Articles

Substituted Benzamide Inhibitors of Human Rhinovirus 3C Protease: Structure-Based Design, Synthesis, and Biological Evaluation

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A series of nonpeptide benzamide-containing inhibitors of human rhinovirus (HRV) 3C protease was identified using structure-based design. The design, synthesis, and biological evaluation of these inhibitors are reported. A Michael acceptor was combined with a benzamide core mimicking the P1 recognition element of the natural 3CP substrate. α,β-Unsaturated cinnamate esters irreversibly inhibited the 3CP and displayed antiviral activity (EC_{50} 0.60 μM, HRV-16 infected H1-HeLa cells). On the basis of cocrystal structure information, a library of substituted benzamide derivatives was prepared using parallel synthesis on solid support. A 1.9 Å cocrystal structure of a benzamide inhibitor in complex with the 3CP revealed a binding mode similar to that initially modeled wherein covalent attachment of the nucleophilic cysteine residue is observed. Unsaturated ketones displayed potent reversible inhibition but were inactive in the cellular antiviral assay and were found to react with nucleophilic thiols such as DTT.

Introduction

The human rhinoviruses (HRV), which are the primary cause of the common cold in man, belong to the Picornovirus family.[1] Picornoviruses, such as HRV, have a single positive-stranded RNA genome,[2] which is translated into a polyprotein of over 2000 amino acids. The 2A and 3C protease (3CP) process this polyprotein into its functional viral proteins in HRV.[3] For the 3CP, the consensus cleavage site in the viral polyprotein substrate is between glutamine (P1) and glycine (P1′) residues. While the 3CP is a cysteine protease, its tertiary structure is reminiscent of trypsin-like serine proteases.[4] The requirement for proteolytic processing of the viral polyprotein, supported by mutagenesis of the active site residues,[5] makes the 3CP a viable target for antirhinoviral therapy. Solution of the HRV 3CP crystal structure has facilitated the design of a number of 3CP inhibitors, which have been previously reported from our laboratories[6a−d] and others.[6e−n] The aim of this research has been to identify a nonpeptide, low molecular weight inhibitor of 3CP with potent antirhinoviral activity. In this report we describe the structure-based design, synthesis, and biological evaluation of a series of benzamide-containing inhibitors of 3CP.

Design of Inhibitors

With the aid of the cocrystal structure of a peptide aldehyde[6b] bound to the 3CP, the design of a novel nonpeptide inhibitor was undertaken. Analysis of the 2.3 Å crystal structure of the HRV-14 3CP enzyme verified that it is structurally related to the trypsin family of proteases, with a catalytic triad composed of the residues cysteine, histidine, and glutamic acid.[4a] Our goal was to ultimately develop orally available 3CP inhibitors; therefore, the design strategy focused on nonpeptide motifs which would be expected to have more favorable pharmacokinetic properties. Initial design was deliberately very simple: positioning an aldehyde group so that it could react with the nucleophilic cysteine and placing a carboxamide group in the S1 recognition pocket. A meta-substituted phenyl ring positioned these two elements with the appropriate distance and relative orientation leading to 3-carbamoyl benzaldehyde as a prototype inhibitor **1a** (Figure 1). This compound was tested and found to be a very weak inhibitor of the 3CP (K_i = 104 μM). In addition to the small size of this inhibitor, we reasoned that its lack of activity could in part be due to the benzaldehyde carbonyl being considerably less reactive than its α-amino aldehyde counterpart found in potent peptide-based aldehyde inhibitors.[6b] Furthermore, the inherent instability of aldehydes made this group undesirable for our purposes. It had been established in our laboratory[6c] and others[6n] that cysteine proteases in general and 3CP in particular are potently inhibited by Michael acceptors when incorporated into a peptidic recognition element. Replacement of the formyl group of inhibitor **1a** with the α,β-unsaturated ethyl ester led to compound **1**, which was found to be an irreversible inhibitor with a weak inactivation constant[7] of 52 s^{-1} M^{-1} (Table 1). Interestingly, compound **1** also showed weak but demonstrable antiviral activity (EC_{50}) when tested in a cytopathic effect assay[6] employing H1-HeLa cells infected with HRV-14 (Table 1). Furthermore, compound **1** was nontoxic (CC_{50}) up to 320 μM.

With this information in hand, additional esters were prepared and tested. It was anticipated that by filling

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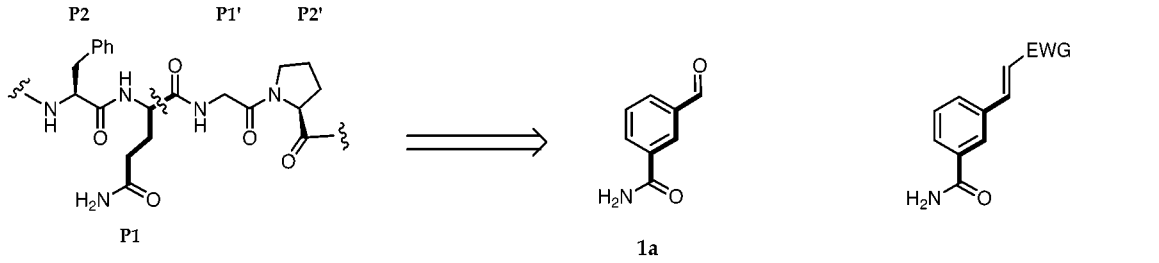
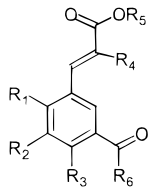


Figure 1. Design of benzamide core based on the 3CP cleavage site.

Table 1. Substituted Benzamides

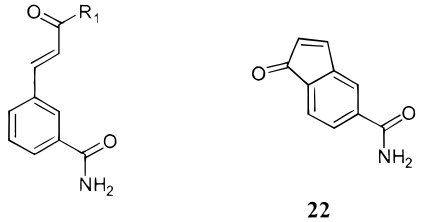


Cmpd No.[a]	R1	R2	R3	R4	R5	R6	K_{obs}/[I] (M^{-1} s^{-1}) [K_i (μM)][b]	EC_{50} (μM)[b]	CC_{50} (μM)[b]
1	H	H	H	H	Et	NH_2	52	15.9	>320
2	H	H	H	H	Me	NH_2	28	150	>320
3	H	H	H	H	CH_2Ph	NH_2	25	5.6	>100
4	H	H	H	H	CH_2CH_2OH	NH_2	42	20	>320
5	H	H	H	H	CH_2CH_2Ph	NH_2	83	100	>320
6	H	H	H	H	CH_2(2-pyridyl)	NH_2	57	10	>320
7	H	H	H	H	Et	OH	NI	ND	ND
8	H	H	H	H	Et	OMe	NI	ND	ND
9	H	H	H	CN	Et	NH_2	[1.5]	>100	>100
10	Me	H	H	H	Et	NH_2	10%@25	>100	>100
11	H	OH	H	H	Et	NH_2	47	>100	>100
12	H	OCH_2Ph	H	H	Et	NH_2	NI	ND	ND
13	H	CH_2OH	H	H	Et	NH_2	54	>100	>100
14	H	H	OH	H	Et	NH_2	NI	ND	ND
15	H	H	OCH_2Ph	H	Et	NH_2	30	>320	>320

[a] Elemental analyses (C, H, N) of all compounds agreed to within ±0.4% of theoretical values. [b] Serotype 14; see Experimental Section for assay Method. NI = no inhibition; ND = not determined.

the active site in the prime direction (S1′-S2′) additional affinity might be obtained. The methyl ester **2**, while showing similarly weak inhibition of the enzyme relative to ethyl ester **1**, was surprisingly 10-fold less active in the antiviral assay. As shown in Table 1, the ester group, in general, was found to have an unremarkable effect on the potency, with the benzyl ester **3** showing the best activity. To determine whether the activity of the parent compound **1** was due to the inherent reactivity of the unsaturated ester, alternative substitutions were examined in the 1-position corresponding to the S1 recognition pocket. A clear preference was observed for the primary carboxamide, consistent with recognition of glutamine in the native substrate peptide.[6a] Increased activation of the Michael acceptor toward nucleophilic addition, by α-cyano substitution, led to a modest but *reversible* inhibitor **9**. Various unsaturated imides, also expected to be more reactive than an unsaturated ester, were poor inhibitors (data not shown). Exploration of α,*β*-unsaturated ketones led to some interesting findings (Table 2). A simple methyl ketone showed only modest but, again, reversible inhibition. However, phenyl ketone **17** was considerably more potent against 3CP. When tested in the antiviral assay, ketones in general had greater toxicity (lower CC_{50}) and no measurable antiviral effect. Upon incubation with DTT, the α,*β*-unsaturated ketones were completely inactivated, suggesting that their lack of antiviral activity may be due to interaction with endogenous thiols in cells (glutathione).[6c] Electron-donating substituents at the 4-position of the phenyl ketone (4-Me_2N-, 4-MeO-), were expected to make the ketones less

Table 2. α,*β*-Unsaturated Keto Benzamides



22

Cmpd No.[a]	R1	K_i (μM)[b]	DTT inhib.[c]	EC_{50} (μM)[b]	CC_{50} (μM)[b]
16	Me	25	yes	32	40
17	Ph	0.40	yes	28	>28
18	Ph(4-NMe_2)	9	yes	>15	>15
19	Ph(4-OMe)	1.8	yes	>22	>22
20	2-pyridyl	1.8	yes	50	>50
21	2-furyl	1.9	yes	>71	>71
22	-	0.12	yes	>20	>20

[a] Elemental analyses (C, H, N) of all compounds agreed to within ±0.4% of theoretical values. [b] Serotype 14; see Experimental Section for assay method. [c] Indicates loss of 3CP inhibitory activity after exposure of compound to 5 mM DTT for 2−3 min at 23 °C.

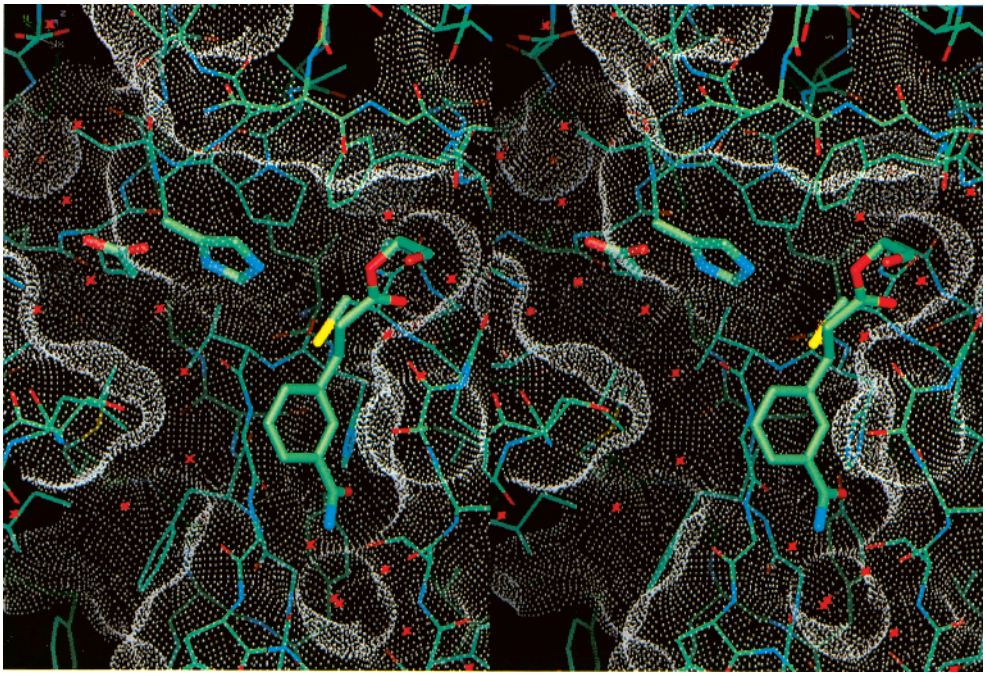


Figure 2. Stereoview of the 1.9 Å cocrystal structure of benzamide inhibitor **4** in complex with HRV-14 3CP. The catalytic triad residues (S2 subsite, Glu-71, His-40, and Cys-146) and inhibitor **4** are shown with thicker bonds. The carboxamide group occupies the S1 specificity pocket. Protein and inhibitor atoms are shown with atoms in green (carbon), blue (nitrogen), red (oxygen), and yellow (sulfur). Ordered water molecules are represented as red crosses. The protein's solvent accessible surface is shown in white.

reactive; however, no effect on their inactivation with DTT was observed. Similarly, heterocyclic ketones (2-pyridyl, 2-furyl) maintained their reactivity with DTT. In an attempt to make the inhibitors more compact, an indenone scaffold was designed using crystal structure information. Bicyclic indenone **22** was the most potent of the ketones; however, it too was inactivated by DTT and had no antiviral activity. It became clear that while small nonpeptidic α,β-unsaturated ketones such as **22** afforded potent reversible inhibition of 3CP, this increased reactivity was incompatible with the more complex cellular milieu present in the cytopathic effect assay.

One of the more soluble unsaturated ester inhibitors, hydroxyethyl ester **4**, was successfully cocrystallized with 3CP, and a 1.9 Å crystal structure was solved. The bound conformation was similar to that of the original model in terms of the phenyl core and 3-carboxamide (Figure 2). Interestingly, the orientation of the Michael ester adduct observed in the crystal structure was opposite of that predicted based on modeling studies. That is, the carboethoxy group was rotated *away* from the 3-carboxamide group in the crystal structure, whereas the modeled compound has the carboethoxy group oriented toward the 3-carboxamide function. This is consistent with the good hydrogen bond (2.98 Å) observed between the ester carbonyl and Cys147 NH which may activate the ester toward 1,4-addition in the transition state and also appears to be a good interaction in the final complex. In addition, this orientation was subsequently observed in the peptide-based Michael acceptors as well.[6c] Other key interactions between the protein and inhibitor were also observed. The 3-carboxamide is found to make three hydrogen bonds to His161 and Thr 142. The hydrogen bond from the amide NH to the Thr142 carbonyl is slightly longer (3.12 Å) and in a less optimal orientation than that observed with peptide-based Michael acceptors.[6c] While the carboxamide is 23° out of the aromatic plane, apparently to secure these hydrogen bonds, further rotation out of plane would be required to more closely mimic the hydrogen bonds observed in the P1 glutamine of peptidic inhibitors, with an associated energetic penalty. This molecular recognition explains the specificity for the primary carboxamide over the other groups tested in this position and is consistent with the binding interactions observed with other inhibitors.[6a−c] Prior to solution of the cocrystal structure of the benzamide core, we began to explore additional substituents on the phenyl ring to increase affinity. We reasoned that substitution from the 6-position might allow access to the S2 subsite, which was unoccupied, and based on other classes of 3CP inhibitors should improve binding significantly. 4-Methyl compound **10**, however, proved to be less active than the parent unsubstituted compound **1**. In retrospect, the ortho-methyl substitution in **10** would be expected to prevent the favorable orientation of the Michael acceptor away from the carboxamide as observed in the cocrystal structure of **1**. Substitution of the Michael acceptor in either the α- or β-position by a methyl group resulted in complete loss of activity (data not shown). Finally, substitution at the 6-position also resulted in loss of activity.

Analysis of the cocrystal structure of **1** indicated that substitution at the 5-position looked particularly promising. The aryl-H vector in the 5-position was directed along the β-sheet toward the S3-S4 pockets, and furthermore, with the appropriate substitution, it appeared that the S2 pocket might also be accessible (see Figure 2). The phenol **11** had comparable activity to that of **1**, yet the corresponding phenol ethers tested showed a universal lack of activity (e.g., **12**). Hydroxymethylene

compound **13**, however, was found to retain all of the potency of the unsubstituted parent **1**.

Parallel Synthesis at the 5-Position

With the tolerance for a hydroxymethyl group at the 5-position observed in **13** along with cocrystal structure information, a parallel synthesis approach was undertaken to explore substitution at this position. Using the primary carboxamide group as a handle for attachment to solid support, it was felt that derivatives could be readily accessed through nucleophilic substitution of the corresponding bromomethyl compound (see Chemistry section). A search of the Available Chemicals Directory (ACD) for primary amines and mercaptans suitable for synthesis yielded 3087 possible final compounds.[8] A structure-based computational approach was used to rank and select a subset of molecules from the total number that could be synthesized. From the precursor fragments, a virtual library of 5-substituted benzamides was created. This library of 3D structures was then run through a partially fixed docking procedure, where the benzamide "core" of the molecule was kept fixed to its position as observed in the cocrystal structure, and the remaining atoms were adjusted to find their optimal position in the active site. Once these molecules were docked, they were analyzed and ranked by low energy interactions with the protein, number of additional protein−ligand hydrogen bonds made, and the degree to which they filled the S2, S3, and/or the S4 pocket. The best candidate compounds from this screening and ranking procedure were then selected for synthesis. Of the 784 compounds prepared and tested, about 30 having greater than 80% inhibition of 3CP at 20 μM[9] were selected for resynthesis and full characterization. As shown in Table 3, a clear preference for branched aminomethylene groups was observed. The rates of inactivation (K_{obs}/I), which are modest, do not correlate with the antiviral potency. In fact, for the most potent compound, **30**, the antiviral EC_{50} of 600 nM is exceptional given the modest K_{obs}/I. This lack of a correlation between the rate of inactivation and the antiviral activity prompted us to investigate whether the antiviral effect of these compounds was in fact due to inhibition of the 3CP. Examination of proteolytic processing by 3CP in the cytopathic effect assay using polyacrylamide gel electrophoresis with compound **1** clearly showed a dose dependent reduction in proteolytic fragments consistent with inhibition of 3CP.

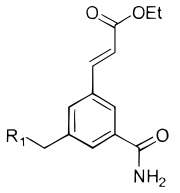
To determine the nature of the binding interactions with the more highly substituted derivatives, a cocrystal structure was solved of compound **26** bound to 3CP (data not shown). In this instance, a significant change in protein conformation was observed which is very likely a result of crystal packing forces.[10] However, the benzamide core of **26** binds essentially in the same space and orientation as the unsubstituted compound **4**. The piperizine ring lays directly over the backbone of β-sheet with the pyridine ring buried deeply into a larger rearranged P4 subsite (see Figure 2). While the pyridine ring is largely buried in the protein, the energetic cost of adopting this new protein conformation is unclear.

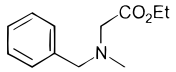
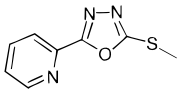
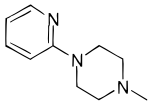
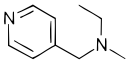
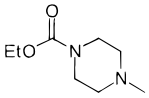
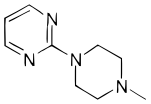
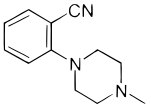
Synthesis

The original synthesis of parent compound **1** is outlined in Scheme 1 involving functional group modification of 3-formyl benzoic acid followed by Wittig olefination (method A). A more versatile route was later devised employing a palladium catalyzed Heck reaction[11] between an acrylate ester and iodobenzamide **32** (method B). Preparation of the various cinnamyl ester derivatives **3**−**6** utilizes cinnamic acid derivative **33** as a key intermediate. Scheme 2 details the straightforward preparation of compounds **7**−**10** using similar chemistry. The synthesis of 5-substituted compounds **11** and **12** began with selective reduction of 3,5-dinitroanisole and conversion to iodobenzamide **40** followed by standard Heck coupling as before (Scheme 3). Phenol **14** and phenolic ether **15** were prepared from commercially available 3-cyanoanisaldehyde (Scheme 4).

The synthesis of α,β-unsaturated ketones **16**−**21** began with the preparation of Weinreb amide **43**, again via a Heck coupling (Scheme 5). Reaction of the required organolithium species with amide **43** afforded ketones in good yield. The organolithium reagents were commercial or were obtained through metalation of either the corresponding bromide or, in the case of furan,[12] via direct metalation of the heterocycle. Finally, indenone **22** was obtained by palladium catalyzed cyanation of

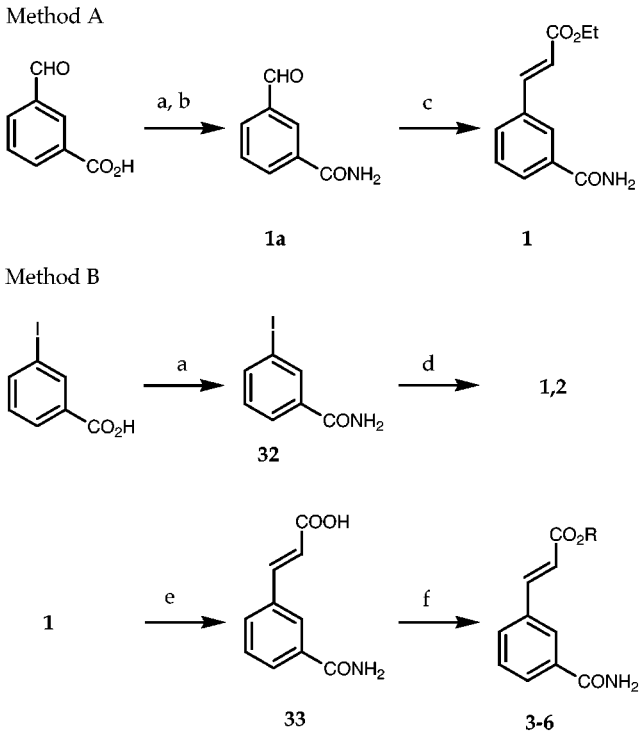
Table 3. 5-Substituted Benzamides



Cmpd No.[a]	R1	K_{obs}/[I] ($M^{-1}s^{-1}$)[b]	EC_{50} (μM)[b]	CC_{50} (μM)[b]
13	OH	54	>100	>100
23		568	10.0	>100
25		286	5.6	>100
26		269	2.5	>100
27		96	5.8	>100
28		130	1.8	>100
29		163	1.0	>100
30		139	0.6	79

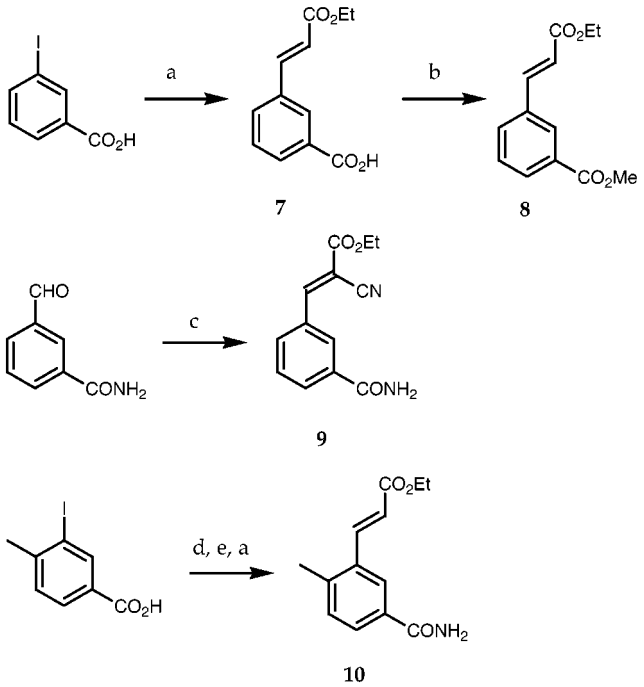
[a] HRMS, NMR, and HPLC purity were all consistent with the indicated structures; see Experimental Section. [b] Serotype 14; see Experimental Section for assay method.

Scheme 1[a]



[a] (a) $(COCl)_2$, cat. DMF; (b) NH_4OH, THF; (c) $Ph_3PCH_2CO_2Et$, $PhCH_3$, reflux; (d) $CH{=}CHCO_2Me$, $Pd(OAc)_2$, Et_3N, 100 °C; (e) NaOH/MeOH; (f) EDC, DMF, ROH, Et_3N, DMAP.

Scheme 2[a]



[a] (a) $CH{=}CHCO_2Et$, $Pd(OAc)_2$, Et_3N, 100 °C; (b) CH_2N_2; (c) $NCCH_2CO_2Et$, EtOH, piperidine; (d) $(COCl)_2$, cat. DMF; (e) NH_4OH, THF.

5-bromoindenone, followed by hydrolysis to the primary amide. Attempted manipulation of the unprotected indenone carboxamide **45** led only to decomposition products. Protection of **45** as the trityl amide,[13] however, permitted α-bromination with NBS followed by elimination and TFA deprotection to afford the desired indenone **22**.

Parallel Synthesis

The construction of a library of 5-substituted benzamides began with the preparation of the benzylic bromide intermediate **57** as outlined in Scheme 6. Protection of commercially available diethyl-5-(hydroxymethyl)isophthalate **50** and selective hydrolysis of one ester gave benzoic acid derivative **52**. Selective reduction of the ester with Li-triethylborohydride afforded hydroxymethyl acid **53**. Oxidation to the aldehyde with TPAP followed by Horner−Emmons condensation and silyl deprotection gave penultimate compound **56** in good yield. Last, conversion of the hydroxymethyl group to the benzyl bromide with PBr_3 produced key intermediate **57** ready for attachment to solid support. Monomer **57** was coupled to Rink amide resin (either free resin or Chiron Crowns) using a standard DIC/HOBt coupling procedure (Scheme 7). Nucleophilic displacement of bromide **58** in DMF occurred with no indication of Michael addition products. TFA deprotection of coupled products **59** afforded final products of high purity.

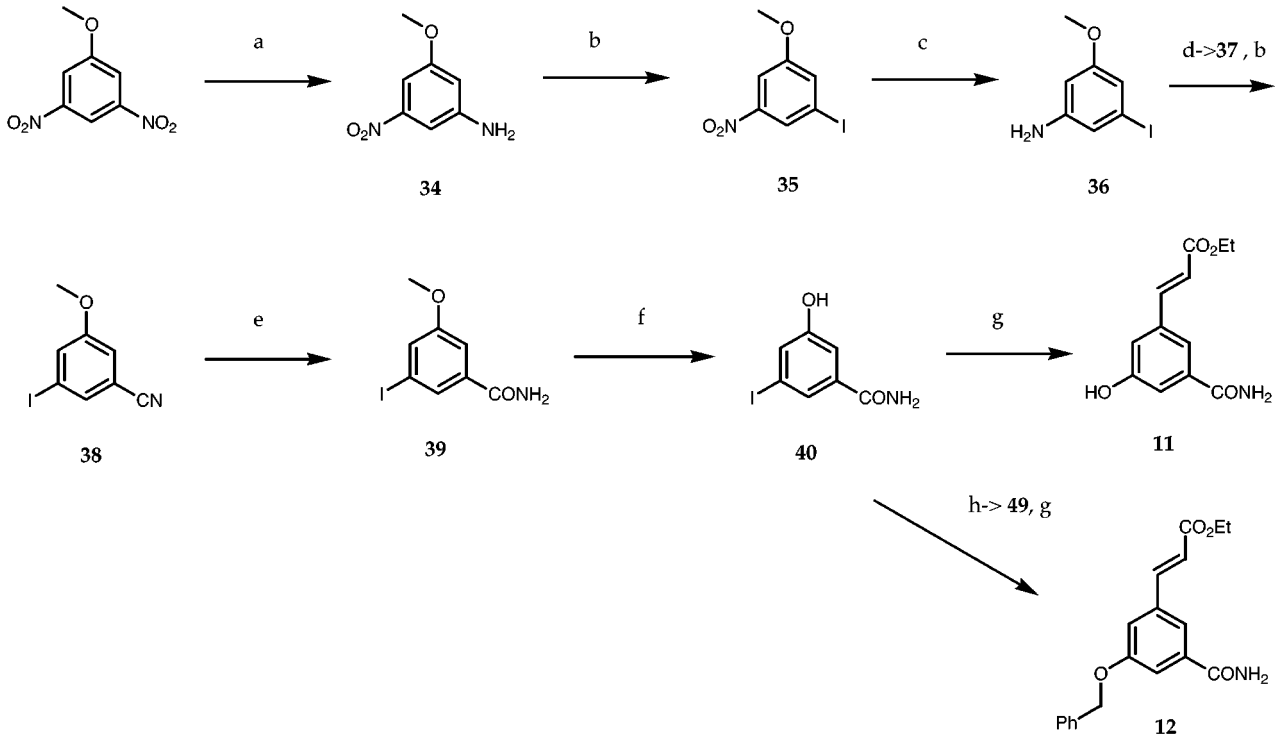
Conclusions

The 3CP is a promising and challenging target for the design of nonpeptide inhibitors. We have identified a series of nonpeptidic benzamide-containing inhibitors using the 3CP crystal structure as a guide. Solution of the cocrystal structure of this new series of inhibitors in complex with the 3CP confirms that they bind essentially as modeled. The α,β-unsaturated ester group suffers irreversible covalent 1,4-addition by the nucleophilic catalytic cysteine on the protein, which is confirmed in the cocrystal structure. Structural feedback facilitated the optimization of compounds attempting to access S2 and S3 subsites of the enzyme. Unfortunately, access to the S2 subsite was not achieved with this class of inhibitors. A related series of α,β-unsaturated ketones display potent reversible inhibition. However, they suffer from inactivation by free thiols and, presumably as a result, exhibit no antiviral activity. It has been shown in previous studies that recognition in the S2 pocket can lead to significant enhancements in binding. A parallel synthesis effort on solid phase allowed for the preparation of a large number of benzamide derivatives substituted in the 5-position to access the S3-S4 subsites of the enzyme. These derivatives were confirmed to occupy the S3-S4 pockets through crystallographic analysis, yet, only modest improvement in enzyme inactivation was realized. Despite very modest inactivation constants (K_{obs}/I), submicromolar antiviral activity was observed with compound **30**. It appears clear from our work and that of others that recognition at S1−S3 subsites and selective covalent binding is necessary to achieve potent 3CP inactivation and antiviral activity.

Experimental Section

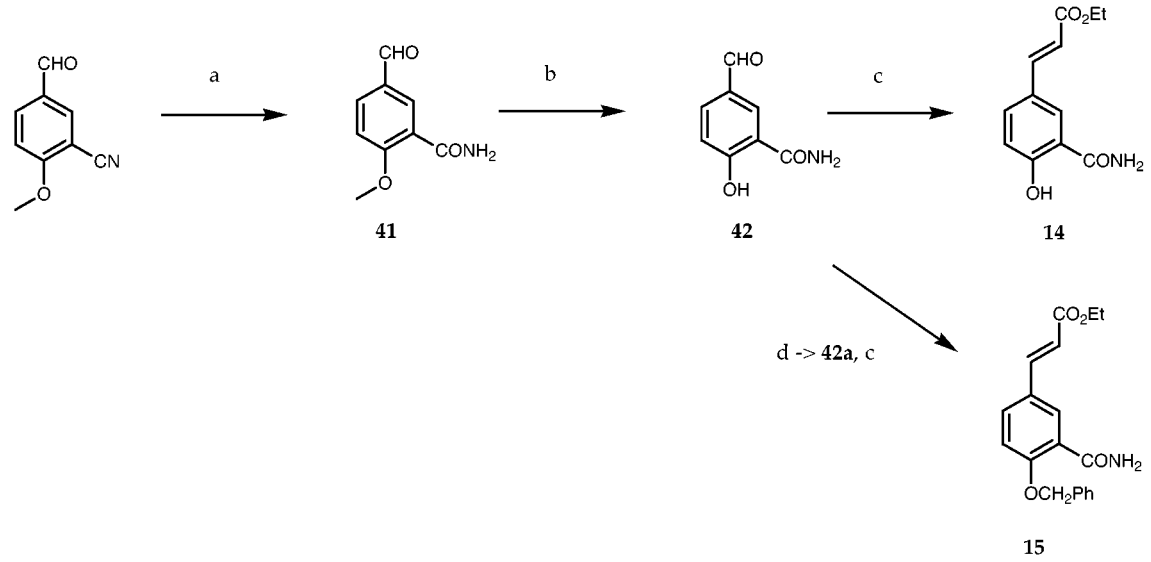
Melting points were determined on a Mel-Temp apparatus and are uncorrected. The structure of all compounds were confirmed by proton magnetic resonance spectroscopy, infrared spectroscopy, and either elemental microanalysis or mass spectrometry. Proton magnetic resonance spectra were determined using a General Electric QE-300 spectrometer operating at a field strength of 300 MHz. Chemical shifts are reported in parts per million (ppm) and by setting the references such

Scheme 3[a]



[a] (a) NaSH, MeOH; (b) $NaNO_2$, HCl, KI; (c) $Fe_3(CO)_{12}$, EtOH, reflux; (d) $Zn(CN)_2$, $Pd(PPh_3)_4$, DMF; (e) H_2O_2, KOH, H_2O; (f) BBr_3, CH_2Cl_2; (g) $CH{=}CHCO_2Et$, $Pd(OAc)_2$, Et_3N, 100 °C; (h) BzBr, K_2CO_3, DMF.

Scheme 4[a]

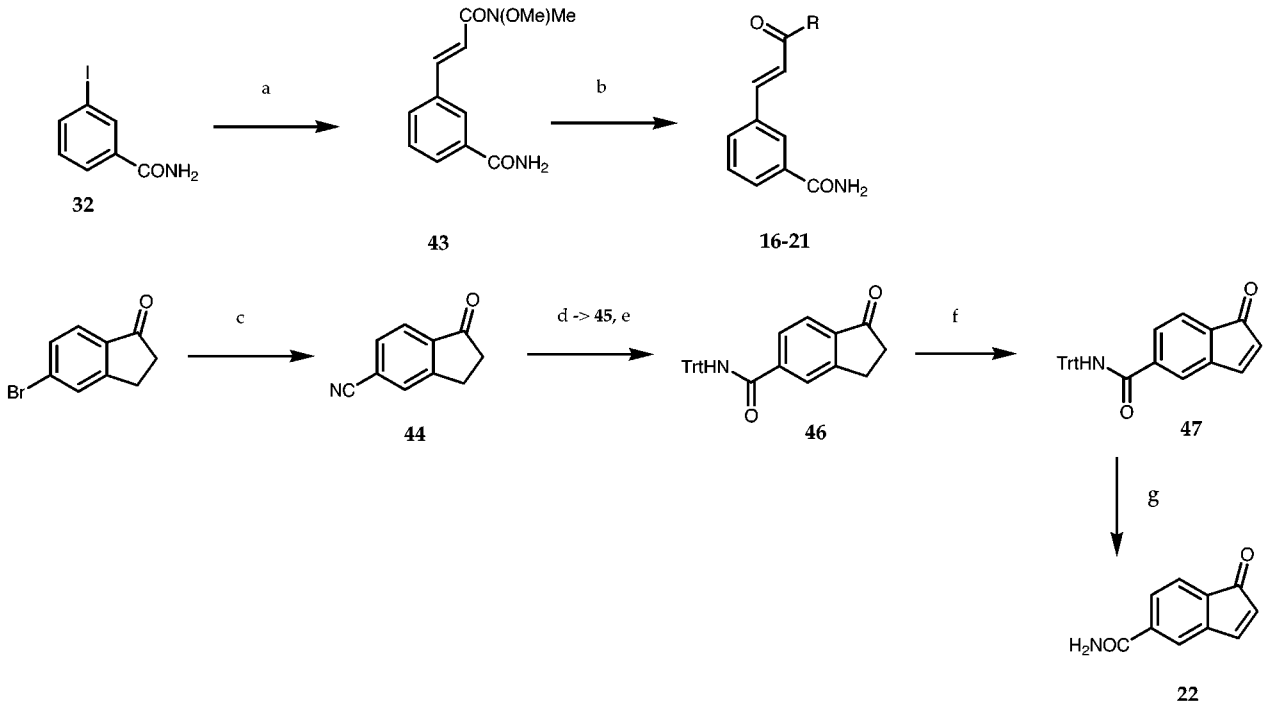


[a] (a) H_2SO_4, 100 °C; (b) BBr_3, CH_2Cl_2; (c) $Ph_3P{=}CHCO_2Et$, DMF; (d) BzBr, K_2CO_3, DMF.

that, in $CDCl_3$, the $CHCl_3$ peak is at 7.26 ppm and, in DMSO-d_6, the DMSO peak is at 2.49 ppm, and, in acetone-d_6, the acetone peak is at 2.04 ppm. Standard and peak multiplicities are designated as follows: s, singlet; d, doublet; dd, doublet of doublets; t, triplet; brs, broad singlet; brd, broad doublet; br, broad signal; and m, multiplet. Mass spectra were determined at Scripps Research Institute, San Diego, CA, Mass Spectrometric Facilities. Infrared absorption spectra were taken on a Perkin-Elmer 457 spectrometer or a MIDAK high resolution FT IR, and values are reported in cm^{-1}. Elemental microanalyses were performed by Atlantic Microlabs Inc., Norcross, GA, and gave results for the elements stated within ±0.4% of the theoretical values. *N*,*N*-Dimethylformamide and *N*,*N*-dimethylacetamide were used as is from Aldrich. Tetrahydrofuran (THF) was distilled from sodium benzophenone ketyl or CaH_2 under nitrogen. Flash chromatography was performed using silica gel 60 (Merck Art 9385), unless stated otherwise. Thin layer chromatographs (TLC) were performed on precoated sheets of silica 60 F254 (Merck Art 5719). Abbreviations: NMO, 4-methylmorpholine *N*-oxide; TPAP, tetrapropylammonium perruthenate; TBAF, tetrabutylammonium fluoride; HATU, *O*-(7-azabenzotriazol-1-yl)-*N*,*N*,*N*′,*N*′-tetramethyluronium; FMOC, 9-fluorenylmethoxycarbonyl; DIEA, diisopropylethylamine; HOBT, 1-hydroxybenzotriazole hydrate; DIC, 1,3-diisopropylcarbodiimide.

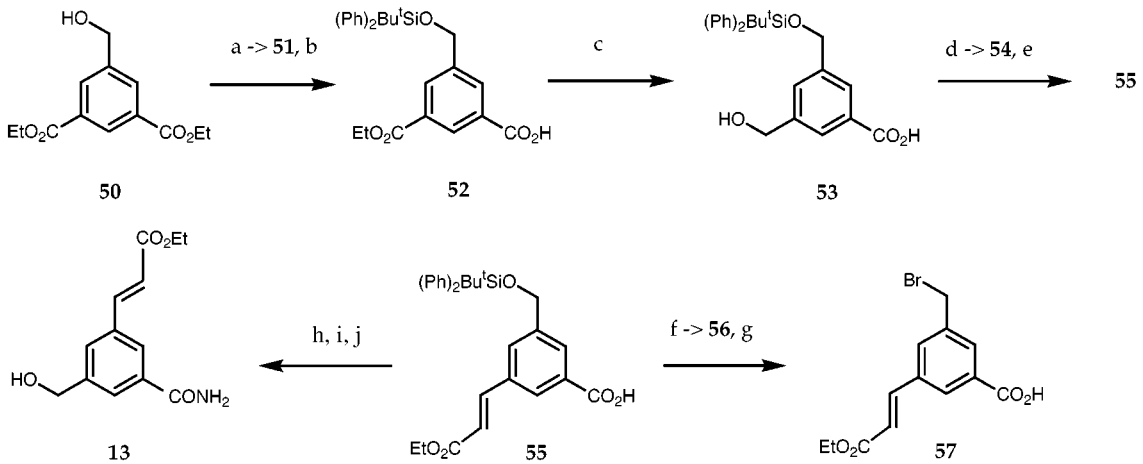
3CP Inhibition Assays and Antiviral Assays. 1. Enzyme Inhibition Assays. The general conditions of the fluorescence resonance energy transfer assay utilized to assess 3CP activity and procedures for reversible inhibitor K_i determinations are described in ref 6b. Continuous fluorometric

Scheme 5[a]



[a] (a) CH═CHCON(OMe)Me, $Pd(OAc)_2$, Et_3N, 100 °C; (b) RLi, −78 °C; (c) $Zn(CN)_2$, $Pd(PPh_3)_4$, DMF; (d) H_2O_2, KOH, H_2O; (e) Ph_3COH, Ac_2O, AcOH, cat. H_2SO_4; (f) NBS, *hν*; then Et_3N; (g) TFA, CH_2Cl_2.

Scheme 6. Preparation of Monomer **57** for Parallel Synthesis[a]

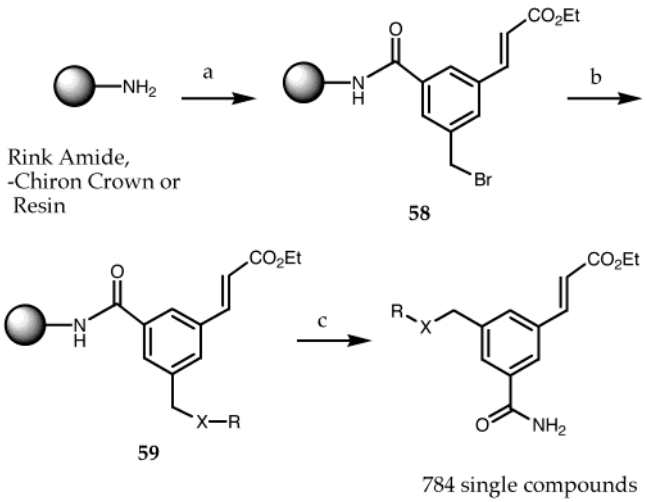


[a] (a) TBDPS-Cl, imidazole, DMF; (b) MeOH, 1 equiv NaOH; (c) $LiHB(Et_3)$; (d) TPAP; (e) $EtO_2CCH_2P(O)(OEt)_2$, NaH; (f) TBAF; (g) PBr_3; (h) RINK-NH_2, HATU, DMF; (i) TBAF, THF; (j) TFA-H_2O.

enzyme assays were conducted using a Perkin-Elmer LS50B spectrofluorimeter with a four place motorized cuvette holder. The K_{obs}/[I] values for the irreversible inhibitors were obtained from reactions initiated by addition of 50 nM 3CP, containing 1 μM substrate, and varying inhibitor concentrations. Typically, three to five concentrations were examined, and three related methods were utilized for subsequent data analysis. For reactions in which less than 20% of the substrate was processed when 100% inactivation was observed, data from the continuous assay were directly analyzed with the nonlinear regression analysis program ENZFITTER to obtain first-order rate constants for enzyme inactivation at each inhibitor concentration. The slope of a graph of K_{obs}/(I) vs [I] was calculated using ENZFITTER and reported as kobs/[I]. The error associated with this determination is less than 10% of a given value and is often less than 5%. For very slow irreversible inhibitors (kobs/[I] < 1000 M^{-1} s^{-1}) and/or when >20% consumption of substrate was observed at 100% 3CP inactivation, the primary data were adjusted to account for expected changes in velocity due to changes in substrate concentration. The transformed data were used to calculate kobs/[I] values and the kobs/[I] figure was determined utilizing the slope method described above.

2. Antiviral Assays. All strains of human rhinovirus (HRV) were purchased from American Type Culture Collection (ATCC). HRV stocks were propagated, and antiviral assays were performed in H1-HeLa cells (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. Briefly, H1-HeLa cells were infected with HRV-14 at a multiplicity of infection (moi) of 0.08 or mock-infected with medium only. Infected or uninfected cells were resuspended at 8 × 105 cells per milliliter and incubated with appropriate concentrations of drug. Two days later, XTT/PMS was added to the test plates, and the amount of formazan produced was quantified spec-

Scheme 7. Parallel Synthesis of 5-Substituted Benzamides on Solid Support[a]



[a] (a) **57**, DIC, HOBt; (b) RR1NH or RSH, DIEA, DMF, 70 °C; (c) TFA.

trophotometrically at 450/650 nm. The EC_{50} was calculated as the concentration of drug that increased the percentage of formazan production in drug-treated, virus-infected cells to 50% of that by drug-free, uninfected cells. The reported values were obtained from either a single antiviral determination or the mean of two or more experiments. To avoid false positives due to toxicity, only compounds displaying CC_{50}'s greater than 10 times the observed EC_{50}'s were considered to be truly active antirhinoviral agents. Using the above method, the EC_{50} of the known antirhinoviral agent Pirodavir was determined to be 0.02 ± 0.01 μM (range 0.18−1 μM), comparable to the 0.03 μM minimal inhibitory concentration value previously reported.

Protein Crystallagraphy. Serotype 2 human rhinovirus 3C protease was incubated with a 3-fold molar excess of compound **4** in the presence of 2% DMSO for 24 h at 4 °C. The complex was concentrated to 10 mg/mL and then passed through a 0.22 μm cellulose-acetate filter. Crystals were grown at 13 °C using a hanging drop vapor diffusion method in which equal volumes (3 μL) of the protein/ligand complex and reservoir solution were mixed on plastic coverslips and sealed over individual wells filled with 1 mL of reservoir solution containing 1.2 M ammonium sulfate, 0.325 M sodium phosphate, 0.325 M potassium phosphate, 0.1 M ADA pH 6.6, and 2.5% (v/v) 1,4-dioxane. A single crystal measuring $0.6 \times 0.4 \times 0.2$ mm (space group $P2_12_12$; $a = 61.22$, $b = 77.71$, $c = 34.35$ Å) was prepared for low temperature data collection by a 2 min immersion in an artificial mother liquor solution consisting of 400 μL of the reservoir solution mixed with 125 μL of glycerol, followed by flash freezing in a stream of N_2 gas at −170 °C. X-ray diffraction data were collected using a MAR imaging plate and processed with DENZO. Diffraction data were 74% complete to a resolution of 1.85 Å with R(sym) = 1.9%. Protein atomic coordinates from the cocrystal structure determination (ref 6c) were used to initiate rigid body refinement in X-PLOR followed by simulated annealing and conjugate gradient minimization protocols. Placement of the inhibitor, addition of ordered solvent, and further refinement proceeded as described in ref 6c. The final R factor was 20.8% (10 680 reflections with $F > 2\_(F)$). The root-mean-square deviations from ideal bond lengths and angles were 0.013 Å and 2.6°, respectively. The final model consisted of all atoms for residues 1−180 (excluding the side chain of residue 21) plus 104 water molecules.

Calculations Used in Virtual Library Analysis. We selected 3087 primary amines and mercaptans from the ACD.[14] The library was built with PEONY (an in-house program which synthesizes the 2D virtual library from its fragments) attaching the compounds at the 5-position of the benzamide core. Three-dimensional coordinates of the library were generated with CORINA Ver. 1.7.[15] The structures were then energy minimized in the Batchmin module of MacroModel[16] Ver. 5.5 using the AMBER* force field (atomic charges were the default from the force field).

Compounds with favorable predicted binding modes to either the P2 or P4 pocket were selected by a partially fixed docking routine as implemented in AGDOCK.[17] Each compound was run through the docking process eight times against the target protein active site from the cocrystal structure of compound **1**, with the benzamide core and protein atoms fixed at the coordinates of the cocrystal structure.[18] All torsions of the 5-position benzamide substituents were flexible during docking. To remove docked structures with highly unfavorable contacts with the target, the fixed ligand atom constraints were removed, and the ligand structures were energy minimized in the context of the rigid protein. Only those structures which after minimization in the protein maintained the coordinates of the benzamide core close to the cocrystal structure (RMS deviation ≤ 1.0 for heavy atoms) were kept for further analysis. The docked molecules were then scored and ranked using HTS,[19] a program developed in-house for rapidly estimating the free energy of protein−ligand association. Structures with poor HTS scores were discarded; and among the multiple docked conformations of the same compound, the one with the best HTS score was retained. Final ranking was made on the basis of which compounds satisfied at least one specific hydrogen bond donor or acceptor interaction with protein atoms which are known to form β-strand type interactions with the native peptide substrate and which fit well into the P2 or P4 pockets, as judged by ligand−protein atom distance criteria to selected protein atoms in the subsite.

Chemistry. 3-Carbamoyl-benzaldehyde (1a). Oxalyl chloride (13.3 mL, 152 mmol) and DMF (50 μL) were added to a suspension of 3-carboxybenzaldehyde (11.4 g, 76.2 mmol) in 200 mL of CH_2Cl_2 and stirred at 23 °C for 18 h. The resulting clear solution was concentrated, dissolved in 100 mL of CH_2Cl_2, and concentrated again. The crude acid chloride was dissolved in 20 mL of THF, poured into a mixture of concentrated NH_4OH (26 mL, 381 mmol) with 100 mL of crushed ice, and allowed to warm to 23 °C with stirring. The mixture was acidified with concentrated HCl to pH ∼3, then concentrated to remove THF. The resulting aqueous suspension was filtered and the white solid product dried under vacuum to give 7.4 g (65%) of 3-carbamoyl-benzaldehyde **1a**: ^{1}H NMR (DMSO-d_6) δ 10.06 (1 H, s), 8.40 (1 H, s), 8.19 (1 H, s), 8.17 (1 H, d, $J = 6.6$ Hz), 8.04 (1 H, d, $J = 7.7$ Hz), 7.69 (1 H, t, $J = 7.7$ Hz), 7.56 (1 H, s); IR (KBr pellet) 3391, 3205, 1711, 1694, 1664, 1385, 1217. Anal. ($C_8H_7NO_2 \cdot 0.1H_2O$) C, H, N.

3-(3-Carbamoylphenyl)-acrylic Acid Ethyl Ester (1). Method A: A solution of 3-carbamoyl-benzaldehyde **1a** (7.38 g, 49.5 mmol) and (carbethoxymethylene)triphenylphosphorane (17.23 g, 49.5 mmol) in 200 mL of toluene was heated to reflux for 24 h. After cooling to room temperature, the reaction mixture was partitioned between 750 mL of CH_2Cl_2 and a mixture of 200 mL of water with 200 mL of brine. The organic layer was washed again with a mixture of 100 mL of water with 100 mL of brine, then dried over $MgSO_4$, filtered, and concentrated to a crude yellow oil which crystallized on standing. The combined aqueous layers were filtered, and the insoluble material was combined with the crude solid obtained from the organic layer. The crude product was purified by recrystallization from methanol. A 5.25 g (48%) yield of **1** was isolated in two crops as yellow needles: mp 168−170 °C; ^{1}H NMR ($CDCl_3$) δ 7.99(1 H, s), 7.80 (1 H, d, $J = 7.7$ Hz), 7.71 (1 H, d, $J = 11.8$ Hz), 7.68 (1 H, s), 7.49 (1 H, t, $J = 7.7$ Hz), 6.52 (1 H, d, $J = 16.2$ Hz), 6.10 (1 H, br s, NH), 5.75 (1 H, br s, NH), 4.28 (2 H, q, $J = 7.0$ Hz), 1.35 (3 H, t, $J = 7.0$ Hz); IR (neat film) 3414, 3177, 1695, 1682, 1639, 1400, 1313, 1215, 1192. Anal. ($C_{12}H_{13}NO_3$) C, H, N.

Compound **1** was also prepared in 78% yield as a white solid using ethyl acrylate in the same procedure (Method B) for the preparation of **2** described below.

3-Iodo-benzamide (32). 3-Iodo-benzamide was prepared

using a modification of the procedure by Remsen.[20] 3-Iodobenzoic acid (28.68 g, 116 mmol) was stirred in CH_2Cl_2 (200 mL) at 23 °C under argon. Oxalyl chloride (30.2 mL, 349 mmol) was added slowly, and slow gas evolution was observed. DMF (0.1 mL) was then added, accelerating gas evolution considerably, and the reaction was stirred for 2 h. Solvent was removed, and the brown oily residue was dissolved in THF (50 mL) and added to a solution of 18% aqueous NH_4OH (260 mL) at 0 °C. After the mixture was stirred for 15 min, the liquid was decanted off, and the remaining sludge was acidified with 1 N HCl. The white solid was collected by filtration, washed with water, and dried under vacuum to give 25.68 g (90%) of 3-iodo-benzamide **32**: ^{1}H NMR (DMSO-d_6) δ 8.21 (1 H, t, J = 1.5 Hz), 8.05 (1 H, s), 7.87 (2 H, dd, J = 8.1, 1.5 Hz), 7.47 (1 H, s), 7.25 (1 H, t, J = 7.8 Hz); IR (KBr) 3343, 3164, 1661, 1628, 1561, 1424, 1389, 1125. Anal. (C_7H_6INO) C, H, N.

3-(3-Carbamoyl-phenyl)-acrylic Acid Methyl Ester (2). Method B: 3-Iodo-benzamide **32** (1.16 g, 4.7 mmol), methyl acrylate (530 μL, 5.87 mmol), palladium(II) acetate (16 mg, 0.071 mmol), and triethylamine (820 μL, 5.87 mmol) were stirred in 10 mL of acetonitrile under argon in a sealed tube at 100 °C for 5 h. The reaction was cooled to 0 °C, and the gray precipitate was collected. Recrystallization from methanol gave 425 mg (44%) **2** as a white solid: ^{1}H NMR (DMSO-d_6) δ 8.20 (1 H, s), 8.04 (1 H, s), 7.87 (2 H, dd, J = 7.8, 18 Hz), 7.69 (1 H, d, J = 16.2 Hz), 7.50 (1 H, t, J = 7.8 Hz), 7.47 (1 H, s), 6.73 (1 H, d, J = 15.9 Hz), 3.73 (3 H, s); IR (KBr) 3424, 3366, 3173, 2957, 1728, 1659, 1578, 1431, 1399, 1317. Anal. ($C_{11}H_{11}NO_3 \cdot 0.2H_2O$) C, H, N.

3-(3-Carbamoyl-phenyl)-acrylic Acid (33). Compound **1** (1.75 g, 7.99 mmol) was hydrolyzed in 2:1 0.8 N aqueous NaOH/methanol (48 mL), stirring at 23 °C for 5 h. The solution was then concentrated to ~20 mL volume, cooled to 0 °C, and acidified to pH = 4 with 1 N HCl. The resulting crystals were collected, washed with 5 mL of cold H_2O, and dried under vacuum to give 1.47 g (96%) of 3-(3-carbamoyl-phenyl)-acrylic acid **33** as a white solid: ^{1}H NMR (DMSO-d_6) δ 12.50 (1 H, br s), 8.17 (1 H, s), 8.05 (1 H, s), 7.85 (2 H, dd, J = 23.7, 7.8 Hz), 7.61 (1 H, d, J = 16.2 Hz), 7.49 (1 H, t, J = 7.5 Hz), 7.47 (1 H, s), 6.62 (1 H, d, J = 15.6 Hz); IR (KBr) 3451, 3202, 2924, 1690, 1640, 1443, 1395, 1316, 1219. Anal. ($C_{10}H_9NO_3 \cdot 0.25H_2O$) C, H, N.

3-(3-Carbamoyl-phenyl)-acrylic Acid Benzyl Ester (3). 3-(3-Carbamoyl-phenyl)-acrylic acid (212 mg, 1.11 mmol) and benzyl alcohol (172 μL, 1.66 mmol) were stirred in DMF (3 mL) at 0 °C. EDC (318 mg, 1.66 mmol), triethylamine (170 μL, 1.22 mmol), and DMAP (14 mg, 0.11 mmol) were added, and the reaction was allowed to stir for 3 h while warming to 23 °C. The solution was concentrated, and the residue was purified by flash chromatography (3% EtOH/$CHCl_3$) to give 90 mg (29%) of **3** as a white solid: ^{1}H NMR (DMSO-d_6) δ 8.22 (1 H, s), 8.04 (1 H, s), 7.94 (2 H, dd, J = 12.0, 7.8 Hz), 7.61 (1 H, d, J = 16.2 Hz), 7.34−7.50 (7 H, m), 6.79 (1 H, d, J = 16.2 Hz), 5.23 (2 H, s); IR (KBr) 3420, 3316, 3150, 1711, 1665, 1630, 1402, 1308, 1167. Anal. ($C_{17}H_{15}NO_3$) C, H, N.

3-(3-Carbamoyl-phenyl)-acrylic Acid 2-Hydroxyethyl Ester (4). This compound was prepared in 33% yield as a white solid using ethylene glycol in the same procedure for the preparation of **3** described above: ^{1}H NMR (DMSO-d_6) δ 8.21 (1 H, s), 8.05 (1 H, br s), 7.90 (1 H, d, J = 7.8 Hz), 7.86 (1 H, d, J = 7.8 Hz), 7.71 (1 H, d, J = 16.2 Hz), 7.51 (1 H, t, J = 7.8 Hz), 7.47 (1 H, br s), 6.74 (1 H, d, J = 16.2 Hz), 4.86 (1 H, t, J = 5.1 Hz), 4.17 (2 H, t, J = 5.1 Hz), 3.64 (2 H, q, J = 5.1 Hz); IR (KBr) 3418, 3212, 1705, 1676, 1626, 1574, 1399, 1280. Anal. ($C_{12}H_{13}NO_4$) C, H, N.

3-(3-Carbamoyl-phenyl)-acrylic Acid Phenethyl Ester (5). This compound was prepared in 49% yield as a white solid using phenethyl alcohol in the same procedure for the preparation of **3** described above: ^{1}H NMR ($CDCl_3$) δ 7.98 (1 H, s), 7.79 (1 H, d, J = 7.8 Hz), 7.68 (1 H, d, J = 16.2 Hz), 7.66 (1 H, d, J = 7.8 Hz), 7.48 (1 H, t, J = 7.5 Hz), 7.23−7.36 (5 H, m), 6.50 (1 H, d, J = 16.2 Hz), 6.14 (1 H, br s), 5.83 (1 H, br s), 4.44 (2 H, t, J = 6.9 Hz), 3.02 (2 H, t, J = 6.9 Hz); IR (KBr) 3410, 3165, 1705, 1678, 1630, 1576, 1393, 1373, 1283, 1261. Anal. ($C_{18}H_{17}NO_3$) C, H, N.

3-(3-Carbamoyl-phenyl)-acrylic Acid Pyridin-3-yl Methyl Ester (6). This compound was prepared in 68% yield as a white solid using 3-pyridyl carbinol in the same procedure for the preparation of **3** described above: ^{1}H NMR ($CDCl_3$) δ 8.68 (1 H, s), 8.60 (1 H, d, J = 4.8 Hz), 7.99 (1 H, s), 7.66−7.81 (4 H, m), 7.48 (1 H, t, J = 7.5 Hz), 7.26−7.35 (1 H, m), 6.55 (1 H, d, J = 16.2 Hz), 6.16 (1 H, br s), 5.79 (1 H, br s), 5.28 (2 H, s); IR (KBr) 3418, 3160, 1700, 1676, 1630, 1576, 1397, 1285, 1259. Anal. ($C_{16}H_{14}N_2O_3$) C, H, N.

3-(2-Ethoxycarbonyl-vinyl)-benzoic Acid (7). Using procedure B described above, compound **7** was prepared in 72% yield as a white solid: ^{1}H NMR ($CDCl_3$) δ 11.22 (1 H, s), 8.29 (1 H, s), 8.13 (1 H, d, J = 7.8 Hz), 7.71−7.78 (2 H, m), 7.52 (1 H, t, J = 7.5 Hz), 6.54 (1 H, d, J = 16.2 Hz), 4.29 (2 H, q, J = 7.2 Hz), 1.36 (3 H, t, J = 7.2 Hz); IR (KBr) 2984, 2672, 2564, 1725, 1642, 1445, 1302, 1209, 1177 cm^{-1}. Anal. ($C_{12}H_{12}O_4 \cdot 0.2H_2O$) C, H.

3-(2-Ethoxycarbonyl-vinyl)-benzoic Acid Methyl Ester (8). Compound **7** (225 mg, 1.02 mmol) was dissolved in a mixture of dichloromethane (2 mL) and methanol (2 mL). (Trimethylsilyl)diazomethane (2 M solution in hexanes, ~0.8 mL, ~1.6 mmol) was added dropwise until gas evolution ceased and a faint yellow color remained for 10 min. The reaction was concentrated and purified by flash chromatography (1% MeOH/$CHCl_3$) to give 196 mg (82%) of **8** as a white solid: ^{1}H NMR ($CDCl_3$) δ 8.21 (1 H, s), 8.05 (1 H, d, J = 7.8 Hz), 7.71 (1 H, d, J = 16.2 Hz), 7.70 (1 H, d, J = 7.8 Hz), 7.47 (1 H, t, J = 7.8 Hz), 6.50 (1 H, d, J = 16.3), 4.28 (2 H, q, J = 7.2 Hz), 3.94 (s, 3H), 1.35 (3 H, t, J = 7.2 Hz); IR (KBr) 3399, 3094, 3065, 3034, 2980, 2907, 1717, 1638, 1447 cm^{-1}. Anal. ($C_{13}H_{14}O_4$) C, H.

(*E*)-3-(3-Carbamoyl-phenyl)-2-cyano-acrylic Acid Ethyl Ester (9). Piperidine (150 μL, 1.52 mmol) was added to a solution of 3-formyl benzamide **30** (111 mg, 0.74 mmol) and ethyl cyanoacetate (79 μL, 0.74 mmol) in ethanol (2 mL) at 0 °C. After the mixture was stirred for 5 h at 23 °C, solvent was removed. The residue was dissolved in CH_2Cl_2 and washed with 0.1 N HCl, H_2O, and then brine. Organics were dried (Na_2SO_4) and concentrated. Purification by flash chromatography (3% MeOH/$CHCl_3$) gave 86 mg (47%) of **9** as a white solid: ^{1}H NMR ($CDCl_3$) δ 8.37 (1 H, s), 8.31 (1 H, s), 8.18 (1 H, d, J = 7.8 Hz), 8.04 (1 H, d, J = 7.8 Hz), 7.63 (1 H, t, J = 7.8 Hz), 6.09 (1 H, br s), 5.71 (1 H, br s), 4.41 (2 H, q, J = 7.2 Hz), 1.42 (3 H, t, J = 7.2 Hz); IR (KBr) 3435, 3351, 3306, 3167, 2224, 1724, 1701, 1626, 1601, 1578, 1433, 1383, 1275, 1211 cm^{-1}. Anal. ($C_{13}H_{12}N_2O_3 \cdot 0.5H_2O$) C, H, N.

6-Methyl-3-(3-carbamoylphenyl)-acrylic Acid Ethyl Ester (10). 3-Iodo-4-methyl benzoic acid was converted to the corresponding benzamide according to the procedure described for compound **1a** above ($(COCl)_2$, NH_4OH), to provide 3-iodo-4-methyl benzamide in 93% yield. Further conversion according to procedure B described above provided compound **10** in 48% yield: ^{1}H NMR ($CDCl_3$) δ 8.02 (1 H, d, J = 1.6 Hz), 7.95 (1 H, d, J = 15.9 Hz), 7.68 (1 H, d, J = 7.9 Hz), 7.29 (1 H, d, J = 7.9 Hz), 6.46 (1 H, d, J = 15.9 Hz), 6.10 (1 H, br s), 5.75 (1 H, br s), 4.08 (2 H, q, J = 7.1 Hz), 2.48 (3 H, s), 1.42 (3 H, t, J = 7.1 Hz). Anal. ($C_{13}H_{15}N_2O_3 \cdot 0.2H_2O$) C, H, N.

3-Methoxy-5-nitro-phenylamine (34). Sodium bicarbonate (6.07 g, 72.3 mmol) was added to sodium sulfide nonahydrate (18.2 g, 75.9 mmol) in deionized water (50 mL). When the sodium bicarbonate was completely dissolved, methanol (50 mL) was added, and the solution cooled to 0 °C. A precipitate formed, which was removed by filtration through a Celite pad; the filtered solution was added to 3,5-dinitroanisole (8.02 g, 40.5 mmol) in methanol (50 mL). After heating at reflux for 30 min, the solution was concentrated in vacuo to remove methanol. The aqueous residue was poured into 200 mL of ice water, and the resulting orange precipitate was collected by suction filtration. Chromatography (1:2 EtOAc/hexanes) of the crude solid yielded unreacted 3,5-dinitroanisole (0.98 g, 12%) and aniline product **34** (4.96 g, 73%; 83% based on recovered 3,5-dinitroanisole) as an orange solid: mp = 117−119 °C; ^{1}H NMR ($CDCl_3$) δ 7.12 (s, 2H), 6.48 (s, 1H), 3.98 (br

s, 2H), 3.83 (s, 3H); IR (KBr pellet) 3447, 3364, 1637, 1523, 1344 cm^{-1}; Anal. ($C_7H_8N_2O_3$) C, H, N.

1-Iodo-3-methoxy-5-nitro-benzene (35). Concentrated HCl (15 mL) was added to a solution of aniline **34** (5.25 g, 31.2 mmol) in water (15 mL) at 0 °C. To this was added a chilled solution of sodium nitrite (3.88 g, 56.2 mmol) in water (20 mL), dropwise, with vigorous mechanical stirring. Stirring was continued at 0 °C for 15 min after the addition was complete, and then a solution of potassium iodide (10.37 g, 62.4 mmol) in water (20 mL) was added carefully. The cooling bath was removed, and the reaction heated to boiling. When the production of purple vapor ceased, the mixture was cooled to 23 °C and extracted with CH_2Cl_2 (3 × 200 mL). The combined organic extracts were dried ($MgSO_4$), filtered, and concentrated in vacuo. Purification by silica gel chromatography (1:9 EtOAc/hexanes) gave pure iodide **35** (7.35 g, 84%) as a colorless solid: mp = 81−82 °C; ^{1}H NMR ($CDCl_3$) δ 8.15 (s, 1H), 7.70 (s, 1H), 7.56 (s, 1H), 3.88 (s, 3H); IR (KBr pellet) 1527, 1342 cm^{-1}; Anal. ($C_7H_6INO_3$) C, H, N.

3-Iodo-5-methoxy-phenylamine (36). A mixture of triirondodecacarbonyl (16.24 g, 32.2 mmol), **35** (7.50 g, 26.9 mmol), methanol (15 mL), and toluene (200 mL) was heated at reflux for 3.5 h. After cooling, filtration, concentration in vacuo, and silica gel chromatography (1:4 EtOAc/hexanes), aniline **36** (6.11 g, 91%) was obtained as a yellow oil which crystallized on standing: mp = 84−86 °C; ^{1}H NMR ($CDCl_3$) δ 6.64 (s, 2H), 6.15 (s, 1H), 3.71 (s, 3H), 3.66 (br s, 2H); IR (KBr pellet) 3414, 3308, 3208, 1572 cm^{-1}; Anal. (C_7H_8INO) C, H, N.

3-Amino-5-methoxy-benzonitrile (37). By the same method used to make compound **44**, iodide **36** (2.51 g, 10.1 mmol) was converted to nitrile **37** (1.24 g, 83%), a yellow solid: mp = 81−85 °C; ^{1}H NMR ($CDCl_3$) δ 6.53 (m, 2H), 6.39 (t, J = 2.0 Hz, 1H), 3.87 (br s, 2H), 3.77 (s, 3H); IR (neat film) 3408, 3333, 3221, 2228, 1597 cm^{-1}; Anal. ($C_8H_8N_2O$) C, H, N.

3-Iodo-5-methoxy-benzonitrile (38). By the same method used to prepare **35**, nitrile **37** (1.12 g, 7.6 mmol) was converted to iodide **38** (1.25 g, 64%): ^{1}H NMR ($CDCl_3$) δ 7.55 (s, 1H), 7.47 (s, 1H), 7.12 (s, 1H), 3.82 (s, 3H); IR (neat film) 2231, 1587, 1284 cm^{-1}; Anal. (C_8H_6INO) C, H, N.

3-Iodo-5-methoxy-benzamide (39). By the same method used to prepare amide **45**, nitrile **38** (1.245 g, 4.81 mmol) was converted to amide **39** (1.039 g, 78%), a white solid: mp = 175−176 °C; ^{1}H NMR ($CDCl_3$) δ 7.65 (s, 1H), 7.40 (s, 1H), 7.34 (s, 1H), 6.0−5.8 (2 br S, 2H), 3.83 (s, 3H); IR (KBr pellet) 3389, 3194, 1658, 1568, 1390 cm^{-1}; Anal. ($C_8H_8INO_2$) C, H, N.

3-Hydroxy-5-iodo-benzamide (40). Amide **39** (1.032 g, 3.72 mmol) suspended in CH_2Cl_2 (100 mL) was cooled to −78 °C. Boron tribromide solution (1.0 M in CH_2Cl_2, 7.45 mL, 7.45 mmol) was added. After being stirred at −78 °C for 30 min, the solution was heated to reflux for 4 h. Another equivalent of boron tribromide (3.7 mL) was added, and the solution was stirred at 23 °C for 16 h. The reaction mixture was quenched with water (50 mL), causing a white precipitate to form. Ether (50 mL) was added to dissolve the precipitate, and the layers separated. The aqueous layer was discarded, and the ether layer was washed with 2 N NaOH (2 × 100 mL). The combined basic washes were treated with 6 N HCl until pH ∼4, then extracted with ether (2 × 150 mL). These ether extracts were dried ($MgSO_4$), filtered, and concentrated in vacuo to give phenol **40** (803.3 mg, 82%) as a white solid: mp = 192−195 °C; ^{1}H NMR (DMSO-d_6) δ 9.99 (s, 1H), 7.93 (s, 1H), 7.61 (s, 1H), 7.37 (s, 1H), 7.24 (s, 2H); IR (KBr pellet) 3483, 3396, 3232, 1680, 1649, 1433 cm^{-1}; Anal. ($C_7H_6INO_2$) C, H, N.

3-(3-Carbamoyl-5-hydroxy-phenyl)-acrylic Acid Ethyl Ester (11). Iodide **40** (24.6 mg, 0.093 mmol) was coupled with ethyl acrylate (23.4 mg, 0.234 mmol) under the standard conditions described above to give **11** (17.3 mg, 79%) as a white solid: mp = 200−202 °C; ^{1}H NMR (DMSO-d_6) δ 9.86 (s, 1H), 7.92 (s, 1H), 7.67 (s, 1H), 7.57 (d, J = 16.8 Hz, 1H), 7.36 (s, 1H), 7.31 (s, 1H), 7.15 (s, 1H), 6.61 (d, J = 16.2 Hz, 1H), 4.18 (q, J = 7.0 Hz, 2H), 1.25 (t, J = 7.0 Hz, 3H); IR (KBr pellet) 3402, 3209, 1680, 1593, 1296 cm^{-1}; Anal. ($C_{12}H_{13}NO_4 \cdot 0.4H_2O$) C, H, N.

3-Benzyloxy-5-iodo-benzamide (49). A solution of benzyl bromide (128 mg, 0.75 mmol), phenol **40** (131.5 mg, 0.50 mmol), and potassium carbonate (138 mg, 1.00 mmol) in DMF (3.0 mL) was heated to 60 °C for 1.5 h. After cooling to 23 °C, the solution was filtered, concentrated in vacuo, and purified by silica gel chromatography (1:1 EtOAc/hexanes) to give benzyl ether **49** (132.5 mg, 75%) as a white solid: mp = 124−125 °C; ^{1}H NMR ($CDCl_3$) δ 7.67 (s, 1H), 7.49 (s, 1H), 7.40 (m, 6H), 6.0−5.8 (2 br s, 2H), 5.08 (s, 2H); IR (KBr pellet) 3369, 3192, 1660, 1564 cm^{-1}; Anal. ($C_{14}H_{12}INO_2$) C, H, N.

3-(3-Benzyloxy-5-carbamoyl-phenyl)-acrylic Acid Ethyl Ester (12). Iodide **49** (51.6 mg, 0.146 mmol) was coupled with ethyl acrylate (36.6 mg, 0.365 mmol) under the standard conditions to give **12** (18.5 mg, 39%) as an off-white solid: mp = 193−194 °C; ^{1}H NMR (DMSO-d_6) δ 8.01 (s, 1H), 7.81 (s, 1H), 7.63 (d, J = 15.8 Hz, 1H), 7.54 (d, J = 4.8 Hz, 2H), 7.45 (m, 6H), 6.75 (d, J = 15.8 Hz, 1H), 5.18 (s, 2H), 4.19 (q, J = 7.0 Hz, 2H), 1.26 (t, J = 7.0 Hz, 3H); IR (KBr pellet) 3418, 3173, 1705, 1670, 1589, 1288 cm^{-1}; Anal. ($C_{19}H_{19}NO_4 \cdot 0.2H_2O$) C, H, N.

3-Carbamoyl-4-methoxy-benzaldehyde (41). 3-Bromoanisaldehyde (8.0 g, 37.2 mmol) and copper cyanide (4.0 g, 44.67 mmol) were stirred in DMF (100 mL) at 150 °C for 16 h. To this was added an iron nitrate solution (20 g of iron(III) nitrate, 6 mL of concetrated HCl, 40 mL of H_2O), and the mixture was stirred 10 min before it was allowed to cool to 23 °C. The reaction was then diluted with H_2O (200 mL) and extracted with $CHCl_3$ (3 × 80 mL). Organic layers were combined and solvents were removed, taking care to pump off the residual DMF. The brown/green residue was once again dissolved in $CHCl_3$ (100 mL) and washed with 1 N HCl (50 mL) and brine (50 mL). The material was dried (Na_2SO_4), and solvent was removed to give the crude nitrile as a tan solid. The nitrile was stirred in concentrated H_2SO_4 (60 mL) at 100 °C for 1 h. The solution was cooled, poured into H_2O (250 mL), and extracted with $CHCl_3$ (8 × 50 mL). Organics were dried (Na_2SO_4) and concentrated. The resulting solid was recrystallized from methanol to give 2.98 g (45%) of 3-carbamoyl-4-methoxy-benzaldehyde **41** as a white solid: ^{1}H NMR ($CDCl_3$) δ 9.92 (1 H, s), 8.28 (1 H, s), 8.00 (1 H, d, J = 9.0 Hz), 7.74 (1 H, br s), 7.69 (1 H, br s), 7.33 (1 H, d, J = 9.0 Hz), 3.98 (3 H, s); IR (KBr) 3399, 3183, 1676, 1589, 1433, 1262, 1204 cm^{-1}. Anal. ($C_9H_9NO_3$) C, H, N.

5-Formyl-2-hydroxy-benzamide (42). 3-Carbamoyl-4-methoxy-benzaldehyde **41** (1.065 g, 5.95 mmol) was stirred in dry CH_2Cl_2 (120 mL) at −78 °C under argon. Boron tribromide (10.71 mL, 1.0 M, 10.71 mmol) was added, and the reaction stirred 18 h while warming to 23 °C. The reaction was quenched with 0.05 N HCl (80 mL) and was allowed to stir for 15 min. The organic layer was collected, and the aqueous layer was further extracted with EtOAc (2 × 50 mL). Organics were combined, dried (Na_2SO_4), and concentrated. Purification by flash chromatography (60% EtOAc/$CHCl_3$) gave 540 mg (55%) of 5-formyl-2-hydroxy-benzamide **42** as a white solid: ^{1}H NMR (DMSO-d_6) δ 14.00 (1 H, s), 9.88 (1 H, s), 8.73 (1 H, br s), 8.55 (1 H, d, J = 1.5 Hz), 8.21 (1 H, br s), 8.00 (1 H, dd, J = 8.7, 1.5 Hz), 7.13 (1 H, d, J = 8.7 Hz); IR (KBr) 3420, 3237, 1686, 1618, 1493, 1375, 1279, 1196 cm^{-1}. Anal. ($C_8H_7NO_3$) C, H, N.

3-(3-Carbamoyl-4-hydroxy-phenyl)-acrylic Acid Ethyl Ester (14). 5-Formyl-2-hydroxy-benzamide **42** (65 mg, 0.40 mmol) and (carbethoxymethylene)triphenylphosphorane (281 mg, 0.81 mmol) were stirred in DMF (3 mL) at 23 °C for 2 h. Solvent was removed, and the residue was purified by flash chromatography (2% MeOH/$CHCl_3$) to give 44 mg (48%) of **14** as a white solid: ^{1}H NMR (DMSO-d_6) δ 13.50 (1 H, s), 8.52 (1 H, br s), 8.29 (1 H, s), 8.07 (1 H, br s), 7.77 (1 H, d, J = 8.7 Hz), 7.56 (1 H, d, J = 15.6 Hz), 6.92 (1 H, d, J = 8.7 Hz), 6.57 (1 H, d, J = 15.6 Hz), 4.18 (2 H, q, J = 7.2 Hz), 1.26 (3 H, t, J = 7.2); IR (KBr) 3387, 3198, 2988, 2359, 1688, 1620, 1491, 1441, 1372, 1279 cm^{-1}. Anal. ($C_{12}H_{13}NO_4$) C, H, N.

2-Benzyloxy-5-formyl-benzamide (42a). Compound **42** (105 mg, 0.64 mmol) and benzyl bromide (114 μL, 0.95 mmol) were stirred in DMF (3 mL) with K_2CO_3 (176 mg, 1.27 mmol)

at 60 °C for 1 h. Solvent was removed, and the residue was purified by flash chromatography (2% MeOH/$CHCl_3$) to give 135 mg (83%) of **42a** as a white solid: ^{1}H NMR (DMSO-d_6) δ 9.91 (1 H, s), 8.24 (1 H, s), 7.98 (1 H, d, J = 8.7 Hz), 7.67 (2 H, br s), 7.51 (2 H, d, J = 7.2 Hz), 7.41−7.34 (4 H, m), 5.36 (2 H, s); IR (KBr) 3387, 3192, 2849, 1690, 1649, 1599, 1437, 1389, 1265, 1206 cm^{-1}. Anal. ($C_{15}H_{13}NO_3 \cdot 0.2H_2O$) C, H, N.

3-(4-Benzyloxy-3-carbamoyl-phenyl)-acrylic Acid Ethyl Ester (15). 2-Benzyloxy-5-formyl-benzamide **42a** (105 mg, 0.412 mmol) and (carbethoxymethylene)triphenylphosphorane (287 mg, 0.824 mmol) were stirred in DMF (3 mL) at 40 °C for 2 h. Solvent was removed, and the residue was purified by flash chromatography (1% MeOH/$CHCl_3$) to give 95.8 mg (72%) of **15** as a white solid: ^{1}H NMR ($CDCl_3$) δ 8.45 (1 H, d, J = 1.8 Hz), 7.70−7.60 (3 H, m), 7.43 (5 H, br s), 7.08 (1 H, d, J = 8.7 Hz), 6.43 (1 H, d, J = 15.6 Hz), 5.75 (1 H, br s), 5.23 (2 H, s), 4.25 (2 H, q, J = 7.2 Hz), 1.33 (3 H, t, J = 7.2 Hz); IR (KBr) 3441, 3154, 1689, 1676, 1593, 1500, 1431, 1371 cm^{-1}. Anal. ($C_{19}H_{19}NO_4$) C, H, N.

3-[2-(Methoxy-methyl-carbamoyl)-vinyl]benzamide (43). 3-Iodo-benzamide **32** (3.0 g, 12.1 mmol), *N*-methoxy-*N*-methylacrylamide[21] (1.8 g, 15 mmol), palladium(II) acetate (40 mg, 0.18 mmol), and triethylamine (2.1 mL, 15 mmol) were stirred in acetonitrile (12 mL) under argon in a sealed tube at 100 °C for 2.5 h. The reaction was allowed to cool, and solvent was removed. The residue was dissolved in CH_2Cl_2, washed with 0.1 N HCl and then with brine, dried ($MgSO_4$), and concentrated. Recrystallization twice from methanol gave 1.64 g (58%) of 3-[2-(methoxy-methyl-carbamoyl)-vinyl]benzamide **43** as a white solid: ^{1}H NMR ($CDCl_3$) δ 8.06 (1 H, s), 7.78 (1 H, d, J = 7.8 Hz), 7.73 (1 H, d, J = 16.2 Hz), 7.69 (1 H, d, J = 7.8 Hz), 7.46 (1 H, t, J = 7.8 Hz), 7.10 (1 H, d, J = 16.2 Hz), 6.33 (1 H, br s), 5.97 (1 H, br s), 3.77 (3 H, s), 3.31 (3 H, s); IR (Kbr) 3385, 3173, 1680, 1649, 1613, 1582, 1476, 1433, 1397, 1182, 1105 cm^{-1}. Anal. ($C_{12}H_{14}N_2O_3 \cdot 0.33H_2O$) C, H, N.

3-(3-Oxo-but-1-enyl)-benzamide (16). Method C: 3-[2-(Methoxy-methyl-carbamoyl)-vinyl]benzamide **43** (100 mg, 0.427 mmol) was stirred in dry THF (4 mL) at 0 °C under argon. Methyllithium (1.6 mL, 1.5 M in ether, 2.4 mmol) was added, and the reaction stirred for 1.5 h. The reaction was poured over 0.1 N HCl and extracted with CH_2Cl_2. Organics were dried ($MgSO_4$) and concentrated. Purification by flash chromatography (2 to 5% EtOH/CH_2Cl_2) gave 54 mg (67%) of **16** as a white solid: ^{1}H NMR ($CDCl_3$) δ 8.03 (1 H, s), 7.80 (1 H, d, J = 7.8 Hz), 7.70 (1 H, d, J = 7.8 Hz), 7.54 (1 H, d, J = 16.2 Hz), 7.50 (1 H, t, J = 7.8 Hz), 6.80 (1 H, d, J = 16.2 Hz), 6.14 (1 H, br s), 5.82 (1 H, br s), 2.39 (3 H, s); IR (KBr) 3345, 3160, 2363, 1667, 1400, 1264 cm^{-1}. Anal. ($C_{11}H_{11}NO_2$) C, H, N.

3-(3-Oxo-3-phenyl-prop-1-enyl)-benzamide (17). Compound **17** was prepared in 20% yield as a white solid using phenyllithium according to method C described above: ^{1}H NMR ($CDCl_3$) δ 8.15 (1 H, s), 8.03 (2 H, d, J = 7.2 Hz), 7.76−7.84 (3 H, m), 7.48−7.64 (5 H, m), 6.26 (1 H, br s), 5.85 (1 H, br s); IR (KBr) 3383, 3192, 3057, 2361, 1653, 1609, 1580, 1447 cm^{-1}. Anal. ($C_{16}H_{13}NO_2$) C, H, N.

3-[3-(4-Dimethylamino-phenyl)-oxo-propenyl]-benzamide (18). 4-Bromo-*N*,*N*-dimethylaniline (770 mg, 3.85 mmol) was stirred in dry THF (6 mL) at −40 °C under argon. *n*-Butyllithium (1.5 mL, 2.5 M in hexanes, 3.75 mmol) was added dropwise, and the solution was stirred for 15 min. A solution of 3-[2-(methoxy-methyl-carbamoyl)-vinyl]benzamide **43** (150 mg, 0.64 mmol) in THF (3 mL) was added slowly, and the reaction was stirred for 1 h while warming to 23 °C. The reaction was poured over saturated NH_4Cl and was then extracted with $CHCl_3$. Organics were washed with brine, dried (Na_2SO_4), and concentrated. Purification by flash chromatography (1 to 4% MeOH/$CHCl_3$) gave 140 mg of **18** (74%) as a bright orange solid: ^{1}H NMR ($CDCl_3$) δ 8.14 (1 H, s), 8.02 (2 H, d, J = 9.0 Hz), 7.83−7.75 (3 H, m), 7.67 (1 H, d, J = 15.6 Hz), 7.50 (1 H, t, J = 7.8 Hz), 6.71 (2 H, d, J = 9.0 Hz), 6.13 (1 H, br s), 5.66 (1 H, br s), 3.10 (6 H, s); IR (KBr) 3372, 3192, 1671, 1609, 1578, 1377, 1188 cm^{-1}. Anal. ($C_{18}H_{18}N_2O_2 \cdot 0.1H_2O$) C, H, N.

3-[3-(4-Methoxy-phenyl)-oxo-propenyl]-benzamide (19). This compound was prepared in 47% yield as a white solid using 4-bromoanisole in the same procedure for the preparation of **18** described above: ^{1}H NMR ($CDCl_3$) δ 8.15 (1 H, s), 8.06 (2 H, d, J = 8.7 Hz), 7.84−7.75 (3 H, m), 7.63 (1 H, d, J = 16.2 Hz), 7.51 (1 H, t, J = 7.8 Hz), 6.99 (2 H, d, J = 9.0 Hz), 6.18 (1 H, br s), 5.73 (1 H, br s), 3.90 (3 H, s); IR (KBr) 3376, 3192, 1659, 1607, 1439, 1227 cm^{-1}. Anal. ($C_{17}H_{15}NO_3 \cdot 0.2H_2O$) C, H, N.

3-(3-Oxo-3-pyridin-2-yl-propenyl)-benzamide (20). This compound was prepared in 17% yield as a white solid using 2-bromopyridine in the same procedure for the preparation of **18** described above: ^{1}H NMR ($CDCl_3$) δ 8.76 (1 H, d, J = 4.8 Hz), 8.39 (1 H, d, J = 16.2 Hz), 8.19 (2 H, t, J = 1.5 Hz), 7.95 (1 H, d, J = 16.2 Hz), 7.91−7.82 (3 H, m), 7.52 (2 H, t, J = 7.8 Hz), 6.26 (1 H, br s), 5.91 (1 H, br s); IR (KBr) 3416, 3207, 3055, 1672, 1607, 1580, 1391,1332, 1221 cm^{-1}. Anal. ($C_{15}H_{12}N_2O_2 \cdot 0.2H_2O$) C, H, N.

3-(3-Furan-2-yl-3-oxo-propenyl)-benzamide (21). To a solution of freshly distilled furan (425 μL, 5.85 mmol) in dry THF (8 mL) at −10 °C under argon was added *n*-butyllithium (1.56 mL, 2.5 M in hexanes, 3.9 mmol).[13] After being stirred for 2 h at 0 °C, the solution was cooled to −50 °C, and a solution of 3-[2-(methoxy-methyl-carbamoyl)-vinyl]benzamide (114 mg, 0.487 mmol) in THF (1 mL) was added slowly. The reaction was stirred 1 h while warming to 0 °C and was then poured over saturated NH_4Cl and extracted with $CHCl_3$. Organics were washed with brine, dried (Na_2SO_4), and concentrated. Purification by flash chromatography (2 to 6% MeOH/$CHCl_3$) gave 49 mg (42%) of **21** as a white solid: ^{1}H NMR ($CDCl_3$) δ 8.16 (1 H, s), 7.89 (1 H, d, J = 15.6 Hz), 7.83−7.76 (2 H, m), 7.68 (1 H, t, J = 0.9 Hz), 7.57−7.48 (2 H, m), 7.37 (1 H, d, J = 3.6 Hz), 6.62 (1 H, dd, J = 2.1, 0.9 Hz), 6.15 (1 H, br s), 5.69 (1 H, br s); IR (KBr) 3474, 3354, 3191, 1668, 1605, 1466, 1393, 1325 cm^{-1}. Anal. ($C_{14}H_{11}NO_3 \cdot 0.2H_2O$) C, H, N.

1-Oxo-indan-5-carbonitrile (44). A solution of 5-bromo-1-indanone (5.28 g, 25 mmol, zinc cyanide (1.76 g, 15 mmol), and tetrakis(triphenylphosphine) palladium(0) (1.15 g, 1.0 mmol) in DMF (25 mL) was heated to 80 °C for 2 h. After cooling to 23 °C, the solution was diluted with toluene (50 mL), washed with 2 N NH_4OH (2 × 50 mL) and brine (50 mL), dried over $MgSO_4$, filtered, and concentrated in vacuuo. Chromatography of the residue (1:1 EtOAc/hexanes) yielded 3.33 g (85%) of **44** as a yellow solid: ^{1}H NMR ($CDCl_3$) δ 7.85 (d, J = 7.7 Hz, 1H), 7.82 (s, 1H), 7.67 (d, J = 7.7 Hz, 1H), 3.21 (t, J = 5.9 Hz, 2H), 2.77 (dd, J = 6.3, 5.9 Hz, 2H); IR (KBr pellet) 2226, 1715 cm^{-1}. Anal. ($C_{10}H_7NO \cdot 0.1H_2O$) C, H, N.

1-Oxo-indan-5-carboxylic Acid Amide (45). A solution of nitrile **44** (3.02 g, 20.4 mmol) in 3% aqueous H_2O_2 (110 mL) was heated at 50 °C for 4 h. The mixture was then cooled to 0 °C for 1 h, and the resulting precipitate was collected by suction filtration and dried under vacuum to give 2.40 g (67%) of **45** as a yellow solid. The aqueous mother liquor was concentrated to dryness and triturated with hot methanol. The methanol solubles were concentrated and purified by silica gel chromatography (5% MeOH in CH_2Cl_2) to give 184.5 mg (5%) more **45** as a white solid: mp = 207 °C; ^{1}H NMR (DMSO-d_6) δ 8.14 (s, 1H), 8.02 (s, 1H), 7.85 (d, J = 7.7 Hz, 1H), 7.67 (d, J = 7.7 Hz, 1H), 7.57 (s, 1H), 3.13 (t, J = 5.7 Hz, 2H), 2.68 (m, 2H); IR (KBr pellet) 1697, 1660, 1622 cm^{-1}; Anal. ($C_{10}H_9NO_2$) C, H, N.

1-Oxo-indan-5-carboxylic Acid Trityl-amide (46). Acetic anhydride (1.05 mL, 11.1 mmol) and concentrated H_2SO_4 (0.01 mL) were added to a solution of amide **45** (620.5 mg, 3.54 mmol) and triphenylmethyl alcohol (615 mg, 2.36 mmol) in glacial acetic acid (12 mL).[12] The solution was heated at 40 °C for 3.5 h, cooled to 23 °C, and concentrated in vacuuo. The residue was purified by silica gel chromatography (1:1 EtOAc/hexanes to 10:1 EtOAc/hexanes) to give 432 mg (44% based on trityl alcohol) of **46** as a yellow solid: ^{1}H NMR ($CDCl_3$) δ 7.92 (s, 1H), 7.78 (q, J = 8.5 Hz, 2H), 7.30 (m, 16H), 3.18 (t, J = 5.9 Hz, 2H), 2.74 (m, 2H). Anal. ($C_{29}H_{23}NO_2 \cdot 0.25H_2O$) C, H, N.

1-Oxo-1*H*-indene-5-carboxylic Acid Trityl-amide (47). A solution of *N*-Bromosuccinimide (67.3 mg, 0.38 mmol) and amide **46** (157.8 mg, 0.38 mmol) in CCl_4 (10 mL) was heated to reflux for 2 h while irradiated with a 200 W lamp. After the solution cooled to 23 °C, the succinimide precipitate was filtered off. The clear solution was cooled to 0 °C and treated with triethylamine (0.055 mL, 0.40 mmol) for 2 h, then concentrated in vacuuo. Chromatography of the residue (1:1 EtOAc/hexanes) afforded 73.6 mg (47%) of enone **47** as a yellow solid: ^{1}H NMR ($CDCl_3$) δ 7.66 (d, J = 7.3 Hz, 1H), 7.61 (d, J = 5.9 Hz, 1H), 7.51 (s, 1H), 7.47 (d, J = 7.3 Hz, 1H), 7.28 (m, 16H), 5.98 (d, J = 5.9 Hz, 1H); IR (neat film) 1711, 1670, 1493 cm^{-1}.

1-Oxo-1*H*-indene-5-carboxylic Acid Amide (22). Trifluoroacetic acid (3 mL) was added to a solution of **47** (68.3 mg, 0.16 mmol) in CH_2Cl_2 (3.0 mL). After 30 min at 23 °C, the solution was concentrated in vacuuo and purified by silica gel chromatography (5% MeOH in CH_2Cl_2) to give 16.6 mg (60%) of **22** as a yellow solid: mp = 275 °C (decomposes); ^{1}H NMR (DMSO-d_6) δ 8.08 (s, 1H), 7.95 (d, J = 5.9 Hz, 1H), 7.78 (d, J = 7.4 Hz, 1H), 7.66 (s, 1H), 7.53 (s, 1H), 7.44 (d, J = 7.4 Hz, 1H), 6.08 (d, J = 5.9 Hz, 1H); IR (KBr pellet) 3383, 1705, 1658 cm^{-1}; HRMS (M + H$^+$) Calcd. for $C_{10}H_8NO_2$: 174.0555; Found 174.0559. Anal. ($C_{10}H_7NO_2 \cdot 0.1H_2O$) C, H, N.

5-(*tert*-Butyl)-diphenyl-silanyloxymethyl)-isophthalic Acid Diethyl Ester (51). A solution of 5-hydroxymethyl-isophthalic acid diethyl ester **50** (20.0 g, 79.4 mmol) and imidazole (10.81 g, 158.8 mmol) in DMF (265 mL) was treated with *tert*-butylchlorodiphenyl silane (19.6 mL, 75.4 mmol) at 23 °C. This solution was held at 23 °C overnight, then saturated aqueous ammonium chloride (200 mL) and EtOAc (200 mL) are added. The EtOAc layer was washed with additional ammonium chloride solution (100 mL) and then washed with brine (100 mL). Evaporation of the organics yielded 39.9 g of product as colorless oil: ^{1}H NMR ($CDCl_3$) δ 8.57 (1H, s), 8.22 (1H, s), 7.70−7.67 (5H, m), 7.46−7.35 (6H, m), 4.84 (2H, s), 4.40 (4H, q, J = 7.1), 1.42 (6H, t, J = 7.1), 1.12 (9H, s).

5-(*tert*-Butyl-diphenyl-silanyloxymethyl)-isophthalic Acid Monomethyl Ester (52). A solution of **51** (39.9 g, 81.3 mmol) in MeOH (1.2 L) was treated with 0.95 N aqueous NaOH (83.6 mL, 79.4 mmol) at 23 °C. The resulting solution was held at 23 °C for 3 d, then acidified with saturated aqueous citric acid. MeOH was removed by evaporation, and the remaining aqueous solution was cooled to 5 °C and held overnight, upon which precipitation occurred. The precipitate was collected by filtration, washed with ice water (3 × 50 mL), then dried to yield 31.0 g (87% overall) of **52** as a white powder: ^{1}H NMR ($CDCl_3$) δ (1H, s), 8.28 (1H, s), 8.25 (1H, s), 7.70−7.67 (4H, m), 7.45−7.36 (6H, m), 4.84 (2H, s), 3.96 (3H, s), 1.12 (9H, s).

3-(*tert*-Butyl-diphenyl-silanyloxymethyl)-5-hydroxymethyl-benzoic Acid (53). A solution of **52** (27.2 g, 60.6 mmol) in THF (400 mL) was treated with lithium triethylborohydride (212.0 mL of a 1 M solution in THF, 212.0 mmol) at 23 °C. The resulting solution was held overnight at 23 °C and then quenched with a saturated aqueous citric acid solution (200 mL). This mixture was evaporated to dryness, then redissolved in EtOAc (500 mL), and washed with brine (3 × 100 mL). Evaporation of the organic layer yielded 26.8 g of alcohol as a white powder: ^{1}H NMR ($CDCl_3$) δ 8.01 (2H, s), 7.74−7.68 (4H, m), 7.59 (1H, s), 7.50 (1H, br s), 7.44−7.36 (6H, m), 4.82 (2H, s), 4.77 (2H, s), 3.50 (1H, br s), 1.12 (9H, s); MS (FAB) 553 (MCs^+).

3-(*tert*-Butyl-diphenyl-silanyloxymethyl)-5-(2-ethoxycarbonyl-vinyl)-benzoic Acid (55). A mixture of **53** (24.8 g, 59.0 mmol,), NMO (10.4 g, 88.5 mmol), and powdered 3 Å molecular sieves (5 g) in methylene chloride (118 mL) was treated with TPAP (1.04 g, 2.95 mmol), then stirred vigorously for 2 h at 23 °C. The mixture was then treated with saturated aqueous citric acid (100 mL) and EtOAc (500 mL). The organic layer was washed with brine (200 mL) then evaporated to yield 3-(*tert*-butyl-diphenyl-silanyloxymethyl)-5-formyl-benzoic acid **54** (21.0 g): ^{1}H NMR ($CDCl_3$) δ 10.08 (1H, s), 8.49 (1H, s), 8.33 (1H, s), 8.09 (1H, s), 7.73−7.66 (4H, m), 7.50 (1H, br s), 7.44−7.36 (6H, m), 4.87 (2H, s), 1.13 (9H, s).

A solution of triethyl phosphonoacetate (58.0 mL, 295.0 mmol) in DMF (300 mL) was cooled to 0 °C and treated with sodium hydride (11.8 g of 60% in mineral oil, 295 mmol). This mixture is held at 0 °C for 30 min, then treated with a solution of **54** (21.0 g, 50.2 mmol) in DMF (300 mL) at 0 °C. This mixture was allowed to warm to 23 °C over a period of 3 h, then held at 23 °C overnight. The mixture was then acidified with saturated aqueous citric acid (500 mL), then extracted with EtOAc (1 L). The organic layer was washed with brine (3 × 150 mL), then evaporated to yield 54.0 g of a dark oil. Purification by silica gel chromatography (EtOAc−hexanes elutant) yielded 14.8 g (51% overall) of a colorless oil that solidified upon standing at 23 °C: ^{1}H NMR ($CDCl_3$) δ 8.17 (1H, s), 8.06 (1H, s), 7.74−7.67 (6H, m), 7.50 (1H, br s), 7.45−7.39 (6H, m), 6.50 (1 H, d, J = 16.2), 4.82 (2H, s), 4.29 (2H, q, J = 7.0), 1.36 (3H, t, J = 7.0), 1.12 (9H, s).

3-Bromomethyl-5-(2-ethoxycarbonyl-vinyl)-benzoic Acid (57). A solution of **55** (5.0 g, 10.2 mmol) in THF (34 mL) was treated with TBAF (15.3 mL of a 1 M solution in THF, 15.3 mmol) and allowed to stand at 23 °C overnight. The solution was then treated with a saturated aqueous sodium bicarbonate solution and extracted with diethyl ether (2 × 20 mL). The aqueous layer was acidified with a saturated citric acid solution (50 mL), then extracted with EtOAc (2 × 50 mL). Evaporation of the organics yielded 2.6 g of a white powder **56**: ^{1}H NMR ($CDCl_3$) δ 8.18 (1H, s), 8.10 (1H, s), 7.79 (1H, s), 7.72 (1H, d, J = 16.0), 7.50 (1H, br s), 6.55 (1H, d, J = 16.0), 4.81 (2H, s), 4.28 (2H, q, J = 7.0), 1.35 (3H, t, J = 7.0), 1.20 (1H, br s).

This material (**56**) was dissolved in methylene chloride (50 mL), treated with phosphorus tribromide (2.91 mL, 30.6 mmol), and held at 23 °C overnight. The solution was then treated with saturated aqueous sodium bicarbonate (50 mL) and extracted with diethyl ether (2 × 30 mL). The aqueous layer was acidified with saturated aqueous citric acid (50 mL), then extracted with citric acid (3 × 30 mL). Evaporation of the organic layer yielded 2.2 g (70% overall) of **57** as a white powder: ^{1}H NMR ($CDCl_3$) δ 8.20 (1H, s), 8.13 (1H, s), 7.77 (1H, s), 7.71 (1H, d, J = 16.2), 7.50 (1H, br s), 6.56 (1H, d, J = 16.2), 4.54 (2H, s), 4.29 (2H, q, J = 7.0), 1.35 (3H, t, J = 7.0).

3-(3-Carbamoyl-5-hydroxymethyl-phenyl)-acrylic Acid Ethyl Ester (13). FMOC-Rink polystyrene resin (0.50 g, 0.16 mmol) in a shaking vessel was treated with a 1:1 mixture of piperidine and DMF (15 mL) and shaken for 30 min. The resin was washed with DMF (3 × 15 mL) and CH_2Cl_2 (3 × 15 mL). Acid **55** (122 mg, 0.25 mmol) in DMF (10 mL) was added to the resin, followed by DIEA (0.09 mL, 0.50 mmol) and HATU (95 mg, 0.25 mmol). The mixture was shaken 1 h, then drained and washed with DMF (3 × 15 mL) and CH_2Cl_2 (3 × 15 mL). TBAF (0.8 mL of 1 M solution in THF, 0.80 mmol) and THF (10 mL) were added to the resin and shaken 4 h. The vessel was drained and washed with THF (3 × 15 mL), MeOH (3 × 15 mL), H_2O (3 × 15 mL), MeOH (3 × 15 mL), and CH_2Cl_2 (3 × 15 mL). The linker was cleaved with 95:5 TFA−H_2O (20 mL). Evaporation of the solvent, followed by purification of the residue on silica gel (EtOAC elutant) yielded 37 mg (90%) of **13**: ^{1}H NMR (CD_3OD) δ (1H, s), 7.94 (1H, s), 7.80 (1H, s), 7.77 (1H, d, J = 16.2), 6.66 (1H, d, J = 16.1), 4.72 (2H, s), 4.29 (2H, q, J = 7.0) 1.36 (3H, t, J = 7.0); MS (FAB) 250 (MH^+), 272 (MNa^+).

Resin 58 (Functionalized with 3-Bromomethyl-5-(2-ethoxycarbonyl-vinyl)-benzoic Acid 57). FMOC-Rink amide polystyrene resin (3.00 g, 1.97 mmol) was treated with a 1:1 mixture of piperidine and DMF (30 mL) and shaken 30 min. The vessel was drained, and the resin was washed with DMF (3 × 25 mL) and then CH_2Cl_2 (3 × 25 mL). In another flask, **57** (0.93 g, 2.96 mmol) and HOBT (0.40 g, 2.96 mmol) in CH_2Cl_2 (30 mL) were treated with DIC (0.93 mL, 5.91 mmol) and held at 23 °C for 45 min. This solution was then added to the resin and shaken for 6 h. The vessel was drained, and the resin was washed with CH_2Cl_2 (3 × 25 mL), MeOH (3 × 25 mL),

then CH_2Cl_2 (3 × 25 mL). The resin **58** was dried under vacuum and stored in a desiccator.

3-[3-Carbamoyl-5-(5-pyridin-2-yl-[1,3,4]oxaldiazol-2-yl-sulfanylmethyl-phenyl]-acrylic Acid Ethyl Ester (25). Resin **58** (100 mg, 0.063 mmol) in DMF (1 mL) and DIEA (0.11 mL, 0.63 mmol) in a screw-top vial was treated with 2-(2-pyridyl)-5-thiol-1,3,4-oxadiazole (50 mg, 0.28 mmol) and heated at 70 °C overnight. The resin was then transferred to a fritted vessel and washed with DMF (3 × 10 mL), MeOH (3 × 10 mL), and CH_2Cl_2 (3 × 10 mL). The resin was treated with 95:5 TFA−CH_2Cl_2 (10 mL), shaken 1 h, and filtered and the filtrate evaporated. The residue was treated with 10% Et_3N−MeOH (3 mL), then evaporated again. The resulting material was purified by silica gel chromatography (EtOAc elutant) to yield 10 mg (38%) of **25**: ^{1}H NMR ($CDCl_3$) δ 8.75 (1H, d, J = 4.0), 8.18 (1H, d, J = 7.7), 8.04−7.88 (3H, m), 7.80 (1H, s), 7.65 (1H, d, J = 16.2), 7.51−7.47 (1H, m), 6.51 (1H, d, J = 16.2), 6.40 (1H, br s), 5.70 (1H, br s), 4.54 (2H, s), 4.25 (2H, q, J = 7.0), 1.32 (3H, t, J = 7.0); MS (FAB) 411 (MH^+), 433 (MNa^+).

3-[3-Carbamoyl-5-(4-pyridin-2-yl-piperizin-1-methyl)-phenyl]-acrylic Acid Ethyl Ester (26). This was prepared with 1-(2-pyridyl)piperazine using conditions described for the synthesis of **25** to yield 12 mg (48%) of **26**: ^{1}H NMR ($CDCl_3$) δ (1H, m), 7.91 (1H, s), 7.79 (1H, s), 7.64 (1H, d, J = 16.0), 7.62 (1H, s), 7.47−7.41 (1H, m), 6.62−6.57 (2H, m), 6.48 (1H, d, J = 16.0), 4.21 (2H, q, J = 7.0), 3.55 (2H, s), 3.49−3.45 (4H, m), 2.55−2.51 (4H, m), 1.32 (3H, t, J = 7.0); MS (FAB) 395 (MH^+), 417 (MNa^+).

3-(3-{[Benzyl-(2-ethoxycarbonyl-ethyl)-amino]-methyl}-5-carbamoyl-phenyl)-acrylic Acid Ethyl Ester (23). This was prepared with *N*-benzyl-3-aminopropionic acid ethyl ester using conditions described for the synthesis of **25** to yield 18 mg (67%) of **23**: ^{1}H NMR ($CDCl_3$) δ 7.95 (1H, s), 7.85 (1H, s), 7.70 (1H, d, J = 16.0), 7.50 (1H, s), 7.38−7.20 (5H, m), 6.95 (1H, s), 6.60 (1H, d, J = 16.0), 5.70 (1H, s), 4.25 (2H, q, J = 7.0), 4.15 (2H, q, J = 7.0), 3.75 (2H, s), 3.63 (2H, s), 2.82 (2H, t, J = 5.0), 2.55 (2H, t, J = 5.0), 1.36 (3H, t, J = 7.0); MS (ES) 439 (MH^+), 461 (MNa^+).

3-{3-Carbamoyl-5-[(ethyl-pyridin-4-ylmethyl-amino)-methyl]-phenyl}-acrylic Acid Ethyl Ester (27). This was prepared with 4-(ethylaminomethyl)pyridine using conditions described for the synthesis of **25** to yield 10 mg (43%) of **27**: ^{1}H NMR ($CDCl_3$) δ 8.75 (2H, d, J = 4.0), 8.05 (1H, s), 7.95 (1H, s), 7.75−7.55 (4H, m), 6.50 (1H, d, J = 16.0), 4.30 (2H, q, J = 7.0), 4.18 (2H, q, J = 8.0), 3.95 (2H, s), 3.90 (2H, s), 1.40−1.20 (6H, m); MS (ES) 368 (MH^+), 390 (MNa^+).

4-[3-Carbamoyl-5-(2-ethoxycarbonyl-vinyl)-benzyl]-piperazine-1-carboxylic Acid Ethyl Ester (28). This was prepared with ethyl-1-piperazine carboxylate using conditions described for the synthesis of **25** to yield 15 mg (63%) of **28**: ^{1}H NMR ($CDCl_3$) δ 8.07 (1H, s), 7.90 (1H, s), 7.66 (1H, s), 4.19 (2H, q, J = 7.4), 4.14 (2H, s), 4.07 (2H, q, J = 7.4), 3.68 (4H, m), 3.55 (4H, m), 1.26 (3H, t, J = 7.4), 1.19 (3H, t, J = 7.4). MS (ES) 390 (MH^+), 412 (MNa^+).

3-{3-Carbamoyl-5-(4-pyrimidin-2-yl-piperazin-1-ylmethyl)-phenyl]-acrylic Acid Ethyl Ester (29). This was prepared with 1-(2-pyrimidyl)piperazine using conditions described for the synthesis of **25** to yield 15 mg (60%) of **29**: ^{1}H NMR ($CDCl_3$) δ (2H, d, J = 4.8), 7.88 (1H, s), 7.80 (1H, s), 7.71 (1H, d, J = 16.2), 7.70 (1H, s), 6.53 (1H, d, J = 16.2), 6.48 (1H, t, J = 4.8), 4.27 (2H, q, J = 7.0), 3.84 (4H, t, J = 5.2), 3.59 (2H, s), 2.51 (4H, t, J = 4.8), 1.34 (3H, t, J = 7.0); MS (FAB) 396 (MH^+), 418 (MNa^+).

3-{3-Carbamoyl-5-[4-(2-cyano-phenyl)-piperazin-1-yl-methyl]-phenyl}-acrylic Acid Ethyl Ester (30). This was prepared with 1-(2-cyanophenyl)-piperazine using conditions described for the synthesis of **25** to yield 19 mg (73%) of **30**: ^{1}H NMR ($CDCl_3$) δ 7.90 (1H, s), 7.81 (1H, s), 7.70 (1H, d, J = 15.8), 7.69 (1H, s), 7.57−7.54 (1H, m), 7.51−7.45 (1H, m), 7.03−6.98 (2H, m), 6.53 (1H, d, J = 15.8), 4.27 (2H, q, J = 7.4), 3.63 (2H, s), 3.24 (4H, q, J = 4.8), 2.68 (4H, q, J = 4.8), 1.34 (3H, t, J = 7.0); MS (FAB) 419 (MH^+), 441 (MNa^+).

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