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Isothiazolidinone heterocycles as inhibitors of protein tyrosine phosphatases: Synthesis and structure–activity relationships of a peptide scaffold

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Abstract—The structure-based design and discovery of the isothiazolidinone (IZD) heterocycle as a mimic of phosphotyrosine (pTyr) has led to the identification of novel IZD-containing inhibitors of protein tyrosine phosphatase 1B (PTP1B). The structure–activity relationships (SARs) of peptidic IZD-containing inhibitors of PTP1B are described along with a novel synthesis of the aryl-IZD fragments via a Suzuki coupling. The SAR revealed the saturated IZD heterocycle (**42**) is the most potent heterocyclic pTyr mimetic compared to the unsaturated IZD (**25**), the thiadiazolidinone (TDZ) (**38**), and the regioisomeric unsaturated IZD (**31**). The X-ray crystal structures of **11c** and **25** complexed with PTP1B were solved and revealed nearly identical binding interactions in the active site. Ab initio calculations effectively explain the strong binding of the (*S*)-IZD due to the preorganized binding of the IZD in its low energy conformation.

1. Introduction

The prevalence and rising incidence of diabetes[1,2] have provided researchers with the impetus to find new and improved treatments for this disease. Many existing therapies aim to increase the level of or sensitivity to the hormone insulin in recognition of its central role in regulating the production and metabolism of glucose.[3–5] Further research in these areas has led to the validation of several proteins that have the potential for being new drug targets.[6–9] One target, protein tyrosine phosphatase 1B (PTP1B),[10–12] has attracted considerable attention in recent years because of genetic and biochemical evidence demonstrating that PTP1B is a negative regulator of the insulin signaling pathway. A primary role of PTP1B is to dephosphorylate the insulin receptor (IR) and IR substrates thus abrogating insulin signaling. Inhibiting the activity of PTP1B should therefore potentiate the action of the insulin receptor and lead to improved responsiveness to the hormone. Compelling evidence for this rationale was independently provided by two laboratories that showed PTP1B knock-out mice had significantly lower glucose and insulin levels, improved insulin sensitivity, and resistance to high-fat diet induced weight gain.[13,14] In addition, the knockout mice showed increased phosphorylation of IR and enhanced glucose uptake in the muscle that confirmed an inhibitory role of PTP1B on insulin receptor signaling.

The research efforts in academia and industry over the past decade have led to the discovery of several novel classes of small molecule PTP1B inhibitors.[15–21] The design of inhibitors of PTP1B has focused on binding to the active site and discovery of mimetics of phosphotyrosine (pTyr). Phosphonates, carboxylic acids, and difluoromethylphosphonates (DFMPs) have been effectively used as replacements of pTyr. These highly

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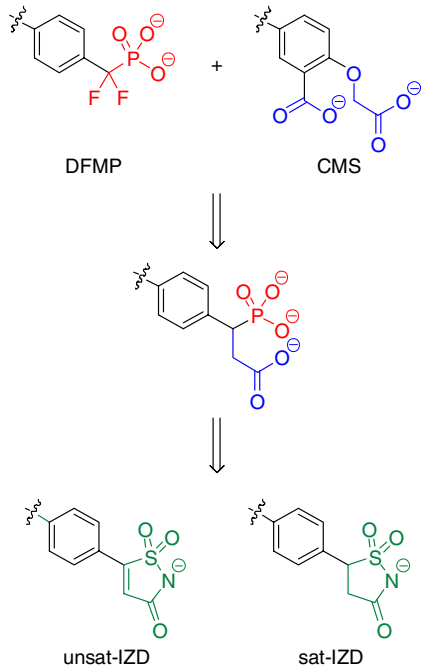


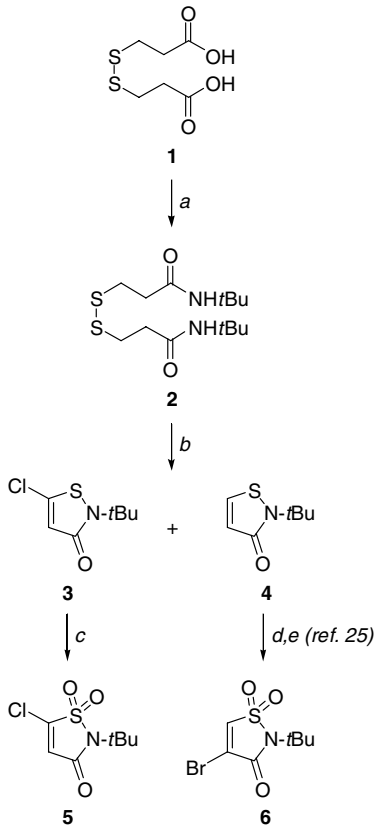
Figure 1. The graphical overlay of the DFMP and CMS crystal structures led to the design and discovery of the five-membered IZD heterocycles.

charged groups, however, have proven difficult to develop into effective pharmaceuticals due to their low cell permeability and oral bioavailability. We recently reported[22] the use of de novo structure-based design to derive a heterocycle, isothiazolidinone (IZD), that mimics pTyr and binds competitively to the active site of PTP1B. The structure-based design of the IZD was made possible by the existing plethora of data available in the PDB for PTP1B inhibitors containing phosphonates, carboxylic acids, and DFMPs. As previously described, our efforts began with a thorough analysis of the known PTP1B inhibitors which led to the realization, by overlaying the DFMP and the carboxymethyl salicylic acid (CMS) moieties, that a five-membered ring heterocycle could mimic both binding modes (Fig. 1). The IZDs were synthesized and exhibited strong binding affinity by displacement of all three conserved water molecules deep in the catalytic site along with the entropic gains derived from a rigid heterocycle compared with the acyclic DFMP and CMS. The newly discovered IZD heterocycle was also able to capitalize on eight important hydrogen bonds from the strategically placed carbonyl and sulfonamide groups. The IZD heterocycle showed a marked improvement (~10-fold) in potency over the DFMP, providing strong evidence that the IZD is the most potent pTyr mimetic known to date. In this paper, we present structure activity relationships (SARs) discovered with respect to the IZD heterocycle and peptide scaffold.

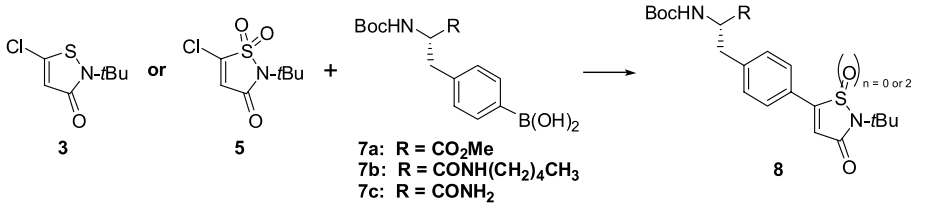
2. Chemistry

Synthesis of the designed aryl-IZD ring fragments was achieved by a novel Suzuki reaction between the IZD heterocycles and an aryl boronic acid. The synthesis of the appropriate halo-IZD Suzuki partners **3** and **5** followed reports in the literature[23,24] (Scheme 1). The commercially available di-acid **1** was converted to the bisamide **2** via the di-acid chloride. The *tert*-butyl group was chosen as the nitrogen protecting group due to its stability to a variety of reaction conditions. The bisamide **2** was treated with sulfuryl chloride which converted **2** to both the chloro-IZD **3** and the non-chlorinated IZD **4** with variable yields (37–70%). The oxidation of **3** to **5** gave the best result when *m*-CPBA was used, but the reaction was very slow. The rate-limiting step appeared to be the second oxidation (sulfinamide to sulfonamide) as the initial oxidation of **3** to the sulfinamide was quite fast. The conversion of **4** to the bromo-IZD **6** proceeded according to a published report.[25]

Initial attempts at the Suzuki coupling began with the unoxidized IZD **3** and the boronic acid of BocPhe methyl ester.[26] The Suzuki reaction required substantial optimization and selected examples are shown in Chart 1. Although the Suzuki reaction worked with other palladium catalysts to couple **3** and simpler boronic acids (data not shown), the $PdCl_2(dppf)CH_2Cl_2$ catalyst provided the highest yields with 4-boronoBocPhe (**7**). The catalysts used in entries A and B did not give coupled



Scheme 1. Synthesis of IZD heterocycles **3**, **5**, and **6**. Reagents: (a) i—$(COCl)_2$, CH_2Cl_2, DMF, 1 h; ii—*t*-$BuNH_2$, $ClCH_2CH_2Cl$, 0 → 25 °C, 1 h, 94%; (b) SO_2Cl_2, $ClCH_2CH_2Cl$, 0 → 25 °C, 1 h, 37–70% (**3**), 38–69% (**4**); (c) *m*-CPBA, CH_2Cl_2, 0 → 25 °C, 3 d, 40%; (d) *m*-CPBA, CH_2Cl_2, 15 h, 97%; (e) i—Br_2, CCl_4, Δ, 20 h; ii—pyr, $CHCl_3$, 96%.



3 5

7a: R = CO_2Me
7b: R = $CONH(CH_2)_4CH_3$
7c: R = $CONH_2$

8

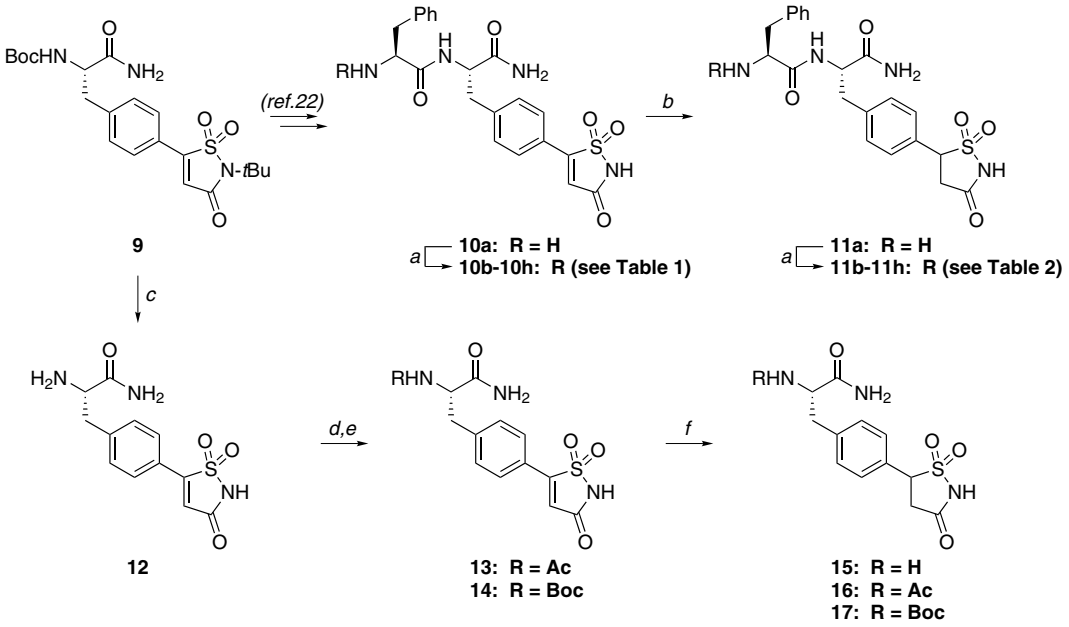
Entry	Heterocycle	Boronic acid	Catalyst	Base	Solvent	Temp.	Time	Yield
A	**3** (1.0 eq.)	**7a** (1.0 eq.)	$PdCl_2(PCy_3)_2$ (0.05 eq.)	CsF (2 eq.)	NMP	100 °C	16 h	0%
B	**3** (1.0 eq.)	**7a** (1.1 eq.)	$Pd(Ph_3P)_4$ (0.05 eq.)	Et_3N (5 eq.)	EtOH	180 °C in μW	10 min	0%
C	**3** (1.0 eq.)	**7a** (1.0 eq.)	$PdCl_2(dppf)\cdot CH_2Cl_2$ (0.07 eq.)	K_2CO_3 (5 eq.)	DMF	180 °C in μW	10 min	35%
D	**3** (1.1 eq.)	**7a** (1.0 eq.)	$PdCl_2(dppf)\cdot CH_2Cl_2$ (0.07 eq.)	K_2CO_3 (5 eq.)	DMF	80 °C	60 h	21%
E	**3** (1.1 eq.)	**7a** (1.0 eq.)	$PdCl_2(dppf)\cdot CH_2Cl_2$ (0.1 eq.)	K_2CO_3 (5 eq.)	DME	80 °C	22 h	65%
F	**5** (1.0 eq.)	**7a** (1.1 eq.)	$PdCl_2(dppf)\cdot CH_2Cl_2$ (0.15 eq.)	K_2CO_3 (5 eq.)	DME	80 °C	16 h	78%
G	**5** (1.1 eq.)	**7b** (1.0 eq.)	$PdCl_2(dppf)\cdot CH_2Cl_2$ (0.1 eq.)	K_2CO_3 (5 eq.)	DME	80 °C	24 h	60%
H	**5** (1.1 eq.)	**7c** (1.0 eq.)	$PdCl_2(dppf)\cdot CH_2Cl_2$ (0.1 eq.)	K_2CO_3 (5 eq.)	DME	80 °C	16 h	65%
I	**5** (1.2 eq.)	**7c** (1.0 eq.)	$PdCl_2(dppf)\cdot CH_2Cl_2$ (0.17 eq.)	K_2CO_3 (5 eq.)	1,4-dioxane	80 °C	16 h	78%

Chart 1. Suzuki reactions of five-membered ring heterocycles (**3, 5**) and boronic acids (**7**).

product. With the $PdCl_2(dppf)CH_2Cl_2$ catalyst, the yield was slightly higher when performed with microwave heating (entry C) compared with heating on the benchtop (entry D). A breakthrough occurred when the solvent was changed to DME which provided a 3-fold improvement in the yield (entry E vs entry D). Use of the oxidized IZD **5** also led to a noticeable increase in the yield (entry F vs entry E). The sensitivity of this reaction is illustrated with entries G and H where the methyl ester of the boronic acid was replaced with a pentyl amide and primary amide, respectively, which gave lower yields. Another beneficial solvent effect was discovered with 1,4-dioxane (entry I) which provided a small increase in yield compared with DME (entry H). Ultimately, oxidized IZD **5** was chosen as the preferred heterocycle coupling partner not only because of the higher yields observed, but it also alleviated the need to oxidize the IZD for compounds derived from **3**.

With the Suzuki reaction optimized, the focus turned toward the synthesis of peptides containing the saturated and unsaturated IZDs (Scheme 2). Starting from primary amine **10a**, derived from **9** via standard amide manipulations,[22] the unsaturated IZD analogs **10b**–**10h** were readily prepared using the appropriate carboxylic acids. Reduction of the unsaturated IZD **10a** afforded the saturated IZD **11a** as a mixture of diastereoisomers. Compound **11a** was converted into the saturated IZD analogs **11b**–**11h** with the same carboxylic acids used to prepare **10b**–**10h**. N-Terminally truncated analogs **15**–**17** were also prepared from **9**. Removal of both the Boc and *tert*-butyl groups of **9** with TFA and microwave heating gave the primary amine **12**. Acylation of **12** with acetic anhydride and Boc anhydride gave **13** and **14**, respectively. Compounds **12**, **13**, and **14** were hydrogenated to give the saturated analogs **15**, **16**, and **17**, respectively, as mixtures of diastereoisomers.

Synthesis of the pentyl amide analogs **25**, **31**, and **38** is shown in Scheme 3. Carboxylic acid **18**[27] was converted to the key intermediate pentyl amide-boronic acid **19**. Suzuki coupling of **19** with **5** gave primary amine **21** after Boc deprotection. Coupling of **21** with Boc-Phe afforded **22**. Removal of the Boc group followed by coupling with *p*-methoxyphenylacetic acid gave **24**. Finally, cleavage of the *tert*-butyl group of the heterocycle provided the desired product **25**. The regioisomeric IZD analog **31** was synthesized in the same fashion using



Scheme 2. Synthesis of unsaturated IZD analogs **10**, saturated IZD analogs **11**, and N-terminal truncated analogs **15**–**17**. Reagents: (a) $R'CO_2H$, HOAt, EDAC, *i*-Pr_2NEt, DMF, CH_2Cl_2, 16 h, 10–81% (**10a** → **10c**–**10h**), 12–52% (**11a** → **11c**–**11h**); (b) 10% Pd/C, H_2, 16 h, 84%; (c) TFA, 140 °C in microwave, 2 min, 63% (**12**); (d) **12** → **13**: AcOH, HOAt, EDAC, *i*-Pr_2NEt, DMF, CH_2Cl_2, 16 h, 84%; (e) **12** → **14**: Boc_2O, pyr, 62%; (f) 10% Pd/C, H_2, 16 h, 82% (**12** → **15**), 17% (**13** → **16**), 53% (**14** → **17**).

heterocycle **6** as the Suzuki coupling partner with boronic acid **19**. The N-linked thiadiazolidinone (TDZ) analog **38** was synthesized via a copper-mediated N-arylation of **32**[28] with boronic acid **19** to yield **33** which was further elaborated to the desired TDZ analog **38**.

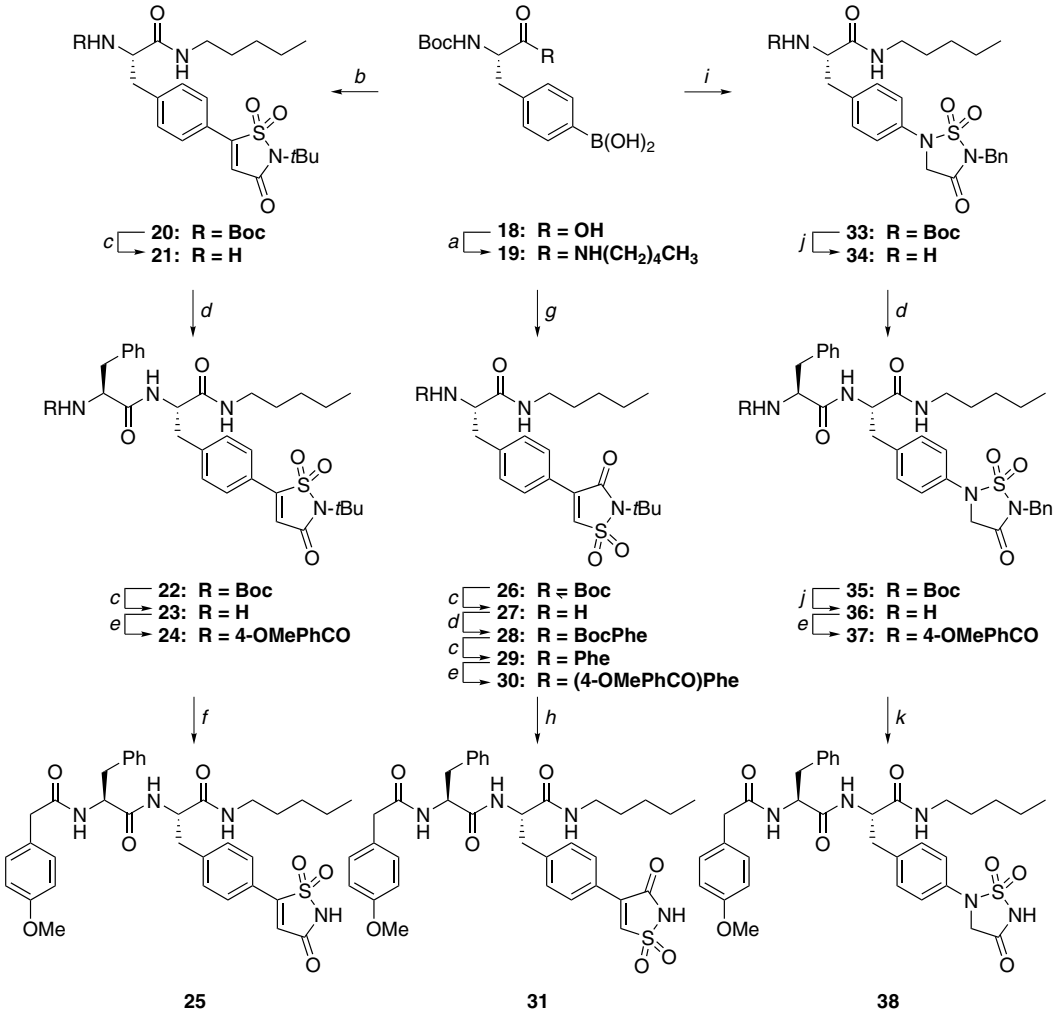
3. Results and discussion

The design and discovery of the IZD heterocycle as an effective mimic of pTyr provided a solid foundation for the development of novel inhibitors of PTP1B. The IZD heterocycle was synthesized on a peptide scaffold to facilitate comparisons with the other known pTyr mimetics. DFMP-containing peptides have been reported as extremely potent inhibitors of PTP1B.[29,30] These compounds incorporated the DFMP moiety on a Phe residue binding in the active site of PTP1B and were the most potent pTyr mimetics known prior to the discovery of the (*S*)-IZD.

Initially, unsaturated IZD analogs were synthesized and evaluated as inhibitors of PTP1B. The compound without any amide capping group at the N-terminus, **10a**, is not effective in inhibiting PTP1B (Table 1). A large improvement in the binding affinity is observed with the acetamide **10b** compared to its precursor **10a**. The benzamide capped analog, **10c**, is ~2-fold more active than **10b**. N-Terminal acylation is clearly beneficial presumably due to this carbonyl hydrogen bonding with the protein and attenuating the basicity of the terminal amino group. Additional amide analogs were synthesized to probe the best position of the phenyl group. The one carbon-linked analog **10d** shows a slight boost in binding affinity compared with **10c**, however, the two carbon-linked analog (**10h**) is less active. Analogs bearing substituents at the 4-position of the terminal phenyl ring of **10d** did not improve the binding potency over the parent compound (**10e**, **10f**, and **10g**).

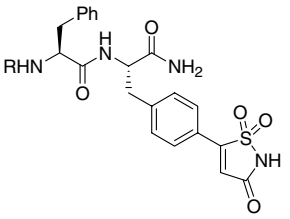
A breakthrough in PTP1B binding affinity came with the synthesis of the saturated IZD. In general, the saturated IZDs, as mixtures of diastereoisomers, exhibited a 5- to 12-fold increase in binding affinity compared to the unsaturated compounds (Table 2). As with the unsaturated IZD analog **10a**, the saturated IZD primary amine **11a** is inactive. The acetamide (**11b**) is again less potent compared to the benzamide (**11c**) and the phenylacetamide (**11d**). Similarly, the phenethyl capped analog (**11h**) is also a less potent inhibitor of PTP1B compared with **11c**. The trend continues with the *p*-hydroxy (**11e**), *p*-methoxy (**11f**), and *p*-trifluoromethyl (**11g**) capped phenylacetamides which are equipotent with the parent compound (**11d**). The selectivity of the saturated IZDs was evaluated in other phosphatases and, as expected, they were non-selective over TCPTP whose active site is quite homologous to PTP1B. However, there was very good selectivity (>300-fold) over other tyrosine phosphatases such as SHP1 and SHP2.

The saturated IZDs were clearly better PTP1B inhibitors than the unsaturated IZDs though it was unclear how other related five-membered ring heterocycles compared with the IZDs. An SAR analysis of other five-membered ring heterocycles was initiated on a pentyl amide scaffold that has been used with the literature CMS analogs.[31] Comparison of the regioisomeric IZD analog **31** with the designed IZD analog **25** shows a decrease in binding affinity consistent with predictions that the carbonyl



Scheme 3. Synthesis of pentylamide analogs **25**, **31**, and **38**. Reagents: (a) $CH_3(CH_2)_4NH_2$, BOP, Et_3N, DMF, 2 h, 56%; (b) **5**, $PdCl_2(dppf)\cdot CH_2Cl_2$, K_2CO_3, DME, 80 °C, 24 h, 60%; (c) HCl in 1,4-dioxane, 16 h, quantitative; (d) Boc-Phe-OH, HOAt, EDAC, *i*-Pr_2NEt, DMF, CH_2Cl_2, 16 h, 85% (**22**), 64% (**28**), 78% (**35**); (e) 4-OMe–$PhCH_2CO_2H$, HOAt, EDAC, *i*-Pr_2NEt, DMF, CH_2Cl_2, 16 h, 96% (**24**), 92% (**30**), 61% (**37**); (f) TFA, *i*-Pr_3SiH, 70 °C, 16 h, 38%; (g) **6**, $PdCl_2(dppf)\cdot CH_2Cl_2$, K_2CO_3, DME, 80 °C, 24 h, 27%; (h) TFA, 150 °C in microwave, 60 s, 38%; (i) 2-benzyl-1,2,5-thiadiazolidin-3-one 1,1-dioxide (**32**), $Cu(OAc)_2$, Et_3N, CH_2Cl_2, 24 h, 42%; (j) TFA, CH_2Cl_2, 2 h, 98% (**34**), 97% (**36**); (k) 10% Pd/C, ammonium formate, 60 °C, 1.5 h, 65%.

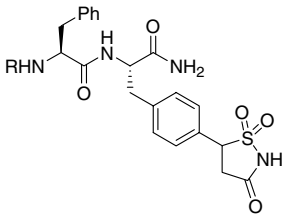
Table 1. Unsaturated IZD SAR



Compound	R	PTP1B[a] IC_{50} (nM)
10a	H	>100,000
10b	CH_3CO	3000
10c	PhCO	1800
10d	$PhCH_2CO$	1000
10e	4-OH–$PhCH_2CO$	790
10f	4-OMe–$PhCH_2CO$	1600
10g	4-CF_3–$PhCH_2CO$	1400
10h	$PhCH_2CH_2CO$	2700

[a] pNPP enzyme assay.

Table 2. Saturated IZD SAR

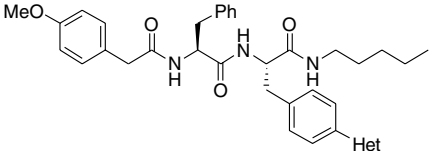


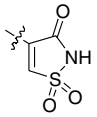
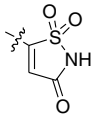
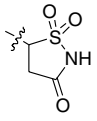
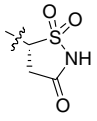
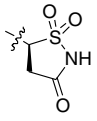
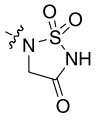
Compound[a]	R	PTP1B[b] IC_{50} (nM)
11a	H	>100,000
11b	CH_3CO	660
11c	PhCO	150
11d	$PhCH_2CO$	180
11e	4-OH–$PhCH_2CO$	170
11f	4-OMe–$PhCH_2CO$	170
11g	4-CF_3–$PhCH_2CO$	170
11h	$PhCH_2CH_2CO$	340

[a] Compounds tested as mixture of diastereoisomers.
[b] pNPP enzyme assay.

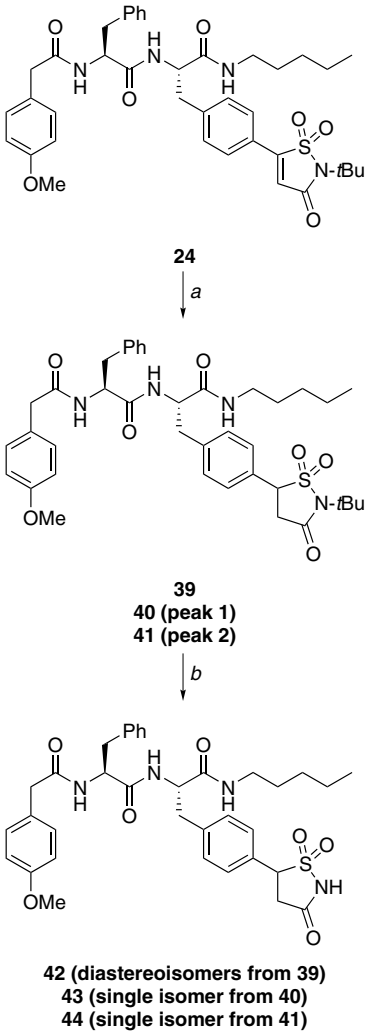
groups of the IZD in **31** could not bind optimally in the active site (Table 3). In agreement with our previous report, the saturated IZD **42**, as a mixture of diastereoisomers, is ~15-fold more potent than the unsaturated IZD **25**. In an attempt to understand the importance of the stereogenic center in the saturated IZD heterocycle, the diastereoisomers of **42** were prepared as illustrated in Scheme 4. Hydrogenation of **24** afforded **39** as a mixture of diastereoisomers which was separated on a chiral HPLC column to give the discrete isomers **40** (first peak to elute) and **41** (second peak to elute). Compounds **40** and **41** were deprotected to give the individual diastereoisomers **43** and **44**. The PTP1B enzyme inhibition data demonstrated that the isomer corresponding to the first peak to elute from the chiral HPLC column (**43**) is 375-fold less active when compared with the isomer from the second peak (**44**). The more active isomer **44** is about 2-fold more potent compared with the diastereoisomeric mixture **42**. Modeling and X-ray crystallography studies (vide infra: X-ray structure of **11c**) indicate the (*S*)-isomer of the saturated IZD (**44**) is the more active isomer consistent with our previous report.[22] The TDZ heterocycle (**38**) is ~37-fold less active compared to the saturated IZD (**44**), and about equipotent compared with the unsaturated IZD (**25**).

Table 3. Five-membered heterocycle SAR



Compound		Het	PTP1B[b] IC_{50} (nM)
31	Regioisomeric IZD		6000
25	Unsaturated IZD		1200
42[a]	Saturated IZD		80
43	Saturated (*R*)-IZD		15,000
44	Saturated (*S*)-IZD		40
38	TDZ		1500

[a] Compound tested as mixture of diastereoisomers.
[b] pNPP enzyme assay.



Scheme 4. Synthesis of pentyl amide analog **42** and its diastereoisomers **43** and **44**. Reagents: (a) 10% Pd/C, H_2, 16 h, 37%; (b) TFA, *i*-Pr_3SiH, 70 °C, 16 h, 39% (**39** → **42**), 12% (**40** → **43**), 21% (**41** → **44**).

The X-ray crystal structure of **11c** complexed with PTP1B (Fig. 2A) was solved at a resolution of 2.3 Å and even though **11c** contained a mixture of diastereoisomers, only the (*S*)-isomer of the saturated IZD crystallized with PTP1B. The X-ray structure of **11c** complexed with PTP1B revealed several important binding interactions similar to the X-ray structure of **11b** complexed with PTP1B[22] which reinforced our earlier observations of the orientation of the saturated IZD in the active site (Figs. 2A and 3). The two oxygens of the sulfone that mimic the phosphonate oxygens of the DFMP displace the two water molecules normally found at these locations (the two water molecules are seen in the CMS structure[22]). The carbonyl of the IZD mimics the CMS inhibitor carboxylic acid and displaces the third conserved water molecule in the

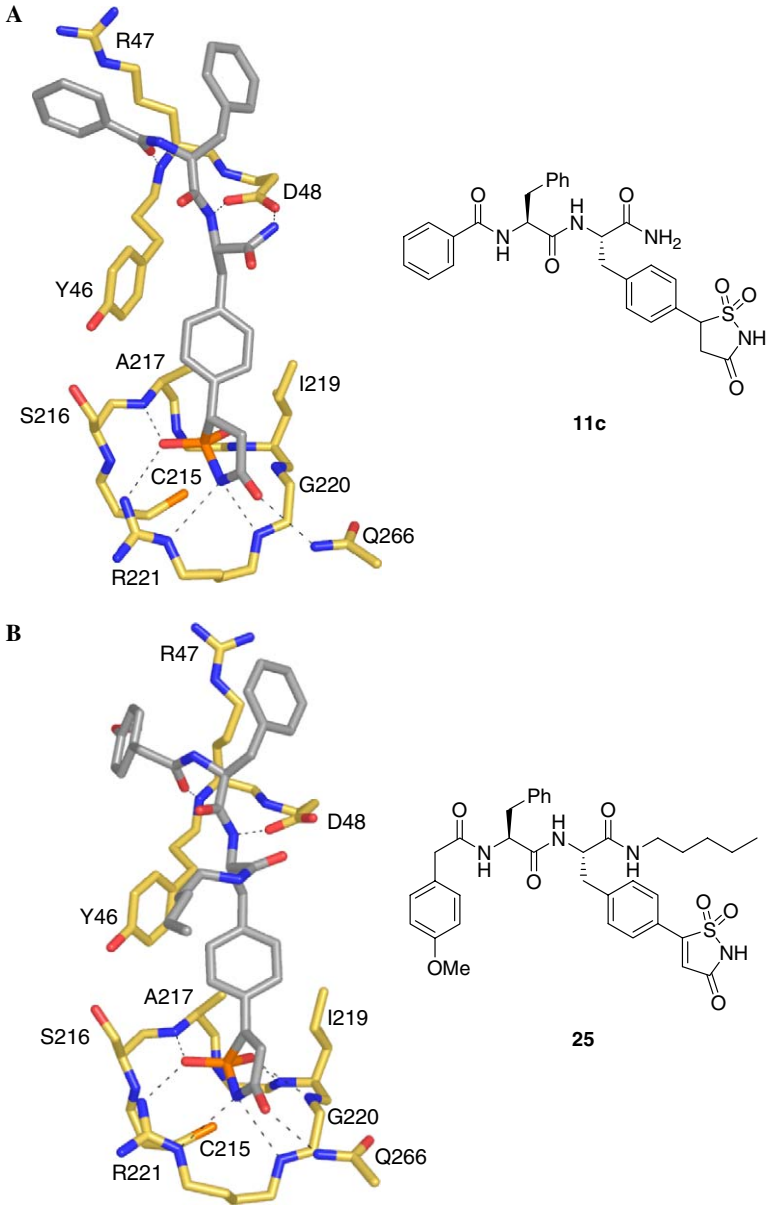


Figure 2. The X-ray crystal structures of **11c** bound to PTP1B (**A**) and of **25** bound to PTP1B (**B**). The saturated IZD of **11c** and the unsaturated IZD of **25** form the same hydrogen bonds in the active site. The phenyl ring attached to the IZD interacts with Tyr46 and Phe182 (not shown). The primary amide of **11c** forms a bidentate hydrogen bonding interaction with Asp48 of PTP1B. The pentyl amide of **25** is rotated 180° compared to the primary amide of **11c** which disrupts the bidentate interaction with Asp48. The carbonyl of the N-terminal amide cap forms a hydrogen bond with the amine of Tyr46. The inhibitors are shown in gray and the enzyme is shown in gold.

active site (the water molecule is seen in the DFMP structure[22]). The peptide backbone of **11c** extends along the surface of PTP1B making specific interactions with the enzyme. The primary amide and α-amino functionalities of **11c** form a bidentate interaction with Asp48, while the carbonyl of the benzamide cap hydrogen bonds with Tyr46. The X-ray crystal structure of **25** complexed with PTP1B was solved at a resolution of 2.0 Å and demonstrated that the unsaturated IZD of **25** binds very similarly to the saturated IZD of **11c** (Figs. 2B and 3). All three conserved water molecules are displaced and the IZD of **25** forms hydrogen bonds with the protein in the same fashion as with **11c**. As expected, the saturated IZD of **11c** does not align perfectly with the unsaturated IZD of **25** due to the stereogenic center in **11c**. One interesting distinction observed is the bidentate interaction of **11c** with Asp48 is absent with pentyl amide **25**, though Asp48 still forms a hydrogen bond with the nitrogen in **25** alpha to the pentyl amide. It appears the region of space occupied by the pentyl amide of **25** has room to accommodate groups other than amides extending further into that region of PTP1B.

The X-ray crystallographic results suggest that the weaker binding of the unsaturated IZD is not likely due to hydrogen bond misalignment. We therefore turned to ab initio calculations on inhibitor fragments

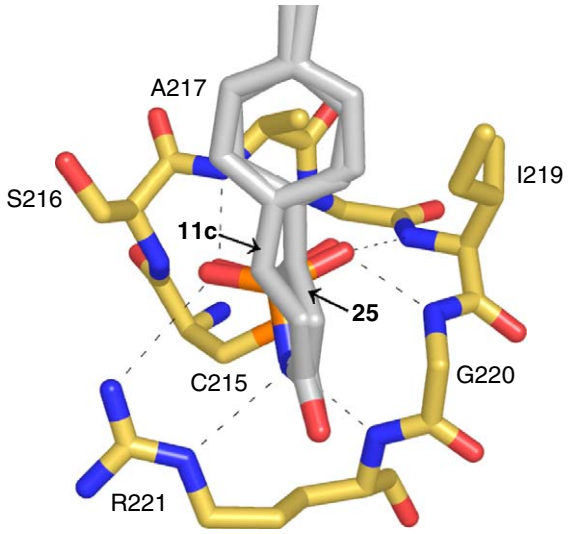


Figure 3. The X-ray crystal structures of the saturated IZD of **11c** and the unsaturated IZD of **25** bound to PTP1B. Both heterocycles form identical hydrogen bonds to PTP1B. One of the oxygens attached to sulfur hydrogen bonds to Gly218 and Gly220. The other oxygen hydrogen bonds to Ala217 and the sidechain of Arg221. The nitrogen in the heterocycles forms hydrogen bonds to the sidechain of Arg221 and to the α-amine of Arg221. The IZD carbonyl oxygen forms hydrogen bonds with Phe182 and Gln266 (not shown).

aryl-(*S*)-IZD and aryl-unsat-IZD to elucidate the affinity differences by calculating energy profiles for rotation of the bond connecting the IZD and phenyl rings (Fig. 4). Superposition of the minimum energy saturated fragment on the analogous crystallographic moiety shows that the saturated (*S*)-IZD binds in a conformation close to the minimum. This preorganization is thought to be the reason for the optimal binding of the (*S*)-IZD (Figs. 4A and 5B).

In the case of the unsaturated IZD, the minimum energy conformation is farther from the crystallographic geometry so that the five- and six-membered rings cannot be simultaneously overlayed (Fig. 5C). However, after bond rotation of about 40° the structural agreement is excellent (Fig. 5D), suggesting that the energetic cost of ring rotation (Fig. 4B) is responsible for the 5- to 20-fold weaker binding of the unsaturated IZD compounds. It is current dogma that inhibitors bind in their minimum energy conformation, or at least at a local minimum of the potential energy surface. The binding of unsaturated-IZDs is a clear demonstration that this need not always be the case. In the case of the aryl-(*R*)-IZD, no such rotation can effect a similar overlay, thus the (*R*)-IZD is inactive.

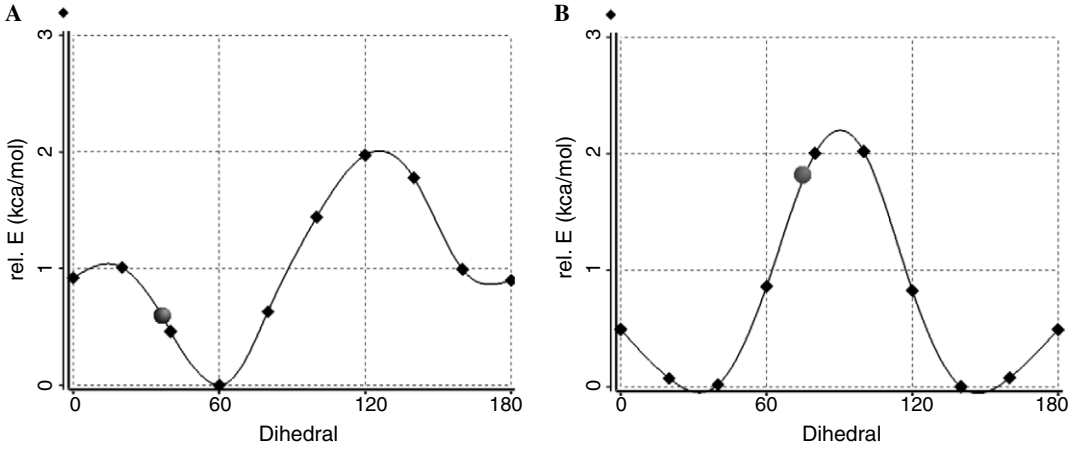


Figure 4. Calculated energy profiles for rotation of the bond connecting the IZD and phenyl rings. (A) Saturated IZD. (B) Unsaturated IZD. The point at which calculated and crystal structure geometries are most similar is denoted by a sphere (●).

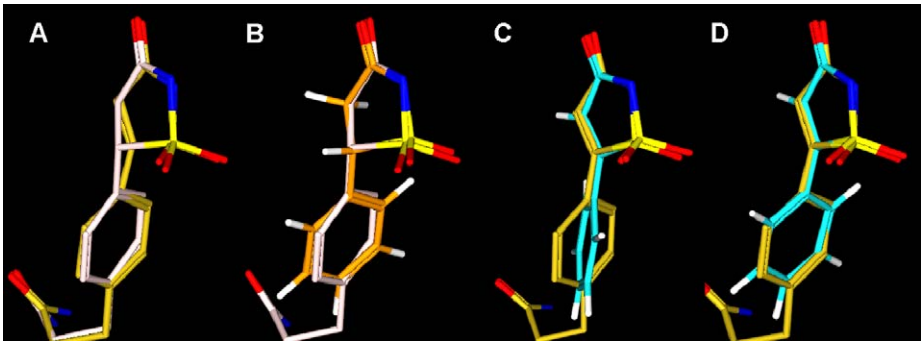


Figure 5. Superposition of X-ray crystallographic and calculated geometries. (A) X-ray crystallographic aryl-(*S*)-IZD (white) and aryl-unsat-IZD (gold), overlaid by superimposing C-alphas of the protein active sites of the two crystal structures (protein not shown for clarity). (B) X-ray crystallographic (white) and calculated minimum energy (orange) of aryl-(*S*)-IZD. (C) X-ray crystallographic (gold) and calculated minimum energy (cyan) of aryl-unsat-IZD. (D) X-ray crystallographic (gold) and calculated minimum energy (cyan) of aryl-unsat-IZD after rotation of about 40°.

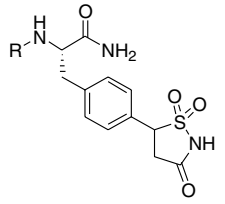
For both saturated and unsaturated IZD heterocycles, energy profiles and minima for neutral and anionic fragments are very similar (data not shown). However, this is not the case for N-linked TDZs. The neutral fragment's minimum shows the rings are almost perpendicular, as in all the crystal structures we have determined. But for the TDZ anion, at the minimum the rings are almost coplanar and the rotational barrier is higher than for IZDs. We conjecture that within the positively charged active site, the barrier of rotation for the anion is lowered and the profile becomes more similar to that of a neutral compound. If the TDZ indeed prefers to be coplanar with the phenyl ring, then this can potentially explain the lower potency of the TDZ compared with the saturated IZD which prefers to be perpendicular to the phenyl ring as seen from the X-ray and computational structures.

Black et al.[32] have also reported a coplanar calculated conformation for TDZ anions. They pointed out a shallow dip in the energy curve at about 110°, and suggest the fragment binds at this high energy local minimum. They attribute enhanced activity for ortho-substituted compounds to enforced orthogonality of the rings. Our data indicate TDZ compounds without ortho-substituents are good inhibitors, albeit not as potent as the saturated IZDs (**38** vs **42**, Table 3). Furthermore, we demonstrate in a separate paper that ortho-substitution effects a small improvement in binding even for the pre-organized saturated IZD.[33]

Several examples of saturated IZD analogs with truncated N-termini were synthesized and evaluated to determine the SAR of the peptide scaffold (Table 4). Compared with the compounds from Table 2, a large decrease in binding affinity is evident when the N-terminal Phe is removed. Compounds **15**, **16**, and **17** are >450-fold less active compared with **11c**.

The use of structure-based design facilitated our objective to discover novel mimics of pTyr as inhibitors of PTP1B. Detailed analysis of the X-ray data available for existing inhibitors in the PDB provided important insights toward the design of the IZD inhibitors. The SAR demonstrated that the saturated IZD is the most potent pTyr mimetic known to date when compared to other related heterocycles such as the unsaturated IZD, the TDZ, and the regioisomeric unsaturated IZD. Furthermore, the (*S*)-isomer of the saturated IZD is the preferred isomer with a binding affinity that is much greater than the (*R*)-isomer. The X-ray crystallography and modeling studies assisted in the understanding of the binding mode of the IZD heterocycle and in ways to further optimize the current compounds. The peptide SAR provided an important foundation for the development of non-peptidic compounds with enhanced pharmaceutical and biological activity.[33]

Table 4. N-terminal truncated peptide scaffold SAR



Compound[a]	R	PTP1B[b] IC_{50} (nM)
15	H	>100,000
16	Ac	100,000
17	Boc	70,000

[a] Compounds tested as mixture of diastereoisomers.
[b] pNPP enzyme assay.

4. Experimental

All reactions were run under an atmosphere of dry nitrogen. Unless otherwise noted, all reactions were performed at ambient temperature which averaged 21 °C. All solvents were used without further purification as acquired from commercial sources. NMR spectra were obtained using either a Varian Mercury-300, Mercury-400, or Inova-500 spectrometer. Chemical shifts are reported in parts per million relative to tetramethylsilane (TMS) as internal standard. All final products were characterized by ^{1}H NMR, ^{13}C NMR, HRMS or LC–MS, and two HPLC methods.

Purifications by flash chromatography were performed on RediSep columns using an Isco CombiFlash SG100c. Preparative LC–MS purifications were performed on a Waters FractionLynx system using mass directed fractionation and compound-specific method optimization.[34] The LC method utilized a Waters SunFire column (19 × 100 mm, 5 μM particle size), with a water/0.1% TFA and acetonitrile/0.1% TFA gradient at a flow rate of 30 mL/min over a total run time of 5 min.

4.1. Ab initio calculations

Ab initio calculations were performed with program SPARTAN (Spartan'04, Wavefunction, Inc., Irvine, CA) using a 6-31G* basis set. The constrained bond angle extends from the aryl ortho-carbon to the IZD carbon. Overlays were performed with program MOE (Chemical Computing Group, Montreal, 2004).

4.2. PTP1B expression and biochemical assays

Human PTP1B, SHP1, SHP2, and TC PTP were expressed in *Escherichia coli* essentially as described in the literature.[35–38] PTP enzymatic assays were performed as described in the literature.[39]

4.3. X-ray crystallography

Details of the methods are described in a separate disclosure.

Compounds **10a** and **10b** were previously reported.[22]

4.4. Representative procedure for the preparation of 10c–10h from 10a and 11c–11h from 11a. *N*-[(1*S*)-2-({(1*S*)-2-Amino-1-[4-(1,1-dioxido-3-oxo-2,3-dihydroisothiazol-5-yl)benzyl]-2-oxoethyl}amino)-1-benzyl-2-oxoethyl]benzamide (10c)

A solution of **10a**[22] (80 mg, 0.14 mmol) and benzoic acid (23 mg, 0.19 mmol) in CH_2Cl_2 (1.6 mL) and DMF (0.5 mL) was treated with *N*,*N*-diisopropylethylamine (83 μL, 0.47 mmol) and 2.0 M 1-hydroxy-7-azabenzotriazole in DMF (14 μL, 29 μmol) followed by EDAC (69 mg, 0.36 mmol). The reaction mixture was stirred for 16 h, concentrated, and purified by preparative LC–MS (CH_3CN/H_2O/TFA) to yield **10c** (20 mg, 25%) as a white solid. ^{1}H NMR (500 MHz, DMSO-d_6, 30 °C): δ 8.51 (d, *J* = 8.3 Hz, 1H), 8.08 (d, *J* = 8.4 Hz, 1H), 7.73 (d, *J* = 7.3 Hz, 2 H), 7.70 (d, *J* = 8.2 Hz, 2H), 7.48 (dd, *J* = 7.3 Hz, 1H), 7.42 (m, 2H), 7.40 (m, 2H), 7.36 (s, 1H), 7.27 (d, *J* = 7.3 Hz, 2H), 7.21 (dd, *J* = 7.4, 7.4 Hz, 2H), 7.15 (s, 1H), 7.13 (dd, *J* = 7.5, 7.5 Hz, 1H), 7.13 (s, 1H), 4.66 (m, 1H), 4.52 (m, 1 H), 3.11 (dd, *J* = 13.7, 4.9 Hz, 1H), 3.04 (dd, *J* = 14.2, 4.5 Hz, 1H), 2.95 (dd, *J* = 14.0, 7.2 Hz, 1H), 2.93 (dd, *J* = 13.8, 5.3 Hz, 1H); ^{13}C NMR (125 MHz, DMSO-d_6, 30 °C): δ 172.0, 170.9, 166.3, 162.9, 152.2, 142.5, 138.3, 133.8, 131.1, 130.3, 129.0, 128.0, 127.8, 127.5, 127.3, 126.1, 122.8, 119.7, 54.7, 53.1, 37.3, 36.6; HRMS calcd for $C_{28}H_{26}N_4O_6SNa$ $(M+Na)^+$: *m*/*z* = 569.1491.

Compounds **10d**–**10h** were prepared according to the procedure of **10c** using **10a** and the appropriate acids with yields ranging from 10% to 81%.

4.5. (2*S*)-*N*-{(1*S*)-2-Amino-1-[4-(1,1-dioxido-3-oxo-2,3-dihydroisothiazol-5-yl)benzyl]-2-oxoethyl}-3-phenyl-2-[(phenylacetyl)amino]propanamide (10d)

^{1}H NMR (500 MHz, DMSO-d_6, 30 °C): δ 8.20 (d, *J* = 8.4 Hz, 1H), 8.05 (d, *J* = 8.2 Hz, 1H), 7.76 (d, *J* = 8.3 Hz, 2H), 7.39 (d, *J* = 8.4 Hz, 2H), 7.31 (s, 1H), 7.27 (s, 1H), 7.18 (m, 2H), 7.17 (m, 6H), 7.15 (m, 1H), 7.12 (s, 1H), 7.02 (d, *J* = 7.5 Hz, 2 H), 4.49 (m, 2H), 3.39 (d, *J* = 13.9 Hz, 1H), 3.30 (d, *J* = 13.9 Hz, 1H), 3.08 (dd, *J* = 13.8, 5.1 Hz, 1H), 2.96 (dd, *J* = 13.9, 4.5 Hz, 1H), 2.90 (dd, *J* = 13.8, 8.6 Hz, 1H), 2.72 (dd, *J* = 13.8, 9.9 Hz, 1H); ^{13}C NMR (125 MHz, DMSO-d_6, 30 °C): δ 172.1, 170.8, 169.8, 162.8, 152.2, 142.5, 137.6, 136.0, 130.3, 129.0, 128.8, 127.9, 127.8, 127.6, 126.2, 126.0, 122.8, 119.8, 53.6, 53.0, 41.7, 37.1, 37.0; HRMS calcd for $C_{29}H_{28}N_4O_6SNa$ $(M+Na)^+$: *m*/*z* = 583.1613.

4.6. (2*S*)-*N*-{(1*S*)-2-Amino-1-[4-(1,1-dioxido-3-oxo-2,3-dihydroisothiazol-5-yl)benzyl]-2-oxoethyl}-2-{[(4-hydroxyphenyl)acetyl]amino}-3-phenylpropanamide (10e)

^{1}H NMR (500 MHz, DMSO-d_6, 30 °C): δ 9.10 (s, 1H), 8.06 (d, *J* = 8.4 Hz, 1H), 7.94 (d, *J* = 8.3 Hz, 1H), 7.69 (d, *J* = 8.3 Hz, 2H), 7.30 (s, 1H), 7.26 (d, *J* = 8.0 Hz, 2H), 7.19 (m, 2H), 7.14 (m, 4H), 7.07 (s, 1H), 6.82 (d, *J* = 8.2 Hz, 2H), 6.59 (s, 1H), 6.56 (d, *J* = 8.4 Hz, 2H), 4.46 (m, 1H), 4.44 (m, 1H), 3.27 (d, *J* = 14.6 Hz, 1H), 3.18 (d, *J* = 14.5 Hz, 1H), 3.02 (dd, *J* = 13.6, 5.2 Hz, 1H), 2.95 (dd, *J* = 13.7, 4.7 Hz, 1 H), 2.86 (dd, *J* = 13.4, 8.0 Hz, 1H), 2.72 (dd, *J* = 13.7, 9.6 Hz, 1H); ^{13}C NMR (125 MHz, DMSO-d_6, 30 °C): δ 172.2, 170.8, 170.5, 169.2, 155.6, 153.6, 139.6, 137.7, 129.8, 129.5, 129.0, 127.8, 127.3, 126.1, 126.0, 125.7, 123.2, 114.8, 53.8, 53.3, 41.1, 37.2, 36.9; HRMS calcd for $C_{29}H_{28}N_4O_7SNa$ $(M+Na)^+$: *m*/*z* = 599.1583.

4.7. (2*S*)-*N*-{(1*S*)-2-Amino-1-[4-(1,1-dioxido-3-oxo-2,3-dihydroisothiazol-5-yl)benzyl]-2-oxoethyl}-2-{[(4-methoxyphenyl)acetyl]amino}-3-phenylpropanamide (10f)

^{1}H NMR (500 MHz, DMSO-d_6, 30 °C): δ 8.10 (d, *J* = 8.6 Hz, 1H), 8.03 (d, *J* = 8.3 Hz, 1H), 7.76 (d, *J* = 8.3 Hz, 2H), 7.39 (d, *J* = 8.3 Hz, 2H), 7.31 (s, 1H), 7.26 (s, 1H), 7.19 (m, 2H), 7.15 (m, 3H), 7.11 (s, 1H), 6.95 (d, *J* = 8.9 Hz, 2H), 6.73 (d, *J* = 8.6 Hz, 2H), 4.49 (m, 1H), 4.46 (m, 1H), 3.68 (s, 3H), 3.31 (d, *J* = 13.5 Hz, 1H), 3.23 (d, *J* = 13.5 Hz, 1H), 3.07 (dd, *J* = 13.6, 5.0 Hz, 1H), 2.95 (dd, *J* = 13.8, 4.6 Hz, 1H), 2.90 (dd, *J* = 13.6, 8.6 Hz, 1H), 2.71 (dd, *J* = 13.8, 10.0 Hz, 1H); ^{13}C NMR (125 MHz, DMSO-d_6, 30 °C): δ 172.0, 170.9, 170.2, 162.9, 157.8, 152.2, 142.4, 137.6, 130.3, 129.8, 129.0, 128.0, 127.8, 127.6, 126.1, 122.8, 119.8, 113.4, 54.8, 53.7, 53.2, 41.1, 37.3, 37.1; HRMS calcd for $C_{30}H_{30}N_4O_7SNa$ $(M+Na)^+$: *m*/*z* = 613.1734.

4.8. (2*S*)-*N*-{(1*S*)-2-Amino-1-[4-(1,1-dioxido-3-oxo-2,3-dihydroisothiazol-5-yl)benzyl]-2-oxoethyl}-3-phenyl-2-({[4-(trifluoromethyl)phenyl]acetyl}amino)propanamide (10g)

^{1}H NMR (500 MHz, DMSO-d_6, 30 °C): δ 8.35 (d, *J* = 8.4 Hz, 1H), 8.07 (d, *J* = 8.3 Hz, 1H), 7.74 (d, *J* = 8.4 Hz, 2H), 7.55 (d, *J* = 8.4 Hz, 1H), 7.35 (s, 1H), 7.33 (d, *J* = 8.5 Hz, 2H), 7.26 (d, *J* = 8.1 Hz, 1H), 7.18 (m, 2H), 7.17 (m, 2H), 7.15 (m, 1H), 7.13 (m, 1H), 6.79 (s, 1H), 4.53 (m, 1H), 4.49 (m, 1H), 3.53 (d, *J* = 14.2 Hz, 1H), 3.44 (d, *J* = 14.2 Hz, 1H), 3.08 (dd, *J* = 13.7, 4.8 Hz, 1H), 3.00 (dd, *J* = 13.9, 4.4 Hz, 1H), 2.90 (dd, *J* = 13.8, 8.8 Hz, 1H), 2.73 (dd, *J* = 13.8, 10.3 Hz, 1H); ^{13}C NMR (125 MHz, DMSO-d_6, 30 °C): δ 172.1, 170.8, 169.1, 167.6, 153.2, 141.0, 140.4, 137.6, 129.9, 129.7, 129.6, 127.8, 127.3, 126.8, 126.1, 125.0, 124.7, 124.2, 122.3, 53.7, 53.3, 41.4, 37.2, 37.0; HRMS calcd for $C_{30}H_{27}F_3N_4O_6SNa$ $(M+Na)^+$: *m*/*z* = 651.1486.

4.9. (2*S*)-*N*-{(1*S*)-2-Amino-1-[4-(1,1-dioxido-3-oxo-2,3-dihydroisothiazol-5-yl)benzyl]-2-oxoethyl}-3-phenyl-2-[(3-phenylpropanoyl)amino]propanamide (10h)

^{1}H NMR (500 MHz, DMSO-d_6, 30 °C): δ 8.03 (d, *J* = 8.2 Hz, 1H), 8.02 (d, *J* = 8.2 Hz, 1H), 7.77 (d, *J* = 8.3 Hz, 2H), 7.41 (d, *J* = 8.2 Hz, 2H), 7.28 (s, 1H), 7.27 (s, 1H), 7.21 (m, 2H), 7.19 (m, 2H), 7.16 (m, 2H), 7.15 (m, 1H), 7.12 (m, 1H), 7.11 (s, 1H), 7.07 (d, *J* = 7.1 Hz, 2H), 4.49 (m, 1H), 4.47 (m, 1H), 3.09 (dd, *J* = 13.8, 5.0 Hz, 1H), 2.93 (dd, *J* = 13.6, 4.7 Hz, 1H), 2.90 (dd, *J* = 13.9, 7.8 Hz, 1H), 2.69 (dd, *J* = 13.7, 9.8 Hz, 1H), 2.64 (t, *J* = 8.4 Hz, 2H), 2.29 (t, *J* = 8.2 Hz, 2H); ^{13}C NMR (125 MHz, DMSO-d_6, 30 °C): δ 172.1, 171.3, 171.0, 162.6, 152.1, 142.7, 141.2, 137.9, 130.3, 129.0, 128.0, 127.9, 127.9, 127.7, 126.1, 125.6, 122.8, 119.7, 53.7, 53.1, 37.3, 37.0, 36.5, 30.7;

HRMS calcd for $C_{30}H_{30}N_4O_6SNa$ $(M+Na)^+$: m/z = 597.1792.

4.10. (2*S*)-2-Amino-*N*-{(1*S*)-2-amino-1-[4-(1,1-dioxido-3-oxoisothiazolidin-5-yl)benzyl]-2-oxoethyl}-3-phenylpropanamide trifluoroacetate (11a)

A solution of **10a** (0.40 g, 0.72 mmol) in MeOH (20 mL) was treated with 10% Pd/C, Degussa type (0.30 g, 75wt%) and stirred under an atmosphere of hydrogen (50 psi) on a Parr shaker for 16 h. The reaction mixture was filtered and the filtrate concentrated to a crude residue which was purified by preparative LC–MS (CH_3CN/H_2O/TFA) to yield **11a** (0.34 g, 84%) as a solid. ^{1}H NMR (500 MHz, DMSO-d_6, 30 °C): δ 8.73 (d, J = 8.3 Hz, 1H), 8.02 (d, J = 3.9 Hz, 2H), 7.54 (s, 1H), 7.41 (d, J = 8.2 Hz, 2H), 7.34 (m, 2H), 7.30 (m, 2H), 7.26 (m, 3H), 7.15 (s, 1H), 5.27 (dd, J = 9.7, 8.2 Hz, 1H), 4.54 (m, 1H), 4.04 (m, 1H), 3.41 (dd, J = 17.0, 10.0 Hz, 1H), 3.22 (dd, J = 17.1, 8.2 Hz, 1H), 3.13 (dd, J = 14.6, 4.6 Hz, 1H), 3.05 (dd, J = 13.6, 4.9 Hz, 1H), 2.90 (m, 2H); ^{13}C NMR (125 MHz, DMSO-d_6, 30 °C): δ 171.4, 168.2, 167.5, 138.4, 134.3, 129.2, 129.1, 128.8, 128.0, 126.7, 126.7, 63.9, 53.5, 52.7, 37.1, 37.0, 36.4; HRMS calcd for $C_{21}H_{24}N_4O_5SNa$ $(M+Na)^+$: m/z = 467.1378.

Compound **11b** was previously reported.[22] Compounds **11c**–**11h** were prepared according to the procedure of **10c** using **11a** and the appropriate acids with yields ranging from 12% to 52%.

4.11. *N*-[(1*S*)-2-({(1*S*)-2-Amino-1-[4-(1,1-dioxido-3-oxoisothiazolidin-5-yl)benzyl]-2-oxoethyl}amino)-1-benzyl-2-oxoethyl]benzamide (11c)

^{1}H NMR (500 MHz, DMSO-d_6, 30 °C): δ 8.54 (d, J = 8.3 Hz, 0.5H), 8.52 (d, J = 8.3 Hz, 0.5H), 7.98 (d, J = 7.3 Hz, 0.5H), 7.96 (d, J = 7.3 Hz, 0.5H), 7.75 (d, J = 8.2 Hz, 2H), 7.50 (dd, J = 7.3, 7.3 Hz, 1H), 7.43 (dd, J = 7.3, 7.3 Hz, 2H), 7.36 (s, 1H), 7.28 (d, J = 8.5 Hz, 2H), 7.21 (dd, J = 7.6, 7.4 Hz, 2H), 7.15 (m, 2H), 7.12 (m, 3H), 7.07 (s, 1H), 4.66 (m, 1H), 4.44 (m, 1H), 4.41 (m, 1H), 3.05 (m, 1H), 3.01 (m, 1 H), 2.96 (dd, J = 11.1 Hz, 0.5H), 2.93 (d, J = 11.1 Hz, 0.5H), 2.86 (m, 1H), 2.85 (m, 1H), 2.71 (dd, J = 15.2, 8.4 Hz, 0.5H), 2.69 (dd, J = 15.6, 8.3 Hz, 0.5H); ^{13}C NMR (125 MHz, DMSO-d_6, 30 °C): δ 173.7, 172.5, 170.9, 166.3, 138.3, 136.9, 133.8, 132.3, 131.1, 129.0, 129.0, 128.1, 128.1, 127.9, 127.3, 126.0, 64.5, 54.8, 53.5, 41.5, 36.9, 36.6; HRMS calcd for $C_{28}H_{28}N_4O_6SNa$ $(M+Na)^+$: m/z = 571.1629.

4.12. (2*S*)-*N*-{(1*S*)-2-Amino-1-[4-(1,1-dioxido-3-oxoisothiazolidin-5-yl)benzyl]-2-oxoethyl}-3-phenyl-2-[(phenylacetyl)amino]propanamide (11d)

^{1}H NMR (500 MHz, DMSO-d_6, 30 °C): δ 8.20 (d, J = 8.4 Hz, 1H), 7.96 (d, J = 8.2 Hz, 1H), 7.29 (s, 1H), 7.19 (m, 6H), 7.18 (m, 1H), 7.16 (m, 4H), 7.15 (m, 1H), 7.06 (s, 1H), 7.03 (d, J = 7.2 Hz, 2H), 4.48 (m, 1H), 4.43 (m, 1H), 4.39 (m, 1H), 3.40 (d, J = 13.9 Hz, 1H), 3.32 (d, J = 13.9 Hz, 1H), 2.99 (dd, J = 8.2, 5.1 Hz, 1H), 2.96 (dd, J = 7.3, 4.0 Hz, 1H), 2.87 (dd, J = 16.2, 8.9 Hz, 1H), 2.82 (m, 1H), 2.71 (m, 1H), 2.70 (m, 1H); ^{13}C NMR (125 MHz, DMSO-d_6, 30 °C): δ 174.4, 172.3, 170.9, 169.9, 137.8, 136.7, 136.0, 133.0, 129.0, 128.9, 128.8, 128.8, 128.8, 128.2, 127.9, 126.1, 64.6, 53.8, 53.5, 42.2, 41.9, 37.1, 37.0; HRMS calcd for $C_{29}H_{30}N_4O_6SNa$ $(M+Na)^+$: m/z = 585.1806.

4.13. (2*S*)-*N*-{(1*S*)-2-Amino-1-[4-(1,1-dioxido-3-oxoisothiazolidin-5-yl)benzyl]-2-oxoethyl}-2-{[(4-hydroxyphenyl)acetyl]amino}-3-phenylpropanamide (11e)

^{1}H NMR (500 MHz, DMSO-d_6, 30 °C): δ 9.15 (s, 0.5H), 9.14 (s, 0.5H), 8.05 (d, J = 7.6 Hz, 0.5H), 8.03 (d, J = 7.6 Hz, 0.5H), 7.92 (d, J = 7.6 Hz, 1H), 7.28 (s, 1H), 7.19 (m, 2H), 7.15 (m, 1H), 7.14 (m, 6H), 7.05 (s, 1H), 6.83 (d, J = 8.4 Hz, 2H), 6.57 (d, J = 8.6 Hz, 2H), 4.44 (m, 1H), 4.41 (m, 1H), 4.38 (m, 1H), 3.26 (d, J = 14.5 Hz, 1H), 3.19 (d, J = 14.6 Hz, 1H), 2.98 (m, 1H), 2.95 (m, 1H), 2.87 (m, 1H), 2.82 (m, 1H), 2.71 (m, 1 H), 2.68 (m, 1H); ^{13}C NMR (125 MHz, DMSO-d_6, 30 °C): δ 174.1, 172.3, 170.8, 170.5, 155.6, 137.7, 136.6, 133.2, 129.8, 129.0, 128.8, 128.2, 128.1, 126.2, 126.1, 114.8, 64.6, 53.8, 53.5, 42.1, 41.0, 37.0, 36.9; HRMS calcd for $C_{29}H_{30}N_4O_7SNa$ $(M+Na)^+$: m/z = 601.1709.

4.14. (2*S*)-*N*-{(1*S*)-2-Amino-1-[4-(1,1-dioxido-3-oxoisothiazolidin-5-yl)benzyl]-2-oxoethyl}-2-{[(4-methoxyphenyl)acetyl]amino}-3-phenylpropanamide (11f)

^{1}H NMR (500 MHz, DMSO-d_6, 30 °C): δ 8.10 (d, J = 8.8 Hz, 1H), 7.93 (d, J = 8.8 Hz, 1H), 7.28 (s, 1H), 7.19 (m, 2H), 7.18 (m, 2H), 7.15 (m, 3H), 7.14 (m, 2H), 7.06 (s, 1H), 6.96 (d, J = 8.2 Hz, 2H), 6.74 (d, J = 8.1 Hz, 2H), 4.46 (m, 1H), 4.41 (m, 1H), 4.39 (m, 1H), 3.68 (s, 3H), 3.30 (m, 1H), 3.23 (m, 1H), 2.98 (m, 1H), 2.95 (m, 1H), 2.87 (dd, J = 16.3, 9.0 Hz, 1H), 2.82 (dd, J = 14.0, 8.1 Hz, 1H), 2.72 (m, 1H), 2.70 (m, 1H); ^{13}C NMR (125 MHz, DMSO-d_6, 30 °C): δ 174.1, 172.4, 170.8, 170.3, 157.6, 137.7, 136.8, 133.0, 129.8, 129.1, 128.9, 128.2, 128.0, 127.9, 126.1, 113.4, 64.6, 54.9, 53.8, 53.4, 42.3, 41.1, 36.9, 36.9; HRMS calcd for $C_{30}H_{32}N_4O_7SNa$ $(M+Na)^+$: m/z = 615.1880.

4.15. (2*S*)-*N*-{(1*S*)-2-Amino-1-[4-(1,1-dioxido-3-oxoisothiazolidin-5-yl)benzyl]-2-oxoethyl}-3-phenyl-2-({[4-(trifluoromethyl)phenyl]acetyl}amino)propanamide (11g)

^{1}H NMR (500 MHz, DMSO-d_6, 30 °C): δ 8.31 (d, J = 9.2 Hz, 1H), 8.08 (d, J = 4.2 Hz, 0.5H), 8.06 (d, J = 4.2 Hz, 0.5H), 7.53 (d, J = 8.0 Hz, 2H), 7.36 (d, J = 8.2 Hz, 2H), 7.32 (s, 1H), 7.27 (d, J = 8.2 Hz, 2H), 7.24 (d, J = 8.2 Hz, 2H), 7.16 (m, 4H), 7.13 (m, 1H), 7.09 (s, 1H), 5.20 (dd, J = 8.8, 8.8 Hz, 1H), 4.51 (m, 1H), 4.46 (m, 1H), 3.51 (d, J = 14.2 Hz, 1H), 3.41 (d, J = 14.2 Hz, 1H), 3.37 (dd, J = 9.9, 5.1 Hz, 0.5H), 3.34 (dd, J = 9.8, 5.0 Hz, 0.5H), 3.20 (dd, J = 8.4, 2.7 Hz, 0.5H), 3.17 (dd, J = 8.3, 2.5 Hz, 0.5H), 3.04 (dd, J = 14.1, 5.0 Hz, 1H), 2.98 (dd, J = 13.6, 4.2 Hz, 1H), 2.86 (dd, J = 14.0, 8.5 Hz, 1H), 2.70 (m, 1H); ^{13}C NMR (125 MHz, DMSO-d_6, 30 °C): δ 172.4, 170.9, 169.2, 168.8, 140.9, 138.9, 137.7, 129.6, 129.5, 129.5,

129.0, 128.9, 127.9, 127.2, 126.1, 124.7, 124.0 (J_{CF} = 250.6 Hz), 64.1, 53.6, 53.4, 41.5, 37.4, 37.2, 37.0; LC–MS calcd for $C_{30}H_{30}F_3N_4O_6S$ $(M+H)^+$: m/z = 631.2.

4.16. (2*S*)-*N*-{(1*S*)-2-Amino-1-[4-(1,1-dioxido-3-oxoisothiazolidin-5-yl)benzyl]-2-oxoethyl}-3-phenyl-2-[(3-phenylpropanoyl)amino]propanamide (11h)

^{1}H NMR (500 MHz, DMSO-d_6, 30 °C): δ 8.04 (dd, J = 8.1, 1.5 Hz, 1H), 7.91 (dd, J = 8.0, 2.1 Hz, 1H), 7.28 (s, 1H), 7.22 (m, 2H), 7.20 (m, 2H), 7.18 (m, 2H), 7.16 (m, 4H), 7.14 (m, 1H), 7.12 (m, 1H), 7.08 (m, 2H), 7.06 (s, 1H), 4.48 (m, 1H), 4.41 (m, 1H), 4.37 (m, 1H), 2.99 (m, 1H), 2.94 (m, 1H), 2.83 (m, 1H), 2.82 (m, 1H), 2.68 (m, 1H), 2.67 (m, 1H), 2.66 (m, 2H), 2.31 (m, 2H); ^{13}C NMR (125 MHz, DMSO-d_6, 30 °C): δ 175.2, 173.1, 172.1, 171.6, 141.8, 138.6, 137.6, 133.9, 129.8, 129.7, 128.8, 128.8, 128.7, 128.7, 126.7, 126.5, 65.4, 54.5, 54.2, 42.9, 37.8, 37.6, 37.3, 31.6; HRMS calcd for $C_{30}H_{32}N_4O_6SNa$ $(M+Na)^+$: m/z = 599.1955.

4.17. (2*S*)-2-Amino-3-[4-(1,1-dioxido-3-oxo-2,3-dihydroisothiazol-5-yl)phenyl]propanamide trifluoroacetate (12)

A solution of **9**[22] (0.45 g, 1.0 mmol) in TFA (5 mL) was heated in a microwave at 140 °C for 2 min. The reaction mixture was concentrated and the crude solid was diluted with 3:1 MeOH/DMSO (10 mL) and filtered to yield **12** (0.12 g, 29%). The filtrate was concentrated and the crude residue was purified by preparative LC–MS (CH_3CN/H_2O/TFA) to yield additional **12** (0.14 g, 34% [63% combined]) as a white solid. ^{1}H NMR (500 MHz, DMSO-d_6): δ 8.10 (br s, 3H), 7.90 (s, 1H), 7.81 (d, J = 7.8 Hz, 2H), 7.63 (s, 1H), 7.37 (d, J = 7.8 Hz, 2H), 6.68 (s, 1H), 4.01–3.95 (m, 1H), 3.14 (dd, J = 14.2, 5.9 Hz, 1H), 3.00 (dd, J = 13.7, 7.8 Hz, 1H); LC–MS calcd for $C_{12}H_{14}N_3O_4S$ $(M+H)^+$: m/z = 296.0.

4.18. (2*S*)-2-(Acetylamino)-3-[4-(1,1-dioxido-3-oxo-2,3-dihydroisothiazol-5- yl)phenyl]propanamide (13)

Compound **13** was prepared from **12** and AcOH according to the procedure of **10c** in 84% yield. ^{1}H NMR (400 MHz, CD_3OD): δ 7.82 (d, 8.4 Hz, 2H), 7.45 (d, J = 8.2 Hz, 2H), 6.98 (s, 1H), 4.67 (dd, J = 9.2, 5.5 Hz, 1H), 3.23 (dd, J = 13.9, 5.5 Hz, 1H), 2.96 (dd, J = 13.9, 9.4 Hz, 1H), 1.90 (s, 3H); LC–MS calcd for $C_{14}H_{16}N_3O_5S$ $(M+H)^+$: m/z = 337.9.

4.19. *tert*-Butyl {(1*S*)-2-amino-1-[4-(1,1-dioxido-3-oxo-2,3-dihydroisothiazol-5-yl)benzyl]-2-oxoethyl}carbamate (14)

A solution of **12** (30 mg, 73 μmol) in pyridine (2 mL) was treated with Boc_2O (32 mg, 0.15 mmol) and stirred for 16 h. Unreacted starting material was treated with additional Boc_2O (0.16 g, 0.73 mmol) and stirred for 4 h. More Boc_2O (0.16 g, 0.73 mmol) was added and the reaction mixture was heated at 50 °C for 3 h. The reaction mixture was cooled to 0 °C, quenched with H_2O dropwise, and concentrated to a crude residue which was co-evaporated with toluene. The crude material was purified by preparative LC–MS (CH_3CN/H_2O/TFA) to yield **14** (18 mg, 62%) as a solid. ^{1}H NMR (400 MHz, CD_3OD): δ 7.82 (d, J = 8.2 Hz, 2H), 7.46 (d, J = 8.2 Hz, 2H), 7.00 (s, 1H), 4.35 (dd, J = 9.4, 5.1 Hz, 1H), 3.21 (dd, J = 13.7, 5.3 Hz, 1H), 2.90 (dd, J = 13.5, 9.6 Hz, 1H), 1.35 (s, 9H); LC–MS calcd for $C_{17}H_{21}N_3O_6SNa$ $(M+Na)^+$: m/z = 418.0.

4.20. (2*S*)-2-Amino-3-[4-(1,1-dioxido-3-oxoisothiazolidin-5- yl)phenyl]propanamide trifluoroacetate (15)

Compound **15** was prepared from **12** according to the procedure of **11a** in 82% yield. ^{1}H NMR (500 MHz, DMSO-d_6): δ 8.13 (s, 2H), 7.86 (s, 1H), 7.54 (s, 1H), 7.45 (d, J = 8.3 Hz, 2H), 7.31 (d, J = 8.3 Hz, 2H), 5.23 (dd, J = 8.9, 8.9 Hz, 1H), 3.97 (m, 1H), 3.37 (dd, J = 17.2, 9.8 Hz, 1H), 3.20 (dd, J = 17.1, 8.3 Hz, 1H), 3.10 (dd, J = 14.2, 6.0 Hz, 1H), 2.99 (dd, J = 14.1, 7.5 Hz, 1H); LC–MS calcd for $C_{12}H_{16}N_3O_4S$ $(M+H)^+$: m/z = 298.1.

4.21. (2*S*)-2-(Acetylamino)-3-[4-(1,1-dioxido-3-oxoisothiazolidin-5-yl)phenyl]propanamide (16)

Compound **16** was prepared from **13** according to the procedure of **11a** in 17% yield. ^{1}H NMR (500 MHz, DMSO-d_6, 30 °C): δ 8.00 (d, J = 8.5 Hz, 1H), 7.40 (s, 1H), 7.37 (d, J = 8.4 Hz, 2H), 7.29 (d, J = 8.4 Hz, 2H), 6.99 (s, 1H), 5.26 (dd, J = 8.9, 8.9 Hz, 1H), 4.42 (m, 1H), 3.41 (dd, J = 17.1, 9.8 Hz, 1H), 3.22 (dd, J = 17.3, 8.1 Hz, 1H), 3.01 (dd, J = 13.9, 4.7 Hz, 1H), 2.76 (m, 1H), 1.76 (s, 3H); ^{13}C NMR (125 MHz, DMSO-d_6, 30 °C): δ 173.0, 169.0, 168.6, 139.5, 129.3, 128.9, 126.8, 64.1, 53.5, 37.1, 36.9, 22.4; LC–MS calcd for $C_{14}H_{18}N_3O_5S$ $(M+H)^+$: m/z = 340.0.

4.22. *tert*-Butyl {(1*S*)-2-amino-1-[4-(1,1-dioxido-3-oxoisothiazolidin-5-yl)benzyl]-2-oxoethyl}carbamate (17)

Compound **17** was prepared from **14** according to the procedure of **11a** in 53% yield. ^{1}H NMR (500 MHz, DMSO-d_6, 30 °C): δ 7.84 (s, 1H), 7.55 (s, 1H), 7.45 (d, J = 8.2 Hz, 2H), 7.30 (d, J = 8.4 Hz, 2 H), 6.77 (d, J = 8.8 Hz, 1H), 5.18 (dd, J = 14.5, 8.6 Hz, 1H), 4.09 (m, 1H), 3.36 (dd, J = 12.1, 10.4 Hz, 1H), 3.19 (m, 1H), 3.10 (dd, J = 14.2, 5.9 Hz, 1H), 2.98 (dd, J = 13.9, 7.8 Hz, 1H), 1.29 (s, 9H); ^{13}C NMR (125 MHz, DMSO-d_6, 30 °C): δ 169.6, 169.0, 155.0, 136.0, 129.5, 129.4, 128.7, 77.8, 64.1, 55.1, 37.6, 36.4, 27.9; HRMS calcd for $C_{17}H_{23}N_3O_6SNa$ $(M+Na)^+$: m/z = 420.1202.

4.23. *N*α-(*tert*-Butoxycarbonyl)-4-(dihydroxyboryl)-*N*-pentyl-L-phenylalaninamide (19)

A solution of **18**[27] (1.6 g, 4.1 mmol) and triethylamine (1.8 mL, 13 mmol) in DMF (20 mL) was treated with benzotriazol-1-yloxytris(dimethylamino)phosphonium hexafluorophosphate (3.3 g, 7.4 mmol). After 5 min, 1-pentanamine (0.96 mL, 8.3 mmol) was added and stirring was continued for 2 h. The reaction mixture was diluted with ethyl acetate (50 mL) and washed with H_2O (50 mL) and 1 N HCl (50 mL). The organic layer

was dried over Na_2SO_4, filtered, and concentrated to a crude residue which was purified by preparative LC–MS (CH_3CN/H_2O/TFA) to yield **19** (1.6 g, 56%) as a white solid. ^{1}H NMR (500 MHz, CD_3OD): δ 7.90 (t, J = 5.6 Hz, 1H), 7.70 (d, J = 7.9 Hz, 2H), 7.57 (d, J = 7.6 Hz, 2H), 7.26 (d, J = 8.14 Hz, 1H), 7.22 (d, J = 8.3 Hz, 2H), 6.70 (d, J = 8.3 Hz, 1H), 6.24 (bs, 1H), 4.28–4.26 (m, 1H), 3.20–3.06 (m, 3H), 2.86 (dd, J = 13.6, 8.1 Hz, 1H), 1.40 (s, 9H), 1.38–1.24 (m, 6H), 0.92 (t, J = 6.7 Hz, 3H); LC–MS calcd for $C_{16}H_{24}N_4O_4SNa$ $(M+Na)^+$: m/z = 379.

4.24. *tert*-Butyl [(1*S*)-1-[4-(2-*tert*-butyl-1,1-dioxido-3-oxo-2,3-dihydroisothiazol-5-yl)benzyl]-2-oxo-2-(pentylamino)ethyl]carbamate (20)

A solution of **19** (1.0 g, 2.6 mmol), **5**[22] (0.65 g, 2.9 mmol), and K_2CO_3 (1.8 g, 13 mmol) in 1,2-dimethoxyethane (9.0 mL) was degassed with a stream of nitrogen gas for 5 min. The reaction mixture was treated with [1,1′-bis(diphenylphosphino)ferrocene]dichloropalladium(II): CH_2Cl_2 (1:1) (0.19 g, 0.26 mmol), degassed for another 5 min, and heated in a sealed tube at 80 °C for 24 h. The reaction mixture was filtered over a pad of silica gel and washed with EtOAc. The filtrate was concentrated and the crude residue was purified by flash column chromatography (100% hexane to 50% EtOAc/hexane) to yield **20** (0.82 g, 60%) as a tan solid. ^{1}H NMR (500 MHz, $CDCl_3$): δ 7.71 (d, J = 8.3 Hz, 2H), 7.37 (d, J = 8.3 Hz, 2H), 6.62 (s, 1H), 5.87–5.85 (m, 1H), 5.04–5.02 (m, 1H), 4.32–4.30 (m, 1H), 3.20–3.13 (m, 3H), 3.11–3.07 (m, 1H), 1.73 (s, 9H), 1.41 (s, 9H), 1.32–1.24 (m, 2H), 1.23–1.17 (m, 2H), 0.87 (t, J = 7.3 Hz, 3H); LC–MS calcd for $C_{26}H_{39}N_3O_6SNa$ $(M+Na)^+$: m/z = 544.1.

4.25. (2*S*)-2-Amino-3-[4-(2-*tert*-butyl-1,1-dioxido-3-oxo-2,3-dihydroisothiazol-5-yl)phenyl]-*N*-pentylpropanamide hydrochloride (21)

A solution of **20** (0.82 g, 1.6 mmol) in 4 M HCl in 1,4-dioxane (10 mL) was stirred for 16 h. The reaction mixture was concentrated to yield **21** (0.72 g, quantitative) as a light tan solid. ^{1}H NMR (500 MHz, CD_3OD): δ 8.28 (t, J = 5.4 Hz, 1H), 7.91 (d, J = 8.3 Hz, 2H), 7.50 (d, J = 8.3 Hz, 2H), 7.10 (s, 1H), 4.07 (t, J = 7.6 Hz, 1H), 3.27–3.17 (m, 3H), 3.13–3.06 (m, 1H), 1.72 (s, 9H), 1.43–1.37 (m, 2H), 1.36–1.27 (m, 2H), 1.22–1.16 (m, 2H), 0.90 (t, J = 7.3 Hz, 3H); LC–MS calcd for $C_{21}H_{32}N_3O_4S$ $(M+H)^+$: m/z = 422.1.

4.26. *tert*-Butyl ((1*S*)-1-benzyl-2-{[(1*S*)-1-[4-(2-*tert*-butyl-1,1-dioxido-3-oxo-2,3-dihydroisothiazol-5-yl)benzyl]-2-oxo-2-(pentylamino)ethyl]amino}-2-oxoethyl)carbamate (22)

Compound **22** was prepared from **21** and Boc-Phe-OH according to the procedure of **10c** in 85% yield. ^{1}H NMR (500 MHz, $CDCl_3$): δ 7.66 (d, J = 8.3 Hz, 2H), 7.34–7.27 (m, 2H), 7.22–7.21 (m, 2H), 7.18–7.16 (m, 3H), 6.61 (s, 1H), 6.29–6.21 (m, 1H), 6.09–6.01 (m, 1H), 4.85–4.80 (m, 1 H), 4.69–4.61 (m, 1H), 4.26 (dd, J = 13.2, 6.8 Hz, 1H), 3.25 – 3.18 (m, 1H), 3.18–3.12 (m, 1H), 3.11–2.96 (m, 4H), 1.73 (s, 9 H), 1.41–1.34 (m, 2H), 1.33 (s, 9H), 1.30–1.24 (m, 2H), 1.21–1.15 (m, 2H), 0.87 (t, J = 7.1 Hz, 3H); LC–MS calcd for $C_{30}H_{41}N_4O_5S$ $([M-Boc+H]+H)^+$: m/z = 569.2.

4.27. (2*S*)-2-Amino-*N*-[(1*S*)-1-[4-(2-*tert*-butyl-1,1-dioxido-3-oxo-2,3-dihydroisothiazol-5-yl)benzyl]-2-oxo-2-(pentylamino)ethyl]-3-phenylpropanamide hydrochloride (23)

Compound **23** was prepared from **22** according to the procedure of **21** in quantitative yield. ^{1}H NMR (500 MHz, CD_3OD): δ 8.12 (t, J = 5.6 Hz, 1H), 7.84 (d, J = 8.3 Hz, 2H), 7.49 (d, J = 8.8 Hz, 2H), 7.39–7.34 (m, 2H), 7.34–7.28 (m, 3H), 7.06 (s, 1H), 4.66 (dd, J = 7.8, 7.3 Hz, 1H), 4.13 (dd, J = 8.3, 5.9 Hz, 1H), 3.27 (dd, J = 14.6, 5.9 Hz, 1H), 3.21–3.13 (m, 2H), 3.11–3.02 (m, 3H), 1.72 (s, 9H), 1.43–1.37 (m, 2H), 1.35–1.28 (m, 2 H), 1.23–1.16 (m, 2H), 0.90 (t, J = 7.3 Hz, 3H); LC–MS calcd for $C_{30}H_{41}N_4O_5S$ $(M+H)^+$: m/z = 569.2.

4.28. (2*S*)-3-[4-(2-*tert*-Butyl-1,1-dioxido-3-oxo-2,3-dihydroisothiazol-5-yl)phenyl]-2-[((2*S*)-2-{[(4-methoxyphenyl)acetyl]amino}-3-phenylpropanoyl)amino]-*N*-pentylpropanamide (24)

Compound **24** was prepared from **23** and 4-methoxyphenylacetic acid according to the procedure of **10c** in 96% yield. ^{1}H NMR (500 MHz, $CDCl_3$): δ 7.66 (d, J = 8.3 Hz, 2H), 7.23–7.19 (m, 5H), 6.97–6.87 (m, 4H), 6.85 (d, J = 8.8 Hz, 2H), 6.61 (s, 1H), 6.41 (d, J = 8.3 Hz, 1H), 5.96 (t, J = 5.6 Hz, 1H), 5.82 (d, J = 6.8 Hz, 1H), 4.61 (dd, J = 15.1, 6.8 Hz, 1H), 4.53 (d, J = 13.7, 6.8 Hz, 1H), 3.83 (s, 3H), 3.43 (d, J = 16.6 Hz, 1H), 3.36 (d, J = 16.6 Hz, 1H), 3.16–3.09 (m, 2H), 3.10–3.06 (m, 1H), 3.05–3.00 (m, 1H), 2.94–2.91 (m, 2H), 1.73 (s, 9H), 1.41–1.34 (m, 2H), 1.32–1.24 (m, 2H), 1.21–1.14 (m, 2H), 0.87 (t, J = 7.3 Hz, 3H); LC–MS calcd for $C_{39}H_{49}N_4O_7S$ $(M+H)^+$: m/z = 717.2.

4.29. (2*S*)-3-[4-(1,1-Dioxido-3-oxo-2,3-dihydroisothiazol-5-yl)phenyl]-2-[((2*S*)-2-{[(4-methoxyphenyl)acetyl]amino}-3-phenylpropanoyl)amino]-*N*-pentylpropanamide (25)

A solution of **24** (0.10 g, 0.14 mmol) in TFA was treated with triisopropylsilane (0.10 mL, 0.49 mmol). The reaction mixture was heated at 70 °C for 16 h, concentrated, and purified by preparative LC–MS (CH_3CN/H_2O/TFA) to yield **25** (35 mg, 38%) as a white solid. ^{1}H NMR (500 MHz, DMSO-d_6, 30 °C): δ 8.09 (d, J = 8.6 Hz, 1H), 8.02 (d, 8.3 Hz, 1H), 7.78 (t, J = 5.7 Hz, 1H), 7.69 (d, J = 8.3 Hz, 2H), 7.24 (d, J = 8.1 Hz, 2 H), 7.17 (m, 2H), 7.14 (m, 3H), 6.97 (d, J = 8.5 Hz, 2H), 6.74 (d, J = 8.5 Hz, 2H), 6.57 (s, 1H), 4.48 (m, 1H), 4.46 (m, 1H), 3.67 (s, 3H), 3.31 (d, J = 13.8 Hz, 1H), 3.25 (d, J = 13.8 Hz, 1H), 2.98 (m, 2H), 2.96 (m, 1H), 2.91 (m, 1H), 2.84 (dd, J = 13.7, 8.1 Hz, 1H), 2.72 (dd, J = 13.7, 9.5 Hz, 1H), 1.32 (m, 2H), 1.23 (m, 2H), 1.16 (m, 2H), 0.83 (t, J = 7.3 Hz, 3H); ^{13}C NMR (125 MHz, DMSO-d_6, 30 °C): δ 170.7, 170.3, 169.9,

169.2, 157.7, 153.6, 139.4, 137.6, 129.9, 129.5, 129.0, 128.0, 127.8, 127.3, 126.2, 125.8, 123.2, 113.4, 54.8, 53.7, 53.6, 41.0, 38.2, 37.7, 37.1, 28.4, 28.3, 20.6, 13.5; HRMS calcd for $C_{35}H_{40}N_4O_7SNa$ $(M+Na)^+$: m/z = 683.2535.

4.30. (2*S*)-3-[4-(2-*tert*-Butyl-1,1-dioxido-3-oxoisothiazolidin-5-yl)phenyl]-2-[((2*S*)-2-{[(4-methoxyphenyl)acetyl]amino}-3-phenylpropanoyl)amino]-*N*-pentylpropanamide (39)

Compound **39** was prepared from **24** according to the procedure of **11a** in 37% yield. ^{1}H NMR (500 MHz, $CDCl_3$): δ 7.29 (d, J = 7.8 Hz, 2H), 7.23–7.17 (m, 5H), 6.98–6.95 (m, 4H), 6.85–6.82 (m, 2H), 6.43 (dd, J = 8.3, 4.9 Hz, 1H), 5.85–5.81 (m, 1H), 5.79–5.78 (m, 1H), 4.77–4.73 (m, 1H), 4.61–4.57 (m, 1H), 4.52–4.48 (m, 1 H), 3.82 (s, 3H), 3.43–3.35 (m, 2H), 3.23–3.18 (m, 2H), 3.16–3.09 (m, 2H), 3.06–3.02 (m, 2H), 3.00–2.95 (m, 1H), 2.88–2.82 (m, 1H), 1.66 (s, 9H), 1.40–1.33 (m, 2H), 1.32–1.25 (m, 2H), 1.23–1.15 (m, 2H), 0.88 (t, J = 7.1 Hz, 3H); LC–MS calcd for $C_{39}H_{51}N_4O_7S$ $(M+H)^+$: m/z = 719.2.

4.31. (2*S*)-3-[4-(1,1-Dioxido-3-oxoisothiazolidin-5-yl)phenyl]-2-[((2*S*)-2-{[(4-methoxyphenyl)acetyl]amino}-3-phenylpropanoyl)amino]-*N*-pentylpropanamide (42)

Compound **42** was prepared from **39** according to the procedure of **25** in 39% yield. ^{1}H NMR (500 MHz, DMSO-d_6, 30 °C): δ 8.08 (d, J = 8.2 Hz, 1H), 8.01 (d, J = 8.2 Hz, 1H), 7.76 (t, J = 5.7 Hz, 1H), 7.22 (m, 2H), 7.21 (m, 2H), 7.16 (m, 3H), 7.15 (m, 2H), 6.97 (d, J = 8.8 Hz, 2H), 6.75 (d, J = 8.6 Hz, 2H), 4.52 (dd, J = 8.1, 8.1 Hz, 1H), 4.47 (m, 1H), 4.42 (m, 1H), 3.69 (s, 3H), 3.32 (d, J = 14.1 Hz, 1H), 3.25 (d, J = 14.1 Hz, 1H), 2.99 (m, 2H), 2.96 (m, 1H), 2.94 (m, 1H), 2.92 (m, 1H), 2.91 (m, 1H), 2.81 (dd, J = 13.8, 7.9 Hz, 1H), 2.71 (dd, J = 13.8, 10.0 Hz, 1H), 1.32 (m, 2H), 1.24 (m, 2H), 1.18 (m, 2H), 0.84 (t, J = 7.1 Hz, 3H); ^{13}C NMR (125 MHz, DMSO-d_6, 30 °C): δ 173.3, 170.7, 170.3, 170.1, 157.7, 137.6, 137.0, 132.2, 129.8, 129.1, 128.9, 128.3, 128.2, 127.9, 126.1, 113.5, 64.5, 54.9, 53.7, 53.7, 41.4, 41.0, 38.3, 37.3, 37.0, 28.4, 28.2, 21.5, 13.8; HRMS calcd for $C_{35}H_{42}N_4O_7SNa$ $(M+Na)^+$: m/z = 685.2659.

4.32. Separation of diastereoisomeric mixture of 39

The diastereoisomeric mixture **39** was separated by normal phase chiral HPLC (ChiralCel OD-H [20 × 250 mm, 5 μm], 30% EtOH/70% hexane, 12 mL/min, ambient temperature) to yield **40** (peak 1) (21 mg, 28%) and **41** (peak 2) (27 mg, 36%). Compound **40** (peak 1): ^{1}H NMR (500 MHz, $CDCl_3$): δ 7.29 (d, J = 7.8 Hz, 2H), 7.22–7.17 (m, 5H), 6.98–6.95 (m, 4H), 6.83 (d, J = 8.8 Hz, 2H), 6.50 (d, J = 8.3 Hz, 1H), 5.90 (dd, J = 5.9, 5.4 Hz, 1H), 5.83 (d, J = 6.8 Hz, 1H), 4.75 (dd, J = 9.3, 8.8 Hz, 1H), 4.59 (dd, J = 15.1, 7.3 Hz, 1H), 4.51 (dd, J = 13.7, 6.3 Hz, 1H), 3.82 (s, 3H), 3.41 (d, J = 16.1 Hz, 1H), 3.37 (d, J = 16.1 Hz, 1H), 3.23–3.15 (m, 2H), 3.11 (dd, J = 15.1, 9.3 Hz, 2H), 3.04 (d, J = 6.8 Hz, 2H), 2.97 (dd, J = 14.2, 5.9 Hz, 1H), 2.84 (dd, J = 14.2, 7.8 Hz, 1H), 1.66 (s, 9H), 1.42–1.34 (m, 2H), 1.33–1.25 (m, 2H), 1.22–1.16 (m, 2H), 0.87 (t, J = 7.3 Hz, 3H); LC–MS calcd for $C_{39}H_{51}N_4O_7S$ $(M+H)^+$: m/z = 719.3.

Compound **41** (peak 2): ^{1}H NMR (500 MHz, $CDCl_3$): δ 7.29 (d, J = 8.3 Hz, 2H), 7.23–7.17 (m, 5H), 6.98–6.95 (m, 4H), 6.83 (d, J = 8.3 Hz, 2H), 6.40 (d, J = 8.3 Hz, 1H), 5.84 (dd, J = 5.9, 5.4 Hz, 1H), 5.78 (d, J = 6.8 Hz, 1 H), 4.75 (dd, J = 9.3, 8.8 Hz, 1H), 4.58 (dd, J = 15.1, 6.8 Hz, 1H), 4.50 (dd, J = 14.2, 6.3 Hz, 1H), 3.82 (s, 3H), 3.41 (d, J = 16.1 Hz, 1H), 3.37 (d, J = 15.6 Hz, 1H), 3.23–3.14 (m, 2H), 3.14–3.09 (m, 2H), 3.04 (dd, J = 6.8, 2.9 Hz, 2H), 2.97 (dd, J = 14.2, 5.9 Hz, 1H), 2.85 (dd, J = 14.2, 7.8 Hz, 1H), 1.65 (s, 9H), 1.40–1.34 (m, 2H), 1.31–1.25 (m, 2H), 1.23–1.17 (m, 2H), 0.88 (t, J = 7.3 Hz, 3H); LC–MS calcd for $C_{39}H_{51}N_4O_7S$ $(M+H)^+$: m/z = 719.3.

Compound **43** was prepared from **40** (peak 1) according to the procedure of **25** in 12% yield. Compound **43**: ^{1}H NMR (500 MHz, DMSO-d_6 + 5 μL TFA-d, 30 °C): δ 8.08 (d, 2H), 7.79 (t, J = 5.4 Hz, 1H), 7.37 (d, J = 8.0 Hz, 2H), 7.25 (d, J = 7.9 Hz, 2H), 7.19 (m, 2 H), 7.16 (m, 1H), 7.15 (m, 2H), 6.97 (d, J = 8.7 Hz, 2H), 6.75 (d, J = 8.4 Hz, 2H), 5.26 (dd, J = 9.1, 9.1 Hz, 1 H), 4.47 (m, 1H), 4.44 (m, 1H), 3.69 (s, 3H), 3.38 (dd, J = 17.3, 9.8 Hz, 1H), 3.32 (d, J = 14.4 Hz, 1H), 3.24 (d, J = 14.4 Hz, 1H), 3.20 (dd, J = 17.6, 8.2 Hz, 1H), 2.99 (m, 2H), 2.98 (m, 1H), 2.93 (m, 1H), 2.84 (dd, J = 13.9, 8.3 Hz, 1H), 2.71 (dd, J = 14.0, 9.1 Hz, 1H), 1.32 (m, 2H), 1.24 (m, 2H), 1.18 (m, 2H), 0.84 (t, J = 7.1 Hz, 3H); ^{13}C NMR (125 MHz, DMSO-d_6 + 5 μL TFA-d, 30 °C): δ 170.7, 170.2, 170.0, 168.3, 157.7, 138.9, 137.6, 129.8, 129.5, 129.0, 128.9, 127.9, 127.8, 126.9, 126.1, 113.4, 64.0, 54.8, 53.7, 53.5, 41.0, 38.3, 37.2, 37.1, 37.0, 28.4, 28.2, 21.5, 13.9; HRMS calcd for $C_{35}H_{42}N_4O_7S$-Na $(M+Na)^+$: m/z = 685.2686.

Compound **44** was prepared from **41** (peak 2) according to the procedure of **25** in 21% yield. Compound **44**: ^{1}H NMR (500 MHz, DMSO-d_6 + 5 μL TFA-d, 30 °C): δ 8.09 (m, 2H), 7.78 (t, J = 5.7 Hz, 1H), 7.36 (d, J = 8.2 Hz, 2H), 7.25 (d, J = 8.0 Hz, 2H), 7.19 (m, 2 H), 7.16 (m, 1H), 7.15 (m, 2H), 6.98 (d, J = 8.4 Hz, 2H), 6.75 (d, J = 8.5 Hz, 2H), 5.26 (dd, J = 9.4, 8.4 Hz, 1 H), 4.46 (m, 1H), 4.43 (m, 1H), 3.69 (s, 3H), 3.39 (dd, J = 17.0, 9.6 Hz, 1H), 3.32 (d, J = 14.5 Hz, 1H), 3.24 (d, J = 14.5 Hz, 1H), 3.20 (dd, J = 17.1, 8.0 Hz, 1H), 2.99 (m, 2H), 2.98 (m, 1H), 2.94 (dd, J = 14.4, 4.3 Hz, 1H), 2.84 (dd, J = 13.8, 8.3 Hz, 1H), 2.72 (dd, J = 14.0, 9.9 Hz, 1H), 1.31 (m, 2H), 1.24 (m, 2H), 1.18 (m, 2H), 0.84 (t, J = 7.1 Hz, 3H); ^{13}C NMR (125 MHz, DMSO-d_6 + 5 μL TFA-d, 30 °C): δ 170.8, 170.2, 170.1, 168.4, 157.7, 138.9, 137.6, 129.8, 129.5, 129.0, 128.9, 128.0, 127.8, 126.9, 126.1, 113.3, 64.1, 54.8, 53.7, 53.5, 41.0, 38.2, 37.3, 37.1, 37.0, 28.4, 28.3, 21.5, 13.7; HRMS calcd for $C_{35}H_{42}N_4O_7SNa$ $(M+Na)^+$: m/z = 685.2654.

4.33. *tert*-Butyl [(1*S*)-1-[4-(2-*tert*-butyl-1,1-dioxido-3-oxo-2,3-dihydroisothiazol-4-yl)benzyl]-2-oxo-2-(pentylamino)ethyl]carbamate (26)

Compound **26** was prepared from **19** and **6**[25] according to the procedure of **20** in 27% yield. ^{1}H NMR (500 MHz, $CDCl_3$): δ 7.70 (d, J = 8.3 Hz, 2H), 7.32 (d, J = 7.8 Hz, 2H), 7.18 (s, 1H), 5.80–5.77 (m, 1H), 5.08–5.00 (m, 1H), 4.31–4.27 (m, 1H), 3.17 (dd, J = 13.2, 6.8 Hz, 2 H), 3.11 (s, 2H), 1.73 (s, 9H), 1.42 (s, 9H), 1.42–1.37 (m, 2H), 1.35–1.24 (m, 2H), 1.21–1.16 (m, 2H), 0.86 (t, J = 7.1 Hz, 3H); LC–MS calcd for $C_{26}H_{39}N_3O_6SNa$ $(M+Na)^+$: m/z = 544.2.

4.34. (2*S*)-2-Amino-3-[4-(2-*tert*-butyl-1,1-dioxido-3-oxo-2,3-dihydroisothiazol-4-yl)phenyl]-*N*-pentylpropanamide hydrochloride (27)

Compound **27** was prepared from **26** according to the procedure of **21** in quantitative yield. ^{1}H NMR (300 MHz, DMSO-d_6): δ 8.56 (s, 1H), 8.40–8.25 (m, 3H), 7.81 (d, J = 7.9 Hz, 2H), 7.35 (d, J = 7.9 Hz, 2H), 3.97 (dd, J = 7.3, 6.7 Hz, 1H), 3.17–3.00 (m, 3H), 2.93–2.82 (m, 1H), 1.62 (s, 9H), 1.30–1.10 (m, 4H), 1.10–0.98 (m, 2 H), 0.77 (t, J = 7.2 Hz, 3H); LC–MS calcd for $C_{21}H_{32}N_3O_4S$ $(M+H)^+$: m/z = 422.1.

4.35. *tert*-Butyl ((1*S*)-1-benzyl-2-{[(1*S*)-1-[4-(2-*tert*-butyl-1,1-dioxido-3-oxo-2,3-dihydroisothiazol-4-yl)benzyl]-2-oxo-2-(pentylamino)ethyl]amino}-2-oxoethyl)carbamate (28)

Compound **28** was prepared from **27** and Boc-Phe-OH according to the procedure of **10c** in 64% yield. ^{1}H NMR (500 MHz, $CDCl_3$): δ 7.66 (d, J = 8.3 Hz, 2H), 7.36–7.32 (m, 2H), 7.30–7.27 (m, 1H), 7.18–7.15 (m, 5H), 6.28–6.22 (m, 1H), 6.08–6.01 (m, 1H), 4.83–4.80 (m, 1H), 4.68–4.60 (m, 1H), 4.26 (dd, J = 12.7, 6.3 Hz, 1H), 3.27–3.20 (m, 1 H), 3.19–3.12 (m, 1H), 3.10–2.94 (m, 4H), 1.73 (s, 9H), 1.40–1.34 (m, 2H), 1.32 (s, 9H), 1.30–1.24 (m, 2H), 1.21–1.15 (m, 2 H), 0.86 (t, J = 7.1 Hz, 3H); LC–MS calcd for $C_{35}H_{49}N_4O_7S$ $(M+H)^+$: m/z = 669.2.

4.36. (2*S*)-2-Amino-*N*-[(1*S*)-1-[4-(2-*tert*-butyl-1,1-dioxido-3-oxo-2,3-dihydroisothiazol-4-yl)benzyl]-2-oxo-2-(pentylamino)ethyl]-3-phenylpropanamide hydrochloride (29)

Compound **29** was prepared from **28** according to the procedure of **21** in quantitative yield. ^{1}H NMR (400 MHz, DMSO-d_6): δ 8.88 (d, J = 8.2 Hz, 1H), 8.53 (s, 1H), 8.12–8.04 (m, 3H), 7.77 (d, J = 8.2 Hz, 2H), 7.36 (d, J = 8.4 Hz, 2H), 7.30–7.20 (m, 5H), 4.54 (d, J = 15.0, 8.0 Hz, 1H), 4.01 (dd, J = 6.6, 6.1 Hz, 1H), 3.09 (dd, J = 14.3, 5.3 Hz, 1H), 3.02 (dd, J = 13.9, 6.8 Hz, 1H), 2.97–2.86 (m, 4H), 1.61 (s, 9H), 1.32–1.15 (m, 4H), 1.12–1.06 (m, 2H), 0.79 (t, J = 7.2 Hz, 3H); LC–MS calcd for $C_{30}H_{40}N_4O_5SNa$ $(M+Na)^+$: m/z = 591.2.

4.37. (2*S*)-3-[4-(2-*tert*-Butyl-1,1-dioxido-3-oxo-2,3-dihydroisothiazol-4-yl)phenyl]-2- [((2*S*)-2-{[(4-methoxyphenyl)acetyl]amino}-3-phenylpropanoyl)amino]-*N*-pentylpropanamide (30)

Compound **30** was prepared from **29** and 4-methoxyphenylacetic acid according to the procedure of **10c** in 92% yield. ^{1}H NMR (300 MHz, DMSO-d_6): δ 8.51 (s, 1 H), 8.13 (dd, J = 18.7, 8.2 Hz, 2H), 7.83 (dd, J = 5.6, 5.3 Hz, 1H), 7.76 (d, 8.2 Hz, 2H), 7.31 (d, J = 8.2 Hz, 2H), 7.24–7.10 (m, 5H), 6.96 (d, J = 8.5 Hz, 2H), 6.74 (d, J = 8.8 Hz, 2H), 4.52–4.42 (m, 2H), 3.68 (s, 3H), 3.29–3.21 (m, 2H), 3.10–2.80 (m, 5H), 2.78–2.67 (m, 1H), 1.61 (s, 9H), 1.34–1.08 (m, 6H), 0.80 (t, J = 7.2 Hz, 3H); LC–MS calcd for $C_{39}H_{49}N_4O_7S$ $(M+H)^+$: m/z = 717.2.

4.38. (2*S*)-3-[4-(1,1-Dioxido-3-oxo-2,3-dihydroisothiazol-4-yl)phenyl]-2-[((2*S*)-2-{[(4-methoxyphenyl)acetyl]amino}-3-phenylpropanoyl)amino]-*N*-pentylpropanamide (31)

A solution of **30** (60 mg, 84 μmol) in TFA (2.5 mL) was heated in a microwave at 150 °C for 60 sec. The reaction mixture was concentrated and the crude residue was purified by preparative LC–MS ($CH_3CN/H_2O/NH_4OH$) to yield **31** (21 mg, 38%) as a white solid. ^{1}H NMR (500 MHz, DMSO-d_6, 30 °C): δ 8.43 (s, 1 H), 8.10 (d, J = 8.1 Hz, 1H), 8.07 (d, J = 8.1 Hz, 1 H), 7.80 (m, 1H), 7.78 (d, J = 8.2 Hz, 2H), 7.30 (d, J = 8.1 Hz, 2H), 7.18 (m, 2H), 7.16 (m, 1H), 7.14 (m, 2H), 6.96 (d, J = 8.8 Hz, 2H), 6.74 (d, J = 8.7 Hz, 2H), 4.48 (m, 1H), 4.47 (m, 1H), 3.68 (s, 3H), 3.31 (d, J = 14.1 Hz, 1 H), 3.24 (d, J = 14.1 Hz, 1H), 3.02 (m, 1H), 2.98 (m, 1H), 2.95 (m, 1H), 2.94 (m, 1H), 2.86 (dd, J = 13.7, 8.4 Hz, 1H), 2.72 (dd, J = 13.9, 9.9 Hz, 1H), 1.29 (m, 2H), 1.21 (m, 2H), 1.13 (m, 2H), 0.81 (t, J = 7.3 Hz, 3H); ^{13}C NMR (125 MHz, DMSO-d_6, 30 °C): δ 170.7, 170.2, 169.9, 162.2, 157.7, 141.3, 138.0, 137.6, 132.5, 129.8, 129.6, 129.1, 128.8, 128.0, 127.8, 126.2, 125.5, 113.5, 54.9, 53.7, 53.5, 41.1, 38.3, 37.8, 37.2, 28.5, 28.4, 21.6, 13.9; HRMS calcd for $C_{35}H_{40}N_4O_7SNa$ $(M+Na)^+$: m/z = 683.2484.

4.39. *tert*-Butyl [(1*S*)-1-[4-(5-benzyl-1,1-dioxido-4-oxo-1,2,5-thiadiazolidin-2-yl)benzyl]-2-oxo-2-(pentylamino)ethyl]carbamate (33)

A solution of **19** (580 mg, 1.5 mmol), 2-benzyl-1,2,5-thiadiazolidin-3-one 1,1-dioxide (**32**)[28] (170 mg, 0.8 mmol), copper (II) acetate (210 mg, 1.1 mmol), and triethylamine (0.32 mL, 2.3 mmol) in CH_2Cl_2 (10 mL) was stirred for 24 h open to the air. The reaction mixture was filtered and the filtrate was concentrated to a crude residue which was purified by preparative LC–MS (CH_3CN/H_2O/TFA) to yield **33** (180 mg, 42%) as a white solid. ^{1}H NMR (500 MHz, CD_3OD): δ 7.48 (d, J = 7.1 Hz, 1H), 7.40–7.33 (m, 7H), 4.88 (s, 2H), 4.62 (s, 2H), 4.26 (dd, J = 8.4, 6.8 Hz, 1H), 3.15–3.06 (m, 3H), 2.86 (dd, J = 13.0, 8.8 Hz, 1H), 1.47–1.43 (m, 2H), 1.39 (s, 9H), 1.37–1.33 (m, 2H), 1.31–1.25 (m, 2H), 0.92 (t, J = 7.0 Hz, 3H); LC–MS calcd for $C_{28}H_{39}N_4O_6S$ $(M+H)^+$: m/z = 559.

4.40. 4-(5-Benzyl-1,1-dioxido-4-oxo-1,2,5-thiadiazolidin-2-yl)-*N*-pentyl-L-phenylalaninamide trifluoroacetate (34)

A solution of **33** (70 mg, 0.13 mmol) in CH_2Cl_2 (3 mL) was treated with TFA (1 mL) and stirred for 2 h. The reaction mixture was concentrated and azeotroped with toluene (2 × 10 mL) to yield **34** (72 mg, 98%) which was used immediately in the next step. LC–MS calcd for $C_{23}H_{31}N_4O_4S$ $(M+H)^+$: $m/z = 459$.

4.41. *N*-(*tert*-Butoxycarbonyl)-L-phenylalanyl-4-(5-benzyl-1,1-dioxido-4-oxo-1,2,5-thiadiazolidin-2-yl)-*N*-pentyl-L-phenylalaninamide (35)

Compound **35** was prepared from **34** and Boc-Phe-OH according to the procedure of **10c** in 78% yield. ^1H NMR (500 MHz, CD_3OD): δ 7.47 (d, $J = 6.9$ Hz, 2H), 7.36–7.10 (m, 12H), 4.88 (s, 2H), 4.61 (s, 2H), 4.55 (t, $J = 7.5$ Hz, 1H), 4.26 (dd, $J = 9.4$, 5.4 Hz, 1H), 3.13–2.98 (m, 5H), 2.80–2.75 (m, 1H), 1.40–1.38 (m, 2H), 1.38 (s, 9H), 1.34–1.22 (m, 4H), 0.91 (t, $J = 7.1$ Hz, 3H); LC–MS calcd for $C_{32}H_{40}N_5O_5S$ $(M(-Boc+H)+H)^+$: $m/z = 606$.

4.42. L-Phenylalanyl-4-(5-benzyl-1,1-dioxido-4-oxo-1,2,5-thiadiazolidin-2-yl)-*N*-pentyl-L-phenylalaninamide trifluoroacetate (36)

Compound **36** was prepared from **35** according to the procedure of **34** in 97% yield.

4.43. *N*-[(4-Methoxyphenyl)acetyl]phenylalanyl-4-(5-benzyl-1,1-dioxido-4-oxo-1,2,5-thiadiazolidin-2-yl)-*N*-pentylphenylalaninamide (37)

Compound **37** was prepared from **36** and 4-methoxyphenylacetic acid according to the procedure of **10c** in 61% yield. ^1H NMR (500 MHz, CD_3OD): δ 8.18 (t, $J = 8.3$ Hz, 2H), 7.87 (t, $J = 5.7$ Hz, 1H), 7.42–7.18 (m, 14H), 6.98 (d, $J = 8.8$ Hz, 2H), 6.76 (d, $J = 8.8$ Hz, 2H), 4.84 (s, 2H), 4.82 (s, 2H), 4.50–4.44 (m, 2H), 3.70 (s, 3H), 3.27 (dd, $J = 15.5$, 8.8 Hz, 2H), 3.03–2.97 (m, 4H), 2.83 (dd, $J = 14.0$, 8.6 Hz, 1H), 2.73 (dd, $J = 13.9$, 9.7 Hz, 1H), 1.41–1.38 (m, 2H), 1.36–1.33 (m, 2H), 1.26–1.20 (m, 2H), 0.86 (t, $J = 7.2$ Hz, 3H); LC–MS calcd for $C_{41}H_{48}N_5O_7S$ $(M+H)^+$: $m/z = 754$.

4.44. *N*-[(4-Methoxyphenyl)acetyl]phenylalanyl-4-(1,1-dioxido-4-oxo-1,2,5-thiadiazolidin-2-yl)-*N*-pentylphenylalaninamide (38)

A solution of **37** (30 mg, 40 μmol) in EtOH (5 mL) was treated with ammonium formate (170 mg, 2.7 mmol) and degassed. 10% Palladium on carbon (25 mg, 0.24 mmol) was added and the reaction mixture was stirred for 1.5 h at 60 °C, filtered, and the filtrate was concentrated to a residue which was purified by preparative LC–MS (CH_3CN/H_2O/TFA) to yield **38** (17 mg, 65%). ^1H NMR (500 MHz, DMSO-d_6, 30 °C): δ 8.08 (d, $J = 8.4$ Hz, 1H), 7.95 (d, $J = 8.6$ Hz, 1H), 7.75 (t, $J = 5.7$ Hz, 1H), 7.18 (m, 2 H), 7.16 (m, 2H), 7.14 (m, 1H), 7.09 (d, $J = 8.8$ Hz, 2H), 6.99 (d, $J = 8.7$ Hz, 2H), 6.96 (m, 2H), 6.75 (d, $J = 8.6$ Hz, 2H), 4.47 (m, 1H), 4.38 (m, 1H), 4.09 (s, 2H), 3.69 (s, 3H), 3.32 (d, $J = 14.2$ Hz, 1H), 3.25 (d, $J = 14.1$ Hz, 1H), 2.99 (m, 2H), 2.94 (dd, $J = 14.0$, 4.6 Hz, 1H), 2.88 (dd, $J = 13.7$, 5.7 Hz, 1H), 2.74 (m, 1H), 2.72 (m, 1H), 1.33 (m, 2H), 1.24 (m, 2H), 1.18 (m, 2H), 0.84 (t, $J = 7.0$ Hz, 3 H); ^{13}C NMR (125 MHz, DMSO-d_6, 30 °C): δ 170.7, 170.2, 170.1, 169.7, 157.7, 137.8, 137.1, 129.9, 129.8, 129.6, 128.9, 128.0, 127.6, 126.1, 115.8, 113.4, 54.7, 54.0, 53.7, 53.5, 40.9, 38.3, 37.0, 36.9, 28.3, 28.3, 21.7, 13.5; HRMS calcd for $C_{34}H_{41}N_5O_7SNa$ $(M+Na)^+$: $m/z = 686.2646$.

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Supplementary data

Analytical HPLC purity data for final products. Supplementary data associated with this article can be found in the online version, at doi:10.1016/j.bmc.2006.05.032.

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