Design, Synthesis, and Evaluation of Nonpeptidic Inhibitors of Human Rhinovirus 3C Protease

Stephen E. Webber,* Jayashree Tikhe, Stephen T. Worland, Shella A. Fuhrman, Thomas F. Hendrickson, David A. Matthews, Robert A. Love, Amy K. Patick, James W. Meador, Rose Ann Ferre, Edward L. Brown, Dorothy M. DeLisle, Clifford E. Ford, and Susan L. Binford

Agouron Pharmaceuticals, Inc., 3565 General Atomics Court, San Diego, California 92121

Received August 21, 1996[⊗]

The design, synthesis, and biological evaluation of reversible, nonpeptidic inhibitors of human rhinovirus (HRV) 3C protease (3CP) are reported. A novel series of 2,3-dioxindoles (isatins) were designed that utilized a combination of protein structure-based drug design, molecular modeling, and structure−activity relationship (SAR). The C-2 carbonyl of isatin was envisioned to react in the active site of HRV 3CP with the cysteine responsible for catalytic proteolysis, thus forming a stabilized transition state mimic. Molecular-modeling experiments using the apo crystal structure of human rhinovirus-serotype 14 (HRV-14) 3CP and a peptide substrate model allowed us to design recognition features into the P_1 and P_2 subsites, respectively, from the 5- and 1-positions of isatin. Attempts to optimize recognition properties in the P_1 subsite using SAR at the 5-position were performed. In addition, a series of *ab initio* calculations were carried out on several 5-substituted isatins to investigate the stability of sulfide adducts at C-3. The inhibitors were prepared by general synthetic methods, starting with commercially available 5-substituted isatins in nearly every case. All compounds were tested for inhibition of purified HRV-14 3CP. Compounds **8**, **14**, and **19** were found to have excellent selectivity for HRV-14 3CP compared to other proteolytic enzymes, including chymotrypsin and cathepsin B. Selected compounds were assayed for antiviral activity against HRV-14-infected HI-HeLa cells. A 2.8 Å cocrystal structure of derivative **19** covalently bound to human rhinovirus-serotype 2 (HRV-2) 3CP was solved and revealed that the isatin was situated in essentially the same conformation as modeled.

Introduction

HRVs are a major cause of the common cold in the United States and Western Europe in the late spring and early fall seasons.[1] They are small icosahedral RNA viruses that belong to the picornaviridae family.[2] More than 100 different serotypes of HRVs have been indentified.[3] In humans, the primary site of virus infection and replication is the nasal epithelium. After the virus binds to cell surface receptors, the plus-sense genome is uncoated and translated by the host cell machinery. The single open reading frame codes for a 230 kD polyprotein which is co- and posttranslationally processed by virally encoded 2A and 3C proteases to yield structural and enzymatic proteins essential for viral replication.[1a,2,4] The enzymatic activity of the 3CP has been thoroughly studied.[2,5,6a] It is a very specific cysteine protease responsible primarily for the catalytic cleavage of glutamine−glycine peptide bonds. Only minimal homology exists between HRV 3CPs and prevalent mammalian enzymes. The X-ray crystal structure of HRV-14 3CP revealed that, unlike thiol proteases such as papain, this enzyme has an overall backbone fold similar to trypsin-like serine proteases.[6]

Currently, there are no adequate drugs to circumvent rhinoviral infections. Remedies which, at best, relieve the symptoms of a cold are the only treatments available. Since there are numerous rhinoviral serotypes, a vaccine seems impractical.[1a,1d] However, the quest to discover efficacious therapeutic agents continues. The use of interferon to prevent rhinoviral infections has been studied.[1a,1c,1d] Other approaches being investigated include the disruption of attachment of the intracellular adhesion molecule (ICAM-1) to human cells using antibodies or a soluble ICAM-1 extracellular domain.[1b] Most of the research has focused on the discovery and development of capsid-binding antipicornaviral agents.[1,7] Alternatively, the selective inhibition of the HRV 3CP is also considered a valid strategy for an antirhinoviral drug discovery program. Examples of HRV 3CP inhibitors have been reported in the literature.[8] Having the coordinates for the apo X-ray crystal structure of HRV-14 3CP, our research group initiated a structure-based drug design program with this as our target.

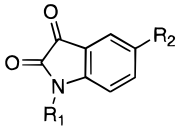
Molecular Design

A major goal of our research was to develop selective, low molecular weight, nonpeptidic inhibitors of several serotypes of the HRV 3CPs. Our investigation began with the examination of the 2.3 Å X-ray crystal structure of human HRV-14 3CP.[6] Several three-dimensional features of this cysteine protease closely resemble those of serine proteases in the trypsin family.[6] The HRV-14 3CP catalytic triad is comprised of a cysteine, histidine, and glutamic acid, whereas in bovine trypsin, the catalytic region is made up of serine, histidine, and aspartic acid. Not having an X-ray structure with a bound inhibitor, peptide substrates, including the octamer Glu-Thr-Leu-Phe-Gln-Gly-Pro-Val (representing the 2C−3A viral cleavage site), were modeled into the P_5−P_3' region in order to establish possible atomic interactions which may be critical for recognition.[6a,9]

[⊗] Abstract published in *Advance ACS Abstracts,* November 15, 1996.

S0022-2623(96)00603-6 CCC: $12.00

Table 1. 1,5-Disubstituted Isatins



1-27

no.	R_1	R_2	mp (°C)	formula[a]	HRV-14 K_i (μM)[b]
1[c]	CH_3	H	130−133	$C_9H_7NO_2$	>100
2[d]	CH_3	Cl	171−173	$C_9H_6ClNO_2$	59.0 ± 8.0
3[e]	CH_3	I	160−162	$C_9H_6INO_2$	30.0 ± 0.6
4[f]	CH_3	NO_2	197−199	$C_9H_6N_2O_4$	9.7 ± 0.7
5	CH_3	CO_2H	290−292 dec	$C_{10}H_7NO_4$[a]	10.0 ± 3.0
6	CH_3	CO_2CH_3	196−198	$C_{11}H_9NO_4$	30.0 ± 2.0
7	CH_3	CN	213−215	$C_{10}H_6N_2O_2$	51.0 ± 3.0
8	CH_3	$CONH_2$	258−260	$C_{10}H_8N_2O_3 \cdot 0.5H_2O$	0.051 ± 0.006
9	CH_3	$CONHCH_3$	268−270	$C_{11}H_{10}N_2O_3 \cdot 0.75H_2O$	12.0 ± 2.0
10	CH_3	$CON(CH_3)_2$	190−192	$C_{12}H_{12}N_2O_3$	33.0 ± 7.0
11	CH_3	$CSNH_2$	229−230	$C_{10}H_8N_2O_2S$	11.0 ± 2.0
12	CH_3	$COCH_3$	153−155	$C_{11}H_9NO_3 \cdot 0.1H_2O$	1.2 ± 0.07
13	CH_3	$SOCH_3$	193−195	$C_{10}H_9NO_3S$	8.2 ± 1.2
14	(*E*)-$CH_2CH{=}CHPh$	$CONH_2$	178−180	$C_{18}H_{14}N_2O_3$	0.011 ± 0.002
15	$(CH_2)_3Ph$	$CONH_2$	176−178	$C_{18}H_{16}N_2O_3 \cdot 0.25H_2O$	0.027 ± 0.005
16	CH_2Ph	$CONH_2$	186−187	$C_{16}H_{12}N_2O_3 \cdot 0.55H_2O$	0.019 ± 0.002
17	CH_2(4-Ph-C_6H_4)	$CONH_2$	114−116	$C_{22}H_{16}N_2O_3 \cdot 1.4H_2O$	0.048 ± 0.008
18	CH_2-β-naphthyl	$CONH_2$	289−290	$C_{20}H_{14}N_2O_3 \cdot 0.25H_2O$	0.004 ± 0.003
19	CH_2-2-benzo[*b*]thiophene	$CONH_2$	261−263	$C_{18}H_{12}N_2O_3S$	0.002 ± 0.002
20	CH_2(4-Me-C_6H_4)	$CONH_2$	220−222	$C_{17}H_{24}N_2O_3 \cdot 0.5H_2O$	0.010 ± 0.002
21	CH_2(3,4-di-MeC_6H_3)	$CONH_2$	230−231	$C_{18}H_{16}N_2O_3 \cdot 0.75H_2O$	0.006 ± 0.002
22	CH_2(3-$OMeC_6H_4$)	$CONH_2$	230−232	$C_{17}H_{14}N_2O_4 \cdot 0.6H_2O$	0.029 ± 0.009
23	CH_2(3,5-di-$OMeC_6H_3$)	$CONH_2$	269−271	$C_{18}H_{16}N_2O_5$	0.025 ± 0.004
24	CH_2(6-OMe-β-naphthyl)	$CONH_2$	235−237	$C_{21}H_{16}N_2O_4 \cdot 0.5H_2O$	0.003 ± 0.001
25	CH_2(3-OHC_6H_4)	$CONH_2$	136−138	$C_{16}H_{12}N_2O_4 \cdot 0.7H_2O$[a]	0.011 ± 0.003
26	CH_2(3,5-di-OHC_6H_3)	$CONH_2$	205−207	$C_{16}H_{12}N_2O_5 \cdot 1.7H_2O$[a]	0.004 ± 0.002
27	CH_2(6-OH-β-naphthyl)	$CONH_2$	190−192	$C_{20}H_{14}N_2O_4 \cdot 1.5H_2O$	0.004 ± 0.002

[a] All compounds were analyzed for C, H, N or C, H, N, S; the results agreed to within ±0.4% of the theoretical values except for **5**; analysis passed only with 1.5HCl. **25**: N, calcd 9.07, found 8.42. **26**: N, calcd 8.17; found 7.59. [b] See Experimental Section for method. [c] Commercially available. [d] See ref 29. [e] See ref 28. [f] See ref 30.

Since a primary goal was to keep the molecular weight of the inhibitors as low as possible, we limited our attention to the scissile cleavage, P_1 recognition, and S_2 sites. The HRV-14 3CP specifically recognizes the primary carboxamide of glutamine in the P_1 subsite and cleaves the Gln (P_1)−Gly (P_1') amide bond by nucleophilic attack of the Cys-146 (serotype 14 numbering). This protease most often distinguishes the aromatic amino acids Phe and Tyr at P_2. With these points in mind, we envisioned the cyclic α-keto amide isatin structure as a good core for molecular design.[10] Peptidyl α-keto amides are known reversible inhibitors of cysteine and serine proteases.[11] In a similar fashion, the isatin heterocycle possesses an electrophilic carbonyl, but in a conformationally restricted form. Thus, the 2-keto group of isatin was superimposed upon the scissile amide carbonyl of our octapeptide substrate model.[12] From this orientation, a carboxamide group could be positioned nicely into the P_1 recognition site from C-5, whereas the S_2 site could be accessed via substitution at N-1. Our first target, 1-methylisatin-5-carboxamide, **8**, was synthesized and found to be a fairly potent inhibitor of HRV-14 3CP having a K_i of 51 nM.

We became concerned that the activity of **8** may be attributed solely to the inherent reactive nature of isatin toward thiols after an important observation was made. The excess addition of the antioxidant dithiothreitol (DTT) to the assay inactivated **8**, but not the protease. Therefore, 1-methylisatin, **1**, in the absence of exogenous thiol, was tested next and shown to be a poor inhibitor of HRV-14 3CP having a K_i of >100 μM. Not entirely convinced that the activity of **8** versus **1** was due only to P_1 recognition of the primary carboxamide, we decided to examine whether different substituents at C-5 would have any significant effect on reactivity and protease inhibition. To evaluate any SAR, various 1-methyl-5-substituted-isatins (Table 1) were prepared. From this set of analogs, it was concluded that a carbonyl or a carbonyl isostere was required at C-5. The primary amide was found to be the best group, having a positive effect of >200× on activity when compared to the secondary and tertiary amides and the thioamide, >20× when compared to the methyl ketone, and >150× when compared to either the nitro or sulfoxide derivative.

In addition to SAR, a series of *ab initio* quantum mechanical calculations were performed to help probe whether substituents at C-5 were contributing to K_i enhancement by influencing thiol reactivity at C-3, as opposed to molecular recognition factors in the P_1 subsite. According to this hypothesis, if the thermodynamic stability of the thiol adduct was the predominate factor, the enthalpy change for the most active C-5 derivative should be the lowest. In Table 2 we have tabulated the calculated absolute and relative enthalpy changes for the reaction of methyl mercaptan to six isatins and acetaldehyde. The electronic energies were computed at both the Hartee-Fock 6-31G(d) and MP2 6-31(d) levels.[12,13,14] Since electronic correlations are included, we believe the MP2 calculations are more accurate. Listed in Table 2 are the absolute gas phase

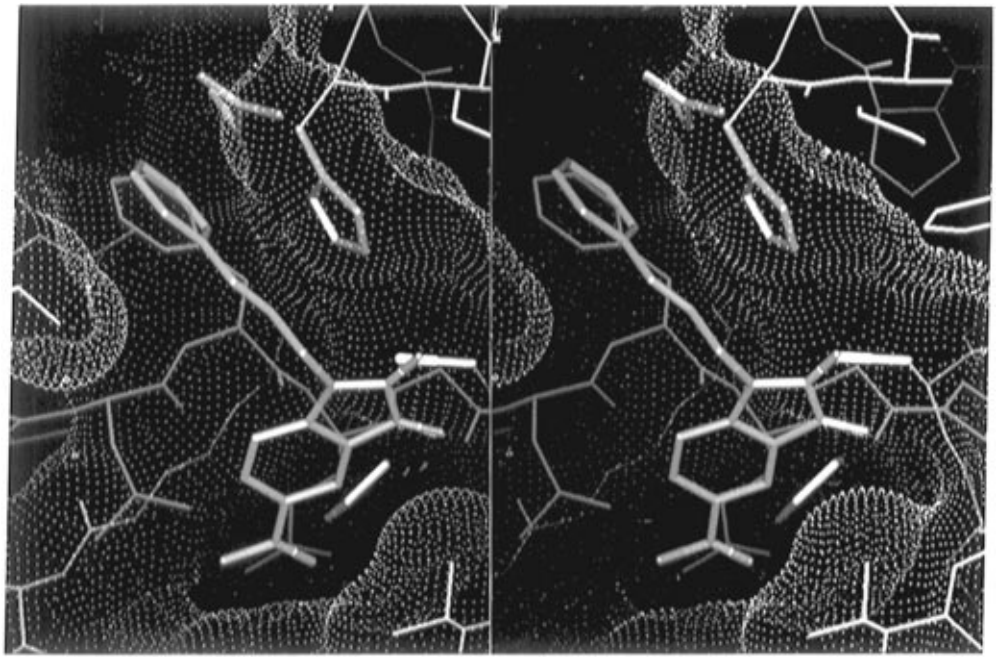
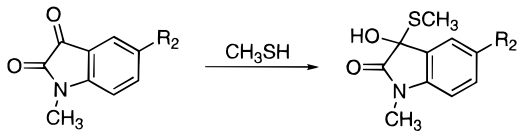
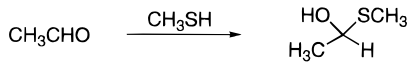


Figure 1. Stereoview of compound **14** modeled into the active site of HRV-14 3CP. The 2.3 Å apo crystal structure of HRV-14 3CP is colored orange, with the catalytic triad (Cys-146, His-40, and Glu-71) and the S_1 His-160 functionalites highlighted. A solvent accessible surface on the protease is colored white. The modeled octapeptide substrate is colored blue. The C-3 carbonyl and C-5 carboxamide of isatin **14** were superimposed upon the octapeptide Gln α-carbonyl and γ-carboxamide, respectively. The S_2 site is occupied by the N-1 cinnamyl group. Individual atoms are displayed in green (carbon), red (oxygen), blue (nitrogen), and yellow (sulfur).

Table 2. *Ab Initio* Quantum Mechanics Calculations[a]

1. 

2. 

R_2	$\Delta H^{b,c}$ (HF)[d]	$\Delta\Delta H$ (HF)	$\Delta H^{b,c}$ (MP2)[e]	$\Delta\Delta H$ (MP2)
H	8.39	7.68	−7.63	7.29
$CONH_2$	7.12	6.42	−9.13	5.78
CO_2CH_3	10.39	9.68	−11.18	3.73
CO_2H	7.42	6.71	−9.02	5.89
CN	0.71	0	−14.92	0
NO_2	6.66	5.96	−9.67	5.25
CH_3CHO	3.17	2.46	−5.95	8.96

[a] See the Experimental Section for details. [b] All energies are in kcal/mol. [c] $\Delta H = \Delta E_{electronic} + \Delta E_{vibrational} + \Delta E_{translation,\ rotation} + RT$. [d] Hartree−Fock electronic energy. [e] Second-order Møller-Plesset perturbation energy.

enthalpy changes and the enthalpy changes relative to the lowest energy reaction. The data indicates that the large enthalpic differences are dependent on the level of *ab initio* theory used, possibly due in part to the relatively large size of the system, the high degree of polarizability of the aromatic ring and its' substituents, and the fact that a polarizable sulfur atom is involved. In both sets of calculations the lowest enthalpic change is for the cyano derivative. If one were to predict the most active compound based on calculated thermodynamic stability, the cyano derivative would be the best. The calculated data at the MP2 level shows that the nitrile analog is 5.79 kcal/mol more stable than the carboxamide analog even though the K_i for nitrile is 1 order of magnitude greater. From this result we infer that molecular recognition in the P_1 subsite is the predominant factor for inhibition of HRV-14 3CP.

We now turned our attention to the S_2 site. Using our original model as described previously, a propyl or allyl group placed at N-1 could reach into this confined hydrophobic pocket. A phenyl ring substituted at the γ-position of either of these two groups lies almost exactly upon the phenyl ring of the octapeptide Phe at P_2.[12] The *trans*-cinnamyl derivative **14** was prepared and was found to be ∼5× (11 nM) more active against HRV-14 3CP than **8**. Compound **14**, superimposed upon the peptide substrate model, is illustrated in Figure 1. The *n*-propylphenyl analog **15**, a 27 nM inhibitor of HRV-14 3CP, was easily derived from compound **14**. Modeling the less extended N-1 benzyl group positions the phenyl ring snugger into the S_2 pocket and more adjacent to the imidazole of His-40 as compared to compound **14**. Several N-1 arylmethylene derivatives were therefore proposed and synthesized. Of these, the benzo[*b*]thiophene derivative, **19**, was found to be the most potent inhibitor of HRV-14 3CP.

A 2.8 Å cocrystal structure of **19** and HRV-2 3CP was solved.[15] Displayed in Figure 2 is an electron density map of this complex. Close examination of this X-ray structure revealed several important atomic interactions between the protein and small molecule. A covalent bond between Cys-147 and the electrophilic C-3 of isatin was evident, positioning the oxygen in a classical "oxyanion hole" arrangement. The carboxamide makes H-bonds to the imidazole of His-161 and the hydroxyl and α-carbonyl of Thr-142. The benzo[*b*]thiophene is tightly situated in the S_2 pocket, although the orientation of this bicyclic ring is ambiguous. The electron density maps reveal an equal population of two conformations differing by 180°. One conformation exposes the sulfur atom to solvent, while the other directs the sulfur toward the β-sheet of the protease.

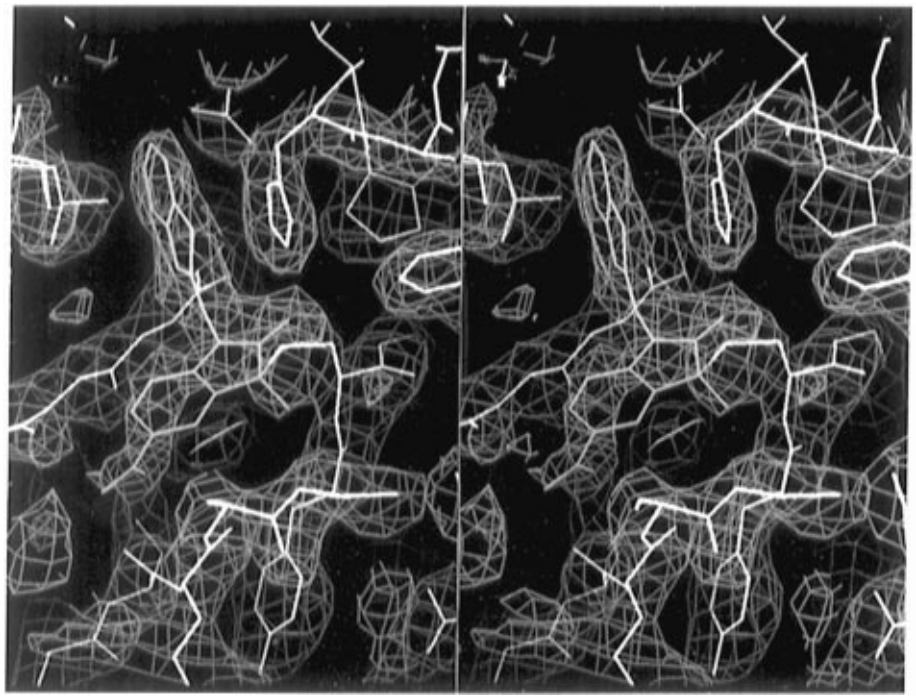
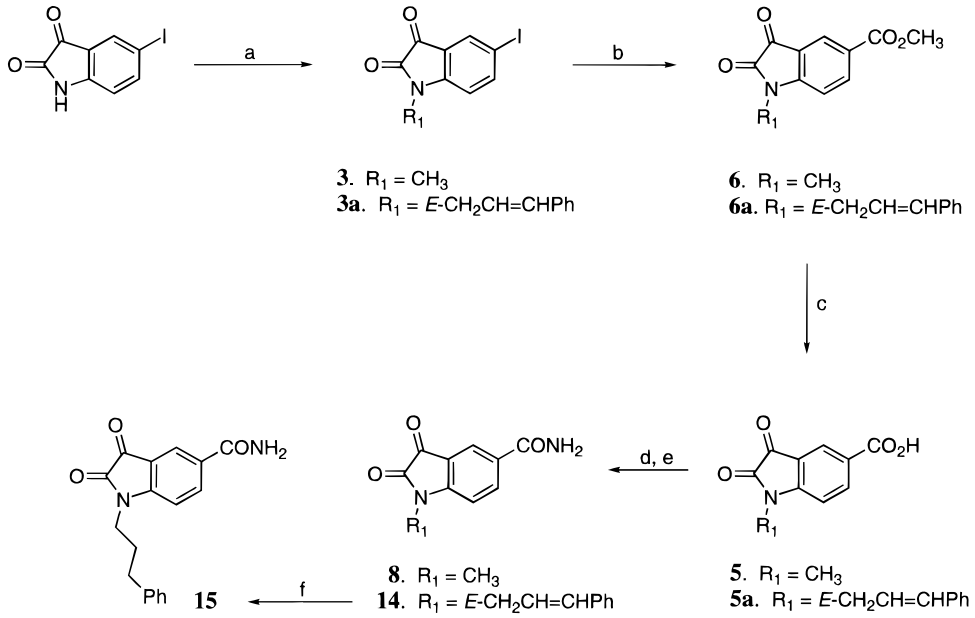


Figure 2. Stereoview of the electron density map of the crystal structure of compound **19** complexed with HRV-2 3CP at 2.8 Å resolution computed with coefficients $2F_{obs} - F_{calc}$ and phases from the protein structure alone (without **19**). The electron density is consistent for a covalent bond formed between Cys-147 and C-3 of **19**.

Scheme 1. Original Synthesis: Preparation of **3**, **5**, **6**, **8**, **14**, and **15**[a]



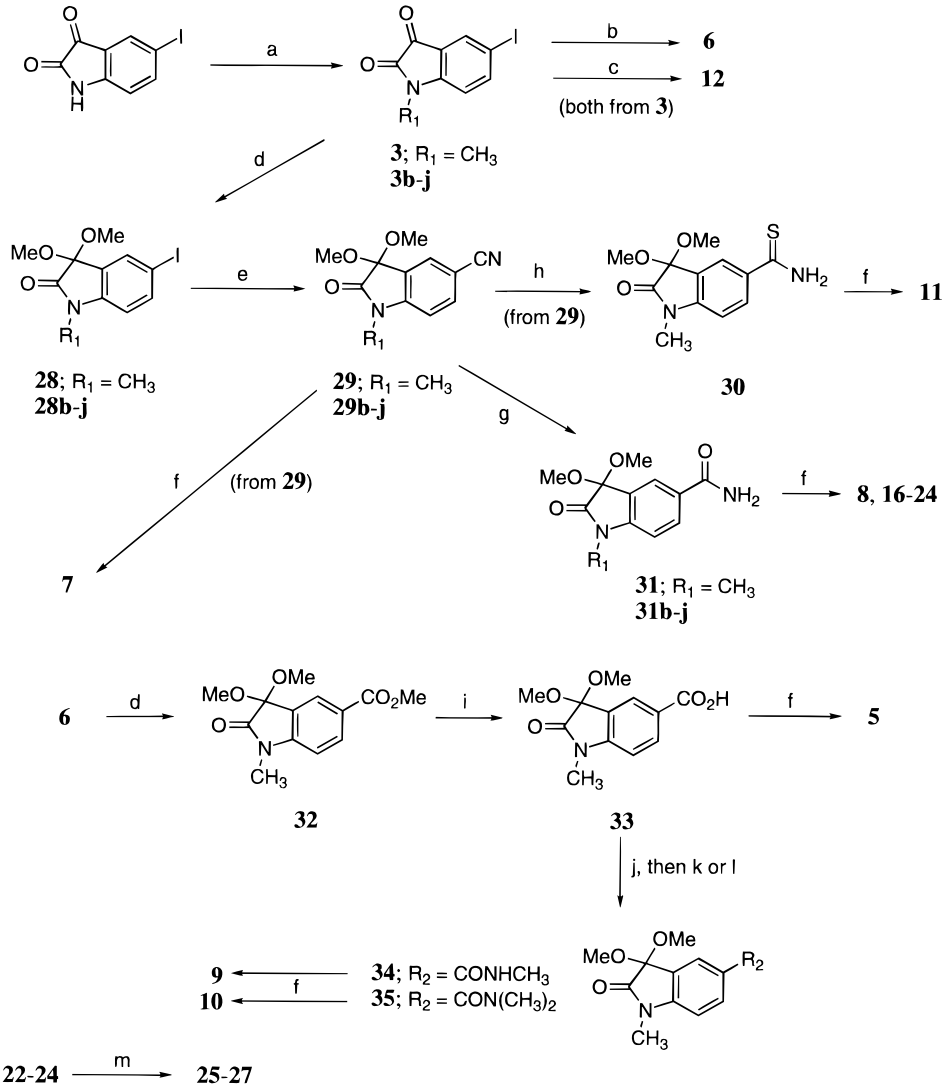
[a] (a) NaH, DMF, MeI, or (*E*)-cinnamyl bromide; (b) CO, MeOH, DMF, Et_3N, $(Ph_3P)_2PdCl_2$; (c) K_2CO_3, MeOH, H_2O; (d) *N*-hydroxysuccinimide, EDC, CH_2Cl_2; (e) Et_3N, H_2O, $CH_2(NH_2)_2 \cdot 2HCl$; (f) (1) H_2, Pd/C, (2) MnO_2, CH_2Cl_2.

Chemistry

Isatin analogs **1**−**27**, listed in Table 1, were prepared as illustrated in Schemes 1−3. We originally devised a five-step synthetic route to **8** starting with commercially available 5-iodoisatin (Scheme 1). This procedure was also used to prepare **14**, but was not very practical due to the following reasons. The cyclic keto amide of **6** was sensitive to ring cleavage under standard saponification conditions with hydroxide. The hydrolysis of the methyl ester **6** to carboxylic acid **5** was eventually achieved by using potassium carbonate. Conversion of **5** to amide **8** was problematic. The synthesis and isolation of the acid chloride of **5** failed. Attempts to quickly generate the acid chloride and immediately expose it to ammonia were also unsuccessful. We finally resorted to preparing the activated *N*-hydroxysuccinimide ester, which was subsequently treated with diaminomethane.[16] The yield for this two-step process was a poor 20%.

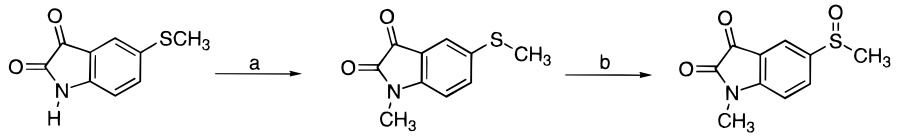
A second, more general synthetic route was devised. It involved the same number of steps as our original plan, but turned out to be much more effective. As illustrated in Scheme 2, ketal protection of the C-3 isatin carbonyl alleviated some of the synthetic difficulties.[17] The primary carboxamide was efficiently introduced via a palladium-catalyzed aryl cyanation[18] of the iodide at

Scheme 2. General Synthesis of 1,5-Disubstituted Isatins[a]



[a] (a) NaH, DMF, R_1X; (b) CO, MeOH, DMF, $(Ph_3P)_2PdCl_2$, NEt_3; (c) $H_2C{=}C(OEt)Sn(n\text{-}Bu)_3$, $(Ph_3P)_4Pd$, BHT, 1,4-dioxane; (d) MeOH, $(MeO)_3CH$, *p*-TsOH; (e) KCN, THF, $(Ph_3P)_4Pd$; (f) H_2SO_4, H_2O, MeOH; (g) H_2O_2, Na_2CO_3, EtOH; (h) H_2S, NEt_3, pyridine; (i) LiOH, MeOH, H_2O; (j) $ClC(O)C(O)Cl$, CH_2Cl_2, DMF; (k) $MeNH_2$, THF; (l) Me_2NH, THF; (m) BBr_3, CH_2Cl_2.

Scheme 3. Synthesis of **13**[a]



[a] (a) NaH, DMF, CH_3I; (b) *m*-CPBA, CH_2Cl_2.

C-5, followed by basic peroxide hydrolysis. Having the carbonyl protected also allowed us to prepare the acid chloride of **33**, which was a common intermediate for amides **9** and **10**.

The methyl ketone, **12**, was prepared directly from **3** by a Stille-type palladium-catalyzed coupling of tributyl-(1-ethoxyvinyl)tin,[19] whereas sulfoxide **13** was synthesized from 5-(methylthio)isatin[20] as shown in Scheme 3.

Enzyme Inhibition and Antiviral Assessment

The HRV 3CP inhibition constants for compounds **1**−**27** were measured using a continuous fluorescence resonance energy transfer assay.[21] The data is reported in Tables 1 and 3. Although inconclusive, a comparison of the K_i values for isatins **8** and **19** for HRV-2, -14, -16, and -89 3CPs shown in Table 3 suggests that this class of inhibitors may have a broad range of activity against many serotypes.

Table 3. Isatins **8** and **19** versus Different Serotypes[a]

	K_i (nM)			
no.	HRV-14	HRV-89	HRV-2	HRV-16
8	51.0 ± 6.0	35.0 ± 3.0	77.0 ± 6.0	40.0 ± 2.0
19	1.7 ± 1.8	1.1 ± 0.02	5.2 ± 0.07	12.0 ± 11.0

[a] See the Experimental Section for details.

Isatins **8**, **14**, and **19** were found to have excellent selectivity for HRV 3CPs over other readily available

Table 4. Protease Selectivity

enzyme	% inhibition (compound concentration)[a] 8	14	19
trypsin (bovine)	0 (100 μM)	0 (100 μM)	0 (100 μM)
chymotrypsin (bovine)	0 (100 μM)	67 (100 μM)	25 (50 μM)
thrombin (human)	0 (100 μM)	34 (100 μM)	0 (100 μM)
elastase (human neutrophil)	0 (100 μM)	40 (100 μM)	0 (100 μM)
cathepsin B (human liver)	0 (100 μM)	27 (100 μM)	0 (10 μM)

[a] See the Experimental Section and ref 22 for details.

Table 5. Antiviral Activity (HRV-14)[a]

no.	EC_{50} (μM)[b]	TC_{50} (μM)[c]	TI[d]	K_i (μM)
3	71	>100	>1	30.0
5	>100	>100		10.0
6	56	>100	>2	30.0
8	66	270	4	0.051
14	6.3	9.6	1.5	0.011
15	>7.1	7.1		0.027
16	14.1	50.1	3.5	0.019
18	>10	10		0.004
19	>5.6	5.6		0.002
22	>20	20		0.029
25	>20	20		0.011
26	>100	>100		0.004
pirodavir[e]	0.03	>10	>300	

[a] See the Experimental Section for assay conditions. [b] 50% effective concentration. [c] 50% toxic concentration. [d] Therapeutic index. [e] Control compound; Janssen Pharmaceuticals.

cysteine and serine proteases as depicted in Table 4.[22] Against the enzymes tested, compound **8** was inactive, whereas **14** and **19** displayed meager inhibition of chymotrysin, possibly due to aromatic S_1 recognition.

The antirhinoviral activity was measured for twelve isatin analogs using a cell protection assay. The data is recorded in Table 5. These compounds were checked for their ability to protect HI-Hela cells against HRV infection using the known technique of measuring XTT dye reduction.[23] Unfortunately these compounds were either relatively inactive or did not display efficacy significantly below their toxic concentrations (TC_{50}). In certain cases the poor antiviral activity may arise from inadequate cell permeability; however, it nevertheless remains unclear whether the reactive nature of these molecules is responsible for cell toxicity.[24]

Concluding Remarks

In summary, we have designed and prepared a novel class of very active, reversible, nonpeptidic HRV 3CP inhibitors joining protein structure-based drug design with molecular modeling, SAR, and *ab initio* quantum mechanical calculations. Our research demonstrates that molecular recognition, particularly in the P_1 subsite, is a crucial aspect when considering the design of nonpeptidic HRV 3CP inhibitors. The cocrystal structure of **19** covalently bound to HRV-2 3CP has validated much of our modeling and design process. Disappointedly, the lack of *in vitro* antiviral potency has raised several puzzling and unanswered questions concerning the toxicity and physiochemical properties of these molecules. The solutions to these problems, together with the further utilization of protein structure-based drug design may lead to the discovery of an improved class of nonpeptidic HRV 3CP inhibitors.

Experimental Section

Proton magnetic resonance spectra (NMR) were determined using a Tech-Mag spectrometer operating at a field strength of 300 MHz. 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 DMSO-d_6 the DMSO is at 2.49 ppm. Standard and peak multiplicities are designated as follows: s, singlet; d, doublet; dd, doublet of doublets; t, triplet; q, quartet; br s, broad singlet; 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 ±0.4% of the theoretical values. Flash column chromatography was performed using silica gel 60 (Merck Art 9385). Thin layer chromatographs were performed on precoated sheets of silica 60 F_{254} (Merck Art 5719). Melting points were determined on a Mel-Temp apparatus and are uncorrected. Tributyl(1-ethoxyvinyl)tin was purchased from Aldrich Chemical Co. Anhydrous *N,N*-dimethylformamide (DMF), dichloromethane (CH_2Cl_2), and dimethyl sulfoxide (DMSO) were used as is. Tetrahydrofuran (THF) was distilled from sodium benzophenone ketyl under nitrogen. Et_2O refers to diethyl ether. Pet ether refers to petroleum ether of bp 36−53 °C.

Inhibition Assays. All strains of human rhinovirus (HRV) were purchased from American Type Culture Collection (ATCC) except for HRV serotype-14 which was obtained from the supernatant of HeLa cells transfected with an RNA transcript derived from a cDNA clone constructed and supplied to us by Drs. R. Rueckert and W.-M. Lee at the Institute for Molecular Virology, University of Wisconsin, Madison, WI.[25] HRV-14 3CP was expressed and purified as described in the literature.[6a] To obtain 3CPs from other serotypes, cytoplasmic RNA was prepared from HeLa cells infected with HRV-2, -16, or -89.[4] cDNAs were synthesized using a primer coincident with the 3′ terminus of the 3C coding regions. A region of the genome corresponding to the carboxy terminus of 3A, all of 3B, and all of 3CP was amplified from the cDNA by PCR and cloned into an *Escherichia coli* expression vector. Protein was purified from the insoluble fraction of *E. coli* as described[6a] or purified from the lysate supernatant by chromatography on Blue Sepharose and Sephacryl 100, both supplied by Pharmacia. Human liver cathepsin B and human neutrophil elastase were purchased from Calbiochem. Bovine chymotrypsin and human thrombin were purchased from Boehringer Mannheim. Bovine trypsin was purchased from Sigma.

The HRV 3CP activity was measured by a continuous fluorescence resonance energy transfer assay using a dimodified decapeptide substrate (*s*), DABCYL-Gly-Arg-Ala-Val-Phe-Gln-Gly-Pro-Val-Gly-EDANS ($k_{cat}/K_m = 3.4 \times 10^4$ M^{-1} s^{-1}) or a dimodified nonapeptide substrate (*s*), DABCYL-Gly-Gln-Val-Leu-Phe-Gln-Gly-Pro-Val-EDANS ($k_{cat}/K_m = 6.7 \times 10^4$ M^{-1} s^{-1}).[21] Inhibition was measured as a change in initial velocity (V_0) as a function of inhibitor (I) concentration in substrate start assays after a 10 min preincubation of enzyme (E) and inhibitor. Assays (3 mL at 30 °C) contained 50 mM Tris, pH 7.6, 1 mM EDTA, 1 μM substrate, 50 nM HRV-14 3CP, 2% DMSO, and inhibitor as needed. Fluorescence was monitored at 336 nm (excitation) and 490 nm (emission). Other serotypes were assayed at 100−200 nM enzyme concentrations. Data was analyzed with the nonlinear regression analysis program ENZFITTER[26] using the equation

$$V(I) = V_0(EI/E_{total}) \text{ where } K_i = (E_{total} - EI)(I_{total} - EI)/EI$$

Because the K_m values for the fluorescent substrates are 12 μM for the 9-mer and 18 μM for the 10-mer ($s \ll K_m$), no corrections for an s/K_m term were used.

Antiviral Assays. HRV stocks were propagated, and antiviral assays were performed in HI-HeLa cells, purchased from ATCC. Cells were grown in minimal essential medium with 10% fetal bovine serum.

The ability of compounds to protect cells against HRV infection was measured by the XTT dye reduction method.[23] HI-Hela cells were infected with HRV-14 at a multiplicity of infection (moi) of 0.13 or mock-infected with medium only. Infected or uninfected cells were resuspended at 8×10^5 cells/mL and incubated with appropriate concentrations of compounds. Two days later, XTT/PMS was added to test plates, and the amount of formazan produced was quantified spectrophotometrically at 450/650 nm. The EC_{50} was calculated as the concentration of compound that increased the percentage of formazan production in compound-treated, virus-infected cells to 50% of that produced by compound-free, uninfected cells. The 50% cytotoxic dose (CC_{50}) was calculated as the concentration of compound that decreased the percentage of formazan produced in compound-treated, uninfected cells to 50% of that produced in compound-free, uninfected cells. The therapeutic index (TI) was calculated by dividing the CC_{50} by the EC_{50}.

***Ab-Initio* Quantum Mechanics Calculations.** The energy changes for two types of reactions were studied; the addition of methyl mercaptan to a series of substituted isatins and the addition of methyl mercaptan to acetaldehyde. A protonated thiol was used as a model since there was evidence to suggest that the active site cysteine in HRV-14 3CP is not ionized in the resting state of the enzyme.[27] Therefore, the calculations should reflect the inherent stability of the adducts with respect to the isolated components without any complicating factors of thiol ionization, and the ion's relative stability in the environment of the enzyme versus the gas phase. All *ab initio* calculations were performed using the GAUSSIAN92[13] program package. Input structures were created using the MacroModel[12] program and were energy minimized using the AMBER* force field. These structures were then converted to the Z-matrix format using the utility program NewZmat and then geometry optimized in GAUSSIAN at the restricted Hartree−Fock (RHF) 6-31G(d) level. Harmonic vibrational frequencies were calculated at this level of theory (RHF/6-31G(d)//RHF/6-31G(d)), and from these calculations were derived vibrational and zero point energies and entropies at 298.15 K. The effects of electron correlation on the electronic energies were obtained by performing second-order Møller−Plesset perturbation theory[14] at the MP2/6-31G(d)//RHF/6-31G(d) level.

Original Synthesis: 1-Methyl-5-iodoisatin (3).[28] To a solution of 5-iodoisatin (3.00 g, 10.99 mmol) in 30 mL of anhydrous DMF at 0 °C was added NaH (0.528 g, 13.19 mmol; 60% dispersion in mineral oil). The heterogeneous mixture was stirred for 1 h at room temperature. To the reaction mixture was added iodomethane (0.89 mL, 14.29 mmol). The reaction mixture was stirred at room temperature overnight. Ice cold water was added to the reaction mixture whereupon a red solid precipitates. The solid was filtered, washed with water and petroleum ether, and dried under vacuum to yield 2.69 g (85%) of a red solid. The product was used without further purification: mp 160−162 °C; ^{1}H NMR (DMSO-d_6) δ 3.10 (s, 3H), 7.00 (d, J = 9.0 Hz, 1H), 7.79 (s, 1H), 7.99 (d, J = 9.0 Hz, 1H); IR (KBr) 1747, 1731, 1615, 1441, 1325, 1102 cm^{-1}. Anal. ($C_9H_6INO_2$) C, H, N.

1-Methyl-5-carbomethoxyisatin (6). A mixture of **3** (0.5 g, 1.74 mmol) and bis(triphenylphosphine)palladium chloride (0.055 g, 0.0783 mmol) in 10 mL of anhydrous DMF, 20 mL of methanol, and triethylamine (0.76 mL, 5.48 mmol) was heated at 50−60 °C for 18 h in a carbon monoxide atmosphere. The resulting solution was evaporated to dryness. The residue was taken up in 1 N HCl. The mixture was extracted with EtOAc, and the combined organic extracts were washed with brine, dried over $MgSO_4$, and concentrated. The crude product was chromatographed on a silica column using $CHCl_3$. The product was isolated as a yellowish orange solid (0.190 g; 99%): mp 196−198 °C; ^{1}H NMR (DMSO-d_6) δ 3.18 (s, 3H), 3.86 (s, 3H), 7.27 (d, J = 9.0 Hz, 1H), 7.94 (s, 1H), 8.24 (d, J = 9.0 Hz, 1H); IR (KBr) 1751, 1709, 1631, 1309, 771, 473 cm^{-1}. Anal. ($C_{11}H_9NO_4$) C, H, N.

1-Methylisatin-5-carboxylic Acid (5). Methyl ester **6** (0.2 g, 0.913 mmol) was dissolved in 100 mL of methanol and 25 mL of water. To this solution was added potassium carbonate (1.67 g, 12 mmol). The reaction mixture was stirred at 55−60 °C for 24 h. Upon evaporation of the reaction solvents, the residue was acidified with 1 N HCl and extracted with EtOAc. The organic layers were dried over anhydrous $MgSO_4$, filtered, and concentrated. (Further purification of the product could be done by flash chromatography on silica using EtOAc/MeOH, 70:30.) An orange solid (0.171 g) was isolated in 91% yield: mp 290−292 °C dec; ^{1}H NMR (DMSO-d_6) δ 3.24 (s, 3H), 7.06 (d, J = 6.0 Hz, 1H), 7.98 (s, 1H), 8.19 (d, J = 6.0 Hz, 1H); IR (KBr) 3400, 1739, 1733, 1618, 1408 cm^{-1}. Anal. ($C_{10}H_7NO_4$·1.5HCl) C, H, N.

1-Methylisatin-5-carboxylic Acid *N*-hydroxysuccinimide Ester. To a solution of the carboxylic acid **5** (51 mg, 0.249 mmol) in 8 mL of anhydrous DMF at 0 °C were added *N*-hydroxysuccinimide (35 mg, 0.3 mmol) and EDC (58 mg, 0.3 mmol). The solution was stirred for 2 h at room temperature. The reaction mixture was evaporated to dryness, and the resulting residue was taken up in EtOAc and washed with water. The organic layers were dried over anhydrous $MgSO_4$ and concentrated. The compound was purified by flash column chromatography on silica with EtOAc to give a yellow solid (42 mg, 56%): mp 230−232 °C; ^{1}H NMR (DMSO-d_6) δ 2.89 (s, 4H), 3.21 (s, 3H), 7.38 (d, J = 9.0 Hz, 1H), 8.04 (s, 1H), 8.36 (d, J = 9.0 Hz, 1H); IR (KBr) 1761, 1751, 1734, 1622, 1207 cm^{-1}.

1-Methylisatin-5-carboxamide (8). Triethylamine (0.17 mL, 1.2 mmol) and diaminomethane dihydrochloride[16] (24 mg, 0.2 mmol; dissolved in minimum amount of water) were added at room temperature to a solution of the 1-methylisatin-5-carboxylic acid *N*-hydroxysuccinimide ester (0.121 g, 0.401 mmol) in 40 mL of 1,4-dioxane. The solution was stirred for 24 h at room temperature. The residue obtained upon evaporation of all reaction solvent was chromatographed on a silica column using EtOAc. The product was isolated as an orange solid (30 mg) in 37% yield: mp 258−260 °C; ^{1}H NMR (DMSO-d_6) δ 3.19 (s, 3H), 7.21 (d, J = 9.0 Hz, 1H), 7.44 (br s, 2H), 8.04 (s, 2H), 8.18 (d, J = 9.0 Hz, 1H); IR (KBr) 3439, 3348, 1741, 1662, 1622, 1391, 1334, 1094 cm^{-1}; MS (EI) m/z 204 (M$^+$), 176, 148, 132, 104, 77, 63, 51. Anal. ($C_{10}H_8N_2O_3$·0.5H_2O) C, H, N.

1(*E*)-Cinnamylisatin-5-carboxamide (14): mp 178−180 °C; ^{1}H NMR ($CDCl_3$) δ 4.59 (d, J = 6.0 Hz, 2H), 5.75 (br s, 2H), 6.18 (dt, J = 15.0, 6.0 Hz, 1H), 6.70 (d, J = 15.0 Hz, 1H), 7.08 (d, J = 9.0 Hz, 1H), 7.32 (m, 5H), 8.01 (s, 1H), 8.18 (d, J = 9.0 Hz, 1H); IR (KBr) 3448, 3375, 1743, 1662, 1653, 1618, 1391, 1342 cm^{-1}; MS (EI) m/z 306 (M$^+$), 189, 117, 105, 91, 83. Anal. ($C_{18}H_{14}N_2O_3$) C, H, N.

1-[1-(3-Phenyl-*n*-propyl)]isatin-5-carboxamide (15). A mixture of **14** (0.101 g, 0.330 mmol) and 10% Pd/C (10.2 mg) in 50 mL of EtOAc was stirred at room temperature for 15 min under an atmosphere of hydrogen (balloon). The resulting black suspension was filtered through a pad of Celite. The yellow filtrate was concentrated to give a 1:1 mixture (86.0 mg) of the desired product along with 1-[1-(3-phenyl-*n*-propyl)]-3-hydroxy-2-oxindole-5-carboxamide. This mixture was dissolved in 15 mL of CH_2Cl_2 and oxidized with MnO_2 (0.040 g, 0.461 mmol). The black suspension was stirred at room temperature for 45 min and then filtered through Celite. The yellow filtrate was concentrated to give a pure product as a yellowish orange solid (0.074 g; 86%): mp 176−178 °C; ($CDCl_3$) δ 2.07 (dt, J = 7.5, 7.5 Hz, 2H), 2.74 (t, J = 7.5 Hz, 2H), 3.80 (t, J = 7.5 Hz, 2H), 5.85 (br s, 2H), 6.83 (d, J = 9.0 Hz, 1H), 7.25 (m, 5H), 7.93 (s, 1H), 8.17 (d, J = 9.0 Hz, 1H); IR (KBr) 3479, 3346, 1739, 1664, 1608, 1383 cm^{-1}; MS (EI) m/z 308 (M$^+$), 280, 204, 175, 149, 118, 91. Anal. ($C_{18}H_{16}N_2O_3$·0.25H_2O) C, H, N.

General Synthesis: 1-(2-Naphthylmethyl)-5-iodoisatin Dimethyl Ketal (28d). A suspension of 1-(2-naphthylmethyl)-5-iodoisatin (**3d**) (prepared as described for **3** from 5-iodoisatin and 2-(bromomethyl)naphthalene; 0.186 g, 0.451 mmol) and *p*-toluene sulfonic acid (8 mg, 0.0415 mmol) in 7 mL of anhydrous methanol and trimethyl orthoformate (1.5 mL, 13.5 mmol) was refluxed for 44 h at 80 °C. Upon evaporation of all solvent, the residue was taken up in CH_2Cl_2 and washed with saturated aqueous $NaHCO_3$. The organic layers were dried and concentrated. When dried under vacuum, a yellow-

ish orange solid (0.189 g, 92%) was formed. The product was used without further purification: mp 118−119 °C; ^{1}H NMR ($CDCl_3$) δ 3.62 (s, 6H), 5.00 (s, 2H), 6.52 (d, J = 9.0 Hz, 1H), 7.35 (d, J = 9.0 Hz, 1H), 7.45−7.53 (m, 3H), 7.69 (s, 2H), 7.76−7.81 (m, 3H); IR (KBr) 2932, 1732, 1606, 1481, 1423, 1176, 1138 cm^{-1}. Anal. ($C_{21}H_{18}INO_3$) C, H, N.

1-(2-Naphthylmethyl)-5-cyanoisatin Dimethyl Ketal (29d). To a solution of the 1-(2-naphthylmethyl)-5-iodoisatin dimethyl ketal (**28d**) (0.108 g, 0.235 mmol) in 2 mL of anhydrous THF were added KCN (31 mg, 0.470 mmol) and tetrakis(triphenylphosphine)palladium (5 mg, 4.0 μmol). The suspension was refluxed for 21 h. The reaction mixture was evaporated to remove all solvent and the resultant residue was taken up in CH_2Cl_2 and washed with water. The organic layers were dried over anhydrous $MgSO_4$, filtered, and concentrated. The product was purified by flash chromatography on silica using EtOAc/hexanes, 30:70. A tan solid (71 mg, 94%) was isolated: mp 120−121 °C; ^{1}H NMR ($CDCl_3$) δ 3.64 (s, 6H), 5.05 (s, 2H), 6.83 (d, J = 9.0 Hz, 1H), 7.35 (d, J = 9.0 Hz, 1H), 7.48−7.54 (m, 3H), 7.67 (s, 1H), 7.71 (s, 1H), 7.78−7.84 (m, 3H); IR (KBr) 3055, 2989, 2951, 2226, 1738, 1616, 1595, 1493, 1346, 1263, 1165, 1033, 827, 504 cm^{-1}. Anal. ($C_{22}H_{18}N_2O_3$) C, H, N.

1-(2-Naphthylmethyl)isatin-5-carboxamide Dimethyl Ketal (31d). To a suspension of the 1-(2-naphthylmethyl)-5-cyanoisatin dimethyl ketal (**29d**) (0.630 g, 1.685 mmol) in 60 mL of EtOH at room temperature were added 6 mL of 3 N aqueous Na_2CO_3 and 5 mL of 30% H_2O_2. The reaction mixture was stirred at room temperature for 4 h. The white suspension was diluted with water and extracted with EtOAc. The combined organic layers were dried over anhydrous $MgSO_4$, filtered, and evaporated to dryness. A white solid (0.618 g, 97%) was isolated which was used without further purification: mp 191−193 °C; ^{1}H NMR ($CDCl_3$) δ 3.65 (s, 6H), 5.06 (s, 2H), 5.20−5.60 (br s, 2H), 7.37 (d, J = 9.0 Hz, 1H), 7.46−7.49 (m, 2H), 7.70 (d, J = 9.0 Hz, 1H), 7.73 (s, 1H), 7.77−7.83 (m, 3H), 7.90 (s, 1H); IR (KBr) 3412, 3181, 2949, 1730, 1668, 1616, 1373, 1143, 1044, 812, 755 cm^{-1}. Anal. ($C_{22}H_{20}N_2O_4$) C, H, N.

1-(2-Naphthylmethyl)isatin-5-carboxamide (18). A slightly turbid solution of 1-(2-naphthylmethyl)isatin-5-carboxamide dimethyl ketal (**31d**) (8.8 mg, 0.023 mmol) in 1.5 mL of MeOH and 0.5 mL of H_2O was cooled to 0 °C. To this cold solution was gradually added concentrated H_2SO_4 (4 drops). Heat (70−75 °C) was applied for 7 h. Upon completion of reaction a yellow solid precipitated out of solution. This yellow solid was filtered, washed with water, and dried under vacuum to yield 4 mg (57%) of pure product: mp 289−290 °C; ^{1}H NMR (DMSO-d_6) δ 5.10 (s, 2H), 7.05 (d, J = 9.0 Hz, 1H), 7.40 (br s, 1H), 7.47−7.50 (m, 3H), 7.56 (d, J = 9.0 Hz, 1H) 7.82−7.91 (m, 3H), 8.01 (s, 1H), 8.03 (d, J = 9.0 Hz, 1H), 8.09 (s, 1H); IR (KBr) 3474, 3369, 1749, 1732, 1664, 1622, 1587, 1369, 1340 cm^{-1}. Anal. ($C_{20}H_{14}N_2O_3 \cdot 0.25\ H_2O$) C, H, N.

1-Methyl-5-carbomethoxyisatin Dimethyl Ketal (32). This compound was prepared from **6** using the procedure to prepare 1-(2-naphthylmethyl)-5-iodoisatin dimethyl ketal (**28d**) in 85% yield: mp 83−85 °C; ^{1}H NMR ($CDCl_3$) δ 3.17 (s, 3H), 3.56 (s, 6H), 3.88 (s, 3H), 6.85 (d, J = 9.0 Hz, 1H), 8.04 (s, 1H), 8.09 (d, J = 9.0 Hz, 1H); IR (KBr) 29157, 2833, 1733, 1700, 1609, 1303, 1228, 1113, 1071, 769, 740 cm^{-1}. Anal. ($C_{13}H_{15}NO_5$) C, H, N.

1-Methylisatin-5-carboxylic Acid Dimethyl Ketal (33). To a solution of 1-methyl-5-carbomethoxyisatin dimethyl ketal (**32**) (0.53 g, 2.0 mmol) in 18 mL of MeOH was added an aqueous solution of LiOH (1.02 g, 2.43 mmol; 8 mL of H_2O). After being stirred at room temperature for 12 h, the mixture was washed twice with 20 mL of Et_2O. The aqueous layer was carefully neutralized at 0 °C with dilute HCl and extracted three times with 40 mL of EtOAc. The organic layers were combined, dried over $MgSO_4$, filtered, and concentrated to give 0.46 g (91% yield) of the crude acid as an off-white solid: mp 181−183 °C; ^{1}H NMR ($CDCl_3$) δ 3.21 (s, 3H), 3.59 (s, 6H), 6.92 (d, J = 9.0 Hz, 1H), 8.13 (s, 1H), 8.16 (d, J = 9.0 Hz, 1H); MS (EI) m/z 251 (M^+), 223, 208, 178, 149.

***N,N*-Dimethyl-1-methylisatin-5-carboxamide Dimethyl Ketal (35).** A solution of dry 1-methylisatin-5-carboxylic acid dimethyl ketal (**33**) (0.251 g, 1.0 mmol) in 15 mL of anhydrous CH_2Cl_2 was cooled to 0 °C. One drop of DMF was added to this solution preceded by the slow addition of oxalyl chloride (0.14 g, 1.1 mmol). The stirred solution was brought to room temperature over a period of 1 h. At this time the solution was concentrated under vacuum to give the crude acid chloride which was used immediately. The acid chloride was taken up in 15 mL of anhydrous THF. The solution was cooled to 0 °C, and an excess of dimethylamine gas was bubbled into the reaction vessel. The mixture was warmed to room temperature and concentrated to give a residue which was purified by column chromatography on silica with 80% EtOAc in pet. ether to give a tan solid in 72% yield: mp 70−72 °C; ^{1}H NMR ($CDCl_3$) δ 3.06 (br s, 6H), 3.18 (s, 3H), 3.56 (s, 6H), 6.85 (d, J = 9.0 Hz, 1H), 7.47−7.50 (m, 2H); IR (KBr) 2941, 1732, 1631, 1620, 1491, 1390, 1205, 1114, 1045, 829, 769 cm^{-1}. MS (EI) m/z 278 (M^+), 247, 206, 188, 160, 104.

***N,N*-Dimethyl-1-methylisatin-5-carboxamide (10):** mp 190−192 °C; ^{1}H NMR ($CDCl_3$) δ 3.06 (br s, 6H), 3.29 (s, 3H), 6.96 (d, J = 9.0 Hz, 1H), 7.67 (s, 1H), 7.78 (d, J = 9.0 Hz, 1H); IR (KBr) 1736, 1624, 1612, 1334, 1168, 1102 cm^{-1}: MS (EI) m/z 232 (M^+), 203, 188, 160, 77. Anal. ($C_{12}H_{12}N_2O_3$) C, H, N.

1-Methyl-5-iodoisatin dimethyl ketal (28): mp 88−90 °C; ^{1}H NMR ($CDCl_3$) δ 3.14 (s, 3H), 3.56 (s, 6H), 6.63 (d, J = 9.0 Hz, 1H), 7.68−7.70 (m, 2H); IR (KBr) 1738, 1607, 1474, 1358, 1251, 1102, 1052 821 cm^{-1}. Anal. ($C_{11}H_{12}NO_3I$) C, H, N.

1-Methyl-5-cyanoisatin dimethyl ketal (29): mp 130−131 °C; ^{1}H NMR ($CDCl_3$) δ 3.20 (s, 3H), 3.58 (s, 6H), 6.92 (d, J = 9.0 Hz, 1H), 7.66 (s, 1H), 7.71 (d, J = 9.0 Hz, 1H); IR (KBr) 2954, 2217, 1738, 1615, 1507, 1358, 1110, 821 cm^{-1}. Anal. ($C_{12}H_{12}N_2O_3$) C, H, N.

1-Methylisatin-5-thiocarboxamide Dimethyl Ketal (30). In a glass pressure tube, equipped with a threaded Teflon cap, a solution of 1-methyl-5-cyanoisatin dimethyl ketal (**29**) (0.23 g, 1.0 mmol) in 7 mL of pyridine and 3 mL of NEt_3 is cooled to 0 °C. The solution is saturated with H_2S gas, sealed, and brought to ambient temperature. (*CAUTION: This procedure should be carried out in a fume hood using a safety shield!*) After 48 h the vessel is cooled to 0 °C and vented. Excess H_2S is displaced by argon, and the solvents are remove under vacuum. The green residue is purified by column chromatography on silica with 25% EtOAc in pet. ether to give a pale yellow solid in 96% yield: 180−181 °C; ^{1}H NMR ($CDCl_3$) δ 3.21 (s, 3H), 3.59 (s, 6H), 6.85 (d, J = 9.0 Hz, 1H), 7.12 (br s, 1H), 7.51 (br s, 1H), 7.98−8.02 (m, 2H); IR (KBr) 3379, 3171, 1722, 1615, 1350, 1109, 1059 cm^{-1}. Anal. ($C_{12}H_{14}N_2O_3S$) C, H, N, S.

1-Methylisatin-5-thiocarboxamide (11): mp 229−230 °C; ^{1}H NMR (DMSO-d_6) δ 3.16 (s, 3H), 7.19 (d, J = 9.0 Hz, 1H), 8.11 (s, 1H), 8.31 (d, J = 9.0 Hz, 1H), 9.55 (br s, 1H), 9.89 (br s, 1H); IR (KBr) 3414, 3310, 3225, 1745, 1618, 1342, 1085 cm^{-1}. MS (EI) m/z 220 (M^+), 186, 158, 130, 103, 76, 50. Anal. ($C_{10}H_8N_2O_2S$) C, H, N, S.

1-Methyl-5-acetylisatin (12). To a solution of **3** (0.4 g, 1.39 mmol) in 20 mL of anhydrous 1,4-dioxane was added tributyl(1-ethoxyvinyl)tin (0.61 mL, 1.81 mmol), tetrakis(triphenylphosphine)palladium (80 mg, 0.07 mmol), and few crystals of 2,6-di-*tert*-butyl-4-methylphenol. The reaction mixture was heated at 90−95 °C for 21 h. The reaction mixture was evaporated to remove all solvent. The resultant residue was acidified with 1 N HCl and extracted with EtOAc. The combined organic layers were dried over anhydrous $MgSO_4$ and concentrated. The product was purified by flash chromatography on a silica column using hexane/EtOAc, 60:40, to give an orange solid. In order to get rid of residual tin byproducts, the orange solid was dissolved in CH_3CN and washed with hexanes and the layers were separated. The CH_3CN layer was dried and evaporated to dryness to give a pure orange solid (60 mg): mp 153−155 °C; ^{1}H NMR ($CDCl_3$) δ 2.59 (s, 3H), 3.13 (s, 3H), 7.00 (d, J = 9.0 Hz, 1H), 8.17 (s, 1H), 8.30 (d, J = 9.0 Hz, 1H); IR

(KBr) 1743, 1682, 1606, 1375, 1317, 1259, 1102 cm^{-1}; MS (EI) *m/z* 203 (M^+), 160, 132, 91 63. Anal. ($C_{11}H_9NO_3 \cdot 0.1H_2O$) C, H, N.

1-Methyl-5-(methylthio)isatin (36). This intermediate was prepared from 5-(methylthio)isatin[20] and iodomethane using the N-alkylation procedure describe above: mp 156−158 °C; ^1H NMR ($CDCl_3$) δ 2.53 (s, 3H), 3.29 (s, 3H), 6.84 (d, $J = 9.0$ Hz, 1H), 7.53 (m, 2H); IR (KBr) 3421, 1736, 1616, 1464, 1332, 1119, 490 cm^{-1}.

1-Methyl-5-(methylsulfinyl)isatin (13). To a stirred solution of the 1-methyl-5-(methylthio)isatin (**36**) (30 mg, 0.145 mmol), in 10 mL of CH_2Cl_2 at room temperature was added *m*-CPBA (39 mg, 0.145 mmol, 65%). The reaction mixture was stirred at room temperature for 1 h. Upon completion, the reaction mixture was diluted with CH_2Cl_2 and washed with saturated aqueous $NaHCO_3$. The organic phase was dried over anhydrous $MgSO_4$ and evaporated to dryness. Purification of the product by flash chromatography on a silica column using $CHCl_3$/MeOH, 99:1, gave 20 mg of an orange solid (63%): mp 193−195 °C; ^1H NMR ($CDCl_3$) δ 2.75 (s, 3H), 3.32 (s, 3H), 7.10 (d, $J = 9.0$ Hz, 1H), 7.81 (s, 1H), 8.02 (d, $J = 9.0$ Hz, 1H); IR (KBr) 3429, 1734, 1604, 1476, 1062, 475 cm^{-1}; MS (EI) *m/z* 223 (M^+), 208, 180, 152, 97, 63. Anal. ($C_{10}H_9NO_3S$) C, H, N, S.

1-Methyl-5-chloroisatin (2):[29] mp 171−173 °C; ^1H NMR (DMSO-d_6) δ 3.13 (s, 3H), 7.18 (d, $J = 9.0$ Hz, 1H), 7.60 (s, 1H), 7.73 (d, $J = 9.0$ Hz, 1H); IR (KBr) 1749, 1726, 1608, 1471, 1327 cm^{-1}. Anal. ($C_9H_6NO_2Cl$) C, H, N.

1-Methyl-5-nitroisatin (4):[30] mp 197−199 °C; ^1H NMR (DMSO-d_6) δ 3.21 (s, 3H), 7.36 (d, $J = 9.0$ Hz, 1H), 8.23 (s, 1H), 8.55 (d, $J = 9.0$ Hz, 1H); IR (KBr) 3057, 2941, 1757, 1743, 1610, 1516, 1467, 1338, 1292, 1111 cm^{-1}. Anal. ($C_9H_6N_2O_4$) C, H, N.

1-Methyl-5-cyanoisatin (7): mp 213−215 °C; ^1H NMR ($CDCl_3$) δ 3.32 (s, 3H), 7.04 (d, $J = 9.0$ Hz, 1H), 7.89 (d, $J = 9.0$ Hz, 1H), 7.92 (s, 1H); IR (KBr) 2238, 1757, 1743, 1734, 1620, 1589, 1489, 1331, 1109, 837 cm^{-1}. Anal. ($C_{10}H_6N_2O_2$) C, H, N.

1-Methylisatin-5-(*N*-methyl)-carboxamide (9): mp 268−270 °C; ^1H NMR (DMSO-d_6) δ 2.76 (d, $J = 6.0$ Hz, 3H), 3.13 (s, 3H), 7.21 (d, $J = 9.0$ Hz, 1H), 7.99 (s, 1H), 8.15 (d, $J = 9.0$ Hz, 1H), 8.52 (br s, 1H); IR (KBr) 3536, 3437, 3325, 1734, 1633, 1616, 1558, 1482, 1317, 1110 cm^{-1}; MS (EI) *m/z* 218 (M^+), 160, 132, 104, 77, 63. Anal. ($C_{11}H_{10}N_2O_3 \cdot 0.75H_2O$) C, H, N.

1-Benzylisatin-5-carboxamide (16): mp 186−187 °C; ^1H NMR ($CDCl_3$) δ 4.98 (s, 2H), 5.90 (br s, 2H), 6.90 (d, $J = 9.0$ Hz, 1H), 7.32 (m, 5H), 7.99 (s, 1H), 8.10 (d, $J = 9.0$ Hz, 1H); IR (KBr) 3429, 3214, 1734, 1662, 1618, 1325, 688 cm^{-1}; MS (EI) *m/z* 280 (M^+), 223, 189, 180, 105, 91, 65. Anal. ($C_{16}H_{12}N_2O_3 \cdot 0.55H_2O$) C, H, N.

1-(4-Phenylbenzyl)-isatin-5-carboxamide (17): mp 114−116 °C; ^1H NMR (DMSO-d_6) δ 4.85 (s, 2H), 6.58 (d, $J = 9.0$ Hz, 1H), 7.28−7.51 (m, 9H), 8.03 (m, 4H); IR (KBr) 3377, 1743, 1736, 1676, 1618 cm^{-1}; MS (EI) *m/z* 356 (M^+), 189, 165, 152, 141, 105. Anal. ($C_{22}H_{16}N_2O_3 \cdot 0.25H_2O$) C, H, N.

2-(Bromomethyl)benzo[*b*]thiophene.[31] (This alkylating agent was used in the first step of the five-step general synthesis of **19**.) To a solution of alcohol (0.210 g, 1.28 mmol) in 10 mL of CH_2Cl_2 at 0 °C was added CBr_4 (0.552 g, 1.67 mmol). The reaction mixture was stirred for 30 min at room temperature. The reaction mixture was diluted with CH_2Cl_2 and washed with water. The organic layers were dried over anhydrous $MgSO_4$, filtered, and concentrated. Purification of the crude by flash chromatography on silica using hexanes/EtOAc, 95:5, yielded an oil (0.107 g; 36%). The product being unstable at room temperature was used immediately: ^1H NMR ($CDCl_3$) δ 4.79 (s, 2H), 7.33−7.36 (m, 3H), 7.73 (t, $J = 4.5$ Hz, 1H), 7.81 (t, $J = 4.5$ Hz, 1H).

1-(2-Benzo[*b*]thiophenylmethyl)isatin-5-carboxamide (19): mp 261−263 °C; ^1H NMR (DMSO-d_6) δ 5.23 (s, 2H), 7.34 (m, 3H), 7.44 (br s, 1H), 7.57 (s, 1H), 7.77 (d, $J = 9.0$ Hz, 1H), 7.91 (d, $J = 9.0$ Hz, 1H), 8.04 (br s, 1H), 8.09 (s, 1H), 8.15 (d, $J = 9.0$ Hz, 1H); IR (KBr) 3470, 3371, 1736, 1668, 1624, 1591, 1371, 763 cm^{-1}: MS (EI) *m/z* 336 (M^+), 308, 279, 189, 147, 134, 103. Anal. ($C_{18}H_{12}N_2O_3S$) C, H, N, S.

1-(4-Methylbenzyl)isatin-5-carboxamide (20): mp 220−222 °C; ^1H NMR (DMSO-d_6) δ 2.26 (s, 3H), 4.89 (s, 2H), 7.01 (d, $J = 9.0$ Hz, 1H), 7.14 (d, $J = 9.0$ Hz, 2H), 7.32 (d, $J = 9.0$ Hz, 2H), 7.41 (s, 1H), 8.01(s, 1H), 8.03−8.08 (m, 2H); IR (KBr) 3387, 3181, 1736, 1662, 1616, 1400, 1350 cm^{-1}; MS (EI) *m/z* 294 (M^+), 237, 189, 105, 77. Anal. ($C_{17}H_{14}N_2O_3 \cdot 0.5H_2O$) C, H, N.

1-(3,4-Dimethylbenzyl)isatin-5-carboxamide (21): mp 230−231 °C; ^1H NMR (DMSO-d_6) δ 2.16 (s, 6H), 4.83 (s, 2H), 7.00 (d, $J = 9.0$ Hz, 1H), 7.13 (m, 3H), 7.40 (br s, 1H), 8.01 (br s, 1H), 8.07 (m, 2H); IR (KBr) 3468, 3377, 3170, 1741, 1668, 1616, 1406, 1338 cm^{-1}; MS (EI) *m/z* 308 (M^+), 189, 133, 119, 89, 59. Anal. ($C_{18}H_{16}N_2O_3 \cdot 0.75\ H_2O$) C, H, N.

1-(3-Methoxybenzyl)isatin-5-carboxamide (22): mp 230−232 °C; ^1H NMR (DMSO-d_6) δ 3.71 (s, 3H), 4.89 (s, 2H), 6.84 (d, $J = 9.0$ Hz, 1H), 6.97−7.02 (m, 3H), 7.01 (t, $J = 7.5$ Hz, 1H), 7.41 (br s, 1H), 8.02 (br s, 1H), 8.08 (m, 2H); IR (KBr) 3475, 3213, 1739, 1662, 1616, 1585, 1491, 1346 cm^{-1}; MS (EI) *m/z* 310 (M^+), 253, 210, 189, 121, 91, 78, 65. Anal. ($C_{17}H_{14}N_2O_4 \cdot 0.6\ H_2O$) C, H, N.

1-(3,5-Dimethoxybenzyl)isatin-5-carboxamide (23): mp 269−271 °C; ^1H NMR (DMSO-d_6) δ 3.81 (s, 6H), 4.83 (s, 2H), 6.39 (s, 1H), 6.60 (s, 2H), 6.99 (d, $J = 9.0$ Hz, 1H), 7.40 (br s, 1H), 8.01 (br s, 1H), 8.08 (m, 2H); IR (KBr) 3468, 3344, 1747, 1676, 1616, 1602, 1356, 1209, 1151, 1068 cm^{-1}; MS (EI) *m/z* 340 (M^+), 189, 151, 121, 105, 77. Anal. ($C_{18}H_{16}N_2O_5$) C, H, N.

1-[[2-(6-Methoxynaphthyl)]methyl]isatin-5-carboxamide (24). This compound was prepared by the five-step general synthesis outlined above. 2-(Chloromethyl)-6-methoxynaphthalene, used in the N-alkylation step, was prepared as described in the literature:[32] mp 235−237 °C; ^1H NMR (DMSO-d_6) δ 3.84 (s, 3H), 5.05 (s, 2H), 7.06 (d, $J = 9.0$ Hz, 1H), 7.15 (d, $J = 9.0$ Hz, 1H), 7.29 (s, 1H), 7.40 (br s, 1H), 7.50 (d, $J = 9.0$ Hz, 1H), 7.75 (d, $J = 9.0$ Hz, 1H), 7.80 (d, $J = 9.0$ Hz, 1H), 7.90 (br s, 1H), 8.05 (m, 3H); IR (KBr) 3412, 1730, 1676, 1635, 1616, 1408 cm^{-1}; MS (EI) *m/z* 360 (M^+), 189, 171, 128. Anal. ($C_{21}H_{16}N_2O_4 \cdot 0.5\ H_2O$) C, H, N.

1-(3-Hydroxybenzyl)isatin-5-carboxamide (25). (The following procedure was used to prepare compounds **25**−**27**.) To a solution of **22** (0.050 g, 0.162 mmol) in 16 mL of anhydrous CH_2Cl_2 was added BBr_3 (1 M in CH_2Cl_2; 0.4 mL, 0.4 mmol) at 0 °C. The reaction mixture was stirred at room temperature for 18 h whereupon the resulting white suspension was diluted with CH_2Cl_2 and washed with saturated aqueous $NaHCO_3$. The organic layers were dried and concentrated to give the product as an orange solid (46 mg; 96%): mp 136−138 °C; ^1H NMR (DMSO-d_6) δ 4.83 (s, 2H), 6.65 (d, $J = 9.0$ Hz, 1H), 6.77 (s, 1H), 6.83 (d, $J = 6.0$ Hz, 1H), 7.04 (d, $J = 9.0$ Hz, 1H), 7.11 (t, $J = 7.5$ Hz, 1H), 7.41 (br s, 1H), 8.02 (br s, 1H), 8.09 (m, 2H), 9.42 (s, 1H); IR (KBr) 3396, 3256, 1739, 1662, 1618, 1591, 1449 cm^{-1}; MS (EI) *m/z* 296 (M^+), 239, 189, 121, 107, 77, 61. Anal. ($C_{16}H_{12}N_2O_4 \cdot 0.7H_2O$) C, H; N: calcd, 9.07; found, 8.42.

1-(3,5-Dihydroxybenzyl)isatin-5-carboxamide (26): mp 205−207 °C; ^1H NMR (DMSO-d_6) δ 4.74 (s, 2H), 6.10 (s, 1H), 6.22 (s, 2H), 7.05 (d, $J = 9.0$ Hz, 1H), 7.42 (br s, 1H), 8.05 (br s, 1H), 8.08 (s, 1H), 8.11 (d, $J = 9.0$ Hz, 1H), 9.25 (s, 2H); IR (KBr) 3383, 3211, 1741, 1662, 1618, 1350, 1176 cm^{-1}. Anal. ($C_{16}H_{12}N_2O_5 \cdot 1.7\ H_2O$) C, H; N: calcd, 8.17; found, 7.59.

1-[[2-(6-Hydroxynaphthyl)]methyl]isatin-5-carboxamide (27): mp 190−192 °C; ^1H NMR (DMSO-d_6) δ 5.02 (s, 2H), 7.06 (m, 3H), 7.42 (m, 2H), 7.67 (t, $J = 9.0$ Hz, 2H), 7.84 (s, 1H), 8.04 (m, 3H), 9.73 (s, 1H); IR (KBr) 3387, 3208, 1739, 1664, 1616, 1400 cm^{-1}; MS (EI) *m/z* 346 (M^+), 289, 189, 157, 128, 61. Anal. ($C_{20}H_{14}N_2O_4 \cdot 1.5\ H_2O$) C, H, N.

Acknowledgment. We are very grateful to Drs. Wai-Ming Lee and Roland Rueckert of the Institute for Molecular Virology, University of Wisconsin, Madison, WI, for supplying us with the infectious cDNA clone for HRV serotype-14. We also thank Dr. P. Dragovich of the medicinal chemistry group at Agouron for suggesting, and providing details for, the palladium-catalyzed

coupling reaction of tributyl(1-ethoxyvinyl)tin with 1-methyl-5-iodoisatin.

References

(1) (a) Couch, R. B. Rhinoviruses. In *Virology*; Fields, B. N., Knipe, D. M., Eds.; Raven Press: New York, 1990; Vol. 1, Chapter 22, pp 607−629. (b) See: McKinlay, M. A.; Pevear, D. C.; Rossman, M. G. Treatment of the Picornavirus Common Cold by Inhibitors of Viral Uncoating and Attachment. *Annu. Rev. Microbiol.* **1992**, *46*, 635−654 and references cited therein. (c) Phillpotts, R. J.; Tyrell, D. A. J. Rhinovirus Colds. *Br. Med. Bull.* **1985**, *41*, 386−390. (d) Gwaltney, J. M. Rhinoviruses. In *Viral Infections of Humans*; Evans, A. S., Ed.; Plenum Publishing Corp.: New York, 1982; Chapter 20, pp 491−517. (e) Gwaltney, J. M. The Common Cold. In *Principles and Practices of Infectious Diseases*; Mandell, G. L., Douglas, R. G., Bennett, J. E., Eds.; John Wiley & Sons: New York, 1985; Chapter 38, pp 351−355.

(2) See: Kräusslich, H.-G.; Wimmer, E. Viral Proteinases. *Annu. Rev. Biochem.* **1988**, *57*, 701−754 and references cited therein.

(3) Hamparian, V. V.; Colonno, R. J.; Cooney, M. K.; Dick, E. C.; Gwaltney, J. M.; Hughes, J. H.; Jordan, W. S.; Kapikian, A. Z.; Mogabgab, W. J.; Monto, A.; Phillips, C. A.; Rueckarts, R. R.; Schieble, J. H.; Stott, E. J.; Tyrrell, D. A. J.; Benade, L. E.; Riker, D. K. A Collaborative Report: Rhinoviruses - Extension of the Numbering System from 89 to 100. *Virology* **1987**, *159*, 191−192.

(4) (a) Callahan, P. L.; Mizutani, S.; Colonno, R. J. Molecular cloning and complete sequence determination of the RNA genome of human rhinovirus type 14. *Proc. Natl. Acad. Sci. U.S.A.* **1985**, *82*, 732−736. (b) Stanway, G.; Hughes, P. J.; Mountford, R. C.; Minor, P. D.; Almond, J. W. The complete nucleotide sequence of the common cold virus: human rhinovirus 14. *Nucleic Acids Res.* **1984**, *12*, 7859−7875. (c) Lee, W.-M.; Wang, W.; Rueckart, R. R. Complete sequence of the RNA genome of human rhinovirus 16, a clinically useful common cold virus belonging to the ICAM-1 receptor group. *Virus Genes* **1995**, *9*, 177−181.

(5) (a) Orr, D. C.; Long, A. C.; Kay, J.; Dunn, B. M.; Cameron, J. M. Hydrolysis of a Series of Synthetic Peptide Substrates by the Human Rhinovirus 14 3C Protease, Cloned and Express in *Escherichia coli*. *J. Gen. Virol.* **1989**, *70*, 2931−2942. (b) Cordingly, M. G.; Register, R. B.; Callahan, P. L.; Garsky, V. M.; Colonno, R. J. Cleavage of Small Peptides In Vitro by Human Rhinovirus 14 3C Protease Expressed in *Escherichia coli*. *J. Virol.* **1989**, *63*, 5037−5045.

(6) (a) Matthews, D. A.; Smith, W. A.; Ferre, R. A.; Condon, B.; Budahazi, G.; Sisson, W.; Villafranca, J. E.; Janson, C. A.; McElroy, H. E.; Gribskov, C. L.; Worland, S. Structure of Human Rhinovirus 3C Protease Reveals a Trypsin-like Polypeptide Fold, RNA-Binding Site, and Means for Cleaving Precursor Polyprotein. *Cell* **1994**, *77*, 761−771. (b) Bazan, J. F.; Fletterick, R. J. Viral cysteine proteases are homologous to the trypsin-like family of serine proteases: Structural and functional implications. *Proc. Natl. Acad. Sci. U.S.A.* **1988**, *85*, 7872−7876. (c) Gorbalenya, A. E.; Blinov, V. M.; Donchenko, A. P. Poliovirus-encoded Proteinase 3C: A Possible Evolutionary Link Between Cellular Serine and Cysteine Proteinase Families. *FEBS Lett.* **1986**, *194*, 253−257. (d) Allaire, M.; Chernala, M. M.; Malcolm, B. A.; James, M. N. G. Picornaviral 3C cysteine proteinases have a fold similar to chymotrypsin-like serine proteinases. *Nature* **1994**, *369*, 72−76.

(7) See Oren, D. A.; Zhang, A.; Nesvadba, H.; Rosenwirth, B.; Arnold, E. Synthesis and Activity of Piperizine-containing Antirhinoviral Agents and Crystal Structure of SDZ 880−061 Bound to Human Rhinovirus 14. *J. Mol. Biol.* **1996**, *259*, 120−134 and references cited therein.

(8) (a) Skiles, J. W.; McNeil, D. Spiro Indolinone Beta-lactams, Inhibitors of Poliovirus and Rhinovirus 3C-Proteinases. *Tetrahedron Lett.* **1990**, *31*, 7277−7280. (b) Kadam, S.; Poddig, J.; Humphrey, P.; Karwowski, J.; Jackson, M.; Tennent, S.; Fung, L.; Hochlowski, J.; Rasmussen, R.; McAlpine, J. Citrinin Hydrate and Radicinin: Human Rhinovirus 3C-Protease Inhibitors Discovered in a Target-directed Microbial Screen. *J. Antibiot.* **1994**, *47*, 836−839. (c) Singh, S. B.; Cordingley, M. G.; Ball, R. G.; Smith, J. L.; Dombrowski, A. W.; Goetz, M. A. Structure and Stereochemistry of Thysanone: A Novel Human Rhinovirus 3C-Protease Inhibitor from Thysanophora penicilloides. *Tetrahedron Lett.* **1991**, *32*, 5279−5282. (d) Kaldor, S. W.; Hammond, M.; Dressman, B. A.; Labus, J. M.; Chadwell, F. W.; Kline, A. D.; Heinz, B. A. Glutamine-derived Aldehydes for the Inhibition of Human Rhinovirus 3C Protease. *Bioorg. Med. Chem. Lett.* **1995**, *5*, 2021−2026. (e) Malcolm, B. A.; Lowe, C.; Shechosky, S.; McKay, R. T.; Yang, C. C.; Shah, V. J.; Simon, R. J.; Vederas, J. C.; Santi, D. V. Peptide Aldehyde Inhibitors of Hepatitis A Virus 3C Proteinase. *Biochemistry* **1995**, *34*, 8172−8179. (f) Sham, H. L.; Rosenbrook, W.; Kati, W.; Betebenner, D. A.; Wideburg, N. E.; Saldivar, A.; Plattner, J. J.; Norbeck, D. W. Potent inhibitor of the human rhinovirus (HRV) 3C protease containing a backbone modified glutamine. *J. Chem. Soc., Perkin Trans. 1* **1995**, 1081−1082. (g) Brill, G. M.; Kati, W. M.; Montgomery, D.; Karwowski, J. P.; Humphrey, P. E.; Jackson, M.; Clement J. J.; Kadam, S.; Chen, R. H.; McAlpine, J. B. Novel Triterpene Sulfates from *Fusarium compactum* Using a Rhinovirus 3C Protease Inhibitor Screen. *J. Antibiot.* **1996**, *49*, 541−546.

(9) Models of peptides bound to HRV-14 3CP were created from a combination of constrained molecular dynamics and molecular mechanics minimizations using the CHARMM software: Brooks, B. R.; Bruccoleri, R. E.; Olafson, B. D.; States, D. J.; Swaminathan, S.; Kaplus, M. CHARMM: A Program for Macromolecular Energy, Minimization, and Dynamics Calculations. *J. Comput. Chem.* **1983**, *4*, 187−217.

(10) During the course of our research, isatin derivatives have been reported to be inhibitors of serine and cysteine proteases. (a) This class of compounds was discovered by a screening program; Spitzer, W. A. Lilly Research Laboratories, Indianapolis, IN 46285, personal communication. (b) Iyers, R. A.; Hanna, P. E. N-(Carbobenzyloxy)isatin: A Slow Binding α-Keto Lactam Inhibitor of α-Chymotrypsin. *Bioorg. Med. Chem. Lett.* **1995**, *5*, 89−92.

(11) (a) Harbeson, S. L.; Abelleira, S. M.; Akiyama, A.; Barrett, R.; Carroll, R. M.; Straub, J. A.; Tkacz, J. N.; Wu, C.; Musso, G. F. Stereospecific Synthesis of Peptidyl α-Keto Amides as Inhibitors of Calpain. *J. Med. Chem.* **1994**, *37*, 2918−2929. (b) Li, Z.; Patil, G. S.; Golubski, Z. E.; Hori, H.; Tehrani, K.; Foreman, J. E.; Eveleth, D. D.; Bartus, R. T.; Powers, J. C. Peptide α-Keto Ester, α-Keto Amide, and α-Keto Acid Inhibitors of Calpains and Other Cysteine Proteases. *J. Med. Chem.* **1993**, *36*, 3472−3480. (c) Fusetani, N.; Matsunaga, S.; Matsumoto, H.; Takebayashi, Y. Cyclotheonamides, Potent Thrombin Inhibitors, from a Marine Sponge *Theonella* sp. *J. Am. Chem. Soc.* **1990**, *112*, 7053−7054.

(12) Modeling exercises were performed with MacroModel: Mohamadi, F.; Richards, N. G. J.; Guida, W. C.; Liskamp, R.; Lipton, M.; Caufield, C.; Chang, G.; Hendrickson, T.; Still, W. C. MacroModel - An Integrated Software System for Modeling Organic and Bioorganic Molecules Using Molecular Mechanics. *J. Comput. Chem.* **1990**, *11*, 440−467.

(13) See the Experimental Section for methods and details of the *ab initio* quantum mechanical calculations.

(14) (a) Møller, C.; Plesset, M. S. Note on the Approximation Treatment for Many-Electron Systems. *Phys. Rev.* **1934**, *46*, 618−622. (b) Pople, J. A.; Seeger, R.; Krishnan, R. Variational Configuration Interaction Methods and Comparison with Perturbation Theory. *Int. J. Quant. Chem. Symp.* **1977**, *11*, 149−163. (c) Krishnan, R.; Frisch, M. J.; Pople, J. A. Contribution of triple substitutions to the electron correlation energy in fourth order perturbation theory. *J. Chem. Phys.* **1980**, *72*, 4244−4245.

(15) Excess **18** was incubated with HRV-2 3CP and crystallized using protocols to be described elsewhere. Diffraction data from a single crystal grown in space group $P2_12_12_1$ were collected at 4 °C on a Rigaku AFC-5 diffractometer equipped with a graphite monochromator and a dual chamber area detector system of the Xuong-Hamlin design. Cell parameters are $a = 34.43$, $b = 66.48$, $c = 77.86$; $\alpha = \beta = \gamma = 90°$. These data are 94% complete to 2.8 Å resolution (average redundancy is 4.5) with a scaling R factor on intensities of 0.113.

(16) Galaverna, G.; Corradini, R.; Dossena, A.; Marchelli, R. Diaminomethane dihydrochloride, a novel reagent for the synthesis of primary amides of amino acids and peptides from active esters. *Int. J. Pept. Protein Res.* **1993**, *42*, 53−57.

(17) Wenkert, E.; Hudlicky, T. Reactions of Isatin Dimethyl Ketal and its Ethyl Imino Ether with Methyllithium. *Synth. Commun.* **1977**, *7*, 541−547.

(18) Heck, R. F. In *Palladium Reagents in Organic Syntheses;* Katritky, A. R., Meth-Cohn, O., Rees C. W., Eds.; Academic Press: London, 1985; Chapter 6, pp 308−311.

(19) McKean, D. R.; Parrinello, G.; Renaldo, A. F.; Stille, J. K. Synthesis of Functionalized Styrenes via Palladium-Catalyzed Coupling of Aryl Bromides with Vinyl Tin Reagents. *J. Org. Chem.* **1987**, *52*, 422−424.

(20) Brand, K.; Völcker, E. Über Methylmerkaptoatophane. *Arch. Pharm.* **1934**, *272*, 257−268.

(21) (a) Fuhrman, S. A.; Worland, S.; Meador, J. W.; DeLisle, D.; Brown, E. L.; Okano, K. A Continuous Assay for Rhinovirus 3C Protease and its use in Drug Discovery. Presented at the IXth Meeting of the European Study Group on the Molecular Biology of Picornaviruses, May 18−24, 1996, abstr. D25. (b) Wang, G. T.; Matayoshi, E.; Huffaker, H. J.; Krafft, G. A. Design and Synthesis of New Fluorogenic HIV Protease Substrates Based on Resonance Energy Transfer. *Tetrahedron Lett.* **1990**, *31*, 6493−6496.

(22) Assays were performed using commercially available proteases essentially as described by the supplier.

(23) Weislow, O. S.; Kiser, R.; Fine, D. L.; Bader, J.; Shoemaker, R. H.; Boyd, M. R. New Soluble-Formazan Assay for HIV-1 Cytopathic Effects: Application to High-Flux Screening of Synthetic and Natural Products for Aids-Antiviral Activity. *J. Natl. Cancer Inst.* **1989**, *81*, 577−586.

(24) See: Zoltán, G.; Klaassen, C. D. Mechanisms of Toxicology. In *Toxicology*; *The Basic Science of Poisons*; Klaassen, C. D., Amdur, M. O., Doull, J., Eds.; McGraw-Hill: New York, 1996; Chapter 3, pp 35−74.
(25) Lee, W.-M.; Monroe, S. S.; Rueckart, R. R. Role of Maturation Cleavage in Infectivity of Picornaviruses: Activation of an Infectosome. *J. Virol.* **1993**, *67*, 2110−2122.
(26) Leatherbarrow, R. J. ENZFITTER, Biosoft, Ferguson, MO 63135.
(27) The details pertaining to this protonated thiol model will be presented elsewhere.
(28) Borsche, W.; Weussmann, H.; Fritzsche, A. Untersuchungen über Isatin und verwandte Verbindungen, VI: Über 5-Jod-isatin und 5.5′-Dijod-indigo. *Chem. Ber.* **1924**, *57*, 1770−1775.
(29) Mangini, A.; Passerini, R. Ricerche sugli eterociclici: spettri di assorbimento u.v. e proprieta cromoforiche. - Nota II. Isatine. *Gazz. Chim. Ital.* **1955**, *85*, 840−880.
(30) Borsche, W.; Weussmann, H.; Fritzsche, A. Untersuchungen über Isatin und verwandte Verbindungen, V: Über Nitro-isatine. *Chem. Ber.* **1924**, *57*, 1149−1152.
(31) Yamamoto, M.; Kikinuma, M.; Kohmoto, S.; Yamada, K. Sommelet-Hauser Rearrangement of Oxygen and Sulfur Containing Heteroaromatic Sulfonium Ylides. *Bull. Chem. Soc. Jpn.* **1989**, *62*, 958−960 and references cited therein.
(32) Haydock, D. B.; Mulholland, T. P. C.; Telford, B.; Thorp, J. M.; Wain, J. S. Analogues of clofibrate and clobuzarit containing fluorine in the side chains. *Eur. J. Med. Chem.* **1984**, *19*, 205−214.

JM960603E