Discovery of CGS 27023A, a Non-Peptidic, Potent, and Orally Active Stromelysin Inhibitor That Blocks Cartilage Degradation in Rabbits

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Structure−activity relationships of a lead hydroxamic acid inhibitor of recombinant human stromelysin were systematically defined by taking advantage of a concise synthesis that allowed diverse functionality to be explored at each position in a template. An *ex vivo* rat model and an *in vivo* rabbit model of stromelysin-induced cartilage degradation were used to further optimize these analogs for oral activity and duration of action. The culmination of these modifications resulted in CGS 27023A, a potent, orally active stromelysin inhibitor that blocks the erosion of cartilage matrix.

Introduction

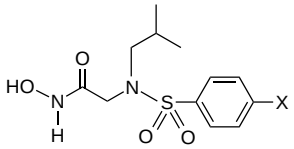
Stromelysin (SLN; MMP-3), a member of the matrix metalloprotease (MMP) family of enzymes,[1,2] is produced by chondrocytes and synovial fibroblasts in joints and plays an important role in the normal turnover and remodeling of cartilage and bone.[3] Stromelysin can hydrolyze a number of key structural proteins that comprise more than 70% of cartilage, such as aggrecan, link protein, and different types of collagens.[4,5] More importantly, however, elevated levels of stromelysin and its degradation products have been measured in cartilage and synovial fluid from patients with osteoarthritis[6,7] and in animal models of osteoarthritis.[8,9] For these reasons, stromelysin (along with collagenase and gelatinase) has become an important pharmaceutical target,[10−15] particularly because the design and development of MMP inhibitors has the potential of becoming a new therapy that may actually prevent the underlying pathology of arthritis. In this report, we summarize our own efforts to identify a potent, orally active stromelysin inhibitor for the treatment of chronic joint disease. The discovery and optimization of our sulfonamide-based hydroxamic acid series is particularly noteworthy because it represents a marked departure from previously described peptide-based hydroxamic acids.

Chemistry

Targeted screening of Ciba's collection of metalloprotease inhibitors was carried out with an enzyme assay using recombinant human stromelysin.[16] One of the compounds tested from this pool was hydroxamic acid **3** (Table 1), a novel template which we had originally designed with molecular modeling from the X-ray structure of the bacterial metalloprotease thermolysin as part of our neutral endopeptidase inhibitor (NEP) program.[17] Although this compound was only a micromolar inhibitor of NEP, it proved to be a potent inhibitor of stromelysin and formed the basis of the medicinal chemistry program outlined here.

The synthesis of analogs with no substituent adjacent to the hydroxamic acid was straightforward, as shown in Scheme 1. The combination of numerous alkyl- or arylalkylamines with a variety of substituted benzenesulfonyl chlorides yielded general intermediate **1**. Alkylation with ethyl bromoacetate, followed by conversion to the hydroxamic acid with base and hydroxylamine, efficiently provided the compounds listed in Tables 1 and 2. Modifications of the *p*-alkyl ether group were generated by a standard deprotection and alkylation sequence as indicated.

Table 1. Inhibition of Recombinant Human Stromelysin by Modifications of the Aryl Substituent



compd	X	SLN K_i (μM)
3	OCH_3	0.133
4	Cl	0.943
5	H	1.76
6	CH_3	3.27
7	F	3.27
8	$N(CH_3)_2$	4.40
9	NH_2	5.00
10	CF_3	5.35
11	NO_2	inactive
12	tBu	inactive
13	SO_2CH_3	inactive
16	$O(CH_2)_3CH_3$	0.058
17	$OCH_2CH_2CH(CH_3)_2$	0.078
18	$O(CH_2)_5CH_3$	0.063
19	$OCH_2(C_6H_{11})$	0.061
20	$OCH(CH_3)_2$	0.336
21	$OCH_2CH_2OCH_2CH_3$	1.13

The compounds listed in Table 3 were derived from readily available amino acids as shown in Scheme 2, which allowed for variations in the side chain next to the hydroxamic acid. Due to the presence of a chiral center sensitive to racemization, the synthesis of these analogs was carried out with acid labile protecting groups to avoid the use of methoxide/hydroxylamine, and potassium carbonate in DMF was used to mildly alkylate the sulfonamide. Chiral purity was established by comparing enantiomeric pairs, which yielded rotations equal in magnitude but opposite in sign (Table 3). Moreover, compound **70** exhibited a single peak by chiral HPLC. This route was particularly well-suited

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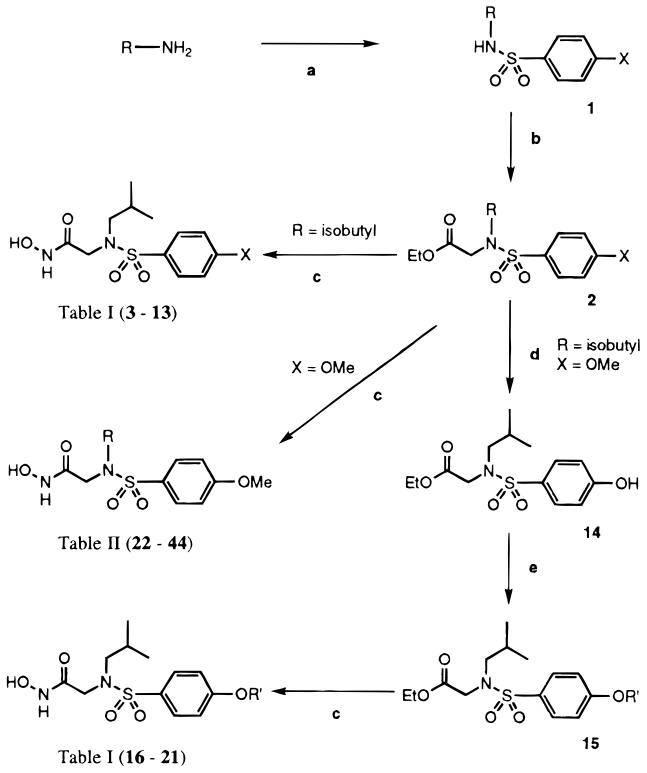
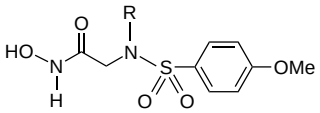
Scheme 1. Synthesis of Hydroxamic Acid Analogs[a]



[a] (a) $ClS(O)_2PhX$, Et_3N; (b) NaH, $BrCH_2CO_2Et$; (c) NaOMe, $HONH_2 \cdot HCl$; (d) EtSH, $AlCl_3$; (e) R′I, Cs_2CO_3.

Table 2. Inhibition of Recombinant Human Stromelysin by Modifications of the *N*-Alkyl Substituent

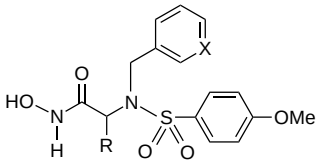


compd	R	SLN K_i (μM)
22	CH_2Ph	0.070
23	CH_2CH_2Ph	0.066
24	$CH_2CH_2CH_2Ph$	0.125
25	$CH_2CH_2CH_2CH_2Ph$	0.100
26	$CH_2CH_2CH(CH_3)_2$	0.094
27	$CH_2(C_6H_{11})$	0.061
28	$CH_2CH_2(C_6H_{11})$	0.038
29	C_6H_{11}	1.26
30	$CH(CH_3)CH_2CH_3$	1.82
31	$C(CH_3)_3$	2.50
32	CH_2Ph-4-Cl	0.094
33	CH_2Ph-4-OMe	0.104
34	CH_2Ph-4-Ph	0.107
35	CH_2CF_3	0.305
36	H	4.40
37	$CH_2CH_2N(CH_2CH_2)_2O$	0.236
38	$CH_2CH_2N(C_5H_{10})$	1.13
39	CH_2Ph-3-OMe	0.070
40	CH_2Ph-3,4,5-OMe	0.151
41	CH_2(4-pyridine)	0.192
42	CH_2(3-pyridine)	0.113
43	CH_2(2-pyridine)	0.090
44	CH_2(2-quinoline)	0.134

for making any compound that contained a heterocycle attached to the sulfonamide nitrogen.

It is important to point out that the longest reaction sequence illustrated in this work is six steps, a feature that permitted the synthesis of large quantities of compound needed early in the project for chronic osteoarthritis studies in 3 kg rabbits and other larger animals.

Table 3. Inhibition of Recombinant Human Stromelysin by Modifications of the Group Next to the Hydroxamic Acid (Stereochemistry Indicated as *R* or *S*)



compd	R	SLN K_i (μM)
X = C		
49	CH_3	0.086
50 (*S*)[a]	CH_3	0.141
51 (*R*)[b]	CH_3	0.036
52	CH_2Ph	0.071
53 (*R*)	CH_2Ph	0.029
54 (*R*)	$CH_2CH(CH_3)_2$	0.023
55 (*R*)	Ph	0.104
56	$C(CH_3)_3$	0.700
57	$CH(CH_3)_2$	0.092
58 (*R*)	$CH(CH_3)_2$	0.034
59 (*R*)	$CH_2CH_2SCH_3$	0.031
60 (*R*)	$CH_2CH_2S(O)_2CH_3$	0.085
61 (*R*)	$(CH_2)_4$NCBZ	0.070
62 (*R*)	$(CH_2)_4N(CH_3)_2$	0.069
63 (*R*)	CH_2OtBu	0.048
64	CH_2Ph-4-OH	0.065
65 (*R*)	$CH_2C(CH_3)_3$	0.028
66 (*R*)	$CH_2(C_6H_{11})$	0.044
67 (*R*)	$CH(CH_3)CH_2CH_3$	0.039
68	$CH_2CH_2N(CH_2CH_2)_2O$	0.050
X = N		
69 (*R*)	$CH_2CH(CH_3)_2$	0.046
70 (*R*)[c]	$CH(CH_3)_2$	0.043
71 (*S*)[d]	$CH(CH_3)_2$	1.30
72 (*R*)	$(CH_2)_4N(CH_3)_2$	0.081
73 (*R*)	$CH_2C(CH_3)_3$	0.042

[a] $[\alpha]_D = -20.80°$ ($c = 7.4$ mg/mL, MeOH). [b] $[\alpha]_D = +23.28°$ ($c = 7.9$ mg/mL, MeOH). [c] $[\alpha]_D = +38.17°$ ($c = 7.9$ mg/mL, MeOH). [d] $[\alpha]_D = -35.43°$ ($c = 10.1$ mg/mL, MeOH).

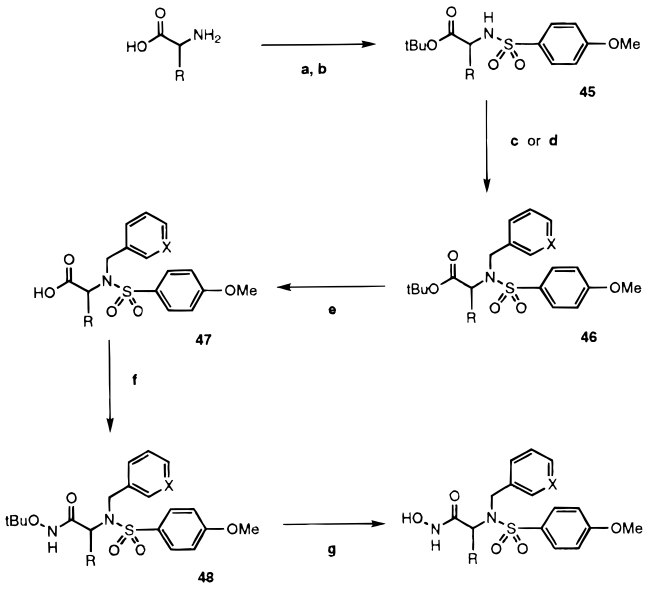
Results and Discussion

Analysis of *in Vitro* Data. The optimization of **3** was explored systematically, mapping out the binding topography at each position in the template. It should be mentioned at this point that compound design and SAR analysis were accomplished without the benefit of enzyme structural information.

First, it was established that the hydroxamic acid group, which acts as the zinc-binding ligand, could not be modified in any way whatsoever, as summarized in Figure 1. For example, the parent carboxylic acid of **3**, as well as the homologated acid, were both inactive. N-Methylation, which removes the ionizable N-H that presumably leads to such high affinity for the active site zinc atom, also abolished all inhibitory activity. Replacement of the carbonyl group by an imine, deletion of the carbonyl group, or substitution of the N-H by CH_2 all yielded inactive analogs, even though in each example only one atom was modified.

Next, our chemistry program focused on the *p*-methoxybenzenesulfonamide group. As shown in Figure 1, replacement of the benzene ring by other heterocycles, such as pyridine, dimethylthiazole, and thiophene yielded inactive inhibitors. The results listed in Table 1 indicate that many modifications of the para substituent led to a loss of activity as well. The preference for the methoxy group could be due to its electron-donating property, which would strengthen the hydrogen-bonding

Scheme 2. Synthesis of Amino Acid Derived Hydroxamic Acids[a]



[a] (a) $ClS(O)_2PhOMe$, Et_3N; (b) $(CH_3)_2NCH[OC(CH_3)_3]_2$; (c) K_2CO_3, BnBr; (d) K_2CO_3, 3-picolyl chloride; (e) HCl; (f) EDC, HOBT, NMM, $tBuONH_2 \cdot HCl$; (g) HCl.

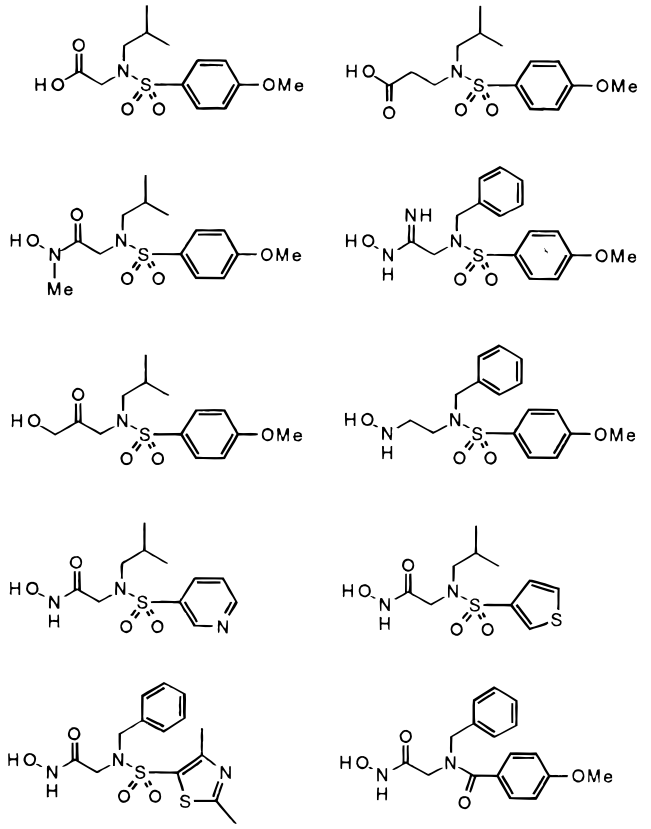


Figure 1. Template modifications that produced inactive SLN inhibitors.

ability of the distal sulfonamide oxygens to the enzyme. Although compounds **8** and **9** might then also be expected to have good potency, protonation of the amino group would actually reduce its donating ability, yielding weak activity similar to that observed with authentic electron-withdrawing substituents as in compounds **10**, **11**, and **13**. Substituents at either the ortho or meta positions on the benzene ring, such as methyl or even an electron-donating methoxy, were not tolerated and led to inactive compounds (data not shown), presumably due to steric interference in the active site.

The sulfonamide bond was also crucial for potency, since replacement by an amide bond (Figure 1) caused the loss of all activity, either because both sulfonamide oxygens make strong H-bonds to the enzyme or because the geometry of the active sulfonamide rotamer in the enzyme cannot be attained by the planar amide linker. In any event, the sulfonamide bond was preferred on the basis of *in vivo* stability.

On the basis of the good potency of the *p*-methyl ether, additional alkoxy residues were synthesized as shown by compounds **16**−**21**. Most groups were acceptable, with the longer chains giving rise to increased potency, while branching, as in the isopropyl analog **20**, attenuated activity possibly due to steric effects. Remarkably, the insertion of just one ether oxygen as in **21** induced a dramatic loss of activity, indicating that this region of the enzyme is quite hydrophobic.

The implication of all the above observations is that the substituted benzene sulfonamide group would appear to be binding in the S1′ pocket of stromelysin, since structural studies have shown that this region is a deep but relatively narrow hydrophobic channel.[18] As further support for this hypothesis, extended hydrophobic groups at the P1′ position in other templates, structurally similar to the side chains in compounds **16**−**19**, have also yielded potent stromelysin inhibitors.[19,20] (The binding mode of this series in SLN was ultimately confirmed near the end of our program when the detailed structure of an enzyme/inhibitor complex was solved by protein NMR.[21])

Initially, a large number of side chains were examined at the sulfonamide nitrogen attachment point with the emphasis on hydrophobic groups, as listed in Table 2. For the most part, all alkyl and arylalkyl groups yielded potent inhibitors, even the large biphenylylmethyl analog **34**. Diminished activity was observed only with substituents containing branching next to the nitrogen (**29**−**31**) which might interfere with the H-bonding of the sulfonamide oxygens, or with strongly basic substituents (piperidine **38**), or with no substituent at all (**36**). The diversity of allowed side chains at this position implies binding to a somewhat ill-defined enzyme pocket, which would implicate the rather shallow and solvent-exposed S2′ pocket.

Our attention then turned to substitution next to the hydroxamic acid, as listed in Table 3. The first analogs synthesized contained aliphatic or aromatic side chains (**49, 52, 56, 57, and 64**). Although these substituents were generally well tolerated, a limitation was encountered with β-trisubstitution (**56**), where steric bulk became too demanding and/or the *tert*-butyl group precluded attainment of the bioactive rotamer. On the other hand, most other structural modifications were allowed, suggesting that these side chains do not reside in a discrete pocket, but rather in a nondiscriminating domain, such as the spacious S1 region of the enzyme.

The next objective was to establish the enantiomeric preference of the newly created chiral center with respect to SLN potency. Accordingly, it was found that the preferred stereochemistry at this position corresponded to the *R*-isomer, which was more potent than

the *S*-isomer or racemate (**50** vs **51**; **52** vs **53**; **57** vs **58**). Note that the *R*-isomer of these inhibitors arises from the unnatural D-enantiomer of the starting amino acid.

A number of D-amino acid derived analogs were then synthesized, many of which were more active than the unsubstituted lead compound **3**. Moreover, it was speculated that substitution α to the hydroxamic acid might hinder *in vivo* hydrolysis of this critical zinc binding ligand, thus prolonging the duration of action of an inhibitor.

At this stage of our optimization program, the most potent inhibitor identified was **54**, a fairly hydrophobic compound derived from D-leucine. Our drug discovery efforts were now ready to enter the next phase of the project, optimization of oral activity and duration of action *in vivo*.

***In Vivo* Pharmacology.** A major goal of the project was to select a compound for evaluation in chronic osteoarthritis animal model studies. Since these experiments could last anywhere from 3 to 16 months, two profiling assays were established to help select those analogs that generated high plasma drug levels after oral dosing and demonstrated good efficacy in preventing cartilage degradation in an acute *in vivo* setting.

The first assay involved measuring drug levels indirectly by carrying out an *ex vivo* SLN assay on a plasma sample taken at timed intervals after orally dosing rats. In the second model,[22] rabbits were first dosed orally or intravenously with a compound. After a specified time, depending on the duration of action being studied, recombinant human stromelysin was injected intraarticularly into each knee joint and cartilage degradation proceeded for an additional 2 h. The animals were then sacrificed, and the synovial fluid was collected by lavage and analyzed for cartilage fragments released due to erosion.

Compounds devoid of any substituent adjacent to the hydroxamic acid were too short-acting in both profiling assays after oral dosing, consistent with our hypothesis that a side chain at that position was important in slowing down metabolic inactivation of that key functionality. Inactivation would include hydrolysis, reduction, or glucuronidation of the hydroxamic acid group, but no attempt was made at this stage of the project to screen analogs in metabolism assays.[23] Instead, our chemistry strategy focused on the amino acid derived analogs described in Table 3 as *in vivo* properties were improved. For example, compound **54** worked well in the rabbit model after iv dosing at 12.5 μmol/kg, inhibiting the release of keratan sulfate (KS) and sulfated glycosaminoglycans (S-GAG) by 65% in a 1 h experiment. However, compound **54** did not work in this model after oral dosing, and this result correlates well with the relatively low drug levels measured in the rat *ex vivo* assay after oral dosing (Figure 2). In fact, hydrophobic analogs such as **54** typically did not generate good drug levels after oral dosing and, as a consequence, did not exhibit good efficacy in our acute cartilage degradation model.

It was hypothesized that improvements in oral activity could be addressed by incorporating polar functionality into the template to increase water solubility. A similar strategy has been followed using peptide-based hydroxamic acid inhibitors.[24] Noteworthy features of cartilage include its high degree of hydration and its

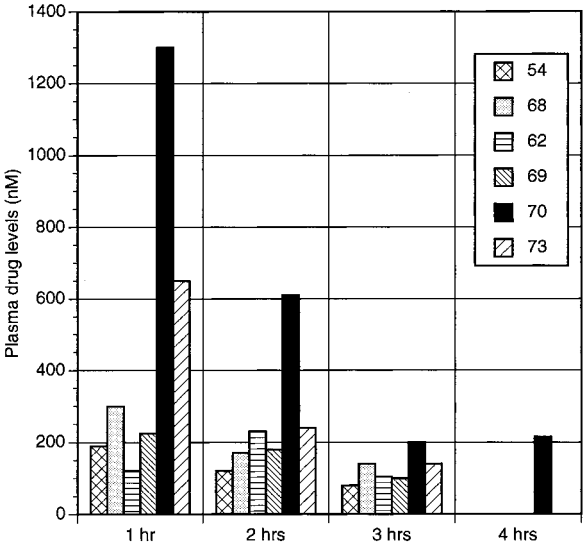


Figure 2. Drug concentrations in rats by *ex vivo* assay. Compounds dosed at 75 μmol/kg po.

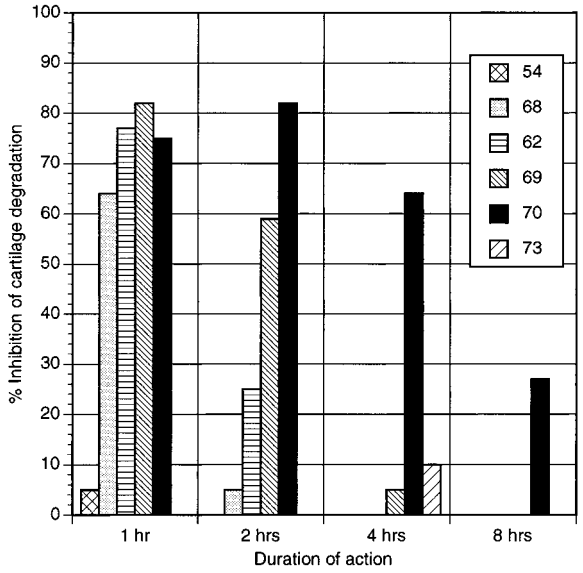
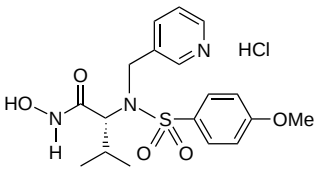


Figure 3. Inhibition of SLN-induced cartilage degradation in rabbits. Compounds dosed at 75 μmol/kg po.

extremely high concentration of negative charges due to all the sulfated sugar residues on the aggrecan protein. These physical characteristics of cartilage influenced our decision to include basic amino groups in the template, since these would be positively charged *in vivo* and might improve drug partitioning into cartilage.

A milestone in the project was achieved with the relatively hydrophilic morpholine-based derivative **68**, since it was the first compound to demonstrate efficacy in the rabbit model after oral dosing, although only for 1 h (Figure 3). Futher improvement was seen with lysine-based derivatives such as compound **62**, which prevented cartilage degradation for 2 h. Given the substantial improvements gained as a result of polar substitution, the focus of our synthetic efforts were redirected toward polar/hydrophilic substitution on the sulfonamide nitrogen. In our early SAR studies, hydrophobic residues were originally emphasized at this position because it was felt that this region of the template mimicked leucines or phenylalanines occupying the P1′ position in SLN substrates. However, it was

Table 4. Potency of CGS 27023A in Four Recombinant Human MMP Assays



enzyme assay	K_i, nM
stromelysin (MMP-3)	43
collagenase (MMP-1)	33
72K gelatinase (MMP-2)	20
92K gelatinase (MMP-9)	8

soon determined that mildly basic heterocycles such as morpholine **37**, pyridines **41**−**43**, or quinoline **44** were perfectly acceptable side chains at this location. On the basis of these observations, numerous analogs derived from D-amino acids that contained heterocycles on the sulfonamide nitrogen were synthesized, such as the ones listed at the end of Table 3.

The pharmacokinetics data presented in Figure 2 reveal that the highest and most persistent drug levels were achieved with the D-valine-derived analog, compound **70** (CGS 27023A), presumably because the sterically bulky isopropyl group efficiently protects the hydroxamic acid from metabolic deactivation. Furthermore, the picolyl group was also critical, because the benzyl version of this analog (**58**) had no oral activity. In general, it was observed that only hydroxamic acids containing one polar appendage exhibited very good pharmacokinetics, presumably due to the proper balance of good water solubility and good absorption permeability. When two or more polar substituents were incorporated into the template (such as incorporating a picolyl group into a lysine derivative, compound **72**), the *in vivo* profile worsened.

Figure 3 highlights the best results from the rabbit model. As the graph clearly shows, the most effective and longest-acting inhibitor identified in this model was compound **70**, CGS 27023A, which prevented cartilage degradation for up to 8 h.

On the basis of these encouraging results, CGS 27023A was then produced in kilogram quantities so that long-term studies could be carried out in the rabbit meniscectomy OA model[25] and the guinea pig model of spontaneous OA.[26] In each of these chronic settings, CGS 27023A preserved cartilage matrix and chondrocyte viability as measured by histological evaluation, supporting the potential of this approach for OA therapy. After the selection of CGS 27023A as a candidate for clinical development, additional recombinant matrix metalloproteases were cloned and expressed to further profile this inhibitor. As shown in Table 4, CGS 27023A is a potent, broad-based inhibitor of the key MMPs implicated in arthritis.

Conclusion

A structurally novel series of non-peptidic sulfonamide-based hydroxamic acid metalloprotease inhibitors was discovered and optimized. A very efficient and versatile synthesis of these analogs was developed from readily available amino acids to facilitate scaling up to kilogram quantities. Incorporation of polar side chains and sterically demanding α-substituents led to continuous optimization of *in vitro* and *in vivo* properties. By relying on an acute rabbit model that provided information on bioavailability, drug accessibility to a joint, and duration of action, CGS 27023A was identified as a compound suitable for evaluation in long-term osteoarthritis animal models and clinical development.

Experimental Section

General. Solvents and reagents were used as received. Flash chromatography was carried out with a forced flow of the indicated solvent system on EM Science silica gel 60 (230−400 mesh). ^{1}H NMR spectra were recorded on a Bruker AC-250 (250 MHz) or a Varian XL-300 (300 MHz) in the indicated solvent. Mass spectra were determined on a Hewlett-Packard 5985B quadrupole mass spectrometer using desorption chemical ionization (DCI) with methane. Melting points were determined on a Hoover capillary melting point apparatus. Optical rotations were determined on a Jasco DIP-370 polarimeter. Microanalyses were carried out at Robertson Laboratory in Madison, NJ.

General Procedure for Compounds in Tables 1 and **2.** The procedures used to synthesize these analogs are described in detail for compound **22**.

N-[(4-Methoxyphenyl)benzenesulfonyl]benzylamine (1, R = CH_2Ph, X = OMe). Benzylamine (16.0 mL, 145.2 mmol) is dissolved in chloroform (110 mL), and the solution is cooled to 0 °C. To this solution is added 4-methoxybenzenesulfonyl chloride (10.0 g, 48.4 mmol). The reaction is stirred at room temperature for 1 h and then refluxed for 1 h. After being cooled back to room temperature, the reaction mixture is washed three times with 4 N hydrochloric acid (200 mL), twice with water (100 mL), and once with brine (50 mL) and then dried (Na_2SO_4), and the solvent is evaporated to give **1** (13.0 g, 97% yield): NMR (300 MHz, $CDCl_3$) ∂ 7.7 (d, 2 H), 7.3 (m, 5 H), 7.0 (d, 2 H), 4.65 (t, 1 H), 4.2 (d, 2 H), 3.8 (s, 3 H); MS (DCI, CH_4) *m*/*e* 278 (M + 1).

Ethyl 2-[[(4-Methoxyphenyl)sulfonyl]benzylamino]-acetate (2, R = CH_2Ph, X = OMe). Sodium hydride (1.56 g of a 50% oil dispersion, 33.0 mmol) is suspended in tetrahydrofuran (85 mL). To this is added a solution of sulfonamide **1** (9.0 g, 32.5 mmol) also in tetrahydrofuran (85 mL), and the reaction mixture is stirred for 30 min at room temperature. Then ethyl bromoacetate (5.40 mL, 48.8 mmol) is added, and the reaction mixture is stirred overnight at room temperature. The reaction is quenched with a small amount of water, and all the solvent is removed. The crude mixture is partitioned between ethyl acetate and water, the aqueous phase is extracted several times with ethyl acetate, the combined organic layers are dried (Na_2SO_4), and the solvent is evaporated. The product is purified by silica gel chromatography (30% ethyl acetate/hexane) to give **2** (11.25 g, 95% yield): NMR (300 MHz, $CDCl_3$) ∂ 7.8 (d, 2 H), 7.3 (m, 5 H), 7.0 (d, 2 H), 4.5 (s, 2 H), 4.0 (q, 2 H), 3.95 (s, 2 H), 3.9 (s, 3 H), 1.15 (t, 3 H); MS (DCI, CH_4) *m*/*e* 364 (M + 1).

N-Hydroxy-2-[[(4-methoxyphenyl)sulfonyl]benzylamino]acetamide (22). Ester **2** (11.20 g, 30.9 mmol) is dissolved in methanol (100 mL). To this solution is added hydroxylamine hydrochloride (4.31 g, 62.0 mmol), followed by the addition of sodium methoxide, freshly prepared from sodium (2.14 g, 93.0 mmol) dissolved in methanol (55 mL). The reaction mixture is stirred overnight at room temperature. The reaction is worked up by partitioning between dilute hydrochloric acid (pH = ∼3) and ethyl acetate. The aqueous phase is extracted well with ethyl acetate, the combined organic layers are dried (Na_2SO_4), and the solvent is evaporated. The product is purified by silica gel chromatography (75% ethyl acetate/hexane) to give **22** (7.40 g, 68% yield): mp 112−114 °C; NMR (300 MHz, MeOH) ∂ 7.8 (d, 2 H), 7.3 (broad s, 5 H), 7.1 (d, 2 H), 4.4 (s, 2 H), 3.85 (s, 3 H), 3.7 (s, 2 H); MS (DCI, CH_4) *m*/*e* 351 (M + 1). Anal. ($C_{16}H_{18}N_2O_5S$) C, H, N.

Ethyl 2-[[(4-Hydroxyphenyl)sulfonyl]isobutylamino]-acetate (14). A solution of ethanethiol (15 mL) and methylene chloride (15 mL) is cooled to 0 °C. Aluminum trichloride (9.62 g, 72.2 mmol) is added (the solution turns green), and the

reaction mixture is warmed to room temperature. Ethyl 2-[[(4-methoxyphenyl)sulfonyl]isobutylamino]acetate (4.75 g, 14.44 mmol) is added in methylene chloride (5 mL), and the reaction mixture is stirred for 3.5 h at room temperature. The reaction is then slowly quenched with water, and the crude reaction mixture is partitioned between water and methylene chloride. The aqueous layer is extracted well with methylene chloride, the combined organic layers are dried (Na_2SO_4), and the solvent is evaporated. The product is purified by silica gel chromatography (25% to 50% ethyl acetate/hexane) to give **14** (2.6 g, 72% yield): NMR (300 MHz, $CDCl_3$) ∂ 7.80 (d, 2 H), 7.0 (d, 2 H), 6.4 (broad s, 1 H), 4.15 (q, 2 H), 3.9 (s, 2 H), 3.0 (d, 2 H), 1.80 (m, 1 H), 1.2 (t, 3 H), 0.85 (d, 6 H); MS (DCI, CH_4) *m/e* 316 (M + 1).

Ethyl 2-[[[4-(Hexyloxy)phenyl]sulfonyl]isobutylamino]-acetate (15, R′ = Hexyl). Phenol **14** (1.0 g, 3.17 mmol) is dissolved in dimethylformamide (16 mL). Cesium carbonate (1.03 g, 3.17 mmol) is added, followed by 1-iodohexane (0.47 mL, 3.17 mmol), and the reaction mixture is stirred overnight at room temperature. The reaction mixture is then partitioned between water and ethyl acetate, the aqueous layer is extracted well with ethyl acetate, the combined organic layers are dried (Na_2SO_4), and the solvent is evaporated. The product is purified by silica gel chromatography (10% ethyl acetate/hexane) to give **15** (1.22 g, 96% yield): NMR (300 MHz, $CDCl_3$) ∂ 7.75 (d, 2 H), 6.9 (d, 2 H), 4.1 (q, 2 H), 4.0 (t, 2 H), 3.95 (s, 2 H), 3.0 (d, 2 H), 1.85 (m, 3 H), 1.5 (m, 2 H), 1.35 (m, 4 H), 1.2 (t, 3 H), 0.95 (t, 3 H), 0.85 (d, 6 H); MS (DCI, CH_4) *m/e* 400 (M + 1).

***N*-Hydroxy-2-[[[4-(hexyloxy)phenyl]sulfonyl]isobutylamino]acetamide (18).** Ester **15** (1.22 g, 3.05 mmol) is dissolved in methanol (15 mL). To this solution is added hydroxylamine hydrochloride (0.43 g, 6.11 mmol), followed by the addition of sodium methoxide, freshly prepared from sodium (0.35 g, 15.3 mmol) dissolved in methanol (5 mL). The reaction mixture is stirred for 36 h at room temperature. The reaction is worked up by partitioning between dilute hydrochloric acid (pH = ~3) and ethyl acetate. The aqueous phase is extracted well with ethyl acetate, the combined organic layers are dried (Na_2SO_4), and the solvent is evaporated. The product is crystallized from hexane/ethyl acetate and collected by filtration to give **18** (0.90 g, 68% yield): mp 108−110 °C; NMR (300 MHz, MeOH) ∂ 7.8 (d, 2 H), 7.05 (d, 2 H), 4.05 (t, 2 H), 3.75 (s, 2 H), 2.95 (d, 2 H), 1.8 (m, 3 H), 1.5 (m, 2 H), 1.35 (m, 4 H), 0.95 (t, 3 H), 0.85 (d, 6 H); MS (DCI, CH_4) *m/e* 387 (M + 1). Anal. ($C_{18}H_{30}N_2O_5S$) C, H, N.

General Procedure for Compounds in Table 3. The procedures used to synthesize the analogs derived from commercially available amino acids are described in detail for compound **70**, CGS 27023A.

N-[(4-Methoxyphenyl)sulfonyl]-D-valine *tert*-Butyl Ester (45, R = Isopropyl). To a solution of D-valine (15.0 g, 128.0 mmol) in 1:1 dioxane/water (200 mL) containing triethylamine (19.4 g, 192.0 mmol) at room temperature is added 4-methoxybenzenesulfonyl chloride (29.0 g, 141.0 mmol), and the reaction mixture is stirred at room temperature overnight. The mixture is then diluted with methylene chloride and washed with 1 N aqueous hydrochloric acid and water. The organic layer is washed again with brine and dried (Na_2SO_4), and the solvent is evaporated to provide *N*-[(4-methoxyphenyl)-sulfonyl]-D-valine (33.0 g) as a crude product. A solution of this crude product (15.0 g) in toluene (100 mL) containing *N*,*N*-dimethylformamide di-*tert*-butyl acetal (50 mL, 206.5 mmol) is heated to 95 °C for 3 h. The solvent is then evaporated. The crude product is purified by silica gel chromatography (30% ethyl acetate/hexanes) to give **45** (7.0 g, 35% overall yield): NMR (250 MHz, $CDCl_3$) ∂ 7.75 (d, 2 H), 6.9 (d, 2 H), 5.0 (d, 1 H), 3.8 (s, 3 H), 3.55 (dd, 1 H), 2.0 (m, 1 H), 1.2 (s, 9 H), 1.0 (d, 3 H), 0.8 (d, 3 H); MS (DCI, CH_4) *m/e* 344 (M + 1).

***tert*-Butyl 2(*R*)-[[(4-Methoxyphenyl)sulfonyl](3-picolyl)amino]-3-methylbutanoate (46, R = Isopropyl, X = N).** To a solution of ester **45** (4.38 g, 13.0 mmol) in dimethylformamide (200 mL) is added 3-picolyl chloride hydrochloride (2.3 g, 14.0 mmol) followed by potassium carbonate (17.94 g, 130.0 mmol). The reaction mixture is stirred at room temperature for 2 days. The mixture is then diluted with water and extracted with ethyl acetate. The combined organic extracts are washed with brine and dried (Na_2SO_4), and the solvent is evaporated. The crude product is purified by silica gel chromatography (ethyl acetate) to give **46** (5.35 g, 95% yield): NMR (250 MHz, $CDCl_3$) ∂ 8.6 (broad s, 1 H), 8.5 (d, 1 H), 7.8 (d, 1 H), 7.6 (d, 2 H), 7.15 (m, 1 H), 6.8 (d, 2 H), 4.65 (dd, 2 H), 4.1 (d, 1 H), 3.8 (s, 3 H), 1.8 (m, 1 H), 1.3 (s, 9 H), 0.85 (d, 3 H), 0.75 (d, 3 H); MS (DCI, CH_4) *m/e* 435 (M + 1).

2(*R*)-[[(4-Methoxyphenyl)sulfonyl](3-picolyl)amino]-3-methylbutanoic Acid Hydrochloride (47, R = Isopropyl, X = N). Ester **46** (5.3 g, 12.2 mmol) is dissolved in methylene chloride (150 mL) and cooled to −10 °C. Hydrochloric acid gas is bubbled into the solution for 10 min. The reaction mixture is then sealed, warmed to room temperature, and stirred for 4 h. The solvent is then evaporated to provide **47** (5.25 g, 100% yield): NMR (250 MHz, $CDCl_3$) ∂ 9.1 (broad s, 1 H), 9.0 (d, 1 H), 8.6 (d, 1 H), 7.95 (t, 1 H), 7.75 (d, 2 H), 6.85 (d, 2 H), 5.1 (d, 1 H), 4.5 (d, 1 H), 4.2 (d, 1 H), 3.8 (s, 3 H), 1.7 (m, 1 H), 0.9 (d, 3 H), 0.7 (d, 3 H); MS (DCI, CH_4) *m/e* 379 (M + 1).

***N*-(*tert*-Butyloxy)-2(*R*)-[[(4-methoxyphenyl)sulfonyl]-(3-picolyl)amino]-3-methylbutanamide (48, R = Isopropyl, X = N).** Acid **47** (5.0 g, 12.06 mmol), 1-hydroxybenzotriazole (1.63 g, 12.06 mmol), 4-methylmorpholine (6.6 mL, 60.31 mmol), and *O*-*tert*-butylhydroxylamine hydrochloride (54.55 g, 36.19 mmol) are dissolved in methylene chloride (200 mL). *N*-[(Dimethylamino)propyl]-*N*′-ethylcarbodiimide hydrochloride (3.01 g, 15.68 mmol) is added, and the reaction mixture is stirred overnight. The reaction mixture is then diluted with water and extracted with methylene chloride. The combined organic extracts are washed with brine and dried (Na_2SO_4), and the solvent is evaporated. The crude product is purified by silica gel chromatography (2% methanol/methylene chloride) to give **48** (4.23 g, 75% yield): NMR (250 MHz, $CDCl_3$) ∂ 8.6 (broad s, 1 H), 8.5 (d, 1 H), 8.2 (s, 1 H), 7.7 (dt, 1 H), 7.55 (d, 2 H), 7.25 (m, 1 H), 6.85 (d, 2 H), 4.6 (dd, 2 H), 3.85 (s, 3 H), 3.65 (d, 1 H), 2.1 (m, 1 H), 1.2 (s, 9 H), 0.85 (d, 3 H), 0.5 (d, 3 H); MS (DCI, CH_4) *m/e* 450 (M + 1).

***N*-Hydroxy-2(*R*)-[[(4-methoxyphenyl)sulfonyl](3-picolyl)amino]-3-methylbutanamide Hydrochloride (70).** The protected hydroxamic acid **48** (4.1 g, 9.13 mmol) is dissolved in dichloroethane (150 mL) containing ethanol (0.53 mL, 9.13 mmol) in a round bottom flask, and the reaction mixture is cooled to −10 °C. Hydrochloric acid gas (from a lecture bottle) is bubbled through for 30 min. The reaction flask is sealed, and the mixture is allowed to slowly warm to room temperature and stirred for 2 days. The solvent is reduced to 1/3 volume by evaporation and triturated with ether. The mixture is filtered, and the filter cake is removed, and dried *in vacuo* to provide **70** as a white solid (3.3 g, 84% yield): mp 169−170 °C; NMR (250 MHz, DMSO) ∂ 8.75 (broad s, 2 H), 8.3 (d, 1 H), 7.8 (dd, 1 H), 7.7 (d, 2 H), 7.0 (d, 2 H), 4.9 (d, 1 H), 4.7 (d, 1 H), 3.85 (s, 3 H), 3.8 (d, 1 H), 2.0 (m, 1 H), 0.8 (d, 3 H), 0.6 (d, 3 H); MS (DCI, CH_4) *m/e* 394 (M + 1); $[\alpha]_D$ = +38.17° (*c* = 7.9 mg/mL, MeOH). Anal. ($C_{18}H_{23}N_3O_5S \cdot HCl \cdot H_2O$) C, H, N.

Compound **68**, derived from an unnatural amino acid, is synthesized as follows.

Diethyl [2-(4-Morpholino)ethyl]acetamidomalonate. *N*-(2-Chloroethyl)morpholine hydrochloride (~12.0 g) is dissolved in water (200 mL) and made basic with ammonium hydroxide (100.0 mL) to a pH = ~11. The aqueous layer is then extracted several times with ether, the combined organic layers are dried (Na_2SO_4), and the solvent is evaporated to yield an oil which is used immediately.

Diethyl acetamidomalonate (11.4 g, 57.08 mmol) is added to a freshly prepared solution of sodium ethoxide in ethanol (made from Na (1.32 g, 57.1 mmol) added to ethanol (34.0 mL)), and the reaction mixture is refluxed for 30 min. The reaction mixture is then adjusted to 55 °C, and potassium iodide (0.14 g, 0.8 mmol) and dimethylformamide (0.2 mL) are added. Finally, the *N*-(2-chloroethyl)morpholine (8.9 g, 59.6 mmol) prepared above is added in ethanol (14.0 mL), and the reaction mixture is maintained at 55 °C for 24 h.

The reaction mixture is diluted with ethyl acetate and filtered through Celite to remove salts. The filtrate is evaporated and then partitioned between ethyl acetate and brine.

The organic layer is dried (Na_2SO_4), and the solvent is evaporated. The product is purified by silica gel chromatography (first 50% ethyl acetate, then 5% methanol/methylene chloride) to give diethyl [2-(4-morpholino)ethyl]acetamidomalonate (8.0 g, 45% yield): NMR (250 MHz, $CDCl_3$) ∂ 7.1 (s, 1 H), 4.2 (m, 4 H), 3.6 (m, 4 H), 2.35 (m, 8 H), 2.0 (s, 3 H), 1.3 (m, 6 H).

Ethyl 2-Acetamido-2-[2-(4-morpholino)ethyl]acetate. Diethyl [2-(4-morpholino)ethyl]acetamidomalonate (8.0 g, 25.6 mmol) is dissolved in ethanol (128.0 mL). Sodium hydroxide (4.55 mL of a 6 N aqueous solution, 27.35 mmol) is added, and the reaction mixture is stirred at room temperature for 24 h. The ethanol is then evaporated, the residue is diluted in water and washed several times with ether, and then the aqueous phase is acidified with concentrated hydrochloric acid to pH = ~5. The solution is evaporated to dryness, suspended in toluene (300.0 mL), and refluxed for 3 h. After being cooled to room temperature, the reaction mixture is diluted with chloroform (300.0 mL) and the mixture is filtered through Celite. The filtrate is evaporated to give ethyl 2-(acetamido)-2-[2-(4-morpholino)ethyl]acetate (6.0 g, 91% yield): NMR (300 MHz, $CDCl_3$) ∂ 7.5 (d, 1 H), 4.6 (m, 1 H), 4.2 (m, 2 H), 3.7 (m, 4 H), 2.5 (m, 6 H), 2.0 (s, 3 H), 1.9 (m, 2 H), 1.3 (t, 3 H).

2-Amino-2-[2-(4-morpholino)ethyl]acetic Acid Dihydrochloride. Ethyl 2-acetamido-2-[2-(4-morpholino)ethyl]-acetate (4.2 g, 16.28 mmol) is dissolved in 6 N hydrochloric acid (100.0 mL), and the reaction mixture is refluxed for 4.5 h. The water is then evaporated, and the product is azeotroped dry using toluene to give 2-amino-2-[2-(4-morpholino)ethyl]-acetic acid dihydrochloride (4.0 g, 94% yield): NMR (250 MHz, D_2O) ∂ 4.1 (m, 3 H), 3.85 (t, 2 H), 3.6 (t, 2 H), 3.45 (m, 2 H), 3.2 (m, 2 H), 2.4 (m, 2 H).

This amino acid is then carried through the general scheme as described above to provide **68**.

Biological Assays. Stromelysin inhibitory activity is based on the hydrolysis of substance P using a modified procedure of Harrison.[27] In this assay, substance P is hydrolyzed by recombinant human stromelysin to generate a fragment, Substance P 7−11, which can be quantitated by HPLC. In a typical assay, a 10 mM stock solution of a compound to be tested is diluted in the assay buffer to 50 μM, mixed 1:1 with 8 μg of recombinant human stromelysin (mol wt 45−47 kDa, 2 units, where 1 unit produces 20 nmol of substance P 7−11 in 30 min) and incubated along with 0.5 mM substance P in a final volume of 0.125 mL for 30 min at 37 °C. The reaction is stopped by adding 10 mM EDTA, and substance P 7−11 is quantified on RP-8 HPLC. The IC_{50} is calculated from control reaction without the inhibitor. The mean K_i values reported in the tables have been derived from the mean IC_{50} according to the method of Waley.[28] The mean IC_{50} was obtained from two independent measurements, and the standard deviation of the mean was less than 10%. To help validate this assay, the best Merck lead was made and found to have a K_i of 361 nM (versus a reported K_i = 470 nM).[29]

Ex vivo pharmacokinetic studies were usually carried out in rats. Compounds were dosed in DMSO/cornstarch at 75 μmol/kg po, after which the rats were bled into heparinized tubes at various time points (typically 1, 2, and 3 h later). After centrifugation at 2000 rpm, the plasma was removed, and the drug was extracted with acetonitrile. The drug level was then determined by incubating this sample with recombinant human stromelysin and substrate and comparing the inhibition observed with a standard curve. It should be emphasized that this method does not directly quantitate the amount of parent compound.

The efficacy of compounds *in vivo* is determined by studying them in rabbits. Typically, four rabbits are dosed orally with a compound at a dose of 75 μmol/kg in 5 mL of fortified corn starch per kilogram of body weight, up to 8 h before intra-articular injection in both knees (N = 8) with 40 units of recombinant human stromelysin (dissolved in 20 mM Tris, 10 mM $CaCl_2$, and 0.15 M NaCl at pH 7.5). Two hours later the rabbits are sacrificed, synovial lavage is collected, and keratan sulfate (KS) and sulfated glycosaminoglycan (S-GAG) fragments released into the joint fluid are quantitated. Keratan sulfate is measured by an inhibition ELISA using the method of Thonar.[30] Sulfated glycosaminoglycans are measured by first digesting the synovial lavage with streptomyces hyaluronidase and then measuring DMB dye binding using the method of Goldberg.[31]

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