM EDTA, 10 μM Ac-YVAD-AMC (caspase 1); 20 mM Na^+ HEPES (pH 7.4), 10% sucrose, 100 mM NaCl, 0.1% CHAPS, 2 mM EDTA, 10 μM Ac-DEVD-AMC (caspase 3); 20 mM Na^+ HEPES (pH 7.4), 10% sucrose, 100 mM NaCl, 0.1% CHAPS, 2 mM EDTA, 10 μM Ac-VEID-AMC (caspase 6); 20 mM Na^+ HEPES (pH 7.4), 100 mM NaCl, 10% sucrose, 0.1% CHAPS, 5 mM 2-mercaptoethanol, 2 mM EDTA, 10 μM Ac-DEVD-AMC (caspase 7); 20 mM Na^+ HEPES (pH 7.4), 10% sucrose, 100 mM NaCl, 0.1% CHAPS, 2 mM EDTA, 10 μM Ac-IETD-AMC (caspase 8).

Recombinant caspases were first assayed to determine the optimal concentration for each experiment. Optimal concentrations were based in the linear range of the enzyme activation curves. Peptide inhibitors with known IC_{50} values were tested together with the compounds as a control for each caspase assay. Peptide inhibitors, Ac-DEVD-CHO (caspase-3 and -7), Ac-VEID-CHO (caspase-6), and Ac-IETD-CHO (caspase-8) were purchased from Sigma-Aldrich (St. Louis, MO) with exception of caspase-1 specific inhibitor (Ac-YVAD-CHO) which was acquired from BIOMOL Research Laboratories (Plymouth Meeting, PA). Peptide and nonpeptide inhibitors were dissolved in DMSO, and a 2× serial dilution was performed prior to screening in order to obtain desired concentrations. 10 μL was added to each well containing 100 μL caspase solution and allowed to incubate on ice for 30 min. A 100 μL substrate solution was added to each well, and plates were incubated for 1−2 h at 37 °C. The final concentration of DMSO in all wells was 5% of the total volume. In caspase-1 and caspase-7 assays, 10 mM 2-mercaptoethanol was added to the substrate solution for full activation of the enzymes.

The amount of AMC released was determined by using a Victor^3 microplate fluorometer (Perkin-Elmer Life Sciences, Boston, MA) at excitation and emission wavelengths 355 nm and 460 nm, respectively. Compounds were tested in duplicate, and IC_{50} curves were calculated for all inhibitors assayed. Final IC_{50}s were the average of three independent experiments.

Enzyme Kinetic Studies. The inhibition profile for compound **21c** was determined for caspase-3 in the assay buffer. The concentration of Ac-DEVD-AMC was varied from 6.25 to 100 μM, and the concentration of **21c** was varied from 0 to 20 nM. The kinetic parameters of **21c** were obtained by fitting initial-rate data to

$$v = \frac{V_m S}{K_m\left(1 + \frac{1}{K_i}\right) + S} \quad (1)$$

where v is the observed velocity, S is the substrate concentration, V_m is the velocity at saturating substrate, K_m is the Michaelis constant of the substrate, I is the inhibitor concentration, and K_i is the dissociation constant of the inhibitor from the E·I complex. The data were analyzed using GraFit 4.0 (Erithacus Software, Staines, U.K.)

Docking Studies. Preparation of Caspase-3 Protein Structure. The crystal structure of caspase-3 complexed with a pyrazinone-based inhibitor[12] (1RHJ) was extracted from the Research Collaboratory for Structural Bioinformatics Protein Data Bank (RCSB-PDB).[22] The structure was read in and manipulated with the program MOE (2004.03).[23] Duplicate chains, empty residues (found at termini of chains), solvent molecules, and counterions were removed leaving the A and B chains as well as the bound inhibitor. The heavy atoms were fixed in position, hydrogens were added, and the structure was minimized using the Charmm22 force field to a gradient of 0.01.

Preparation of Ligand Structures. The inhibitors were built in MOE and minimized using the MMFF94 force field, and the structures were then exported into MacroModel[24] and subjected to 2500 iterations of a Low-Mode conformational search (LMCS) with a 25 kJ/mol energy window using the MMFF94 force field. The lowest energy structures found were then exported back into MOE and modified for covalent docking into the protein. The inhibitors were modified to allow for covalent docking by adding a sulfur to position 3 of the isatin moieties, forming a tetrahedral carbon with sulfur and oxyanion substituents. As this generates a chiral center, both enantiomers were prepared and used in docking studies. The sulfur serves as the link between the inhibitor and Cys285 of the enzyme.

Docking. The ligands were then docked into the enzyme using covalent constraints as implemented in Gold 2.2. A substructure-based template mapping was used in order to setup the covalent constraints so that multiple compounds could be docked within one study. The template utilized consisted of the isatin core with a sulfur covalently attached at C3. Gold utilizes an evolutionary genetic algorthithm to optimize the docked pose of the inhibitor within the enzyme.[25] Each inhibitor was docked into the binding site, through a covalent attachment between the isatin moiety and Cys285. Each molecule was docked 25 times with early termination if the top three poses are within 1.5 Å rmsd. Each pose was ranked according to its GoldScore which consists of protein−ligand hydrogen bond energy, protein−ligand van der Waals (vdw) energy, ligand internal vdw energy, and ligand torsional strain energy. A total of four docking runs were done with each ligand, both enantiomers of the covalently modified ligands as well as both enantiomers of the template utilized for the substructure mapping. The fitness value from all runs were combined and the highest scoring poses used for further analysis.

Acknowledgment. This work was supported by grants HL1385 awarded by the National Heart, Lung and Blood Institute and EB1729 and EB00340 awarded by the National Institute of Biomedical Imaging and Bioengineering.

Supporting Information Available: Elemental analysis data. The structures of the molecules in their best poses are also available as a mol2 file. This material is available free of charge via the Internet at http://pubs.acs.org.

References

(1) Jacobson, M. D.; Weil, M.; Raff, M. C. Programmed Cell Death in Animal Development. *Cell* **1997**, *88*, 347−354.
(2) Reed, J. C. Apoptosis-based Therapies. *Nature Rev. Drug Discovery* **2002**, *1*, 111−121.
(3) Rodriguez, I.; Matsuura, K.; Ody, C.; Nagata, S.; Vassalli, P. Systemic injection of a tripeptide inhibits the intracellular activation of CPP32-like proteases in vivo and fully protects mice against Fas-mediated fulminant liver destruction and death. *J. Exp. Med.* **1996**, *184*, 2067−2072.
(4) O'Brien, T.; Lee, D. Prospects for caspase inhibitors. *Mini Rev. Med. Chem.* **2004**, *4*, 153−165.
(5) Denault, J.-B.; Salvesen, G. S. Capsases: keys in the ignition of cell death. *Chem. Rev.* **2002**, *102*, 4489−4499.
(6) Garcia-Calvo, M.; Peterson, E. P.; Leiting, B.; Ruel, R.; Nicholson, D. W.; Thornberry, N. A. Inhibition of human caspases by peptide-based and macromolecular inhibitors. *J. Biol. Chem.* **1998**, *273*, 32608−32613.
(7) Hotchkiss, R. S.; Chang, K. C.; Swanson, P. E.; Tinsley, K. W.; Hui, J. J.; Klender, P.; Xanthoudakis, S.; Roy, S.; Black, C.; Grimm, E.; Aspiotis, R.; Han, Y.; Nicholson, D. W.; Karl, I. E. Caspase inhibitors improve survival in sepsis: a critical role of the lymphocyte. *Nature Immunol.* **2000**, *1*, 496−501.
(8) Choong, I. C.; Lew, W.; Lee, D.; Pham, P.; Burdett, M. T.; Lam, J. W.; Wiesmann, C.; Luong, T. N.; Fahr, B.; DeLano, W. L.; McDowell, R. S.; Allen, D. A.; Erlanson, D. A.; Gordon, E. M.; O'Brien, T. Identification of potent and selective small-molecule inhibitors of caspase-3 through the use of extended tethering and structure-based drug design. *J. Med. Chem.* **2002**, *45*, 5005−5022.
(9) Linton, S. D.; Karanewsky, D. S.; Ternansky, R. J.; Wu, J. C.; Pham, B.; Kodandapani, L.; Smidt, R.; Diaz, J.-L.; Fritz, L. C.; Tomaselli, K. J. Acyl peptides as reversible caspase inhibitors. Part 1: Initial lead optimization. *Bioorg. Med. Chem. Lett.* **2002**, *12*, 2969−2971.

(10) Linton, S. D.; Karanewsky, D. S.; Ternansky, R. J.; Chen, N.; Guo, X.; Jahangiri, K. G.; Kalish, V. J.; Meduna, S. P.; Robinson, E. D.; Ullman, B. R.; Wu, J. C.; Pham, B.; Kodandapani, L.; Smidt, R.; Diaz, J.-L.; Fritz, L. C.; von Krosigk, U.; Roggo, S.; Schmitz, A.; Tomaselli, K. J. Acyl peptides as reversible caspase inhibitors. Part 2: Further optimization. *Bioorg. Med. Chem. Lett.* **2002**, *12*, 2969−2971.

(11) Han, Y.; Giroux, A.; Grimm, E. L.; Aspiotis, R.; Francoeur, S.; Bayly, C. I.; Mckay, D. J.; Roy, S.; Xanthoudakis, S.; Vallancourt, J. P.; Rasper, D. M.; Tam, J.; Tawa, P.; Thornberry, N. A.; Paterson, E. P.; Garcia-Calvo, M.; Becker, J. W.; Rotonda, J.; Nicholson, D. W.; Zamboni, R. J. Discovery of novel aspartyl ketone dipeptides as potent and selective caspase-3 inhibitors. *Bioorg. Med. Chem. Lett.* **2004**, *14*, 805−808.

(12) Becker, J. W.; Rotonda, J.; Soisson, S. M.; Aspiotis, R.; Bayly, C.; Francoeur, S.; Gallant, M.; Garcia-Calvo, M.; Giroux, A.; Grimm, E.; Han, Y.; McKay, D.; Nicholson, D. W.; Peterson, E.; Renaud, J.; Roy, S.; Thornberry, N.; Zamboni, R. Reducing the peptidyl features of caspase-3 inhibitors: a structural analysis. *J. Med. Chem.* **2004**, *47*, 2466−2474.

(13) Han, B. H.; Xu, D.; Choi, J.; Han, Y.; Xanthoudakis, S.; Roy, S.; Tam, J.; Vaillancourt, J.; Colucci, J.; Siman, R.; Giroux, A.; Robertson, G. S.; Zamboni, R.; Nicholson, D. W.; Holtzman, D. M. Selective, reversible caspase-3 inhibitor is neuroprotective and reveals distinct pathways of cell death after neonatal hypoxia-ischemia brain injury. *J. Biol. Chem.* **2002**, *277*, 30128−31036.

(14) Lee, D.; Long, S. A.; Adams, J. L.; Chan, G.; Vaidya, K. S.; Francis, T. A.; Kikly, K.; Winkler, J. D.; Sung, C.-M.; Debouck, C.; Richardson, S.; Levy, M. A.; DeWolf, W. E., Jr.; Keller, P. M.; Tomaszek, T.; Head, M. S.; Ryan, M. D.; Haltiwanger, R. C.; Liang, P.-H.; Janson, C. A.; McDevitt, P. J.; Johanson, K.; Concha, N. O.; Chan, W.; Abdel-Meguid, S. S.; Badger, A. M.; Lark, M. W.; Nadeau, D. P.; Suva, L. J.; Gowen, M.; Nuttall, M. E. Potent and selective nonpeptide inhibitors of caspases 3 and 7 inhibit apoptosis and maintain cell functionality. *J. Biol. Chem.* **2000**, *275*, 16007−16104.

(15) Chapman, J. G.; Magee, W. P.; Stukenbrok, H. A.; Beckius, G. E.; Milici, A. J.; Tracey, W. R. A novel nonpeptidic caspase 3/7 inhibitor, (S)-(+)-5-[1-(2-methoxymethylpyrrolidinyl)sulfonyl]-isatin reduces myocardial ischemic injury. *Eur. J. Pharmacol.* **2002**, *456*, 59−68.

(16) Lee, D.; Long, S. A.; Murray, J. H.; Adams, J. L.; Nutall, M. E.; Nadeau, D. P.; Kikly, K.; Winkler, J. D.; Sung, C.-M.; Ryan, D.; Levy, M. A.; Keller, P. M.; DeWolf, W. E., Jr. Potent and selective nonpeptide inhibitors of caspases 3 and 7. *J. Med. Chem.* **2001**, *44*, 2015−2026.

(17) Abreo, M. A.; Lin, N.; Garvey, D. S.; Gunn, D. E.; Hettinger, A.; Wasicak, J. T.; Paulik, P. A.; Martin, Y. C.; Dannelly-Roberts, D. L.; Anderson, D. J.; Sullivan, J. P.; Williams, M.; Arneric, S. P.; Holladay, M. W. Novel 3-pyridyl ethers with subnanomolar affinity for central nicotinic acetylcholine receptors. *J. Med. Chem.* **1996**, *39*, 817−825.

(18) Wildman, S. A.; Crippen, G. M. Prediction of Physicochemical Parameters by Atomic Contributions. *J. Chem. Inf. Comput. Sci.* **1999**, *39*, 868−873.

(19) Stanton, D. T.; Jurs, P. C. Development and Use of Charged Partial Surface Area Structural Descriptors in Computer-Assisted Quantitative Structure−Property Relationship Studies. *Anal. Chem.* **1990**, *62*, 2323−2329.

(20) Wallace, A. C.; Laskowski, R. A.; Thornton, J. M. LIGPLOT: A Program to generate schematic diagrams of protein − ligand interactions. *Protein Eng.* **1995**, *8*, 127−124.

(21) Sullivan, P. T.; Sullivan, C. B.; Norton, S. J. α-Fluoro- and α-hydroxypyridylalanines. *J. Med. Chem.* **1971**, *14*, 211−214.

(22) Berman, H. M.; Westbrook, J.; Feng, Z.; Gilliland, G.; Bhat, T. N. et al. The Protein Data Bank. *Nucleic Acids Res.* **2000**, *28*, 235−242.

(23) *Molecular Operating Environment (MOE)*; 2004.03 ed.; Chemical Computing Group: Montreal, Canada.

(24) Mohamadi, F.; Richards, N. G. J.; Guida, W. C.; Liskamp, R.; Lipton, M. et al. Macromodel − an Integrated Software System for Modeling Organic and Bioorganic Molecules Using Molecular Mechanics. *J. Comput. Chem.* **1990**, *11*, 440.

(25) Jones, G.; Willett, P.; Glen, R. C.; Leach, A. R.; Taylor, R. Development and Validation of a Genetic Algorithm for Flexible Docking. *J. Mol. Biol.* **1997**, *267*, 727−748.

JM0506625