Discovery of Potent, Selective, and Orally Active Carboxylic Acid Based Inhibitors of Matrix Metalloproteinase-13†

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The matrix metalloproteinase enzyme MMP-13 plays a key role in the degradation of type II collagen in cartilage and bone in osteoarthritis (OA). An effective MMP-13 inhibitor would therefore be a novel disease modifying therapy for the treatment of arthritis. Our efforts have resulted in the discovery of a series of carboxylic acid inhibitors of MMP-13 that do not significantly inhibit the related MMP-1 (collagenase-1) or tumor necrosis factor-α (TNF-α) converting enzyme (TACE). It has previously been suggested (but not proven) that inhibition of the latter two enzymes could lead to side effects. A promising carboxylic acid lead **9** was identified and a convergent synthesis developed. This paper describes the optimization of **9** and the identification of a compound **24f** for further development. Compound **24f** is a subnanomolar inhibitor of MMP-13 (IC_{50} value 0.5 nM and K_i of 0.19 nM) having no activity against MMP-1 or TACE (IC_{50} of >10000 nM). Furthermore, in a rat model of MMP-13-induced cartilage degradation, **24f** significantly reduced proteoglycan release following oral dosing at 30 mg/kg (75% inhibition, $p < 0.05$) and at 10 mg/kg (40% inhibition, $p < 0.05$).

Introduction

The matrix metalloproteinases (MMPs)[a] are a large family of zinc-dependent endopeptidases that can degrade the extracellular matrix,[1] including many of the key structural proteins that comprise cartilage. MMPs play an important role in the remodeling and repair of normal tissue and continue to be an interesting target for drug discovery.[2] However, MMP activity can lead to tissue destruction, a component of diseases such as tumor growth and metastasis, periodontal disease, and arthritis. MMPs are upregulated in osteoarthritis and are largely responsible for the destruction of collagens and proteoglycans in cartilage and bone, leading to loss of joint function. Because the preferred substrate of MMP-13 is type II collagen, MMP-13 is believed to play a key role in articular cartilage degradation.[3−5]

Extensive animal model data supports the role of MMP inhibition in the treatment of cartilage degradation. The hydroxamic acid **1**[6] (CGS 27023) demonstrated efficacy in a surgical partial lateral meniscectomy OA model in rabbits[7] and in spontaneous OA in guinea pigs. Another hydroxamic acid, **2**[8] (RS 130830), has shown efficacy in dog and rabbit models of OA. The carboxylic acid **3**[9] (BAY 12,9566) was effective in both dog and guinea pig meniscectomy models (Figure 1).

Efforts within the pharmaceutical industry to target MMPs as a therapeutic approach initially used broad-based hydroxamic acid inhibitors **1**, **4**[10] (cipemastat), **5**[11] (marimastat), and **6**[12] (AG3340). These approaches failed to demonstrate efficacy in the clinic primarily because of musculoskeletal side effects,[13−18] the exact nature of which is not completely clear.[19] These side effects are described for **5** as a dose-limiting symptomatic inflammatory polyarthritis.

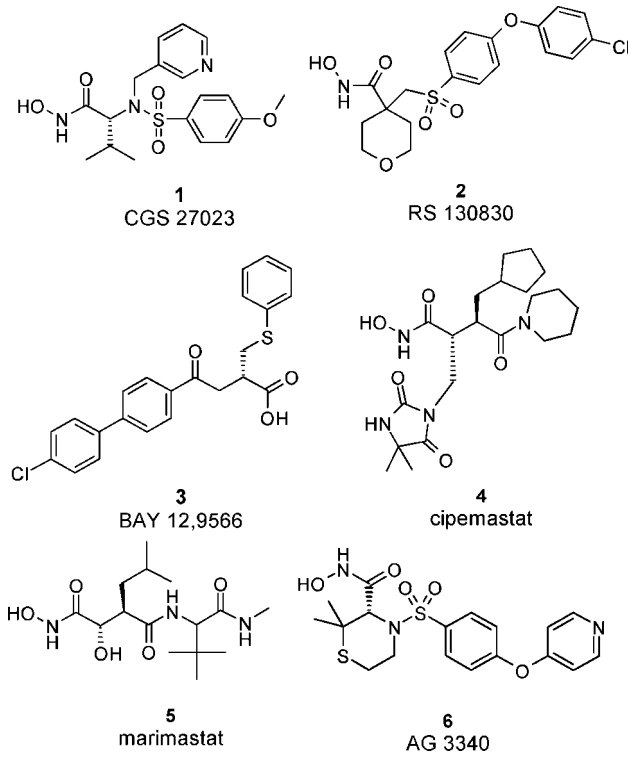

Figure 1. Structures of reference MMP inhibitors.

† The coordinates and X-ray data for the crystal structure of MMP-13 complexed with compound **24f** are available from the Protein Data Bank, deposition code is 3ELM.

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[a] Abbreviations: MMPs, matrix metalloproteinases; OA, osteoarthritis; IA, intra-articular; TACE, Tumor Necrosis Factor-α (TNF-α) converting enzyme.

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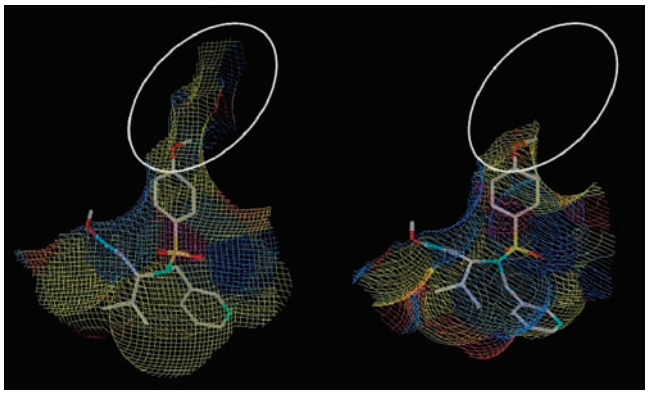


Figure 2. Compound **1** cocrystallized with MMP-13 (X-ray, left) and modeled into MMP-1 (right).

Two hypotheses were proposed to explain the musculoskeletal side effects observed in the clinic. The first hypothesis focused on the inhibition of TNF-α converting enzyme (TACE),[20−24] and/or other sheddases,[25] by hydroxamates **1**, **4**, and **5**.[26]

Another hypothesis attributed the musculoskeletal side-effects to nonselective inhibition within the MMP family. Specifically MMP-1, which is linked to normal tissue turnover and repair,[27] was implicated as an antitarget.

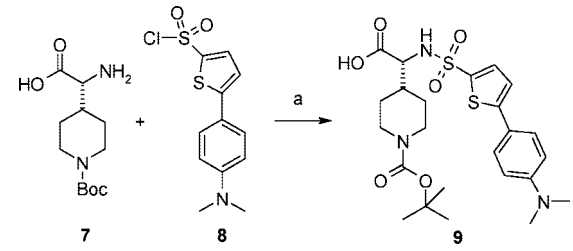
Considering the high affinity of the hydroxamate moiety for zinc relative to other coordinating moieties,[28] we hypothesized that selective inhibition of MMP-13 versus other metalloproteinases[29] (specifically TACE and MMP-1) could be achieved with alternate[30,31] or no[32] zinc-binding moiety. Thus, a primary goal of the current research was to replace the hydroxamic acid zinc-binding moiety common to **1**, **2**, and **4**−**6** in order to reduce the potential for pan-MMP and off-target sheddase inhibition.

Direct replacement of the hydroxamic acid zinc binder of **1** with a carboxylic acid moiety results in a 50-fold loss of potency versus MMP-13 (IC_{50}s from 1.9 to 89 nM), reflecting the relatively weaker affinity of the carboxylic acid for zinc. As is suggested by Figure 2, the open, predominantly hydrophobic (yellow) S1′ region (circled) within the X-ray structure of **1** complexed to MMP-13 can be filled with hydrophobic substituents. Indeed, installing hydrophobes to interact with the S1′ site of MMP-13 can generate carboxylic acid MMP-13 inhibitors having low nanomolar potency, regaining potency lost with the removal of the hydroxamic acid group. As the open S1′ region is absent in MMP-1, the addition of bulky substituents attenuates MMP-1 activity, giving selective MMP-13 inhibitors. The inhibitory potency of carboxylic acid MMP-13 inhibitors benefit from additional hydrophobic contacts with the S1′ region. However, many of these lipophilic carboxylates are completely inactivated in the presence of serum proteins, such as bovine serum albumin (BSA), as a result of both high lipophilicity and anionic character.[33−35] Modification of the side chain to the piperidine carbamate yielded orally active BOC-piperidinyl glycine derivative **9** (Scheme 1), which was less affected by BSA. Herein we report the optimization of **9**, leading to the discovery of potent, orally active, nonhydroxamic acid MMP-13 inhibitors lacking sheddase and MMP-1 activities.[36]

Chemistry. An efficient modular synthesis of compound **9** was developed by coupling an appropriate biarylsulfonyl chloride **8** and *N*-BOC-4-piperidylglycine **7** (Scheme 1).

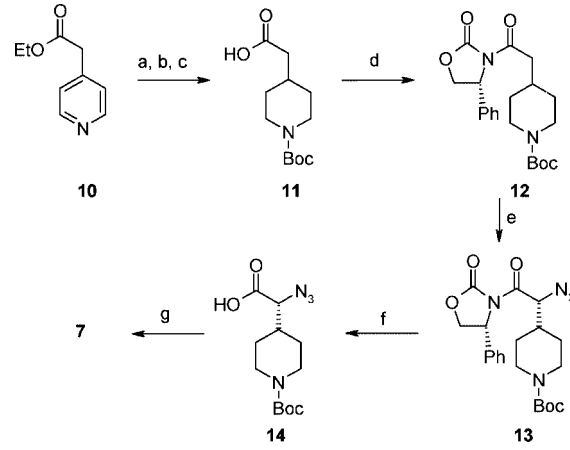
Although the unnatural amino acid **7** is commercially available, the quantity of material required necessitated a large-scale asymmetric synthesis as illustrated in Scheme 2. The efficient synthesis of 40 g of this amino acid **7** was accomplished in 38% overall yield with minimal chromatography. Ethyl

Scheme 1. Modular Synthesis of **9**[a]



[a] Reagents: (a) Et_3N, CH_2Cl_2.

Scheme 2. Synthesis of *R*-(−)-*N*-BOC-Piperidinyl Glycine[a]



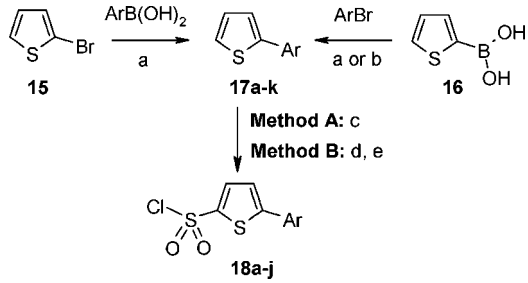
[a] Reagents: (a) H_2, PtO_2, 4 N HCl; (b) Na_2CO_3; (c) BOC_2O; (d) PivCl, THF, *n*-BuLi, (*R*)-4-phenyl-oxazolidin-2-one; (e) KHMDS, trisyl azide, THF, −78 °C to RT then HOAc, KOAc; (f) LiOH, H_2O_2, THF/H_2O; (g) H_2 Pd/C, EtOH/water.

4-pyridyl acetate **10** was converted into the *N*-BOC protected piperidine intermediate via a three-step, one-pot process. Thus, hydrogenation of the pyridine using Adam's catalyst provided the piperidine acetate with partial concomitant hydrolysis of the ethyl ester. Complete hydrolysis was effected by the addition of sodium carbonate. Reaction with di-*t*-butyl dicarbonate afforded the BOC-protected piperidyl glycine **11** in 70% overall yield. The carboxylic acid was activated by the addition of pivaloyl chloride and then reacted with the lithium salt of the Hruby oxazolidinone derived from L-phenylglycine to afford acyl oxazolidinone **12**.[37] Asymmetric introduction of the α-azido moiety of **13** was accomplished by reaction of the potassium enolate with trisyl azide. [Use of the commercially available material was not optimal due to the presence of trisyl chloride in this material. Instead, trisyl azide was prepared by reaction of trisyl chloride with sodium azide in acetone.]

Lithium peroxide-mediated removal of the chiral auxiliary provided the α-azido acid **14** as a white crystalline solid in 81% yield from **13**. Conversion to the desired amino acid **7** was achieved by hydrogenation of the azide using 10% palladium on charcoal. The product could be used for the conversion into the desired sulfonamides without further purification.

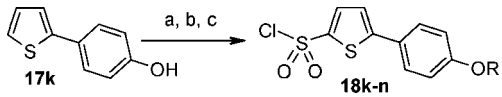
The sulfonyl chlorides required for the convergent synthesis of target compounds were prepared using routes outlined in Schemes 3−6. From either 2-bromo thiophene **15** or thiophene-2-boronic acid **16**, Suzuki couplings[38] were used to prepare the thiophene-phenyl intermediates **17**. Coupling of thiophene 2-boronic acid **16** to haloarenes at room temperature afforded the most convenient and flexible protocol. Chlorosulfonylation of the thiophenes **17** was effected directly using excess $ClSO_3H$ or by selective 2-lithiation of the thiophene, followed by trapping

Scheme 3. General Synthesis of Thiophene Sulfonyl Chlorides[a]



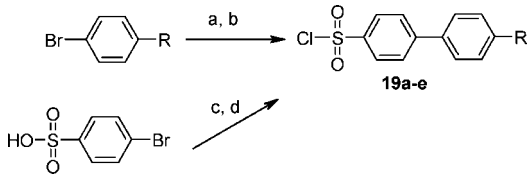
[a] Reagents: (a) $PdCl_2$(dppf), DME; (b) $Pd_2(dba)_3$, (t-Bu)$_3$P, KF, THF; (c) $ClSO_3H$; (d) n-BuLi, SO_2 (g); (e) chlorination with NCS, SO_2Cl_2, or PCl_5.

Scheme 4. Synthesis of 4-Ethers[a]



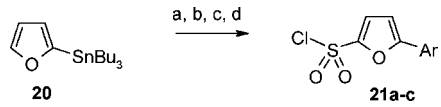
[a] Reagents: (a) PPh_3, ROH, DEAD; (b) n-BuLi, SO_2 (g); (c) NCS.

Scheme 5. General Synthesis of Biphenyl Sulfonyl Chlorides[a]



[a] Reagents: (a) $PhB(OH)_2$, $PdCl_2$(dppf); (b) $ClSO_3H$; (c) $RPhB(OH)_2$, $PdCl_2$(dppf); (d) oxalyl chloride, DMF.

Scheme 6. Stille Coupling to Furan Sulfonyl Chlorides[a]



[a] Reagents: (a) $Pd(PPh_3)_4$, PhMe, 4-bromotrifluoro-methylbenzene; (b) n-BuLi, −78 °C; (c) SO_2 (g); (d) NCS, CH_2Cl_2.

with gaseous SO_2 and subsequent chlorination to afford sulfonyl chlorides **18a**−**j**.

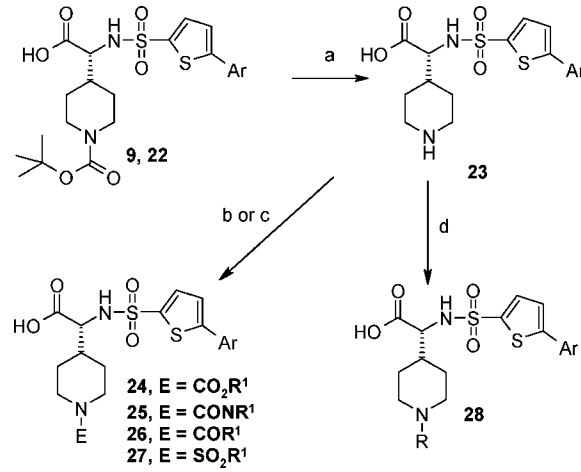
Introduction of selected ethers was conducted by Mitsunobu reaction prior to formation of the sulfonyl chloride (Scheme 4). Phenol **17k**, generated according to Scheme 3, was treated with PPh_3 and DEAD in the presence of an alcohol to afford the desired ether. Selective lithiation of the thiophene at the 2-position, followed by trapping with gaseous SO_2, afforded the corresponding 2-sulfonic acid. Conversion to the sulfonyl chloride was effected by treatment with a chlorinating agent, such as NCS.

Suzuki coupling between phenyl boronic acid and an appropriately substituted bromobenzene, followed by chlorosulfonylation, gave biphenyl sulfonyl chlorides **19** (Scheme 5). Alternatively, sulfonyl chlorides **19** were generated by Suzuki coupling of commercially available 4-bromobenzene sulfonic acid with the requisite aryl boronic acid, followed by chlorination.

Availability of the 2-(tri-*n*-butylstannyl) furan **20** enabled the Stille coupling for the synthesis of the required furan analogues (Scheme 6). As above, selective lithiation of the furan, trapping with SO_2 (g), and chlorination yielded the desired furyl sulfonyl chlorides **21a**−**c**.

By the standard method outlined in Scheme 1, sulfonyl chloride fragments were coupled to amino acid **7** using

Scheme 7. General Approach to BOC-Replacements[a]



[a] Reagents: (a) HCl, CH_2Cl_2; (b) NMM (3 equiv), DMF, electrophile; (c) bis-(trimethylsilyl)acetamide (4 equiv), THF, electrophile; (d) NaH-B(OAc)$_3$, THF, RCHO.

triethyl amine in CH_2Cl_2 to give sulfonamides in generally acceptable yields. These approaches allowed access to compounds **9** and **22** containing a BOC group on the piperidine nitrogen (Scheme 7).

Analogues lacking the BOC were prepared by treating **9** and **22a**−**g** with anhydrous HCl in CH_2Cl_2. Treatment of the resultant piperidine **23** with an appropriate electrophile yielded the desired analogues as illustrated in Scheme 7. In several cases, the reaction between **23** and highly reactive electrophiles, such as chloroformates, led to adducts incorporating more than one electrophile.

Because **23** contains three potential nucleophiles, a method was needed to better differentiate the piperidine nucleophile. The poor organic solubility of the starting material relative to the acylated products disfavored the production of the desired monoacylated material. In theory, in situ polysilylation of **23** could afford the silylated ester, sulfonamide, and piperidine, thereby masking the nucleophilic character of all but the piperidine. Simultaneously, the silyl ester and silyl piperidine effectively mask the zwitterionic character deleterious to organic solubility. In accordance with the above hypothesis, treatment of compounds **23** with excess silylating agent improved solubility in THF. Monocarbamoylation of compounds **23** was achieved by treatment with excess bis(trimethylsilyl)-acetamide and subsequent treatment with a chloroformate electrophile to afford **24**.[39] We found general use for the silylation technique to aid with solubilizing the starting material, even in cases where the electrophiles used were not chloroformates, to afford ureas **25**, amides **26**, and sulfonamides **27**. Also, alkyl piperidine analogues **28** were readily accessed from piperidine **23** by Borch reduction. For large-scale production, these extra steps could be avoided by use of the appropriately derivatized amino acid.

Results and Discussion

Starting Point for Optimization. Previous in-house attempts to identify carboxylic acid MMP inhibitors yielded comparatively lipophilic compounds with MMP-13 IC_{50}s between 5 and 100 nM lacking in vivo activity in the IA models. Further, the inhibitory activity of these compounds against MMP-13 shifted to above 5 μM in the presence of serum proteins. This contrasts with hydroxamic acid MMP inhibitors, which retained in vitro activity in the presence of serum proteins and were active in vivo. As a result of the empirical observation that activity in

Table 1. Initial Carboxylic Acid Leads and Hydroxamic Acid Based MMP-13 Inhibitors

	IC_{50} (nM)			rat IA % inh[a]
compd	MMP-13	MMP-13 w/BSA	MMP-1	(30 mg/kg po)
1	1.9	4.4	42	73 ± 18 (12)
4	3.5	4.7	2.3	70 ± 17 (31)
2	0.3	2	356	99 ± 2 (40)
9	0.3	12	3350	77 ± 17 (17)

[a] Results are expressed as mean ± SD (*n*), where *n* is the number of animals, 4 h post 30 mg/kg dose in 20% PEG400/80% CMC (0.5% CMC containing 0.25% Tween 80) at 10 mL/kg.

the presence of serum proteins was important for activity in the IA model, the in vitro MMP-13 assay was routinely carried out both in the presence and absence of 1% bovine serum albumin (BSA). [Early in our work, a subset of compounds was tested in the presence of human, rat, and rabbit serum and the results were similar to those with BSA. Given the easy availability of BSA, we continued to use it routinely.] In this context, we discovered that suitably substituted piperidinyl glycine analogues exhibited enhanced inhibition of MMP-13. The first of the series, compound **9** (MMP-13 IC_{50} < 1 nM), mimics hydroxamic acids **1**, **2**, and **4** with respect to in vitro potency and especially in vivo efficacy (see Table 1).

At screening doses of 30 mg/kg po, all four compounds inhibited MMP-13 degradation of rat IA cartilage similarly. This model is a version of the rabbit intra-articular (IA) injection model,[7] which had been successfully used to profile compounds. The model was adapted for rats to allow for more convenient profiling, and the high capacity and rapid turnaround made the resulting model the key driver of our optimization efforts. In this modified model, MMP-13 was injected into knee joints of rats that had been treated with test compounds, and inhibition of proteoglycan breakdown, as measured by chondroitin sulfate ELISA, was then measured.

It should also be noted that the biaryl hydroxamate **2** and carboxylate **9** show, respectively, 1000- and 10000-fold selectivity for MMP-13 over MMP-1. This is consistent with the larger S1′ pocket of MMP-13 relative to that of MMP-1.[40] The added hydrophobic contact contributes to the hydroxamate **2** and carboxylic acid **9** having 5- to 10-fold better MMP-13 potency than the hydroxamates **1** and **4**. However, carboxylate **9** shows a 40-fold loss of MMP-13 potency in the presence of as little as 1% BSA, eliminating any improvement in intrinsic MMP-13 potency relative to **1**. Similarly, the biphenyl ether hydroxamic acid **2** shows an approximately 10-fold loss of MMP-13 inhibition when 1% BSA is present, whereas the smaller hydroxamates **1** and **4** are unaffected by the addition of serum. Carboxylic acids are known to bind to plasma proteins,[41] and this can be particularly extensive for lipophilic compounds. A consistent trend for the carboxylates to lose potency in the presence of serum was verified with serum drawn from other species.

While compound **9** is a selective MMP-13 inhibitor and has the desired in vitro and in vivo activity, it was considered to pose an unacceptable risk for development due to its dimethyl aniline moiety, which is known to be metabolically dealkylated in vitro to a potential carcinogenic amine.[42,43] The BOC group was also targeted for replacement to reduce potential instability problems during manufacture and use. Thus, the optimization of **9** focused on the removal/replacement of the 4-dimethylamino group, exploration of different substituents on the piperidine nitrogen, and variation of the biaryl group. The overall goal was to maintain the in vitro potency, selectivity (MMP-13 over

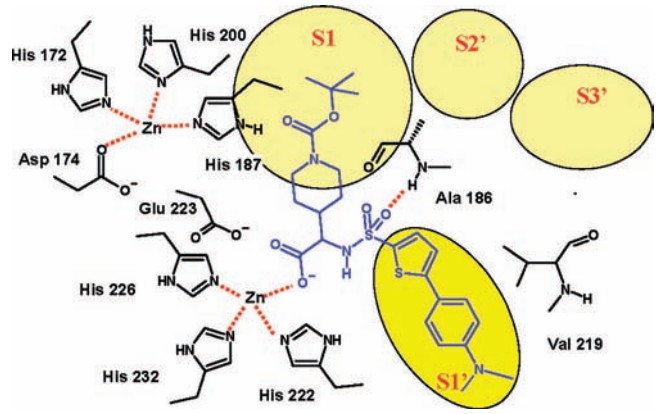
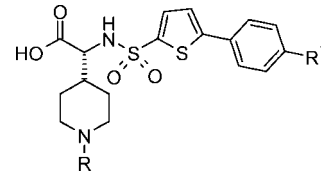


Figure 3. Predicted binding of **9** indicating binding into the S1 and S1′ pockets.

Table 2. Pairwise Changes to the BOC and *N*,*N*-Dimethylamine Moieties



			IC_{50} (nM)		
compd	R	R^1	MMP-13	MMP-13 w/ BSA	MMP-1
9	*t*-BuO_2C	Me_2N	0.3	12	3350
23	H	Me_2N	11	47	>10000
22a	*t*-BuO_2C	1-piperidinyl	0.75	63	>10000

MMP-1 and TACE), and the in vivo activity of **9** in the absence of the BOC and dimethyl aniline moieties.

Optimization of Binding in the S1′ Pocket. Figure 3 illustrates the key residues and interactions in the MMP-13 active site. The carboxylate binds the catalytic zinc atom and the biaryl portion of the molecule extends into the S1′ pocket, where it makes significant interactions. Interestingly, the *tert*-butyl carbamate extends into the S1 pocket, potentially making additional hydrophobic interactions. Hence our strategy was to exploit this observation and scan other derivatives that could benefit from this binding interaction as well as the aforementioned physical property considerations.

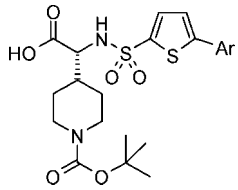
Removal of the BOC group reduced the activity in the enzyme assay by 30-fold (Table 2). Piperidine **23** also exhibited a lower fold loss of MMP-13 inhibitory activity in the presence of serum albumin, lower plasma levels 4 h after a 30 mg/kg po dose, and no efficacy in the rat IA model. Replacing the *para*-dimethylamino moiety with a 1-piperidinyl group (**22a**), which is noted in some cases to reduce carcinogenetic potential,[44] preserved the subnanomolar potency and in vivo activity of **9**.

To increase the hydrophilicity of the series, we explored substitutions of the terminal dimethylaminophenyl moiety. Removal of the *para*-dimethylamino group (**22b**) reduced the MMP-13 potency by more than 1 order of magnitude (Table 3).

In general, *ortho*- or *para*- substitution of the phenyl ring was poorly tolerated (**22c**,**d**). Replacing the terminal phenyl group with pyridine (**22e**−**g**) decreased in vitro activity further. However, incorporating nitrogen reduced the loss in MMP-13 inhibitory activity in the presence of BSA from 1000-fold (**22b**) to less than 100-fold (**22e**−**g**).

Unfortunately, the most potent pyridyl analogue **22f** exhibited poor activity after oral dosing in the rat IA model. The

Table 3. Replacement of Dimethylaminophenyl Moiety



compd	Ar	IC_{50} (nM) MMP-13	MMP-13 w/BSA	MMP-1	rat IA%inh (30 mg/kg po)
22b	Ph	6.8	7200	3600	42
22c	3-EtOPh	1058	14500	>10000	nd
22d	3,4-diMeOPh	575	>10000	>10000	nd
22e	4-pyr	90	1975	>10000	nd
22f	2-pyr	18	1850	>10000	0
22g	2-MeO-5-pyr	50	2500	>10000	nd

unsubstituted phenyl compound **22b** had superior in vitro activity relative to the pyridine containing compounds **22e**−**g**. Replacements for the inner aryl ring are summarized in Table 4. Fused aromatic derivatives, such as benzofuran **29** and benzothiophene **30**, resulted in significant loss of activity (>100-fold). By contrast, replacing the thiophene moiety of **9** with phenyl, produced an equipotent compound **31**. Similarly, biphenyls **32**−**35** are potent MMP-13 inhibitors, with the alkoxy and dialkylamino analogues exhibiting the best potencies in the presence of BSA. While phenyls were found to be potent, furans were much less active in vitro (e.g., **36**−**38**).

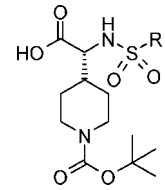
By analogy to their respective in vitro potencies, compounds **31** and **34** gave 98% and 85% inhibition of proteoglycan degradation in the rat IA model, whereas fluoride **33** achieved only 18% inhibition. Therefore, the data suggests that the substituent on the terminal phenyl ring is important for both in vitro and in vivo potency.

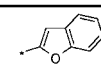
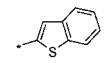
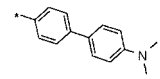
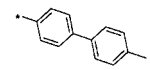
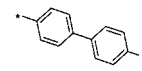
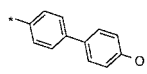
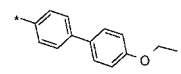
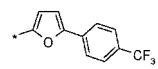
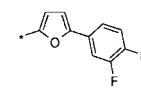
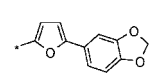
To reduce the potential for hydrolysis of the *tert*-butyl carbamates **9** and **22a** at gastric pH, the *iso*-propyl carbamate was employed as a uniformly effective method for maintaining in vitro potency. *iso*-Propyl carbamate **24a** exhibits 1 nM potency for MMP-13, similar to BOC analogue **22a** (Table 2), although the activity in the rat IA model is lost. In this context, we varied the 4-substituent of the phenyl ring (Table 5). Ether-containing analogues trended toward better potency, versus MMP-13, than nonether analogues. Further, the fold-loss of activity in the presence of BSA was comparatively smaller for the ether subseries (∼100×) versus analogues **24b**, toluene **24j**, and trifluoromethane **24k** (∼1000×). Last, the methyl **24d** and ethyl ethers **24f** gave the best results in vivo.

Replacements for the BOC Group. To further optimize the piperidine nitrogen substituent, a range of linear and branched carbamates were prepared while maintaining the 4-ethoxyphenyl substituent of **24f** above. Generally, carbamates had MMP-13 IC_{50}s below 1 nM with selectivity over MMP-1 (Table 6). Little change in MMP-13 potency with or without BSA was observed across the series. Several of these compounds exhibited MMP-13 IC_{50} values less than 100 nM in the presence of BSA, with the analogues **24q**, **24s**, **24t**, and **24u** trending toward the best activities. The in vivo potency in the rat IA model was similar for the most efficacious compounds (**24m**, **24q**, **24s**, and **24t**), with the striking exception of the THP analogue **24u**. Confirming that MMP-13 activity in the presence of BSA was necessary, but not sufficient, for in vivo activity.

Having explored the SAR of the carbamate series, we explored noncarbamate substituents on the piperidine nitrogen (Table 7). Neutral analogues, such as ureas **25**, produced

Table 4. Additional Aromatic Ring Replacements



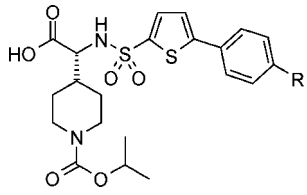
Cmpd	R	MMP-13 IC_{50} (nM)	MMP-13 IC_{50} w/BSA (nM)
29		1325	>10000
30		145	>10000
31		0.3	9
32		0.7	72
33		2.4	48
34		0.5	11
35		0.5	9.8
36		61.5	>10000
37		9000	>10000
38		197	>10000

excellent in vitro MMP-13 potency. However even **25c**, the closest analogue of carbamate **24f** above, failed to inhibit MMP-13-induced cartilage degradation at a 30 mg/kg po dose.

Likewise amides **26** and sulfonamides **27** lacked activity in vivo despite promising results in vitro. Of the alkylated piperidines **28**, only **28a** gave modest in vivo activity. Consequently, carbamate **24f** was selected for further in vivo profiling.

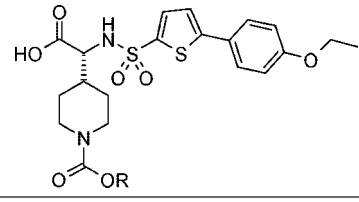
Activity in the rat IA model drove compound optimization in the work reported here and the high capacity and rapid turnaround of the model allowed in vivo testing of potent MMP-

Table 5. 4-Phenyl Substitutions



compd	R	IC$_{50}$ (nM) MMP-13	MMP-13 w/ BSA	MMP-1	rat IA % inh (30 mg/kg po)
24a	1-piperidinyl	1.3	100	>10000	0 ± 13
24b	H	12.1	11290	3230	45 ± 13
24c	OH	3.3	300	680	22 ± 18
24d	OMe	0.6	68	950	70 ± 6
24e	OCF_3	2.2	1800	nd	21 ± 16
24f	OEt	0.5	37.8	4540	81 ± 17
24g	O^{n}Pr	0.8	57	>10000	23 ± 15
24h	O^{i}Pr	4.0	390	>10000	19 ± 11
24i	$O(CH_2)_2OMe$	0.9	25	>10000	33 ± 11
24j	CH_3	1.3	1160	2020	37 ± 16
24k	CF_3	3.9	4475	6370	22.5 ± 12

Table 6. SAR of Carbamate Analogues



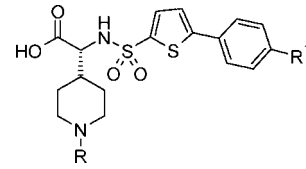
compd	R	IC$_{50}$ (nM) MMP-13	MMP-13 w/ BSA	MMP-1	rat IA % inh (30 mg/kg po)
24l	Me	3.5	242	>10000	22 ± 21
24m	Et	2.0	142	>10000	59 ± 7
24n	^{n}Pr	1.0	81.2	9330	36 ± 11
24o	^{n}Bu	0.8	90	>10000	48 ± 16
24p	^{i}Bu	0.6	105	>10000	40 ± 14
24q	$CHEt_2$	0.4	60	>10000	78 ± 13
24r	$CH_2{}^t$Bu	0.9	172	>10000	16 ± 16
24s	^{t}Bu	0.6	14.4	>10000	78 ± 10
24t	cyclopentyl	0.5	60.6	7050	66 ± 10
24u	4-THP	0.4	7.0	1740	19 ± 11

13 inhibitors in an efficacy model integrating diverse biophysical parameters. Plasma drug levels were however obtained at 4 and 6 h postdose for all compounds tested in the model. Low plasma exposure (≤200 nM at 4 h, data not shown) likely contributed to the poor in vivo activity observed for MMP-13 inhibitors lacking the urethane moiety, such as ureas **25**, amides **26**, sulfonamides **27**, and amines **28** listed in Table 7.

Characterization of Compound 24f: In Vitro Profile. Kinetic and thermodynamic parameters for **24f**, **2**, **1**, and **4** are presented in Table 8. Consistent with the MMP-13 inhibition, the equilibrium constant measured for carboxylate **24f** and hydroxamate **2**, both competitive MMP-13 inhibitors, is 1 order of magnitude lower than that of the pan-MMP hydroxamic acid comparators **1** and **4**. The lower K_i value for **24f** results from an association rate (5×10^6 M^{-1} s^{-1}) 10 times faster than that observed with the comparator hydroxamic acids. Although reversible, both classes of compounds are tight binding inhibitors with extremely slow off rates. Thus, all can be classified as pseudoirreversible inhibitors.

Compound **24f** is a subnanomolar inhibitor of MMP-13 activity with an IC$_{50}$ value of 0.5 nM. It is 3−7 times more potent against MMP-13 than the hydroxamic acids **4** and **1**.

Table 7. Piperidine Substitutions



compd	R	R^1	IC$_{50}$ (nM) MMP-13	MMP-13 w/ BSA	MMP-1
25a	CONH^{t}Bu	OMe	1.4	110	1290
25b	CONH^{i}Pr	OMe	1.6	19.5	770
25c	CONH^{i}Pr	OEt	0.8	13.5	6560
25d	$CONEt_2$	OMe	2.8	212	2300
26a	CO^{i}Pr	OMe	16.0	245	4180
26b	CO^{t}Bu	OEt	8.0	250	>10000
26c	$COCH_2{}^t$Bu	OEt	3.6	163	>10000
26d	COcyclohexyl	OEt	0.9	79.5	>10000
26e	CO-4-THP	OEt	1.7	20	>10000
27a	SO_2Ph-4-Me	OMe	1.4	65	4300
27b	SO_2Ph	OMe	1.2	580	650
27c	SO_2Ph	OEt	0.7	181	12900
27d	SO_2CH_2Ph	OEt	0.7	25	>10000
27e	$SO_2{}^i$Bu	OEt	5.5	230	>10000
27f	$SO_2{}^n$Bu	OEt	4.0	107	>10000
27g	$SO_2{}^i$Pr	OEt	4.7	280	>10000
27h	$SO_2{}^n$Pr	OEt	6.9	143	>10000
27i	SO_2Me	OEt	12.7	123	>10000
28a	Bn	OEt	7.6	65	>10000
28b	^{i}Bu	OEt	28	188	>10000
28c	$CH_2{}^t$Bu	OEt	49	325	>10000

Table 8. MMP-13 Dissociation Constants for Compound **24f**, **2**, **4**, and **1**

compd	K_i (nM)
24f	0.19
2	0.28
4	0.53
1	1.27

Table 9. Partial Metalloproteinase Profile of Compound **24f**, **2**, **1**, and **4**

IC$_{50}$ (nM)	**24f**	**2**	**1**	**4**
MMP-1	>10000	347	42	2.3
MMP-2	0.2	0.3		
MMP-3	18	7.1	19	87
MMP-7	3025	3100	736	18.5
MMP-9	10	2.0	6.8	2.4
MMP-13	0.5	0.3	2.9	3.5
MMP-14	91	14	7.5	23
TACE	>1000	440	40.9	230.3

Compound **24f**, like most of the compounds described in the present study, did not inhibit MMP-1 (IC$_{50}$ values >10000 nM) or TACE (>1000). The high selectivity for MMP-13 over MMP-1 suggests that inhibition of MMP-1 will not be significant in vivo at concentrations needed to effectively inhibit MMP-13. The selectivity within the MMP family contrasts that of **1** and **4**, which have dual MMP-13 and MMP-1 activity and greater activity for TACE (Table 9). Achieving the goals of the present study, compound **24f** is a subnanomolar competitive inhibitor of MMP-13 (IC$_{50}$ 0.5 nM, K_i of 0.19 nM) showing greater than 20000-fold selectivity for MMP-13 over MMP-1 and no inhibition of TACE activity.

X-ray crystal structure determination of compound **24f** in MMP-13 confirmed our initial hypothesis, that biaryl carboxylic acids such as **9** benefited from extensive interactions with the S1′ binding pocket. Also the *iso*-propyl carbamate of **24f** makes an additional hydrophobic interaction with a

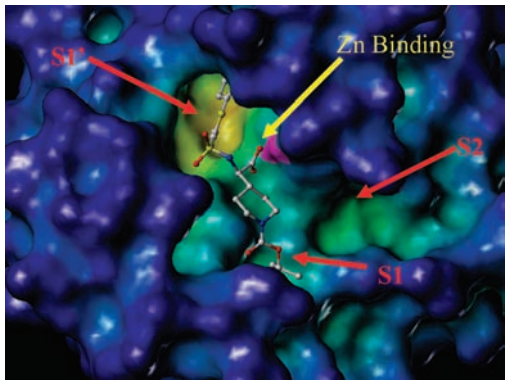


Figure 4. X-ray structure of **24f** indicating binding into the S1 and S1′ pockets.

small depression at the base of the S1 pocket (as indicated by the arrow in Figure 4).

In addition to MMP-1 and -13, other members of the MMP family, including MMPs-3, -7, -9, and -14, are implicated in the degradation of cartilage matrix in OA.[45−49] Thus, the inhibitory activity of **24f** and hydroxamate comparators were evaluated against a panel of MMP isozymes. Of the MMP isozymes implicated in cartilage degradation, acid **24f** inhibits MMPs-13 (IC_{50} 0.5 nM), -3 (IC_{50} 18 nM), -9 (IC_{50} 10 nM), and -14 (IC_{50} 91 nM), while sparing MMPs -1 and -7 (IC_{50}s >10000 and 3000 nM, respectively). The inhibitory activities of **24f** against MMPs -3 and -9 were similar to that of **2**, **1**, and **4**. Carboxylate **24f** shows appreciably weaker MMP-7 activity than **4** and weaker MMP-14 activity than **1**. In addition to the primary target MMP-13, compound **24f** exhibits significant activity toward MMPs -3, -9, -13, and -14, a subset of MMPs implicated in cartilage degradation. Carboxylate **24f** exhibits weaker MMP-1 and TACE activity (IC_{50}s >10000 nM and >1000 nM, respectively) by comparison to all the hydroxamates, while achieving equivalent or better MMP-13 potency.

Because the enzyme assays in these studies utilized synthetic peptides as substrates, it was important to confirm the activity of compounds against the native substrate and, where possible, in a more physiologically relevant setting. To determine the efficacy of MMP-13 inhibitors in blocking the degradation of type II collagen, an in vitro model using bovine nasal cartilage explants was established. The cartilage explants were treated with a combination of two cytokines found in arthritic joints, IL-1α and oncostatin M, to induce production of endogenous MMPs from chondrocytes and the degradation and release of type II collagen fragments into the tissue culture fluid.

Acid **24f** inhibited type II collagen degradation and release of hydroxyproline by greater than 98% ($p < 0.05$) at 200 and 2000 nM, with only very small amounts of hydroxyproline being detected in the culture media (Figure 5). Compound **24f** was as potent as the hydroxamic acid **1** in this in vitro cartilage degradation assay where MMP-13 degrades its native substrate under physiologically relevant conditions.

Characterization of Compound 24f. In vivo Parameters. The pharmacokinetic time course following oral doses of **24f** is represented in Figure 6, and the corresponding parameters are summarized in Table 10. In the conscious rat model used, the absolute oral bioavailability of **24f** was 46.6%. Compound **24f** was rapidly absorbed with the C_{max} of 0.58 μg/mL at 0.56 h. From the intravenous dose, the mean terminal half-life was 7.0 h and the clearance was high (112 mL/min/kg). Further compound optimization will target higher sustained plasma drug levels, however, previous hydroxamic acid MMP inhibitors, such as **1**, have shown in vivo activity even after plasma drug levels have dropped close to or below the limits of detection. Given that compound **24f** is a pseudoirreversible inhibitor, further studies may ultimately show a longer duration of action than predicted by plasma drug levels alone.

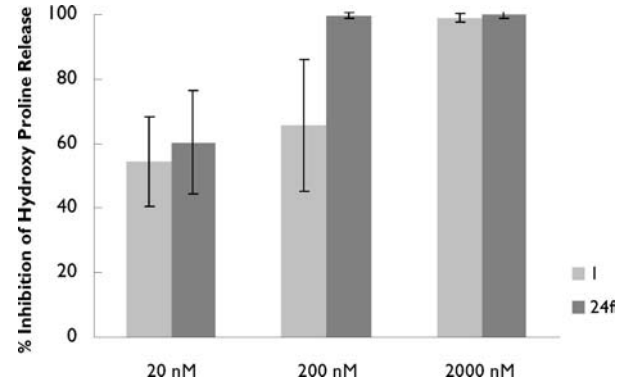


Figure 5. Inhibition of the release of hydroxyproline from bovine nasal cartilage, stimulated with IL-1/oncostatin M, by compound **24f** and **1**. The amount of hydroxyproline released by the stimulated and unstimulated cartilage, in the absence of inhibitor, is taken as being equivalent to 0% and 100% inhibition.

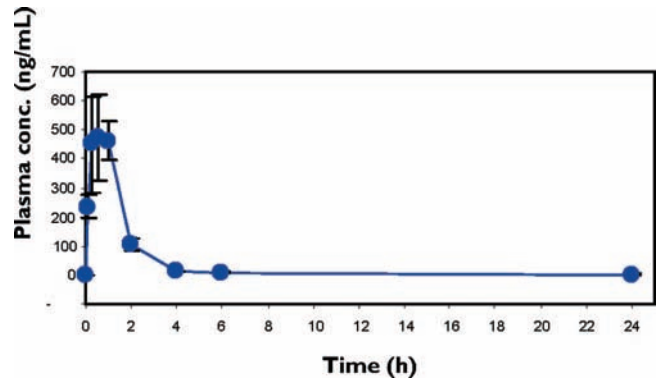


Figure 6. Pharmacokinetic time course for **24f** following oral (10 mg/kg) dosing in 20% PEG400/80% CMC (0.5% CMC containing 0.25% Tween 80).

Table 10. Summary of Pharmacokinetic Parameters for **24f**

parameter	value
C_{max} (μg/mL)	0.58 ± 0.14
T_{max} (h)	0.56 ± 0.16
Terminal $t_{1/2}$ (h)	7.0 ± 4.6
CL (mL/min/kg)	111.8 ± 30.0
V_{SS} (l/kg)	23.7 ± 13.0
$AUC_{(0-\infty)}$ po[a] (μg/mL·h)	0.09 ± 0.01
$AUC_{(0-\infty)}$ iv[a] (μg/mL·h)	0.20 ± 0.06
BA (%)	46.6 ± 4.8

[a] Results are expressed as the mean ± SEM, $n = 4$. Dose normalized.

In the in vivo rat model of MMP-13-induced cartilage degradation (Figure 7), **24f** reduced proteoglycan release significantly compared to the vehicle control following oral dosing at 100 mg/kg ($p < 0.05$), 30 mg/kg ($p < 0.05$), and 10 mg/kg ($p < 0.05$). The inhibition of 79% at 30 mg/kg and 45% at 10 mg/kg achieved with **24f** was comparable to that attained by **4**. However, biaryl hydroxamate **2** gives maximal inhibition at the lower 10 mg/kg dose. Therefore, **24f** compared favorably with respect to efficacy and selectivity/side effect potential with

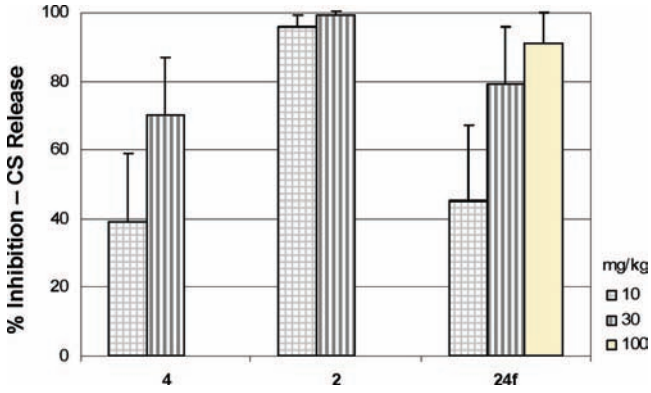


Figure 7. Effect of compounds **4**, **2**, and **24f** on the rat IA MMP-13 model.

4 but showed 10-fold greater compound requirement (100 mg/kg vs 10 mg/kg) relative to hydroxamate **2** to achieve equivalent efficacy.

Conclusion

Orally active carboxylic acid-derived MMP-13 inhibitors with subnanomolar potency were identified and tested in a rat intra-articular injection model. Greater than 20000-fold in vitro selectivity for MMP-13 over MMP-1 was achieved with these compounds. Carboxylic acids such as **24f** are as potent as hydroxamic acids in a bovine nasal cartilage explant model and an in vivo model of MMP-13 activity. This is the first time that carboxylic acid based MMP inhibitors have been shown to be equipotent to hydroxamic acid based inhibitors in models of cartilage protection.

Experimental Section

Determination of MMP-1,-3, and -9 Activity. Stock enzyme solutions (345 μg/mL) were diluted (1:125 for MMP-1, 1:1250 for MMP-3, and 1:555 for MMP-9) in assay buffer (20 mM Tris at pH 7.5 containing 0.15 M NaCl, 10 mM $CaCl_2$, 0.05% Brij-35, and 0.02% NaN_3) to obtain MMP buffer solutions. A 50 μL aliquot of a stock inhibitor solution (10 mM in DMSO) was added to 450 μL of DMSO in deep well plates. Test compounds were serially diluted 1:10 in deep well plates to obtain 10, 1, and 0.1 uM final concentrations. A 108 μL aliquot of one of the inhibitor solutions was transferred to a Biomek deep well plate containing 12 μL of a MMP-1, -3, or -9 buffer solution described above. After 30 min at room temperature, 90 μL of the inhibitor enzyme mixture was transferred to a 96-well black plate containing 10 μL of a solution of the NFF-2 substrate (Mca-RPKPYA-Nva-WM-K(Dnp)-NH_2).[50] The NFF-2 substrate solution was prepared fresh daily from a 4% of 4 mM NFF-2 solution (cat. no. SMO-3167-v, Peptide International), 8% DMSO, and 88% assay buffer. The increase in fluorescence (λ_{ex} = 340 nm; λ_{em} = 400 nm) was measured using the Cytofluor between 5 and 60 at 5 min intervals. The % inhibition (%*I*) was defined as below where *B* is the slope in the linear region and *C* is the inhibitor concentration giving 50% inhibition (IC_{50}).

$$\%I = \frac{100\%}{1 + (x/C)^B}$$

Determination of MMP-13 Activity. A stock enzyme solution (345 μg/mL) was diluted 1:400 in assay buffer (20 mM Tris at pH 7.5 containing 0.15 M NaCl, 10 mM $CaCl_2$, 0.005% Brij-35, and 0.002% NaN_3). Stock inhibitor solution (10 mM in DMSO) was diluted to 100 μM in DMSO and then 7.5 μL was diluted to 300 μL in assay buffer to yield a 2.5 μM inhibitor solution. The final inhibitor solution was diluted serially 1:3 in the assay buffer. In each well of a 96-well microtiter plate (Immunofluor B, Dynatech), 50 μL of the inhibitor solution and 50 μL of the diluted enzyme were combined at room temperature. After 10 min, the activity was assayed by adding 25 μL/well of assay buffer containing 8 μM MCA-Pro-Leu-Gly-Leu-Dpa-Ala-Arg-$CONH_2$ (1 μL of 1 mM stock solution in DMSO; Peptides International, Louisville, KY) and continuously monitoring the increase in fluorescence (λ_{ex} = 380 nm; λ_{em} = 440 nm) of the hydrolysis product, MCA-Pro-Leu. The enzyme activity was defined as the change in fluorescence value measured in a 6 min interval divided by 6. The IC_{50} value was determined by plotting % inhibition of activity vs inhibitor concentration.

Inhibition of Type II Collagen Degradation in Bovine Nasal Explants. Bovine nasal explants were stimulated with rh-IL-1α and rh-OSM in media, in the presence or absence of an inhibitor at various concentrations, for 5 or 6 days. After incubation, the medium was harvested and replaced with fresh stimuli and inhibitors. On day 11, the experiment was terminated by harvesting media and digesting the remainder of cartilage with papain. The harvested fluid and papain digested cartilage were then subjected to HyPro analysis. The percent inhibition of type II collagen degradation was calculated by the following formulas:

$$\%\ \text{inhibition} = 100 - (100\%\ \text{HyP released in drug treated sample}/\%\ \text{HyP released in the IL-1 plus OSM treated sample})$$

where % HyP released = 100(μg HyP in the media/μg HyP in the media + μg HyP in the papain digested cartilage).

The *N*-hydroxyproline (HyPro) released into the tissue culture fluids during the experiment was measured; with untreated cartilage explants releasing only negligible amounts of these type II collagen fragments (0.1−0.4% of total HyPro). In contrast, cytokine stimulated cartilage explants released large amounts of type II collagen fragments, representing 30−80% of the total HyPro content of the cartilage, into the media. The total type II collagen content of the cartilage explants was measured by digesting the explants with papain and measuring the HyPro released.

Rat IA Model. Test compound was dissolved in PEG400 (20% of final volume) and diluted in 0.5% carboxymethylcellulose containing 0.25% Tween 80 (80% of final volume). Fasted rats were dosed orally with compounds and after 4 h were challenged with an IA injection of rh-tMMP-13. After a further 2 h, the knees were lavaged to collect synovial fluid. CS was measured by ELISA. Multiple experiments were carried out and the data combined. The % inhibition was calculated as follows: % inhibition by CS release = 100%(1 − (CS_{C+E} − CS_B)/(CS_E − CS_B)), where CS is the concentration of CS released into the SF lavage (μg/mL), CS_E is the average of the enzyme alone injected group (maximum), CS_B is the average of the buffer alone injected group (background), and CS_{C+E} is the average of the group given compound then challenged with the enzyme. Each test compound was tested in 1−5 experiments with 3−4 animals per group in an experiment. The percent inhibition for each animal was based on the average of the percent inhibition present in each knee. The calculated % inhibition ± SD for each treatment was derived by pooling data from all of the experiments.

Pharmacokinetics. Cannulated male Sprague−Dawley rats were used in these studies. Compound was administered orally (10 mg/kg) after an overnight fast and as an intravenous bolus (3 mg/kg). For oral administration, compound was dissolved in PEG400 (20% of final volume) and diluted in 0.5% carboxymethylcellulose containing 0.25% Tween 80 (80% of final volume). Compound **24f** was prepared for intravenous (iv) administration by the addition of 1 M Tris (1M, pH about 9) at 15% of the final volume, diluted in water and adjusted to pH ∼ 7 with 1 N HCl. Plasma samples were analyzed for parent compound by HPLC/MS/MS. Pharmacokinetic parameters were derived from curves fitted by noncompartmental modeling. Oral bioavailability (BA) was calculated as follows:

$$\text{BA}(\%) = \frac{\text{AUC}_{\text{po}(0-\infty)} \times \text{Dose}_{\text{iv}}}{\text{AUC}_{\text{iv}(0-\infty)} \times \text{Dose}_{\text{po}}} \times 100$$

Crystallography. The catalytic domain of MMP-13 was prepared as described previously.[51] Protein was concentrated to 10 mg/mL in 50 mM NaCl, 15 mM $CaCl_2$, 0.01 mM Zn $(Ac)_2$, 20 mM Tris, pH 7.0. The MMP-13 protein and compound **24f** were combined using a molar ratio of 1:2. Crystals were grown with the hanging drop vapor diffusion method at +4 °C. The well solution contained 13% PEG 6K, 1.2 M NaOAc, 2% glycerol, 100 mM Tris, pH 8.5. Crystals were cryoprotected in well solution containing an additional 4% PEG 6K and 21% glycerol. After flash freezing in liquid N_2, a 1.9 Å diffraction data set was collected using an RU2HR generator with a Raxis IIc detector. The structure was refined to an *R* factor of 16.4% (R_{free} = 20.2%) with good geometry. The final structure contained two complexes of MMP-13 with compound **24f**. Ramachandran analysis revealed two residues in disallowed regions, which has been routinely observed in the MMP-13 structures and reflects residues involved in coordination of the structural Ca^{2+} atoms.

Chemistry. 5-Pyridin-2-yl-thiophene-2-sulfonyl chloride, benzofuran-2-sulfonyl chloride, and benzo[*b*]thiophene-2-sulfonyl chloride were purchased from commercial sources. Melting points were determined on a Thomas−Hoover melting point apparatus and are uncorrected. ^{1}H NMR spectra were recorded on a Bruker DPX-300 spectrometer. Mass spectroscopy (MS) analyses were performed on a Micromass platform electrospray mass spectrometer, using positive and negative ionization. Microanalyses were performed at Robertson Laboratory, Inc., Madison, NJ.

4-[2-*oxo*-2-((*R*)-2-*oxo*-4-Phenyloxazolidin-3-yl)-ethyl]-piperidine-1-carboxylic Acid *tert*-Butyl Ester (12). A slurry of 4-pyridyl acetate (204 g, 1.21 mol) in 4 M HCl (800 mL) was flushed with N_2 and charged with PtO_2 (2.40 g, 10.6 mmol). The mixture was hydrogenated using a Parr apparatus at 50 psi H_2. Periodically over a 72 h period, fresh catalyst was added (3 × 2.40 g) and the reaction was recharged with H_2 to maintain 50 psi. Upon completion, the reaction was filtered through Hyflo. The filter cake was washed with water (400 mL). The filtrate was adjusted to pH 9 with Na_2CO_3 and heated at 65 °C for 2 h. After cooling to 0 °C, the filtrate was diluted with dioxane (400 mL) and treated with di *tert*-butyldicarbonate (264 g, 1.21 mol). After 24 h, the reaction was diluted with water to a volume of 4000 mL and washed with pentane (3 × 1000 mL). The separated aqueous phase was washed and acidified to pH 4 with 6 M HCl before extraction with ether (3 × 1000 mL). The combined ether extracts were dried (Na_2SO_4), filtered, and concentrated in vacuo to give 4-carboxymethyl-piperidine-1-carboxylic acid *tert*-butyl ester (**11**) (207 g, 70%).

To 4-carboxymethyl-piperidine-1-carboxylic acid *tert*-butyl ester (**11**) prepared above (48.66 g, 200 mmol) in THF (650 mL) at −78 °C was added Et_3N (21.24 g, 210 mmol) followed by pivaloyl chloride (25.32 g, 210 mmol). The thick, white suspension was stirred for 10 min and then warmed to 0 °C for 30 min. The reaction was cooled to −60 °C before a mixture prepared by dropwise addition of *n*-BuLi (137 mL, 220 mmol, 1.6 M in hexane) to (−)-(*R*)-phenyl-2-oxazolidinone (35.88 g, 220 mmol) at −60 °C was added via cannula. The reaction was allowed to warm to room temperature and stirred for 3 days before quenching with a saturated aqueous solution of $NaHCO_3$ (250 mL). The reaction was extracted with Et_2O (3 × 500 mL), and the combined organic phases were washed with a saturated aqueous solution of NaCl (250 mL), dried with 50 g of $MgSO_4$, and concentrated in vacuo. The residue was diluted with hexanes (750 mL) and stirred with heating to 50 °C at ambient pressure for 30 min. The desired acyloxazolidinone was isolated by filtration to afford 70.7 g (90.9%) of a white solid. ^{1}H NMR (300 MHz, $CDCl_3$) δ ppm 1.04−1.24 (m, 2 H), 1.43 (s, 9 H), 1.53−1.67 (m, 3 H), 1.87−2.04 (m, 1 H), 2.65 (apparent t, J = 12.4 Hz, 2 H), 2.80 (dd, J = 16.6, 7.5 Hz, 1 H), 2.93 (dd, J = 16.6, 6.0 Hz, 1 H), 3.95−4.11 (m, 2 H), 4.29 (dd, J = 9.0, 3.8 Hz, 1 H), 4.69 (t, J = 8.7 Hz, 1 H), 5.42 (dd, J = 8.7, 3.8 Hz, 1 H), 7.22−7.45 (m, 5 H).

(*R*)-α-*N*-BOC-4-Piperidinylglycine (7): Method A. To a 5 L, three-necked flask fitted with a mechanical stirrer charged with 4-[2-*oxo*-2-((*R*)-2-*oxo*-4-phenyloxazolidin-3-yl)-ethyl]-piperidine-1-carboxylic acid *tert*-butyl ester (**12**) (70.7 g, 182 mmol) in THF (910 mL) at −60 °C was added potassium bis(trimethylsilyl)amide (473 mL of a 0.5 M solution in toluene, 237 mmol). After 0.5 h, triisopropylbenzene azide (61.87 g, 200 mmol) was added as a solution in THF (700 mL) and the reaction was maintained at −60 °C. After 1 h, the reaction was quenched with glacial acetic acid (38 mL) and allowed to warm to room temperature. KOAc (66.06 g, 674 mmol) was added and stirred an additional 1.5 h. A saturated aqueous solution of $NaHCO_3$ (910 mL) was added, and the reaction was extracted with Et_2O (2 × 910 mL). The combined ether extracts were washed with water (500 mL) then a saturated aqueous solution of NaCl (500 mL), dried with $MgSO_4$ (300 g), and concentrated under reduced pressure. The residue was dissolved in CH_2Cl_2 (500 mL) and adsorbed onto silica gel (100 g) before it was applied to a silica gel column (600 g) using 10% EtOAc/hexane as the eluent to afford 4-[(*R*)-1-azido-2-oxo-2-((*R*)-2-*oxo*-4-phenyl-oxazolidin-3-yl)-ethyl]-piperidine-1-carboxylic acid *tert*-butyl ester (**13**) (69.1 g, 88%). ^{1}H NMR (300 MHz, $CDCl_3$) δ ppm 1.32−1.41 (m, 2 H), 1.45 (s, 9 H), 1.53−1.63 (m, 1 H), 1.74−1.84 (m, 1 H), 1.97−2.12 (m, 1 H), 2.58−2.76 (m, 2 H), 4.05−4.24 (m, 2 H), 4.34 (dd, J = 9.0, 4.1 Hz, 1 H), 4.77 (t, J = 8.7 Hz, 1 H), 4.98 (d, J = 7.9 Hz, 1 H), 5.45 (dd, J = 8.7, 4.1 Hz, 1 H), 7.29−7.46 (m, 5 H).

A solution of 4-[(*R*)-1-azido-2-*oxo*-2-((*R*)-2-oxo-4-phenyl-oxazolidin-3-yl)-ethyl]-piperidine-1-carboxylic acid *tert*-butyl ester (**13**) (7.40 g, 16.7 mmol) in THF (186 mL) and water (47 mL) was cooled to 0 °C before $LiOH \cdot H_2O$ (1.39 g, 33.1 mmol) and H_2O_2 (6.77 mL of a 30% solution in water) were added. Upon complete reaction, the solvents were removed under reduced pressure, and the residue was partitioned between water and CH_2Cl_2. The separated CH_2Cl_2 layer was set aside and the aqueous layer was placed in an ice bath and acidified with 4 N HCl. The acidified aqueous layer was extracted with EtOAc (2×). The combined EtOAc layers were washed with brine, dried (Na_2SO_4), and concentrated to afford 3.85 g (81%) of the carboxylic acid **14**.

To a degassed solution of 4-(*R*)-(azidocarboxymethyl)-piperidine-1-carboxylic acid *tert*-butyl ester (**14**) (8.79 g, 30.9 mmol) in EtOH (590 mL) and AcOH (8.2 mL) was added 10% Pd/C. The mixture was treated with H_2 (45 psi) using a Parr shaker apparatus. The reaction was filtered through celite, and the filter cake was washed with water (600 mL). The reaction was concentrated to half volume under reduced pressure and freeze-dried to afford the title amino acid (6.71 g, 84%). ^{1}H NMR (300 MHz, D_2O) δ ppm 1.08−1.32 (m, 2 H), 1.35 (s, 9 H), 1.50−1.60 (m, 1 H), 1.60−1.71 (m, 1 H), 1.94−2.08 (m, 1 H), 2.63−2.78 (m, 2 H), 3.54 (d, J = 4.9 Hz, 1 H), 3.95−4.11 (m, 2 H).

Method B. Tetramethylguanidine (4.5 g, 0.039 mol) was added to a solution of 9.94 g (0.030 mol) of *N*-CBZ-α-phosphonoglycine trimethyl ester in THF (20 mL) under nitrogen and the solution stirred for 15 min. A solution of *N*-BOC-4-piperidone (16.74 g, 0.084 mol) in THF was then added via addition funnel over 5 min and the solution stirred at room temperature for 21 h. THF was removed in vacuo and EtOAc (100 mL) was added. The organic solution was washed with 5% aqueous citric acid solution (150 mL), saturated sodium bicarbonate solution (50 mL), and saturated brine (50 mL), dried over $MgSO_4$, and evaporated to give an oil, which was dissolved in EtOAc (12 mL). Hexane (50 mL) was added to precipitate the crude product, which was filtered off and recrystallized from EtOAc/hexane (1:4 ratio) to yield 4-(benzyloxycarbonylamino-methoxycarbonyl-methylene)-piperidine-1-carboxylic acid *tert*-butyl ester as a white solid: mp 101.5−102.6 °C. IR (KBr) 3312, 2972, 1725, 1703, 1684, 1512, 1477, 1451, 1426, 1365, 1327, cm^{-1}. ^{1}H NMR (500 MHz, $CDCl_3$) δ 7.49−6.30 (m, 5H), 6.17 (br s, 1H), 5.13 (s, 2H), 3.76−3.68 (m, 3H), 3.56−3.40 (m, 4H), 2.92−2.78 (m, 2H), 2.39 (t, J = 5.9 Hz, 2H), 1.47 (s, 9H). ^{13}C NMR (125 MHz, $CDCl_3$) δ 165.4, 155.1, 147.6, 136.4, 128.9, 128.8, 128.7, 128.6, 120.6, 80.2, 67.8, 53.3, 44.2, 43.43, 30.8, 30.1, 28.9, 28.8. Anal. $C_{21}H_{28}N_2O_6$ (C, H, N).

A Parr bottle was charged with 4-(benzyloxycarbonylamino-methoxycarbonyl-methylene)-piperidine-1-carboxylic acid *tert*-butyl ester (0.37 g, 0.9 mmol) and degassed MeOH (40 mL) under nitrogen purge. To this colorless solution, (*R*,*R*)-Me-BPE-Rh catalyst (10 mg) was quickly added. The resulting solution

evacuated, and then the vessel refilled with nitrogen for three cycles. The solution was stirred under 90 psi of hydrogen gas at room temperature for 72 h. The mixture was then concentrated on a rotary evaporator to remove MeOH. The residue was redissolved in EtOAc (20 mL) and filtered through a pad of silica gel (3 g) to remove the catalyst, and the filter cake was rinsed with EtOAc (20 mL). The combined filtrate was concentrated to afford *N*-CBZ-(*R*)-α-(*N*-BOC-4-piperidinyl)-glycine methyl ester as an oil: $R_f = 0.36$ (hexane/EtOAc 1:1); $[\alpha]^{25}_D$ −20.7 ($c = 1.05$, $CHCl_3$); chiral HPLC 94% ee: (+)-enantiomer, 3%, Rt 7.21 min, (−)-enantiomer, 97%, RT 10.04 min (Chiralcel OD column, hexane/IPA/TFA 9/1/0.1%, flow rate 1.5 mL/min). IR (KBr) 3323, 2950, 1691, 1529 cm^{-1}. ^1H NMR (300 MHz, $CDCl_3$) 7.32−7.42 (m, 5H), 5.32 (d, $J = 8.7$ Hz, 1H), 5.12 (s, 2H), 4.33−4.44 (m, 1H), 4.08−4.21 (m, 2H), 3.77 (s, 3H), 2.57−2.76 (m, 2H), 1.85−2.02 (m, 1H), 1.40−1.70 (m, 2H), 1.46 (s, 9H), 1.19−1.39 (m, 2H). ^{13}C NMR (125 MHz, $CDCl_3$) 171.8, 156.0, 154.6, 136.0, 128.5, 128.2, 128.1, 79.5, 67.1, 57.8, 52.3, 43.4, 39.6, 28.3, 27.1. Anal. calcd for $C_{21}H_{30}O_6N_2$: C, 62.05; H, 7.44; N, 6.89. Found: C, 61.99; H, 7.09; N, 7.04.

A solution of *N*-CBZ-(*R*)-α-(*N*-BOC-4-piperidinyl)-glycine methyl ester (2.8 g, 6.9 mmol) in MeOH (103 mL) was cooled to 5 °C with an ice bath. A solution of 1 M LiOH (35 mL, 35 mmol, prepared from 1.5 g of $LiOH \cdot H_2O$ in 33.5 mL of H_2O) was added and the mixture allowed to warm up to room temperature and stirred for another 20 h. The reaction mixture was neutralized with 1 N $KHSO_4$ solution, concentrated in vacuo to remove MeOH, and redissolved into EtOAc. The pH of the aqueous layer was adjusted to 2 with 2 N $KHSO_4$ and the organic layers separated. The aqueous layer was further extracted with EtOAc (2 × 50 mL). The combined EtOAc layers were then washed with 50 mL of brine, dried over $MgSO_4$, filtered through celite, and concentrated under vacuum to give (*N*-BOC-4-piperidinyl)-glycine as a white foamy solid: $[\alpha]^{25}_D$ −18.6 ($c = 1.07$, $CHCl_3$) chiral HPLC 90% ee: (+)-enantiomer, 5%, RT 5.72 min, (−)-enantiomer, 95%, RT 8.54 min (Chiralcel OD Hexane/IPA/TFA 9/1.0.1%, flow rate 1.5 mL/min).

A Parr bottle was charged with 5% Pd/C (0.27 g) under nitrogen atmosphere. A solution of *N*-CBZ-(*R*)-(*N*-BOC-4-piperidinyl)-glycine (1.25 g, 3.2 mmol) in MeOH (14 mL) and H_2O (8 mL) was added under nitrogen purge. The mixture was evacuated and then refilled with nitrogen three times, then evacuated and refilled with hydrogen another three times. The mixture was hydrogenolyzed under 52 psi hydrogen gas at room temperature for 3 h. The mixture was then filtered and the catalyst cake rinsed with EtOH (100 mL). The filtrate was concentrated under vacuum to azeotropically remove H_2O. The gray solid residue was suspended in MeOH (20 mL), stirred at 60 °C for 2 h, cooled to 0 °C, and stirred for an additional 1 h. The mixture was filtered and the solid cake rinsed with cold MeOH (10 mL). The solid was dried under vacuum to obtain (*R*)-α-(*N*-BOC-4-piperidinyl)-glycine (0.7 g, 85% yield) as gray solid: $[\alpha]^{25}_D$ −4.2 ($c = 0.51$, H_2O); chiral HPLC 98% ee: (−)-enantiomer, 99%, RT 13.98 min, (+)-enantiomer, 1%, RT 23.37 min, (Crownpak CR+, perchloric acid pH 1.5/MeOH 85/15, flow rate 1 mL/min). ^1H NMR (500 MHz, D_2O) δ 4.18 (m, 2H), 3.68 (d, $J = 4.9$ Hz, 1H), 2.76−2.92 (m, 2H), 2.09−2.21 (m, 1H), 1.74−1.83 (m, 1H), 1.64−1.72 (m, 1H), 1.48 (s, 9H), 1.25−1.53 (m, 2H). ^{13}C NMR (125 MHz, $CDCl_3$) δ 173.2, 156.4, 81.7, 58.9, 43.6, 37.0, 27.6, 26.9. Anal. ($C_{12}H_{22}O_4N_2$) C, H, N.

4-{Carboxy-[5-(4-*N*,*N*-dimethylaminophenyl)-thiophene-2-sulfonylamino]-methyl}-piperidine-1-carboxylic Acid *tert*-Butyl Ester (9). A mixture amino acid **7** (500 mg, 1.94 mmol), sulfonyl chloride **8** (508 mg, 1.69 mmol), and Et_3N (427 mg, 4.23 mmol) in CH_2Cl_2 were combined and stirred at room temperature for 18 h. An aqueous solution of citric acid was added. The separated aqueous phase was extracted with CH_2Cl_2. The combined CH_2Cl_2 layers were dried over Na_2SO_4 and concentrated in vacuo. The residue was purified by column chromatography, eluting with 2% then 5% MeOH/CH_2Cl_2 to afford the title compound (570 mg, 64%). ^1H NMR (300 MHz, DMSO-d_6) δ ppm 1.02−1.22 (m, 2 H), 1.35 (s, 9 H), 1.41−1.54 (m, 2 H), 1.73−1.90 (m, 1 H), 2.54−2.76 (m, 2 H), 2.96 (s, 6 H), 3.63−3.72 (m, 1 H), 3.84−3.98 (m, 2 H), 6.76 (d, $J = 8.7$ Hz, 2 H), 7.28 (d, $J = 3.9$ Hz, 1 H), 7.44 (d, $J = 3.9$ Hz, 1 H), 7.51 (d, $J = 8.7$ Hz, 2 H), 8.26 (d, $J = 9.4$ Hz, 1 H). Anal. ($C_{24}H_{33}N_3O_6S_2$) C, H, N.

4-{(*R*)-Carboxy-[5-(4′-piperidin-1-yl-phenyl)-thiophene-2-sulfonylamino]-methyl}piperidine-1-carboxylic Acid *tert*-Butyl ester (22a). Amino acid **7** was coupled to 5-(4′-piperidin-1-ylphenyl)-thiophene-2-sulfonyl chloride (**18a**) prepared above according to the procedure outlined for the preparation of sulfonamide **9**. ^1H NMR (300 MHz, DMSO-d_6) δ ppm 1.07−1.27 (m, 2 H), 1.28−1.67 (m, 17 H), 1.74−1.92 (m, 1 H), 2.53−2.73 (m, 2 H), 3.27−3.42 (m, 5 H), 3.48−3.57 (m, 1 H), 3.84−3.99 (m, 2 H), 6.96 (d, $J = 8.7$ Hz, 2 H), 7.31 (d, $J = 4.1$ Hz, 1 H), 7.46 (d, $J = 4.1$ Hz, 1 H), 7.51 (d, $J = 8.7$ Hz, 2 H). Anal. ($C_{27}H_{37}N_3O_6S_2$) C, H, N.

4-[(*R*)-Carboxy-(5-phenylthiophene-2-sulfonylamino)-methyl]-piperidine-1-carboxylic Acid *tert*-Butyl Ester Potassium Salt (22b). Amino acid **7** was coupled to 5-phenyl-thiophene-2-sulfonyl chloride (**18b**). A solution of the product carboxylic acid (175 mg, 0.36 mmol) in THF (10 mL) was cooled to 0 °C before a solution of *t*-BuOK (0.36 mL of a 1 M solution in THF) was added. The reaction was stirred 0.5 h before the volatiles were removed in vacuo. The residue was taken up in Et_2O and filtered and dried in vacuo to afford the title potassium salt. ^1H NMR (300 MHz, MeOH) δ ppm 1.17−1.35 (m, 1 H), 1.36−1.54 (m, 10 H), 1.38−1.53 (m, 10 H), 1.53−1.71 (m, 2 H), 1.81−2.02 (m, 1 H), 2.58−2.86 (m, 2 H), 3.59 (d, $J = 4.9$ Hz, 1 H), 4.02−4.20 (m, 2 H), 7.33−7.49 (m, 4 H), 7.54 (d, $J = 3.8$ Hz, 1 H), 7.65 (s, 2 H). Anal. ($C_{20}H_{25}KN_2O_6S_2 \cdot 0.75H_2O$) C, H, N.

4-{(*R*)-Carboxy-[5-(3-ethoxyphenyl)-thiophene-2-sulfonylamino]-methyl}-piperidine-1-carboxylic Acid *tert*-Butyl Ester (22c). Amino acid **7** was coupled to 3-ethoxyphenylthiophene-2-sulfonyl chloride (**18c**). ^1H NMR (300 MHz, DMSO-d_6) δ ppm 0.97−1.56 (m, 15 H) 1.76−1.92 (m, 1 H) 2.53−2.80 (m, 2 H) 3.27−3.45 (m, 1 H) 3.65−3.78 (m, 1 H) 3.83−4.01 (m, 2 H) 4.10 (q, $J = 6.8$ Hz, 2 H) 6.91−7.03 (m, 1 H) 7.20−7.30 (m, 2 H) 7.32−7.41 (m, 1 H) 7.48−7.63 (m, 2 H) 8.45 (d, $J = 9.4$ Hz, 1 H) 12.86 (br s, 1 H). Anal. ($C_{24}H_{32}N_2O_7S_2$) C, H, N.

4-{(*R*)-Carboxy-[5-(3,4-dimethoxyphenyl)-thiophene-2-sulfonylamino]-methyl}-piperidine-1-carboxylic Acid *tert*-Butyl Ester Potassium Salt (22d). Amino acid **7** was coupled to 3,4-dimethoxyphenyl-thiophene-2-sulfonyl chloride (**18d**). A solution of the product carboxylic acid (75 mg, 0.14 mmol) in THF (5 mL) was cooled to 0 °C before a solution of *t*-BuOK (0.14 mL of a 1 M solution in THF) was added. The reaction was stirred 0.5 h before the volatiles were removed in vacuo. The residue was taken up in Et_2O, filtered, and dried in vacuo to afford the title potassium salt. ^1H NMR (300 MHz, MeOD) δ ppm 1.16−1.33 (m, 1 H) 1.32−1.51 (m, 10 H) 1.52−1.69 (m, 2 H) 1.83−1.99 (m, 1 H) 2.55−2.86 (m, 3 H) 3.52−3.67 (m, 1 H) 3.79−3.93 (m, 6 H) 4.00−4.20 (m, 2 H) 6.99 (d, $J = 7.9$ Hz, 1 H) 7.16−7.32 (m, 3 H) 7.50 (d, $J = 4.1$ Hz, 1 H). Anal. ($C_{24}H_{31}KN_2O_8S_2 \cdot 0.75H_2O$) C, H, N.

4-[(*R*)-Carboxy-(5-pyridin-4-yl-thiophene-2-sulfonylamino)-methyl]-piperidine-1-carboxylic Acid *tert*-Butyl Ester (22e). Amino acid **7** was coupled to 5-pyridin-4-yl-thiophene-2-sulfonyl chloride (**18e**). ^1H NMR (300 MHz, DMSO-d_6) δ ppm 1.06−1.27 (m, 2 H), 1.37 (s, 9 H), 1.41−1.54 (m, 2 H), 1.79−1.94 (m, 1 H), 2.55−2.76 (m, 2 H), 3.69−3.79 (m, 1 H), 3.85−3.99 (m, 2 H), 7.59−7.65 (m, 1 H), 7.69−7.76 (m, 2 H), 7.81−7.87 (m, 1 H), 8.55 (d, $J = 9.4$ Hz, 1 H), 8.60−8.70 (m, 2 H), 12.82 (br s, 1 H). Anal. ($C_{21}H_{27}N_3O_6S_2$) C, H, N.

4-[(*R*)-Carboxy-(5-pyridin-2-yl-thiophene-2-sulfonylamino)-methyl]-piperidine-1-carboxylic Acid *tert*-Butyl Ester (22f). Amino acid **7** was coupled to commercial 5-pyridin-2-yl-thiophene-2-sulfonyl chloride. ^1H NMR (300 MHz, MeOD) δ ppm 1.13−1.30 (m, 2 H) 1.30−1.48 (m, 10 H) 1.54−1.69 (m, 2 H) 1.81−2.00 (m, 1 H) 2.58−2.81 (m, 2 H) 3.75−3.85 (m, 1 H) 3.98−4.17 (m, 2 H) 7.24−7.42 (m, 1 H) 7.51−7.62 (m, 1 H) 7.61−7.74 (m, 1 H) 7.77−7.98 (m, 2 H) 8.40−8.60 (m, 1 H). Anal. ($C_{21}H_{27}N_3O_6S_2 \cdot H_2O$) C, H, N.

4-{Carboxy-[5-(6-methoxy-pyridin-3-yl)-thiophene-2-sulfonylamino]-methyl}-piperidine-1-carboxylic Acid *tert*-Butyl Ester (22g). Amino acid **7** was coupled to 5-(6-methoxy-pyridin-3-yl)-thiophene-2-sulfonyl chloride (**18f**). ^1H NMR (300 MHz, DMSO-d_6) δ ppm

1.06−1.20 (m, 2 H) 1.32−1.40 (m, 8 H) 1.53 (s, 1 H) 2.47−2.54 (m, 6 H) 2.69 (s, 1 H) 3.70−3.86 (m, 4 H) 3.87−3.93 (m, 4 H) 3.96 (s, 1 H) 7.47−7.57 (m, J = 4.14, 4.14, 4.14, 4.14 Hz, 1 H) 8.55 (d, J = 1.88 Hz, 1 H). Anal. ($C_{22}H_{29}N_3O_7S_2$) C, H, N.

4-[(*R*)-(Benzofuran-2-sulfonylamino)-carboxy-methyl]-piperidine-1-carboxylic Acid *tert*-Butyl Ester (29). Amino acid **7** was coupled to commercial benzofuran-2-sulfonyl chloride. ^{1}H NMR (300 MHz, DMSO-d_6) δ ppm 1.03−1.26 (m, 2 H), 1.31−1.50 (m, 11 H), 1.79−1.95 (m, 1 H), 2.54−2.73 (m, 2 H), 3.71−3.79 (m, 1 H), 3.82−3.96 (m, 2 H), 7.34−7.42 (m, 1 H), 7.48−7.55 (m, 2 H), 7.68 (d, J = 8.3 Hz, 1 H), 7.78 (d, J = 7.5 Hz, 1 H), 8.73 (br s, 1 H), 12.80 (br s, 1 H). Anal. ($C_{20}H_{26}N_2O_7S$) C, H, N.

4-[(*R*)-(Benzo[*b*]thiophene-2-sulfonylamino)-carboxy-methyl]-piperidine-1-carboxylic Acid *tert*-Butyl Ester (30). Amino acid **7** was coupled to commercial benzo[*b*]thiophene-2-sulfonyl chloride. ^{1}H NMR (300 MHz, DMSO-d_6) δ ppm 1.02−1.26 (m, 2 H), 1.30−1.40 (m, 10 H), 1.40−1.53 (m, 2 H), 1.75−1.93 (m, 1 H), 2.54−2.73 (m, 2 H), 3.65−3.74 (m, 1 H), 3.81−3.97 (m, 2 H), 7.45−7.56 (m, 2 H), 7.94 (s, 1 H), 7.97−8.03 (m, 1 H), 8.05−8.10 (m, 1 H), 12.72 (br s, 1 H). Anal. ($C_{20}H_{26}N_2O_6S_2$) C, H, N.

4-[(*R*)-Carboxy-(4′-dimethylamino-biphenyl-4-sulfonylamino)-methyl]-piperidine-1-carboxylic Acid *tert*-Butyl Ester (31). Amino acid **7** was coupled to chloride **19a**. ^{1}H NMR (300 MHz, DMSO-d_6) δ ppm 1.11−1.27 (m, 3 H) 1.33−1.47 (m, 12 H) 1.75−1.89 (m, 1 H) 2.50 (d, J = 1.88 Hz, 3 H) 2.96 (s, 6 H) 3.38 (d, J = 4.14 Hz, 2 H) 3.90 (s, 2 H) 6.81 (d, J = 9.04 Hz, 2 H) 7.60 (d, J = 9.04 Hz, 2 H) 7.69−7.79 (m, 4 H). Anal. ($C_{26}H_{35}N_3O_6S\cdot H_2O$) C, H, N.

4-[(*R*)-Carboxy-(4′-methylbiphenyl-4-sulfonylamino)-methyl]-piperidine-1-carboxylic Acid *tert*-Butyl Ester (32). Amino acid **7** was coupled to chloride **19b**. ^{1}H NMR (300 MHz, DMSO-d_6) δ ppm 1.01−1.29 (m, 2 H) 1.28−1.43 (m, 10 H) 1.49 (s, 1 H) 1.72−1.95 (m, 1 H) 2.36 (s, 3 H) 2.52−2.65 (m, 2 H) 3.14−3.29 (m, 2 H) 3.80−4.06 (m, 2 H) 7.26−7.35 (m, 2 H) 7.62 (d, J = 7.9 Hz, 2 H) 7.80 (s, 4 H). Anal. ($C_{25}H_{32}N_2O_6S\cdot 0.5H_2O$).

4-[(*R*)-Carboxy-(4′-fluorobiphenyl-4-sulfonylamino)-methyl]-piperidine-1-carboxylic Acid *tert*-Butyl Ester (33). Amino acid **7** was coupled to chloride **19c**. ^{1}H NMR (300 MHz, $CDCl_3$) δ ppm 1.22−1.44 (m, 3 H) 1.36 (s, 9 H) 1.57 (m, 1 H) 1.92 (m, 1 H) 2.48−2.62 (m, 2 H) 3.76 (m, 1 H) 4.01 (m, 2 H) 7.12 (m, 2 H) 7.53 (m, 2 H) 7.62 (m, 2 H) 7.90 (m, 2 H). Anal. ($C_{24}H_{29}FN_2O_6S\cdot 0.5H_2O$).

4-[(*R*)-Carboxy-(4′-methoxybiphenyl-4-sulfonylamino)-methyl]-piperidine-1-carboxylic Acid *tert*-Butyl Ester (34). Amino acid **7** was coupled to chloride **19d**. ^{1}H NMR (300 MHz, $CDCl_3$) δ ppm 1.13−1.28 (m, 2 H) 1.32 (m, 1 H) 1.40 (s, 9 H) 1.45 (m, 1 H) 1.86 (m, 1 H) 2.56 (m, 2 H) 3.79 (m, 1 H) 3.86 (s, 3 H) 4.08 (m, 2 H) 5.46 (m, 1 H) 7.00 (d, J = 8.7 Hz, 2 H) 7.53 (d, J = 8.7 Hz, 2 H) 7.63 (d, J = 8.3 Hz, 2 H) 7.83 (d, J = 8.3 Hz, 2 H). Anal. ($C_{25}H_{32}N_2O_7S$).

4-[(*R*)-Carboxy-(4′-ethoxybiphenyl-4-sulfonylamino)-methyl]-piperidine-1-carboxylic Acid *tert*-Butyl Ester (35). Amino acid **7** was coupled to chloride **19e**. ^{1}H NMR (300 MHz, DMSO-d_6) δ ppm 1.35 (t, 3 H) 1.37 (s, 9 H) 1.40−1.55 (m, 2 H) 1.83 (m, 1 H) 2.62 (m, 1 H) 2.82 (m, 1 H) 3.35 (m, 3 H) 3.90 (m, 2 H) 4.08 (q, J = 7.2 Hz, 2 H) 7.04 (d, J = 8.7 Hz, 2 H) 7.55−7.62 (m, 2 H) 7.67 (m, 2 H) 7.80−7.84 (m, 2 H). Anal. ($C_{26}H_{34}N_2O_7S$).

4-{(*R*)-Carboxy-[5-(4′-trifluoromethylphenyl)-furan-2-sulfonylamino]-methyl}-piperidine-1-carboxylic Acid *tert*-Butyl Ester Potassium Salt (36). Amino acid **7** was coupled to chloride **21a**. To a solution of the carboxylic acid (27 mg, 0.05 mmol) in THF (2 mL) was added KO*t*-Bu (0.05 mL, 1 M in THF). The reaction was diluted with Et_2O and filtered. The solid was dried in vacuo to afford the title compound. ^{1}H NMR (300 MHz, DMSO-d_6) δ ppm 1.09−1.40 (m, 13 H) 1.41−1.54 (m, 1 H) 1.76−1.94 (m, 1 H) 2.51−2.68 (m, 1 H) 3.13−3.23 (m, 2 H) 3.82−4.05 (m, 2 H) 7.18 (d, J = 3.8 Hz, 1 H) 7.28 (d, J = 3.8 Hz, 1 H) 7.83 (d, J = 8.3 Hz, 2 H) 7.97 (d, J = 8.3 Hz, 2 H). Anal. ($C_{23}F_3H_{26}KN_2O_7S\cdot 2H_2O$).

4-{(*R*)-Carboxy-[5-(3′,4′-difluorophenyl)-furan-2-sulfonylamino]-methyl}-piperidine-1-carboxylic Acid *tert*-Butyl Ester (37). Amino acid **7** was coupled to chloride **21b**. ^{1}H NMR (300 MHz, MeOD) δ ppm 1.15−1.51 (m, 12 H) 1.53−1.69 (m, 2 H) 1.83−2.01 (m, 1 H) 2.56−2.86 (m, 2 H) 3.58 (d, J = 4.9 Hz, 1 H) 4.00−4.16 (m, 2 H) 6.55 (dd, J = 3.4, 1.9 Hz, 1 H) 6.92 (d, J = 3.4 Hz, 1 H) 7.61 (d, J = 1.5 Hz, 1 H) 7.74−7.84 (m, 1 H) 7.87−7.92 (m, 1 H). Anal. ($C_{22}F_2H_{25}KN_2O_7S\cdot 0.75H_2O$).

4-{(*R*)-Carboxy-[5-(3′,4′-methylenedioxyphenyl)-furan-2-sulfonylamino]-methyl}-piperidine-1-carboxylic Acid *tert*-Butyl Ester (38). Amino acid **7** was coupled to chloride **21c**. ^{1}H NMR (300 MHz, MeOD) δ ppm 1.25−1.51 (m, 12 H), 1.52−1.69 (m, 2 H), 1.83−2.00 (m, 1 H), 2.56−2.80 (m, 2 H), 3.61−3.69 (m, 1 H), 3.99−4.17 (m, 2 H), 6.00 (s, 2 H), 6.70 (d, J = 3.8 Hz, 1 H), 6.88 (d, J = 8.3 Hz, 1 H), 7.04 (d, J = 3.4 Hz, 1 H), 7.21−7.37 (m, 2 H). Anal. ($C_{22}F_2H_{27}KN_2O_7S\cdot 0.75H_2O$).

4-{(*R*)-Carboxy-[5-(4′-*N*,*N*-dimethylaminophenyl)-thiophene-2-sulfonylamino]-methyl}-piperidine-1-carboxylic Acid (23). A suspension of *tert*-butyl ester **9** in ether was treated with HCl (g) at 0 °C for 5 min. The reaction was sealed, allowed to stir at room temperature for 1 h, and concentrated under reduced pressure to a solid. ^{1}H NMR (300 MHz, DMSO-d_6) δ ppm 1.33−1.75 (m, 4 H) 1.89−2.09 (m, 1 H) 2.70−2.94 (m, 3 H) 2.97 (s, 6 H) 3.18−3.31 (m, 2 H) 3.64−3.78 (m, 1 H) 6.76−6.88 (m, 2 H) 7.32 (d, J = 3.9 Hz, 1 H) 7.47 (d, J = 3.9 Hz, 1 H) 7.54 (d, J = 9.0 Hz, 2 H) 8.27−8.46 (m, 2 H) 8.78−8.91 (m, 1 H). Anal. ($C_{19}Cl_2H_{27}N_3O_4S_2\cdot 0.5H_2O$) C, H, N.

In the preparation of compounds **24a**−**k**, the intermediate formed from the couple of amino acid **7** and the sulfonyl chloride was treated as in the synthesis of **24f** to remove the BOC group and introduce *iso*-propyl carbamate unless otherwise stated.

4-{(*R*)-Carboxy-[5-(4′-piperidin-1-yl-phenyl)-thiophene-2-sulfonylamino]-methyl}piperidine-1-carboxylic Acid *iso*-Propyl Ester (24a). Compound **22a** was deprotected with HCl (g) in CH_2Cl_2 at room temperature over 3 h. The volatiles were removed under reduced pressure to afford (*R*)-piperidin-4-yl-[5-(4-piperidin-1-yl-phenyl)-thiophene-2-sulfonylamino]-acetic acid as the hydrochloride salt, which was treated as in the synthesis of **24f** to yield **24a**. ^{1}H NMR (300 MHz, DMSO-d_6) δ ppm 1.01−1.29 (m, 8 H), 1.39−1.67 (m, 8 H), 1.78−1.95 (m, 1 H), 2.54−2.79 (m, 2 H), 3.19−3.28 (m, 4 H), 3.48−3.63 (m, 1 H), 3.85−4.05 (m, 2 H), 4.60−4.80 (m, 1 H), 6.98 (d, J = 9.0 Hz, 2 H), 7.32 (d, J = 3.4 Hz, 1 H), 7.41−7.56 (m, 3 H), 8.00 (br s, 1 H). Anal. ($C_{26}H_{35}N_3O_6S_2\cdot 0.5H_2O$) C, H, N.

4-{(*R*)-Carboxy-[5-phenylthiophene-2-sulfonylamino]-methyl}piperidine-1-carboxylic Acid *iso*-propyl Ester (24b). Amino acid **7** was coupled to chloride **18b**, which was treated as described for **24f**. ^{1}H NMR (300 MHz, MeOD) δ ppm 1.12−1.50 (m, 9 H) 1.52−1.73 (m, 2 H) 1.87−2.06 (m, 1 H) 2.61−2.92 (m, 2 H) 3.79−3.91 (m, 1 H) 4.05−4.24 (m, 2 H) 4.77−4.87 (m, 1 H) 7.31−7.49 (m, 4 H) 7.50−7.60 (m, 1 H) 7.62−7.77 (m, 2 H). Anal. ($C_{21}H_{26}N_2O_6S_2$) C, H, N.

4-{(*R*)-Carboxy-[5-(4′-hydroxyphenyl)-thiophene-2-sulfonylamino]-methyl}-piperidine-1-carboxylic Acid *iso*-Propyl Ester (24c). Amino acid **7** was coupled to sulfonyl chloride **18l**. Treatment of 4-{carboxy-[5-(4′-ethoxyphenyl)-thiophene-2-sulfonylamino]-methyl}-piperidine-1-carboxylic acid *tert*-butyl ester (0.26 g, 0.50 mmol) with HBr (1 mL of a 48% solution in water) at 100 °C for 5 h yielded 4-{carboxy-[5-(4′-hydroxyphenyl)-thiophene-2-sulfonylamino]-methyl}-piperidine as the hydrobromide salt after removal of the volatiles under reduced pressure. Introduction of the *iso*-propyl carbamate was effected from the hydrobromide salt by the procedure described for **24f**. ^{1}H NMR (300 MHz, MeOD) δ ppm 1.18−1.46 (m, 8 H), 1.54−1.70 (m, 2 H), 1.86−2.04 (m, 1 H), 2.65−2.87 (m, 2 H), 3.79−3.85 (m, 1 H), 4.06−4.19 (m, 2 H), 4.77−4.86 (m, 1 H), 6.83 (d, J = 8.7 Hz, 2 H), 7.20 (d, J = 4.1 Hz, 1 H), 7.50 (s, 3 H). Anal. ($C_{21}H_{26}N_2O_7S_2$) C, H, N.

4-{(*R*)-Carboxy-[5-(4′-methoxyphenyl)-thiophene-2-sulfonylamino]-methyl}-piperidine-1-carboxylic Acid *iso*-Propyl Ester (24d). Amino acid **7** was coupled to sulfonyl chloride **18g**, which was treated as described for **24f**. ^{1}H NMR (300 MHz, DMSO-d_6) δ ppm 1.03−1.31 (m, 8 H), 1.40−1.56 (m, 2 H), 1.79−1.96 (m, 1 H), 2.56−2.80 (m, 2 H), 3.55−3.67 (m, 1 H), 3.80 (s, 3 H), 3.87−4.04 (m, 2 H), 4.65−4.85 (m, 1 H), 7.02 (d, J = 8.1 Hz, 2

H), 7.40 (d, $J = 3.4$ Hz, 1 H), 7.49 (d, $J = 3.4$ Hz, 1 H), 7.64 (d, $J = 8.1$ Hz, 2 H). Anal. ($C_{22}H_{28}N_2O_7S_2$) C, H, N.

4-{(*R*)-Carboxy-[5-(4′-trifluoromethoxyphenyl)-thiophene-2-sulfonylamino]-methyl}-piperidine-1-carboxylic Acid *iso*-Propyl Ester (24e). Amino acid **7** was coupled to sulfonyl chloride **18h**, which was treated as described for **24f**. ^{1}H NMR (300 MHz, MeOH) δ ppm 1.15−1.29 (m, 7 H) 1.33−1.46 (m, 1 H) 1.55−1.69 (m, 2 H) 1.75−1.84 (m, 1 H) 2.67−2.83 (m, 2 H) 3.81−3.95 (m, 2 H) 4.04−4.18 (m, 2 H) 7.35 (d, $J = 7.9$ Hz, 2 H) 7.41 (d, $J = 4.1$ Hz, 1 H) 7.55 (d, $J = 3.8$ Hz, 1 H) 7.77 (d, $J = 8.7$ Hz, 2 H). Anal. ($C_{22}H_{25}FN_2O_7S_2$) C, H, N.

4-{(*R*)-Carboxy-[5-(4′-ethoxyphenyl)-thiophene-2-sulfonylamino]-methyl}-piperidine-1-carboxylic Acid *iso*-Propyl Ester (24f). (*R*)-[5-(4′-Ethoxyphenyl)-thiophene-2-sulfonylamino]-piperidin-4-yl-acetic acid *tert*-butyl ester was prepared by coupling of amino acid **7** and 4-(4′-ethoxyphenyl)phenylsulfonyl chloride by analogy to the preparation of sulfonamide **9** above. The BOC group was removed under acidic conditions according to the method outlined for **23** above. To a slurry of the resulting hydrochloride salt (15 g, 32.5 mmol) in THF (325 mL) was added *N*,*O*-bistrimethylsilylacetamide (32.1 mL, 130 mmol). After 2 h, *iso*-propyl chloroformate (65 mL of a 1 M solution in toluene, 65 mmol) was added. The reaction was concentrated in vacuo and the residue diluted with CH_2Cl_2 and 1 M HCl. The separated aqueous phase was extracted with fresh CH_2Cl_2 (2×). The combined organic phases were dried with $MgSO_4$, filtered, and concentrated under reduced pressure. The residue was purified by flash chromatography, eluting with 2%, then 5% MeOH in CH_2Cl_2 to afford the title compound as a solid (8.66 g, 52%). ^{1}H NMR (300 MHz, DMSO-d_6) δ ppm 1.05−1.25 (m, 8 H), 1.34 (t, $J = 6.8$ Hz, 3 H), 1.42−1.55 (m, 2 H), 1.74−1.94 (m, 1 H), 2.56−2.82 (m, 2 H), 3.65−3.75 (m, 2 H), 3.88−4.01 (m, 2 H), 4.07 (q, $J = 6.8$ Hz, 2 H), 4.74 (quin, $J = 6.4$ Hz, 1 H), 7.01 (d, $J = 8.7$ Hz, 2 H), 7.40 (d, $J = 3.8$ Hz, 1 H), 7.49 (d, $J = 3.8$ Hz, 1 H), 7.63 (d, $J = 8.7$ Hz, 2 H), 8.35 (d, $J = 9.4$ Hz, 2 H), 12.79 (s, 1 H). Anal. ($C_{23}H_{30}N_2O_7S_2$) C, H, N.

4-{(*R*)-Carboxy-[5-(4′-*n*-propoxyphenyl)-thiophene-2-sulfonylamino]-methyl}-piperidine-1-carboxylic Acid *iso*-Propyl Ester (24g). Amino acid **7** was coupled to chloride **18k**, which was treated as described for **24f**. ^{1}H NMR (300 MHz, DMSO-d_6) δ ppm 0.98 (t, $J = 7.4$ Hz, 3 H) 1.17 (s, 9 H) 1.40−1.55 (m, 2 H) 1.66−1.80 (m, 2 H) 1.79−1.97 (m, 1 H) 2.64 (br s, 2 H) 3.50−3.69 (m, 1 H) 3.86−4.04 (m, 4 H) 4.61−4.82 (m, 1 H) 7.01 (d, $J = 8.7$ Hz, 2 H) 7.39 (d, $J = 3.8$ Hz, 1 H) 7.49 (d, $J = 3.8$ Hz, 1 H) 7.62 (d, $J = 9.0$ Hz, 2 H) 8.08 (br s, 1 H). Anal. ($C_{24}H_{32}N_2O_7S_2$) C, H, N.

4-{(*R*)-Carboxy-[5-(4′-*iso*-propoxyphenyl)-thiophene-2-sulfonylamino]-methyl}-piperidine-1-carboxylic Acid *iso*-Propyl Ester (24h). Amino acid **7** was coupled to chloride **18m**, which was treated as described for **24f**. ^{1}H NMR (300 MHz, DMSO-d_6) δ ppm 0.98−1.34 (m, 9 H) 1.28 (d, $J = 6.0$ Hz, 6 H) 1.40−1.55 (m, 2 H) 1.79−1.94 (m, 1 H) 2.55−2.79 (m, 2 H) 3.59 (d, $J = 5.3$ Hz, 1 H) 3.85 − 4.01 (m, 2 H) 4.61−4.78 (m, 2 H) 6.99 (d, $J = 8.7$ Hz, 2 H) 7.39 (d, $J = 3.8$ Hz, 1 H) 7.49 (d, $J = 3.8$ Hz, 1 H) 7.61 (d, $J = 8.7$ Hz, 2 H) 8.06 (br s, 1 H). Anal. ($C_{24}H_{32}N_2O_7S_2$) C, H, N.

4-((*R*)-Carboxy-{5-[4′-(2-methoxyethoxy)-phenyl]-thiophene-2-sulfonylamino}-methyl)-piperidine-1-carboxylic Acid *iso*-Propyl Ester (24i). Amino acid **7** was coupled to chloride **18n**, which was treated as described for **24f**. ^{1}H NMR (300 MHz, DMSO-d_6) δ ppm 1.02−1.28 (m, 8 H) 1.40−1.54 (m, 2 H) 1.76−1.95 (m, 1 H) 2.55−2.80 (m, 2 H) 3.32 (s, 3 H) 3.61−3.76 (m, 3 H) 3.85−4.01 (m, 2 H) 4.07−4.19 (m, 2 H) 4.64−4.80 (m, 1 H) 7.03 (d, $J = 8.7$ Hz, 2 H) 7.41 (d, $J = 3.8$ Hz, 1 H) 7.49 (d, $J = 3.8$ Hz, 1 H) 7.63 (d, $J = 8.7$ Hz, 2 H) 8.36 (d, $J = 9.4$ Hz, 1 H) 12.81 (br s, 1 H). Anal. ($C_{24}H_{32}N_2O_8S_2$) C, H, N.

4-{(*R*)-Carboxy-[5-(4′-methylphenyl)-thiophene-2-sulfonylamino]-methyl}-piperidine-1-carboxylic Acid *iso*-Propyl Ester (24j). Amino acid **7** was coupled to chloride **18i**, which was treated as described for **24f**. ^{1}H NMR (300 MHz, $CDCl_3$) δ ppm 1.04−1.68 (m, 10 H) 1.77−2.02 (m, 1 H) 2.39 (s, 3 H) 2.53−2.79 (m, 2 H) 3.81−4.02 (m, 1 H) 4.03−4.27 (m, 2 H) 4.71−4.95 (m, 1 H) 5.34−5.52 (m, 1 H) 7.06−7.24 (m, 3 H) 7.38−7.58 (m, 3 H). Anal. ($C_{22}H_{28}N_2O_6S_2$) C, H, N.

4-{(*R*)-Carboxy-[5-(4′-trifluoromethylphenyl)-thiophene-2-sulfonylamino]-methyl}-piperidine-1-carboxylic Acid *iso*-Propyl Ester (24k). Amino acid **7** was coupled to sulfonyl chloride **19n**, which was treated as described for **24f**. ^{1}H NMR (300 MHz, $CDCl_3$) δ ppm 1.15−1.34 (m, 8 H) 1.35−1.51 (m, 1 H) 1.57−1.71 (m, 2 H) 1.88−2.03 (m, 1 H) 2.59−2.78 (m, 2 H) 3.89−4.04 (m, 1 H) 4.07−4.27 (m, 2 H) 4.77−4.93 (m, 1 H) 5.42−5.56 (m, 1 H) 7.31 (d, $J = 3.8$ Hz, 1 H) 7.57 (d, $J = 3.8$ Hz, 1 H) 7.69 (s, 4 H). Anal. ($C_{22}H_{25}F_3N_2O_6S_2$) C, H, N.

4-{(*R*)-Carboxy-[5-(4′-ethoxyphenyl)-thiophene-2-sulfonylamino]-methyl}-piperidine-1-carboxylic Acid Methyl Ester (24l). By the methods described for the preparation of **24f**, compound **24s** was treated with HCl (g) to afford the piperidine hydrochloride salt. Silylation of the piperidine hydrochloride salt with *N*,*O*-bistrimethylsilylacetamide in THF, followed by reaction with methyl chloroformate, afforded the title compound. ^{1}H NMR (300 MHz, $CDCl_3$) δ ppm 1.16−1.72 (m, 7 H) 1.82−2.02 (m, 1 H) 2.56−2.79 (m, 2 H) 3.64 (s, 3 H) 3.85−3.98 (m, 1 H) 3.99−4.24 (m, 4 H) 5.42 (d, $J = 9.42$ Hz, 1 H) 6.92 (d, $J = 8.7$ Hz, 2 H) 7.07 (d, $J = 3.8$ Hz, 1 H) 7.40−7.56 (m, 3 H). Anal. ($C_{21}H_{26}N_2O_7S_2$) C, H, N.

4-{(*R*)-Carboxy-[5-(4′-ethoxyphenyl)-thiophene-2-sulfonylamino]-methyl}-piperidine-1-carboxylic Acid Ethyl Ester (24m). By the method described for the preparation of **24f**, compound **24s** was treated with HCl (g) to afford the piperidine hydrochloride salt. Silylation of the piperidine hydrochloride salt with *N*,*O*-bistrimethylsilylacetamide in THF, followed by reaction with ethyl chloroformate, afforded the title compound. ^{1}H NMR (300 MHz, $CDCl_3$) δ ppm 1.05−1.48 (m, 9 H) 1.46−1.64 (m, 2 H) 1.78−2.02 (m, 1 H) 2.50−2.77 (m, 2 H) 3.76−3.92 (m, 1 H) 3.96−4.23 (m, 6 H) 5.67−5.90 (m, 1 H) 6.87 (d, $J = 8.7$ Hz, 1 H) 7.06 (d, $J = 4.1$ Hz, 1 H) 7.38−7.57 (m, 3 H). Anal. ($C_{22}H_{28}N_2O_7S_2$) C, H, N.

4-{(*R*)-Carboxy-[5-(4′-ethoxyphenyl)-thiophene-2-sulfonylamino]-methyl}-piperidine-1-carboxylic Acid Propyl Ester (24n). By the methods described for the preparation of **24f**, compound **24s** was treated with HCl (g) to afford the piperidine hydrochloride salt. Silylation of the piperidine hydrochloride salt with *N*,*O*-bistrimethylsilylacetamide in THF, followed by reaction with propyl chloroformate, afforded the title compound. ^{1}H NMR (300 MHz, $CDCl_3$) δ ppm 0.91 (t, $J = 7.2$ Hz, 3 H) 1.17−1.34 (m, 2 H) 1.41 (t, $J = 7.2$ Hz, 3 H) 1.51−1.69 (m, 4 H) 1.78−2.05 (m, 1 H) 2.53−2.77 (m, 2 H) 4.02 (s, 7 H) 5.53−5.65 (m, 1 H) 6.90 (d, $J = 9.0$ Hz, 2 H) 7.08 (d, $J = 3.8$ Hz, 1 H) 7.42−7.53 (m, 3 H).Anal. ($C_{23}H_{30}N_2O_7S_2$) C, H, N.

4-{(*R*)-Carboxy-[5-(4′-ethoxyphenyl)-thiophene-2-sulfonylamino]-methyl}-piperidine-1-carboxylic Acid Butyl Ester (24o). By the methods described for the preparation of **24f**, compound **24s** was treated with HCl (g) to afford the piperidine hydrochloride salt. Silylation of the piperidine hydrochloride salt with *N*,*O*-bistrimethylsilylacetamide in THF, followed by reaction with butyl chloroformate, afforded the title compound. ^{1}H NMR (300 MHz, $CDCl_3$) δ ppm 0.81−1.01 (m, 3 H) 1.15−1.69 (m, 11 H) 1.84−2.00 (m, 1 H) 2.51−2.83 (m, 2 H) 3.69−4.33 (m, 7 H) 5.49 (d, $J = 9.8$ Hz, 1 H) 6.93 (d, $J = 8.7$ Hz, 2 H) 7.08 (d, $J = 3.8$ Hz, 1 H) 7.44−7.56 (m, 3 H). Anal. ($C_{24}H_{32}N_2O_7S_2$) C, H, N.

4-{(*R*)-Carboxy-[5-(4′-ethoxyphenyl)-thiophene-2-sulfonylamino]-methyl}-piperidine-1-carboxylic Acid *iso*-Butyl Ester (24p). By the methods described for the preparation of **24f**, compound **24s** was treated with HCl (g) to afford the piperidine hydrochloride salt. Silylation of the piperidine hydrochloride salt with *N*,*O*-bistrimethylsilylacetamide in THF, followed by reaction with *iso*-butyl chloroformate, afforded the title compound. ^{1}H NMR (300 MHz, MeOH-d_4) δ ppm 0.94 (d, $J = 6.8$ Hz, 6 H) 1.16−1.32 (m, 1 H) 1.34−1.50 (m, 4 H) 1.55−1.73 (m, 2 H) 1.82−1.99 (m, 2 H) 2.68−2.92 (m, 2 H) 3.78−3.89 (m, 3 H) 4.02−4.22 (m, 5 H) 6.98 (d, $J = 9.0$ Hz, 2 H) 7.27 (d, $J = 4.1$ Hz, 1 H) 7.49 (d, $J = 3.8$ Hz, 1 H) 7.56 (d, $J = 8.7$ Hz, 2 H). Anal. ($C_{24}H_{32}N_2O_7S_2$) C, H, N.

4-{(*R*)-Carboxy-[5-(4′-ethoxyphenyl)-thiophene-2-sulfonylamino]-methyl}-piperidine-1-carboxylic Acid 1-Ethyl-propyl Ester (24q). By the methods described for the preparation of **24f**, compound **24s** was treated with HCl (g) to afford the piperidine hydrochloride salt. Silylation of the piperidine hydrochloride salt with *N*,*O*-bistrimethylsilylacetamide in THF, followed by reaction with 3-pentyl chloroformate, afforded the title compound. ^{1}H NMR (300 MHz, DMSO-d_6) δ ppm 0.80 (t, J = 7.54 Hz, 6 H) 1.04−1.28 (m, 2 H) 1.33 (t, J = 6.78 Hz, 3 H) 1.39−1.57 (m, 6 H) 1.79−1.94 (m, 1 H) 2.58−2.80 (m, 2 H) 2.83−3.06 (m, 1 H) 3.55−3.71 (m, 1 H) 3.88−4.01 (m, 2 H) 4.07 (q, J = 6.78 Hz, 2 H) 4.41−4.54 (m, 1 H) 6.97 (d, J = 8.67 Hz, 2 H) 7.38 (d, J = 3.77 Hz, 1 H) 7.47 (d, J = 4.14 Hz, 1 H) 7.61 (d, J = 8.67 Hz, 2 H) 12.79 (br s, 1 H). Anal. ($C_{25}H_{34}N_2O_7S_2$) C, H, N.

4-{(*R*)-Carboxy-[5-(4′-ethoxyphenyl)-thiophene-2-sulfonylamino]-methyl}-piperidine-1-carboxylic Acid Neopentyl Ester (24r). By the methods described for the preparation of **24f**, compound **24s** was treated with HCl (g) to afford the piperidine hydrochloride salt. Silylation of the piperidine hydrochloride salt with *N*,*O*-bistrimethylsilylacetamide in THF, followed by reaction with neopentyl chloroformate, afforded the title compound. ^{1}H NMR (300 MHz, DMSO-d_6) δ ppm 0.85 (s, 9 H) 1.04−1.39 (m, 5 H) 1.41−1.57 (m, 2 H) 1.81−1.98 (m, 1 H) 2.56−2.87 (m, 2 H) 3.18−3.47 (m, 2 H) 3.57−3.70 (m, 2 H) 3.91−4.13 (m, 4 H) 7.00 (d, J = 8.7 Hz, 2 H) 7.39 (d, J = 3.8 Hz, 1 H) 7.49 (d, J = 3.8 Hz, 1 H) 7.62 (d, J = 8.7 Hz, 2 H) 8.17 (br s, 1 H). Anal. ($C_{25}H_{34}N_2O_7S_2$) C, H, N.

4-{(*R*)-Carboxy-[5-(4′-ethoxyphenyl)-thiophene-2-sulfonylamino]-methyl}-piperidine-1-carboxylic Acid *tert*-Butyl Ester (24s). By the method described for the preparation of **9**, amino acid **7** was coupled to sulfonyl chloride **19i** to afford the title sulfonamide. ^{1}H NMR (300 MHz, DMSO-d_6) δ ppm 1.02−1.26 (m, 2 H) 1.28−1.55 (m, 14 H) 1.75−1.95 (m, 1 H) 2.55−2.79 (m, 2 H) 3.61−3.76 (m, 1 H) 3.83−3.98 (m, 2 H) 4.07 (q, J = 6.4 Hz, 2 H) 7.02 (d, J = 7.9 Hz, 2 H) 7.40 (d, J = 3.8 Hz, 1 H) 7.49 (d, J = 3.8 Hz, 1 H) 7.64 (d, J = 7.9 Hz, 2 H) 8.34 (br s, 1 H) 12.64 (br s, 1 H). Anal. ($C_{24}H_{32}N_2O_7S_2$) C, H, N.

4-{(*R*)-Carboxy-[5-(4′-ethoxyphenyl)-thiophene-2-sulfonylamino]-methyl}-piperidine-1-carboxylic Acid Cyclohexyl Ester (24t). By the methods described for the preparation of **24f**, compound **24s** was treated with HCl (g) to afford the piperidine hydrochloride salt. Silylation of the piperidine hydrochloride salt with *N*,*O*-bistrimethylsilylacetamide in THF, followed by reaction with cyclohexyl chloroformate, afforded the title compound. ^{1}H NMR (300 MHz, $CDCl_3$) δ ppm 1.06−1.43 (m, 13 H) 1.47−1.62 (m, 3 H) 1.63−1.79 (m, 2 H) 1.83−1.98 (m, 1 H) 2.44−2.68 (m, 2 H) 3.72−3.86 (m, 1 H) 3.90−4.15 (m, 4 H) 4.47−4.61 (m, 1 H) 6.79 (d, J = 7.5 Hz, 2 H) 6.95−7.03 (m, 1 H) 7.34−7.50 (m, 3 H). Anal. ($C_{26}H_{34}N_2O_7S_2$) C, H, N.

4-{(*R*)-Carboxy-[5-(4′-ethoxyphenyl)-thiophene-2-sulfonylamino]-methyl}-piperidine-1-carboxylic Acid 4-Tetrahydropyranyl Ester (24u). By the methods described for the preparation of **24f**, compound **24s** was treated with HCl (g) to afford the piperidine hydrochloride salt. Silylation of the piperidine hydrochloride salt with *N*,*O*-bistrimethylsilylacetamide in THF, followed by reaction with 4-tetrahydropyranyl chloroformate, afforded the title compound. ^{1}H NMR (300 MHz, DMSO-d_6) δ ppm 1.11−1.58 (m, 9 H) 1.77−1.96 (m, 3 H) 2.54−2.80 (m, 2 H) 3.36−3.50 (m, 3 H) 3.68−3.81 (m, 2 H) 3.91−4.13 (m, 4 H) 4.62−4.78 (m, 1 H) 7.00 (d, J = 9.0 Hz, 2 H) 7.38 (d, J = 3.8 Hz, 1 H) 7.49 (d, J = 3.8 Hz, 1 H) 7.62 (d, J = 9.0 Hz, 2 H). Anal. ($C_{24}H_{32}N_2O_8S_2\cdot H_2O$) C, H, N.

(*R*)-[5-(4′-Methoxyphenyl)-thiophene-2-sulfonylamino]-(1-*tert*-butylcarbamoyl-piperidin-4-yl)-acetic Acid (25a). Amino acid **7** was coupled to sulfonyl chloride **18g**. Removal of the BOC group was effected by the procedure described for **24f**. Elaboration of the piperidine nucleophile was conducted in parallel with a series of isocyanates. To a solution of (*R*)-[5-(4′-methoxyphenyl)-thiophene-2-sulfonylamino]-piperidin-4-yl-acetic acid hydrochloride (0.025 g, 0.061 mmol) in DMF (0.5 mL) was added *N*-methylmorpholine (NMM) (0.013 mL, 0.122 mmol) and *tert*-butyl isocyanate (0.007 mL, 0.061 mmol). The crude reaction mixture was quenched by the addition of TFA and purified by HPLC to afford the title urea. MS (ESI) *m*/*z* 510.4 (M + 1).

(*R*)-[5-(4′-Methoxyphenyl)-thiophene-2-sulfonylamino]-(1-*iso*-propylcarbamoyl-piperidin-4-yl)-acetic Acid (25b). The title compound was prepared by the analogy to the method described in **25a** from (*R*)-[5-(4′-methoxyphenyl)-thiophene-2-sulfonylamino]-piperidin-4-yl-acetic acid hydrochloride and *iso*-propyl isocyanate. MS (ESI) *m*/*z* 496.2 (M + 1).

(*R*)-[5-(4′-Ethoxyphenyl)-thiophene-2-sulfonylamino]-(1-*iso*-propylcarbamoyl-piperidin-4-yl)-acetic Acid (25c). Compound **24s** was treated with HCl (g) to afford the piperidine hydrochloride salt. To a solution of (*R*)-[5-(4′-ethoxyphenyl)-thiophene-2-sulfonylamino]-piperidin-4-yl-acetic acid hydrochloride (0.183 g, 0.40 mmol) in DMF (5 mL) was added *N*-methylmorpholine (NMM) (0.100 mg, 0.99 mmol) and *iso*-butyl isocyanate (0.034 mL, 0.40 mmol). After 18 h, the reaction was diluted with a 10% aqueous solution of citric acid and water. The resulting white precipitate was collected by filtration. The filter cake was rinsed with water and EtOAc and dried in vacuo to a constant weight of 170 mg (84%). ^{1}H NMR (300 MHz, DMSO-d_6) δ ppm 1.00 (d, J = 6.8 Hz, 6 H) 1.06−1.26 (m, 2 H) 1.34 (t, J = 7.2 Hz, 3 H) 1.38−1.48 (m, 2 H) 1.70−1.92 (m, 1 H) 2.37−2.60 (m, 2 H) 3.61−3.77 (m, 2 H) 3.87−4.00 (m, 2 H) 4.07 (q, J = 7.2 Hz, 2 H) 6.06 (d, J = 7.5 Hz, 1 H) 7.00 (d, J = 8.7 Hz, 2 H) 7.38 (d, J = 3.8 Hz, 1 H) 7.49 (d, J = 4.1 Hz, 1 H) 7.63 (d, J = 8.7 Hz, 2 H) 8.34 (d, J = 9.4 Hz, 1 H) 12.63 (br s, 1 H). Anal. ($C_{23}H_{31}N_3O_6S_2$) C, H, N.

(*R*)-[5-(4′-Methoxyphenyl)-thiophene-2-sulfonylamino]-(1-diethylcarbamoylpiperidin-4-yl)-acetic Acid (25d). Amino acid **7** was coupled to sulfonyl chloride **18g**. Removal of the BOC group was effected by the procedure described for **24f**. To a suspension of (*R*)-[5-(4′-methoxyphenyl)-thiophene-2-sulfonylamino]-piperidin-4-yl-acetic acid hydrochloride (0.20 g, 0.45 mmol) in THF (5 mL) was added *N*,*O*-bistrimethylsilylacetamide (0.44 mL, 1.80 mmol). After 1 h, diethyl carbamoyl chloride (0.114 mL, 0.90 mmol) was added. After 18 h, the reaction was concentrated under reduced pressure. The residue was taken up in CH_2Cl_2 and washed with 1 N HCl. The organic phase was dried ($MgSO_4$), filtered, and concentrated in vacuo. The residue was purified by column chromatography (10% MeOH/CH_2Cl_2) to afford to the title compound. ^{1}H NMR (300 MHz, DMSO-d_6) δ ppm 1.01 (t, J = 6.8 Hz, 6 H) 1.09−1.37 (m, 3 H) 1.40−1.52 (m, 2 H) 1.75−1.89 (m, 1 H) 2.53−2.68 (m, 2 H) 3.05 (q, J = 7.2 Hz, 4 H) 3.39−3.52 (m, 2 H) 3.52−3.60 (m, 1 H) 3.78 (s, 3 H) 7.02 (d, J = 8.7 Hz, 2 H) 7.39 (d, J = 4.1 Hz, 1 H) 7.48 (d, J = 3.8 Hz, 1 H) 7.65 (d, J = 8.7 Hz, 2 H). Anal. ($C_{23}H_{31}N_3O_6S_2\cdot 0.75H_2O$) C, H, N.

(*R*)-[5-(4′-Methoxyphenyl)-thiophene-2-sulfonylamino]-(1-*iso*-butyl-piperidin-4-yl)-acetic Acid (26a). Amino acid **7** was coupled to sulfonyl chloride **19g**. Removal of the BOC group was effected by the procedure described for **24f**. Elaboration of the piperidine nucleophile was conducted in parallel with a series of acid chlorides. To a solution of (*R*)-[5-(4′-methoxyphenyl)-thiophene-2-sulfonylamino]-piperidin-4-yl-acetic acid hydrochloride (0.025 g, 0.061 mmol) in DMF (0.5 mL) was added *N*-methylmorpholine (NMM) (0.013 mL, 0.122 mmol) and *iso*-butyryl chloride (0.007 mL, 0.061 mmol). The crude reaction mixture was quenched by the addition of TFA and purified by HPLC to afford the title amide: MS (ESI) *m*/*z* 481.3 (M + 1).

(*R*)-[5-(4′-Ethoxyphenyl)-thiophene-2-sulfonylamino]-[1-(2,2-dimethylpropionyl)-piperidin-4-yl]-acetic Acid (26b). Compound **24s** was treated with HCl (g) to afford the piperidine hydrochloride salt. Silylation of the piperidine hydrochloride salt with *N*,*O*-bistrimethylsilylacetamide in THF, followed by reaction with pivaloyl chloride, afforded the title amide. ^{1}H NMR (300 MHz, DMSO-d_6) δ ppm 1.06−1.24 (m, 11 H) 1.29−1.41 (m, 3 H) 1.44−1.59 (m, 2 H) 1.88−1.99 (m, 1 H) 2.60−2.80 (m, 2 H) 3.62−3.75 (m, 1 H) 4.00−4.18 (m, 2 H) 4.18−4.36 (m, 2 H) 6.93−7.10 (m, 2 H) 7.37−7.44 (m, 1 H) 7.46−7.54 (m, 1 H) 7.57−7.73 (m, 2 H) 8.39 (d, J = 7.5 Hz, 1 H) 12.81 (br s, 1 H). Anal. ($C_{24}H_{32}N_2O_6S_2\cdot 0.75H_2O$) C, H, N.

(*R*)-[5-(4′-Ethoxyphenyl)-thiophene-2-sulfonylamino]-[1-(3,3-dimethylbutyryl)-piperidin-4-yl]-acetic Acid (26c). Compound **24s** was treated with HCl (g) to afford the piperidine hydrochloride salt. Silylation of the piperidine hydrochloride salt with *N*,*O*-bistrimethylsilylacetamide in THF, followed by reaction with 3,3-dimethyl-butyryl chloride, afforded the title amide. ^{1}H NMR (300 MHz, DMSO-d_6) δ ppm 0.95 (s, 9 H) 1.00−1.62 (m, 7 H) 1.84−2.01 (m, 1 H) 2.03−2.45 (m, 3 H) 2.66−2.99 (m, 3 H) 3.89−4.01 (m, 1 H) 4.07 (q, J = 6.8 Hz, 2 H) 4.38−4.53 (m, 1 H) 7.00 (d, J = 9.0 Hz, 2 H) 7.38 (d, J = 3.8 Hz, 1 H) 7.49 (d, J = 4.14 Hz, 1 H) 7.62 (d, J = 8.7 Hz, 2 H). Anal. ($C_{25}H_{34}N_2O_6S_2$) C, H, N.

(*R*)-[5-(4′-Ethoxyphenyl)-thiophene-2-sulfonylamino]-(1-cyclohexanecarbonyl-piperidin-4-yl)-acetic Acid 26d). Compound **24s** was treated with HCl (g) to afford the piperidine hydrochloride salt. Silylation of the piperidine hydrochloride salt with *N*,*O*-bistrimethylsilylacetamide in THF, followed by reaction with cyclohexanecarbonyl chloride, afforded the title amide. ^{1}H NMR (300 MHz, DMSO-d_6) δ ppm 0.96−1.40 (m, 10 H) 1.41−1.71 (m, 7 H) 1.83−2.02 (m, 1 H) 2.22−2.47 (m, 2 H) 2.76−3.04 (m, 1 H) 3.48−3.65 (m, 1 H) 3.85−3.97 (m, 1 H) 4.06 (q, J = 6.8 Hz, 2 H) 4.24−4.48 (m, 1 H) 6.99 (d, J = 8.7 Hz, 2 H) 7.38 (d, J = 3.8 Hz, 1 H) 7.48 (d, J = 3.8 Hz, 1 H) 7.61 (d, J = 8.7 Hz, 2 H) 7.97 (br s, 1 H). Anal. ($C_{26}H_{34}N_2O_6S_2$) C, H, N.

(*R*)-[5-(4′-Ethoxyphenyl)-thiophene-2-sulfonylamino]-[1-(tetrahydropyran-4-carbonyl)-piperidin-4-yl]-acetic Acid (26e). Compound **24s** was treated with HCl (g) to afford the piperidine hydrochloride salt. Silylation of the piperidine hydrochloride salt with *N*,*O*-bistrimethylsilylacetamide in THF, followed by reaction with tetrahydropyran-4-carbonyl chloride, afforded the title amide. ^{1}H NMR (300 MHz, DMSO-d_6) δ ppm 0.96−1.27 (m, 2 H) 1.34 (t, J = 6.4 Hz, 3 H) 1.41−1.65 (m, 6 H) 1.85−2.01 (m, 1 H) 2.31−2.50 (m, 2 H) 2.74−3.05 (m, 3 H) 3.61−3.86 (m, 3 H) 3.92−4.03 (m, 1 H) 4.08 (q, J = 6.8 Hz, 2 H) 4.29−4.45 (m, 1 H) 7.02 (d, J = 8.7 Hz, 2 H) 7.40 (d, J = 4.1 Hz, 1 H) 7.49 (d, J = 4.1 Hz, 1 H) 7.64 (d, J = 8.3 Hz, 2 H) 8.36 (d, J = 9.0 Hz, 1 H) 12.67 (br s, 1 H). Anal. ($C_{25}H_{32}N_2O_7S_2 \cdot 0.5H_2O$) C, H, N.

(*R*)-[5-(4′-Methoxyphenyl)-thiophene-2-sulfonylamino]-[1-(toluene-4-sulfonyl)-piperidin-4-yl]-acetic Acid (27a). Amino acid **7** was coupled to sulfonyl chloride **19g**. Removal of the BOC group was effected by the procedure described for **24f**. Elaboration of the piperidine nucleophile was conducted in parallel with a series of acid chlorides. To a solution of (*R*)-[5-(4′-methoxyphenyl)-thiophene-2-sulfonylamino]-piperidin-4-yl-acetic acid hydrochloride (0.025 g, 0.061 mmol) in DMF (0.5 mL) was added *N*-methylmorpholine (NMM) (0.013 mL, 0.122 mmol) and *para*-toluene sulfonyl chloride (0.011 mg, 0.061 mmol). The crude reaction mixture was quenched by the addition of TFA and purified by HPLC to afford the title sulfonamide. MS (ESI) *m*/*z* 565.4 (M + 1).

(*R*)-[5-(4′-Methoxyphenyl)-thiophene-2-sulfonylamino]-[1-(benzenesulfonyl)-piperidin-4-yl]-acetic Acid (27b). Amino acid **7** was coupled to sulfonyl chloride **19g**. Removal of the BOC group was effected by the procedure described for **24f**. Elaboration of the piperidine nucleophile was conducted in parallel with a series of acid chlorides. To a solution of (*R*)-[5-(4′-methoxyphenyl)-thiophene-2-sulfonylamino]-piperidin-4-yl-acetic acid hydrochloride (0.025 g, 0.061 mmol) in DMF (0.5 mL) was added *N*-methylmorpholine (NMM) (0.013 mL, 0.122 mmol) and phenyl sulfonyl chloride (0.007 mL, 0.061 mmol). The crude reaction mixture was quenched by the addition of TFA and purified by HPLC to afford the title sulfonamide. MS (ESI) *m*/*z* 551.4 (M + 1).

(*R*)-[5-(4′-Ethoxyphenyl)-thiophene-2-sulfonylamino]-[1-(benzenesulfonyl)-piperidin-4-yl]-acetic Acid (27c). Compound **24s** was treated with HCl (g) to afford the piperidine hydrochloride salt. Silylation of the piperidine hydrochloride salt with *N*,*O*-bistrimethylsilylacetamide in THF, followed by reaction with benzenesulfonyl chloride, afforded the title sulfonamide. ^{1}H NMR (300 MHz, DMSO-d_6) δ ppm 1.24−1.69 (m, 8 H), 2.01−2.18 (m, 2 H), 3.38−3.49 (m, 1 H), 3.57−3.69 (m, 2 H), 4.07 (q, J = 7.2 Hz, 2 H), 7.00 (d, J = 9.0 Hz, 2 H), 7.38 (d, J = 4.1 Hz, 1 H), 7.47 (d, J = 4.1 Hz, 1 H), 7.58−7.67 (m, 4 H), 7.66−7.74 (m, 3 H). Anal. ($C_{25}H_{26}N_2O_7S_3$) C, H, N.

(*R*)-[5-(4′-Ethoxyphenyl)-thiophene-2-sulfonylamino]-[1-(phenylmethanesulfonyl)-piperidin-4-yl]-acetic Acid (27d). Compound **24s** was treated with HCl (g) to afford the piperidine hydrochloride salt. Silylation of the piperidine hydrochloride salt with *N*,*O*-bistrimethylsilylacetamide in THF, followed by reaction with phenylmethanesulfonyl chloride, afforded the title sulfonamide. ^{1}H NMR (300 MHz, DMSO-d_6) δ ppm 1.09−1.41 (m, 5 H) 1.53 (s, 2 H) 1.65−1.81 (m, 1 H) 2.53−2.72 (m, 2 H) 3.45−3.58 (m, 2 H) 3.70 (dd, J = 9.42, 6.40 Hz, 1 H) 4.07 (q, J = 6.8 Hz, 2 H) 4.35 (s, 2 H) 7.00 (d, J = 8.7 Hz, 2 H) 7.29−7.44 (m, 6 H) 7.48 (d, J = 3.8 Hz, 1 H) 7.63 (d, J = 9.0 Hz, 2 H) 8.41 (d, J = 9.4 Hz, 1 H). Anal. ($C_{26}H_{30}N_2O_7S_3$) C, H, N.

(*R*)-[5-(4′-Ethoxyphenyl)-thiophene-2-sulfonylamino]-[1-(2-methylpropane-1-sulfonyl)-piperidin-4-yl]-acetic Acid (27e). Compound **24s** was treated with HCl (g) to afford the piperidine hydrochloride salt. ^{1}H NMR (300 MHz, MeOD) δ ppm 1.08 (d, J = 6.4 Hz, 6 H) 1.26−1.61 (m, 5 H) 1.64−1.80 (m, 2 H) 1.80−1.95 (m, 1 H) 2.11−2.30 (m, 1 H) 2.63−2.89 (m, 4 H) 3.65−3.78 (m, 2 H) 3.86 (d, J = 5.7 Hz, 1 H) 4.07 (q, J = 7.2 Hz, 2 H) 6.96 (d, J = 9.0 Hz, 2 H) 7.24 (d, J = 3.8 Hz, 1 H) 7.50 (d, J = 3.8 Hz, 1 H) 7.57 (d, J = 8.7 Hz, 2 H). Anal. ($C_{23}H_{32}N_2O_7S_3$) C, H, N.

(*R*)-[5-(4′-Ethoxyphenyl)-thiophene-2-sulfonylamino]-[1-(propane-2-sulfonyl)-piperidin-4-yl]-acetic Acid (27f). Compound **24s** was treated with HCl (g) to afford the piperidine hydrochloride salt. Silylation of the piperidine hydrochloride salt with *N*,*O*-bistrimethylsilylacetamide in THF, followed by reaction with propane-2-sulfonyl chloride, afforded the title sulfonamide. ^{1}H NMR (300 MHz, DMSO-d_6) δ ppm 1.16 (d, J = 6.8 Hz, 6 H) 1.23−1.41 (m, 5 H) 1.43−1.63 (m, 2 H) 1.78−1.95 (m, 1 H) 2.67−2.88 (m, 2 H) 3.13−3.31 (m, 3 H) 3.50−3.70 (m, 3 H) 4.07 (q, J = 7.2 Hz, 2 H) 7.00 (d, J = 9.0 Hz, 1 H) 7.39 (d, J = 4.1 Hz, 1 H) 7.50 (d, J = 4.1 Hz, 1 H) 7.62 (d, J = 8.7 Hz, 2 H). Anal. ($C_{22}H_{30}N_2O_7S_3$) C, H, N.

(*R*)-[5-(4-Ethoxyphenyl)-thiophene-2-sulfonylamino]-[1-(butane-1-sulfonyl)-piperidin-4-yl]-acetic Acid (27g). Compound **24s** was treated with HCl (g) to afford the piperidine hydrochloride salt. Silylation of the piperidine hydrochloride salt with *N*,*O*-bistrimethylsilylacetamide in THF, followed by reaction with butane-1-sulfonyl chloride, afforded the title sulfonamide. ^{1}H NMR (300 MHz, $CDCl_3$) δ ppm 0.93 (t, J = 7.5 Hz, 2 H) 1.34−1.55 (m, 7 H) 1.62−1.83 (m, 5 H) 1.83−1.98 (m, 1 H) 2.59−2.78 (m, 2 H) 2.78−3.05 (m, 2 H) 3.75−3.87 (m, 2 H) 3.90−3.98 (m, 1 H) 4.08 (q, J = 6.8 Hz, 2 H) 5.54 (d, J = 9.4 Hz, 1 H) 6.92 (d, J = 8.7 Hz, 2 H) 7.09 (d, J = 3.4 Hz, 1 H) 7.46−7.54 (m, 3 H). Anal. ($C_{23}H_{32}N_2O_7S_3 \cdot H_2O$) C, H, N.

(*R*)-[5-(4′-Ethoxyphenyl)-thiophene-2-sulfonylamino]-[1-(propane-1-sulfonyl)-piperidin-4-yl]-acetic Acid (27h). Compound **24s** was treated with HCl (g) to afford the piperidine hydrochloride salt. Silylation of the piperidine hydrochloride salt with *N*,*O*-bistrimethylsilylacetamide in THF, followed by reaction with propane-1-sulfonyl chloride, afforded the title sulfonamide. ^{1}H NMR (300 MHz, DMSO-d_6) δ ppm 0.96 (t, J = 7.54 Hz, 3 H) 1.18−1.42 (m, 5 H) 1.52−1.73 (m, 4 H) 1.72−1.87 (m, 1 H) 2.61−2.79 (m, 2 H) 2.91−3.00 (m, 2 H) 3.49−3.62 (m, 2 H) 3.67−3.76 (m, 1 H) 4.07 (q, J = 7.16 Hz, 2 H) 7.00 (d, J = 9.04 Hz, 2 H) 7.40 (d, J = 4.14 Hz, 1 H) 7.50 (d, J = 3.77 Hz, 1 H) 7.63 (d, J = 9.04 Hz, 2 H) 8.41 (d, J = 9.42 Hz, 1 H) 12.76 (br s, 1 H). Anal. ($C_{22}H_{30}N_2O_7S_3 \cdot 0.5H_2O$) C, H, N.

(*R*)-[5-(4′-Ethoxyphenyl)-thiophene-2-sulfonylamino]-[1-(methane-1-sulfonyl)-piperidin-4-yl]-acetic Acid (27i). Compound **24s** was treated with HCl (g) to afford the piperidine hydrochloride salt. ^{1}H NMR (300 MHz, DMSO-d_6) δ ppm 1.19−1.44 (m, 5 H) 1.51−1.67 (m, 2 H) 1.69−1.86 (m, 1 H) 2.54−2.75 (m, 2 H) 2.82 (s, 3 H) 3.53 (d, J = 11.0 Hz, 2 H) 3.73 (dd, J = 9.4, 6.4 Hz, 1 H) 4.07 (q, J = 7.2 Hz, 2 H) 7.00 (d, J = 9.0 Hz, 2 H) 7.41 (d, J = 4.1 Hz, 1 H) 7.50 (d, J = 3.8 Hz, 1 H) 7.61 (d, J = 8.7 Hz, 2 H) 8.43 (d, J = 9.4 Hz, 1 H) 12.75−12.98 (br s, 1 H). Anal. ($C_{20}H_{26}N_2O_7S_3$) C, H, N.

(*R*)-[5-(4′-Ethoxyphenyl)-thiophene-2-sulfonylamino]-[1-benzylpiperidin-4-yl]-acetic Acid (28a). Compound **24s** was treated with HCl (g) to afford the piperidine hydrochloride salt. To a mixture of (*R*)-[5-(4′-ethoxyphenyl)-thiophene-2-sulfonylamino]-piperidin-4-yl-acetic acid hydrochloride (80 mg, 0.17 mmol) and benzaldehyde (20 mg, 0.19 mmol) in THF (10 mL) was added sodium triacetoxyborohydride (55 mg, 0.26 mmol). The reaction was stirred at room temperature under N_2. After stirring for 36 h, additional sodium triacetoxyborohydride (18 mg, 0.08 mmol) acetic acid (two drops) were added, and the reaction was stirred for another 12 h. The reaction was diluted with a saturated aqueous solution of sodium bicarbonate (10 mL) and extracted with CH_2Cl_2 (3 × 10 mL). The combined organic layers were washed with brine (10 mL), dried over Na_2SO_4, filtered, and concentrated to give a brownish solid. The solid was dissolved in CH_2Cl_2, filtered through celite, and recrystallized from EtOAc to provide the title acid as a solid (58 mg, 65%). ^{1}H NMR (400 MHz, $CDCl_3$) δ ppm 1.32−1.54 (m, 4 H) 1.73−1.85 (m, 1 H) 1.91−2.11 (m, 2 H) 2.35−2.62 (m, 3 H) 3.07−3.21 (m, 1 H) 3.57−3.63 (m, 1 H) 3.64−3.74 (m, 2 H) 3.96−4.15 (m, 3 H) 6.37 (d, J = 4.5 Hz, 1 H) 6.83 (d, J = 8.7 Hz, 2 H) 7.08 (d, J = 3.8 Hz, 1 H) 7.12−7.24 (m, 5 H) 7.44 (d, J = 8.7 Hz, 2 H) 7.52 (d, J = 3.8 Hz, 1 H). Anal. ($C_{26}H_{30}N_2O_5S_2 \cdot 0.5H_2O$) C, H, N.

(*R*)-[5-(4′-Ethoxyphenyl)-thiophene-2-sulfonylamino]-[1-*iso*-butylpiperidin-4-yl]-acetic Acid (28b). Compound **24s** was treated with HCl (g) to afford the piperidine hydrochloride salt. To a mixture of (*R*)-[5-(4′-ethoxyphenyl)-thiophene-2-sulfonylamino]-piperidin-4-yl-acetic acid hydrochloride (150 mg, 0.33 mmol) and isobutyraldehyde (35.19 mg, 0.49 mmol) in dichloroethane (10 mL) was added sodium triacetoxyborohydride (140 mg, 0.66 mmol), followed by two drops of acetic acid. After stirring at RT under N_2 overnight, the solvent was removed under reduced pressure. To the residue was added H_2O (10 mL) to afford a gray−white solid precipitate. The solid was collected, washed with H_2O (10 mL) and EtOH (1 mL), and dried under vacuum to provide the title acid as a solid (140 mg, 89.6%). ^{1}H NMR (400 MHz, $CDCl_3$) δ ppm 0.64−0.89 (m, 6 H) 1.36−1.51 (m, 5 H) 1.79−1.90 (m, 1 H) 1.91−2.08 (m, 3 H) 2.37−2.53 (m, 3 H) 2.54−2.74 (m, 2 H) 3.37−3.46 (m, 1 H) 3.56−3.62 (m, 1 H) 3.63−3.72 (m, 1 H) 4.05 (q, J = 6.8 Hz, 2 H) 6.31 (d, J = 4.5 Hz, 1 H) 6.88 (d, J = 8.7 Hz, 1 H) 7.04 (d, J = 3.8 Hz, 1 H) 7.39−7.51 (m, 3 H) 13.68 (br s, 1 H). Anal. ($C_{23}H_{32}N_2O_5S_2$) C, H, N.

(*R*)-[5-(4′-Ethoxyphenyl)-thiophene-2-sulfonylamino]-[1-(2,2-dimethylpropyl)-piperidin-4-yl]-acetic Acid (28c). Compound **24s** was treated with HCl (g) to afford the piperidine hydrochloride salt. To a mixture of (*R*)-[5-(4′-ethoxyphenyl)-thiophene-2-sulfonylamino]-piperidin-4-yl-acetic acid hydrochloride (150 mg, 0.33 mmol) and trimethylacetaldehyde (42 mg, 0.49 mmol) in THF (10 mL) was added sodium triacetoxyborohydride (140 mg, 0.66 mmol), followed by two drops of acetic acid. The reaction was stirred overnight at room temperature under N_2. Additional sodium triacetoxyborohydride (140 mg, 0.66 mmol) and two drops of acetic acid were added, and the resulting mixture was stirred for another 12 h. Additional trimethylacetaldehyde (40 mg, 0.46 mmol) was added. After stirring for 12 h, the solvent was removed under reduced pressure. To the residue was added H_2O (10 mL), and the solid was collected by filtration. The solid was purified by chromatography on diol silica gel using 100% CH_2Cl_2 as the eluent to provide the title acid as a yellow solid (80 mg, 50%). ^{1}H NMR (400 MHz, $CDCl_3$) δ ppm −0.01 (s, 9 H) 0.39−0.59 (m, 1 H) 0.83−1.00 (m, 1 H) 1.37−1.50 (m, 3 H) 1.83−2.15 (m, 3 H) 2.46−2.79 (m, 4 H) 3.48−3.71 (m, 3 H) 4.05 (q, J = 7.2 Hz, 2 H) 6.20−6.31 (m, 1 H) 6.89 (d, J = 8.7 Hz, 2 H) 7.06 (d, J = 4.1 Hz, 1 H) 7.42−7.53 (m, 3 H) 14.05 (br s, 1 H). Anal. ($C_{24}H_{34}N_2O_5S_2$) C, H, N.

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Supporting Information Available: Experimental procedures for the synthesis of sulfonyl chlorides **8**, **18a**−**n**, **19a**−**e**, and **21a**−**c** and elemental analysis for all target compounds. This material is available free of charge via the Internet at http://pubs.acs.org.

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