4,5-Diarylisoxazole Hsp90 Chaperone Inhibitors: Potential Therapeutic Agents for the Treatment of Cancer

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Inhibitors of the Hsp90 molecular chaperone are showing considerable promise as potential chemotherapeutic agents for cancer. Here, we describe the structure-based design, synthesis, structure−activity relationships and pharmacokinetics of potent small-molecule inhibitors of Hsp90 based on the 4,5-diarylisoxazole scaffold. Analogues from this series have high affinity for Hsp90, as measured in a fluorescence polarization (FP) competitive binding assay, and are active in cancer cell lines where they inhibit proliferation and exhibit a characteristic profile of depletion of oncogenic proteins and concomitant elevation of Hsp72. Compound **40f** (VER-52296/NVP-AUY922) is potent in the Hsp90 FP binding assay ($IC_{50} = 21$ nM) and inhibits proliferation of various human cancer cell lines in vitro, with GI_{50} averaging 9 nM. Compound **40f** is retained in tumors in vivo when administered i.p., as evaluated by cassette dosing in tumor-bearing mice. In a human colon cancer xenograft model, **40f** inhibits tumor growth by ∼50%.

Introduction

Molecular chaperones are proteins which play a key role in the conformational maturation, stability and function of other "client"protein substrates within the cell.[1,2] Many of the client proteins of heat shock protein 90 (Hsp90)[3–6] are involved in signal transduction, cell cycle regulation and apoptosis, including kinases, transcription factors and hormone receptors. The dysregulation of pathways involving these proteins are commonly associated with cancer pathology,[7] and Hsp90 thus plays a key role in assisting survival, proliferation, invasion, metastasis and angiogenesis, which represent the hallmark traits of malignancy. However, Hsp90 is ubiquitously expressed in normal cells, comprising 1–2% of the total cellular protein, and has important functions in normal cell homeostasis. Consequently, Hsp90 was not pursued as a drug target until the discovery and characterization of natural product inhibitors of Hsp90 function such as geldanamycin[8–10] (GM) and radicicol[11,12] (RD) (Figure 1, **1a** and **2**). In the cell, Hsp90 function is complex, involving homodimerization and binding to client proteins, co-chaperones and other accessory proteins operating in a chaperone cycle.[13] The chaperone cycle is driven by hydrolysis of ATP to ADP and by ATP/ADP exchange,[14,15] and it was shown that both GM and RD are able to inhibit Hsp90 function by binding to an ATP binding pocket in the N-terminal domain of Hsp90.[16] The subsequent loss of chaperone function causes client proteins to be degraded by the ubiquitin−proteasome pathway. The prevalence of a high-affinity form of Hsp90 in tumor cells, the "addiction"of cancer cells to oncogenic client proteins and their greater dependency on Hsp90 have been proposed as rationales for selectivity of Hsp90 inhibitors for cancer versus normal cells.[17] Further validation of Hsp90 as a protein target for drug discovery comes from pharmacological and clinical studies with the GM analogue 17-allylaminogeldanamycin (17-AAG, Figure 1, **1b**).[18] This first-in-class Hsp90 inhibitor to enter clinical trials has generated encouraging results in Phase-I trials.[19–21] Further clinical trials have been initiated with 17-AAG used as a single agent and in drug combinations in various cancer types. Hsp90 inhibitors are now the focus of significant research and development efforts,[22–25] and their ability to interfere with multiple oncogenic pathways has raised hopes that they will show broad antitumor activity and be less susceptible to acquired drug resistance. However, despite the high cellular activity and clinical progression of 17-AAG, this compound has several potential limitations, including poor solubility, (thereby demanding special formulation), limited bioavailability, hepatotoxicity and extensive metabolism by polymorphic enzymes.[26–28] Some of these issues have been addressed with the development of the water-soluble ansamycin 17-dimethylaminoethylgeldanamycin (17-DMAG Figure 1, **1c**)[29] and the prodrug of 17-AAG, IPI-504[30,31] (Figure 1), which are reported to be in Phase-I/Ib and Phase-I/II clinical trials, respectively. However, these issues and the inherent chemical complexity of these ansamycin inhibitors have led to significant efforts to identify novel small-molecule inhibitors of Hsp90.[22,32–37]

Research from several groups has focused on the purine scaffold and has generated potent inhibitors such as **3** and **4** (Figure 2), which are active in cellular and animal models of cancer.[38–40] We recently disclosed the identification of 4,5-diarylpyrazoles such as **5** (Figure 3) as a new class of Hsp90 inhibitors,[41,42] and the structure-based elaboration of this hit resulted in the amide-substituted pyrazole **6**.[43] Compound **6** binds to the ATP binding site of Hsp90 with an IC_{50} of 25 nM in a FP assay and has antiproliferative activity against HCT116 colon cancer cells, with a GI_{50} of 260 nM. This activity compares favorably with the GI_{50} value for 17-AAG of 160

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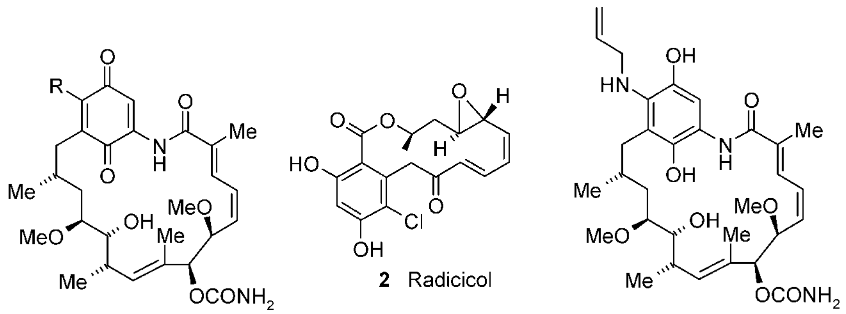
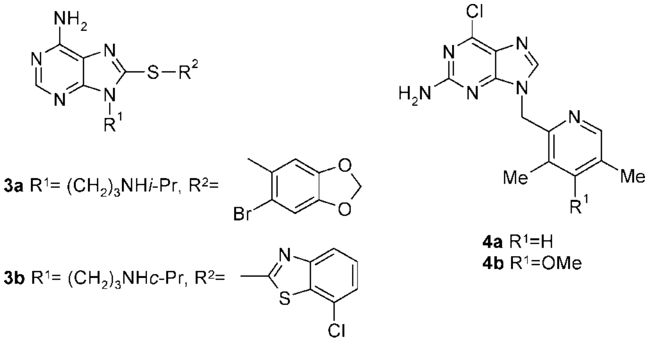


1a Geldanamycin R = OMe
1b 17-AAG R = $NHCH_2CH=CH_2$
1c 17-DMAG R = $NHCH_2CH_2NMe_2$

2 Radicicol

IPI-504

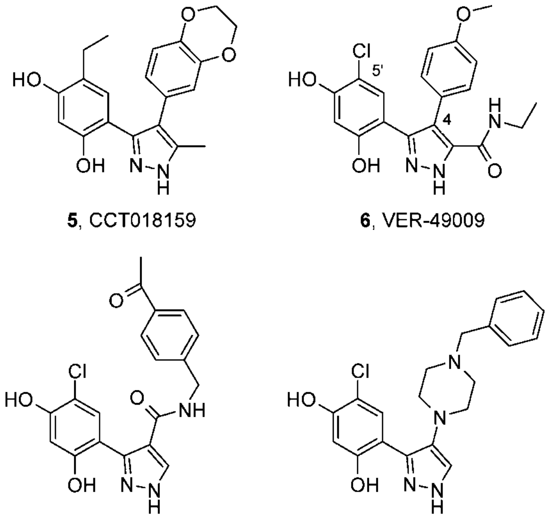
Figure 1. Natural product derived Hsp90 inhibitors.



3a R^1= $(CH_2)_3NH$*i*-Pr, R^2=
3b R^1= $(CH_2)_3NH$*c*-Pr, R^2=

4a R^1=H
4b R^1=OMe

Figure 2. Purine class Hsp90 inhibitors.



5, CCT018159 **6**, VER-49009

7 **8**

Figure 3. Pyrazole class Hsp90 inhibitors.

nM in the same tumor cell line. Further medicinal chemistry efforts in this area have identified 4-substituted pyrazole amides such as **7**[44] and piperazines **8**.[45] These are not as active in the FP assay as compound **6** and, consequently, exhibit reduced activity in tumor cell lines. Here, we report on further medicinal chemistry studies which identified diarylisoxazoles with a greater than 20-fold increase in their ability to inhibit cancer cell proliferation compared with that of previously reported diarylpyrazole compounds. Pharmacokinetic analyses performed by cassette dosing in tumor-bearing animals were used to select compounds showing the highest ratio between tumor levels and cellular GI_{50}. The drug discovery potential of lead compounds was further evaluated by monitoring target modulation in vitro and assessing the ability to inhibit tumor growth in the HCT116 human colon carcinoma murine xenograft model.

Chemistry

Our strategy to optimize the in vivo activity in our series was focused on the structure–activity relationships (SAR) in three distinct areas of the molecule and was guided by structural information of ligands (such as **6**) bound to the ATP binding site of Hsp90α.[46] The binding mode anchor of this family of compounds is the resorcinol moiety, also present in radicicol.[16] The resorcinol binds deep into the pocket, where the 4′-hydroxyl displaces one of the four water molecules that mediate substrate binding in the adenine binding region. Both phenolic hydroxyl groups and the N atoms of the pyrazole ring form a very tight network of hydrogen bonds with the carboxylate of the key residue Asp93 and surrounding polar groups or water molecules. The high complementarities and tight packing between Hsp90 and the inhibitor in this area leave room only for small modifications of

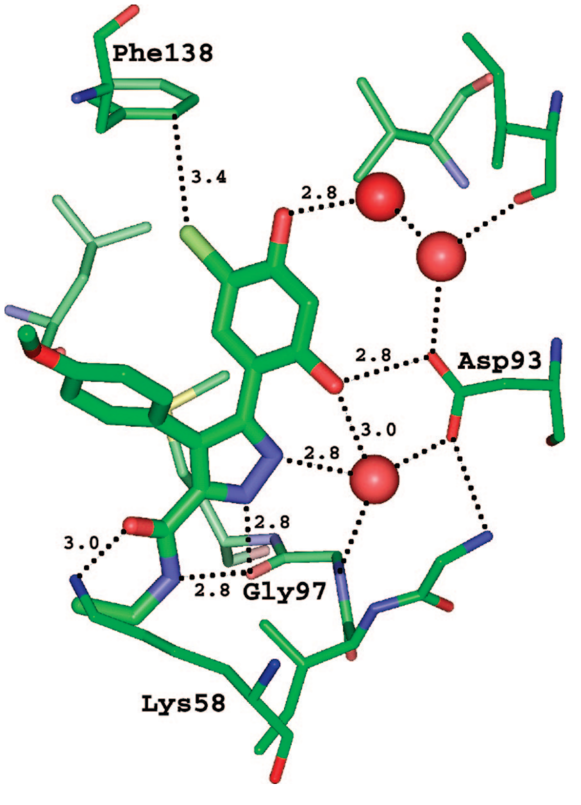
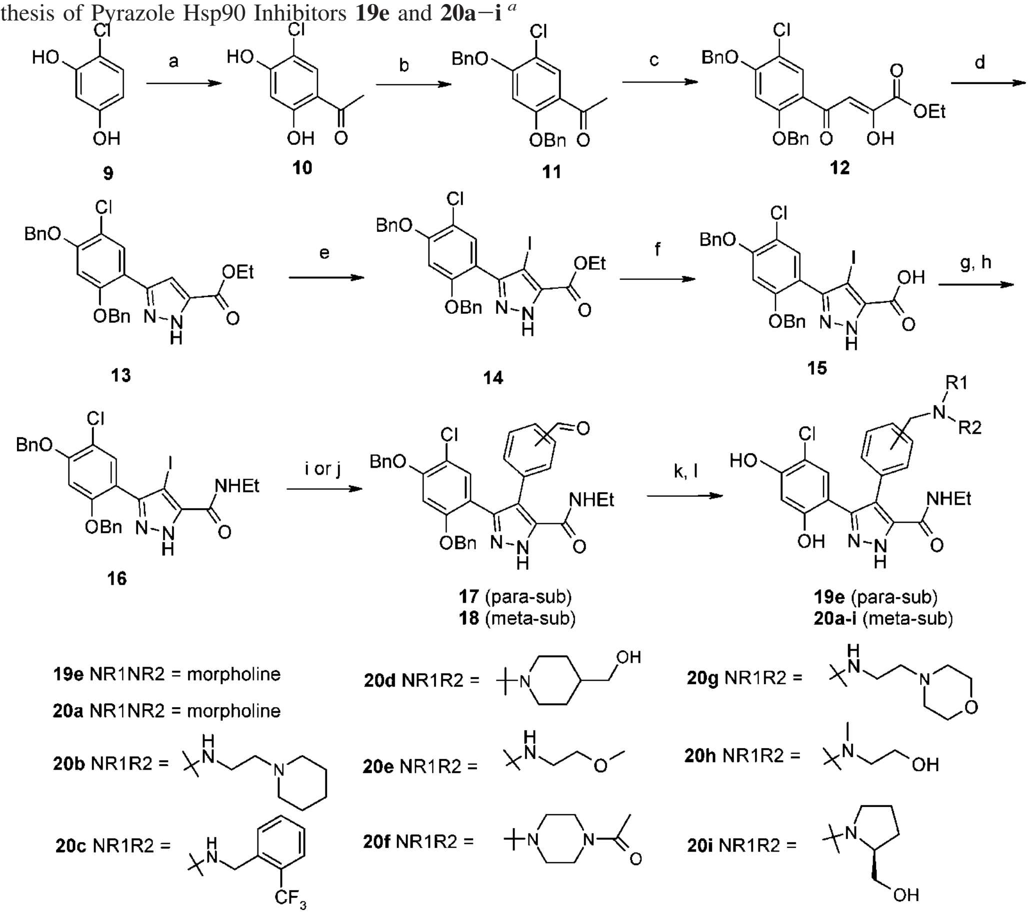


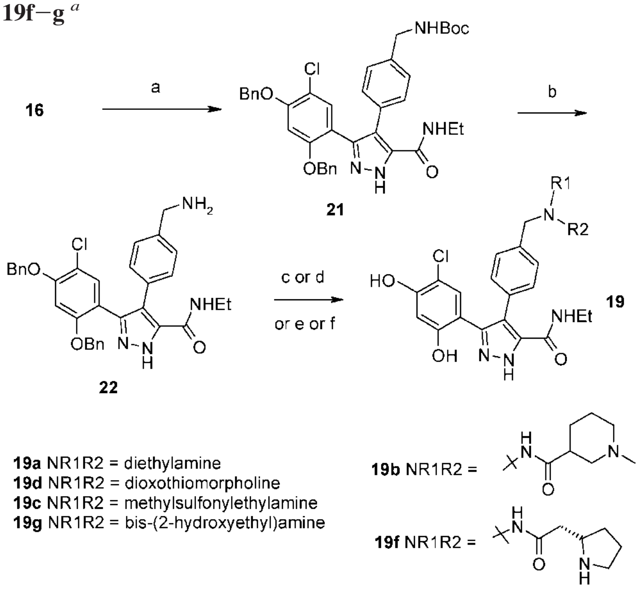
Figure 4. X-ray structure of **6** bound to the ATP binding site of human Hsp90α. Red spheres represent structurally conserved water molecules.

Scheme 1. Synthesis of Pyrazole Hsp90 Inhibitors **19e** and **20a**–**i**[a]



[a] Reagents and conditions: (a) AcOH, $BF_3 \cdot OEt_2$, 58%; (b) BnBr, MeCN, K_2CO_3, 90%; (c) $(CO_2Et)_2$, NaOEt, EtOH, 94%; (d) H_2NNH_2, AcOH, 87%, or $H_2NNH_2 \cdot HCl$, EtOH, Δ, 81%; (e) NIS, CAN (1 mol %), MeCN, Δ, 98%; (f) 2 M NaOH, dioxane; 92%; (g) $(COCl)_2$, DMF, DCM; (h) $EtNH_2 \cdot HCl$, NEt_3, 99%; (i) 4-formylphenylboronic acid, Pd(dppf)Cl_2, K_2CO_3, THF, H_2O, microwave, 68%; (j) 3-formylphenylboronic acid, Pd(dppf)Cl_2, K_2CO_3, THF, H_2O, microwave, 71%; (k) R1R2NH, $NaCNBH_3$, AcOH, MeOH; (l) H_2, Pd/C, AcOH.

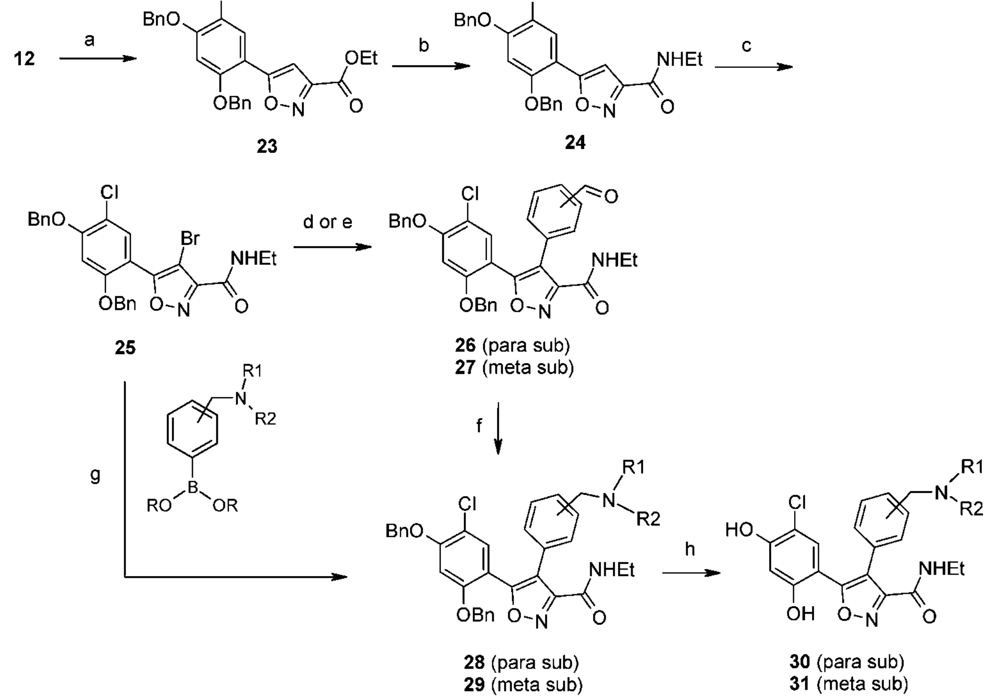
Scheme 2. Synthesis of Pyrazole Hsp90 Inhibitors **19a**–**d** and **19f**–**g**[a]



[a] Reagents and conditions: (a) 4-aminomethylphenyl boronic acid, H_2O, THF, Pd(dppf)Cl_2, K_2CO_3, Boc_2O, Et_3N, DMF, Δ, 26%; (b) (i) TFA; (ii) $NaHCO_3$, 95%; (c) (i) vinylmethylsulfone or divinylsulfone; (ii) BCl_3, DCM; (d) (i) acetaldehyde, $NaBH(OAc)_3$, DCE then $NaBH_4$, EtOH; (ii) cyclohexadiene, Pd/C, MeOH, 16%; (e) (i) HATU, DMF, DIPEA, 1-methylpiperidine-3-carboxylic acid or 2-carboxymethylpyrrolidine-1-carboxylic acid, rt; (ii) BCl_3, DCM; (f) (i) 2-bromoethanol, EtOH, DIPEA; (ii) BCl_3, DCM, 13%.

the inhibitor's core, but a wide range of substituents can be placed off of the pyrazole ring and the 5′ position of the resorcinol. Accordingly, and bearing in mind the experience gained in our previous work,[43–45,47] the areas that we focused on were (i) incorporation of a solubilizing group on the 4-arylpyrazole substituents, (ii) modification of the central pyrazole heterocycle, and (iii) the optimization of the 5′ substituent on the resorcinol ring. Figure 4 shows compound **6** bound to the Hsp90 ATP binding site and illustrates the importance of the key interaction of the resorcinol with Asp93 and the hydrogen bond from the 5-amide substituent to Gly97.[43,48] Further inspection of this structure, and others of the same structural class, indicated that the meta and para positions of the aryl group appended to the 4 position of the pyrazole were pointing toward solvent and should therefore be suitable sites for the incorporation of a solubilizing group. Thus, we initiated the synthesis of pyrazole Hsp90 inhibitors (**19** and **20**), incorporating CH_2-amine moieties as the solubilizing functionality. Several routes to these targets were utilized (Schemes 1 and 2). Key intermediates were the *p*- and *m*-formylphenyl compounds (**17** and **18**), serving as precursors to reductive amination reactions (Scheme 1), or the *p*-aminomethyl derivative **22**, as a versatile reagent for reductive amination, alkylation, conjugate addition and amide-forming reactions. The route used for the synthesis of **19e** and **20a**–**i** is shown in Scheme 1. Acetophenone **10** was made by regioselective Friedel–Crafts acylation of commercially available 4-chlororesorcinol **9**. Both phenolic hydroxyls were protected with benzyl groups, generating **11** prior to the subsequent reaction of an intermediate sodium

Scheme 3. Synthesis of Isoxazole Hsp90 Inhibitors [a]

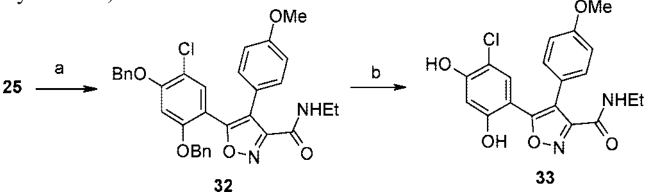


30a NR1R2 = dioxothiomorpholine
30b NR1R2 = methylsulfonylethylamine
30c NR1R2 = 4-methylpiperazine
30d NR1R2 = morpholine
30f NR1R2 = diethylamine
30g NR1R2 = piperidine
30i NR1R2 = 4-hydroxypiperidine
30j NR1R2 = pyrrolidine
30k NR1R2 =dimethylamine

31a NR1R2 = morpholine
31b NR1R2 = 4-methylpiperazine
31c NR1R2 = 4-acetylpiperazine

[a] Reagents and conditions: (a) $H_2NOH \cdot HCl$, EtOH, 86%; (b) $EtNH_2$, MeOH, Δ, 89%; (c) NBS, CAN, MeCN, Δ, 4.5 h, 74%; (d) 4-formylphenylboronic acid, $Pd(PPh_3)_2Cl_2$, $NaHCO_3$, DMF, H_2O, 73%; (e) 3-formylphenylboronic acid, $Pd(PPh_3)_2Cl_2$, $NaHCO_3$, DMF, H_2O; (f) R1R2NH, $NaCNBH_3$, AcOH, MeOH; (g) boronate ester, DME, $NaHCO_3$, $Pd(PPh_3)_4$; (h) BCl_3, DCM, 0 °C.

Scheme 4. Preparation of **33** (the Isoxazole Analogue of Pyrazole **6**) [a]



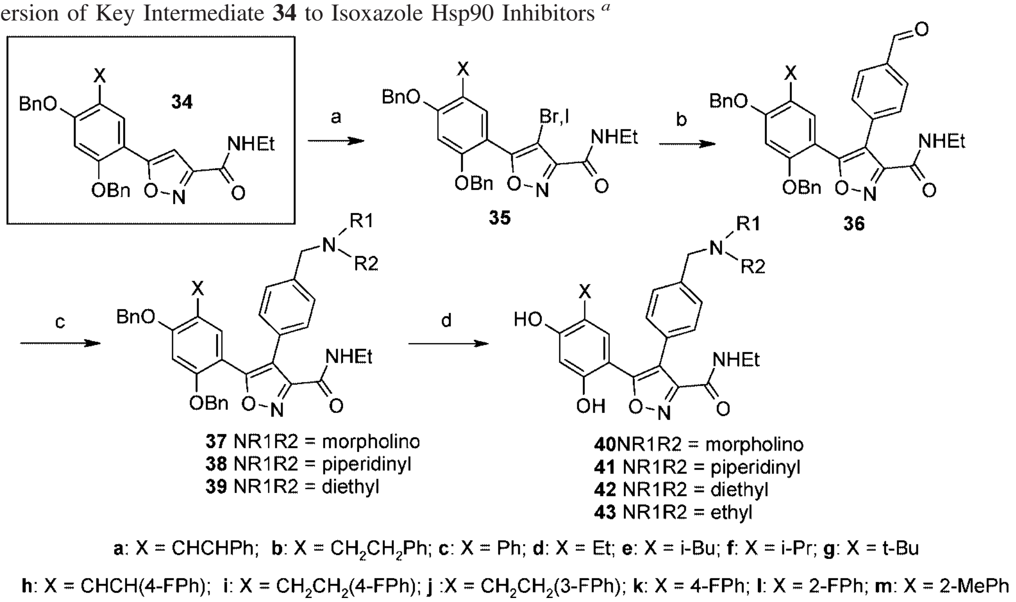
[a] Reagents and conditions: (a) 4-methoxyphenylboronic acid, $NaHCO_3$, H_2O, DMF, $Pd(PPh_3)_2Cl_2$, 63%; (b) BCl_3, DCM, 0 °C, 65%.

enolate with diethyl oxalate, which afforded keto–enol ester **12**. Construction of the pyrazole heterocycle **13** was achieved by reaction of **12** with hydrazine hydrate in acetic acid or hydrazine hydrochloride in ethanol. Iodination at the 4-position of the pyrazole was achieved with *N*-iodosuccinimide, and it was found that the reaction yield was significantly improved with the addition of a catalytic amount of ceric ammonium nitrate. Conversion of the ethyl ester moiety of **14** to the ethylamide of **16** was achieved by ester saponification, activation of resulting acid **15**, and subsequent reaction with ethylamine. Compound **16** underwent a palladium-catalyzed Suzuki cross-coupling reaction with *p*- or *m*-formylphenylboronic acid to generate **17** and **18**, respectively. Reductive amination with various amines, followed by hydrogenation to remove the benzyl groups, furnished the desired amines **19e** and **20 a–i**.

Compounds **19a–d** and **19f**,**g** were made using alternative procedures (Scheme 2) from the key intermediate aminomethyl derivative **22**. Compound **16** was cross-coupled with 4-aminomethylboronic acid and protected in situ as the *t*-butyloxycarbamate derivative to aid in product isolation. Trifluoroacetic-acid-mediated deprotection afforded the amine **22**, which was functionalized via four distinct synthetic methodologies. Diethylamine **19a** was prepared by reductive amination with acetaldehyde and subsequent debenzylation by transfer hydrogenolysis with cyclohexadiene and palladium on carbon. Sulfones **19c** and **19d** were prepared by vinylogous attack of the amine on methylvinylsulfone and divinylsulfone, respectively. Amides **19b** and **19f** were formed by a HATU[49]-mediated coupling procedure, and **19g** utilized *bis* nucleophilic displacement on 2-bromoethanol. With the exception of **19a**, final products were obtained by boron trichloride-mediated deprotection of the dibenzyl resorcinol.

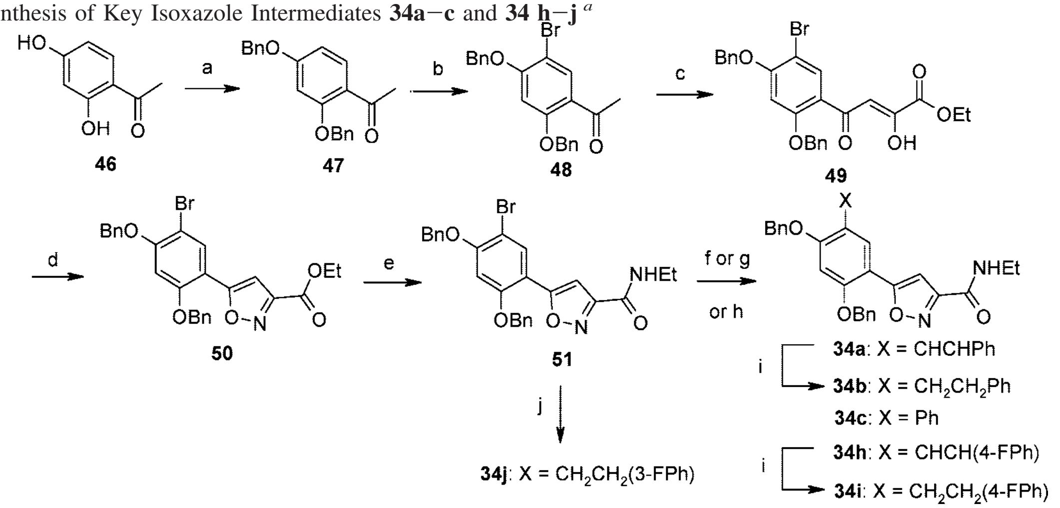
The synthesis of the isoxazoles is analogous to that used for the pyrazole series (Scheme 3). Keto–enol ester **12** was reacted with hydroxylamine hydrochloride to give the isoxazole **23**, and the subsequent conversion to final compounds utilized methods similar to those used for the corresponding pyrazoles, as outlined in Scheme 1. The amide functionality however was introduced directly from the ester prior to halogenation. *N*-bromosuccinimide (NBS)/ceric ammonium nitrate (CAN) proved superior for bromination compared to an earlier method which used bromine in acetic acid. However, compared to pyrazole bromination, a relative increase in the amount of CAN was

Scheme 5. Conversion of Key Intermediate **34** to Isoxazole Hsp90 Inhibitors [a]



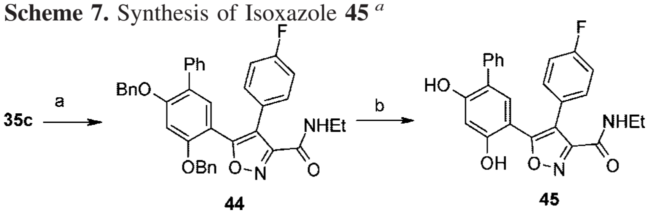
[a] Reagents and conditions: (a) NBS or NIS, CAN, MeCN, Δ; (b) 4-formylphenylboronic acid, $NaHCO_3$, H_2O, DMF, $Pd(PPh_3)_2Cl_2$; (c) R1R2NH, $NaCNBH_3$, AcOH, MeOH; (d) BCl_3, DCM, 0 °C.

Scheme 6. Synthesis of Key Isoxazole Intermediates **34a–c** and **34 h–j** [a]



[a] Reagents and conditions: (a) BnBr, K_2CO_3, MeCN, 97%; (b) NBS, DMF, rt, 97%; (c) $(CO_2Et)_2$, NaOEt, EtOH, 96%; (d) hydroxylamine hydrochloride, EtOH, Δ, 92%; (e) $EtNH_2$, MeOH, Δ, 80%; (f) *trans*-2-phenylvinylboronic acid, DMF, H_2O, $Pd(PPh_3)_2Cl_2$, $NaHCO_3$, Δ, 71%; (g) $PhB(OH)_2$, K_2CO_3, H_2O, THF, $Pd(dppf)Cl_2$, Δ, 68%; (h) *trans*-2-(4-fluorophenyl)vinylboronic acid, DMF, H_2O, $Pd(PPh_3)_2Cl_2$, $NaHCO_3$, 69%; (i) 1,4-dioxane, Pd(C), H_2; (j) (i) 3-fluorostyrene, $Pd(o\text{-}Tol_3P)_2Cl_2$, *n*-BuOH; *i*-$Pr_2NH$, (ii) $TsNH_2NH_2$, NaOAc, 1,2-DME, Δ, 92%.

Scheme 7. Synthesis of Isoxazole **45** [a]

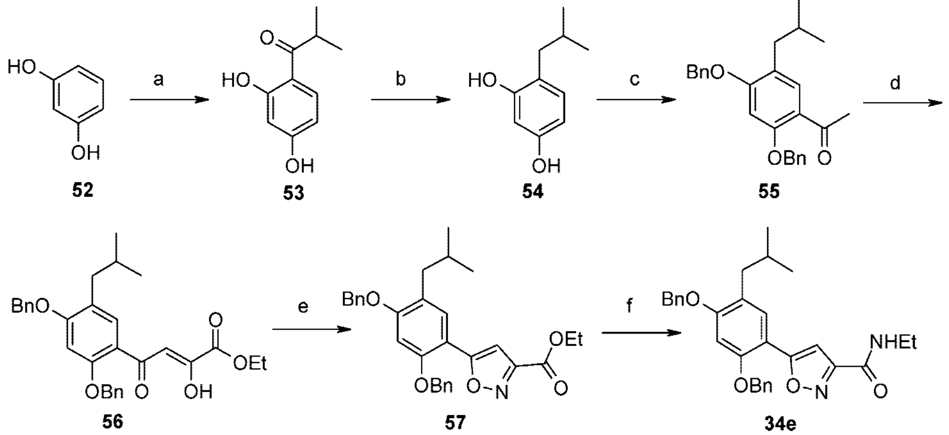


[a] Reagents and conditions: (a) 4-fluorophenylboronic acid, $NaHCO_3$, H_2O, DMF, $Pd(dppf)Cl_2$, 76%; (b) BCl_3, DCM, 0 °C, 65%.

necessary to effect good yields in this case. Debenzylation of **28** and **29** was accomplished with boron trichloride in preference to hydrogenation. This change was due to concerns that hydrogenation may cleave the isoxazole ring and the observation of partial (ca. 5%) 5′-chlorine reduction in several reactions using catalytic hydrogenation. Some compounds of this class were made by a modified sequence, in which **25** was cross-coupled with boronate esters having the amino functionality already present. A modification of this route was also used for making the isoxazole analogue (**33**) of VER-49009 (**6**) using the intermediate **32** (Scheme 4).

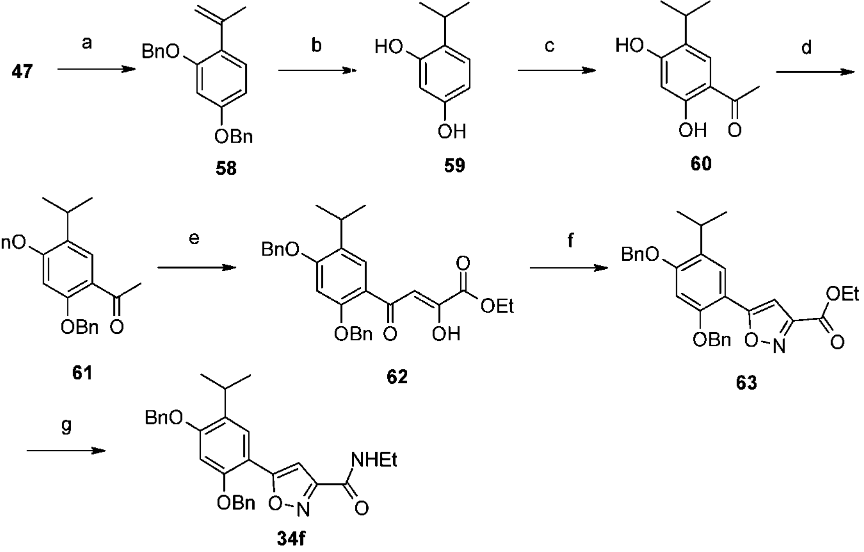
The final area of interest for SAR studies described here was the 5′ position on the resorcinol ring. Substituents at C-5′ have the potential to induce a conformational change in the protein by forming a helix between residues 104 and 111.[50] This structural change creates a lipophilic pocket in the ATP binding site of the Hsp90 protein, and we were keen to explore the influence of substituents at this position on the potency and physicochemical properties of the Hsp90 ligands. All of the 5′ analogues were made from key intermediates **34** by the method outlined in Scheme 5. Differing synthetic methodologies were utilized for the synthesis of variably substituted 5′-resorcinol derivatives of **34**. For 5′-aryl and 5′-phenethyl substituents, the 5′-bromo intermediate **51** (Scheme 6) was used as a substrate for Suzuki or Heck Pd-catalyzed coupling reactions. Styrene

Scheme 8. Synthesis of Isoxazole Intermediate **34e** [a]



[a] Reagents and conditions: (a) isobutyric acid, $BF_3 \cdot OEt_2$ quant; (b) (i) ethylchloroformate, NEt_3, THF; (ii) $NaBH_4$, THF/H_2O; (iii) 10% aq NaOH, 98%; (c) (i) AcOH, $BF_3 \cdot OEt_2$; (ii) BnBr, K_2CO_3, MeCN, 25%; (d) $(CO_2Et)_2$, NaOEt, EtOH, Δ, 58%; (e) hydroxylamine hydrochloride, EtOH, Δ, 74%; (f) $EtNH_2$, MeOH, Δ, quant.

Scheme 9. Synthesis of Isoxazole Intermediate **34f** [a]



[a] Reagents and conditions: (a) Ph_3PMeBr, *n*-BuLi, THF, 80%; (b) H_2, Pd/C, EtOH, 70%; (c) AcOH, $BF_3 \cdot OEt_2$, 88%; (d) BnBr, K_2CO_3, DMF, 88%; (e) $(CO_2Et)_2$, NaOEt, NaH, toluene, Δ, 86%; (f) hydroxylamine hydrochloride, EtOH, Δ, 91%; (g) $EtNH_2$, MeOH, Δ, 85%.

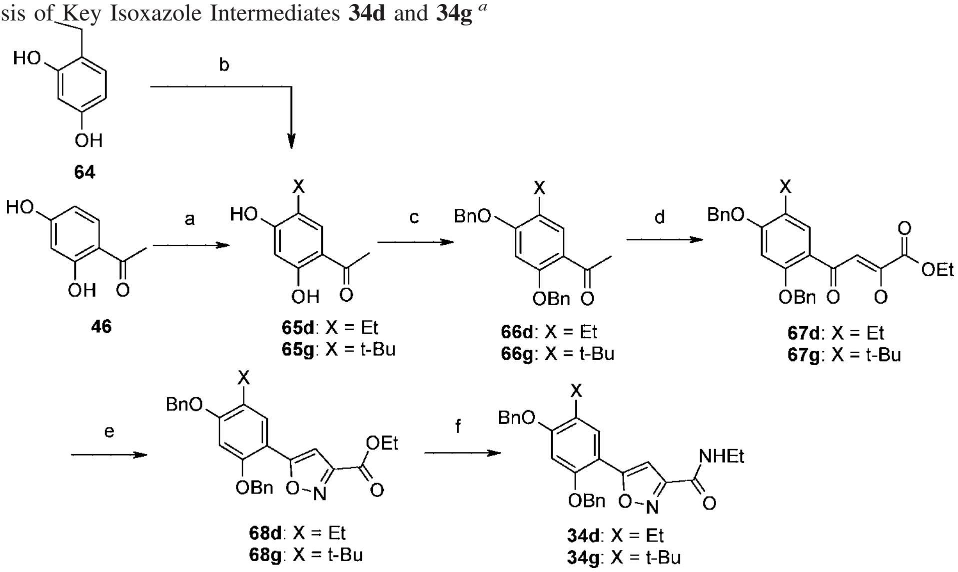
derivatives such as **34a** from Heck or Suzuki reactions of styrenes or vinylogous boronic acids were reduced prior to further manipulation. Compound **35c** (Scheme 6) was subsequently used to make the 5′-phenyl derivative **45** (Scheme 7), which was used for compound profiling comparisons. For the isobutyl-substituted intermediate **34e**, the route outlined in Scheme 8 was used. Starting from resorcinol, a Friedel−Crafts acylation afforded acetophenone derivative **53**, which was reduced[51] to the *sec*-butyl compound **54** prior to a second Friedel−Crafts acylation and benzyl protection. Construction of the key isoxazole **34e** proceeded as described above. An acetophenone derivative was also used in the route to the isopropyl-substituted intermediate **34f** (Scheme 9). In this case however, a Wittig reaction was followed by hydrogenation to generate the 5′-isopropyl substituent (**47**–**59**, Scheme 9). The reduction of the styrene functionality in **58** caused concomitant debenzylation. However, the resorcinol **59** acylated in better yield than the corresponding benzyl-protected substrate; therefore, reprotection was delayed until the subsequent step. The 5′-ethyl-substituted compounds (such as **34d**) were made from 4-ethylresorcinol by the same route as that used for the 5-chlororesorcinol outlined in Scheme 3 (see also Scheme 10).

For the introduction of a 5′-*tert*-butyl group, Friedel−Crafts alkylation methodology was applied (Scheme 10), utilizing the *tert*-butyl cation generated from *tert*-butanol to afford acetophenone **65g**. Conversion to **34g** was accomplished in a similar fashion as that described previously for compound **24** (Scheme 3).

Results and Discussion

Using the routes outlined in Schemes 1 and 2, we synthesized a number of pyrazoles incorporating solubilizing groups on the 4-aryl group (Table 2). For substituents in the para position of the 4-aryl group, in some cases, both enzyme potency and cellular GI_{50} values (**19e** and **19f**) were similar to those of VER-49009 (**6**). However, in most instances, these diverse analogues were less potent than **6**. For compounds with aminomethyl groups at the meta position (**20a**−**i**), none of the examples matched the binding affinity of compound **6** in the FP assay or in the cell growth inhibition assay with HCT116 cells. Thus, although the crystal structure of **6** suggested that there was space to introduce solubilizing groups off of the 4-aryl substituent, it appeared that the regiochemistry and nature of the solubilizing

Scheme 10. Synthesis of Key Isoxazole Intermediates **34d** and **34g** [a]



[a] Reagents and conditions: (a) H_2SO_4, 2-methylpropan-2-ol, TFA, 75 °C, 92%; (b) AcOH, $BF_3\cdot OEt_2$, 90 °C; (c) BnBr, K_2CO_3; (d) $(CO_2Et)_2$; NaOEt, EtOH; (e) $H_2NOH\cdot HCl$, EtOH, Δ; (f) $EtNH_2$, MeOH, Δ.

Table 1. Binding (FP Assay) and Cell Growth Inhibition (SRB Assay) Data for Previously Reported Hsp90 Inhibitors[a]

compound	FP IC_{50}[a] (μM)	GI_{50}[a] HCT116 (μM)
17−AAG **(1b)**	1.27	0.16
5	0.28	5.8
6	0.025	0.26
7	0.258	11.6
8	0.600	6.5

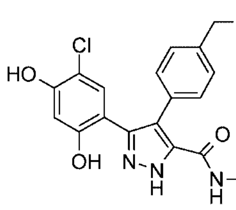
[a] Values are reported as the mean of at least two independent determinations.

group had an effect on how well this substitution was tolerated in these pyrazole analogues.

Examination of the binding for the pyrazole ring in ligands such as **6** demonstrated that the polar interactions deep inside of the binding site are very well matched between the protein and ligand. With this in mind, it was clear to us that very little steric change would be tolerated in this region. The nitrogen adjacent to the resorcinol (N1) is involved in hydrogen bonding with the key Asp93/water network at the base of the pocket as an H-bond acceptor. We felt that an alternative hydrogen-bond acceptor such as oxygen might be advantageous. Pyrazoles can exist in tautomeric forms, and when N1 is protonated, this represents a form which is unlikely to bind well to Hsp90. Further, the interaction between N2 and the amide backbone carbonyl of Gly97 is not in the ideal geometry. Therefore, fixing the position designated N1 as a 100% H-bond acceptor was considered to be a preferred feature, with the N2 position being less critical. We desired to achieve such a subtle change while keeping the other carbon positions already substituted with key functionality in a defined orientation for binding. Isoxazoles and pyrazoles differ in that the former does not bear hydrogen on a heteroatom and can therefore not be substituted. As a consequence, the isoxazole is also smaller in size and should still be accommodated in the binding site. In addition, there is no possibility of the existence of isomeric forms that can exist with the pyrazole. Evaluation of the binding of the isoxazole analogue **33** (Scheme 4) of the pyrazole **6** to Hsp90 protein gave an IC_{50} value of 28 nM, similar to that of compound **6** (25 nM). This shows that the change is tolerated, with the matched pyrazole/isoxazole **6**/**33** exhibiting equipotent binding in the FP assay. However, the advantage of the isoxazole over the pyrazole scaffold became apparent when we looked at the activity of **6** versus **33** in cell growth inhibition assays across a range of human cancer cell lines (Table 3). These data demonstrated a trend for higher potency (with respect to cell growth inhibition) for the isoxazole **33.** The interactions of pyrazole **6** and isoxazole **33** with Hsp90 were studied previously by isothermal calorimetry,[47] which showed that the isoxazole−Hsp90 complex is marginally more stable than the corresponding pyrazole complex and that the difference is predominantly due to the enthalpy difference. Kinetic analysis by surface plasmon resonance (SPR) has shown that, although these compounds have similar K_{on} values (6.4×10^5 and 8.2×10^5 M^{-1} s^{-1}) for **33** and **6**, respectively, the K_{off} for isoxazole **33** of 4.05×10^{-4} s^{-1} is 10-fold slower than that of 5.64×10^{-3} s^{-1} for pyrazole **6**. These combined data can help account for the increased cellular potency of the isoxazole and also help rationalize the measured four-fold higher concentrations of **33** compared to **6** recently shown in HCT116 cells. This was also sufficient for the isoxazole **33** to demonstrate the first signs of in vivo efficacy in a xenograft tumor model, as reported previously.[47]

The pyrazole to isoxazole scaffold change was next combined with solubilizing group incorporation on the 4-aryl ring. Table 4. shows data for a series of diarylisoxazoles, substituted with a range of amine-bearing substituents predominantly on the para position of the 4-aryl ring. It was clear that the pyrazole to isoxazole switch was very well tolerated in this series of compounds and that the preference for substitution at the para position of the 4-aryl ring compared to the meta position seen with analogous pyrazole series was maintained. All of the para-substituted examples had sub-70 nM IC_{50} values in the binding assay and were at least equipotent with analogous pyrazoles (**30a** vs **19d**, **30b** vs **19c**, and **30f** vs **19a**). The meta-substituted compounds **31a**, **31b**, and **31c** were less potent, as was also observed in the pyrazole series, but when comparing analogous pyrazole analogues (**31a** vs **20a** and **31c** vs **20f**), the isoxazoles were more potent. Most importantly, the significant improved inhibition of cell proliferation seen with isoxazole **33** compared to that of pyrazole **6** was retained in this subseries (Table 4). Thus **30d**, **30f**, **30g**, and **30k** have sub-100 nM GI_{50} values against HCT116 cells.

Table 2. Binding (FP Assay) and Cell Growth Inhibition (SRB Assay) Data for Aminomethyl-Functionalized Diarylpyrazoles[a]



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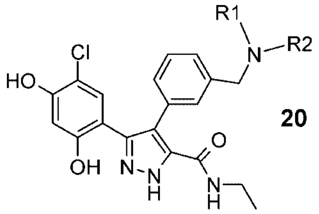
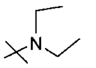
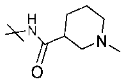
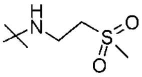
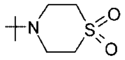
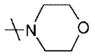
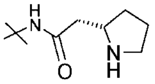
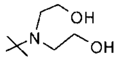
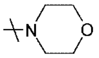
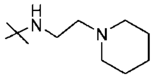
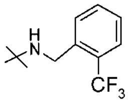
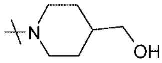
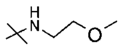
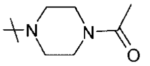
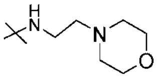
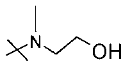
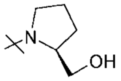


20

Compound Number	NR1R2	FP IC_{50}[a] (μM)	GI_{50}[a] (HCT116) (μM)
19a		0.146	0.315
19b		0.115	3.47
19c		0.035	2.162
19d		0.027	0.826
19e		0.037	0.290
19f		0.057	0.275
19g		0.142	7.80
20a		0.222	2.69
20b		1.00	25.14
20c		0.231	3.11
20d		2.63	>80
20e		0.728	12.93
20f		1.29	32.7
20g		0.431	12.54
20h		0.914	9.24
20i		1.66	11.37

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[a] Values are reported as the mean of at least two independent determinations.

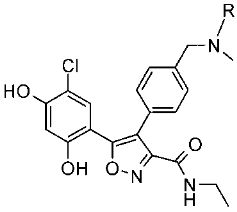
We next turned our attention to the 5′ position of the resorcinol. We had previously shown[43] that the removal of ethyl or chloro from this position resulted in a 20-fold decrease in binding affinity for ligands closely related to **6**. This loss in potency is rationalized by structural information showing that the ethyl and chloro groups are interacting at the edge of a

Table 3. Binding (FP Assay) and Cell Growth Inhibition (SRB Assay) Data in Various Human Cancer Cell Lines for Compounds **6** and **33**[a]

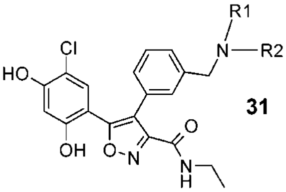
compound number	human Hsp90β FP IC$_{50}$ (μM)	HCT116 (colon) GI$_{50}$ (μM)	DU145 (prostate) GI$_{50}$ (μM)	PC3M (prostate) GI$_{50}$ (μM)	SKMel28 (melanoma) GI$_{50}$ (μM)	SF268 (glioma) GI$_{50}$ (μM)	U87MG (glioma) GI$_{50}$ (μM)
6	0.025	0.36	1.2	2.2	0.48	0.32	1.2
33	0.028	0.12	0.23	0.22	0.045	0.034	0.028

[a] Values are reported as the mean of at least two independent determinations.

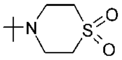
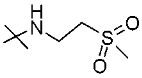
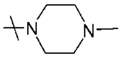
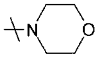
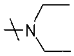
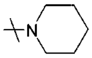
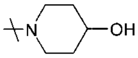
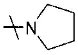
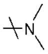
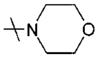
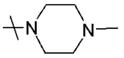
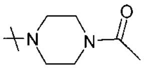
Table 4. Binding (FP Assay) and Cell Growth Inhibition (SRB Assay) Data for Aminomethyl-Functionalized Diarylisoxazoles[a]



30



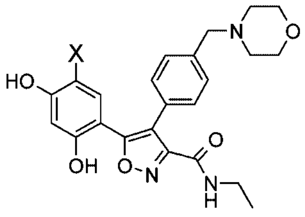
31

Compound Number	NR1R2	FP IC$_{50}$[a] (μM)[a]	GI$_{50}$[a] (HCT116) (μM)[a]
30a		0.021	0.47
30b		0.014	1.98
30c		0.039	0.125
30d		0.021	0.083
30f		0.064	0.056
30g		0.019	0.061
30i		0.028	1.288
30j		0.039	0.118
30k		0.018	0.069
31a		0.127	0.634
31b		0.343	2.78
31c		0.239	6.76

[a] Values are reported as the mean of at least two independent determinations.

lipophilic pocket created by Phe138, Met98, Val150, Leu103, Leu107, Trp162, and Tyr139. This region of the protein is also flexible and able to accommodate larger groups by a conformational movement of the loop defined by Ile104 to Ala111.[50] The data presented in Table 5. clearly demonstrate that a large steric variance is tolerated at the 5′ position. Groups such as phenethyl (**40i**, **40j**, and **41i**) and phenyl (**40c**, **41c**, and **41m)** retain binding affinity similar to those of the chloro analogues, such as **30d**, or small alkyl-substituted analogues, such as **42d** and **42f**. Additionally, these analogues demonstrated excellent

Table 5. Effects of Modifications to the 5′ Position of the Resorcinol on Binding (FP Assay) and Cell Growth Inhibition (SRB Assay)[a]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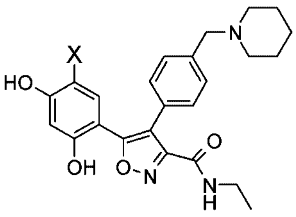
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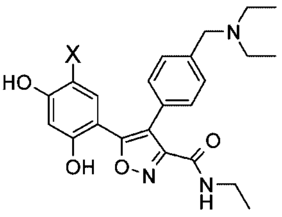
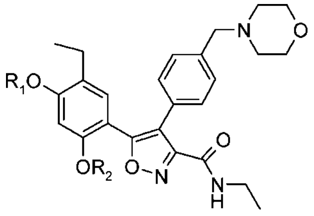

42

compound number	X	amine moiety	FP IC50[a] (μM)	GI_{50}[a] (HCT116) (μM)
40b	$-CH_2CH_2Ph$	morpholino	0.032	0.029
40d	Et	morpholino	0.029	0.025
42d	Et	NEt_2	0.013	0.041
40i	$-CH_2CH_2$(4-FPh)	morpholino	0.020	0.039
41i	$-CH_2CH_2$(4-FPh)	piperidinyl	0.026	0.029
40j	$-CH_2CH_2$(3-FPh)	morpholino	0.026	0.058
40c	Ph	morpholino	0.022	0.152
41c	Ph	piperidinyl	0.014	0.086
40l	2-FPh	morpholino	0.039	0.087
41m	2-MePh	piperidinyl	0.014	0.114
41k	4-FPh	piperidinyl	0.036	0.035
40e	isobutyl	morpholino	0.035	0.133
40g	*tert*-butyl	morpholino	0.008	0.070
41g	*tert*-butyl	piperidinyl	0.011	0.065
40f	isopropyl	morpholino	0.021	0.016
42f	isopropyl	NEt_2	0.006	0.006
41f	isopropyl	piperidinyl	0.006	0.040
43c	Ph	ethyl	0.074	0.091

[a] Values are reported as the mean of at least two independent determinations.

Table 6. Effect of Oxygen-methylation on Binding (FP Assay) and Cell Growth Inhibition (SRB Assay)[a]



compound number	R1	R2	FP IC_{50} (μM)	GI_{50} (HCT116) (μM)
40d	H	H	0.029	0.025
70	Me	H	0.015	0.080
71	H	Me	0.540	2.15
72	Me	Me	1.56	32.0

[a] Values are reported as the mean of at least two independent determinations.

potency in cell growth inhibition assays, as demonstrated by a number of the compounds in Table 5, in particular, the 5′-isopropyl analogues **40f** and **42f**.

To evaluate the importance of the resorcinol OH moieties in the binding of this class of inhibitor, several O-methylated analogues of the 5′-ethyl compound **40d** were synthesized. As demonstrated in Table 6, O-methylation at C-2′ caused a large drop in binding affinity. O-Methylation at C-4′ was tolerated, but compound **70** had reduced potency and cellular activity and thus demonstrated no advantage over the free resorcinol.

At all stages in the optimization process, X-ray crystal structures were obtained with ligands bound to the N-terminal domain of Hsp90α to confirm that the changes made in the elaboration of **6** had not altered the binding mode of the ligands. Figure 5shows an overlay of the X-ray structures of **6** and the isoxazole **40f**.[52] The structural data for **40f** demonstrate an equivalent binding mode as we previously reported[41,43,47] for **5**, **6**, and **33**, with the same network of hydrogen-bonding interactions involving the resorcinol moiety and the heterocyclic ring with Asp93, Thr184, and a cluster of structurally conserved and highly ordered water molecules. The isoxazole 3-amide group in compound **40f** makes the same hydrogen-bond interactions with the protein backbone via Gly97 as that seen with **6** and **33**, as well as a further interaction with Lys58, but as previously reported, the latter reaction does not significantly contribute to the binding free energy.[43,48] Replacement of the chlorine of the resorcinol ring in **6** or **30d**[53] by an isopropyl group in **40f** results in an additional hydrophobic interaction with Leu107 in the flexible lipophilic pocket, and additional hydrophobic interactions are also seen with Thr109 and Gly135 from the morpholine moiety present in **40f**.

It was important to establish that the new ligands retained the well-established molecular signature of known Hsp90 inhibitors with respect to the effect of the ligands on cellular markers. Figure 6shows that the pyrazoles **6** and **19e** and the isoxazoles **33**, **30d**, and **40f** gave the expected depletion of the client proteins Raf-1 and Her2 and induction of Hsp72 in HCT116 cells in vitro, as determined by western blots at 1 and 2 × GI_{50} concentrations. These results support the premise that the effects on cell growth were a consequence of Hsp90 chaperone inhibition.

We had previously shown[54] that the resorcinol moiety was glucuronidated but that in vitro microsomal metabolism rates were not predictive of the plasma clearance of this chemical series. Preferred compounds from the foregoing research were therefore further characterized in vivo using cassette dosing to mice bearing subcutaneous HCT116 human colon cancer xenografts, followed by quantitative LC/MS/MS analysis in various tissues.[54,55] The method was first validated by comparing results for a set of five compounds dosed singly and in mixture

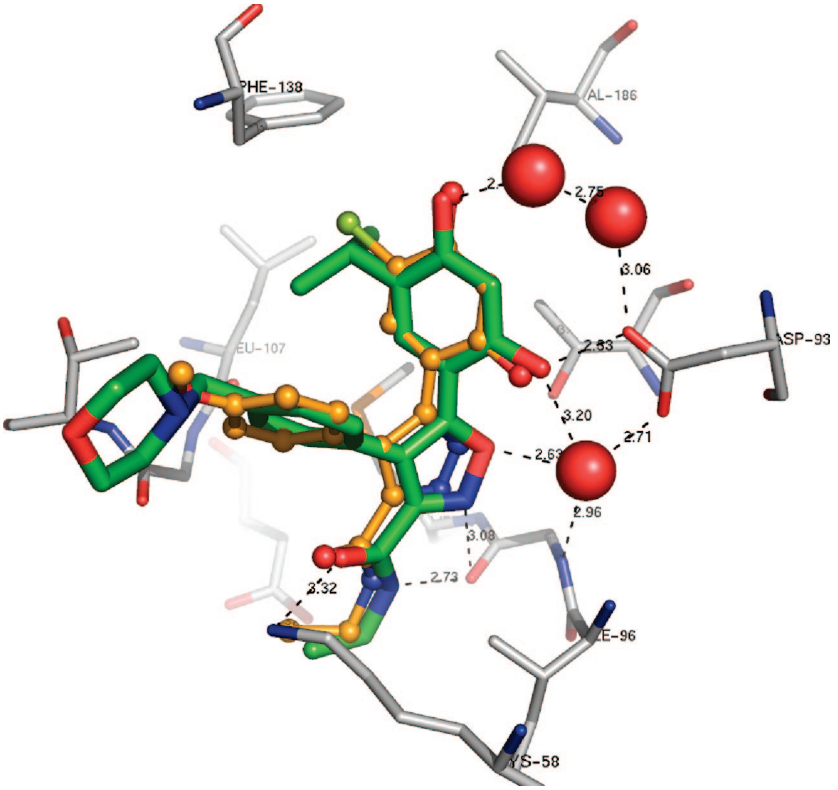


Figure 5. Overlay of X-ray structure of **6** and **40f** bound to the ATP binding site of human Hsp90α. Red spheres represent structurally conserved water molecules.

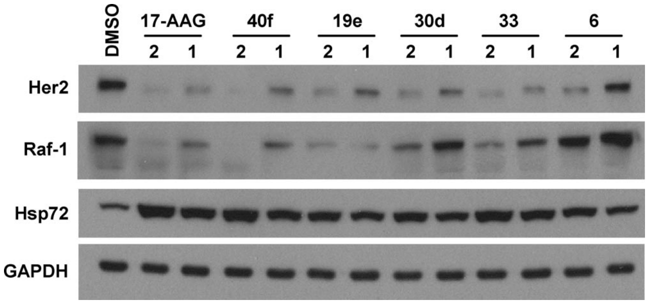


Figure 6. Effects of 17-AAG, pyrazoles **6** and **19e**, and isoxazoles **30d**, **33**, and **40f** on expression of Raf-1, Hsp72, and Her2 in HCT116 cells as determined by western blot. Compounds were dosed for 24 h at concentrations of 1 and 2 times the GI_{50} value. GAPDH was used as a loading control.

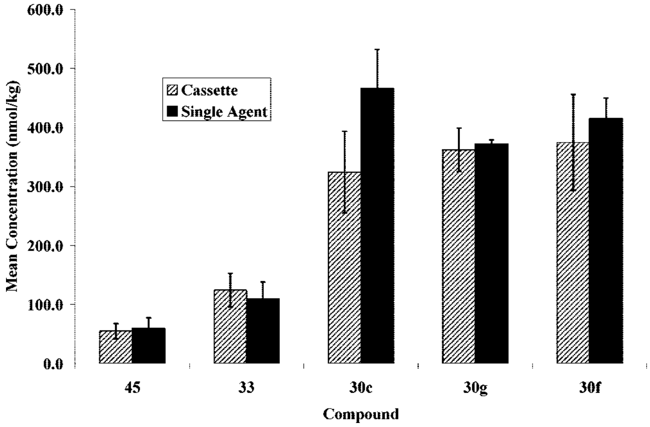


Figure 7. Cassette dosing method validation, showing the compound tumor concentration 6 h post administration in mice bearing HCT116 subcutaneous human colon tumor xenografts dosed at 4 mg per kg i.p. Compounds were dosed as single agents or were cassette dosed with mixtures of five compounds.

by measuring tumor concentrations 6 h post administration. Concentrations in tumors were similar in both experiments (Figure 7), with the same rank order, suggesting that compound–compound interactions were not significant. In subsequent studies, selected compounds were administered i.p. in cassettes of five compounds to tumor-bearing mice at a dose of 4 mg/kg each. Table 7. shows a summary of three cassettes dosed with some of the most potent diarylisoxazole amides. Table 8 shows some additional pharmacodynamic (PK) parameters for cassette dosing experiment 3. The pharmacokinetic standard used, **33**, gave consistent results for tumor levels but exhibited variable plasma concentrations (up to 60% between cassettes 1 and 3). Overall, tumor-to-plasma ratios were found to vary from 0.3 to 20. Compounds with aliphatic substituents such as ethyl (**40d**) and isopropyl (**40f**) in the 5′ position of the resorcinol had higher tumor exposure than compounds with aromatic substituents (such as **40b**, **41i**, and **41m**). The 5′-phenyl derivative **43c** had a comparatively high tumor concentration but still higher plasma levels. The 5′-chloro compounds also achieved high tumor concentrations (**30f**, **30g** and **30c**). The isopropyl analogue **40f** clearly showed the highest ratio of tumor concentration to cellular GI_{50} (up to 35-fold), with a half-life of 9.5 h in the tumor, and this compound was therefore taken forward for further study.

Isoxazole **40f** showed potent antiproliferative effects in a range of human cancer cell lines (Table 9), and the expected depletion of client proteins and induction of Hsp72 were observed in all of the cell lines examined (data not shown). Compound **40f** was administered at 50 mg/kg daily i.p. to

Table 7. Pharmacokinetic Data Obtained by Cassette Dosing with Isoxazole Compounds[a]

cassette	compound	AUClast tumor (hr*nmol/L)	AUClast plasma (hr*nmol/L)	tumor/plasma ratio	max fold above GI$_{50}$
cassette 1	**33**	1332.8	1222.8	1.1	6.5
	30c	2522.9	1542.3	1.6	4.7
	45	597.5	1191.8	0.5	1.5
	30f	2930.4	925.8	3.2	12.0
	30g	2403.5	989.0	2.4	20.0
cassette 2	**33**	1437.6	814.9	1.8	6.0
	40b	655.9	2547.2	0.3	4.2
	40d	1750.9	618.8	2.8	24.0
	41i	668.2	1327.4	0.5	5.6
	41m	685.5	1630.4	0.4	1.9
cassette 3	**33**	1733.0	659.5	2.6	7.0
	43c	1248.0	3708.1	0.3	2.8
	40f	1720.0	754.2	2.3	35.0
	40g	751.1	792.2	0.9	2.9
	41c	994.8	966.7	1.0	2.8

[a] A graphical representation of cassette dosing PK results for cassette 3 is provided in the Supporting Information section.

Table 8. Details of Pharmacokinetic Parameters for Cassette Dosing Experiment 3

compound	T_{max} (hr)	C_{max} (nmol/L)	AUClast (hr*nmol/L)	HL_Lambda_z (hr)	Vz_F_obs (L)	CL_F_obs (L/hr)
33	1	482.1	1733.0	3.9	0.432	0.076
43c	4	252.5	1248.0	14.6	0.736	0.035
40f	1	385.4	1720.0	9.5	0.478	0.035
40g	1	202.9	751.1	5.0	0.809	0.111
41c	1	237.4	994.8	7.3	0.652	0.062

Table 9. Binding (FP Assay) and Cell Growth Inhibition (SRB Assay) in Various Human Cancer Cell Lines for Compound **40f**[a]

compound number	human Hsp90β FP IC$_{50}$ (μM)	HCT116 (colon GI$_{50}$ (μM)	DU145 (prostate) GI$_{50}$ (μM)	PC3M (prostate) GI$_{50}$ (μM)	SKMel28 (melanoma) GI50 (μM)	SF268 (glioma) GI$_{50}$ (μM)	U87MG (glioma) GI$_{50}$ (μM)
40f	0.021	0.016	0.005	0.006	0.005	0.006	0.008

[a] Values are reported as the mean of at least two independent determinations.

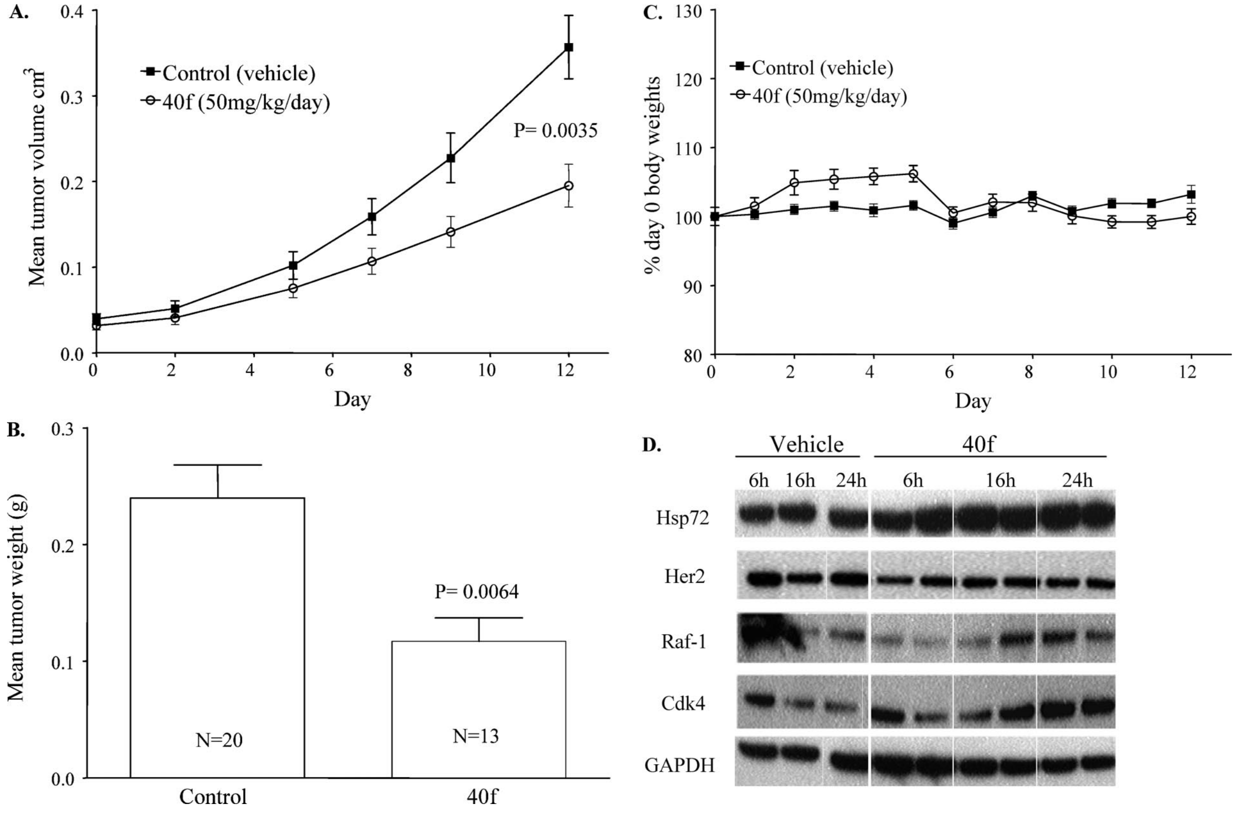


Figure 8. Response of HCT116 human colon carcinoma xenografts to **40f** therapy (50 mg/kg i.p. q.d). (A) Mean tumor volumes (therapy vs vehicle controls). (B) Mean weights of excised tumors after 12 days of therapy. Therapy/control ratio (T/C) = 49.9. (C) Mouse body weights during therapy. (D) Changes in PD markers as measured at 6, 16, and 24 h after the final dose. GAPDH was used as a loading control for the western blots.

athymic mice bearing HCT116 human colon carcinoma xenografts. The rate of tumor growth was significantly inhibited by **40f** administration (Figure 8A), and treated tumor weights measured at day 12 were 49.8% of the control values (Figure 8B). This dose schedule was well-tolerated, with no body weight losses (Figure 8C), and the pharmacodynamic (PD) marker changes at the end of the study were consistent with Hsp90 inhibition (Figure 8D).

Conclusion

A significant reduction of tumor growth in HCT116 colon cancer xenografts was observed with the potent Hsp90 inhibitor VER-52296/NVP-AUY922[59] (**40f**). This compound has excellent potency against Hsp90 in a FP binding assay ($IC_{50} = 21$ nM) and inhibits proliferation in a wide range of human cancer cell lines with an average GI_{50} of 9 nM. Treated cells show the characteristic molecular signature of Hsp90 inhibition, namely, elevation of Hsp72 and degradation of oncogenic client proteins. The compound is retained in HCT116 xenograft tumors at concentrations well above the GI_{50}, with a half-life of 9.5 h following i.p. administration at 50 mg/kg qd. The identification of **40f** as a potential therapeutic agent for treatment of cancer was strongly influenced by X-ray structures of protein−inhibitor complexes and was the culmination of studies that optimized the physical and biological properties of lead compounds, including, in particular, tumor drug levels. In addition, **40f** showed excellent efficacy in a range of subcutaneous and orthotopic human tumor xenograft models covering major cancer types and diverse oncogenic profiles, and these data will be published elsewhere.[56] The overall properties warrant further studies of this compound, and an i.v. formulation of NVP-AUY922 (**40f**) is currently in a Phase-I study in patients with a range of cancer indications.

Experimental Section

Fluorescence Polarization Assay. The ability of compounds to compete with a fluorescently labeled probe for binding to full-length human Hsp90β was determined, as described previously,[57] with a modified probe (VER-00051001). This probe binds with greater affinity to Hsp90 than that described previously, allowing the accurate determination of IC_{50} for higher-affinity compounds. Synthetic procedures for VER-00051001 and details relating to the FP assay can be found in the Supporting Information section.

Cell Growth and Client Protein Degradation. Growth inhibition assays using the SRB method were undertaken as previously described.[27] Client protein degradation was determined by western blotting. In brief, HCT116 cells were exposed to compounds **6**, **19e**, **30d**, **33**, **40f**, or 17-AAG at either 1× or 2× their cellular GI_{50} for 24 h. Cell lysates were prepared in 50 mM tris-HCl and 2% SDS (pH 6.8), and protein levels were determined by the BCA method (Pierce, Rockford, IL). Equal amounts of lysate were separated by SDS-PAGE and proteins transferred to PVDF membrane. Her2 (ab8054, Abcam), Raf-1 (sc-133, Santa Cruz Biotechnology), and Hsp72 (SPA-810, StressGen) were determined using ECL (GE Bioscience). GAPDH (MAB374, Chemicon International) levels were determined to show equal protein loading.

Pharmacokinetics and Metabolism. Cassette Dosing in Tumor-Bearing Mice. All work was performed in accordance with the Home Office regulations under the Animals (Scientific Procedures) Act 1986 and according to the UKCCCR guidelines for animal experimentation.[58] HCT116 human colon cancer cells were cultured in DMEM plus 5% fetal calf serum. When ∼80% confluent, they were released from plastic flasks by a short exposure to trypsin, washed, and re-suspended in PBS. Two million cells were injected s.c. bilaterally in the flanks of female NCr athymic mice. Dosing commenced when tumors were well established, 4–5 mm mean diameter for therapy and 8–9 mm for cassette dosing pharmacokinetic studies.

Compounds were dissolved in DMSO and diluted in sterile saline/Tween 20 such that animals received the dose required in 0.1 mL of final solution per 10 g of body weight. Final concentrations were 10% DMSO, 5% Tween 20, and 85% saline. The compound was stable in this vehicle and, for repeat dosing in the therapy study, was stored for 3–4 days at 4 °C between doses. Controls received an equal volume of vehicle. For cassette dosing, animals received 4 mg/kg of each compound. The approach used was consistent with the guidelines for cassette dosing that we have published previously[55] and our prior experience with early pyrazole−resorcinol Hsp90 inhibitors.[54]

The plasma and tumors were taken as previously described[54] at 0, 0.25, 0.5, 1, 2, 4, and 6 h post administration ($N = 3$ animals per time point) and snap frozen. Quantitative analysis of the plasma and tumors was performed by LC/MS/MS. The LC system comprised a Waters Alliance 2795 separations module (Hertford, U.K.). Chromatography was achieved on a Synergi Polar-RP 4μ 50 × 2.1 mm column (Phenomenex, Macclesfield, U.K.) with an A100-X precolumn filter (Anachem, Luton, Bedfordshire, UK). The mobile phase, initially containing 0.1% formic acid and methanol, 80/20 v/v, was increased linearly to 90% methanol from 0 to 6 min, kept isocratic for 5 min, and then washed with 100% methanol for 1 min before returning to the initial conditions. The flow rate was 0.2 mL/min. Detection was achieved on a Micromass Quattro Ultima triple-quadrupole mass spectrometer (Manchester, U.K.). The electrospray interface was used in negative-ionization mode (ES-) with a capillary voltage of 4 kV and a source temperature of 200 °C. Detection was accomplished by multiple reaction monitoring of the optimized transitions and collision energy for each of the six compounds (five compounds and an analytical internal standard). The program WinNonlin was used to evaluate the half life of test compounds.

Therapeutic Activity in Human Tumor Xenografts. The compound **40f** was prepared as described below. Therapy was initiated when tumors were established with a mean diameter of 4–5 mm and continued for 12 days. The dose selected (50 mg/kg) had been shown in pilot studies to be well-tolerated and to give plasma levels within the therapeutic range. Tumors were measured three times weekly and weighed when excised at the end of the experiment. Animals were weighed daily as an indication of general health. Tumor samples were also collected for analysis of pharmacodynamic biomarkers, as described earlier.

Chemistry. 1-(5-Chloro-2,4-dihydroxyphenyl)ethanone (10). Acetic acid (17.5 mL) was added dropwise to a suspension of 4-chlorobenzene-1,3-diol (42.5 g, 0.294 mol) in $BF_3 \cdot OEt_2$ (200 mL, 1.62 mol) under a nitrogen atmosphere. The reaction mixture was heated at 90 °C for 3.5 h and then allowed to cool to room temperature, causing a solid to precipitate. The mixture was poured into a 10% w/v aqueous sodium acetate solution (700 mL). This mixture was then stirred vigorously for 2.5 h to afford a light-brown solid, which was filtered, washed with water, and air-dried overnight to afford the title compound **10** (31.6 g, 58%): TLC $R_f = 0.40$ (1:3 EtOAc/hexane); LCMS $t_R = 1.75$ min; $m/z = 185[M - H]^-$; ^1H NMR (400 MHz, DMSO-d_6) δ 2.70 (s, 3H), 6.62 (s, 1H), 8.03 (s, 1H), 11.45 (s, 1H), 12.35 (s, 1H); ^{13}C NMR (100.6 MHz, DMSO-d_6) δ 27.0 (CH_3), 103.5 (CH), 111.4 (C), 113.6 (C), 132.6 (CH), 159.9 (CH), 162.1 (C), 202.1 (C); $\nu = 3257, 1608$ cm^{-1}.

1-(2,4-Bis(benzyloxy)-5-chlorophenyl)ethanone (11). Benzyl bromide (30 mL, 0.252 mol) was added to a mixture of 1-(5-chloro-2,4-dihydroxyphenyl)ethanone (**10**) (20 g, 0.107 mol) and potassium carbonate (37 g, 0.27 mol) in acetonitrile (350 mL). The mixture was heated at reflux for 6 h and then allowed to cool to ambient temperature and stirred overnight. The mixture was filtered, and the solids were washed with dichloromethane (3 × 100 mL). The combined organic filtrates were evaporated in vacuo to leave a pale-yellow solid, which was triturated with a mixture of hexane (350 mL)/EtOAc (15 mL) and filtered to give the title compound **11** (35.4 g, 90% yield) as an off-white solid: TLC $R_f = 0.33$ (1:4

EtOAc/hexane); LCMS t_R = 2.66 min; m/z = 389[M + Na]$^+$, 367[M + H]$^+$; ^{1}H NMR (400 MHz, DMSO-d_6) δ 2.55 (s, 3H), 5.06 (s, 2H), 5.13 (s, 2H), 6.55 (s, 1H), 7.3–7.4 (m, 10H), 7.90 (s, 1H); ν = 3035, 1660 cm^{-1}.

4-(2,4-Bis(benzyloxy)-5-chlorophenyl)-2-hydroxy-4-oxobut-2-enoic Acid Ethyl Ester (12). Sodium metal (1.35 g, 58 mmol) was cut into small pieces, washed with hexane to remove mineral oil, and added to anhydrous EtOH (35 mL) under a nitrogen atmosphere over a period of 20 min. The reaction mixture was stirred for a further 10 min until all sodium had reacted. Compound **11** (10 g, 27.26 mmol) was added in portions over 5 min, and the resulting suspension was then stirred for a further 5 min. Diethyl oxalate (6 mL, 43 mmol) was added, resulting in a thicker yellow-colored precipitate. The reaction mixture was heated to reflux for 4 h, affording a dark-colored homogeneous solution, which, upon cooling, produced a solid mass to which acetic acid (6 mL) was added. The mixture was triturated to afford a yellow solid, which was filtered, washed sequentially with water, EtOH, and diethyl ether, and then dried in vacuo to afford the title compound **12** (12 g, 94%) as a yellow solid. LCMS t_R = 2.93 min; m/z = 489[M + Na]$^+$, 467[M + H]$^+$; ^{1}H NMR (400 MHz, $CDCl_3$) δ, 1.29 (t, 3H, J = 7.0 Hz), 4.28 (q, 2H, J = 7.0 Hz), 5.11 (s, 2H), 5.17 (s, 2H), 6.58 (s, 1H), 7.33–7.42 (m, 10H), 8.01 (s, 1H), 15.34 (brs, 1H); ^{13}C NMR (100.6 MHz, $CDCl_3$) δ 14.0 (CH_3), 62.2 (CH_2), 99.5 (CH), 103.0 (CH), 116.1 (C), 118.4 (C), 127.0 (CH), 127.4 (CH), 128.4 (CH), 128.5 (CH), 128.8 (CH), 128.8 (CH), 132.1 (CH), 135.2 (CH), 135.4 (CH), 158.6 (C), 158.7 (C), 162.4 (C), 168.6 (C).

5-(2,4-(Bis)benzyloxy-5-chlorophenyl)-2H-pyrazole-3-carboxylic Acid Ethyl Ester (13). Method a: Compound **12** (20 g, 42.8 mmol) was added portionwise to cooled glacial acetic acid (100 mL) with mechanical stirring under nitrogen, with the temperature maintained at ~14 °C with an ice bath. The suspension was treated dropwise with hydrazine hydrate (2.4 mL, 47.2 mmol). The cooling was removed at the initiation of freezing in order to maintain stirring, and the reaction was allowed to warm to room temperature over 3 h during continued stirring. The resultant suspension was poured into ice/water (500 mL), and the pale-yellow precipitate was filtered and washed with water. The solid was recrystallized from hot EtOH to furnish the title compound **13** (17 g, 86%). Method b: Compound **12** (18.34 g, 39.3 mmol) was added to EtOH (350 mL) and mechanically stirred. Hydrazine hydrochloride (3.41 g, 49.1 mmol) was added, and the mixture was then heated at reflux for 3.75 h and then allowed to cool. The resulting solid was filtered, and the filtered solids were washed with EtOH and then diethyl ether and dried in vacuo to afford the title compound **13** (14.78 g, 87% yield) as a yellow solid: LCMS t_R = 2.82 min; m/z = 463[M + H]$^+$; ^{1}H NMR (400 MHz, DMSO-d_6) δ 1.27 (t, 3H, J = 7.1 Hz), 4.24 (q, 2H, J = 7.1 Hz), 5.29 (s, 2H), 5.30 (s, 2H), 7.10 (s, 1H), 7.16 (s, 1H), 7.35–7.44 (m, 6H), 7.45–7.49 (m, 4H), 7.96 (s, 1H); ^{13}C NMR (100.6 MHz, DMSO-d_6) δ 14.3 (CH_3), 61.0 (CH_2), 71.2 (CH_2), 71.6 (CH_2), 100.1 (C), 104.9 (CH), 111.1 (C), 116.2 (C), 127.0 (CH), 128.0 (CH), 128.3 (CH), 128.8 (CH), 128.9 (CH), 129.1 (CH), 129.2 (CH), 134.9 (C), 135.8 (C), 141.4 (C), 143.3 (C), 154.5 (C), 154.8 (C), 162.2 (C).

5-(2,4-Bis(benzyloxy)-5-chlorophenyl)-4-iodo-2*H*-pyrazole-3-carboxylic Acid Ethyl Ester (14). Compound **13** (30 g, 64.8 mmol) was suspended in acetonitrile (1000 mL) and treated with *N*-iodosuccinimide (16 g, 71 mmol) followed by ceric ammonium nitrate (350 mg, 0.64 mmol), and the mixture was heated to reflux overnight. The reaction mixture was cooled to ambient temperature, and solvents were removed in vacuo to leave a foam-like solid. The residual solid was triturated with water and then filtered and washed with 1% sodium thiosulfate solution and then further water. The solid product was dried, then stirred in EtOH, and filtered, and the filtered solids were stirred with diethyl ether and filtered. The resultant solid was dried in vacuo to afford title the compound **14** (37.5 g, 98%) as a colorless solid: TLC R_f = 0.56 (1:1 EtOAc/hexane); LCMS t_R = 2.66 min; m/z = 589[M + H]$^+$; ^{1}H NMR (400 MHz, $CDCl_3$) δ 1.42 (t, 3H, J = 7.1 Hz), 4.43 (q, 2H, J = 7.1 Hz), 4.99 (s, 2H), 5.16 (s, 2H), 6.66 (s, 1H), 7.22–7.26 (m, 2H), 7.30–7.45 (m, 8H), 7.84 (s, 1H), NH not observed.

5-(2,4-Bis(benzyloxy)-5-chlorophenyl)-4-iodo-2*H*-pyrazole-3-carboxylic Acid (15). Compound **14** (37.5 g, 63.8 mmol) was dissolved in a mixture of 1,4-dioxane (300 mL) and aqueous sodium hydroxide solution (2.0 M, 50 mL, 100 mmol), and the mixture was heated to reflux for 5 h. The reaction mixture was cooled to ambient temperature and then acidified with aqueous HCl (2.0 M). The majority of the 1,4-dioxane was removed in vacuo and the residual mixture diluted with more water. The resulting precipitate was filtered and dried in vacuo, and the solid was stirred with EtOH, filtered, and then stirred with diethyl ether and filtered again. Drying in vacuo afforded the title compound **15** (32.7 g, 92%) as a colorless solid: LCMS t_R = 2.60 min; m/z = 561[M + H]$^+$; ^{1}H NMR (400 MHz, DMSO-d_6) δ 5.17 (s, 2H), 5.30 (s, 2H), 7.16 (brs, 1H), 7.29–7.40 (m, 7H), 7.41–7.47 (m, 2H), 7.48–7.51 (m, 2H), 13.8 (brs, 1H).

5-(2,4-Bis(benzyloxy)-5-chlorophenyl)-4-iodo-2*H*-pyrazole-3-carboxylic Acid Ethylamide (16). Oxalyl chloride (42 μL, 0.50 mmol) was added to a suspension of compound **13** (242 mg, 0.43 mmol) in dichloromethane (10 mL). One drop of DMF was added, and the reaction mixture was stirred at ambient temperature for 1 h; then, the solvents were removed in vacuo to afford a yellow residue, which was suspended in dichloromethane (5 mL) and added dropwise to a stirred solution of ethylamine hydrochloride (350 mg, 4.3 mmol) and triethylamine (600 μL, 4.3 mmol) in dichloromethane (15 mL). After 1 h, the solvents were evaporated in vacuo and the residue dissolved in EtOAc and washed sequentially with aqueous HCl (2N), a 5% w/v Na_2CO_3 solution, and a saturated aqueous NaCl solution. The organic phase was dried over Na_2SO_4 and filtered; the filtrate solvents were removed in vacuo to afford title compound **16** (250 mg, 99% yield) as an off-white solid: TLC R_f = 0.35 (1:1 EtOAc/hexane); LCMS t_R = 2.80 min; m/z = 588[M + H]$^+$; ^{1}H NMR (400 MHz, $CDCl_3$) δ 1.23 (t, 3H, J = 7.4 Hz), 3.47 (dt, 2H, J = 7.4, 5.5 Hz), 5.01 (s, 2H), 5.17 (s, 2H), 6.66 (s, 1H), 6.91 (t, 1H, J = 5.5 Hz), 7.25 (m, 2H), 7.31–7.45 (m, 8H), 7.88 (s, 1H), NH not observed.

5-(2,4-Bis(benzyloxy)-5-chlorophenyl)-4-(4-formylphenyl)-2*H*-pyrazole-3-carboxylic Acid Ethylamide (17). Compound **16** (235 mg, 0.4 mmol) was dissolved in THF (4 mL) and water (0.4 mL). 4-Formylphenylboronic acid (120 mg, 0.8 mmol) was added, followed by potassium carbonate (110 mg, 0.8 mmol). The mixture was degassed,[1,1′-bis(diphenylphosphino)ferrocene]dichloropalladium(II) (5 mol%) was added, and the reaction mixture was heated in a microwave synthesizer at 160 °C for 10 min. The solvents were removed in vacuo to afford a solid residue, which was adsorbed onto silica gel and then purified by flash chromatography on silica gel, eluting with a gradient of 1:2 to 1:1 EtOAc/hexane. This afforded the title compound **17** (153 mg, 68%) as a colorless solid: LCMS t_R = 2.76 min; m/z = 566[M + H]$^+$; ^{1}H NMR (400 MHz, $CDCl_3$) δ 1.06 (t, 3H, J = 7.0 Hz), 3.20 (dt, 2H, J = 7.0, 6.0 Hz), 4.92 (s, 2H), 5.23 (s, 2H), 7.02 (s, 1H), 7.04–7.08 (m, 2H), 7.25 (s, 1H), 7.26–7.46 (m, 11H), 7.70 (d, 2H, J = 8 Hz), 8.24 (brt, 1H, J = 6 Hz), 9.95 (s, 1H), 13.45 (s, 1H).

5-(2,4-Bis(benzyloxy)-5-chlorophenyl)-4-(3-formylphenyl)-2H-pyrazole-3-carboxylic Acid Ethylamide (18). The title compound was made by same synthetic method as that used for compound **17**. Thus, **16** was reacted with 3-formylphenylboronic acid to afford the title compound as a colorless solid: LCMS t_R = 2.78 min; m/z = 566[M + H]$^+$.

General Procedure A: Preparation of Amines 19e and 20a−i from 17 and 18 by Reductive Amination. The appropriate amine (0.265 mmol, 2 equiv) was added to a solution of **17** or **18** (75 mg, 0.133 mmol, 1 equiv) in MeOH (5 mL). Molecular sieves (Aldrich, 3 Å) were added, followed by acetic acid (12 μL, 0.2 mmol) and sodium cyanoborohydride (13 mg, 0.2 mmol, 1.5 equiv). The reaction mixture was stirred for 2–4 h (analyzed by LCMS), and when judged complete, the solvents were removed in vacuo, and the residue was purified by flash chromatography on silica gel (eluting with a gradient of dichloromethane to DMAW 120). Alternatively, the reactions underwent an aqueous workup. The reaction mixture was quenched with MeOH or saturated $NaHCO_3$ solution, solvents were removed in vacuo, and the residue was partitioned between water and EtOAc. The organic phase was washed with saturated aqueous NaCl solution, dried with sodium sulfate, and evaporated in vacuo. The resulting amine products were purified as above or used directly in the debenzylation step. The amine product of reductive amination (0.122 mmol) was dissolved in acetic acid (5 mL), and the solution was degassed by evacuation−nitrogen purge (two cycles). Palladium on carbon catalyst (10%) was added, and the reaction mixture was hydrogenated with shaking for 16 h. The reaction mixture was filtered through a pad of Celite 521 and the filtrate concentrated in vacuo. Final product purification was by preparative HPLC unless otherwise stated.

5-(5-Chloro-2,4-dihydroxyphenyl)-4-(4-morpholin-4-ylmethylphenyl)-2*H*-pyrazole-3-carboxylic Acid Ethylamide (19e). By general procedure A, morpholine was reacted with **17**, and benzyl groups were removed by catalytic hydrogenation to afford the title compound **19e** (11%) as a colorless solid: LCMS t_R = 1.69 min; m/z = 457[M + H]$^+$; ^{1}H NMR (400 MHz, DMSO-d_6) δ 1.05 (t, 3H, J = 7.4 Hz), 2.34 (brm, 4H), 3.18 (m, 2H), 3.41 (brs, 2H), 3.56 (brm, 4H), 6.56 (s, 1H), 6.71 (s, 1H), 7.13 (d, 2H, J = 8.4 Hz), 7.16 (d, 2H, J = 8.4 Hz), 8.00 (brs, 1H); ^{13}C NMR (100.6 MHz, DMSO-d_6) δ 14.9 (CH_3), 33.2 (CH_2), 53.2 (CH_2), 62.3 (CH_2), 66.2 (CH_2), 103.7 (CH), 108.7 (C), 109.5 (C), 119.2 (C), 128.2 (CH), 129.8 (CH), 131.2 (CH), 131.8 (C), 135.4 (C), 137.5 (C), 143.4 (C), 153.9 (C), 155.3 (C), 162.4 (C); HRMS calcd for $C_{23}H_{25}ClN_4O_4$[M + H]$^+$ 457.1642, found 457.1626; HPLC 98.7% (t_R = 2.87 min).

{4-[3-(2,4-Bis(benzyloxy)-5-chlorophenyl)-5-ethylcarbamoyl-1H-pyrazol-4-yl]benzyl}carbamic Acid *tert*-Butyl Ester (21). 4-Aminomethylphenylboronic acid hydrochloride (80 mg, 0.43 mmol) was added to 5-(2,4-bis(benzyloxy)-5-chlorophenyl)-4-iodo-2*H*-pyrazole-3-carboxylic acid ethylamide (**16**) (200 mg, 0.34 mmol) and potassium carbonate (140 mg, 1.01 mmol) in a microwave vial. THF (3 mL) and water (0.3 mL) were added, and the mixture was degassed by bubbling nitrogen through the reaction mixture. 1,1-Bis(diphenylphosphino)ferrocenedichloropalladium was added (10 mol%), and the reaction mixture was heated in a microwave synthesizer at 150 °C for 10 min. The reaction mixture after cooling was filtered through a pad of Celite 521, and the solvents were removed in vacuo. The residue was dissolved in THF (5 mL), and triethylamine (142 μL) was added followed by di-*tert*-butyldicarbonate (222 mg, 1.02 mmol). The reaction mixture was heated for 10 min at 100 °C and then allowed to cool and was partitioned between EtOAc and water. The organic phase was dried over Na_2SO_4, evaporated in vacuo, and purified by flash chromatography on silica gel, eluting 15–75% gradient of EtOAc in hexane, to afford title compound **21** (60 mg, 26%) as a glass-like solid: LCMS t_R = 2.8 min; m/z = 667[M + H]$^+$; ^{1}H NMR (400 MHz, $CDCl_3$) δ 1.23 (t, 3H, J = 7.2 Hz), 1.47 (s, 9H), 3.36 (m, 2H), 4.34 (brd, 2H, J = 5.8 Hz), 4.85 (brs, 1H), 4.94 (s, 2H), 5.07 (s, 2H), 6.56 (s, 1H), 6.6 (brs, 1H), 7.06 (s, 1H), 7.15–7.30 (m, 8H), 7.31–7.43 (m, 6H), pyrazole NH not observed.

4-(4-Aminomethylphenyl)-5-(2,4-bis-benzyloxy-5-chlorophenyl)-2*H*-pyrazole-3-carboxylic Acid Ethylamide (22). Compound **21** (0.191 g, 0.0286 mmol) was dissolved in dichoromethane (5 mL), trifluoroacetic acid (5 mL) was added, and the mixture was stirred at room temperature for 1 h. The mixture was then concentrated to give a yellow oil, which was partitioned between EtOAc and saturated aqueous $NaHCO_3$ solution. The organic phase was dried over Na_2SO_4 and concentrated to give a yellow oil, which was triturated with hexane/EtOAc mixture to afford the title compound **22** (0.154 g, 95%) as a cream-colored powder: LCMS t_R = 2.11 min; m/z = 568[M + H]$^+$.

5-(5-Chloro-2,4-dihydroxyphenyl)-4-(4-diethylaminomethylphenyl)-2*H*-pyrazole-3-carboxylic Acid Ethylamide (19a). Compound **22** (0.03 g, 0.056mMol) was dissolved in dichloroethane (10 mL). To this mixture were added sodium triacetoxyborohydride (0.034 mg, 0.16 mmol) and acetaldehyde (0.07 mL, 0.16 mmol). The mixture was stirred at room temperature for 1 h, and then, sodium borohydride (0.01 g, 0.26 mmol) in EtOH (1 mL) was added, and the mixture was stirred at room temperature for 1 h. The mixture was filtered, and filtrate solvents were removed in vacuo to give a white solid. The solid was suspended in MeOH (10 mL), and cyclohexadiene (1 mL) was added. The mixture was then heated to reflux for 1 h in the presence of a catalytic amount of palladium on carbon. The reaction mixture was filtered, and the filtrate was concentrated to give a brown solid, which was purified by preparative HPLC to give the title compound **19a** (4 mg, 16%) as a colorless solid: LCMS t_R = 1.63 min; m/z = 443[M + H]$^+$; purity > 95%;^{1}H NMR (400 MHz, CD_3OD) δ 1.16 (t, 6H, J = 7.0 Hz), 1.18 (t, 3H, J = 7.0 Hz), 2.73 (q, 4H, J = 7.0 Hz), 3.34 (m, 2 H), 3.87 (s, 2 H), 6.51 (s, 1 H), 6.80 (s, 1 H), 7.16 (d, 2H, J = 7.8 Hz), 7.3 (d, 2H, J = 7.1 Hz).

5-(2,4-Bis(benzyloxy)-5-chlorophenyl)isoxazole-3-carboxylic Acid Ethyl Ester (23). 4-(2,4-Bis(benzyloxy)-5-chlorophenyl)-2-hydroxy-4-oxobut-2-enoic acid ethyl ester (**12**) (12.0 g, 25.7 mmol) was suspended in EtOH (230 mL), and hydroxylamine hydrochloride (2.23 g, 1.25 equiv) was added. The reaction mixture was heated to reflux for 3.5 h and then allowed to cool to ambient temperature. The resulting suspension was filtered, washed sequentially with EtOH (2 × 20 mL), water (2 × 20 mL), and EtOH (2 × 20 mL), and dried in vacuo to afford the title compound **23** (10.23 g, 86%) as a flocculent light-yellow solid: TLC R_f = 0.41 (20:80 EtOAc/hexane); LCMS t_R = 2.94 min; m/z = 466, 464[M + H]$^+$, chlorine isotope pattern observed; ^{1}H NMR (400 MHz, $CDCl_3$) δ 1.40 (s, 3H, J = 7.1 Hz), 4.42 (q, 2H, J = 7.1 Hz), 5.13 (s, 2H), 5.15 (s, 2H), 6.62 (s, 1H), 7.01 (s, 1H), 7.30–7.43 (m, 10H), 8.80 (s, 1H); ^{13}C NMR (100.6 MHz, $CDCl_3$) δ 14.1, 62.0, 71.0, 71.1, 99.8, 103.0, 109.8, 115.7, 127.0, 127.3, 128.3, 128.6, 128.6, 128.8, 128.9, 135.5, 135.7, 155.0, 156.3, 157.0, 160.2, 166.6.

5-(2,4-Bis(benzyloxy)-5-chlorophenyl)isoxazole-3-carboxylic Acid Ethylamide (24). Compound **23** (10.0 g, 21.5 mmol) was suspended in EtOH (120 mL), and ethylamine in MeOH solution (2.0 M, 70 mL, 140 mmol) was added. The reaction mixture was heated to 80 °C with stirring for 18 h, affording a yellow homogeneous solution, which was allowed to cool to ambient temperature. A flocculent colorless solid formed upon cooling, and the reaction mixture was cooled further to 4 °C and then filtered. The filtered product was washed with cold EtOH and dried in vacuo to afford the title compound **24** (8.86 g, 89%) as a colorless solid: TLC R_f = 0.22 (20:80 EtOAc/hexane); LCMS t_R = 2.86 min; m/z = 465, 463[M + H]$^+$; ^{1}H NMR (400 MHz, $CDCl_3$) δ 1.25 (s, 3H, J = 7.4 Hz), 3.47 (dq, 2H, J = 7.3, 5.2 Hz), 5.10 (s, 2H), 5.16 (s, 2H), 6.59 (s, 1H), 6.82 (brt, 1H, J = 5.2 Hz) 7.08 (s, 1H), 7.30–7.43 (m, 10H), 7.96 (s, 1H).

5-(2,4-Bis(benzyloxy)-5-chlorophenyl)-4-bromoisoxazole-3-carboxylic Acid Ethylamide (25). *N*-bromosuccinimide (1.90 g, 10.7 mmol) was added to a suspension of **24** (4.50 g, 9.73 mmol) in acetonitrile (120 mL). Ceric ammonium nitrate (2.66 g, 4.86 mmol) was added, and the resulting orange suspension was heated to reflux for 4.5 h. The mixture was allowed to cool to room temperature, and the acetonitrile was removed in vacuo. The residue was partitioned between EtOAc (120 mL) and water (100 mL), the phases were then separated, and the organic phase was washed with water (50 mL) and saturated aqueous NaCl solution (100 mL) and dried over $MgSO_4$. The mixture was filtered, and the filtrate solvents were removed in vacuo to afford an orange solid, which was triturated with diethyl ether, filtered, and dried in vacuo to give the title compound **25** (3.90 g, 74%) as a cream-colored solid: R_f = 0.22 (30:70 EtOAc/hexane); LCMS t_R = 2.93 min; m/z = 543, 541[M + H]$^+$, chlorine isotope pattern observed; ^{1}H NMR (400 MHz, $CDCl_3$) δ 1.26 (t, 3H); 3.50 (m, 2H); 5.07 (s, 2H); 5.12 (s, 2H); 6.62 (s, 1H); 6.74 (t, 1H), 7.28–7.43 (m, 10H); 7.52 (s, 1H).

5-(2,4-Bis(benzyloxy)-5-chlorophenyl)-4-(4-formylphenyl)-isoxazole-3-carboxylic Acid Ethylamide (26). 4-Formylphenylboronic acid (1.52 g, 10.2 mmol) was added to a mixture of **25** (4.60 g, 8.5 mmol) and $NaHCO_3$ (2.14 g, 25.5 mmol). DMF (105 mL) was added followed by water (21 mL), and the resulting suspension was degassed by evacuation−nitrogen purge (three cycles) and then bubbling nitrogen gas through the reaction mix for 10 min. Dichlorobis(triphenylphosphine)palladium(II) (5 mol%) was added, and the reaction mixture was heated under nitrogen atmosphere at 80 °C for 3 h. The mixture was allowed to cool, and the majority of the solvents was removed in vacuo. The residual mixture was partitioned between EtOAc (200 mL) and water (200 mL), and this mixture was then filtered through a pad of Celite 521. The filtrate phases were separated, and the organic phase was washed with water (2 × 200 mL) and saturated aqueous sodium chloride solution (250 mL) and then dried over anhydrous Na_2SO_4 and filtered. The filtrate solvents were evaporated in vacuo, and the crude was product purified by trituration with diethyl ether to afford the title compound **26** (3.51 g, 73%) as a yellow solid: LCMS t_R = 2.91 min; m/z = 567$[M + H]^+$, chlorine isotope pattern observed; ^1H NMR (400 MHz, $CDCl_3$) δ 1.23 (t, 3H, J = 7.3 Hz), 3.44 (m, 2H), 4.59 (s, 2H), 5.05 (s, 2H), 6.44 (s, 1H), 6.81 (brt, 1H, J = 5.3 Hz), 6.88 (m, 2H), 7.21–7.30 (m, 3H), 7.31–7.41 (7H), 7.48 (s, 1H), 7.69 (d, 2H, J = 8.4 Hz), 9.96 (s, 1H).

5-(5-Chloro-2,4-dihydroxyphenyl)-4-[4-(1,1-dioxo-1-thiomorpholin-4-ylmethyl)phenyl]isoxazole-3-carboxylic Acid Ethylamide (30a). Triethylamine (800 μL, 76 mmol) was added to a solution of 4-aminomethyl phenylboronic acid (710 mg, 3.8 mmol) in EtOH (3 mL). Divinylsulfone (380 μL, 3.8 mmol) was added, and the mixture was then heated at 100 °C for 3 h. The EtOH was removed in vacuo and the residue triturated with a small quantity of water, filtered, and dried in vacuo to afford the crude boronic acid as a solid. To the boronic acid (60 mg, 0.19 mmol) was added **25** (110 mg, 0.19 mmol), followed by DME (3 mL) and saturated $NaHCO_3$(aq) solution (1 mL). The mixture was degassed and tetrakis(triphenylphosphine)palladium(0) (5 mol%) was added, and the mixture was heated in a microwave synthesizer at 130 °C for 10 min. The reaction mixture was partitioned between dichloromethane and water, and the phases were separated. The organic phase was dried over sodium sulfate and filtered, and the filtrate solvents were removed in vacuo to afford the crude dibenzylated product, which was then dissolved in dichloromethane (5 mL), and boron trichloride in dichloromethane solution (2.0 M, 5 mL, 10 mmol) was added and the mixture stirred at room temperature for 10 min. After this time, water (10 mL) was added, the mixture was concentrated, and the residue was purified by preparative HPLC to afford the title compound **30a** (0.016 g, 17%) as a colorless solid: LCMS t_R = 2.09 min; m/z = 528$[M + Na]^+$, 506$[M + H]^+$, chlorine isotope pattern observed; ^1H NMR (400 MHz, CD_3OD) δ 1.23 (t, 3H, J = 7.3 Hz), 3.01 (m, 4H), 3.13 (m, 4H), 3.39 (q, 2H, J = 7.3 Hz), 3.71 (s, 2H), 6.51 (s, 1H), 7.07 (s, 1H), 7.30 (d, 2H, J = 8.6 Hz), 7.35 (d, 2H, J = 8.6 Hz); ^{13}C NMR (100.6 MHz, DMSO-d_6) δ 14.3 (CH_3), 33.7 (CH_2), 50.2 (CH_2), 50.2 (CH_2), 59.1 (CH_2), 103.8 (CH), 106.2 (C), 110.2 (C), 115.8 (C), 128.1 (C), 128.4 (CH), 128.6 (CH), 130.8 (CH), 137.1 (C), 155.8 (C), 155.8 (C), 157.7 (C), 159.5 (C), 164.2 (C); HRMS calcd for $C_{23}H_{24}ClN_3O_6S[M + H]^+$ 506.1152, found 506.1162; HPLC 97.3% (t_R = 3.04 min).

5-(5-Chloro-2,4-dihydroxyphenyl)-4-[4-(4-methylpiperazin-1-ylmethyl)phenyl]isoxazole-3-carboxylic Acid Ethylamide (30c). Methyl iodide (250 μL, 4.6 mmol) was added to a mixture of 1-(4-bromobenzyl)piperazine (1.0 g, 4.15 mmol) and potassium carbonate (1.8 g, 12.45 mmol) in DMF (15 mL) and stirred overnight. The reaction mixture was partitioned between EtOAc and water, the phases were separated, the organic phase was dried over $MgSO_4$, and the filtrate solvents were evaporated in vacuo. The resulting product (750 mg, 6.5 mmol) was dissolved in DMSO (15 mL), and potassium carbonate (900 mg, 3.0 equiv) was added followed by bis(pinnacolato)diboron (1.1 g, 4.5 mmol). The mixture was degassed, 1,1′-bis(diphenylphosphinoferrocene)dichloropalladium(II) (5 mol%) was added, and the reaction mixture was heated at 85 °C for 3 h under a nitrogen atmosphere; another 1.0 equiv of bis(pinnacolato)diboron was added, and heating continued for 3 h. The reaction mixture was allowed to cool and was worked up as above. The crude product was purified by flash chromatography on silica gel (eluting with 0–8% gradient of MeOH in DCM) to afford the product as a colorless solid. To the boronate ester (82.5 mg (0.26 mmol) were added **27** (106 mg, 0.18 mmol) followed by DME (3 mL) and saturated $NaHCO_3$(aq) solution (1 mL). The mixture was degassed, tetrakis(triphenylphosphine)palladium(0) (5 mol%) was added, and the mixture was heated in a microwave synthesizer at 130 °C for 10 min. The reaction mixture was partitioned between DCM and water, and the phases were separated. The organic phase was dried over Na_2SO_4 and filtered, and the filtrate solvents were removed in vacuo to afford the crude dibenzylated product, which was then dissolved in DCM (5 mL); boron trichloride in DCM solution (1.0 M, 2 mL) was added, and the mixture was stirred at room temperature for 10 min. Water (10 mL) was added, the mixture was concentrated, and the residue was purified by preparative HPLC to give the title compound **30c** (0.5 formate salt) as a colorless solid: LCMS t_R = 1.75 min; m/z = 473, 471$[M + H]^+$, chlorine isotope pattern observed; ^1H NMR (400 MHz, CD_3OD) δ 1.23 (t, 3H, J = 7.4 Hz), 2.67 (s, 3H), 2.69 (brs, 4H), 3.00 (brs, 4H), 3.40 (q, 2H, J = 7.4 Hz), 6.51 (s, 1H), 7.06 (s, 1H), 7.30 (d, 2H, J = 8.6 Hz), 7.34 (d, 2H, J = 8.6 Hz), 8.54 (s, 0.5H, formate); HRMS calcd for $C_{24}H_{27}ClN_4O_4[M + H]^+$ 471.1799, found 471.1792; HPLC 98.3% (t_R = 1.46 min).

5-(5-Chloro-2,4-dihydroxyphenyl)-4-(4-diethylaminomethylphenyl)isoxazole-3-carboxylic Acid Ethylamide (30f). AcOH (0.51 mL, 8.85 mmol) was added dropwise to a mixture of **26** (1.0 g, 1.77 mmol), diethylamine (0.55 mL, 5.3 mmol), $NaCNBH_3$ (222 mg, 3.5 mmol), CH_2Cl_2 (30 mL), and 3 Å molecular sieves (1 g). The reaction mixture was left to stir at RT under a nitrogen atmosphere for 16 h. The mixture was then filtered through a sintered funnel, and the filtrate was washed with saturated $NaHCO_3$ solution (40 mL). The aqueous phase was extracted further with CH_2Cl_2 (2 × 40 mL). The combined organics were dried over Na_2SO_4 and evaporated in vacuo to give a crude product, which was purified by flash chromatography on silica gel, eluting with a gradient of 50% EtOAc/hexane − 100% EtOAc, to afford 5-(2,4-bis(benzyloxy)-5-chlorophenyl)-4-(4-diethylaminomethylphenyl-)isoxazole-3-carboxylic acid ethylamide (0.65 g, 59%) as a colorless oil: LCMS t_R = 2.43 min; m/z = 624, 626. To this product (55 mg, 0.09 mmol) in CH_2Cl_2 (1 mL) cooled to 0 °C under N_2 was added BCl_3 (1.0 M in CH_2Cl_2, 0.27 mL, 0.26 mmol). The reaction was allowed to warm to RT and was stirred for 1.5 h. Saturated $NaHCO_3$ solution (5 mL) was added and the CH_2Cl_2 removed in vacuo. The aqueous residue was extracted with EtOAc (4 × 10 mL), passed through a hydrophobic frit, and evaporated in vacuo. Flash chromatography on silica gel, eluting with 10% MeOH/CH_2Cl_2 then 20% MeOH/CH_2Cl_2, afforded the title compound **30f** (0.018 g, 45%) as a yellow solid: LCMS t_R = 1.78 min; m/z = 444, 446$[M + H]^+$; ^1H NMR (400 MHz, DMSO-d_6) δ 0.97 (t, 6H, J = 7.0 Hz), 1.07 (t, 3H, J = 7.0 Hz), 2.41–2.54 (m, 4H), 3.19–3.28 (m, 2H), 3.52 (brs, 2H), 6.61 (s, 1H), 7.07 (s, 1H), 7.18 (d, 2H, J = 8.0 Hz), 7.25 (d, 2H, J = 8.0 Hz), 8.89 (t, 1H, J = 8.0 Hz), 10.15 (s, 1H), 10.67 (brs, 1H); ^{13}C NMR (100.6 MHz, DMSO-d_6) δ 11.4 (CH_3), 14.3 (CH_3), 33.8 (CH_2), 46.12 (CH_2), 56.4, 104.0 (C_H), 106.2 (C), 110.2 (C), 115.8 (C), 127.7 (C), 128.4 (CH), 128.5 (CH), 130.8 (CH), 138.6 (C), 155.9 (C), 156.0 (C), 157.8 (C), 159.6 (C), 164.3 (C); HRMS calcd for $C_{23}H_{26}ClN_3O_4[M + H]^+$ 444.1690, found 444.1693; HPLC 96.4% (t_R = 2.31 min).

1-(2,4-Bis(benzyloxyphenyl)ethanone (47). Potassium carbonate (90.80 g, 0.657 mol) was added to a solution of 2,4-dihydroxyacetophenone **46** (40.00 g, 0.263 mol) in acetonitrile (400 mL), and the suspension was stirred at room temperature. Benzyl bromide (78 mL, 112.00 g, 0.657 mol) was added dropwise over 10 min and the mixture heated at reflux for 18 h. The mixture was cooled, the solvents were evaporated in vacuo to afford a slurry, which was partitioned between water (1 L) and EtOAc (1 L), and the phases were separated. The aqueous layer was further extracted with dichloromethane (250 mL), and the organic extracts were combined, dried over $MgSO_4$, and evaporated in vacuo. The product was triturated with hexane, filtered, washed with cold hexane, and dried in vacuo at 45 °C to give the title compound **47** (84.69 g, 97%) as a white powder: LCMS t_R = 2.87 min; m/z = 333$[M + H]^+$; ^1H NMR (400 MHz, $CDCl_3$) δ 2.55 (s 3H), 5.08 (s, 2H), 5.11 (s, 2H), 6.63–6.60 (m, 2H), 7.44–7.35 (m, 10H), 7.85 (d, 1H, J = 9.3 Hz).

1-(2,4-Bis(benzyloxy)-5-bromophenyl)ethanone (48). 1-(2,4-Bis(benzyloxyphenyl)ethanone (**47**) (84.60 g, 0.255 mol) was dissolved in DMF (250 mL) and stirred at room temperature. *N*-Bromosuccinimide (45.30 g, 0.255 mol) was added portionwise over 10 min and stirred for 3 h, over which time a white precipitate formed. The suspension was poured into water (1 L), and the product was removed by filtration and dried in vacuo at 45 °C to give the title compound **48** (101.37 g, 97%) as a white powder: LCMS $t_R = 2.97$ min; $m/z = 411.2, 413.2[M + H]^+$, bromine isotope pattern observed; ^{1}H NMR (400 MHz, $CDCl_3$) δ 2.52 (s, 3H), 5.07 (s, 2H), 5.14 (s, 2H), 6.52 (s, 1H), 7.42–7.33 (m, 10H), 8.06 (s, 1H).

4-(5-Bromo-2,4-diphenoxyphenyl)-2-hydroxy-4-oxobut-2-enoic Acid Ethyl Ester (49). Sodium (8.38 g, 364.71 mmol) was added portionwise to EtOH (350 mL) over 1 h with mechanical stirring under nitrogen, and the resulting solution was stirred at room temperature for 18 h. 1-(2,4-Bis(benzyloxy)-5-bromophenyl)ethanone (**48**) (49.31 g, 119.9 mmol) was added over 5 min followed by diethyl oxalate (24.77 mL, 26.65 g, 182.4 mmol). The suspension became pale-yellow in color. The mixture was warmed to reflux, whereupon the solids dissolved and a dark orange solution formed. The mixture was heated under reflux for 2 h and then cooled to 65 °C. Glacial acetic acid (30 mL) was added, and the hot solution was poured into 800 mL of cold water. The suspension was stirred while cooling, and the cooled suspension was filtered, washed with water (500 mL), and dried in vacuo at 45 °C to give the title compound **49** (59.59 g, 96%) as a golden-yellow powder: LCMS $t_R = 3.12$ min; $m/z = 511.2, 513.2[M + H]^+$, bromine isotope pattern observed; ^{1}H NMR (400 MHz, $CDCl_3$) δ 1.27 (t, 3H, $J = 7.1$ Hz), 4.27 (q, 2H, $J = 7.1$ Hz), 5.11 (s, 2H), 5.16 (s, 2H), 6.54 (s, 1H), 7.42–7.34 (m, 11H), 8.17 (s, 1H), 13.5–13.0 (brs, 1H).

5-(2,4-Bis(benzyloxy)-5-bromophenyl)isoxazole-3-carboxylic Acid Ethyl Ester (50). Hydroxylamine hydrochloride (1.41 g, 20.4 mmol) was added to a solution of **49** (8.69 g, 17 mmol) in EtOH (170 mL), and the mixture was heated at reflux for 1 h and 10 min. The reaction mixture was allowed to cool to room temperature and was then filtered; the filtered solids were washed with water (2 × 30 mL) and EtOH (2 × 15 mL) and dried in vacuo to afford the title compound **50** (7.98 g, 92%) as a flocculent yellow solid: TLC $R_f = 0.63$ (30:70 EtOAc/hexane); LCMS $t_R = 3.11$ min; $m/z = 508, 510[M + H]^+$, bromine isotope pattern observed; ^{1}H NMR (400 MHz, $CDCl_3$) δ 1.40 (t, 3H, $J = 7.2$ Hz), 4.42 (q, 2H, $J = 7.2$ Hz), 5.13 (s, 2H), 5.15 (s, 2H), 6.58 (s, 1H), 7.00 (s, 1H), 7.32–7.44 (m, 10H), 8.15 (s, 1H); ^{13}C NMR (100.62 MHz, $CDCl_3$) δ 14.1, 62.0, 71.0, 71.0, 99.5, 102.9, 103.8, 110.4, 126.9, 127.3, 128.3, 128.6, 128.7, 128.9, 131.5, 135.4, 135.6, 155.7, 156.9, 157.3, 160.2.

5-(2,4-Bis(benzyloxy)-5-bromophenyl)isoxazole-3-carboxylic Acid Ethylamide (51). Ethylamine in MeOH solution (2.0M, 65 mL, 130 mmol) was added to a suspension of **50** (7.89 g, 15.5 mmol) in EtOH (80 mL), and the mixture was heated to reflux for 18 h, affording a homogeneous solution which was allowed to cool to ambient temperature. A colorless precipitate formed, which was filtered, washed with EtOH, and dried in vacuo to afford the title compound **51** (6.31 g, 80%) as a colorless solid: TLC $R_f = 0.39$ (30:70 EtOAc/hexane); LCMS $t_R = 2.98$ min; $m/z = 509, 507[M + H]^+$, bromine isotope pattern observed; ^{1}H NMR (400 MHz, $CDCl_3$) δ 1.25 (t, 3H, $J = 7.3$ Hz), 3.48 (dt, 2H, $J = 7.3, 5.3$ Hz), 5.10 (s, 2H), 5.16 (s, 2H), 6.56 (s, 1H), 6.82 (brt, 1H, $J = 5.4$ Hz), 7.08 (s, 1H) 7.30–7.42 (m, 10H), 8.12 (s, 1H).

5-(2,4-Bis(benzyloxy)-5-styrylphenyl)isoxazole-3-carboxylic Acid Ethylamide (34a). *trans*-2-Phenylvinylboronic acid (472 mg, 3.2 mmol) was added to **51** (1.079 g, 2.13 mmol) and $NaHCO_3$ (536 mg, 6.39 mmol). Water (5 mL) and DMF (25 mL) were added, and the mixture was degassed by evacuation−nitrogen purge followed by bubbling nitrogen gas through the reaction mixture for 5 min. Dichlorobis(triphenylphosphine)palladium(II) chloride (149 mg, 5 mol%) was added, and the reaction mixture was heated at 80 °C for 7 h. The mixture was allowed to cool, and the majority of solvents was removed in vacuo. The residual mixture was partitioned between dichloromethane (200 mL) and water (200 mL), and this mixture was then filtered through a pad of Celite 521. The filtrate phases were separated, and the organic phase was washed with water (2 × 50 mL) and saturated aqueous NaCl solution (100 mL) and then dried over anhydrous Na_2SO_4 and filtered. The filtrate solvents were evaporated in vacuo, and the crude product was purified by trituration with a hexane/EtOAc mixture (9:1) to afford the title compound **34a** (808 mg, 71%) as a light-brown solid: TLC $R_f = 0.40$ (30:70 EtOAc/hexane); LCMS $t_R = 3.13$ min; $m/z = 531[M + H]^+$; ^{1}H NMR (400 MHz, $CDCl_3$) δ 1.27 (t, 3H, $J = 7.3$ Hz), 3.50 (dq, 2H, $J = 7.3, 5.4$ Hz), 5.11 (s, 2H), 5.19 (s, 2H), 6.57 (s, 1H), 6.83 (brt, 1H, $J = 5.5$ Hz), 7.10 (s, 1H), 7.17 (d, 1H, $J = 16.5$ Hz), 7.23 (m, 1H), 7.31–7.45 (m, 13H), 7.48–7.52 (m, 2H), 8.21 (s, 1H).

5-(2,4-Bis(benzyloxy)-5-phenethylphenyl)isoxazole-3-carboxylic Acid Ethylamide (34b). Compound **34a** (690 mg, 1.30 mmol) was dissolved in 1,4-dioxane, and 10% palladium on carbon catalyst (50 mg) was added. The reaction mixture was hydrogenated at atmospheric pressure for a total of 4 h and 45 min, with further catalyst (50 mg) added at 1.5 and 4.0 h. The reaction mixture was filtered through a pad of Celite 521, and the filter cake was washed with 1,4 dioxane and dichloromethane. The combined filtrate solvents were removed in vacuo to leave a cream-colored solid, which was adsorbed onto silica gel and purified by flash chromatography on silica gel (20 g), eluting with a gradient of 10–50% EtOAc in hexane. This afforded the title compound **34a** (609 mg, 88%) as a pale-yellow solid: TLC $R_f = 0.43$ (30:70 EtOAc/hexane); LCMS $t_R = 3.12$ min; $m/z = 533[M + H]^+$; ^{1}H NMR (400 MHz, DMSO-d_6) δ 1.09 (t, 3H, $J = 7.1$ Hz), 2.79–2.89 (m, 4H), 3.25 (dq, 2H, $J = 7.1, 5.8$ Hz), 5.23 (s, 2H), 5.35 (s, 2H), 6.85 (s, 1H), 7.08 (s, 1H), 7.13–7.19 (m, 2H), 7.21–7.27 (m, 2H), 7.34–7.46 (m, 6H), 7.47–7.53 (m, 4H), 7.66 (s, 1H), 8.75 (brt, 1H, $J = 5.8$ Hz).

5-(2,4-Bis(benzyloxy)-5-phenethylphenyl)-4-bromoisoxazole-3-carboxylic Acid Ethylamide (35b). *N*-bromosuccinimide (207 mg, 1.16 mmol) was added to a stirred suspension of **34b** (564 mg, 1.06 mmol) in acetonitrile (230 mL). Ceric ammonium nitrate (290 mg, 0.53 mmol) was added, and the mixture was heated to reflux for 30 min. Acetonitrile was removed in vacuo, and the residue was partitioned between EtOAc (50 mL) and water (50 mL). The organic phase was washed with saturated aqueous NaCl solution and dried over Na_2SO_4 and filtered. The filtrate solvents were removed in vacuo to afford a yellow oil, which was purified by flash chromatography on silica gel (20 g), eluting with a gradient of 10–30% EtOAc in hexane. This afforded the title compound **35b** (326 mg, 53%) as a colorless solid: LCMS $t_R = 3.09$ min; $m/z = 613, 611[M + H]^+$; ^{1}H NMR (400 MHz, DMSO-d_6) δ 1.11 (t, 3H, $J = 7.3$ Hz), 2.82 (m, 4H), 3.27 (q, 2H, $J = 7.3$ Hz), 5.24 (s, 2H), 5.25 (s, 1H), 7.09 (s, 1H), 7.12–7.10 (m, 3H), 7.22–7.27 (m, 2H), 7.29 (s, 1H), 7.30–7.50 (m, 10H), 8.93 (t, 1H, $J = 5.8$ Hz); ^{13}C NMR (100.62 MHz, $CDCl_3$) δ 14.4, 31.2, 33.7, 35.5, 69.8, 70.1, 90.9, 99.0, 99.9, 106.0, 122.3, 127.9, 128.1, 128.3, 128.3, 128.5, 128.6, 131.3, 136.5, 136.7, 141.6, 156.1, 158.0, 157.6, 159.6, 166.8.

5-(2,4-Dihydroxy-5-phenethylphenyl)-4-(4-morpholin-4-ylmethylphenyl)isoxazole-3-carboxylic Acid Ethylamide (40b). This compound was made using the procedure described for **30c**, utilizing 5-(2,4-bis(benzyloxy)-5-phenethylphenyl)-4-bromoisoxazole-3-carboxylic acid ethylamide (**35b**) and 4-[4-(4,4,5,5-tetramethyl-[1,3,2]dioxaborolan-2-yl)benzyl]morpholine. Thus, **35b** (374 mg, 0.576 mmol) was reacted with boronate ester (215 mg, 0.72 mmol), and the resulting crude product after workup was purified by flash chromatography on silica gel, eluting with a gradient of 50–100% EtOAc in hexane. Deprotection with BCl_3, as described previously, gave a crude product, which was purified by flash chromatography on silica gel, eluting with 10% MeOH in EtOAc, to afford title compound **40b** as a light-brown oil. The HCl salt was made by trituration with 1.0 M HCl in ether solution, affording the title compound **40b**·HCl (19 mg, 6%) as a light-brown solid: LCMS $t_R = 2.02$ min; $m/z = 528[M + H]^+$; purity 90–95%; ^{1}H NMR (400 MHz, DMSO-d_6) δ 1.11 (t, 3H, $J = 6.9$ Hz), 2.60–2.70 (m, 4H), 3.00–3.30 (m, 4H), 3.69 (m, 2H), 3.87 (m, 2H), 4.3 (m, 2H), 6.45 (s, 1H), 6.84 (s, 1H), 7.11–7.32 (m, 6H), 7.46–7.65 (m, 3H), 8.93 (brt, 1H), 9.67 (s, 1H), 9.90 (s, 1H), 10.73 (brs, 1H); HRMS calcd for $C_{31}H_{33}N_3O_5[M + H]^+$ 528.2498, found 528.2486.

1-(2,4-Dihydroxyphenyl)-2-methylpropane-1-one (53). Resorcinol (2.42 g, 22 mmol) was taken up in $BF_3 \cdot OEt_2$ (15 mL), and isobutyric acid (2.04 mL, 22 mmol) was added. The solution was heated for 1.5 h at 90 °C and then allowed to cool to room temperature. The solution was added dropwise to 10% NaOAc(aq) (100 mL) and allowed to stand for 4 h before being extracted in EtOAc. The organic phases were combined and washed with saturated $NaHCO_3$(aq), dried over anhydrous $MgSO_4$, filtered, and concentrated in vacuo to give the title compound **53** (3.96 g, 100%) as a red oil, which was used without additional purification: LCMS $t_R = 2.28$ min; $m/z = 181[M + H]^+$; ^1H NMR (400 MHz, $CDCl_3$) δ 1.18 (d, 6H, $J = 6.8$ Hz), 3.49 (m, 1H), 6.38 (m, 1H), 7.24 (s, 1H), 7.64 (d, 1H, $J = 9.6$ Hz), 13.06 (s, 1H).

4-Isobutylbenzene-1,3-diol (54). Ethyl chloroformate (6.3 mL, 66 mmol) was added slowly to a cooled (0 °C) solution of compound **53** (3.96 g, 22 mmol) and triethylamine (9.2 mL, 66 mmol) in THF (50 mL). The mixture was warmed to ambient temperature and stirred for 3 h before being filtered, and the solids were washed with cold THF (10 mL). The combined filtrates were cooled to 0 °C, and sodium borohydride (3.35 g, 88 mmol) in water (60 mL) was added slowly. The mixture was warmed to ambient temperature, stirred for 3 h, and diluted with water. The mixture was twice extracted with diethyl ether, and the combined extracts were concentrated to dryness and resuspended in 10% aqueous sodium hydroxide solution (40 mL). After refluxing for 90 min, the mixture was cooled, acidified with 5.0 M aqueous HCl, and twice extracted with diethyl ether. The organic extracts were dried over anhydrous $MgSO_4$ and filtered, and the solvents were removed in vacuo to give 4-isobutylbenzene-1,3-diol **54** (3.61 g, 98%) as a cloudy oil, which was used without further purification: ^1H NMR (400 MHz, $CDCl_3$) δ 0.89 (d, 6H, $J = 6.8$ Hz), 1.85 (m, 1H), 2.37 (d, 2H, $J = 7.2$ Hz), 6.31 (m, 2H), 7.24 (s, 1H), 6.87 (d, 1H, $J = 8.1$ Hz).

1-(2,4-Bis(benzyloxy)-5-isobutylphenyl)ethanone (55). 4-Isobutylbenzene-1,3-diol (**54**) (3.61 g, 21.7 mmol) was dissolved in $BF_3 \cdot OEt_2$ (16 mL), and acetic acid (2.48 mL, 43.4 mmol) was added. The solution was heated for 16 h at 90 °C and then allowed to cool to room temperature. The solution was added dropwise to 10% NaOAc(aq) and allowed to stand for 4 h before being extracted twice with diethyl ether. The organic phases were combined and washed with saturated $NaHCO_3$(aq), dried over anhydrous $MgSO_4$, filtered, and concentrated in vacuo to give 6-acetyl-4-isobutylresorcinol, which was dissolved in DMF (50 mL). Potassium carbonate (12.7 g, 92 mmol) and then benzyl bromide (11 mL, 92 mmol) were added. The suspension was heated, with stirring to 150 °C, under nitrogen for 16 h. The solution was cooled to room temperature, filtered, and concentrated in vacuo. The resulting solid was purified by flash column chromatography on silica gel, eluting with hexane/EtOAc (4:1), and then recrystallized from the EtOAc/hexane mixture to give the title compound **55** (2.14 g, 25%) as colorless crystals: LCMS $t_R = 3.03$ min; $m/z = 389.3[M + H]^+$; ^1H NMR (400 MHz, $CDCl_3$) δ 0.91 (d, 6H, $J = 8.8$ Hz), 1.85 (m, 1H), 2.41 (d, 2H, $J = 6.8$ Hz), 2.54 (s, 3H), 6.36 (s, 1H), 7.39 (s, 1H).

4-(2,4-Bis(benzyloxy)-5-isobutylphenyl)-2-hydroxy-4-oxobut-2-enoic Acid Ethyl Ester (56). This compound was made using the procedure described for compound **12**. Thus, **55** (2.14 g, 5.51 mmol) was reacted with sodium ethoxide and diethyl oxalate to afford title compound **56** (1.55 g, 58%) as a yellow solid, used without further purification: LCMS $t_R = 3.25$ min; $m/z = 489.3[M + H]^+$.

5-(2,4-Bis(benzyloxy)-5-isobutylphenyl)isoxazole-3-carboxylic Acid Ethyl Ester (57). This compound was made using the procedure described for **23**. Thus, compound **56** (0.73 g, 1.52 mmol) was reacted with hydroxylamine hydrochloride to afford title compound **57** (0.73 g, 74%) as a colorless solid: LCMS $t_R = 3.26$ min; $m/z = 486.3[M + H]^+$; ^1H NMR (400 MHz, $CDCl_3$) δ 0.90 (d, 6H, $J = 6.6$ Hz), 1.38 (t, 3H, $J = 7.1$ Hz), 1.91 (m, 1H), 2.51 (d, 2H, $J = 7.1$ Hz), 4.40 (q, 2H, $J = 7.1$ Hz), 5.03 (s, 2H), 5.13 (s, 2H), 6.54 (s, 1H), 6.97 (s, 1H), 7.37 (m, 10 H), 7.71 (s, 1H).

5-(2,4-Bis(benzyloxy)-5-isobutylphenyl)isoxazole-3-carboxylic Acid Ethyl Amide (34e). Compound **57** (0.73 g, 1.52 mmol) was dissolved in ethylamine in MeOH solution (2.0 M, 7.6 mL, 15.2 mmol) and heated in a Smith microwave synthesizer at 120 °C for 10 min. The solution was concentrated in vacuo to give the title compound **34e** (0.73 g, 100%) as a colorless solid, which was used without additional purification: LCMS $t_R = 3.11$ min; $m/z = 485.3[M + H]^+$; ^1H NMR (400 MHz, $CDCl_3$) δ 0.89 (d, 6H, $J = 6.6$ Hz), 1.23 (t, 3H, $J = 7.1$ Hz), 1.92 (m, 1H), 2.49 (d, 2H, $J = 7.3$ Hz), 3.46 (dt, 2H, $J = 5.7$ Hz, 7.1 Hz), 5.00 (s, 2H), 5.14 (s, 2H), 6.52 (s, 1H), 6.79 (1H, bt, $J = 5.7$ Hz), 7.04 (s, 1H), 7.35 (m, 10 H), 7.67 (s, 1H).

5-(2,4-Bis(benzyloxy)-5-isobutylphenyl)-4-iodoisoxazole-3-carboxylic Acid Ethyl Amide (35e). Compound **34e** (0.63 g, 1.30 mmol) and *N*-iodosuccinimide (0.58 g, 2.6 mmol) were dissolved in acetonitrile (50 mL), ceric ammonium nitrate (0.07 g, 0.13 mmol) was added, and the solution was stirred at room temperature overnight. The reaction mixture was concentrated in vacuo, and the resulting gum was partitioned between EtOAc and saturated aqueous NaCl solution. The organic phase was dried over $MgSO_4$, filtered, and concentrated in vacuo. The residue was purified by column chromatography on silica gel, eluting with 4:1 hexane/EtOAc, to give the title compound **35e** (0.43 g, 54%) as an oil: LCMS $t_R = 3.09$ min; $m/z = 611.2[M + H]^+$.

5-(2,4-Bis(benzyloxy-5-isobutylphenyl)-4-(4-formylphenyl)-isoxazole-3-carboxylic Acid Ethyl Amide (36e). This compound was made using the procedure described for compound **32**. Thus, compound **35e** (0.42 g, 0.69 mmol) was reacted with 4-formylphenylboronic acid (0.21 g, 1.38 mmol) to afford a crude product after workup, which was purified by flash chromatography on silica gel, eluting with 10% EtOAc in hexane, to afford the title compound **36e** (4.85 g, 58%) as a yellow solid: LCMS $t_R = 5.57$ min (7.5 min run time); $m/z = 589.1[M + H]^+$; ^1H NMR (400 MHz, $CDCl_3$) δ 0.79 (d, 6H, $J = 6.8$ Hz), 1.21 (t, 3H, $J = 7.2$ Hz), 1.81 (m, 1H), 2.39 (d, 2H, $J = 7.0$ Hz), 3.43 (dt, 2H, $J = 3.7$ Hz, 7.2 Hz), 4.67 (s, 2H), 4.96 (s, 2H), 6.41 (s, 1H), 6.87 (brt, 1H, $J = 3.7$ Hz), 6.98 (m, 2H), 7.06 (s, 1H), 7.25 (m, 2 H), 7.33 (m, 6H), 7.38 (d, 2H, $J = 8.2$ Hz), 7.67 (d, 2H, $J = 8.2$ Hz), 9.92 (s, 1H).

5-(2,4-Dihydroxy-5-isobutylphenyl)-4-(4-morpholin-4-ylmethylphenyl)isoxazole-3-carboxylic Acid Ethylamide (40e). This compound was made using the procedure described for compound **30f**, using morpholine in reaction with compound **36e** (0.13 g, 0.22 mmol). The crude product after debenzylation was purified by preparative HPLC to afford the title compound **40e** as a colorless solid: LCMS $t_R = 2.17$ min; $m/z = 480[M + H]^+$; ^1H NMR (400 MHz, $CDCl_3$) δ 0.72 (d, 6H, $J = 6.6$ Hz), 1.17 (t, 3H, $J = 7.3$ Hz), 1.68 (m, 1H), 2.20 (d, 2H, $J = 7.1$ Hz), 2.60 (m, 4H), 3.38 (m, 2H), 3.57 (s, 2H), 3.68 (m, 4H), 6.11 (s, 1H), 6.81 (s, 1H), 6.85 (t, 1H, $J = 5.8$ Hz), 7.20 (d, 2H, $J = 8.1$ Hz), 7.25 (d, 2H, $J = 8.1$ Hz); ^{13}C NMR (100.6 MHz, $CDCl_3$) 14.5 (CH_3), 22.4 (CH_3), 28.4 (CH), 34.5 (CH_2), 38.4 (CH_2), 52.9 (CH_2), 62.6 (CH_2), 65.7 (CH_2), 104.0 (CH), 105.6 (C), 115.8 (C), 120.7 (C), 129.7 (C), 129.9 (CH), 130.2 (CH), 132.1 (CH), 133.5 (C), 153.8 (C), 156.1 (C), 157.8 (C), 159.7 (C), 167.6 (C); HRMS calcd for $C_{27}H_{33}N_3O_5[M + H]^+$ 480.2498, found 480.2485; HPLC 93% ($t_R = 4.61$ min).

2,4-Bis(benzyloxy)-1-isopropenylbenzene (58). Methyltriphenylphosphonium bromide (64.99 g, 0.182 mol) was suspended in anhydrous THF (300 mL), and the mixture was cooled to 0 °C under a nitrogen atmosphere. *n*-Butyllithium (1.6 M solution in hexanes, 114 mL, 0.182 mol) was added dropwise, and the resulting mixture was stirred at 0 °C for 30 min. A solution of 1-(2,4-bis(benzyloxy)phenyl)ethanone (**47**) (46.78 g, 0.14 mol) in anhydrous THF (150 mL) was then added dropwise to the reaction mixture. When the addition was complete, the ice bath was removed, and the reaction mixture was stirred at room temperature under nitrogen for 18 h. MeOH (100 mL) was added to the reaction mixture, and the resulting solution was evaporated in vacuo. Hexane was added to the resulting oil, and the mixture was heated to reflux for 30 min and then filtered through a pad of Celite 521. The liquors were combined and were evaporated in vacuo to give an oil, which was purified by flash column chromatography on silica gel, eluting with 5% EtOAc in hexane, to give the title compound **58** (37.15 g, 80%) as a colorless oil: TLC $R_f = 0.73$ (25:75 EtOAc/hexane); ^{1}H NMR (400 MHz, CDCl$_3$) δ 2.12 (s, 3H), 5.03 (s, 2H), 5.05 (s, 2H), 5.07 (brs, 2H), 6.54 (dd, 1H, $J = 8.3$ Hz, 2.5 Hz), 6.59 (d, 1H, $J = 2.5$ Hz), 7.14 (d, 1H, $J = 8.3$ Hz). 7.29–7.44 (m, 10H).

4-Isopropylbenzene-1,3-diol (59). Compound **58** (37.15 g, 0.113 mol) was dissolved in EtOH (300 mL) and carefully added to 10% palladium on carbon, which had been prewetted with water under a nitrogen atmosphere. Hydrogen was introduced to the flask, and the mixture was allowed to shake under a positive hydrogen atmosphere for 16 h. The catalyst was filtered from the reaction mixture, and the filtrate solvents were removed in vacuo to give the title compound **59** (12.05 g, 70%) as a white crystalline solid: LCMS $t_R = 2.08$ min; $m/z = 153.1[M + H]^+$; ^{1}H NMR (400 MHz, CDCl$_3$) δ 1.22 (d, 6H, $J = 7.1$ Hz), 3.09 (sept, 1H, $J = 7.1$ Hz), 4.63 (brs, 1H), 4.74 (brs, 1H), 6.29 (d, 1H, $J = 2.5$ Hz), 6.38 (dd, 1H, $J = 8.3$, 2.5 Hz), 7.03 (d, 1H, $J = 8.3$ Hz).

1-(2,4-Dihydroxy-5-isopropylphenyl)ethanone (60). 4-Isopropylbenzene-1,3-diol (**59**) (12.05 g, 0.079 mol) was dissolved in BF$_3$·OEt$_2$ (60 mL, 0.486 mol) and acetic acid (9 mL, 0.157 mol) was added. The solution was heated for 16 h at 90 °C, allowed to cool to room temperature, added dropwise to 10% NaOAc(aq) solution (1000 mL), and allowed to stand for 4 h before being extracted with EtOAc (2 × 200 mL). The organic phases were combined and washed with saturated NaHCO$_3$(aq), dried over MgSO$_4$ and filtered, and the filtrate solvents were removed in vacuo. The residual oil was triturated with cold hexane to afford the title compound **60** (13.57 g, 88%) as an off-white solid: LCMS $t_R =$ 2.63 min; $m/z = 195.1[M + H]^+$; ^{1}H NMR (400 MHz, CDCl$_3$) δ 1.25 (d, 6H, $J = 6.8$ Hz), 2.57 (s, 3H), 3.13 (sept, 1H, $J = 6.8$ Hz), 5.64 (s, 1H), 6.30 (s, 1H), 7.50 (s, 1H), 12.56 (s, 1H).

1-(2,4-Bis(benzyloxy)-5-isopropylphenyl)ethanone (61). 1-(2,4-Dihydroxy-5-isopropylphenyl)ethanone (**60**) (13.57 g, 0.070 mol) was dissolved in DMF (120 mL), and potassium carbonate (21.27 g, 0.154 mol) and benzyl bromide (18.3 mL, 0.154 mol) were added. The resulting suspension was heated, with stirring, to 150 °C under a nitrogen atmosphere for 16 h. The solution was cooled to room temperature, and the mixture was poured into 1.0 M HCl(aq) (1000 mL) and then extracted with EtOAc (2 × 300 mL). The organic phases were combined and washed with 1.0 M HCl(aq) and saturated NaCl(aq) solution (3 × 300 mL). The organic phase was dried over MgSO$_4$ and filtered, and the filtrate solvents were removed in vacuo to give a solid, which was purified by trituration with diethyl ether/hexane (1:1) to give the title compound **61** (23.10 g, 88%) as a colorless solid: LCMS $t_R = 3.03$ min; $m/z = 375.2[M + H]^+$; ^{1}H NMR (400 MHz, CDCl$_3$) δ 1.22 (d, 6H, $J = 6.9$ Hz), 2.56 (s, 3H), 3.28 (sept, 1H, $J = 6.9$ Hz), 5.08 (s, 2H), 5.10 (s, 2H), 6.51 (s, 1H), 7.31–7.44 (m, 10H), 7.76 (s, 1H).

4-(2,4-Bis(benzyloxy)-5-isopropylphenyl)-2-hydroxy-4-oxobut-2-enoic Acid Ethyl Ester (62). Sodium hydride (60% in mineral oil) (2.19 g, 54.9 mmol) was added to an ice-cold homogeneous solution of 1-(2,4-bis(benzyloxy)-5-isopropylphenyl)ethanone (**61**) (13.65 g, 36.5 mmol) in toluene (200 mL). Diethyl oxalate (5.0 mL, 36.8 mmol) was added, and the reaction mixture was heated to reflux for 4 h. The mixture was allowed to cool to room temperature, and 1.0 M HCl(aq) (600 mL) was added to acidify the reaction mixture. Solvents were removed in vacuo, and the resulting gum was partitioned between dichloromethane and saturated NaCl(aq) solution. The phases were separated, and the organic phase was dried over MgSO$_4$ and filtered, and the filtrate solvents were removed in vacuo to afford an orange gum, which slowly crystallized. Trituration with hexane and filtration afforded the title compound **62** (14.94 g, 86%) as a yellow−orange crystalline solid: LCMS $t_R = 3.06$ min; $m/z = 475[M + H]^+$; ^{1}H NMR (400 MHz, CDCl$_3$) δ 1.23 (d, 6H, $J = 6.8$ Hz), 1.28 (t, 3H, $J = 7.1$ Hz), 3.29 (sept, 1H, $J = 6.8$ Hz), 4.28 (q, 2H, $J = 7.1$ Hz), 5.11 (s, 2H), 5.14 (s, 2H), 6.53 (s, 1H), 7.32–7.46 (m, 11H), 7.87 (s, 1H).

5-(2,4-Bis(benzyloxy)-5-isopropylphenyl)isoxazole-3-carboxylic Acid Ethyl Ester (63). Compound **62** (14.94 g, 31.5 mmol) was dissolved in EtOH (150 mL), hydroxylamine hydrochloride (2.63 g, 37.8 mmol) was added, and the solution was heated to reflux for 4 h under a nitrogen atmosphere. The reaction mixture was cooled to room temperature and concentrated in vacuo. The residue was partitioned between dichloromethane and saturated NaCl(aq) solution. The organic phase was dried over MgSO$_4$, filtered, and concentrated in vacuo to give a yellow solid, which was triturated with an EtOH/water mix, filtered, and dried to afford the title compound **62** (13.5 g, 91%) as a yellow solid: LCMS t_R = 3.20 min; $m/z = 472[M + H]^+$; ^{1}H NMR (400 MHz, CDCl$_3$) δ 1.26 (d, 6H, $J = 6.8$ Hz), 1.41 (t, 3H, $J = 7.1$ Hz), 3.34 (sept, 1H, $J = 6.8$ Hz), 4.42 (q, 2H, $J = 7.1$ Hz), 5.07 (s, 2H), 5.16 (s, 2H), 6.57 (s, 1H), 7.00 (s, 1H), 7.32–7.43 (m, 10H), 7.83 (s, 1H); ^{13}C NMR (100.6 MHz, DMSO-d_6) δ 13.9 (CH$_3$), 22.4 (CH$_3$), 26.2 (CH), 61.7 (CH$_2$), 69.8 (CH$_2$), 70.4 (CH$_2$), 98.8 (CH), 101.5 (CH), 107.2 (C), 124.0 (CH), 127.5 (CH), 128.0 (CH), 128.0 (CH), 128.2 (CH), 128.6 (CH), 128.6 (CH), 129.3 (C), 136.3 (C), 136.7 (C), 154.9 (C), 156.5 (C), 158.6 (C), 159.4 (C), 167.8 (C).

5-(2,4-Bis(benzyloxy)-5-isopropylphenyl)isoxazole-3-carboxylic Acid Ethylamide (34f). Compound **63** (13.57 g, 28.8 mmol) was suspended in ethylamine in MeOH solution (2.0M, 140 mL, 280 mmol) and heated to 90 °C for 1 h to afford a homogeneous solution. The solution was allowed to cool to room temperature, and a colorless solid precipitated. The solid was collected by filtration, washed with cold MeOH, and dried in vacuo to afford title compound **63** (11.5 g, 85%) as a colorless solid: LCMS $t_R =$ 3.06 min; $m/z = 471[M + H]^+$; ^{1}H NMR (400 MHz, CDCl$_3$) δ.1.25 (d, 6H, $J = 6.8$ Hz), 1.26 (t, 3H, $J = 7.0$ Hz), 3.33 (sept, 1H, $J =$ 6.9 Hz), 3.49 (m, 2H), 5.03 (s, 2H), 5.17 (s, 2H), 6.55 (s, 1H), 6.81 (brt, 1H, $J = 5.7$ Hz), 7.07 (s, 1H), 7.30–7.42 (m, 10H), 7.78 (s, 1H).

5-(2,4-Bis(benzyloxy)-5-isopropylphenyl)-4-iodoisoxazole-3-carboxylic Acid Ethylamide (35f). Compound **34f** (11.94 g, 25.4 mmol) was dissolved in a mixture of anhydrous acetonitrile (150 mL) and dichloromethane (60 mL), *N*-iodosuccinimide (11.44 g, 50.8 mmol) was added followed by ceric ammonium nitrate (0.697 g, 1.27 mmol), and the solution was stirred at room temperature overnight. The reaction mixture was concentrated in vacuo, and the resulting gum was partitioned between EtOAc and saturated NaCl(aq) solution. The organic phase was dried over MgSO$_4$ and filtered, and the filtrate solvents were removed in vacuo. The residue was purified by column chromatography, eluting with 9:1 hexane/EtOAc, to give the title compound **35f** (9.1 g, 60%) as a pale-yellow solid: LCMS $t_R = 2.97$ min; $m/z = 597.2[M + H]^+$; ^{1}H NMR (400 MHz, CDCl$_3$) δ.1.23 (d, 6H, $J = 6.8$ Hz), 1.26 (t, 3H, $J = 7.3$ Hz), 3.35 (sept, 1H, $J = 6.8$ Hz), 3.50 (m, 2H), 5.05 (s, 2H), 5.09 (s, 2H), 6.58 (s, 1H), 6.80 (brt, 1H, $J = 5.7$ Hz), (s, 1H), 7.28–7.41 (m, 11H).

5-(2,4-Bis(benzyloxy)-5-isopropylphenyl)-4-(4-formylphenyl)-isoxazole-3-carboxylic Acid Ethylamide (36f). This compound was made using the procedure described for compound **32**. Thus, compound **35f** (9.0 g, 15.1 mmol) was reacted with 4-formylphenyl boronic acid (4.53 g, 30.2 mmol) to afford a crude product after workup, which was purified by flash chromatography on silica gel, eluting with dichloromethane, to afford the title compound **36f** (6.0 g, 69% yield) as a yellow solid: LCMS $t_R = 2.98$ min; $m/z =$ 575.3$[M + H]^+$; ^{1}H NMR (400 MHz, CDCl$_3$) δ 1.04 (d, 6H, $J =$ 6.8 Hz), 1.08 (t, 3H, $J = 7.1$ Hz), 3.16 (sept, 1H, $J = 6.8$ Hz), 3.23 (m, 2H), 4.95 (s, 2H), 5.18 (s, 2H), 6.93 (s, 1H), 7.05 (m, 2H), 7.12 (s, 1H), 7.25–7.29 (m, 3H), 7.32–7.37 (m, 3H), 7.39–7.47 (m, 4H), 7.78 (d, 2H, $J = 8.3$ Hz), 8.95 (brt, 1H, $J = 5.6$ Hz), 9.98 (s, 1H); ^{13}C NMR (100.6 MHz, DMSO-d_6) δ 14.3 (CH$_3$), 22.3 (CH$_3$), 25.8 (CH), 33.8 (CH$_2$), 69.7 (CH$_2$), 69.9 (CH$_2$), 98.9 (CH), 107.2 (C), 115.0 (C), 127.3 (CH), 127.5 (CH), 127.8 (CH), 127.9 (CH), 127.9 (CH), 128.2 (CH), 128.5 (CH), 128.9 (C), 129.2 (CH), 129.3 (CH), 135.0 (C), 135.8 (C), 136.3 (C), 136.7 (C), 155.2 (C), 157.6 (C), 158.5 (C), 159.2 (C), 165.7 (C), 192.6 (CH).

5-(2,4-Dihydroxy-5-isopropylphenyl)-4-(4-morpholin-4-ylmethylphenyl)isoxazole-3-carboxylic Acid Ethylamide (40f). This compound was made using the procedure described for compound **30f**, using morpholine in reaction with compound **36f**. The crude product after debenzylation was purified by flash chromatography (eluting with a gradient of 2–4% MeOH in dichloromethane) to afford the title compound as a colorless solid: LCMS t_R = 1.99 min; m/z = 466.3$[M + H]^+$; ^{1}H NMR (400 MHz, DMSO-d_6) δ 0.90 (d, 6H, J = 7.1 Hz), 1.07 (t, 3H, J = 7.4 Hz), 2.33 (brm, 4H), 2.97 (sept, 1H, J = 7.1 Hz), 3.22 (m, 2H), 3.42 (s, 2H), 3.55 (brm, 4H), 6.43 (s, 1H), 6.72 (s,1H), 7.18 (d, 2H, J = 8.4 Hz), 7.23 (d, 2H, J = 8.4 Hz), 8.83 (brt, 1H, J = 5.9 Hz), 9.65 (s, 1H), 9.76 (s, 1H); ^{13}C NMR (100.6 MHz, DMSO-d_6) δ 14.4 (CH_3), 22.4 (CH_3), 25.4 (CH), 33.7 (CH_2), 53.1 (CH_2), 62.1 (CH_2), 66.2 (CH_2), 102.7 (CH), 104.5 (C), 114.7 (C), 125.6 (C), 127.7 (CH), 128.5 (C), 128.7 (CH), 128.8 (CH), 136.9 (C), 154.7 (C), 157.4 (C), 157.8 (C), 159.8 (C), 166.3 (C); HRMS calcd for $C_{26}H_{31}N_3O_5[M + Na]^+$ 488.2161, found 488.2162; HPLC 96% (t_R = 4.90 min).

1-(5-Ethyl-2,4-dihydroxyphenyl)ethanone (65d). 4-Ethylresorcinol (**64**) (6.23 g, 45.1 mmol) was dissolved in $BF_3 \cdot OEt_2$ (50 mL) and acetic acid (5.42 g, 90.3 mmol) and stirred at 90 °C for 12 h. The reaction was cooled to room temperature and quenched with water (200 mL), and the reaction mixture pH was adjusted to pH = 3 with Na_2CO_3. The red precipitate was filtered and recrystallized from toluene (53 mL) to yield the title compound **65d** (5.63 g, 69%) as a light-pink crystalline solid: LCMS t_R = 2.26 min; m/z = 181.2$[M + H]^+$; ^{1}H NMR (400 MHz, DMSO-d_6) δ 1.12 (t, 3H, J = 7.5 Hz), 2.48 (q, 2H, J = 7.5 Hz), 2.52 (s, 3H), 6.29 (s, 1H), 7.58 (s, 1H), 10.61 (s, 1H), 12.49 (s, 1H).

1-(2,4-Bis(benzyloxy)-5-ethylphenyl)ethanone (66d). Compound **65d** (5.63 g, 31.24 mmol) was dissolved in DMF (100 mL). K_2CO_3 (9.50 g, 68.7 mmol) and benzyl bromide (11.76 g, 68.7 mmol) were added, and the reaction mixture was stirred at room temperature for 3 h. The mixture was poured into water (300 mL), and the off-white precipitate was filtered off and recrystallized from acetonitrile (50 mL) to yield the title compound **66d** (7.85 g, 70%) as a colorless solid: LCMS t_R = 2.98 min; m/z = 361.2$[M + H]^+$; ^{1}H NMR (400 MHz, $CDCl_3$) δ 1.12 (t, 3H, J = 7.5 Hz), 2.49 (s, 3H), 2.56 (q, 2H, J = 7.5 Hz), 5.01 (s, 2H), 5.02 (s, 2H), 6.43 (s, 1H), 7.26–7.35 (m, 10H), 7.63 (s, 1H); ^{13}C NMR (100.6 MHz, $CDCl_3$) δ 14.2 (CH_3), 22.1 (CH_2), 32.0 (CH_3), 69.7 (CH_2), 70.5 (CH_2), 98.3 (CH), 119.7 (C), 124.22 (C), 127.5 (CH), 128.0 (CH), 128.0 (CH), 128.1 (CH), 128.6 (CH), 128.6 (CH), 130.2 (CH), 136.5 (C), 136.7 (C), 158.4 (C), 160.6 (C), 196.2 (C).

4-(2,4-Bis(benzyloxy)-5-ethylphenyl)-2-hydroxy-4-oxobut-2-enoic Acid Ethyl Ester (67d). Sodium (1.5 g, 65.2 mmol) was added in small pieces to absolute EtOH (200 mL) under nitrogen over a period of 20–30 min. When all of the sodium had reacted, compound **66d** (7.85 g, 21.8 mmol) and diethyl oxalate (4.77 g, 32.67 mmol) were added, and the mixture was heated at reflex for 3 h. The reaction was allowed to cool to room temperature and quenched with 2 N aqueous hydrochloric acid (33 mL). The yellow precipitate was filtered, washed with water, and dried in vacuo to afford the title compound **67d** (9.41 g, 94%) as a yellow solid: LCMS t_R = 3.14 min; m/z = 461.3$[M + H]^+$; ^{1}H NMR (400 MHz, $CDCl_3$) δ 1.46 (t, 3H, J = 7.5 Hz), 1.53 (t, 3H, J = 7.1 Hz), 2.90 (q, 2H, J = 7.5 Hz), 4.53 (q, 2H, J = 7.1 Hz), 5.36 (s, 2H), 5.38 (s, 2H), 6.77 (s, 1H), 7.59–7.70 (m, 11H), 8.06 (s, 1H), 15.62 (s, 1H); ^{13}C NMR (100.6 MHz, $CDCl_3$) δ 13.8 (CH_3), 14.0 (CH_3), 22.1 (CH_2), 61.8 (CH_2), 69.9 (CH_2), 70.8 (CH_2), 98.5 (CH), 102.6 (CH), 115.7 (C), 125.1 (C), 127.6 (CH), 127.9 (CH), 128.1 (CH), 128.1 (CH), 128.6 (CH), 128.6 (CH), 130.2 (CH), 136.0 (C), 136.5 (C), 159.1 (C), 161.9 (C), 162.0 (C), 167.7 (C), 188.8 (C).

5-(2,4-Bis(benzyloxy)-5-ethylphenyl)isoxazole-3-carboxylic Acid Ethyl Ester (68d). Compound **67d** (4.0 g, 8.69 mmol) was suspended in EtOH (90 mL), and hydroxylamine hydrochloride (0.905 g, 13.0 mmol) was added. The reaction mixture was heated to reflux for 1.5 h, allowed to cool to room temperature, and poured into water (100 mL); the resulting off-white precipitate was filtered and recrystallized from EtOH (100 mL) to yield the title compound **68d** (3.51 g, 88%) as a white solid: LCMS t_R = 3.16 min; m/z = 458.3$[M + H]^+$; ^{1}H NMR (400 MHz, $CDCl_3$) δ 1.39 (t, 3H, J = 7.5), 1.56 (t, 3H, J = 7.1 Hz), 2.84 (q, 2H, J = 7.5 Hz), 4.58 (q, 2H, J = 7.1 Hz), 5.22 (s, 2H), 5.32 (s, 2H), 6.73 (s, 1H), 7.16 (s, 1H), 7.48–7.58 (m, 10H), 7.95 (s, 1H); ^{13}C NMR (100.6 MHz, $CDCl_3$) δ 13.9 (CH_3), 14.3 (CH_3), 22.3 (CH_2), 61.7 (CH_2), 69.7 (CH_2), 70.5 (CH_2), 98.6 (CH), 101.5 (CH), 107.1 (C), 125.0 (C), 126.8 (CH), 127.5 (CH), 128.0 (CH), 128.0 (CH), 128.2 (CH), 128.6 (CH), 128.7 (CH), 136.3 (C), 136.7 (C), 155.1 (C), 156.5 (C), 159.1 (C), 159.5 (C), 167.8 (C).

5-(2,4-Bis(benzyloxy)-5-ethylphenyl)isoxazole-3-carboxylic Acid Ethylamide (34d). Compound **68d** (1.8 g, 3.93mmol) was suspended in EtOH (23 mL), ethylamine solution (2.0 M in MeOH, 15.7 mL, 31.4 mmol) was added, and the reaction mixture was heated to reflux for 3 h. The reaction was allowed to cool to room temperature and poured into water (50 mL), and the resulting white precipitate was filtered, washed with water, and recrystallized from EtOH (37 mL) to yield the title compound **34d** (1.53 g, 85%) as a colorless flaky solid: LCMS t_R = 3.01 min; m/z = 457.3$[M + H]^+$; ^{1}H NMR (400 MHz, DMSO-d_6) δ 1.10 (t, 3H, J = 7.2 Hz), 1.14 (t, 3H, J = 7.5 Hz), 2.60 (q, 2H, J = 7.5 Hz), 3.26 (m, 2H), 5.23 (s, 2H), 5.34 (s, 2H), 6.87 (s, 1H), 7.04 (s, 1H), 7.35–7.50 (m, 10H), 7.66 (s, 1H), 8.75 (t, 1H, J = 5.8 Hz); ^{13}C NMR (100.6 MHz, DMSO-d_6) δ 14.3 (CH_3), 14.6 (CH_3), 22.3 (CH_2), 33.7 (CH_2), 69.6 (CH_2), 70.2 (CH_2), 98.7 (CH), 100.7 (CH), 107.5 (C), 124.9 (C), 127.0 (CH), 127.5 (CH), 127.9 (CH), 128.0 (CH), 128.2 (CH), 128.6 (CH), 128.7 (CH), 136.4 (C), 136.7 (C), 154.9 (C), 158.4 (C), 158.95 (C), 159.4 (C), 167.0 (C).

5-(2,4-Bis(benzyloxy)-5-ethylphenyl)-4-iodoisoxazole-3-carboxylic Acid Ethylamide (35d). Compound **34d** (33.96 g, 74.38 mmol) was dissolved in acetonitrile (400 mL) and dichloromethane (250 mL). *N*-Iodosuccinimide (33.47 g, 148.77 mmol) and ceric ammonium nitrate (20.39 g, 37.19 mmol) were added, and the reaction was stirred at room temperature for 6 h. The solvent was removed under reduced pressure, and the residue was partitioned between dichloromethane and aqueous $Na_2S_2O_3$ solution. The organic phase was washed with water and saturated NaCl(aq) and dried over Na_2SO_4. The solvents were removed in vacuo, and the residue was triturated with EtOH to afford title compound **35d** (16.43 g, 38%) as a yellow solid: LCMS t_R = 2.96 min; m/z = 583.2$[M + H]^+$; ^{1}H NMR (400 MHz, $CDCl_3$) δ 1.20 (t, 3H, J = 7.5 Hz), 1.25 (t, 3H, J = 7.3 Hz), 2.65 (q, 2H, J = 7.5 Hz), 3.46–3.53 (m, 2H), 5.01 (s, 2H), 5.04 (s, 2H), 6.58 (s, 1H), 6.80 (t, 1H, J = 5.2 Hz), 7.27 (s, 1H), 7.28–7.39 (m, 10H); ^{13}C NMR (100.6 MHz, $CDCl_3$) δ 14.00 (CH_3), 14.6 (CH_3), 22.5 (CH_2), 34.4 (CH_2), 56.87 (C), 70.0 (CH_2), 71.0 (CH_2), 98.5 (CH), 107.8 (C), 125.6 (C), 127.0 (CH), 127.1 (CH), 127.9 (CH), 128.0 (CH), 128.6 (CH), 128.7 (CH), 131.3 (CH), 136.5 (C), 136.5 (C), 156.0 (C), 156.4 (C), 158.4 (C), 159.5 (C), 172.0 (C).

5-(2,4-Bis(benzyloxy)-5-ethylphenyl)-4-(4-formylphenyl)isoxazole-3-carboxylic Acid Ethylamide (36d). Compound **35d** (1.41 g, 2.42 mmol) was dissolved in THF (20 mL). 4-Formylphenylboronic acid (544 mg, 3.63 mmol),[1,1′-bis(diphenylphospino)ferrocene]dichloropalladium(II) (0.090 g, 0.12 mmol) and $NaHCO_3$ (1.0 M in water, 4.84 mL, 4.84 mmol) were added, and the mixture was degassed with nitrogen. The reaction was stirred at 80 °C for 8 h and then cooled to room temperature. Water and EtOAc were added, the phases were separated, and the organic phase was washed with water (2 ×) and then saturated NaCl(aq) solution. The organic layer was dried over Na_2SO_4 and filtered through a silica plug. The solvent was removed in vacuo, and the residual solid was triturated with diethyl ether, filtered, and dried to afford the title compound **36d** (0.99 g, 74%) as a pink solid: LCMS t_R = 2.90 min; m/z = 561.2$[M + H]^+$; ^{1}H NMR ($CDCl_3$) δ 1.04 (t, 3H, J = 7.5 Hz), 1.11 (t, 3H, J = 7.0 Hz), 2.50 (q, 2H, J = 7.5 Hz), 3.32–3.41 (m, 2H), 4.55 (s, 2H), 4.89 (s, 2H), 6.31 (s, 1H), 6.72 (s, 1H), 6.85–6.87 (m, 2H), 7.12 (s, 1H), 7.15–7.17 (m, 2H), 7.23–7.32 (m, 8H), 7.59 (d, 2H, J = 8.4 Hz), 9.86 (s, 1H); ^{13}C NMR (100.6 MHz, $CDCl_3$) δ 13.9 (CH_3), 14.3 (CH_3), 21.9 (CH_2), 33.8 (CH_2), 69.5 (CH_2), 69.8 (CH_2), 98.7 (CH), 107.1 (C), 115.0 (C), 124.5 (C), 127.2 (CH), 127.5 (CH), 127.7 (CH), 127.9 (CH), 128.2 (CH), 128.5 (CH), 129.1 (CH), 129.1 (CH), 130.3 (CH), 134.9 (C), 135.7 (C), 136.1 (C), 136.6 (C), 155.2 (C), 157.6 (C), 159.0 (C), 159.1 (C), 165.4 (C), 192.5 (CH).

5-(2,4-Dihydroxy-5-ethylphenyl)-4-(4-morpholin-4-ylmethylphenyl)isoxazole-3-carboxylic Acid Ethylamide (40d). This compound was made using the procedure described for compound **30f**, using morpholine in reaction with compound **36d**. The crude product after debenzylation was purified by preparative HPLC to afford the title compound **40d** (15.3 mg, 69%) as an off-white solid: LCMS t_R = 1.78 min; m/z = 452.2[M + H]$^+$; ^{1}H NMR (400 MHz, DMSO-d_6) δ 0.93 (t, 3H, J = 7.5 Hz), 1.07 (t, 3H, J = 7.2 Hz), 2.30–2.35 (m, 6H), 3.23 (m, 2H), 3.42 (s, 2H), 3.55 (m, 4H), 6.42 (d, 1H, J = 2.4 Hz), 6.77 (s, 1H), 7.17–7.23 (m, 4H), 8.86 (t, 1H, J = 5.7 Hz), 9.61 (bs, 1H), 9.74 (bs, 1H); ^{13}C NMR (100.6 MHz, DMSO-d_6) δ 14.0 (CH$_3$), 14.4 (CH$_3$), 21.6 (CH$_2$), 33.8 (CH$_2$), 53.2 (CH$_2$), 62.1 (CH$_2$), 66.2 (CH$_2$), 102.6 (CH), 104.6 (C), 114.9 (C), 121.2 (C), 128.4 (C), 128.6 (2 × CH), 130.3 (CH), 136.8 (C), 154.9 (C), 157.8 (C), 158.0 (C), 159.8 (C), 166.0 (C); HRMS calcd for $C_{25}H_{29}N_3O_5$[M + H]$^+$ 452.2185, found 452.2182; HPLC 97% (t_R = 3.97 min).

1-(5-*tert*-Butyl-2,4-dihydroxyphenyl)ethanone (65g). Sulfuric acid (4 mL, 75 mmol) was added to a suspension of 2,4-dihydroxyacetophenone (**46**) (22.8 g, 150 mmol) in a mixture of 2-methyl-2-propanol (35 g, 470 mmol) and trifluoroacetic acid (80 mL) under a nitrogen atmosphere. The resulting suspension was heated at 75 °C (oil bath temperature) for 3 h, generating a pale-red solution, which was allowed to cool to ambient temperature and then poured into an ice/water mixture (350 mL) to give a pale-pink precipitate. The solids were removed by filtration, washed with water (600 mL) and hexane (200 mL), and then dried in vacuo (at 40 °C) to give the title compound **65g** (28.8 g, 92%) as a pale-orange powder: LCMS t_R = 2.74 min; m/z = 209.1[M + H]$^+$; ^{1}H NMR (400 MHz, CDCl$_3$) δ 1.39 (s, 9H), 2.58 (s, 3H), 6.27 (s, 1H), 7.59 (s, 1H).

1-(2,4-Bis(benzyloxy)-5-*tert*-butylphenyl)ethanone (66g). Benzyl bromide (10 mL, 84 mmol) was added to a solution of **65g** (13.5 g, 65 mmol) in DMF (50 mL). Potassium carbonate (20 g, 145 mmol) was added, and the suspension was stirred at room temperature for 4 h. The resulting suspension was poured into water (200 mL) to give a pale-orange precipitate. The solids were removed by filtration, washed with water, and then dissolved in dichloromethane (150 mL), and the solution was washed with water (2 × 100 mL) and saturated NaCl(aq) solution (100 mL). The solution was dried over anhydrous, and the solvents were removed in vacuo to afford a pale-red oil. Benzylation was not complete at this stage, the crude product was dissolved in 2-methyl-2-propanol (100 mL), and potassium *tert*-butoxide (7.5 g, 67 mmol) was added to give a pale-yellow precipitate. Benzyl bromide (8 mL, 67 mmol) was added, and the mixture was heated at reflux for 1 h. The resulting suspension was allowed to cool and was poured into water (250 mL) to give a pale-orange precipitate. The solids were removed by filtration, washed with water, dissolved in EtOAc (150 mL), and washed sequentially with water (2 × 200 mL) and saturated NaCl(aq) solution (100 mL). The solution was dried over anhydrous Na$_2$SO$_4$ and filtered, and the filtrate solvents were removed in vacuo. Trituration with EtOH afforded a pale-pink solid, which was collected by filtration and dried in vacuo (at 40 °C) to give the title compound **66g** (9.1 g, 36%) as a pale-pink powder: LCMS t_R = 3.03 min; m/z = 389.3[M + H]$^+$; ^{1}H NMR (400 MHz, CDCl$_3$) δ 1.38 (s, 9H), 2.57 (s, 3H), 5.08 (s, 2H), 5.11 (s, 2H), 6.53 (s, 1H), 7.33–7.42 (m, 10 H), 7.85 (s, 1H).

4-(2,4-Bis(benzyloxy)-5-*tert*-butylphenyl)-2-hydroxy-4-oxo-but-2-enoic Acid Ethyl Ester (67g). Sodium ethoxide (2.8 g, 41 mmol) was added to a suspension of **66g** (7.8 g, 20 mmol) in EtOH (40 mL). Diethyl oxalate (4 mL, 29.5 mmol) was added, and the resulting suspension was heated at reflux for 2 h to give a pale-red solution, which was allowed to cool and then poured into water (200 mL). The mixture was acidified with hydrochloric acid (50 mL, 1.0 M) and extracted with dichloromethane (150 mL). The organic extracts were washed with water (2 × 200 mL) and then saturated aqueous NaCl solution (100 mL). The organic solution was dried over anhydrous Na$_2$SO$_4$ and filtered, and the filtrate solvents were removed in vacuo to afford a yellow gum. Trituration with hexane gave a yellow solid, which was filtered, washed with hexane, and then dried in vacuo (at 40 °C) to afford the title compound **67g** (9.1 g, 93%) as a yellow powder: ^{1}H NMR (400 MHz, CDCl$_3$) δ 1.26 (t, 3H, J = 7.1 Hz), 1.39 (s, 9H), 4.28 (q, 2H, J = 7.1 Hz), 5.11 (s, 2H), 5.13 (s, 2H), 6.54 (s, 1H), 7.34–7.45 (m, 11H), 7.96 (s, 1H).

5-(2,4-Bis(benzyloxy)-5-*tert*-butylphenyl)isoxazole-3-carboxylic Acid Ethyl Ester (68g). Hydroxylamine hydrochloride (3.6 g, 52 mmol) was added to a solution of **67g** (9.0 g, 18.5 mmol) in EtOH (75 mL), and the suspension was heated at reflux for 4 h. The resulting solution was allowed to cool and poured into water (200 mL) to give an off-white precipitate. The solids were removed by filtration and then dissolved in dichloromethane (150 mL). The solution was washed with water (150 mL) and then saturated aqueous NaCl solution (50 mL). The organic phase was dried over anhydrous Na$_2$SO$_4$ and filtered, and the filtrate solvents were removed in vacuo to afford an off-white solid, which was washed with hexane and dried in vacuo (at 40 °C) to give the title compound **68g** (8.0 g, 89%) as a pale-brown powder: LCMS t_R = 3.13 min; m/z = 486.5[M + H]$^+$; ^{1}H NMR (400 MHz, CDCl$_3$) δ 1.41 (t, 3H, J = 7.1 Hz), 1.41 (s, 9H), 4.43 (q, 2H, J = 7.1 Hz), 5.09 (s, 2H), 5.15 (s, 2H), 6.59 (s, 1H), 7.00 (s, 1H), 7.33–7.43 (m, 10H), 7.93 (s, 1H).

5-(2,4-Bis(benzyloxy)-5-*tert*-butylphenyl)isoxazole-3-carboxylic Acid Ethylamide (34g). This compound was made using the procedure described for compound **34d**. Thus, **68g** (10.0 g, 20.6 mmol) was reacted with ethylamine in MeOH solution (2.0 M) to afford the title compound as a brown solid (9.9 g, 100%): LCMS t_R = 3.02 min; m/z = 485.3[M + H]$^+$; ^{1}H NMR (400 MHz, CDCl$_3$) δ 1.26 (t, 3H, J = 7.1 Hz), 1.41 (s, 9H), 3.49 (dq, 2H, J = 7.1, 5.4 Hz), 5.06 (s, 2H), 5.15 (s, 2H), 6.57 (s, 1H), 6.85 (brt, 1H, J = 5.4 Hz), 7.08 (s, 1H), 7.3–7.4 (m, 10H), 7.88 (s, 1H).

5-(2,4-Bis(benzyloxy)-5-*tert*-butylphenyl)-4-iodoisoxazole-3-carboxylic Acid Ethylamide (35g). This compound was made using the procedure described for compound **35d**. Thus, **34g** (9.9 g, 20.4 mmol) was reacted with NIS and CAN to afford the title compound **35g** (7.75, 62%) as an off-white powder: LCMS t_R = 3.07 min; m/z = 610[M + H]$^+$; ^{1}H NMR (400 MHz, CDCl$_3$) δ 1.26 (t, 3H, J = 7.1 Hz), 1.41 (s, 9H), 3.50 (dq, 2H, J = 7.1, 5.4 Hz), 5.07 (s, 4H), 6.60 (s, 1H), 6.80 (brt, 1H, J = 5.4 Hz), 7.28–7.44 (m, 11H).

5-(2,4-Bis(benzyloxy)-5-*tert*-butylyphenyl)-4-(4-formylphenyl)isoxazole-3-carboxylic Acid Ethylamide (36g). Aqueous potassium phosphate solution (25 mL, 1.2 M) was added to a solution of **35g** (6.1 g, 10 mmol) and 4-formylphenyl boronic acid (2.35 g, 15.7 mmol) in 1,4-dioxane (75 mL) under a nitrogen atmosphere. Dichlorobis(tri-*O*-tolylphosphine)palladium(II) (cat.) was added, and the mixture was heated at 100 °C (oil bath temperature) for 1 h. The mixture was allowed to cool, and the aqueous layer was separated and extracted with EtOAc (100 mL). The combined organic phases were concentrated in vacuo to give a pale-brown gum, which was purified by flash column chromatography on silica gel, eluting with EtOAc/hexane (1:3), to give the title compound **36g** (5.18 g, 88%) as a pale-yellow foam: LCMS t_R = 3.01 min; m/z = 589.4[M + H]$^+$; ^{1}H NMR (400 MHz, CDCl$_3$) δ 1.26 (t, 3H, J = 7.1 Hz), 1.27 (s, 9H), 3.44 (dq, 2H, J = 7.1, 5.4 Hz), 4.67 (s, 2H), 5.00 (s, 2H), 6.44 (s, 1H), 6.82 (brt, 1H, J = 5.4 Hz), 7.00 (m, 2H), 7.25–7.27 (m, 4H), 7.33–7.39 (m, 5H), 7.43 (d, 2H, J = 6.9 Hz), 7.72 (d, 2H, J = 6.9 Hz), 9.96 (s, 1H).

5-(2,4-Dihydroxy-5-ethylphenyl)-4-(4-morpholin-4-ylmethylphenyl)isoxazole-3-carboxylic Acid Ethylamide (40g). This compound was made using the procedure described for compound **30f**, using morpholine in reaction with compound **36g**. The crude product after debenzylation was purified by preparative HPLC to afford the title compound **40g** (21 mg, 75%) as an off-white solid (formate salt): LCMS t_R = 1.97 min; m/z = 480.5[M + H]$^+$; ^{1}H NMR (400 MHz, DMSO-d_6) δ 1.05 (t, 3H, J = 7.2 Hz), 1.1 (s, 9H), 2.3 (brs, 4H), 3.2 (dq, 2H, J = 7.2 Hz, 5.6 Hz), 3.45 (brs, 4H), 6.45 (s, 1H), 6.7 (s, 1H), 7.15 (d, 2H, $J = 7.2$ Hz), 7.25 (d, 2H, $J = 7.2$ Hz), 8.8 (t, 1H, $J = 5.6$ Hz); HRMS calcd for $C_{27}H_{33}N_3O_5[M + H]^+$ 480.2483, found 480.2500; HPLC 91% ($t_R = 4.65$ min).

5-(5-Ethyl-2-hydroxy-4-methoxyphenyl)-4-(4-morpholin-4-ylmethylphenyl)isoxazole-3-carboxylic Acid Ethylamide (70); 5-(5-Ethyl-4-hydroxy-2-methoxyphenyl)-4-(4-morpholin-4-ylmethylphenyl)isoxazole-3-carboxylic Acid Ethylamide (71); 5-(5-Ethyl-2,4-dimethoxyphenyl)-4-(4-morpholin-4-ylmethylphenyl-)isoxazole-3-carboxylic Acid Ethylamide (72). To an argon-charged flask containing 5-(5-ethyl-2,4-dihydroxyphenyl)-4-(4-morpholin-4-ylmethylphenyl)isoxazole-3-carboxylic acid ethylamide (**42d**) (25 mg, 0.055 mmol) and *N*,*N*-(diisopropyl)aminomethylpolystyrene[PS-DIEA] (35 mg, 3.83 mmol/g, 2.4 equiv) were added anhydrous DCM (2.3 mL) and anhydrous MeOH (0.25 mL). During gentle stirring, 2 M (trimethylsilyl)diazomethane in hexanes (28 μL, 1.0 equiv) was added, and the solution was stirred overnight at room temperature. Argon was bubbled through the solution for 10 min, the resin was filtered off, and the volatiles were removed in vacuo. The crude residue was purified by semipreparative HPLC to isolate the three methylated products. **70** (1.14 mg, 4%): LCMS (method B) $t_R = 5.13$ min; $m/z = 466.3[M + H]^+$; ^{1}H NMR (250 MHz, acetone-d_6) δ 1.10 (t, 3H, $J = 7.5$ Hz), 1.25 (t, 3H, $J = 7.5$ Hz), 2.42 (m, 4H), 2.50 (q, 2H, $J = 7.5$ Hz), 3.10–3.40 (m, 4H), 3.45 (q, 2H, $J = 7.5$ Hz), 3.82 (s, 2H), 3.95 (s, 2H), 4.10–4.40 (m, 4H), 6.65 (s, 1H), 7.05 (s, 1H), 7.40 (d, 2H, $J = 8.2$ Hz), 7.70 (d, 2H, $J = 8.2$ Hz), 7.90 (brs, 1H), 8.75 (s, 1H); HRMS calcd for $C_{26}H_{32}N_3O_5[M + H]^+$, 466.2342, found 466.2342. **71** (5.5 mg, 21%): LCMS (method B) $t_R = 4.88$ min; $m/z = 466.3[M + H]^+$; ^{1}H NMR (250 MHz, acetone-d_6) δ 0.95 (t, 3H, $J = 8.4$ Hz), 1.06 (t, 3H, $J = 7.1$ Hz), 2.25–2.29 (m, 4H), 2.40 (q, 2H $J = 7.6$ Hz), 3.26 (q, 2H, $J = 7.2$ Hz) 3.27 (s, 3H), 3.35 (s, 2H), 3.46–3.3.49 (m, 4H), 6.42 (s, 1H), 6.95 (s, 1H), 7.12 (s, 4H), 8.00 (brs, 1H). **72**: (1.46 mg, 5%): LCMS (method B) $t_R = 5.44$ min; $m/z = 480.3$; ^{1}H NMR (500 MHz, CD_3OD) δ 1.08 (t, 3H, $J = 7.4$ Hz), 1.19 (t, 3H, $J = 8.1$ Hz), 2.48 (brs, 4H), 2.53 (q, 2H, $J = 8.1$ Hz), 3.37 (q, 2H, $J = 7.4$ Hz), 3.48 (s, 3H), 3.54 (s, 2H), 3.69 (t, 4H, $J = 5.4$ Hz), 3.89 (s, 3H), 6.58 (s, 1H), 7.09 (s, 1H), 7.21(d, 2H, $J = 8.1$ Hz), 7.29 (d, 2H, $J = 8.1$ Hz); HRMS calcd for $C_{27}H_{34}N_3O_5$ 480.2498$[M + H]^+$, found 480.2492.

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Supporting Information Available: Experimental procedures for the FP assay and synthesis of the FP assay probe. Methods and parameters relating to X-ray crystallography and protein production are also provided as are graphical representations of PK cassette dosing results for cassette 3. Full chemistry experimental procedures and characterization data are presented in this section. This information is available free of charge via the Internet at http://pubs.acs.org.

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