Structure−Activity Relationship Analysis of the Selective Inhibition of Transglutaminase 2 by Dihydroisoxazoles

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Human transglutaminase 2 (TG2) is believed to play an important role in the pathogenesis of various human disorders including celiac sprue, certain neurological diseases, and some types of cancer. Selective inhibition of TG2 should therefore enable further investigation of its role in physiology and disease and may lead to effective clinical treatment. Recently we showed that certain 3-halo-4-,5-dihydroisoxazole containing compounds are selective inhibitors of human TG2 with promising pharmacological activities. Here, we present definitive evidence that this class of compounds targets the active site of human TG2. Structure−activity relationship studies have provided insights into the structural prerequisites for selectivity and have led to the discovery of an inhibitor with about 50-fold higher activity than a prototypical dihydroisoxazole inhibitor with good in vivo activity. A method for preparing enantiomerically enriched analogues was also developed. Our studies show that the 5-(*S*)-dihydroisoxazole is a markedly better inhibitor of human TG2 than its 5-(*R*) stereoisomer.

Introduction

The transglutaminase family of enzymes plays important roles in many biological functions by cross-linking selected γ-glutaminyl and ϵ-lysine residues in proteins.[1−3] Expressed in many tissues, transglutaminase 2 (TG2) is known to have extracellular functions in fibronectin cross-linking, cell migration, adhesion, and proliferation.[1−3] When bound to GTP inside the cell, TG2 has no known catalytic activity but rather acts as a G protein in the phospholipase C signal transduction cascade.[3] Its many biological and biochemical roles implicate it in a number of neurological diseases including Huntington's, Alzheimer's, and Parkinson's diseases.[4,5] Importantly, TG2 knockout mice suffer no apparent physiological, developmental, or reproductive defects.[6] That the critical functions of TG2 may be absorbed by redundant enzymes in its absence and that its roles in the pathogenesis of disease may be curtailed by selective inhibition make TG2 an attractive therapeutic target. Several recent reports have described small-molecule inhibitors against TG2 (most often using guinea pig TG2 as a surrogate target).[7−11]

Our interest in the inhibition of human TG2 began as a consequence of our interest in celiac sprue, a common disorder for which there is no current nondietary therapy.[12−14] Celiac sprue is an autoimmune-like disease in which genetically susceptible individuals experience gut damage in response to gluten, a group of ubiquitous dietary proteins. Gluten proteins contain proline- and glutamine-rich sequences, some of which are selectively deamidated by TG2. The resultant peptides are presented to immune system cells, leading to a combined T-cell and B-cell mediated inflammatory response in the small intestinal mucosa.[15,16] Thus, the development of specific TG2 inhibitors may lead to compounds capable of limiting deamidation of gluten peptides in the gut, thereby curtailing the immune response to gluten in celiac patients.

We have previously investigated mechanism-based TG2 inhibitors[10,17] based on a 3-halo-, 4,5-dihydroisoxazole pharmacophore, whose structure is derived from the naturally occurring glutamine isostere acivicin. Decarboxylated acivicin derivatives had increased activity as TG2 inhibitors.[18] The inhibitors studied mostly comprised α-amino acids functionalized with *N*-carbamate moieties and coupled to the dihydroisoxazole electrophile. Several trends in the structure−activity relationships of this class of TG2 inhibitors emerged.[17] Increases in affinity (as determined by K_I) correlated with increases in the bulk of carbamate moiety, e.g., OCH_2-2-naphthyl > OCH_2-CH_2Ph > OCH_2Ph (Table 1). Unfortunately, the 10-fold increase in affinity gained by changing to a naphthyl-2-ylmethyl carbamate from a benzyl carbamate was outweighed by a 12-fold decrease in reactivity (as determined by k_{inh}), leading to a virtually unchanged specificity (as determined by k_{inh}/K_I). Increases in affinity were also observed with the existence of heteroatom hydrogen bond acceptors in the carbamate groups, e.g., OCH_2-3-picolyl > OCH_2-*N*-phthalimidyl > OCH_2-3-dioxobenzothiophene. However, the 10-fold increase in affinity observed with the change from a benzyl carbamate moiety to a picolin-3-ylmethyl carbamate moiety was still met with a 4-fold decrease in reactivity (Table 1). Finally, our previous studies varying the amino acid side chain showed an increase in affinity with the presence of heteroatoms, e.g., compounds derived from 5-OH-tryptophan > tryptophan > tyrosine > phenylalanine. Here too, the increase in affinity (7-fold) with the change from 5-OH-tryptophan to phenylalanine corresponded to a decrease (4-fold) in reactivity.

The best inhibitor found in our previous studies was the 5-OH-tryptophan derivative **10** containing a dioxobenzothiophene in the *N*-carbamate moiety. This compound combined a heteroatom-containing amino acid with a bulky, H-bond-acceptor-containing carbamate moiety. However, its metabolic instability made it an unsuitable candidate for in vivo investigations. Therefore, compound **10** served as a starting point for our current study.

Results and Discussion

In this study we have demonstrated directly via mass spectrometry that 3-halo-4,5-dihydroisoxazole inhibitors bind to the active site cysteine of human TG2. Furthermore, we have

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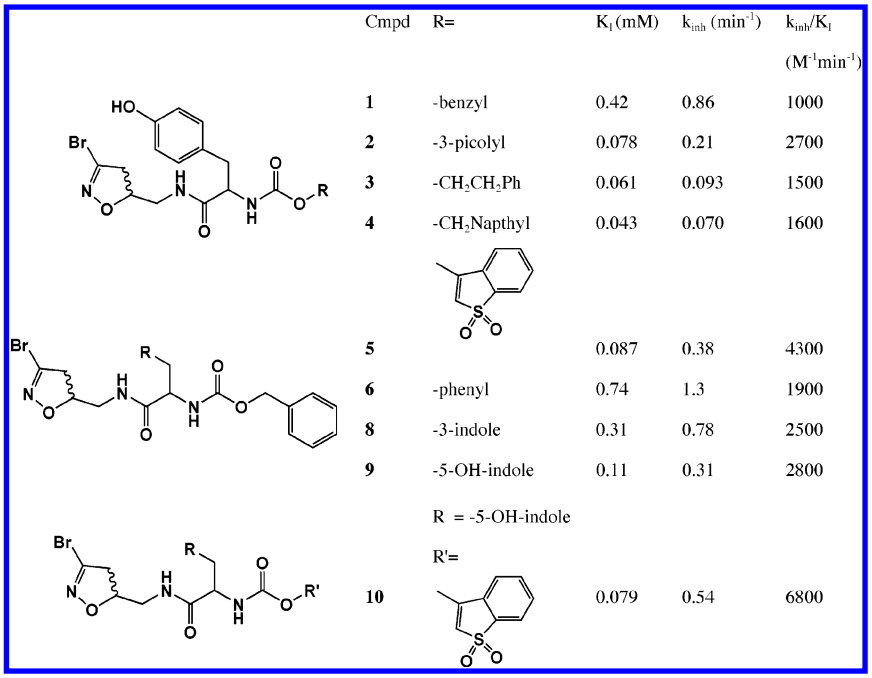
Table 1. Summary of Key Results from Our Previous Study[17]



Cmpd	R=	K_I (mM)	k_{inh} (min^{-1})	k_{inh}/K_I ($M^{-1}min^{-1}$)
1	-benzyl	0.42	0.86	1000
2	-3-picolyl	0.078	0.21	2700
3	$-CH_2CH_2Ph$	0.061	0.093	1500
4	$-CH_2$Napthyl	0.043	0.070	1600
5		0.087	0.38	4300
6	-phenyl	0.74	1.3	1900
8	-3-indole	0.31	0.78	2500
9	-5-OH-indole	0.11	0.31	2800
10	R = -5-OH-indole, R'=	0.079	0.54	6800

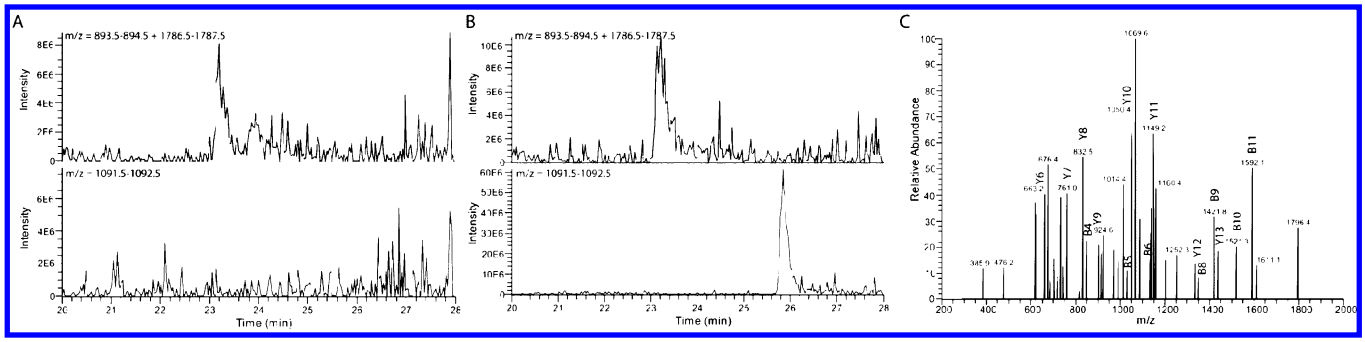


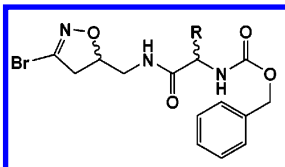
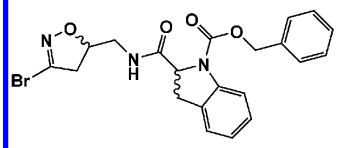
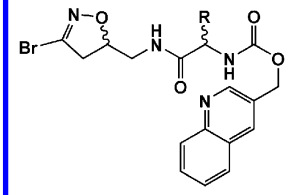
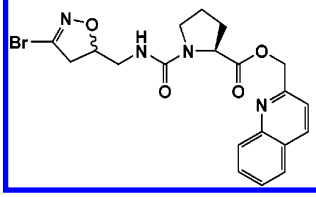
Figure 1. Dihydroisoxazole inhibitors covalently bind the active site cysteine of transglutaminase 2. Human TG2 was incubated with or without inhibitor **1** before being digested with trypsin and analyzed by mass spectrometry. LC−MS chromatogram of uninhibited TG2 (A) and inhibited TG2 (B) was filtered to only display peptide peaks corresponding to the predicted weight of the peptide fragment (YGQ*C*WVFAAVACTVLR) containing the active site cysteine (1787 amu (+1 peak) + 894 amu (+2 peak)) or inhibited active site cysteine (1092 amu (+2 peak)). The fragment containing the active site cysteine is the smaller peak eluting at 23.9 min. Note that there is a log difference in the scale of the 1092 plots. MS/MS spectrum (C) shows the 1092 peak for the inhibited TG2 sample with 15 "y" and "b" peaks identified, proving the identity of the peptide fragment.

systematically investigated (1) the importance of the identity and position of heteroatoms on the amino acid side chain, (2) the significance of alternative hydrogen bond acceptors in a bulky, aromatic carbamate moiety, (3) the effect of stereochemistry in the amino acid side chain, and (4) the stereochemistry of the dihydroisoxazole.

To verify that dihydroisoxazole inhibitors such as the compounds shown in Table 1 do indeed covalently bind to the active site cysteine of TG2, recombinant human TG2 was incubated in the presence or absence of the Cbz-tyrosine derivative **1**, digested with trypsin, and analyzed by LC−MS/MS. While the samples were incubated with (Figure 1B) and without (Figure 1A) derivative **1**, both contained peaks at 1787 (+1) *m*/*z* and 894 (+2) *m*/*z* corresponding to the predicted proteolytic fragment containing the active site cysteine; only the sample incubated with the inhibitor showed a significant 1092 (+2) *m*/*z* peak corresponding to the mass of the active site cysteine containing fragment (1787) plus the mass of the dihydroisoxazole inhibitor without bromine (395). To confirm that these mass peaks did in fact correspond to the TG2 proteolytic fragment containing the active site cysteine, MS/MS analysis (Figure 1C) was performed and 18−22 of the 32 possible b and y peaks were successfully assigned. Similar analysis was repeated for four different dihydroisoxazole inhibitors, including compounds **1**, **4**, **14**, and a dansyl carbamate protected tyrosine derivative (data not shown). All compounds were found to bind the active site cysteine of human TG2. Inhibitor binding to other proteolytic fragments was not observed.

Because the tryptophan derivatives were among the best inhibitors in our previous study, we prepared and tested additional tryptophan derivatives. Commercially available 4-fluoro-, 5-fluoro-, 6-fluoro-, and 7-azaindole derivatives of (D/L)-tryptophan were functionalized with *N*-benzyl carbamates and coupled to the racemic dihydroisoxazole pharmacophore (compounds **13**−**16**). The 5-fluoroindole derivative **14** was the best inhibitor of TG2 with a K_I of 0.019 mM and a k_{inh} of 0.071 min^{-1}. However, it is interesting to note that the 6-fluoro and 7-aza derivatives **15** and **16** had comparable affinity (K_I = 0.019 and 0.022 mM, respectively), but their reactivity was only about half that of the 5-fluoro compound (k_{inh} = 0.046 and 0.033 min^{-1}, respectively). It is also noteworthy that the 4-fluorot-

Table 2. Investigation of Amino Acid Side Chains and Carbamate Protecting Groups

	Cmpd	R=	K_I (mM)	k_{inh} (min^{-1})	k_{inh}/K_I ($min^{-1}M^{-1}$)
	**13**	*(S-/R-)*-CH_2-3-(4-F-indolyl)	.099	.09	925
	14	*(S-/R-)*-CH_2-3-(5-F-indolyl)	.019	.071	3600
	15	*(S-/R-)*-CH_2-3-(6-F-indolyl)	.018	.046	2500
	16	*(S-/R-)*-CH_2-3-(7-aza-indolyl)	.022	.033	1500
	**17**	*(S-/R-)*-2,3-dihydro-indole-1-carbamic acid benzyl ester	.138	.171	1250
	18	*(S-)*-CH_2-p-OH-phenyl	.041	.209	5100
	**19**	*(S-/R-)*-CH_2-3-(5-OH-indolyl)	.034	.136	4000
	20	*(S-/R-)*-CH_2-3-(5-OMe-indolyl)	.051	.211	4100
	21	*(S-/R-)*-CH_2-3-(5-F-indolyl)	.018	.186	10,000
	22	*(S-)*-CH_2-3-(5-F-indolyl)	0.004	.189	45,000
	**23**	*(S-)*-proline	0.062	.377	6100

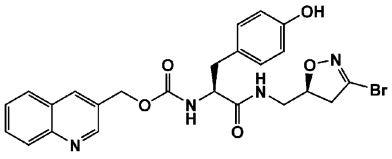
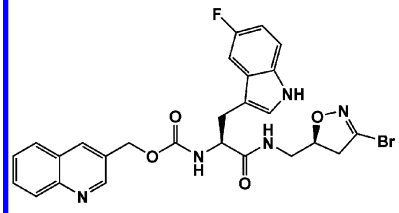
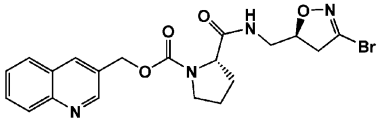
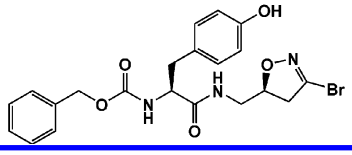
ryptophan derivative **13** is both the least specific and most reactive of these compounds. Even though there is about a 10-fold increase in TG2 affinity of these compounds relative to the Cbz-tryptophan derivative **8**, that increase is met with a nearly equal decrease in reactivity.

We changed the identity of the carbamate moiety from benzyl- to quinoline-3-ylmethyl for several reasons. We had observed the benefit to TG2 affinity of aromatic groups more distal to the amino acid N-terminus; the naphthyl-2-ylmethyl carbamate **4** and the phenethyl carbamate **3** gave greater affinity than the benzyl carbamate **1**. In tests with compounds containing H-bond acceptors in the carbamate moiety, other compounds with second aromatic rings had also shown promise; the OCH_2-3-dioxobenzothiophene carbamate had only half the affinity of the naphthyl-2-ylmethyl compound but 5-fold greater reactivity. From this we surmised that benefits to affinity accruing from the existence of a second aromatic ring could be augmented by H-bond acceptors in the carbamate moiety. This drew into focus the 3-picolyl carbamate compound **2**. Although **2** had only one ring in its carbamate moiety, its affinity was slightly better than the other H-bond-acceptor-containing compounds, and its reactivity was 3-fold better than that of naphthyl-2-ylmethyl compound **4**. Our hypothesis was that the enzyme formed stronger or geometrically more favorable hydrogen bonds to the picolyl protecting group than to the dioxobenzothiophene. Thus, if the binding modes of the picolyl, naphthylmethyl, and dioxobenzothiophene derivatives were similar, a quinoline-3-ylmethyl carbamate moiety might retain the reactivity of the picolyl compound without losing the affinity of the naphthyl compound. To test this hypothesis, quinoline-containing derivatives of tyrosine, **18**, and 5-fluorotryptophan, **21**, were prepared. Also, to further explore the effects of changes in functional groups on the tryptophan side chain, derivatives of 5-hydroxytryptophan **19** and 5-methoxytryptophan **20** were prepared with the quinoline-containing carbamate.

Changing from the picolyl- to the quinoline-3-ylmethyl carbamate increased the TG2 affinity of the tyrosine-derived compounds 2-fold ($K_I^{picolyl} = 0.08$ mM, $K_I^{quinolyl} = 0.04$ mM) with no change in reactivity ($k_{inh}^{picolyl} = 0.21$ min^{-1}, $k_{inh}^{quinolyl} = 0.21$ min^{-1}). No effect was seen on the affinity of the 5-fluorotryptophan derived compounds in the change from the benzyl carbamate to quinolyl carbamate ($K_I^{benzyl} = 0.019$ mM, $K_I^{quinolyl} = 0.018$ mM), but a nearly 3-fold increase in reactivity was observed ($k_{inh}^{benzyl} = 0.071$ min^{-1}, $k_{inh}^{quinolyl} = 0.186$ min^{-1}). The 5-hydroxy and 5-methoxy substituents on the indole moiety in derivatives **19** and **20** gave lower TG2 affinity than the 5-fluoro substituent in **21**, although the reactivity of the MeO-tryptophan compound **20** was ~10% higher. Encouraged by these results and because only racemic mixtures of 5-fluorotryptophan had been used thus far, we prepared a quinoline-containing 5-fluorotryptophan derivative from (*S*)-5-fluorotryptophan. Testing this compound, **22**, showed the importance of the side chain (*S*)-stereochemistry on the activity of the inhibitor. The (*S*)-tryptophan derivative had a K_I of 4-fold better than the (*R*/*S*)-mixture and an equivalent k_{inh} (Table 2).

In the context of celiac sprue, TG2 has high specificity for the second glutamine in a gluten-derived sequence PQPQLPY.[7]

Table 3. TG2 Inhibitory Activity of Enantiomerically Enriched Dihydroisoxazoles

Cmpd	K_I (mM)	k_{inh} (min^{-1})	k_{inh}/K_I ($M^{-1}min^{-1}$)
 **24A**	0.030	0.139	4,600
 **25A**	0.0013	0.072	57,000
 **26A**	0.011	0.110	10,300
 **27A**	0.068	0.155	2,300

Because the dihydroisoxazole pharmacophore is derived from acivicin, a known mimic of glutamine, we prepared the quinolyl carbamate functionalized (*S*)-proline compound **23** to test whether a compound with more structural similarity to the natural substrate might improve inhibition. Although the affinity of **23** was considerably poorer than the (*S*)-5-fluorotryptophan derivative **22** ($K_I^{prolyl} = 0.062$ mM, $K_I^{5-F-tryptophan} = 0.004$ mM), its reactivity was modestly higher ($k_{inh}^{prolyl} = 0.377$ min^{-1}, $k_{inh}^{5-F-tryptophan} = 0.189$ min^{-1}).

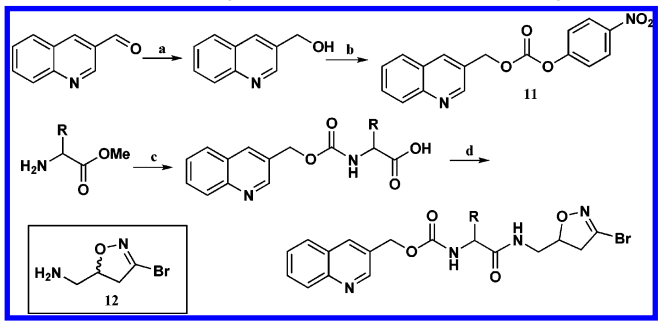
To determine which stereoisomer of the dihydroisoxazole pharmacophore gave better inhibition, enantiomerically enriched versions of the dihydroisoxazole pharmacophore were prepared by enzymatic chiral resolution, as described below, and coupled to several N-protected amino acids. The (*S*)-isomer of the dihydroisoxazole retained its potency as a pharmacophore (Table 3), whereas inhibitors containing the (*R*)-dihydroisoxazole isomer showed little or no inhibition. The quinoline carbamate functionalized proline with the (*R*)-dihydroisoxazole showed <5% of the inhibitory activity of the (*S*)-isomer at 0.048 mM. The quinoline carbamate functionalized tyrosine was indistinguishable from the negative control at 0.048 mM, as was the Cbz-tyrosine compound. Compound **25A**, with a k_{inh}/K_I of 57 000 M^{-1} min^{-1}, is 50-fold more potent than **1**, which has shown promising activity in rodent models of glioblastoma.[11,19] The (*R*)-isomer of quinoline carbamate functionalized 5-fluorotryptophan showed ~5% of the inhibitory activity of its (*S*)-diastereomer. Because the enzymatic resolution of the two isomers of the dihydroisoxazole was imperfect, this inhibition may be an artifact arising from the presence of small amounts of (*S*)-isomer in the sample.

Conclusions

Heteroatom-containing amino acid side chains, hydrogen bond acceptors in the *N*-carbamate moieties of those amino acids, and (*S*)-stereochemistry at the 5-position of the dihydroisoxazole pharmacophore all confer increased activity on TG2 inhibitors of this class. The improvement in inhibition correlating to the change from our previously reported lead compound, Cbz-tyrosylhalodihydroisoxazole **1** ($k_{inh}/K_I = 1000$ min^{-1} M^{-1}), to the quinolinecarbinolic-5-fluorotryptophanhalo-(*S*)-dihydroisoxazole **25A** ($k_{inh}/K_I = 57\,000$ min^{-1} M^{-1}) is significant.

The emerging structure−activity relationships also suggest further opportunities for improvement. The order of specificity among our most promising lead compounds predominantly tracks inversely with reactivity. In the context of the four compounds that have been synthesized with the (*S*)-dihydroisoxazole, the most reactive compound, **27A**, is also the least specific, and the most specific compound, **25A**, is the least reactive. The other two compounds, **24A** and **26A**, also follow this trend. A likely explanation for this phenomenon is that moieties in these compounds that enhance noncovalent interactions with the inhibitor binding site do so in a way that curtails access by the active site nucleophile to the dihydroisoxazole electrophile. Unless the inhibitor shifts out of that binding mode in a specific manner, the irreversible nucleophilic attack cannot occur. The orthogonality between specificity and reactivity modes might be because the binding mode holds the pharmacophore electrophile too far away from the active site nucleophile or because the binding mode orients the electrophile such that an unfavorable rotation barrier must be overcome before it is in position to undergo nucleophilic attack. Future compounds may be designed to test this hypothesis by allowing better access to the active site from the binding mode. For example, it may be feasible to combine the specificity-engendering indole of the quinolyl-5-fluorotryptophan compound **25A** with the reactivity-engendering conformational bias of the quinolylproline compound **26A**. Development of increasingly effective and selective inhibitors of transglutaminase 2 should facilitate illumination

Scheme 1. General Synthesis of Inhibitors in this Study[a]



[a] (a) $LiBH_4$, THF; (b) *p*-nitrophenyl chloroformate, *N*-methylmorpholine, CH_2Cl_2; (c) (1) amino acid methyl ester, **11**, DMF; (2) MeOH, THF, H_2O, LiOH; (d) EDCI, HOBT, DMF, **12**.

of the role of TG2 in a variety of human disorders and may ultimately lead to effective clinical treatments for those disorders.

Experimental Methods

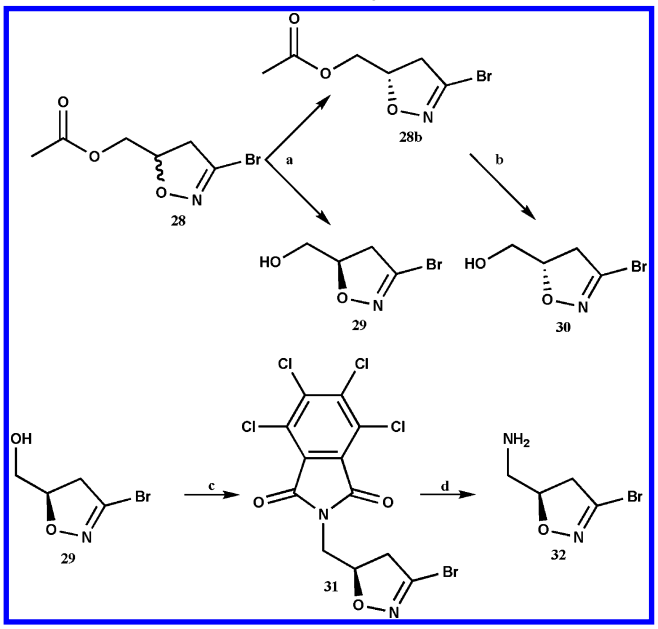
Mass Spectrometry. Recombinant human transglutaminase 2 (50 μL of a 20 μM stock) was diluted 1:1 with Tris buffered saline containing 10 mM calcium chloride, and 1 μL of inhibitor dissolved in DMSO was added to give a final concentration of 100 μM. The sample was incubated 1.5 h at 37 °C to allow for irreversible inhibition. Next, 1 μL of a 15 mg/mL stock of trypsin (Sigma) was added and incubated 1 h at 37 °C to completely digest the protein. The reaction was quenched by diluting the sample 1:1 with formic acid and stored at −20 °C until submission for LC−MS/MS analysis. Fifteen of 20 expected proteolytic fragments from trypsin digestion were found. Of those, only the active site cysteine-containing fragment was labeled with inhibitor.

General Techniques. All reactions were carried out under anhydrous conditions unless otherwise stated. Yields refer to chromatographically and spectroscopically (^{1}H NMR) homogeneous materials. Reagents were purchased at the highest commercial quality and used without further purification. Reactions were monitored by thin-layer chromatography (TLC) carried out on 0.25 mm E. Merck silica gel plates (60F-254) using UV light as a visualizing agent and cerium molybdate stain, ninhydrin stain, permanganate stain, and heat as developing agents. E. Merck silica gel (60, particle size 0.040−0.063 mm) was used for flash column chromatography. Preparative thin-layer chromatography (PTLC) separations were carried out on 0.25 mm E. Merck silica gel plates (60F-254). NMR spectra were recorded on Varian Inova 500 or Varian 200 instruments and calibrated using residual undeuterated solvent as an internal reference. The following abbreviations were used to explain the multiplicities: s = singlet, d = doublet, t = triplet, q = quartet, sept = septet, m = multiplet, b = broad, bs = broad singlet. Mass spectra were recorded on a Finnigan LCQ quadrupole ion trap mass spectrometer using electrospray ionization.

Dihydroisoxazole Synthesis. The synthesis of compounds **1**−**10** was reported earlier.[17] Synthesis of compounds **13**−**17** was accomplished similarly by reaction of commercially available Cbz-Cl with individual amino acids, followed by coupling of the resulting products to the dihydroisoxazole pharmacophore using EDCI/HOBT in DMF. The dihydroisoxazole was synthesized by [3 + 2] cycloaddition of dibromoformaldoxime with allylamine.[20]

Compounds **18**−**23** were synthesized similarly except the amino acids were conjugated to the appropriate quinoline-containing carbamate (Scheme 1). This quinoline-containing carbamate was synthesized by reducing commercially available 3-quinolylcarbaldehyde with lithium borohydride in THF, followed by an aqueous workup and flash chromatography yielding 3-quinolylcarbinol. *p*-Nitrophenyl chloroformate was reacted with 3-quinolylcarbinol in methylene chloride and *N*-methylmorpholine to give a carbonate of 3-quinoline methanol and *p*-nitrophenol. After purification by flash chromatography, this carbonate was reacted with the methyl ester of the amino acid in DMF and *N*-methylmorpholine to give the *N*-quinolylmethyl carbamate protected amino acid methyl ester. Base hydrolysis of the methyl ester followed by EDCI coupling of the resultant acid to the halodihydroisoxazole gave the indicated compounds as a mixture of diastereomers. Compound **17** was synthesized by coupling of the dihydroisoxazole pharmacophore to commercially available 2,3-dihydroindole-1-carbamic acid benzyl ester with EDCI.

Scheme 2. Chiral Resolution of Dihydroisoxazoles[a]



[a] (a) 15% v/v acetone/0.1 M pH7 phosphate buffer, Amano lipase PS; (b) MeOH, 20% aqueous K_2CO_3; (c) DIAD, PPh_3, tetrachlorophthalimide, THF; (d) ethylenediamine, ACN, THF, EtOH.

The preparation of chiral dihydroisoxazoles (Scheme 2) was achieved by [3 + 2] cycloaddition of dibromoformaldoxime with allyl acetate. The racemic product was resolved into enantiomers by treatment with Amano lipase PS in phosphate buffer, pH7, containing 15% acetone. These reaction conditions selectively hydrolyze the ester borne on the (*R*)-dihydroisoxazole, leaving the (*S*)-enantiomer unchanged. The (*R*)-dihydroisoxazole alcohol is readily separated from the (*S*)-dihydroisoxazole ester by flash chromatography. Hydrolysis of the (*S*)-dihydroisoxazole ester in methanol/water/potassium carbonate afforded the second enantiomer of the dihydroisoxazole.[21] The alcohols from the chiral resolution had optical rotations of −135.5° ((*S*)-isomer) and +111.4° ((*R*)-isomer), consistent with published literature.[21] Both compounds were converted to the corresponding amines by means of the Mitsunobu reaction using tetrachlorophthalimide as nucleophile, followed by aminolysis with ethylenediamine in ethanol/acetonitrile/THF. The enantiomerically enriched dihydroisoxazole **32** and its enantiomer were coupled to N-functionalized amino acids under the same conditions as before.

[1-[(3-Bromo-4,5-dihydroisoxazol-5-ylmethyl)carbamoyl]-2-(4-fluoro-1*H*-indol-3-yl)ethyl]carbamic Acid Benzyl Ester (13). An amount of 10 mg (0.045 mmol) of commercially available racemic 4-fluorotryptophan was dissolved in 450 μL of 1:1 dioxane/water (0.1 M) and cooled in a water/ice bath. Excess potassium carbonate was added followed by 2 equiv of Cbz-Cl (15.35 mg) and stirred vigorously overnight. The reaction mixture was diluted to 10 mL with EtOAc, acidified with HCl to pH 1, washed with brine (10 × 1 mL), dried over anhydrous sodium sulfate, concentrated under reduced pressure, and purified by preparative TLC in 90% methylene chloride, 9% methanol, and 1% HOAc (11 mg, 0.03 mmol, 69%). Without further characterization the compound was dissolved in 300 μL of neat DMF (0.1M) and placed in a water/ice bath. An amount of 6 mg of 7-amino-3-bromo-4,5-dihydroisoxazole (0.045 mmol, 1.5 equiv) was added to the reaction mixture followed by 9.8 mg (0.06 mmol, 2 equiv) of hydroxybenzotriazole

hydrate and 14 mg of EDCI (0.06 mmol, 2 equiv). The reaction was allowed to run for 12 h, was followed by an aqueous workup with 1 M phosphate buffer at pH 3, extraction with EtOAc (5 × 5 mL), drying over sodium sulfate, concentration under reduced pressure, and purification by PTLC in 4:1 dichloromethane/acetone (13 mg, 0.025 mmol, 83%). ^{1}H NMR (500 MHz, $CDCl_3$): δ 3.14−3.196 (dd, 1H, J = 10.5, 17.5), 3.23−3.30 (m, 1H), 3.368−3.409 (dd, 1H, J = 6.0, 15.0), 3.452−3.524 (m,2H), 4.483−4.527 (m,-1H), 4.65−4.73 (bm, 1H), 5.046−5.057 (d, 2H, J = 5.5), 5.531−5.545 (d,1H, J = 7.0), 6.544−6.574 (bm, 1H), 6.753−6.796 (m, 1H), 6.998−7.001 (d, 1H, J = 1.5), 7.076−7.118 (m, 1H), 7.140−7.156 (d,1H, J = 8.0), 7.315−7.344 (m, 5H), 8.048 (s, 1H), 8.412 (bs, 1H).

[1-[(3-Bromo-4,5-dihydroisoxazol-5-ylmethyl)carbamoyl]-2-(5-fluoro-1*H*-indol-3-yl)ethyl]carbamic Acid Benzyl Ester (14). Compound **14** (25 mg, 0.049 mmol, 53%) was prepared by the method used for compound **13**. ^{1}H NMR (500 MHz, $CDCl_3$): δ 3.8−3.9 (m, 1H), 3.1−3.2 (m, 1H), 3.3−3.4 (m, 1H), 3.4−3.5 (m, 1H), 4.43−4.505 (m, 1H), 4.46−4.47 (m, 1H), 5.1−5.2 (m, 2H), 5.3−5.5 (m, 1H), 6.1−6.25 (m, 1H), 6.95−7.0 (m, 1H), 7.09−7.15 (d, 1H), 7.17−7.21 (m,1H), 7.27−7.4 (m, 8H), 8.1−8.2 (s, 1H). MS (ESI) m/z 518.9 $[M + H]^+$.

[1-[(3-Bromo-4,5-dihydroisoxazol-5-ylmethyl)carbamoyl]-2-(6-fluoro-1*H*-indol-3-yl)ethyl]carbamic Acid Benzyl Ester (15). Compound **15** (70.0 mg, 0.136 mmol, 68%) was prepared by the method used for compound **13**. ^{1}H NMR (500 MHz, $CDCl_3$): δ 2.783−2.849 (m, 1H), 3.094−3.182 (m, 2H), 3.283−3.440 (m, 2H), 4.45−4.59 (m, 1H), 4.639 (m, 1H), 5.069−5.147 (m, 2H), 5.501−5.549 (dd, 1H, J = 7.0, 17.5), 6.348−6.380 (d,1H, J = 16.0), 6.860−6.892 (dd, 1H, J = 8.5), 7.020−7.056 (m, 2H), 7.3−7.4 (m, 5H), 7.557 (bs, 1H), 8.075 (s, 1H), 8.328 (bs, 1H).

[1-[(3-Bromo-4,5-dihydroisoxazol-5-ylmethyl)carbamoyl]-2-(1*H*-pyrrolo[2,3-*b*]pyridin-3-yl)ethyl]carbamic Acid Benzyl Ester (16). Compound **16** (14.5 mg, 0.029 mmol, 47%) was prepared by the method used for compound **13**. ^{1}H NMR (500 MHz, $CDCl_3$): δ 3.1−3.3 (m, 4H), 4.577 (m, 1H), 4.695 (m, 1H), 5.087−5.118 (m, 2H), 5.548−5.605 (m, 2H), 5.694 (bs, 1H), 7.265−7.279 (m, 2H), 7.3−7.46 (m, 5H), 7.596−7.637 (m, 2H), 8.221−8.276 (m, 1H), 8.514−8.561 (dd, 1H, J = 5.0, 18.5).

2-[(3-Bromo-4,5-dihydroisoxazol-5-ylmethyl)carbamoyl]-2,3-dihydroindole-1-carboxylic Acid Benzyl Ester (17). Commercially available 1-[(benzyloxy)carbonyl]-2-indolinecarboxylic acid (100 mg, 0.336 mmol) was dissolved in neat DMF (3.36 mL, 0.1 M). With rapid stirring, 7-amino-3-bromo-4,5-dihydroisoxazole (90.2 mg, 0.504 mmol, 1.5 equiv) was added followed by HOBT hydrate (68.1 mg, 0.504 mmol, 1.5 equiv) and EDCI (96.6 mg, 0.504 mmol, 1.5 equiv). The mixture was stirred for 12 h, was followed by an aqueous workup with 1 M phosphate buffer at pH 3, and was extracted with EtOAc (5 × 25 mL). The crude product was dried over sodium sulfate, concentrated under reduced pressure, and purified by PTLC in 7:1 dichloromethane/acetone (319 mg, 0.235 mmol, 70%). ^{1}H NMR (500 MHz, $CDCl_3$): δ 2.7−3.6 (m, 6H), 4.5−4.7 (m, 1H), 4.8−4.92 (m, 1H), 5.15−5.35 (m, 2H), 6.4 (bs, 1H), 6.9−7.0 (m, 1H), 7.03−7.2 (m, 2H), 7.25−7.4 (m, 5H), 7.61 (bs, 1H).

4-Nitrophenylquinolin-3-ylmethyl Carbonate (11). Commercially available 2-quinolinecarbaldehyde (651 mg, 4.14 mmol, 1 equiv) was dissolved in ethanol (11.7 mL, 0.1 M) and cooled in a dry ice/acetone bath. Lithium borohydride, 1 M in THF, was added dropwise slowly and followed by TLC. When the reaction was complete, it was quenched with 1 N HCl (1 mL) and stirred for 1 h while warming to room temperature. The reaction mixture was concentrated under reduced pressure, diluted to 50 mL with EtOAc, washed with 1 M pH 7 buffer (3 × 10 mL) and with brine (3 × 5 mL), dried over sodium sulfate, concentrated under reduced pressure, and used without further purification. A 250 mg aliquot of the resulting quinoline carbinol was added dropwise to a solution 0.16 M in *p*-nitrophenyl chloroformate (1.5 equiv) with rapid stirring in a bath of ice/brine. After the mixture was stirred for 2 min, 4-methylmorpholine (1.6 equiv) was added in one aliquot. The mixture was stirred for 10 min and then removed from the bath and allowed to warm to room temperature. The reaction was followed by TLC and was complete in about 7 h. The mixture was diluted with 50 mL of EtOAc and washed with 1 M HCl (2 × 10 mL) to remove the morpholines, then with saturated aqueous bicarbonate (2 × 10 mL) to begin removal of *p*-nitrophenol. The organic phase was dried over sodium sulfate and then pushed through a plug of silica topped by a plug of anhydrous potassium carbonate to remove most of the remaining *p*-nitrophenol. The resultant liquor was concentrated under reduced pressure and purified by flash chromatography in 10−70% EtOAc/hexanes (219 mg, 43%). ^{1}H NMR (200 MHz, $CDCl_3$): δ 5.450 (s, 2H), 7.308−7.354 (d, 2H, J = 23), 7.525−7.605 (dddd, 1H, J = 1, 7), 7.698−7.782 (dddd, 1H, J = 1, 7), 7.806−7.852 (dd, 1H, J = 1.2, 8.0), 8.097−8.141 (d, 1H, J = 8.8), 8.203−8.250 (d, 2H, J = 23), 8.950−8.961 (d, 1H, J = 2.2).

(*S*)-Quinolin-3-ylmethyl 1-((3-Bromo-4,5-dihydroisoxazol-5-yl)methylamino)-3-(5-fluoro-1*H*-indol-3-yl)-1-oxopropan-2-ylcarbamate (22). Commercially available (*S*)-5-F-tryptophan methyl ester (75 mg, 0.315 mmol, 1 equiv) was dissolved in 1 mL of DMF (0.315 M). **11** (219 mg, 0.615 mmol, 2.14 equiv) was added in one aliquot. The mixture was stirred overnight, then diluted with 50 mL of CH_2Cl_2 and 75 mL of EtOAc and washed with saturated aqueous bicarbonate (3 × 25 mL). The organic phase was dried over sodium sulfate, then pushed through a pad of silica topped with a pad of anhydrous potassium carbonate. The resultant liquor was concentrated under reduced pressure and purified by flash chromatography in equal parts EtOAc/hexanes and EtOAc/hexanes + 5% EtOH (79 mg, 0.187 mmol, 60%). The resultant methyl ester was dissolved in 1.5 mL of THF, and 1 equiv of LiOH was added as a 1 M solution in water (187 μL). Methanol was added dropwise till the reaction mixture became homogeneous, and the mixture was stirred for 3 h. The reaction mixture was acidified and extracted with EtOAc (4 × 10 mL), dried over sodium sulfate, and concentrated under reduced pressure (70 mg, 0.172 mmol, 94%). The free acid was dissolved in DMF (1.7 mL, 0.1 M). HOBT (23.24 mg, 0.172 mmol, 1 equiv) was added followed by **12** (30 mg, 0.172 mmol, 1 equiv) and EDCI (33 mg, 0.172 mmol, 1 equiv). The mixture was stirred overnight, diluted with 25 mL of EtOAc, washed with brine (3 × 10 mL), washed with 1 M phosphate buffer, pH 7 (3 × 10 mL), then dried over sodium sulfate, concentrated under reduced pressure, and purified by column chromatography in 10−50% acetone in dichloromethane and 50% acetone/dichloromethane, and 0−3% EtOH (73 mg, 0.128 mmol, 75%). ^{1}H NMR (200 MHz, DMSO-d_6)): δ 2.9−3.55 (m, 4H), 4.387−4.497 (dd, 1H, J = 7.6, 14.8), 4.6−4.76 (m, 1H), 5.199 (s, 2H), 6.541−6.575 (d, 1H, J = 6.8), 6.770−6.873 (ddd, 1H, J = 2.2, 9.0), 7.24−7.38 (m, 4H), 7.515−7.590 (m, 2H), 7.658−7.742 (ddd, 1H, J = 1.6, 7.0), 7.849−7.888 (d, 1H, J = 8.0), 7.973−8.015 (d, 1H, J = 8.4) 8.8 (s, 1H), 10.18 (bs, 1H).

(*S*)-Quinolin-3-ylmethyl 1-((3-Bromo-4,5-dihydroisoxazol-5-yl)methylamino)-3-(4-hydroxyphenyl)-1-oxopropan-2-ylcarbamate (18). Compound **18** (6.8 mg, 0.0129 mmol, 3.2% for three steps) was prepared by the method used to prepare compound **22**. ^{1}H NMR (200 MHz, $CDCl_3$): δ 2.6−3.0 (m, 4H), 4.0−4.6 (m, 1H), 4.49−4.7 (m, 1H), 5.094−5.114 (d, 2H, J = 4.0), 6.543−6.584 (d, 2H, J = 8.2), 6.966−7.008 (d, 2H, J = 8.4), 7.47−7.596 (m, 2H), 7.655−7.738 (ddd, 1H, J = 1.2, 7.0), 7.82−7.978 (m, 2H), 8.1−8.35 (m, 2H), 8.779−8.789 (d, 1H, J = 2.0), 9.124 (s, 1H).

(*S*)-Quinolin-3-ylmethyl 1-((3-Bromo-4,5-dihydroisoxazol-5-yl)methylamino)-3-(5-hydroxy-1*H*-indol-3-yl)-1-oxopropan-2-ylcarbamate (19). Compound **19** (5.6 mg, 0.0099 mmol, 3.6% for three steps) was prepared by the method used to prepare compound **22**. ^{1}H NMR (200 MHz, $CDCl_3$): δ 2.8−3.4 (m, 4H), 4.1−4.2 (m, 1H), 4.45−4.66 (m, 1H), 5.101−5.132 (d, 2H, J = 6.2), 6.492−6.545 (dd, 1H, J = 2.0, 8.8), 6.84−6.9 (m, 1H), 6.96−7.07 (m, 2H), 7.25−7.589 (m, 3H), 7.659−7.729 (dd, 1H, J = 1.2, 7.0), 7.852−7.971 (dd, 2H, J = 8.4, 16.6), 8.15−8.3 (m, 2H), 8.516 (s, 1H), 8.794−8.804 (d, 1H, J = 2.0), 10.440 (bs, 1H).

(*S*)-Quinolin-3-ylmethyl 1-((3-Bromo-4,5-dihydroisoxazol-5-yl)methylamino)-3-(5-methoxy-1*H*-indol-3-yl)-1-oxopropan-2-ylcarbamate (20). Compound **20** (9.0 mg, 0.0155 mmol, 6.2% for

three steps) was prepared by the method used to prepare compound **22**. ^{1}H NMR (200 MHz, $CDCl_3$): δ 2.8−3.4 (m, 4H), 3.680 (s, 3H), 4.1−4.3 (m, 1H), 4.5−4.7 (m, 1H), 5.087−5.121 (d, 2H, J = 6.8), 6.604−6.659 (dd, 1H, J = 2.3, 8.7), 7.116−7.159 (d, 1H, J = 8.6), 7.462−7.588 (m, 2H), 7.661−7.729 (dd, 1H, J = 1, 6.8), 7.838−7.970 (dd, 2H, J = 8.4, 17.8), 8.167 (s, 1H), 8.22−8.38 (m, 1H), 8.781−8.791 (d, 1H, J = 2.0), 10.590 (s, 1H).

Quinolin-3-ylmethyl 1-((3-Bromo-4,5-dihydroisoxazol-5-yl)-methylamino)-3-(5-fluoro-1*H*-indol-3-yl)-1-oxopropan-2-ylcarbamate (21). Compound **21** (4.7 mg, 0.0083 mmol, 2.4% for three steps) was prepared by the method used to prepare compound **22**. ^{1}H NMR (200 MHz, DMSO-d_6): δ 2.9−3.55 (m, 4H), 4.387−4.497 (dd, 1H, J = 7.6, 14.8), 4.6−4.76 (m, 1H), 5.199 (s, 2H), 6.541−6.575 (d, 1H, J = 6.8), 6.770−6.873 (ddd, 1H, J = 2.2, 9.0), 7.24−7.38 (m, 4H), 7.515−7.590 (m, 2H), 7.658−7.742 (ddd, 1H, J = 1.6, 7.0), 7.849−7.888 (d, 1H, J = 8.0), 7.973−8.015 (d, 1H, J = 8.4) 8.8 (s, 1H), 10.18 (bs, 1H).

(*S*)-Quinolin-3-ylmethyl 2-(((3-Bromo-4,5-dihydroisoxazol-5-yl)methyl)carbamoyl)pyrrolidine-1-carboxylate (23). Compound **23** (5.2 mg, 0.0113 mmol, 5.1% for three steps) was prepared by the method used to prepare compound **22**. ^{1}H NMR (200 MHz, $CDCl_3$): δ 1.6−1.8 (m, 4H), 2.8−3.4 (m,6H), 4.05−4.25 (m, 1H), 4.45−4.7 (m, 1H), 5.1−5.25 (m, 2H), 7.522−7.738 (m, 2H), 7.866−7.977 (m, 2H), 8.15−8.3 (m, 2H), 8.765−8.859 (d, 1H, J = 18.4).

Quinolin-3-ylmethyl (*S*)-1-(((*S*)-3-Bromo-4,5-dihydroisoxazol-5-yl)methylamino)-3-(4-hydroxyphenyl)-1-oxopropan-2-ylcarbamate (24a) and Quinolin-3-ylmethyl (*S*)-1-(((*R*)-3-Bromo-4,5-dihydroisoxazol-5-yl)methylamino)-3-(4-hydroxyphenyl)-1-oxopropan-2-ylcarbamate (24b). Compounds **24a** and **24b** (**24a**, 40.6 mg, 0.077 mmol, 77% in the coupling reaction; **24b**, 26.0 mg, 0.049 mmol, 58%) were prepared by the method used to prepare compound **22**, with the exception that the enantiomerically enriched dihydroisoxazoles, **32** and **32b**, were used.

24a. ^{1}H NMR (500 MHz, DMSO-d_6): δ 2.799−2.836 (dd, 1H, J = 4.5, 13.5), 2.935−2.985 (dd, 1H, J = 7.5, 17.5), 3.179−3.228 (ddd, 1H, J = 5.0, 5.0, 14.0), 3.293−3.350 (dd, 1H, J = 11.0, 18.0), 3.378−3.430 (m, 1H), 4.146−4.193 (m, 1H), 4.653−4.709 (m, 1H), 5.144−5.229 (dd, 2H, J = 13.0, 29.5), 6.644−6.661 (d, 2H, J = 8.5), 7.062−7.079 (d, 2H, J = 8.5), 7.952−7.609 (d, 1H, J = 8.5), 7.625−7.655 (ddd, 1.0, 7.0, 7.0), 7.761−7.794 (ddd, 1H, J = 1.0, 8.5, 8.5), 7.952−7.968 (d, 1H, J = 8.0), 8.029−8.046 (d, 1H, J = 8.5), 8.245−8.247 (d, 1H, J = 1.0), 8.331−8.355 (dd, 1H, J = 6.0, 6.0), 8.865−8.869 (d, 1H, J = 1.5), 9.213 (s, 1H); ^{13}C NMR (125 MHz, DMSO-d_6) δ 173.2, 156.5, 156.4, 151.3, 147.8, 138.9, 135.2, 130.8, 130.3, 129.5, 128.7, 127.9, 127.7, 115.6, 80.8, 64.0, 57.4, 44.2, 41.9, 37.6, 28.58, 23.4. $[\alpha]^{22}_D$ +40.0 (*c* 0.5, DMSO-d_6). HRMS (TOF MS ES+) *m*/*z* calcd for $C_{24}H_{23}N_4O_5Br$, 527.0930, found, 527.0915 $(M + H)^+$.

24b. ^{1}H NMR (500 MHz, CD_3OD): δ 2.790−2.835 (dd, 1H, J = 8.5, 13.5), 2.965−3.012 (m, 2H), 3.187−3.243 (dd, 1H, J = 10.5, 17.5), 3.328−3.454 (ddd, 2H, J = 10.2, 14.0, 44.0), 4.279−4.308 (dd, 1H, J = 6.5, 8.0), 4.652−4.709 (m, 1H), 5.294 (s, 2H), 6.688−6.705 (dd, 2H, J = 2.0, 6.5), 7.048−7.066 (d, 2H, 9.0), 7.641−7.671 (dd, 1H, J = 7.0, 7.0), 7.953−7.97 (d, 1H, J = 8.5), 8.041−8.058 (d, 1H, 8.5), 8.304 (s, 1H), 8.859−8.862 (d, 1H, J = 1.5). ^{13}C NMR (125 MHz, CD_3OD): δ 173.7, 156.7, 156.2, 150.2, 147.0, 138.0, 136.0, 130.5, 130.2, 128.2, 128.1, 127.8, 127.3, 115.1, 80.5, 57.2, 43.7, 41.5, 37.1. $[\alpha]^{22}_D$ −38.8 (*c* 0.5, CD_3OD). HRMS (TOF MS ES+) *m*/*z* calcd for $C_{24}H_{23}N_4O_5Br$, 527.0930, found, 527.0942 $(M + H)^+$.

Quinolin-3-ylmethyl (*S*)-1-(((*S*)-3-Bromo-4,5-dihydroisoxazol-5-yl)methylamino)-3-(5-fluoro-1*H*-indol-3-yl)-1-oxopropan-2-ylcarbamate (25a) and Quinolin-3-ylmethyl (*S*)-1-(((*R*)-3-Bromo-4,5-dihydroisoxazol-5-yl)methylamino)-3-(5-fluoro-1*H*-indol-3-yl)-1-oxopropan-2-ylcarbamate (25b). Compounds **25a and 25b** (**25a**, 12.2 mg, 0.0214 mmol, 67% in the coupling reaction; **25b**, 14.6 mg, 0.026 mmol, 81%) were prepared by the method used to prepare compound **22**, with the exception that the enantiomerically enriched dihydroisoxazoles, **32** and **32b**, were used.

25a. ^{1}H NMR (500 MHz, DMSO-d_6): δ 2.87−3.1 (m, 3H), 3.186−3.325 (ddd, 1H, J = 5.0, 5.0, 14.5), 3.257−3.313 (dd, 1H, J = 10.5, 17.5), 3.98−3.54 (m, 1H), 4.247−4.293 (m, 1H), 4.644−4.700 (m, 1H), 5.111−5.226 (dd, 2H, J = 8.0, 44.5), 6.833−6.925 (ddd, 1H, J = 3.0, 9.5, 9.5), 7.261−7.265 (d, 1H, J = 2.0), 7.311−7.338 (dd, 1H, J = 4.5, 4.5), 7.473−7.498 (dd, 1H, J = 2.5, 10.0), 7.604−7.636 (dd, 1H, J = 8.0, 8.0), 7.633−7.652 (dd, 1H, J = 1.5, 7.0), 7.757−7.790 (ddd, 1H, J = 1.5, 7.0, 8.0), 7.927−7.945 (dd, 1H, J = 1.0, 8.0), 8.023−8.040 (d, 1H, J = 8.5), 8.236−8.239 (d, 1H, J = 1.5), 8.441−8.464 (dd, 1H, J = 6.0, 6.0), 8.856−8.860 (d, 1H, J = 2.0), 10.944−10.948 (d, 1H, J = 2.0). ^{13}C NMR (125 MHz, DMSO-d_6): δ 173.4, 158.3, 156.5, 151.3, 147.8, 138.8, 135.2, 133.4, 130.7, 130.3, 129.5, 128.7, 128.1, 127.9, 127.7, 127.0, 112.9, 111.1, 109.8, 109.6, 104.2, 80.8, 64.9, 56.2, 44.1, 42.0, 28.7. $[\alpha]^{22}_D$ +18.7 (*c* 0.5, DMSO-d_6). HRMS (TOF MS ES+) *m*/*z* calcd for $C_{26}H_{23}N_5O_4FBr$, 568.0996, found, 568.0094 $(M + H)^+$.

25b. ^{1}H NMR (500 MHz, DMSO-d_6): δ 2.867−2.916 (dd, 1H, J = 10.0, 14.5), 2.995−3.052 (m, 1H), 3.257−3.337 (m, 1H), 4.236−4.287 (m, 1H), 4.664−4.721 (m,1H), 5.112−5.232 (dd, 2H, J = 13.0, 47.0), 6.880−6.921 (ddd, 1H, J = 2.0, 8.0, 8.0), 7.274 (s, 1H), 7.308−7.335 (dd, 1H, J = 5.0, 9.0), 7.471−7.496 (dd, 1H, J = 2.0, 20.0), 7.578−7.594 (d, 1H, J = 8.0), 7.621−7.651 (dd, 1H, J = 7.0,7.0), 7.757−7.790 (ddd, 1H, J = 1.0, 8.0, 8.0), 7.929−7.945 (d, 1H, J = 8.0), 8.023−8.040 (d, 1H, J = 8.5), 8.427−8.451 (dd, 1H, J = 6.0, 6.0), 8.858−8.861 (d, 1H, J = 1.5), 10.947 (s, 1H). ^{13}C NMR (125 MHz, DMSO-d_6): δ 173.2, 158.3, 156.5, 151.3, 147.8, 139.0, 135.2, 133.4, 130.8, 130.3, 129.5, 128.7, 128.0, 127.7, 127.0, 112.9, 111.1, 109.7, 109.5, 104.0, 80.8, 69.7, 56.1, 44.0, 41.7, 28.5). $[\alpha]^{22}_D$ −30.8 (*c* 0.5, DMSO-d_6). HRMS (TOF MS ES+) *m*/*z* calcd for $C_{26}H_{23}N_5O_4FBr$, 568.0996, found, 568.0980 $(M + H)^+$.

(*S*)-Quinolin-3-ylmethyl 2-((((*S*)-3-Bromo-4,5-dihydroisoxazol-5-yl)methyl)carbamoyl)pyrrolidine-1-carboxylate (26a) and (*S*)-Quinolin-3-ylmethyl 2-((((*R*)-3-Bromo-4,5-dihydroisoxazol-5-yl)methyl)carbamoyl)pyrrolidine-1-carboxylate (26b). Compounds **26a and 26b** (**26a**, 22.2 mg, 0.048 mmol, 48.2% in the coupling reaction; **26b**, 26.0 mg, 0.056 mmol, 66%) were prepared by the method used to prepare compound **22**, with the exception that the enantiomerically enriched dihydroisoxazoles, **32** and **32b**, were used.

26a. ^{1}H NMR (500 MHz, CD_3CN): δ 1.867−1.972 (bm, 3H), 2.909−3.059 (ddd, 1H, J = 7.5, 17.5, 50.0), 3.153−3.209 (dd, 1H, J = 10.0, 17.5), 3.273−3.396 (m, 2H), 3.462−3.538 (m, 2H), 3.573−3.616 (m, 1H), 4.205−4.279 (ddd, 1H, J = 3.0, 8.5, 25.0), 4.587−4.644 (m, 1H), 4.755−4.811 (m, 1H), 5.233−5.371 (dd, 1H, J = 13.0, 57.0), 5.358 (s, 1H), 6.904−6.971 (bd, 1H, J = 33.5), 7.637−7.664 (dd, 1H, J = 7.0, 7.0), 7.781−7.810 (dd, 1H, J = 8.5, 8.5), 7.946−7.991 (dd, 1H, J = 8.0, 14.5), 8.231−8307 (d, 1H, J = 38.0), 8.892−8.969 (d, 1H, J = 38.5). ^{13}C NMR (125 MHz, CD_3CN): δ 173.5, 173.1, 155.2, 154.6, 151.0, 148.0, 137.9, 135.0, 134.8, 130.4, 129.8, 129.3, 128.3, 127.9, 127.2,80.8, 80.6, 64.7, 61.3, 60.7, 47.5, 47.1, 43.9, 43.7, 41.6, 41.2, 31.6, 300.2, 24.4, 23.6. $[\alpha]^{22}_D$ +17.2 (*c* 0.5, CD_3CN). HRMS (TOF MS ES+) *m*/*z* calcd for $C_{20}H_{21}N_4O_4Br$, 461.0824, found, 461.086 $(M + H)^+$.

26b. ^{1}H NMR (500 MHz, CD_3OD): δ 1.900−2.035 (m, 3H), 2.201−2.370 (m, 1H), 2.892−2.943 (dd, 1H, J = 8.0, 17.5), 3.266−3.324 (m, 1H), 3.358−3.434 (dd, 1H, J = 4.5, 33.5), 3.387−3.406 (dd, 1H, J = 4.5, 4.5), 3.522−3.649 (m, 2H), 4.272−4.341 (ddd, 1H, J = 3.5, 8.5, 22.0), 4.618−4.815 (m, 1H), 5.292−5.442 (dd, 1H, J = 13.0, 62.5), 5.388 (s, 1H), 7.651−7.683 (ddd, 1H, J = 1.0, 8.0, 8.0), 7.800−7.833 (ddd, 1H, J = 1.0, 8.0, 8.0), 7.982−8.011 (dd, 1H, J = 7.0, 7.0), 8.052−8.069 (d, 1H, J = 9.0), 8.356−8.416 (d, 1H, J = 30.0), 8.862−8.936 (d, 1H, J = 42.0). ^{13}C NMR (125 MHz, CD_3OD): δ 174.8, 150.4, 150.3, 147.1, 137.6, 136.4, 136.2, 130.2, 128.2, 127.8, 127.4, 80.7, 80.3, 60.9, 43.7, 41.8, 41.3, 31.6, 30.3, 24.3, 23.4. $[\alpha]^{22}_D$ −68.0 (*c* 0.54, CD_3OD). HRMS (TOF MS ES+) *m*/*z* calcd for $C_{20}H_{21}N_4O_4BrNa$, 483.0664, found, 483.0646 $(M + H)^+$.

Benzyl (*S*)-1-(((*S*)-3-Bromo-4,5-dihydroisoxazol-5-yl)methylamino)-3-(4-hydroxyphenyl)-1-oxopropan-2-ylcarbamate (27a) and Benzyl (*S*)-1-(((*R*)-3-Bromo-4,5-dihydroisoxazol-5-yl)methy-

lamino)-3-(4-hydroxyphenyl)-1-oxopropan-2-ylcarbamate (27b). Compounds **27a and 27b** (**27a**, 72.3 mg, 0.152 mmol, 76% in the coupling reaction; **27b**, 80.8 mg, 0.170 mmol, 84.8%) were prepared by the method used to prepare compound **22**, with the exception that the enantiomerically enriched dihydroisoxazoles, **32** and **32b**, were used.

27a. ^{1}H NMR (500 MHz, CD_3OD): δ 2.609−2.657 (dd, 1H, J = 10.5, 13.5), 2.795−2.832 (dd, 1H, J = 4.5, 14.0), 2.939−39.989 (dd, 1H, J = 7.0, 17.5), 3.181−3.230 (ddd, 1H, J = 5.0, 5.0, 14.0), 3.299−3.356 (dd, 1H, J = 11.0, 17.5), 4.134−4.181 (m,1H), 4.659−4.716 (m, 1H), 4.946−4.953 (d, 2H, J = 3.5), 6.648−6.665 (d, 2H, J = 8.5), 7.055−7.071 (d, 2H, J = 8.5), 7.240−7.254 (d, 1H, J = 7.0), 7.282−7.359 (m, 3H), 7.462−7.479 (d, 1H, J = 8.5), 8.294−8.318 (dd, 1H, J = 6.0, 6.0), 9.238 (s, 1H). ^{13}C NMR (125 MHz, DMSO-d_6): δ 173.1, 156.5, 138.9, 137.7, 130.8, 129.0, 128.7, 128.4, 128.2, 115.6, 80.8, 65.9, 57.3, 44.2, 41.9, 37.6. $[\alpha]^{22}_D$ +45.5 (c 0.5, DMSO-d_6). HRMS (TOF MS ES+) m/z calcd for $C_{21}H_{22}N_3O_4$-BrNa, 498.06, found, 498.0641 $(M + Na)^+$.

27b. ^{1}H NMR (500 MHz, CD_3OD): δ 2.607−2.655 (dd, 1H, J = 7.0, 13.5), 2.806−2.843 (dd, 1H, J = 4.5, 13.5), 3.002−3.053 (dd, 1H, J = 7.5, 17.5), 3.303−3.365 (m, 4H), 4.122−4.168 (m, 1H), 4.671−4.720 (m, 1H), 4.965 (s, 2H), 6.646−6.663 (d, 2H, J = 8.5), 7.059−7.076 (d, 2H, J = 8.5), 7.247−7.361 (m, 5H), 7.457−7.474 (d, 1H, J = 8.5), 8.273−8.296 (dd, 1H, J = 6.0, 6.0), 9.207 (s, 1H). ^{13}C NMR (125 MHz, DMSO-d_6): δ 173.1, 156.6, 156.5, 139.0, 137.8, 130.8, 129.0, 128.8, 128.4, 128.2, 115.6, 80.8, 65.9, 57.3, 44.1, 41.8, 37.5. $[\alpha]^{22}_D$ −68.2 (c 0.5, CD_3CN). HRMS (TOF MS ES+) m/z calcd for $C_{21}H_{22}N_3O_4Br$, 498.06, found, 498.0641 $(M + Na)^+$.

(3-Bromo-4,5-dihydroisoxazol-5-yl)methyl Acetate. (28) To 100.1 mg (1mmol) of allyl acetate in 10 mL of 1:1 H_2O/EtOAc was added 202.8 mg (1 mmol) of dibromoformaldoxime with vigorous stirring at room temperature. An amount of 10 mL of 1 M pH7 phosphate buffer was added slowly, and the reaction was followed by TLC. Upon completion, aqueous NaOH was added dropwise to give pH9, and the organic phase was decanted. The aqueous phase was extracted with 50 mL of EtOAc, and the combined organic phases were dried with sodium sulfate. The solvent was removed under reduced pressure. The resultant oil was purified by flash chromatography in 10−40% EtOAc in hexanes (158 mg, 0.72 mmol, 71%). ^{1}H NMR (200 MHz, $CDCl_3$): δ 1.990 (s, 3H), 2.911−3.348 (ddd, 2H, J = 7.6, 17.4, 59.2), 4.052−4.240 (m, 2H), 4.774−4.885 (m, 1H). ^{13}C NMR (125 MHz, $CDCl_3$): δ 170.1, 136.3, 78.2, 63.6, 42.8, 20.1.

(*R*)-(3-Bromo-4,5-dihydroisoxazol-5-yl)methanol (29) and ((*S*)-3-Bromo-4,5-dihydroisoxazol-5-yl)methyl Acetate (28b). To 8.5 g (38.5mmol) of the racemic dihydroisoxazole acetate **27** was added 1.15 L of 15% v/v acetone in 0.1 M pH7 phosphate buffer, followed by 38 mg (1 mg/mmol substrate) of Amano lipase PS. The reaction progress as monitored by TLC stalled after 2 h. NaCl (∼1 teacup) was added, and the reaction mixture was extracted with EtOAc, 10 × 125 mL. The combined organic layers were dried with sodium sulfate and evaporated under reduced pressure. The chiral alcohol and ester were purified and separated by flash chromatography in 40% EtOAc in hexanes. The reaction yielded about 1 g of alcohol, and the remaining acetate was retreated with the lipase under identical conditions. After three rounds of resolution, the total yield of alcohol was 3.26 g (18.23 mmol, 47%) and the yield of acetate was 4.02 g (18.18 mmol, 47%).

29. ^{1}H NMR (200 MHz, $CDCl_3$): δ 1.818−2.1 (bs, 1H), 2.544−2.772 (m, 2H), 4.305−4.475 (m, 2H), 4.79−4.88 (m, 1H). ^{13}C NMR (125 MHz, $CDCl_3$) δ 138.2, 82.4, 63.0, 42.8. $[\alpha]^{22}_D$ −135.7 (c 0.5, $CDCl_3$).

28b. ^{1}H NMR (200 MHz, $CDCl_3$): δ 1.990 (s, 3H), 2.911−3.348 (ddd, 2H, J = 7.6, 17.4, 59.2), 4.052−4.240 (m, 2H), 4.774−4.885 (m, 1H). ^{13}C NMR (125 MHz, $CDCl_3$): δ 170.1, 136.3, 78.1, 63.6, 42.8, 20.1. $[\alpha]^{22}_D$ +101.0 (c 0.5, $CDCl_3$).

(*S*)-(3-Bromo-4,5-dihydroisoxazol-5-yl)methanol (30). Acetate **27b**, 1.21 g, 5.5mmol, was hydrolyzed in 31.5 mL of MeOH and 16 mL of 20% aqueous potassium carbonate. After 15 min, the reaction was complete by TLC, and the reaction mixture was diluted with 200 mL of EtOAc and washed with 10 × 10 mL of brine. The organic phase was dried with sodium sulfate, evaporated under reduced pressure, and purified by flash chromatography in 40% EtOAc/hexanes (808 mg, 4.51 mmol, 82%). Note: The (*S*)-enantiomer of this compound was spectroscopically indistinct from the (*R*)-enantiomer with the exception of its optical rotation: $[\alpha]^{22}_D$ +111.2 (c 0.5, $CDCl_3$).

(*S*)-2-((3-Bromo-4,5-dihydroisoxazol-5-yl)methyl)-4,5,6,7-tetrachloroisoindoline-1,3-dione (31). To 1 g (4.97mmol) of DIAD in 45 mL of THF was added at room temperature 1.3 g (4.97 mmol) of triphenylphosphine. After 15 min of vigorous stirring, the mixture was cooled in an ice/water bath and an amount of 808 mg (4.51mmol) of alcohol **29** was added. The mixture was stirred for 45 min, whereupon 1.42 g (4.97mmol) of tetrachlorophthalimide was added. The mixture was allowed to warm to room temperature and was stirred overnight. The mixture was diluted with 200 mL of EtOAc and washed with brine, 10 × 30 mL. The organic phase was dried with sodium sulfate and evaporated under reduced pressure. The resulting solid was purified by flash chromatography in neat methylene chloride (694 mg, 1.55 mmol, 34%). ^{1}H NMR (500 MHz, $CDCl_3$): δ 3.113−3.430 (ddd, 2H, J = 7.0, 17.5, 130.5), 3.825−4.059 (ddd, 2H, J = 5.5, 14.50, 96.0), 4.876−5.025 (m, 1H). ^{13}C NMR (125 MHz, $CDCl_3$): δ 163.6, 140.8, 137.5, 130.6, 130.3, 127.6, 78.0, 45.4, 41.4, 31.2. $[\alpha]^{22}_D$ +108.6 (c 0.5, $CDCl_3$). Note: The (*R*)-enantiomer of this compound was spectroscopically indistinct from the (*S*)-enantiomer with the exception of its optical rotation: $[\alpha]^{22}_D$ −98.2 (c 0.5, $CDCl_3$).

(*S*)-(3-Bromo-4,5-dihydroisoxazol-5-yl)methanamine (32). An amount of 2.03 g (4.54mmol) of **30** was dissolved in 46 mL of 2:1:1 acetonitrile/THF/ethanol. Ethylenediamine was added in $^1/_{10}$ equivalent aliquots hourly until 2 equiv had been added. The mixture was stirred overnight, diluted with 250 mL of EtOAc, and washed with brine, 3 × 25 mL. The organic phase was dried with sodium sulfate and was evaporated under reduced pressure, and the resultant oil was purified by flash chromatography in 0−60% acetone in methylene chloride (335 mg, 1.87 mmol, 41%). ^{1}H NMR (500 MHz, $CDCl_3$): δ 2.855−2.894 (dd, 1H, J = 6.0, 13.5), 3.013−3.048 (dd, 1H, J = 4.0, 13.5), 3.079−3.130 (dd, 1H, J = 7.0, 17.5), 3.244−3.300 (dd, 1H, J = 10.5, 17.0), 4.73−4.79 (m, 1H). ^{13}C NMR (125 MHz, $CDCl_3$): δ 137.65, 83.22, 45.12, 43.99. The (*R*)-enantiomer of this compound (**32b**) was spectroscopically indistinct by NMR.

TG2 Inhibition Assay. Recombinant human TG2 was expressed in *E. coli* and purified to >90% homogeneity as before.[17] Compounds were assayed in a reaction mixture containing 200 mM MOPS (pH 7.2), 5 mM $CaCl_2$, 1 mM EDTA, 10 mM α-ketoglutarate, 18 U/mL glutamate dehydrogenase, 0.4 mM NADH, 3.3% (v/v) DMSO, 0.5 μM TG2, 11.2 mM Cbz-Gln-Gly (K_M = 6.28 mM, $k_{cat.}$ = 37 min^{-1}). Concentrations of inhibitors were varied from 0.001 to 0.3 mM. The enzyme reaction was started by addition of TG2, and the consumption of NADH was monitored by UV spectroscopy (340 nm, ϵ = 6220 cm^{-1} M^{-1}). Kinetic parameters were obtained by plotting the reaction progress curves against theoretical equations for irreversible enzyme inhibition.[22]

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