Discovery and Structure−Activity Relationships of Imidazole-Containing Tetrahydrobenzodiazepine Inhibitors of Farnesyltransferase

Charles Z. Ding,[§] Roberta Batorsky,[†] Rajeev Bhide,[§] Hannguang J. Chao,[‡] Young Cho,[§] Saeho Chong,[▽] Johnni Gullo-Brown,[†] Peng Guo,[§] Soong Hoon Kim,[§] Frank Lee,[†] Katerina Leftheris,[§] Arthur Miller,[§] Toomas Mitt,[§] Manorama Patel,[§] Becky A. Penhallow,[†] Carol Ricca,[†] William C. Rose,[†] Robert Schmidt,[§] William A. Slusarchyk,[§] Gregory Vite,[§] Ning Yan,[†] Veeraswamy Manne,[†] and John T. Hunt*[,§]

Departments of Oncology Chemistry, Metabolism and Pharmacokinetics, Combinatorial Drug Discovery, and Oncology Drug Discovery, Bristol-Myers Squibb Pharmaceutical Research Institute, Princeton, New Jersey 08543-4000

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2,3,4,5-Tetrahydro-1-(imidazol-4-ylalkyl)-1,4-benzodiazepines were found to be potent inhibitors of farnesyltransferase (FT). A hydrophobic substituent at the 4-position of the benzodiazepine, linked via a hydrogen bond acceptor, was important to enzyme inhibitory activity. An aryl ring at position 7 or a hydrophobic group linked to the 8-position through an amide, carbamate, or urea linkage was also important for potent inhibition. 2,3,4,5-Tetrahydro-1-(1*H*-imidazol-4-ylmethyl)-7-(4-pyridinyl)-4-[2-(trifluoromethoxy)benzoyl]-1*H*-1,4-benzodiazepine (**36**), with an FT IC_{50} value of 24 nM, produced 85% phenotypic reversion of Ras transformed NIH 3T3 cells at 1.25 μM and had an EC_{50} of 160 nM for inhibition of anchorage-independent growth in soft agar of H-Ras transformed Rat-1 cells. Selected analogues demonstrated ip antitumor activity against an ip Rat-1 tumor in mice.

Introduction

Ras proteins are a family of key molecules involved in cellular signal transduction.[1] These proteins act as on−off switches by preventing signal transmission when GDP-bound but allowing it when GTP-bound. The ability to signal is controlled by the intrinsic GTPase activity of the protein and its stimulation by GTPase-activating protein (GAP). Activating mutations of the Ras protein result in reduced GTPase activity through ineffective stimulation of GAP activity. Therefore higher levels of the competent signaling complex are observed in a substantial number of tumor types with Ras mutations, in particular those of the colon, pancreas, and lung. The signaling functions of both normal and oncogenic Ras are dependent upon its membrane association, which is accomplished through a series of enzymatic transformations of cytosolic Ras, namely farnesylation, C-terminal tripeptide hydrolysis, and carboxymethylation. The demonstration that the key step in this sequence of reactions is the farnesylation of ras by the enzyme protein farnesyltransferase (FT) has focused a great deal of attention on interrupting this enzymatic transformation as a potential anticancer therapeutic modality.[2] Interestingly, cellular and in vivo testing of various FT inhibitors has led to the supposition that some of the effects of these compounds are not mediated through inhibition of Ras processing.[3,4] Therefore, although FT was identified as an intriguing drug target due to its involvement in oncogenic Ras processing, it should be kept in mind that the defined target is FT and that FT inhibitors are likely more than just 'Ras inhibitors'.

FT is a zinc metalloenzyme, with the zinc believed to play an important role in the catalytic mechanism.[5] Many of the early inhibitors of FT were peptidomimetics, based on the C-terminal CAAX motif of the Ras protein, containing a thiol as a zinc ligand (see ref 6). Our search for a thiol surrogate which could function as a zinc ligand led to our discovery of high-affinity imidazole-based tetrapeptide inhibitors of FT.[7] While this was the first report in the open literature on the use of an imidazole functionality in a potent FT inhibitor, the discovery by Merck researchers of imidazole-based tetrapeptide FT inhibitors was clearly contemporaneous.[8]

Although selected groups had demonstrated preclinical in vivo antitumor activity using peptide-based inhibitors, we sought potent FT inhibitors which incorporated the key imidazole functionality into a non-peptide framework.[9−11] The preclinical activity, followed by progression into human clinical trials, of Janssen's imidazole-based FT inhibitor R115777 and Schering's tricyclic FT inhibitor SCH 66336 support the wisdom of seeking non-peptide inhibitors.[12,13] In this report we describe the development of potent FT inhibitors based on the attachment of an imidazol-4-ylmethyl chain to the 1-position of the 2,3,4,5-tetrahydro-1,4-benzodiazepine nucleus. The best of these compounds are low double-digit nanomolar inhibitors of FT, produce cell effects at concentrations as low as 100−200 nM, and demonstrate antitumor activity in an ip/ip tumor model.

Chemistry

Compounds **4** and **6** (Table 1) were prepared from 4-phenylmethyl-2(*S*)-(2-methoxyethyl)piperazine by carbodiimide-mediated acylation of the appropriate protected (**6**) or unprotected (**4**) imidazolylalkanoic acid, followed by carbonyl reduction with LAH, hydrogenolysis of the benzyl group, and acylation with 1-naphthoic

* To whom correspondence should be addressed. Tel: (609) 252-4989. Fax: (609) 252-6601. E-mail: john.hunt@bms.com.
[§] Department of Oncology Chemistry.
[▽] Department of Metabolism and Pharmacokinetics.
[‡] Department of Combinatorial Drug Discovery.
[†] Department of Oncology Drug Discovery.

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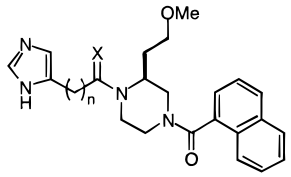
Table 1. Imidazole-Containing Piperazine Inhibitors of FT



compd	*n*	X	FT IC_{50} (μM)
1[b]	0	O	51% at 90 μM
2[b]	0	H,H	1.3 ± 0.18
3[b]	1	O	34 ± 3
4[a]	1	H,H	3.2 ± 0.3
5[a]	2	O	27 ± 3
6[a]	2	H,H	10 ± 1.5

[a] Elemental analysis for C, H, N. [b] HRMS.

acid. The remaining compounds in Table 1 were prepared from 1-[(1,1-dimethylethoxy)carbonyl]-2(*S*)-(2-methoxyethyl)piperazine.[14] Compounds **1**, **3**, and **5** were prepared by carbodiimide-mediated acylation with 1-naphthoic acid, removal of the Boc protecting group, and acylation with the appropriate imidazolylalkanoic acid, followed by imidazole deprotection if necessary (**1**, **5**). For compound **2**, the second acylation was replaced with reductive alkylation with 1-tritylimidazole-4-carboxaldehyde followed by imidazole deprotection.

The 2,3,4,5-tetrahydro-1,4-benzodiazepine ring system **47** was prepared by condensation of the appropriately substituted isatoic anhydride (R = H, 7-Br, 8-Cl, 8-NO_2, 9-Me) with glycine ethyl ester hydrochloride in pyridine at elevated temperature to form the benzodiazepinedione, followed by reduction with lithium aluminum hydride or diborane (Scheme 1). Acylation/sulfonylation of the more basic 4-amine to form **48** was accomplished by condensation with the appropriate carboxylate ester, acid chloride, sulfonyl chloride, or carbamyl chloride or by carbodiimide-mediated coupling of the appropriate carboxylic acid. Imidazole attachment at N-1 to form **49** was generally achieved by reductive alkylation with the optionally protected imidazolylalkanal, followed by deprotection if necessary. Compound **7** was prepared by protection of N-4 to form **50**, acylation with protected imidazoleacetic acid, amide reduction with concomitant double deprotection, and N-4 acylation. Imidazole attachment at N-4 was achieved by acylation of **50**, deprotection of N-4, and reductive alkylation. Compound **11** was prepared from **8** by tritylation of the imidazole π-nitrogen, reaction with benzyl triflate prepared in situ, and deprotection with aqueous acetic acid at reflux.

The 8-amino-1,4-benzodiazepine analogue **35** was prepared from the 8-nitro analogue (data not shown) by reduction with $TiCl_3$ in aqueous acetic acid. Acylated/sulfonylated analogues were prepared from **35** using acid chlorides (**24, 22**), acid anhydrides (**28**), isocyanates (**25**), chloroformates (**23**), or sulfonyl chlorides (**32**).

The 7-phenyl substituent was introduced by palladium-catalyzed coupling of 7-bromo-1,4-benzodiazepine-2,5-dione with phenylboronic acid. A more convergent approach to 7-aryl-substituted analogues involved a palladium coupling of the arylstannane with the final 7-bromo analogue **31** in which the imidazole was protected with either a trityl or trifluoroacetyl group. These couplings were found to not proceed in the presence of an unprotected imidazole, presumably due to the intervention of palladium−imidazole complexation. The unprotected bromo analogue **31** could also be metalated with 2 equiv of *n*-BuLi and reacted with electrophiles such as cyclohexanone. In this case, the resulting tertiary alcohol was reduced with $NaBH_4$/TFA to afford the 7-cyclohexyl analogue **30**.

The 4-naphthylmethyl analogue **44** was prepared by LAH reduction of **26**, while the N-4-aryl analogue **42** was prepared by potassium carbonate-mediated reaction with the appropriate chlorotetrazole.

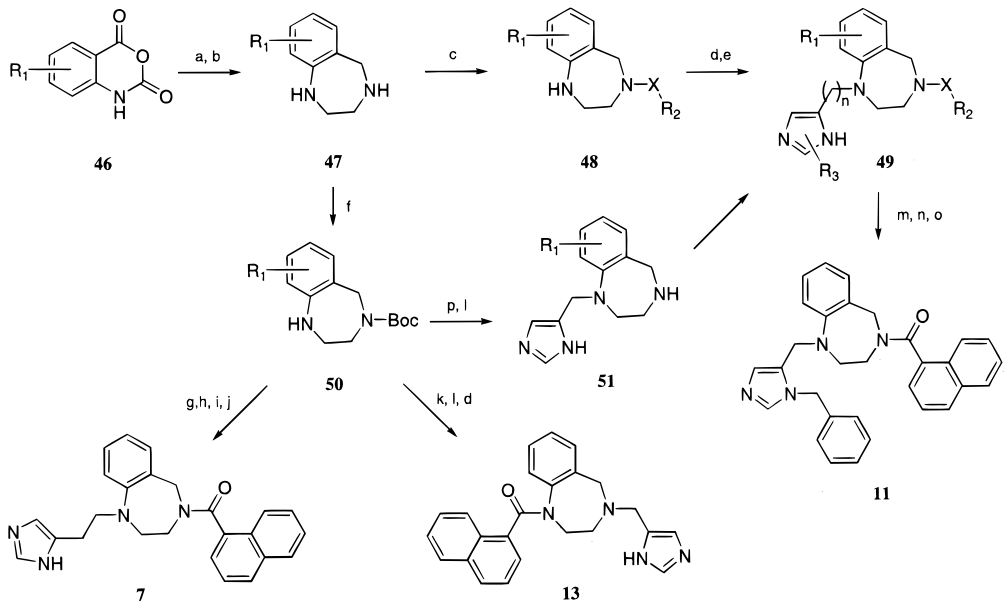
A number of analogues in Tables 3 and 5 were prepared by automated parallel synthesis techniques. For those in Table 3, EDC/HOBT couplings of carboxylic acids were performed on 2,3,4,5-tetrahydro-1-(1*H*-imidazol-4-ylmethyl)-1*H*-1,4-benzodiazepine (**51a**, R_1 = H) which was bound to Merrifield resin through an imidazole nitrogen. The products were cleaved from the resin with HBr/TFA/thioanisole. For most of the 4-substituted 7-phenyl analogues, including some included in Table 5, automated solution syntheses were performed using diisopropylcarbodiimide/HOAT-mediated coupling of carboxylic acids to 2,3,4,5-tetrahydro-1-(1*H*-imidazol-4-ylmethyl)-7-phenyl-1*H*-1,4-benzodiazepine (**51b**, R_1 = 7-phenyl).

Results and Discussion

Biological Testing. Enzyme inhibition assays measured the incorporation of tritium from tritiated farnesyl pyrophosphate into recombinant human H-Ras, using recombinant human FT.[15] Whole cell activity was determined by two methods. Cell-permeable FT inhibitors reverse the spindle-shaped refractile transformed morphology of H-Ras transformed Rat-1 cells into a cobble stone, flat, and nonrefractile normal morphology. Reversion of the cells begins to appear as early as 24 h following drug addition and is complete by 72 h of treatment. The degree of the morphological reversion can be easily scored by microscopic observations. A second cell-based assay measured the ability of compounds to prevent the anchorage-independent growth of H-Ras transformed NIH 3T3 cells.[16] Antitumor testing involved ip or sc implantation of 1×10^6 Rat-1 cells, followed by ip or iv injection of compound (dissolved in 10% ethanol in water) once daily, beginning on day 1 post-implant. Therapeutic results are presented in terms of increases in lifespan reflected by the relative median survival time (MST) of treated (T) versus control (C) groups and is expressed as a maximum %T/C. Adriamycin was included as a positive control.

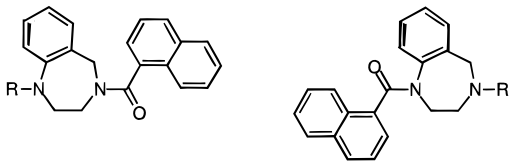
Structure−Activity Relationships. Like many of the groups involved in early efforts to produce inhibitors of FT, some of our initial successes generated extremely potent thiol tetrapeptide inhibitors.[17] However, their enzyme inhibitory potency translated to cellular effects only at much higher concentrations, and the peptidic nature of the inhibitors was considered a liability for in vivo application. Subsequent efforts focused on the discovery of more stable and druglike molecules, with the specific aims of finding a surrogate for the thiol as well as a non-peptidic template upon which to attach this putative zinc ligand. We previously described the discovery that an imidazole attached through various linkers to a tetrapeptide framework provides very potent

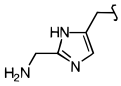
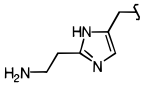
Scheme 1[a]



[a] (a) HCl−$H_2NCH_2CO_2Et$, pyridine, reflux; (b) LAH or BH_3; (c) R_2CO_2Et, R_2COCl, R_2CO_2H or R_2SO_2Cl (X = CO or SO_2); (d) optionally protected imidazolylalkanal, $NaBH(OAc)_3$; (e) deprotection if necessary; (f) $(tBuOCO)_2O$, THF; (g) *N*-trityl-4-imidazoleacetic acid, iBuOCOCl; (h) BH_3; THF; (i) HCl; (j) 1-naphtholic acid, EDC; (k) 1-naphthoyl chloride, pyridine, CH_2Cl_2; (l) TFA, CH_2Cl_2; (m) trityl chloride, Et_3N, acetonitrile; (n) benzyl triflate, THF; (o) aq AcOH, reflux; (p) 4-formylimidazole, $NaBH(OAc)_3$.

Table 2. (Imidazolylalkyl)benzodiazepine Inhibitors of FT



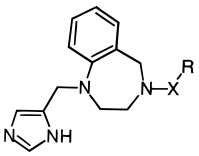
compd	compd class	R	FT IC_{50} (μM)	% rev (10 μM)	SAG EC_{50} (μM)
7[a]	A	imidazol-4-ylethyl	0.34 ± 0.007	20	
8[a]	A	imidazol-4-ylmethyl	0.46 ± 0.13	0	6
9[b]	A	imidazol-2-ylpropyl	0.59 ± 0.05		
10[a]	A		0.84 ± 0.34		
11[b]	A	1-phenylmethylimidazol-5-ylmethyl	1.1 ± 0.02		
12[a]	A		1.3 ± 0.18		
13[a]	B	imidazol-4-ylmethyl	2.8 ± 0.11	0	3
14[b]	A	imidazol-2-ylmethyl	21 ± 3		

[a] Elemental analysis for C, H, N. [b] HRMS.

FT inhibitors.[7] In our search for a suitable template upon which to attach an imidazole ligand, we became aware of a publication from Merck describing potent FT inhibitors resulting from attachment of a thiol functionality to a piperazine.[18] These compounds appeared to fit a pharmacophore model which had evolved within our own group in which a zinc ligand, two hydrophobes, and a hydrogen bond-accepting group, when appropriately positioned, provided good FT inhibition. Attachment of an imidazole via a number of linkers to several substituted piperazines afforded modest inhibitors, with the most potent analogues, containing a methylene or ethylene linker, demonstrating only single-digit micromolar IC_{50} values (Table 1).

Despite the modest potency of these analogues, we continued to investigate templates related to piperazines which might support our pharmacophore model. Extrapolating from the evidence that a hydrophobic side chain at the 2-position was important to the inhibitory potency of the thiol-containing piperazines, we postulated that the critical side chain could be replaced by a fused aryl ring in the same region of the molecule.[18] This change, combined with expansion of the saturated ring from a piperazine to a homopiperazine, afforded a tetrahydrobenzodiazepine template. While tetrahydrobenzodiazepines are somewhat less common than benzodiazepines, the latter has been termed a 'privileged structure' because it has served as the template

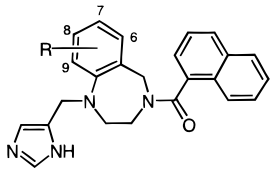
Table 3. N-4 Substituents



compd	X	R	FT IC_{50} (μM)	% rev (10 μM)	SAG EC_{50} (μM)
15[b]	CO	2-biphenylyl	0.32[c]	25	0.7
16[a]	SO_2	1-naphthalenyl	0.48 ± 0.008		
17[b]	CO	2-(phenylmethylamino)phenyl	0.6[c]		2.4
18[b]	CO	2-phenoxyphenylmethyl	0.7[c]		
19[a]	SO_2	2-(methoxycarbonyl)phenyl	0.72 ± 0.09		1.6
20[a]	CO	diphenylamino	1.2 ± 0.33		

[a] Elemental analysis for C, H, N. [b] Prepared by automated solid-phase synthesis. [c] $n = 1$.

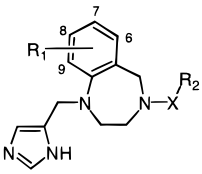
Table 4. Fused Aryl Ring Substituents



compd	R	FT IC_{50} (μM)	% rev (1.25 μM/5 μM)	SAG EC_{50} (μM)
21[b]	7-(pyrid-4-yl)	15.4 ± 0.9	60/95	0.2
22[b]	8-NHCO-cyclohexyl	18.1 ± 0.4	0/45	0.34
23[b]	8-NHCO-cyclohexyl	28 ± 7	40/95	0.11
24[b]	8-NHCO-phenyl	28.5 ± 2.5	0/75	0.3
25[b]	8-NHCONH-cyclohexyl	35.5 ± 0.5	0/0	4
26[a]	7-phenyl	95 ± 13	0/90	1.6
27[b]	7-(pyrid-3-yl)	145 ± 5	0/70	1
28[a]	8-NHCOMe	168 ± 5	0/0	
29[a]	7-(pyrid-2-yl)	177 ± 5	0/0	
30[b]	7-cyclohexyl	186 ± 16	0/20	
31[a]	7-Br	228 ± 7	0/5	
32[b]	8-$NHSO_2$-phenyl	249 ± 9	0/0	11
33[a]	9-Me	350 ± 55	0/35	12
34[a]	8-Cl	540 ± 89		
35[b]	8-NH2	1000 ± 268		2.4

[a] Elemental analysis for C, H, N. [b] HRMS.

Table 5. N-4 Substituents with 7- or 8-Substituents



compd	R_1	X	R_2	FT IC_{50} (μM)	% rev (1.25 μM/5 μM)	SAG EC_{50} (μM)
36[b]	7-pyrid-4-yl	CO	2-trifluoromethoxyphenyl	24 ± 1	85/95	0.16
37[c]	7-phenyl	CO	2-methoxynaphth-1-yl	58 ± 18	85/95	0.55
38[a]	7-phenyl	COCO	4-methoxyphenyl	91 ± 17	0/90	0.9
39[c]	7-phenyl	CO	2-(hydroxyethylthio)phenyl	124 ± 35	0/90	0.2
40[a]	7-phenyl	CO	tetrahydroquinol-1-yl	205 ± 16	0/40	
41[b]	7-phenyl	SO2	naphth-1-yl	426 ± 81	0/25	
42[a]	7-phenyl	–	1-phenyltetrazol-5-yl	702 ± 1	0/0	
43[a]	7-phenyl	C(NCN)NH	4-chlorophenyl	1423 ± 198	0/5	
44[a]	7-phenyl	CH_2	1-naphthyl	2968 ± 105	0/0	
45[a]	7-phenyl	CO2	phenylmethyl	55% at 15	0/0	

[a] Elemental analysis for C, H, N. [b] HRMS. [c] Prepared by automated parallel solution synthesis.

for a variety of small molecules which are biologically active against diverse protein targets.[19] Interestingly, a benzodiazepine was used as a constrained dipeptide mimic in an early series of thiol tetrapeptide inhibitors of FT.[20]

Applying our pharmacophore model to the tetrahydrobenzodiazepine template, we utilized the 1-naphthylcarbonyl group to supply the second hydrophobe as well as the hydrogen bond-accepting group. We then attached the putative zinc ligand as either a 1-(imidazol-

4-ylmethyl) or 4-(imidazol-4-ylmethyl) substituent to afford **8** and **13**, respectively (Table 2). Gratifyingly, the benzodiazepine **8** in which the imidazole was appended to N-1 provided a submicromolar inhibitor. Equally promising was the observation that **8** was an effective inhibitor of anchorage-independent transformed cell growth at concentrations only 10-fold higher than those needed for enzyme inhibition. This suggested that the benzodiazepine template could afford highly cell-permeable compounds.

To understand the nature of FT inhibition by such novel (imidazolylmethyl)benzodiazepine inhibitors, the kinetics of inhibition by compound **8** were analyzed by initial velocity studies at various concentrations of H-Ras and a fixed concentration of FPP. The double-reciprocal plots intercepted the $1/v$ axis at the same point, indicating that the V_{max} remained unchanged with increasing amounts of the inhibitor. Compound **8** increased the apparent K_m for Ras (plot available as Supporting Information). The change in K_m with V_{max} being unaffected is a characteristic feature of a competitive inhibitor for the varied substrate (Ras). Because the replot of the reciprocal plot slopes (K_m/V_{max}) versus the concentration of **8** is linear, **8** can be classified as a pure competitive inhibitor with respect to the Ras substrate ($K_i = 456$ nM; replot available as Supporting Information). Kinetic analysis of **8** inhibition with respect to FPP was also performed. In the presence of the inhibitor, both the K_m and V_{max} for FPP were affected (data not shown). The plots clearly indicated that **8** is not a competitive inhibitor with respect to FPP, but either a noncompetitive or mixed type inhibitor. However, the inhibition kinetics are complex, and the more rigorous analysis required to determine the precise nature of inhibition with respect to FPP was not performed.

Returning to our structure−activity (SAR) investigations, we next prepared a number of N-1-imidazolylalkyl analogues to explore the effects of linker length and site of imidazole attachment. Attachment at C-2 of the imidazole in the methylene linker series was detrimental (**14**). The imidazol-4-yl analogue with an ethylene linker (**7**) was equipotent to the methylene linker, but the more difficult synthesis of the former compound led us to focus on analogues with a methylene linker. Once the chain was extended to propylene, attachment at C-2 of the imidazole became acceptable (**9**). Several attempts to create a putative bidentate ligand for the catalytic zinc by attaching an amino group to the imidazole via an alkyl chain did not lead to more potent inhibitors (**10, 12**). Alternative ligands to the basic, protonatable amine functionality were not pursued. Attachment of a phenylmethyl group on the π-nitrogen did not lead to an increase in potency (**11**) suggesting that this substitution pattern was unable to access the novel aromatic FT binding pocket recently reported.[21] Nevertheless, the retention of inhibitory activity by **11** suggested that the key zinc ligand in the methylene series was the τ-nitrogen.

Limited studies of the N-4 hydrogen bond acceptor and hydrophobe were performed on the parent 1-(imidazol-4-ylmethyl)tetrahydrobenzodiazepine, utilizing either manual or parallel synthesis (Table 3). The sulfonamide analogue **16** was equipotent to the carboxamide **8**, suggesting some flexibility in the nature of the hydrogen bond acceptor. Ureas such as **20** were only slightly less potent than **16** or **8**. From a parallel solution synthesis of carboxamides, a few submicromolar compounds were discovered, each of which contained a phenyl- or phenylmethylamide with a hydrophobic 2-substituent appended either directly or via a linker. In particular, the biphenylamide **15** was a submicromolar inhibitor of anchorage-independent transformed cell growth. Several amides in which a two- or three-atom linker separated the carbonyl and the aryl group were somewhat less potent (data not shown). Nevertheless, a variety of amide substituents appeared to be tolerated without dramatic loss of activity.

Because large improvements in inhibitory potency were not immediately afforded by modification of the 4-substituent, attention was turned toward substitution on the fused aryl ring (Table 4). Although a few 9-substituted analogues were prepared (e.g., **33**), synthetic accessibility led us to focus our studies on 7- or 8-substituted analogues. At the 7-position, a variety of aryl groups led to potent inhibitors. Both enzyme inhibitory activity and cellular activity were improved by replacement of the 7-phenyl substituent with the 7-(pyrid-4-yl) substituent (compare **26** and **21**). However, polar substitutions in the region of the ortho position led to somewhat poorer inhibitors (**29**). A 7-cyclohexyl group (**30**) was somewhat poorer than a 7-phenyl ring (**26**), suggesting either a size constraint to the binding pocket or the need for an aromatic ring. The former hypothesis is suggested by the intermediate activity of the 7-bromo analogue **31**. Several of the most potent 7-aryl analogues (**21**, **26**, **27**) displayed substantial reversion activity in the 1−5 μM range and inhibited anchorage-independent soft agar growth in the 0.2−1 μM range.

At the 8-position, small substituents such as chloro (**34**) led to equipotent analogues, while small polar substituents such as an 8-amino group (**35**) produced weaker FT inhibitors. The effect of larger substituents was primarily explored through the synthesis of analogues of the 8-amino derivative **35**. While modest increases in inhibitory potency were achieved by small acyl groups such as acetyl (**28**), the most potent inhibitors contained a hydrophobic cycloalkyl or aryl group linked to the 8-amine through an amide, urea, or carbamate. An 8-arylsulfonamide (**32**) was substantially less potent. Several of the most potent 8-acylamino analogues (**22**, **23**, **24**) displayed substantial reversion activity in the 1−5 μM range and inhibition of anchorage-independent soft agar growth at concentrations as low as 100−300 nM. It is possible that the poor cell activity of the potent urea **25** is due to reduced cell permeability resulting from its increased hydrogen-bonding capability.

The SAR of the 4-substituent was reinvestigated using substitution patterns at the 7-position (phenyl, pyrid-4-yl) or 8-position (NHCO-cyclohexyl) which had led to potent analogues in the 4-(1-naphthoyl) series. Selected analogues from this study are presented in Table 5. The majority of compounds prepared were carboxamide analogues, most of which were synthesized using an extensive parallel synthesis approach. Analogues with alternate linkers or with direct heterocycle attachment were prepared manually. Again, relatively

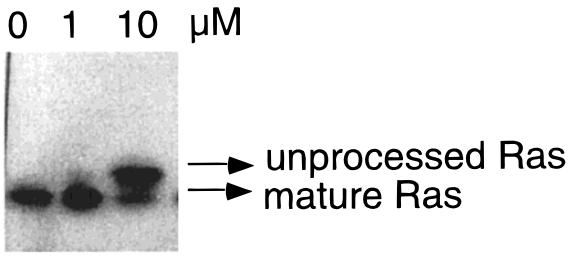


Figure 1. Inhibition of Ras protein processing by **26**. Rat-1 CVLS cells overexpressing 61L activated H-Ras were treated with 0 μM (lane 1), 1 μM (lane 2), and 10 μM (lane 3) **26** for 72 h. Detergent extracts were prepared, clarified by centrifugation, analyzed by 15% low-cross-linking SDS−PAGE, transferred to immobilon P membrane, probed with 146-03E4 antibody, and detected by ECL detection system.

potent inhibitors were afforded by ortho-substituted arylamides and by sulfonamides, and substantial tolerance was observed in the nature of the group attached to the N-4 linker. Nevertheless, the only novel structural modification which was found to maintain potent FT inhibitory activity was an α-ketoamide such as **38**. Alternate linkers such as ureas (**40**), cyanoguanidines (**43**), and carbamates (**45**) led to less potent inhibitors. The importance of the linking hydrogen bond acceptor was highlighted by the poor activity of the methylene-linked compound **44**. Interestingly, moderately potent analogues such as **42** indicate that the putative hydrogen bond acceptor can be incorporated into a heterocyclic ring directly attached to N-4.

Ras Processing Inhibition. Having demonstrated good cellular effects of these potent FT inhibitors in both an assay which measured reversal of a transformed phenotype and an assay which measured anchorage-independent cell growth, it was of interest to examine the relationship between these activities and the inhibition of Ras protein processing. Unprocessed Ras can be differentiated from processed Ras in cell extracts by the slower migrating ability of the former on gels. Tetrahydrobenzodiazepine FT inhibitors such as **26** inhibit Ras protein processing in Rat-1 cells overexpressing activated H-Ras (Figure 1). Concentrations which produced morphological reversion correlated reasonably well with those which provided inhibition of Ras processing as well as inhibition of growth in soft agar (data not shown). We generally observed at least partial reversion and inhibition of Ras processing at 2−5-fold higher concentrations compared to those which produced 50% inhibition of soft agar growth.

In Vivo Antitumor Testing. Selected compounds which demonstrated submicromolar activity in cellular assays were tested for in vivo antitumor activity in the Rat-1 tumor model. Tumor cells were implanted ip, and compounds were administered ip once daily for 7 days. Lifespan increases for the treated versus the untreated controls were measured, and a %T/C value of ≥125 was considered an active result. In the first experiment, **26** and **24** yielded active results with %T/C values of 136 and 128, respectively (Table 6). In a second experiment, **26** yielded an inactive result at the only dose evaluated (30 mpk/inj, %T/C = 96), as did **21** (%T/C = 108), while **22** was active (%T/C = 138). The variability in the activity of **26** was not considered exceptional for a compound of modest in vivo activity.

Table 6. In Vivo Antitumor Testing versus ip Rat-1 Tumors

compd	expt no.	optimal dose (mpk/inj)[a]	maximum %T/C
26	1[b]	10	136
26	2[c]	30	96
24	1[b]	90	128
22	2[c]	10	138
21	2[c]	90	108

[a] qd×7 ip, maximum tolerated dose shown when no activity was observed. [b] Control median survival time (MST) 12.5 days, adriamycin (3 mpk/inj, days 1, 4, 7) %T/C = 148. [c] Control MST 13 days, adriamycin (3 mpk/inj, days 1, 4, 7) %T/C = 158.

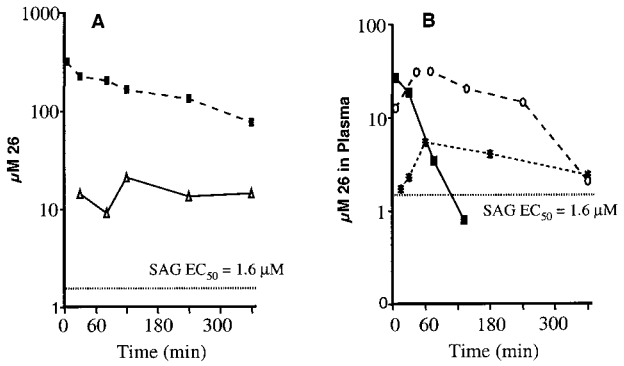


Figure 2. Pharmacokinetics of **26** in mice: A, levels in plasma (△) or ip fluid (■) following ip administration of a 90 mpk dose in 10% ethanol:90% water; B, levels in plasma following administration of an iv dose (■; 30 mpk), ip dose (○; 90 mpk), or po dose (∗, 90 mpk).

26 was also evaluated for distal site activity versus sc implanted Rat-1 tumor cells. Intravenous administration of **26**, at doses as great as 30 mg/kg/inj (higher doses limited by solubility constraints) once daily for 7 days, as well as ip injections of up to 90 mg/kg/inj once daily for 11 days, failed to inhibit Rat-1 primary tumor growth. To explore the lack of distal activity with this minimally ip/ip active compound, a limited pharmacokinetic evaluation of **26** was performed. After an ip dose to tumor-bearing animals, the concentrations of **26** in the intraperitoneal cavity were approximately 5−15-fold higher than those found in plasma up to 6 h after dosing (Figure 2A). In non-tumor-bearing mice, ip administration of **26** led to relatively sustained plasma levels and high bioavailability. However, these levels were still substantially lower than those found in the ip fluid in the ip drug/ip tumor experiment. After iv administration (dosing vehicle 10% ethanol/water), **26** was cleared rapidly from plasma with an apparent $t_{1/2}$ of <30 min (Figure 2B). Insufficient systemic or tumor exposure is therefore likely to be the reason for the lack of distal site activity (ip/sc or iv/sc) of **26** in this model.

Conclusions

2,3,4,5-Tetrahydro-1-(imidazol-4-ylalkyl)-1,4-benzodiazepines were discovered to inhibit FT. SAR studies were focused primarily on imidazol-4-ylmethyl derivatives. These studies demonstrated that a hydrophobic substituent linked to N-4 via a hydrogen bond-accepting group as well as a 7- or 8-hydrophobic substituent were important for potent enzyme inhibition. Low nanomolar inhibitors were identified which are able to revert the transformed phenotype of Ras transformed cells at submicromolar concentrations and which can prevent the anchorage-independent growth in soft agar of Ras

transformed cells at concentrations as low as 160 nM. Selected analogues also demonstrate in vivo antitumor activity in a Rat-1 ip/ip tumor model. These results demonstrate that the tetrahydrobenzodiazepine ring system is a useful template for the attachment of pharmacophores providing potent inhibition of FT. In addition, these studies confirm the utility of the imidazole group as a putative ligand for the active site zinc atom of FT and demonstrate the compatibility of the imidazole functionality with potent cell activity.

Experimental Section

Prenyltransferase Assays. Assays for FT and GGTI inhibition were performed as previously described except that >90% purified recombinant human FT (hFT) or purified recombinant human GGTI (hGGTI) enzymes were used.[15] The amount of enzyme used in the assays was 0.3 nM hFT or 5 nM hGGTI. The assays also contained 10 μM (FT) or 5 μM (GGTI) $ZnCl_2$ in addition to the components described earlier.

Cell-Based Assays. The assays for Ras processing inhibition, inhibition of soft agar growth, and phenotypic reversion were performed as previously described.[16]

In Vivo Antitumor Testing. Rat-1 tumor, an H-Ras transformed rat fibroblast line, with negative murine antibody profile test results, was used as an in vivo tumor model in athymic Balb/c background female mice (18−22 g; Harlan Sprague−Dawley, Indianapolis, IN). The Rat-1 tumors were sourced from in vitro propagated cells. Experiments were initiated by the implantation, ip or sc, of 1×10^6 cells, except in control groups given titrated cell inocula. All experiments began on day 1 post-tumor implant. Compounds were dissolved in 10% ethanol/sterile water, and the solutions were injected ip or iv within 1 h of dissolution. Group sizes were typically 6 mice for ip implants and 8 mice for sc implant experiments. A detailed description of the basic assay and evaluation methods used for the experiments conducted have been reported.[22] Therapeutic results are presented in terms of: increases in lifespan reflected by the relative median survival time (MST) of treated (T) versus control (C) groups (i.e., %T/C values) and any long-term survivors. Statistical evaluations of data were performed using the Gehan's generalized Wilcoxon test.[23] The activity criterion for increased lifespan was a T/C of ≥125% and was applicable for both sc and ip experiments. Group sizes typically consisted of 6 mice in ip-implanted tumor treatment groups, 8 mice in sc-implanted tumor treatment groups, and 8 mice in all control groups. Drug-treated mice dying before the first death in parallel with control mice implanted with the same tumor inoculum were considered to have died from drug toxicity. Groups of mice with more than one death due to drug toxicity were not used in the evaluation of antitumor efficacy, and the highest dose tested that did not cause such lethality was termed the maximum tolerated dose (MTD).

General Chemical Procedures. Melting points were recorded on a Thomas-Hoover capillary apparatus and are reported uncorrected. IR spectra were recorded on a Mattson Sirius 100 spectrometer. Proton NMR (^{1}H NMR) and carbon NMR (^{13}C NMR) spectra were obtained on GE QE-300 or JEOL FX-270 or GX-400 spectrometers and are reported relative to tetramethylsilane (TMS) reference. Analytical and preparative HPLC were performed on YMC columns (A-302, S-5, 120A ODS, 4.6 × 150 mm; SH-345-15, S-15, 120A ODS, 20 × 500 mm) with acetonitrile:water gradients containing 0.1% trifluoroacetic acid. Chromatography was performed under flash conditions using EM Science silica gel, 0.040−0.063-mm particle size. THF was distilled from Na/benzophenone. Solutions were dried with magnesium sulfate unless otherwise noted.

(*S*)-1-[2-(1*H*-Imidazol-4-yl)ethyl]-2-(2-methoxyethyl)-4-(1-naphthalenylcarbonyl)piperazine, Trifluoroacetate (4). To a solution of 4-phenylmethyl-1-[(1,1-dimethylethoxy)-carbonyl]-2(*S*)-(2-methoxyethyl)piperazine (1.0 g, 3 mmol) in methylene chloride (2 mL) was added 4 N HCl in dioxane (10 mL).[14] The solution was stirred for 2 h and concentrated. Ethyl ether was added and the resulting white solid was filtered, washed with ethyl ether and dried under vacuum to yield 800 mg (87%) of 4-phenylmethyl-2(*S*)-(2-methoxyethyl)piperazine, hydrochloride salt (**52**): MS $(M + H)^+$ 235. To a stirred solution of **52** (800 mg, 2.6 mmol), hydroxybenzotriazole (HOBT, 99 mg, 3.7 mmol), 4-imidazoleacetic acid hydrochloride (602 mg, 3.7 mmol), and *N*-methylmorpholine (NMM; 1.21 mL, 11.1 mmol) in DMF (5 mL) under argon was added 1-ethyl-3-(3-dimethylaminopropyl)carbodiimide·HCl (EDC; 711 mg, 3.7 mmol). The solution was stirred for 16 h and concentrated to half-volume. Saturated aqueous sodium bicarbonate solution (40 mL) was added along with ethyl acetate (50 mL). The layers were separated and the organic layer was washed with saturated aqueous sodium bicarbonate solution, dried, and concentrated to a yellowish residue. Chromatography (silica, 85:10:5 chloroform:methanol:acetic acid) followed by passage of a methanol solution of the product through a short column of YMC ODS-AQ 120−550 silica (4.6 × 4 cm) and concentration afforded 600 mg (50%) of 1-[(imidazol-4-yl)acetyl]-4-phenylmethyl-2(*S*)-(2-methoxyethyl)piperazine, acetic acid salt (**53**) as a clear glass: MS $(M + H)^+$ 342. To a solution of **53** (462 mg, 1 mmol) in 15 mL of THF was added 1 M lithium aluminum hydride in THF (LAH; 4 mL, 4 mmol) under argon. The solution was refluxed for 2 h and cooled. Aqueous NaOH was added (0.5 N, 15 mL, dropwise at first). The basic solution was washed with methylene chloride (3 × 40 mL) and the pooled organic layers were dried and filtered. Dry HCl gas was bubbled through the solution for 1 min followed by concentration under high vacuum to give 430 mg (98%) of 1-[2-(imidazol-4-yl)ethyl]-4-phenylmethyl-2(*S*)-(2-methoxyethyl)piperazine, trihydrochloride salt (**54**) as a viscous oil: MS $(M + H)^+$ 329.2. In a Parr bottle, **54** (370 mg, 0.85 mmol) was dissolved in methanol (20 mL) and the vessel was purged with argon. To this was added palladium hydroxide (10% on carbon, 0.5 g) and the mixture was hydrogenated under 50 psi for 16 h. The catalyst was removed by filtration through Celite and the filtrate was evaporated under vacuum to give 290 mg (98%) of 1-[2-(imidazol-4-yl)ethyl]-2(*S*)-(2-methoxyethyl)piperazine, trihydrochloride salt (**55**) as an oil: MS $(M + H)^+$ 239.1. To a stirred solution of **55** (75 mg, 0.22 mmol), HOBT (43.8 mg, 0.32 mmol), naphthoic acid (55.8 mg, 0.32 mmol), and diisopropylethylamine (DIEA; 0.28 mL, 0.32 mmol) in DMF (5 mL) under argon was added EDC (75 mg, 0.32 mmol). The solution was stirred for 3 h followed by the addition of 1 N aqueous HCl (30 mL) and methylene chloride (30 mL). The layers were separated and the aqueous layer was washed with methylene chloride (2 × 20 mL). The aqueous layer was made basic with aqueous 4 N NaOH to pH 10−11 and extracted with methylene chloride (3 × 30 mL). The residue was subjected to preparative HPLC to yield 66 mg (50%) of **4** as a white powder: ^{1}H NMR (CD_3-OD, 270 MHz) δ 8.85 (1H, s), 8.03−7.45 (8H, m), 4.65−4.00 (1H, m), 4.00−2.95 (13H, m), 2.75 (2H, m), 2.28−1.71 (2H, m); ^{13}C NMR (CD_3OD, 100 MHz) 171.65, 136.03, 131.71, 130.62, 130.27, 129.12, 128.37, 126.79, 126.04, 125.70, 118.78, 69.83, 59.28, 59.20, 45.06, 39.42, 26.7, 21.08 ppm; MS $(M + H)^+$ 393.2.

(*S*)-1-[2-(1*H*-Imidazol-4-yl)propyl]-2-(2-methoxyethyl)-4-(1-naphthalenylcarbonyl)piperazine (6): prepared similarly, using 3-(1-triphenylmethylimidazol-4-yl)-propionic acid; ^{1}H NMR (CD_3OD, 400 MHz) δ 8.76 (1H, s), 8.12 (2H, m), 7.95 (1H, m), 7.70 (4H, m), 7.55 (1H, s), 3.99−3.77 (2H, m), 3.73−3.55 (4H, m), 3.50 (3H, s), 3.35 (2H, m), 3.04−2.71 (5H, m), 2.39−2.10 (4H, m).

(*S*)-1-(1*H*-Imidazol-4-ylmethyl)-2-(2-methoxyethyl)-4-(1-naphthalenylcarbonyl)piperazine, Dihydrochloride (2). A solution of 1-naphthoic acid (2.35 g, 13.1 mmol), EDC (2.74 g, 14.3 mmol), and HOBT (1.93 g, 14.3 mmol) was stirred in DMF (10 mL) at room temperature for 20 min. A solution of 1-[(1,1-dimethylethoxy)carbonyl]-2(*S*)-(2-methoxyethyl)piperazine (3.0 g, 11.9 mmol) and DIEA (3.5 g, 4.8 mL, 27.4 mmol) in DMF (10 mL) was added dropwise and the mixture was stirred for 16 h.[14] The mixture was poured into water (200 mL) and extracted with ethyl acetate (3 × 100 mL). The combined ethyl acetate layers were washed with water (2 × 100 mL),

brine (100 mL), dried, and concentrated. Chromatography on silica (40% ethyl acetate, 60% hexane) yielded 1-[(1,1-dimethylethoxy)carbonyl]-2(*S*)-(2-methoxyethyl)-4-(1-naphthalenylcarbonyl)piperazine (**56**) as a slightly brown oil (4.8 g, 99%): MS $(M + H)^+$ 399$^+$. A solution of **56** (240 mg, 0.60 mmol) in dimethyl sulfide (0.3 mL) and 4 N HCl in dioxane (10 mL) was stirred for 30 min. The mixture was concentrated, dissolved in methylene chloride (50 mL), and concentrated. This procedure was repeated five times to yield 2(*S*)-(2-methoxyethyl)-4-(1-naphthalenylcarbonyl)piperazine, hydrochloride (**57**) as a clear glass. Titanium isopropoxide (0.83 mL, 2.8 mmol) was added to a solution of **57** (0.24 g, 0.70 mmol) and 1-triphenylmethyl-4-imidazolecarboxaldehyde (0.25 g, 0.70 mmol) in THF (1 mL) under argon. After stirring for 1 h, ethanol (2 mL) was added, followed by sodium cyanoborohydride (0.04 g, 0.70 mmol). After stirring for 16 h, the mixture was diluted with water (2 mL), stirred for 30 min, and diluted with methylene chloride. The solid was filtered and the layers of the filtrate were separated. The aqueous layer was extracted with methylene chloride (2 × 20 mL). The combined organic layers were washed with $NaHCO_3$ (20 mL) and brine (2 × 20 mL), dried, filtered, and concentrated. Chromatography (1/1 hexane/EtOAc followed by 9/1 $CHCl_3/CH_3OH$) afforded (*S*)-1-(1-triphenylmethylimidazol-4-ylmethyl)-2-(2-methoxyethyl)-4-(1-naphthalenylcarbonyl)piperazine (**58**) (0.31 g, 70%) as a foamy solid: $(M + H)^+$ 621. TFA (1 mL) was added to a solution of **58** (0.30 g, 0.48 mmol) in CH_2Cl_2 (1 mL) under argon. After stirring for 1 h the mixture was concentrated and the residue was triturated with hexane and chromatographed (9/1 $CHCl_3/CH_3OH$). The TFA salt was exchanged for HCl by treating with 1 N HCl followed by lyophilization to afford **2** (0.10 g, 62%): $(M + H)^+$ 379; ^{1}H NMR (400 MHz, CD_3OD) δ 8.8−8.75 (m, 1H) 8.1−7.89 (m, 2H), 7.87−7.72 (m, 1H), 7.61−7.42 (m, 5H), 4.39−3.8 (m, 4H), 3.62−3.49 (m, 1H), 3.43−3.1 (m, 3H), 3.05−2.52 (m, 7H), 2.3−1.55 (m, 2 H).

Compounds **1**, **3**, and **5** were prepared from **57** similarly, by carbodiimide-mediated coupling with 1-trityl-4-imidazolecarboxylic acid, 4-imidazoleacetic acid or 3-(1-trityl-imidazol-4-yl)propionic acid, respectively, followed by deprotection with TFA if necessary.

(*S*)-1-(1*H*-Imidazol-4-ylcarbonyl)-2-(2-methoxyethyl)-4-(1-naphthalenylcarbonyl)piperazine, dihydrochloride (1): ^{1}H NMR (270 MHz, CD_3OD) δ 9.1−9.0 (s, H), 8.12−7.85 (m, 3H), 7.78−7.4 (m, 5 H), 4.7−3.1 (m, 11H), 3.1−3.0 (s, H), 2.9−2.79 (s, H), 2.3−1.49 (m, H); $(M + H)^+$ 393.

(*S*)-1-(1*H*-Imidazol-4-ylacetyl)-2-(2-methoxyethyl)-4-(1-naphthalenylcarbonyl)piperazine, dihydrochloride (3): ^{1}H NMR (CD_3OD, 270 MHz) δ 8.92 (1H, s), 7.9−7.4 (6H, m), 5.1−4.5 (2H, m), 4.4−2.7 (12H, m), 2.3−1.6 (2H, m); ^{13}C NMR (CD_3OD, 67.8 MHz) 133.36, 129.72, 128.69, 128.58, 128.06, 127.40, 126.70, 125.18, 124.37, 117.35, 77.98, 77.23, 76.17, 67.99, 58.63 ppm $(M + H)^+$ 407.

(*S*)-1-(1*H*-Imidazol-4-ylpropanoyl)-2-(2-methoxyethyl)-4-(1-naphthalenylcarbonyl)piperazine, dihydrochloride (5): ^{1}H NMR (400 MHz, CD_3OD) δ 1.2−1.6 (m, 1H), 1.9−2.2 (m, 2H), 2.6−3.3 (m, 6H), 3.3−3.4 (s + t, 5H, OMe and OCH2), 3.45−3.65 (m, 2H), 4.05−4.6 (m, 2H), 7.2−7.4 (m, 2H), 7.5−7.7 (br d, 4H), 7.95−8.01 (t, 2H), 8.75 (d, 1H); $(M + H)^+$ 421.

2,3,4,5-Tetrahydro-1-(1*H*-imidazol-4-ylmethyl)-4-(1-naphthalenylcarbonyl)-1*H*-1,4-benzodiazepine, Hydrochloride (8). A stirred solution of isatoic anhydride (16.4 g, 0.1 mol) and glycine ethyl ester hydrochloride in 40 mL of pyridine was heated under reflux for 7 h. The resulting suspension was cooled to 0 °C for 18 h. The precipitate was collected and washed with ethanol and ether to afford 1,4-benzodiazepine-2,5-dione (**59**) as a light yellow solid. To a stirred suspension of LAH (3.5 g, 90 mmol) in THF (100 mL) at room temperature under argon was slowly added **59** (3.5 g, 20 mmol) portionwise as a solid. The suspension was heated at reflux under argon for 18 h and cooled to 0 °C, and a mixture of NH_4OH (5 mL, concentrated) in 30 mL of THF was added dropwise. The suspension was stirred for 1 h and filtered. The filtrate was concentrated to give 2,3,4,5-tetrahydro-1*H*-1,4-benzodiazepine (**47a**, R_1 = H) as an oil. A mixture of **47a** (500 mg, 3.37 mmol) and 1-naphthoic acid, phenyl ester (750 mg, 3.02 mmol) in a small amount of acetonitrile in the presence of a catalytic amount of (dimethylamino)pyridine (DMAP) was heated at 110 °C for 18 h under argon. The mixture was cooled to room temperature. Chromatography (1:1 ethyl acetate: hexanes) afforded 2,3,4,5-tetrahydro-4-(1-naphthalenylcarbonyl)-1*H*-1,4-benzodiazepine (**60**) as a white solid (520 mg). Anal. C,H,N. (A number of alternative conditions may also be used, including coupling of the acid chloride under standard conditions.) To a stirred solution of **60** (200 mg, 0.66 mmol) and 4-formylimidazole (110 mg, 1.15 mmol) in a mixture of dichloroethane (2 mL) and acetic acid (1.0 mL) was added $NaBH(OAc)_3$ (190 mg) in one portion. The mixture was stirred for 30 min and diluted with ethyl acetate (25 mL) followed by NH_4OH (3 mL, concentrated). The mixture was stirred at room temperature for 18 h and poured into a mixture of ethyl acetate (50 mL) and satd $NaHCO_3$ (50 mL). The aqueous layer was extracted with ethyl acetate (50 mL). The combined organic extracts were washed with satd NH_4Cl solution (50 mL), dried over Na_2SO_4, and concentrated in vacuo. The residue was dissolved in methanol (2 mL), and 1 N HCl solution in ether (2 mL) was added. The solvent was removed in vacuo and the residue was dried under vacuum to give **8** as a light yellow solid (240 mg): ^{1}H NMR (CD_3OD) δ 2.90 (m), 3.30 (m), and 3.90 (m) (2H, H3); 3.35 (m, 2H, H2); 4.40 (dd) and 4.90 (dd) (2H, H5); 4.60 (s) and 5.00 (s) (2H, $ImCH_2N$); 6.00 (d), 6.65 (t), 7.00−7.60 (m) and 7.90 (m) (13H); 8.75 (s) and 8.85 (s) (1H, imidazole H2); ^{13}C NMR (CD_3OD, aliphatic region) δ 46.95, 48.20, 48.30, 49.90, 51.34, 53.62, 54.22, 55.60 ppm; MS $(M + H)^+$ 383.

Compounds **31**, **33**, and **34** were prepared starting from 6-bromoisatoic anhydride, 8-methylisatoic anhydride, and 7-chloroisatoic anhydride, respectively. Diamide reductions in the preparations of **31** and **34** were performed with borane•THF in refluxing ethylene glycol dimethyl ether.

7-Bromo-2,3,4,5-tetrahydro-1-(1*H*-imidazol-4-ylmethyl)-4-(1-naphthalenylcarbonyl)-1*H*-1,4-benzodiazepine, hydrochloride (31): ^{1}H NMR (CD_3OD, 300 MHz) δ 2.95 (br m, 1H), 3.20 (m, 1H), 4.00 (br s, 1H), 4.20 (br s, 1H), 4.40 (br d, 1H), 4.50 (br s, 1H), 4.65 (s, 1H), 5.05 (s, 1H), 6.05 (d, 1H), 7.00 (dd, 1H), 7.20−8.10 (m, 9H), 8.85 (s, 1H), 8.95 (s, 1H).

2,3,4,5-Tetrahydro-1-(1*H*-imidazol-4-ylmethyl)-9-methyl-4-(1-naphthalenylcarbonyl)-1*H*-1,4-diazepine, dihydrochloride (33): ^{1}H NMR (270 MHz, CD_3OD) δ 8.97−8.89 (m, 1H), 8.1−8.0 (m, 4H), 7.8−7.2 (m, 7H), 6.8−6.0 (NH), 4.5−4.3 (m, 4H), 3.75−3.4 (m, 3H), 3.1−3.2 (m, 1H), 2.45−2.41 (m, 3H); IR (KBr) 3007, 2851, 2598, 1719, 1628, 1470; MS $(M + H)^+$ 397.

8-Chloro-2,3,4,5-tetrahydro-1-(1*H*-imidazol-4-ylmethyl)-4-(1-naphthalenylcarbonyl)-1*H*-1,4-benzodiazepine, hydrochloride (34): ^{1}H NMR (CD_3OD, 300 MHz) δ 2.98 (br m, 1H), 3.24 (m, 1H), 3.95 (br s, 1H), 4.16 (br s, 1H), 4.22 (br d, 1H), 4.40 (br s, 1H), 4.60 (s, 1H), 4.95 (s, 1H), 5.85 (d, 1H), 6.55 (dd, 1H), 7.00−8.00 (m, 9H), 8.83 (s, 1H), 8.90 (s, 1H). MS: $(M + H)^+$ 417; mp 160−162 °C.

2,3,4,5-Tetrahydro-1-[3-(1*H*-imidazol-2-yl)propyl]-4-(1-naphthalenylcarbonyl)-1*H*-1,4-benzodiazepine, dihydrochloride (9): prepared from 3-(N-triphenylmethylimidazol-2-yl)propanal; ^{1}H NMR (CD_3OD, 300 MHz) δ 8.05−7.95 (2H, m), 7.6−7.05 (12H, m), 6.71 (0.5H, m), 6.02 (0.5H, m), 4.4 (2H, m), 3.6−3.0 (8H, m), 2.2−2.0 (2H, m); $(M + H)^+$ 411.

Compounds **10** and **12** were prepared from [2-([[(1,1-dimethyl)ethoxycarbonyl]amino]methyl)-1-[(1,1-dimethyl)ethoxycarbonyl]imidazol-4-yl]carboxaldehyde and [2-([[(1,1-dimethyl)-ethoxycarbonyl]amino]ethyl)-1-[(1,1-dimethyl)ethoxycarbonyl]imidazol-4-yl]carboxaldehyde, respectively. Each of these was prepared from the known [2-(2-aminoalkyl)-1*H*-imidazol-4-yl]methanol analogues by Boc protection followed by MnO_2 oxidation in $CHCl_3$ at 50 °C.[24]

1-[[2-(2-Aminomethyl)-1*H*-imidazol-4-yl]methyl]-2,3,4,5-tetrahydro-4-(1-naphthalenylcarbonyl)-1*H*-1,4-benzodiazepine, trihydrochloride (10): mp 155−160 °C; ^{1}H NMR (270 MHz, CD_3OD, as a mixture of rotamers) δ 2.92 (1H, m), 3.45 (1H, m), 3.68 (m) and 4.00 (m) (1H), 4.30 (2H, m), 4.50

(3H, s), 4.66 (1H, s), 5.08 (1H, s), 5.92 (m) and 6.58 (m) (1H), 7.05−7.68 (9H, m), 7.89−8.04 (2H, m); ^{13}C NMR (67.8 MHz, CD_3OD, as a mixture of rotamers) 34.5, 47.6, 52.2, 54.1, 54.9, 56.5, 119.0, 119.2, 120.1, 123.1, 124.1, 124.8, 125.5, 125.7, 126.1, 126.4, 127.7, 128.1, 128.3, 129.6, 129.7, 129.8, 130.0, 130.5, 130.7, 131.1, 131.6, 132.1, 132.6, 134.3, 134.5, 135.0, 135.2, 135.3, 140.4, 151.3, 151.5, 172.1, 173.0 ppm. IR (KBr) 781 cm^{-1}, 804, 1144, 1209, 1248, 1283, 1373, 1437, 1454, 1470, 1495, 1508, 1599, 1632, 2922, 3426; $(M + H)^+$ 412.

1-[[2-(2-Aminoethyl)-1*H*-imidazol-4-yl]methyl]-2,3,4,5-tetrahydro-4-(1-naphthalenylcarbonyl)-1*H*-1,4-benzodiazepine, trihydrochloride (12): mp 165 °C; ^{1}H NMR (270 MHz, CD_3OD, as a mixture of rotamers) δ 2.91 (1H, m), 3.41 (5H, m), 3.66 (m) and 3.92 (m) (1H), 4.30 (2H, m), 4.42 (1H, s), 4.58 (1H, s), 5.01 (1H, s), 5.95 (m) and 6.57 (m) (1H), 7.05−7.18 (2H, m), 7.30−7.56 (5H, m), 7.88−8.02 (2H, m); IR (KBr) 1454, 1470, 1495, 1508, 1599, 1630, 2922 cm^{-1}; $(M + H)^+$ 426.

2,3,4,5-Tetrahydro-1-(1*H*-imidazol-2-ylmethyl)-4-(1-naphthalenylcarbonyl)-1*H*-1,4-benzodiazepine, dihydrochloride (14): prepared from 2-formylimidazole; ^{1}H NMR (CD_3OD, 270 MHz) δ 8.11 (2H, m), 7.7−7.1 (10H, m), 6.71 (0.5H, t, J = 7.05 Hz), 6.07 (0.5H, d, J = 7.05 Hz), 5.01 (1H, m), 4.7−4.0 (2H, m), 3.6−3.4 (4H, m), 3.1 (1H, m). MS $(M + H)^+$ 383.

Compounds **16** and **19** were prepared from **47a** by reaction with 1-naphthalenesulfonyl chloride or 2-methoxycarbonylbenzenesulfonyl chloride, respectively, followed by reductive alkylation.

2,3,4,5-Tetrahydro-1-(1*H*-imidazol-4-ylmethyl)-4-(1-naphthalenylsulfonyl)-1*H*-1,4-benzodiazepine, hydrochloride (16): ^{1}H NMR ($CDCl_3$) δ 3.32 (m, 2H), 3.75 (m, 2 H), 4.70 (s, 2 H), 4.80 (s, 2 H), 7.00 (m, 1 H), 7.10 (m, 3 H), 7.50 (m, 4 H), 7.85 (m, 1 H), 8.10 (m, 1 H), 8.45 (m, 1 H), 9.00 (s, 1 H); MS $(M + H)^+$ 419.

2-[[2,3,4,5-Tetrahydro-1-(1*H*-imidazol-4-ylmethyl)-1*H*-1,4-benzodiazepin-4-yl]sulfonyl]benzoic acid, methyl ester, hydrochloride (19): ^{1}H NMR ($CDCl_3$) δ 3.40−3.90 (m, 4H), 3.85 (s, 3H), 4.90 (br s, 4H), 7.10 (br s, 2H), 7.35 (m, 1H), 7.50 (m, 4H), 7.75 (m, 2H), 9.00 (s, 1H). MS $(M + H)^+$ 427.

2,3,4,5-Tetrahydro-1-(1*H*-imidazol-4-ylmethyl)-*N*,*N*-diphenyl-4*H*-1,4-benzodiazepine-4-carboxamide, hydrochloride (20): prepared from **47a** by reaction with *N*,*N*-diphenylcarbamyl chloride in aqueous THF with sodium hydrogen carbonate, followed by reductive alkylation; ^{1}H NMR ($CDCl_3$) δ 3.15 (br s, 2H), 3.67 (br s, 2H), 4.45 (br s, 2H), 4.60 (br s, 2H), 6.85−7.40 (m, 15H), 8.65 (s, 1H); MS $(M + H)^+$ 424.

2,3,4,5-Tetrahydro-1-[2-(1*H*-imidazol-4-yl)ethyl]-4-(1-naphthalenylcarbonyl)-1*H*-1,4-benzodiazepine, Dihydrochloride (7). To a stirred solution of **47a** (300 mg) in THF was added di-*tert*-butyl dicarbonate (400 mg). The mixture was stirred for 18 h and quenched by the addition of saturated $NaHCO_3$ solution. The solvent was removed and the residue was chromatographed (1:2 ethyl acetate:hexanes) to give 2,3,4,5-tetrahydro-4-[(1,1-dimethylethoxy)carbonyl]-1*H*-1,4-benzodiazepine (**50a**, R_1 = H) as an oil (350 mg). To a solution of 250 mg (0.68 mmol) of N-triphenylmethyl-4-imidazole acetic acid and 94 μL (0.68 mmol) of triethylamine in 3 mL of THF at −30 °C under argon was added dropwise 97 μL (0.75 mmol) of isobutyl chloroformate. Stirring was continued for 10 min and a solution of 253 mg (1.02 mmol) of **50a** in 1 mL of THF was added. The mixture was stirred 7 h as it warmed to room temperature and diluted with ethyl acetate. The solution was washed with brine, saturated $NaHCO_3$, and brine, dried, and concentrated. The resulting oil was chromatographed (75% ethyl acetate:hexanes) to afford 195 mg (0.33 mmol, 48%) of 2,3,4,5-tetrahydro-1-[1-oxo-2-(1-triphenylmethylimidazol-4-yl)-ethyl]-4-[(1,1-dimethylethoxy)carbonyl]-1*H*-1,4-benzodiazepine (**61**) as a white foamy solid. To a solution of 100 mg (0.17 mmol) of **61** in 1 mL of THF under argon was added 1 mL (1 mmol) of 1 M borane in THF. After the initial foaming had ceased, the mixture was heated at 60 °C for 1 h and cooled to room temperature. Concentrated HCl (0.5 mL) was added and the solution was heated at 60 °C for 1 h and evaporated to dryness. The residue was diluted with water and the solution was washed twice with ethyl acetate, rendered alkaline by the dropwise addition of 40% KOH-water, and extracted with methylene chloride (3×). The combined methylene chloride extracts were washed with brine (2×) and dried, and the solvent was removed to give 39 mg of viscous oil. This material was chromatographed ($CHCl_3$:MeOH:NH_4OH, 80:20:2) to afford 16 mg (0.066 mmol, 40%) of 2,3,4,5-tetrahydro-1-[2-(1*H*-imidazol-4-yl)ethyl]-1*H*-1,4-benzodiazepine (**62**) as an oil. Standard EDC/HOBT coupling with naphthoic acid afforded **7** as a foamy solid in 36% yield following preparative HPLC and salt exchange with HCl: ^{1}H NMR (270 MHz, CD_3OD, as a mixture of rotamers) δ 2.99 (2H, m), 3.10 (1H, m), 3.40 (1H, m) 3.58 (2H, m), 3.77 (1H, m), 4.20 (2H, m), 6.01 (m) and 6.62 (m) (1H), 7.10−7.57 (10, m), 7.90−8.01 (2H, m), 8.77 (1H, s); ^{13}C NMR (67.8 MHz, CD_3OD, as a mixture of rotamers) 24.9, 47.1, 51.7, 53.4, 53.6, 54.3, 55.2, 56.6, 118.0, 118.2, 120.1, 120.4, 125.2, 125.7, 125.8, 126.0, 126.5, 126.8, 128.1, 128.2, 128.5, 128.6, 130.0, 130.1, 130.3, 130.5, 131.0, 131.1, 131.7, 132.3, 132.6, 133.6, 135.1, 135.2, 135.4 ppm; MS $(M + H)^+$ 397; IR (neat/film) 781, 1202, 1248, 1431, 1468, 1622, 3430 cm^{-1}.

2,3,4,5-Tetrahydro-4-(1*H*-imidazol-4-ylmethyl)-1-(1-naphthalenylcarbonyl)-1*H*-1,4-benzodiazepine, Dihydrochloride (13). To a stirred solution of **50a** (350 mg, 1.4 mmol) in methylene chloride at 0 °C was added 1-naphthoyl chloride (0.22 mL, 1.4 mmol), followed by pyridine (0.25 mL). The mixture was stirred for 2 h. Saturated $NaHCO_3$ was added and the mixture was stirred for 18 h. The solution was poured into a mixture of methylene chloride and saturated $NaHCO_3$. The organic layer was washed with 10% HCl (2 × 25 mL), dried, and concentrated to give 2,3,4,5-tetrahydro-1-(1-naphthalenylcarbonyl)-4-[(1,1-dimethylethoxy)carbonyl]-1*H*-1,4-benzodiazepine (**63**) as an oil (450 mg, 80%). A solution of **63** in a mixture of methylene chloride and TFA (10 mL, 1:1) was stirred for 2 h. The solvent was removed and the residue was diluted in $CHCl_3$ and made basic with 10 N NaOH solution. The organic layer was separated, dried and concentrated to give 2,3,4,5-tetrahydro-1-(1-naphthalenylcarbonyl)-1*H*-1,4-benzodiazepine (**64**) as an oil (310 mg, 92%). Compound **13** was prepared as a light yellow solid from **64** as described for **8**: ^{1}H NMR (CD_3OD) δ 3.30 (m, 2H, H3), 3.70 (br s, 1H, H2), 4.00 (br s, 1H, H2), 4.85 (br s, 4H, H5, and imidazole CH_2−N), 6.65 (d, 1H), 6.90 (m, 1H), 7.20 (m, 3H), 7.60 (m, 4 H), 7.70 (d, 1H), 7.80 (d, 1H), 8.00 (s, 1H), 8.15 (d, 1H), 9.10 (d, 1H).

2,3,4,5-Tetrahydro-4-(1-naphthalenylcarbonyl)-1-[[1-(phenylmethyl)-1*H*-imidazol-5-yl]methyl]-1*H*-1,4-benzodiazepine, Hydrochloride (11). To a solution of **8** (90 mg, 0.21 mmol) in acetonitrile (1 mL) at room temperature under argon was added triethylamine (0.14 μL, 1 mmol) followed by trityl chloride (56 mg, 0.2 mmol). The resulting mixture was refluxed for 2 h, cooled to room temperature, and stirred for an additional 14 h. The precipitate was filtered and the filtrate was concentrated to afford 2,3,4,5-tetrahydro-4-(1-naphthalenylcarbonyl)-1-[[1-(triphenylmethyl)-1*H*-imidazol-4-yl]methyl]-1*H*-1,4-benzodiazepine (**65**; 110 mg, 92%) which was used without further purification: MS $(M + H)^+ = 625$. To a solution of benzyl alcohol (18 μL, 0.18 mmol) in THF (1 mL) at −78 °C under argon was added triflic anhydride (30 μL, 0.18 mmol) and DIPEA (35 μL, 2 mmol). After 20 min, a THF (1 mL) solution of **65** (100 mg, 0.15 mmol) was added dropwise. The mixture was allowed to warm to room temperature over 3 h and stirred for additional 14 h. Acetic acid (1.5 mL) and water (1 mL) were added and the mixture was refluxed for 30 min. After cooling to room temperature, the volatiles were removed in vacuo, the residue was dissolved in chloroform, and the solution was washed with saturated $NaHCO_3$ solution. The organic extract was dried and concentrated. The purified material from flash column chromatography (9:1 $CHCl_3$:MeOH) was dissolved in ethyl acetate and HCl gas was bubbled through the solution for 30 s. Concentration afforded **11** (33 mg, 33% overall): ^{1}H NMR (CD_3OD) δ 2.7−2.8 (m, 1H), 3.12−3.18 (m, 1H), 4.0−4.2 (m, 2H), 4.85 (s, 4H), 5.3 (s, 2H), 6.85−8.0 (m, 18H); IR (KBr) 3036, 2853, 1630, 1508, 1468 cm^{-1}; MS $(M + H)^+ = 473$.

Automated Solid-Phase Synthesis: Compounds 15, 18, and 17 (Table 3). To a mixture of 2,3,4,5-tetrahydro-4-[(1,1-dimethylethoxy)carbonyl]-1*H*-1,4-benzodiazepine (**50a**) (3.83 g, 15.4 mmol) and 4-imidazolecarboxaldehyde (2.22 g, 23.1 mmol) in 120 mL of CH_2Cl_2 and 3 mL of AcOH at room temperature was added $NaBH(OAc)_3$ (4.89 g, 23.1 mmol). The mixture was stirred for 1.5 h, diluted with 200 mL of CH_2Cl_2, and washed with 5% $NaHCO_3$. The organic phase was dried over Na_2SO_4, filtered, and concentrated in vacuo. Chromatography of the residue (5% MeOH/CH_2Cl_2, trace NH_4OH) afforded 2.01 g (40%) of 2,3,4,5-tetrahydro-4-[(1,1-dimethylethoxy)carbonyl]-1-(1*H*-imidazol-4-ylmethyl)-1*H*-1,4-benzodiazepine (**51a**, R_1 = H). Hydroxymethyl resin (3.5 g, 6.58 mmol, 1.88 mmol/g) was swelled with 50 mL of 1,2-dichloroethane for 45 min in a 125 mL shake flask. To this was added paraformaldehyde (0.15 g, 5.0 mmol). HCl(g) was bubbled through the mixture for 15 min. Then, an additional amount of paraformaldehyde (0.15 g, 5.0 mmol) was added to the mixture. HCl(g) was bubbled through the mixture with shaking for 4 h. The 1,2-dichloroethane was removed and the resin was rinsed with 1,2-dichloroethane (4 × 20 mL).

The resin was suspended in 20 mL of 1,2-dichloroethane and then treated with a solution of **51a** (2.23 g, 6.78 mmol) in 25 mL of 1,2-dichloroethane and 6 mL of DIEA. The mixture was shaken for 12 h. MeOH (2 mL) was added, and the mixture was shaken for an additional 1.5 h. The solvent was removed, and the resin was rinsed sequentially with 1,2-dichloroethane (2 × 20 mL), DMF (2 × 20 mL), and MeOH (2 × 20 mL). The material was dried in vacuo to afford 4.58 g (67%) of resin containing imidazole-bound 2,3,4,5-tetrahydro-4-[(1,1-dimethylethoxy)carbonyl]-1-(1*H*-imidazol-4-yl-methyl)-1*H*-1,4-benzodiazepine (%N = 4.39).

To 150 mg (0.135 mmol, 0.90 mmol/g) of this resin in a 5-mL polypropylene syringe barrel were added 1.5 mL of 3% Et_3SiH in CH_2Cl_2 and 0.5 mL of TFA. The tube was placed in a vac-elute chamber (capacity for 24 syringe barrels), and the entire apparatus was shaken on an orbital shaker for 3 h. The solvent was removed, and the resin was rinsed sequentially with 2 mL each of CH_2Cl_2, 25% TEA/CH_2Cl_2, MeOH, DMF, and CH_2Cl_2. The resin was swelled with 0.5 mL of a DMF solution containing 1 M DIEA and 0.5 M HOBT. To this was added 50 mg of carboxylic acid, followed by 1.5 mL of a CH_2Cl_2 solution containing 0.2 M EDC. The mixture was shaken for 18 h. The solvent was removed, and the resin was rinsed sequentially with 2 mL each of CH_2Cl_2, 25% Et_3N/CH_2Cl_2, MeOH, DMF, and CH_2Cl_2. The coupling procedure was repeated. The products were cleaved from the resin by shaking for 18 h in the presence of a HBr/TFA/thioanisole solution (45 mL TFA/1.25 mL of thioanisole/5 mL of 30% HBr/HOAc). The solvent was removed, and the resin was rinsed with MeOH (3 × 3 mL). The solvent was removed in vacuo, and the residue was purified by HPLC (C18, 50 × 100 mm, 10−90% MeOH with 0.1% TFA, 10 min gradient, 20 mL/min). Target compounds were characterized by analytical HPLC and mass spectrometry.

2,3,4,5-Tetrahydro-1-(1*H*-imidazol-4-ylmethyl)-4-(1-naphthalenylcarbonyl)-7-(4-pyridinyl)-1*H*-1,4-benzodiazepine, Trihydrochloride (21). Trifluoroacetic anhydride (0.4 mmol, 60 μL) was added to a solution of **31** and TEA (1.04 mmol, 150 μL) in CH_2Cl_2 (5 mL). The solution was maintained at room temperature for 1 h and concentrated and the residue passed through a short silica gel column (gradient elution: 30% ethyl acetate/hexanes to neat ethyl acetate) to isolate a fluffy white solid which was taken to the next step without further purification. This material was dissolved in toluene (2.0 mL) together with 4-(tributylstannyl)pyridine (0.52 mmol, 190 mg) and $Pd(PPh_3)_4$ (30 mg, 0.026 mmol) and the solution was purged with argon for 15 min. The homogeneous brown solution was heated to 115 °C for 20 h. The black heterogeneous solution was concentrated, the residue was dissolved in MeOH/2 N NaOH(aq) (5 mL:5 mL), and the mixture was stirred at room temperature for 30 min. Methanol was removed under reduced pressure and the mixture was partitioned between 10% 2-propanol/CH_2Cl_2 and 2 N NaOH/saturated NaCl (1:1, 10 mL) and extracted twice with 10% 2-propanol/CH_2Cl_2 (2 × 5 mL). The pooled organic phase was dried over Na_2SO_4, concentrated, and passed through a short silica gel column (95:5:1 $CHCl_3$:MeOH:TEA) to remove polar impurities. The crude material was taken up in 1,2-dichloroethane:AcOH (1:1, 2 mL total) and treated with 4-formylimidazole (62 mg, 0.65 mmol) and $NaBH(OAc)_3$ (0.78 mmol, 165 mg) and the solution was heated to 55 °C for 2 h and concentrated. The residue was partitioned between 10% 2-propanol/CH_2Cl_2 and 2 N NaOH/saturated NaCl (1:1, 10 mL) and extracted twice with 10% 2-propanol/CH_2Cl_2 (2 × 5 mL). The pooled organic phase was concentrated, dissolved in MeOH/TFA (5 mL:0.5 mL), and purified by preparative HPLC. The trifluoroacetate salt was converted to the HCl salt by lyophilizing in 1 M HCl (2 × 5 mL) to give 75 mg (50% yield over 4 steps) of **21** as a bright yellow solid: ^{1}H NMR (CD_3OD) δ 3.2 (m, 1H), 3.7 (m, 2H), 3.8 (m, 1H), 4.0 (m, 1H), 5.2 (m, 1H), 6.4 (s, 1H), 7.2−8.0 (m, 11H), 8.4 (m, 1H), 8.7 (m, 1H), 8.8 (m, 1H), 8.9 (s, 1H), 9.0 (s, 1H); MS $(M + H)^+$ 460.

2,3,4,5-Tetrahydro-1-(1*H*-imidazol-4-ylmethyl)-4-(1-naphthalenylcarbonyl)-7-(3-pyridinyl)-1*H*-1,4-benzodiazepine, trihydrochloride (27): prepared similarly using 3-(tributylstannyl)pyridine; ^{1}H NMR (CD_3OD) δ 3.2 (m, 2H), 3.4 (m, 3H), 3.9 (m, 1H), 4.4 (m, 1H), 4.5−4.6 (m, 3H), 4.7 (d, 2H, J = 7 Hz), 5.1 (s, 2H), 6.1 (d, 1H, J = 1 Hz), 7.2−8.1 (m, 13H), 8.4 (s, 1H), 8.6 (d, 1H, J = 2 Hz), 8.7 (d, 1H, J = 2 Hz), 8.8 (d, 1H, J = 8 Hz), 8.9 (d, 1H, J = 1 Hz), 9.0 (d, 1H, J = 1 Hz), 9.2 (s, 1H); MS $(M + H)^+$ 460.

2,3,4,5-Tetrahydro-1-(1*H*-imidazol-4-ylmethyl)-4-(1-naphthalenylcarbonyl)-7-(2-pyridinyl)-1*H*-1,4-benzodiazepine, trihydrochloride (29): prepared similarly using 2-(tributylstannyl)pyridine and trityl protection with HCl deprotection; ^{1}H NMR (CD_3OD) δ 3.2 (m, 2H), 3.4 (m, 4H), 3.6 (m, 1H), 3.7 (m, 1H), 4.0 (m, 1H), 4.4 (m, 1H), 4.6 (m, 4H), 4.8 (m, 2H), 5.1 (m, 2H), 6.3 (m, 1H), 7.2−8.0 (m, 13H), 8.1 (m, 1H), 8.5 (m, 2H), 8.6 (m, 2H), 8.7 (dd, 1H, J = 1 Hz, 5 Hz), 8.8 (m, 1 Hz), 8.9 (m, 1H); MS $(M + H)^+$ 460.

2,3,4,5-Tetrahydro-1-(1*H*-imidazol-4-ylmethyl)-4-(1-naphthalenylcarbonyl)-8-amino-1*H*-1,4-benzodiazepine, Dihydrochloride (35). To a solution of 2,3,4,5-tetrahydro-1-(1*H*-imidazol-4-ylmethyl)-4-(1-naphthalenylcarbonyl)-8-nitro-1*H*-1,4-benzodiazepine ((0.10 g, 0.23 mmol; prepared from 7-nitroisatoic anhydride as described for **8**, with diamide reduction using borane/THF in refluxing THF) in acetic acid/water (2 mL, 1:1) was added 16% aqueous $TiCl_3$ (2 mL). After stirring for 15 min, the mixture was made basic with 1 N NaOH and $NaHCO_3$ and stirred for 30 min. The aqueous layer was extracted with $CHCl_3/CH_3OH$ (9/1) and the combined organic layers were dried, filtered, and concentrated to afford the free base of **35** (0.92, 73%). A portion of this material (20 mg) was treated with 1 M HCl in ether (2 mL). A light yellow solid was formed which was triturated several times with ether, and dried to afford **35** (0.23 g): MS $(M + H)^+$ 398.

***N*-[2,3,4,5-Tetrahydro-1-(1*H*-imidazol-4-ylmethyl)-4-(1-naphthalenylcarbonyl)-1*H*-1,4-benzodiazepin-8-yl]cyclohexanamide, Dihydrochloride (22).** Cyclohexanecarbonyl chloride (0.020 mL, 0.15 mmol) was added to a solution of **35** (0.50 g, 0.12 mmol) and TEA (0.021 mL, 0.15 mmol) in CH_2Cl_2 (1 mL). After stirring for 16 h, the mixture was diluted with $CHCl_3$ (10 mL) and $NaHCO_3$ (3 mL), and stirred for 30 min. The aqueous layer was extracted with $CHCl_3$ (2 × 20 mL). The combined organic layers were dried, filtered, and concentrated. The residue was treated with HCl/ether; a yellow solid was formed which was triturated with ether several times and dried to afford **22** (0.647 g, 90%); ^{1}H NMR (270 MHz, CD_3OD) δ 8.85 (d, 1H, J = 13 Hz), 8.05−7.88 (m, 2H), 7.6−7.3 (m, 7H), 7.35 (d, 0.5H, J = 7 Hz), 7.22 (d, 0.5H, J = 7 Hz), 7.1 (d, 0.5H, J = 8 Hz), 6.5 (d, 0.5H, J = 8 Hz), 5.86 (d, 0.5H, J = 8 Hz), 4.9 (s, 1H), 4.6−3.8 (m, 3H), 3.4−3.3 (m, 1H), 3.3−2.8 (m, 1H), 2.49−2.28 (m, 2.5 H), 1.9−1.65 (m, 5H), 1.6−1.2 (m, 5H); MS $(M + H)^+$ 508.

Compounds **23**−**28**, and **32** were prepared similarly from **35**.

[2,3,4,5-Tetrahydro-1-(1*H*-imidazol-4-ylmethyl)-4-(1-naphthalenylcarbonyl)-1*H*-1,4-benzodiazepin-8-yl]carbamic acid, cyclohexyl ester, dihydrochloride (23): ^{1}H NMR (270 MHz, CD_3OD) δ 8.0−6.9 (m, 11H), 6.81 (d, 0.5H, J = 8 Hz), 5.85 (d, 0.5H, J = 9 Hz), 5.85 (m, 1H), 4.4−4.0 (m, 3H), 3.9−3.7 (m, 0.5H), 3.4−3.1 (m, 1.5H), 2.88 (m, 1H), 2.0−1.2 (m, 12H); MS (M + H)$^+$ 524.

***N*-[2,3,4,5-Tetrahydro-1-(1*H*-imidazol-4-ylmethyl)-4-(1-naphthalenylcarbonyl)-1*H*-1,4-benzodiazepin-8-yl]benzamide, dihydrochloride (24):** ^{1}H NMR (270 MHz, CD_3OD) δ 8.83 (d, 1H, J = 20 Hz), 8.06−7.82 (m, 4H), 7.8−7.19 (m, 11H), 6.81 (d, 0.5H, J = 8 Hz), 5.92 (d, 0.5H, J = 8 Hz), 4.95 (m, 1H), 4.6 (s, 1H), 4.52−4.35 (m, 2H), 4.3−4.15 (d, 1H, J = 0.02 Hz), 3.96 (br s, 0.5H), 3.45 (m, 1.5H), 2.96−2.92 (d, 1H, J = 0.16 Hz); IR (KBr) 3434, 2930, 1611, 1508, 1424, 1263 cm^{-1}; MS (M + H)$^+$ 502.

***N*-Cyclohexyl-*N*′-[2,3,4,5-tetrahydro-1-(1*H*-imidazol-4-ylmethyl)-4-(1-naphthalenylcarbonyl)-1*H*-1,4-benzodiazepin-8-yl]urea, dihydrochloride (25):** ^{1}H NMR (270 MHz, CD_3OD) δ 8.83 (d, 1H, J = 19 Hz), 8.0−7.89 (m, 2.5H), 7.63−7.3 (m, 6.5H), 7.23 (d, 0.5H, J = 7 Hz), 6.8 (d, 0.5H, J = 8 Hz), 6.31 (d, 0.5H, J = 7 Hz), 5.83 (d, 0.5H, J = 8 Hz), 4.8 (s, 1H), 4.6−3.8 (m, 4H), 3.6−3.5 (m, 1H), 3.45−3.3 (m, 2H), 3.0−2.8 (m, 1H), 1.9−1.58 (m, 5H), 1.48−1.13 (m, 5H); MS (M + H)$^+$ 523.

***N*-[2,3,4,5-Tetrahydro-1-(1*H*-imidazol-4-ylmethyl)-4-(1-naphthalenylcarbonyl)-1*H*-1,4-benzodiazepin-8-yl]acetamide, dihydrochloride (28):** ^{1}H NMR (270 MHz, DMSO-d_6) 2.00−2.09 (m, 3H), 3.15−3.40 (m, 4H), 4.32 (s, 1H), 4.48 (s, 1H), 4.83 (s, 2H), 5.85 (m, 1H), 6.75 (m, 1H), 7.15−7.70 (m, 6H), 7.90−8.05 (m, 4H), 9.05, 9.10 (s, 1H); MS (M + H)$^+$ 440.

***N*-[2,3,4,5-Tetrahydro-1-(1*H*-imidazol-4-ylmethyl)-4-(1-naphthalenylcarbonyl)-1*H*-1,4-benzodiazepin-8-yl]phenylsulfonamide, dihydrochloride (32):** ^{1}H NMR (270 MHz, CD_3OD) δ 8.8 (d, 1H, J = 20 Hz), 7.23−8.1 (m, 13H), 7.1 (d, 0.5H, J = 8 Hz), 7.0 (d, 0.5H, J = 8 Hz), 6.9 (d, 0.5H, J = 8 Hz), 6.62 (d, 0.5H, J = 8 Hz), 6.12 (d, 0.5H, J = 8 Hz), 5.71 (d, 0.5H, J = 8 Hz), 4.55 (m, 1H), 4.55−3.9 (m, 3H), 3.45−3.25 (m, 2H), 3.0−2.8 (m, 2H); MS (M + H)$^+$ 538.

2,3,4,5-Tetrahydro-1-(1*H*-imidazol-4-ylmethyl)-4-(1-naphthalenylcarbonyl)-7-phenyl-1*H*-1,4-benzodiazepine, Hydrochloride (26). A solution of 7-bromo-1,4-benzodiazepine-2,5-dione (0.834 g, 3.1 mmol; prepared from 6-bromoisatoic anhydride as described for **8**) in DMF (10 mL)/THF (10 mL) was degassed with nitrogen. Tetrakis(triphenylphosphine)palladium (10 mol %) was added. After 30 min, anhydrous sodium cabonate (0.37 g, 3.5 mmol) in water (6 mL) and phenylboronic acid (1.00 g, 8.3 mmol) were added. The suspension was stirred at room temperature overnight, then at 80−90 °C for 2 days. The resulting dark suspension was filtered. The solid was washed with water and then ethyl acetate to give 7-phenyl-1,4-benzodiazepine-2,5-dione (**66**) as a slightly gray solid (0.65 g, 84%): MS (M + H) 253. Reduction to 7-phenyl-1,4-benzodiazepine (**47b**, R_1 = 7-phenyl) was accomplished by LAH reduction as described for **8**. Compound **26** was prepared from this material as described for **8**: mp 158−160 °C; ^{1}H NMR (CD_3OD, 300 MHz) δ 2.95 (br m, 1H), 3.30 (m, 1H), 4.00 (br s, 1H), 4.20 (br s, 1H), 4.40 (br d, 1H), 4.60 (m, 1H), 4.65 (m, 1H), 5.05 (s, 1H), 6.05 (d, 1H), 7.00 (d, 1H), 7.15−8.10 (m, 14H), 8.85 (s, 1H), 8.95 (s, 1H); ^{13}C NMR (CD_3OD, 300 MHz) 48, 50, 52, 54.2, 55.0, 56.0 (CH2), 120−142 (25C, Ar−C), 150, 172/172.5 ppm (C═O); MS (M + H)$^+$ 459.

2,3,4,5-Tetrahydro-1-(1*H*-imidazol-4-ylmethyl)-4-(1-naphthalenylcarbonyl)-7-cyclohexyl-1*H*-1,4-benzodiazepine, 2.5 Hydrochloride (30). *n*-BuLi (2.5 M in THF, 1.4 mL, 3.5 mmol) was added to a solution of **31** (0.68 g, 1.4 mmol) in THF (15 mL) at −78 °C. The resulting brown solution was stirred for 5 min at −78 °C and cyclohexanone (1.5 mL, 14.4 mmol) was added. After stirring at −78 °C for 10 min, saturated NH_4Cl (3 mL) was added followed by saturated $NaHCO_3$ (10 mL). The aqueous solution was extracted with CH_2Cl_2. The organic phase was dried (Na_2SO_4) and evaporated. The residue was chromatographed (10% CH_3OH, 0.5% AcOH in CH_2Cl_2) to give the crude alcohol (80 mg) as well as a 25% yield of **30**. TFA (3 mL) was added to the crude alcohol (40 mg) in CH_2Cl_2 (15 mL) at −78 °C. The resulting blue solution was treated with solid $NaBH_4$ (0.7 g, 18.5 mmol). The mixture was warmed to room temperature, quenched with NH_4OH (10 mL), diluted with CH_2Cl_2 (20 mL), and washed with aqueous NaOH (1 N, 10 mL) and brine (10 mL). Drying over Na_2SO_4 and evaporation of solvent gave a solid which was converted to its HCl salt by lyophilization from 1 M HCl to provide **30** as a yellow solid (30 mg): ^{1}H NMR (CD_3OD) δ 1.50−2.40 (m, 10H), 2.89 (m, 1H), 3.20 (m, 2H), 4.00 (br s, 1H), 4.20 (br s, 1H), 4.40 (br d, 1H), 4.50 (br s, 1H), 4.65 (s, 1H), 4.95 (s, 1H), 6.15 (d, 1H), 7.19−8.10 (m, 11H), 8.85 (s, 1H), 8.95 (s, 1H): MS (M + H$^+$) 465.

2,3,4,5-Tetrahydro-1-(1*H*-imidazol-4-ylmethyl)-7-(4-pyridinyl)-4-[2-(trifluoromethoxy)benzoyl]-1*H*-1,4-benzodiazepine, trihydrochloride (36): prepared from 7-bromo-1*H*-1,4-benzodiazepine by EDC/HOBt-mediated coupling with 2-trifluoromethoxybenzoic acid, trifluoroacetylation of N-1 with trifluoroacetic acid, coupling with 4-(tributylstannyl)pyridine using $Pd(PPh_3)_4$ catalysis as described for **21**, deprotection using aq NaOH, and reductive alkylation; ^{1}H NMR (CD_3OD) δ 3.40 (m, 1H), 3.65 (m, 2H), 3.95 (m, 1H), 4.20 (m, 1H), 4.5−5.0 (m, 3H), 5.1 (d, 1H, J = 10 Hz), 7.05−7.70 (m, 7H), 7.95 (dd, 1H, J = 1 Hz, 9 Hz), 8.10 (s, 1H), 8.39 (d, 1H, J = 6 Hz), 8.75 (dd, 2H, J = 5 Hz, 10 Hz), 8.94 (d, 2H, J = 10 Hz); MS (M + H)$^+$ 494.

2,3,4,5-Tetrahydro-1-(1*H*-imidazol-4-ylmethyl)-4-[2-(4-methoxyphenyl)-1,2-dioxoethyl]-7-phenyl-1*H*-1,4-benzodiazepine, hydrochloride (38): prepared from 4-(methoxyphenyl)-2-oxoacetic acid and **51b** (R_1 = 7-phenyl) using standard 1-hydroxy-7-azotriazole (HOAt)/diisopropylcarbodiimide (DIC)-mediated coupling; ^{1}H NMR (CD_3OD) δ 3.12 (m, 1H), 3.27 (m, 1H), 3.39−3.45 (m, 2H), 3.74, 3.87 (s, 3H), 4.49−4.58 (m, 3H), 4.87 (m, 1H), 6.78−778 (m, 13H), 9.09−9.11 (m, 1 H); MS (M + H$^+$) 467.

2,3,4,5-Tetrahydro-1-(1*H*-imidazol-4-ylmethyl)-7-phenyl-4-[(1,2,3,4-tetrahydro-1-quinolinyl)carbonyl]-1*H*-1,4-benzodiazepine, monohydrochloride (40): prepared from **47b** by reaction with *N*-(tetrahydroquinolinyl)carbonyl chloride in methylene chloride/1 N NaOH followed by reductive alkylation as described for **8**; mp 143−148 °C dec; ^{1}H NMR (400 MHz, CD_3OD) δ 1.87 (m, 2H, C−CH_2−C), 2.77 (m, 2H, Ar−CH_2−C), 3.22 (m, 2H, N−CH_2), 3.52 (m, 4H, 2 N−CH_2), 4.54 (br s, 2H, N−CH_2), 4.58 (br s, 2H, N−CH_2), 6.9−7.6 (m, 13 H, 12 aromatics + 1 imidazole), 8.90 (s, 1H, 1 imidazole); MS (M + H$^+$) 464.

2,3,4,5-Tetrahydro-1-(1*H*-imidazol-4-ylmethyl)-4-(1-naphthalenylsulfonyl)-7-phenyl-1*H*-1,4-benzodiazepine, monohydrochloride (41): prepared from **47b** by reaction with 1-naphthalenesulfonyl chloride followed by reductive alkylation; ^{1}H NMR (CD_3OD, 270 MHz) δ 8.83 (1H, s), 8.5 (1H, m), 8.24 (1H, d, J = 8 Hz), 8.11 (1H, J = 8 Hz), 7.94 (1H, m), 7.61−7.25 (9H, m), 7.02 (1H, d, J = 8 Hz). 4.61 (2H, s), 4.41 (2H, s) 3.52 (2H, m), 3.09 (2H, m); MS (M + H$^+$) 415.

2,3,4,5-Tetrahydro-1-(1*H*-imidazol-4-ylmethyl)-7-phenyl-4-(1-phenyl-1*H*-tetrazol-5-yl)-1*H*-1,4-benzodiazepine, Monohydrochloride (42). To a solution of **47b** (100 mg, 0.45 mmol) and potassium carbonate (60 mg) in DMF (2 mL) was added 5-chloro-1-phenyltetrazole (100 mg, 0.55 mmol). The mixture was stirred at 60 °C for 18 h and partitioned between ethyl acetate and satd. NH_4Cl. The organic layer was washed with satd. $NaHCO_3$, dried, and concentrated. The residue was purified by column chromatography to give 2,3,4,5-tetrahydro-7-phenyl-4-(1-phenyl-1H-tetrazol-5-yl)-1*H*-1,4-benzodiazepine as a white solid (75 mg, 45%): mp 150−151 °C. Anal. C,H,N. Reductive alkylation to form **42** was performed as described for **8**: mp 158 °C. ^{1}H NMR (CD_3OD) δ 3.40 (br s, 2H), 3.75 (br s, 2H), 4.55 (br s, 2H), 4.65 (br s, 2H), 6.75 (s, 1H), 7.15 (s, 1H), 7.30−7.75 (m, 10H), 9.00 (s, 1H); MS (M + H$^+$) 449.

***N*-(4-Chlorophenyl)-*N′*-cyano-1,2,3,5-tetrahydro-1-(1*H*-imidazol-4-ylmethyl)-7-phenyl-4*H*-1,4-benzodiazepin-4-imidamide, Monohydrochloride (43).** To a solution of **47b** (110 mg, 0.5 mmol) in DMF were added sequentially *N*-(4-chlorophenyl)-*N′*-cyanothiourea (130 mg, 0.62 mmol) and EDC (120 mg, 0.61 mmol). The mixture was stirred for 18 h and partititoned between ethyl acetate and satd. NH_4Cl. The organic layer was separated, washed with saturated $NaHCO_3$ solution and brine, dried, and concentrated. The residue was crystalized from MeOH to give *N*-(4-chlorophenyl)-*N′*-cyano-1,2,3,5-tetrahydro-7-phenyl-4*H*-1,4-benzodiazepin-4-imidamide as a solid (150 mg, 75%): MS ($M + H^+$) 402. Reductive alkylation to form **43** was performed as described for **8**: ^1H NMR (CD_3OD) δ 3.35 (br s, 2H), 3.80 (br s, 2H), 4.60 (br s, 2H), 4.70 (br s, 2H), 6.80 (s, 1H), 7.05 (d, 2H), 7.20−7.50 (m, 10 H), 9.00 (s, 1 H); MS ($M + H^+$) 482.

2,3,4,5-Tetrahydro-1-(1*H*-imidazol-4-ylmethyl)-4-(1-naphthalenylmethyl)-7-phenyl-1*H*-1,4-benzodiazepine, Trifluoroacetate (44). LAH (1 M in THF, 15 mL, 15 mmol) was added to a suspension of **26** (0.25 g, 0.55 mmol) in THF (10 mL). The suspension was refluxed for 5 h and cooled to 0 °C. Aqueous NaOH (20%, 10 mL) and H_2O (10 mL) were added. The mixture was saturated with NaCl and extracted with CH_2Cl_2 (2 × 50 mL). Drying (Na_2SO_4) and evaporation of the solvent gave a solid (0.21 g) which was disolved in MeOH/TFA (10:1) and purified by prep HPLC to provide **44** (50 mg): ^1H NMR (DMSO-d_6, 300 MHz) δ 2.75 (br m, 2H), 3.15 (m, 2H), 3.90 (brd, 2H), 4.00 (s, 2H), 4.30 (br s, 2H), 4.65 (m, 1H) (s, 1H), 7.00 (d, 1H), 7.15−8.10 (m, 14H), 8.85 (s, 1H), 8.25 (s, 1H); MS ($M + H^+$) 445.

2,3,4,5-Tetrahydro-1-(1*H*-imidazol-4-ylmethyl)-7-phenyl-1*H*-1,4-benzodiazepine-4-carboxylic Acid, Phenylmethyl Ester (45). 2,3,4,5-Tetrahydro-1-(1*H*-imidazol-4-ylmethyl)-7-phenyl-1*H*-1,4-benzodiazepine (**51b**, R_1 = 7-phenyl) was prepared from **47b** by Boc protection as described for **7**, reductive alkylation with 4-formylimidazole as described for **8**, and Boc removal. Reaction with *p*-nitrophenyl benzyl carbonate in DMF afforded **45**: ^1H NMR (DMSO) δ 2.95−3.56 (m, 4H), 4.29−4.32 (m, 2H), 4.54−4.57 (m, 2H), 5.04 (s, 2H), 6.90−7.60 (m, 15H). MS ($M + H^+$) 439.

Automated Solution Phase Synthesis: Compounds 37 and 39. The coupling of each carboxylic acid to **51b** was carried out using standard HOAt/DIC-mediated coupling. The process was automated by using a Hamilton 2200 liquid handler. A Zymark Benchmate robotic workstation was used to carry out the weighings of the test tubes and for purification of the resulting amide products. An IBM PC was used to run the Zymark Benchmate workstation operating program and to write the Benchmate procedures. The standard protocol for preparation of amides is illustrated by the following examples:

A 16- × 100-mm tube was charged with the appropriate carboxylic acid (0.10 mmol, 1.0 equiv) and the liquid handler then carried out the following steps on the tube: (1) Added 0.5 mL of a 0.2 M HOAt solution in DMF. (2) Added 0.5 mL of **51b** (0.2 M, 0.10 mmol, 1.0 equiv) in DMF. (3) Added 1.0 mL of a methylene chloride solution of DIC (0.016 mL, 0.10 mmol, 1.0 equiv). (4) Mixed tube contents by vortexing at speed 3 for 30 s.

After 24 h, the mixture was concentrated on a Savant speed vac (approximately 2 mmHg for 72 h). The residue was purified by ion exchange chromatography on a solid-phase extraction cartridge mediated by the Benchmate robotic workstation using the following protocol: (1) Added 5.0 mL of methanol/methylene chloride (1:1) to the reaction. (2) Mixed tube contents by vortexing at speed 3 for 60 s. (3) Conditioned a Varian solid-phase extraction column (1.5 g, SCX cation exchange) with 10 mL of methanol/methylene chloride at 0.15 mL/s. (4) Loaded reaction contents onto column at 0.02 mL/s. (5) Washed column with 2 × 7.5 mL of methanol/methylene chloride (1:1) at 0.1 mL/s. (6) Washed column with 1 × 7.5 mL of methanol at 0.1 mL/s. (7) Washed column with 0.01 M ammonia in methanol. (8) Eluted column with 7.5 mL of 1 M ammonia in methanol and collected into a tared receiving tube at 0.05 mL/s.

All solution/solvent deliveries were followed by 1.0 mL of air, and a 5-s push delay was used after loading reaction contents onto the ion-exchange column. The product solution was concentrated on a Savant speed vac (approximately 2 mmHg for 20 h) to afford the target compound.

Syntheses requiring further purification were subjected to preparative HPLC (YMC S3 ODS 50 × 100 mm, 30 mL/min, 10 min gradient of 10−90% aqueous methanol with 0.1%TFA, monitored at 220 nm). The appropriate fractions were combined and concentrated under vacuum. The residues were dissolved in methanol (5 mL) and 1 N HCl (1 mL) and concentrated on a Savant speed vac (approximately 2 mmHg for 20 h) to afford the target compound. Target compounds were characterized by analytical HPLC and mass spectrometry.

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Supporting Information Available: Double-reciprocal plot for compound **8** in the presence of varying concentrations of H-Ras; replot of the reciprocal plot slopes (K_m/V_{max}) versus concentration of compound **8**. This material is available free of charge via the Internet at http://pubs.acs.org.

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