Structure-Based Design and Synthesis of Potent Matrix Metalloproteinase Inhibitors Derived from a 6*H*-1,3,4-Thiadiazine Scaffold

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We describe a new generation of heterocyclic nonpeptide matrix metalloproteinase (MMP) inhibitors derived from a 6*H*-1,3,4-thiadiazine scaffold. A screening effort was utilized to identify some chiral 6-methyl-1,3,4-thiadiazines that are weak inhibitors of the catalytic domain of human neutrophil collagenase (cdMMP-8). Further optimization of the lead compounds revealed general design principles that involve the placement of a phenyl or thienyl group at position 5 of the thiadiazine ring, to improve unprimed side affinity; the incorporation of an amino group at position 2 of the thiadiazine ring as the chelating agent for the catalytic zinc; the placement of a *N*-sulfonamide-substituted amino acid residue at the amino group, to improve primed side affinity; and the attachment of diverse functional groups at position 4 or 5 of the phenyl or thienyl group at the unprimed side, to improve selectivity. The new compounds were assayed against eight different matrix metalloproteinases, MMP-1, cdMMP-2, cdMMP-8, MMP-9, cdMMP-12, cdMMP-13, cdMMP-14, and the ectodomain of MMP-14, respectively. A unique combination of the above-described modifications produced the selective inhibitor (2*R*)-*N*-[5-(4-bromophenyl)-6*H*-1,3,4-thiadiazin-2-yl]-2-[(phenylsulfonyl)amino]propanamide with high affinity for MMP-9 (K_i = 40 nM). X-ray crystallographic data obtained for cdMMP-8 cocrystallized with *N*-allyl-5-(4-chlorophenyl)-6*H*-1,3,4-thiadiazin-2-amine hydrobromide gave detailed design information on binding interactions for thiadiazine-based MMP inhibitors.

Introduction

Matrix metalloproteinases (MMPs) are a superfamily of zinc- and calcium-dependent endopeptidases involved in the degradation and remodeling of connective tissues. They are secreted as proenzymes that are subsequently processed by other proteinases to generate the active forms. The proteolytic activity is directed against most constituents of the extracellular matrix, like proteoglycans, fibronectin, laminin, and interstitial collagens.[1] Under normal physiological conditions, the proteolytic activities are controlled by maintaining a balance between the synthesis of the active forms and their inhibition by tissue inhibitors of matrix metalloproteinases (TIMPs) or by nonspecific α_2-macroglobulin.[2] In pathological conditions this equilibrium is shifted toward increased MMP activity leading to tissue degradation,[3] which has been linked to several disease states such as osteoarthritis,[4] rheumatoid arthritis,[5] periodontal disease,[6] multiple sclerosis,[7] and tumor metastasis.[8] Consequently, there has been significant interest in the development of drugs to control the aberrent regulation of MMP production. Recent efforts by a number of laboratories working in the area have provided several classes of MMP inhibitors that have been extensively reviewed.[9] One key issue in the clinical development of MMP inhibitors relates to whether the development of broad-spectrum inhibitors, active against a range of different enzymes, or of selective inhibitors, targeted against a particular subset of the MMPs, represents the optimal strategy. Some orally active compounds for the treatment of cancer and/or arthritis are currently under clinical investigation. Representative examples include succinamides[10] (Marimastat), linear sulfonamides[11] (CGS-27023A), heterocyclic sulfonamides[12] (Prinomastat), and biphenylbutanoic acid derivatives[13] (BAY-129566) (Figure 1).

Within three of these compound classes the hydroxamate moiety plays a decisive role in achieving inhibitor potency. Because of the biologically labile nature of hydroxamates, we undertook additional efforts toward the discovery of new chelating groups suitable for use in MMP inhibitor templates. In recent years, interest in 1,3,4-thiadiazines has increased due to their high biological activity and the broad-spectrum action of their derivatives.[14] The value of thiadiazines as MMP inhibitors has, however, not hitherto been recognized. As part of a collaborative effort, a series of chiral 6-methyl-1,3,4-thiadiazines previously reported[15] was assayed utilizing the catalytic domain of human neutrophil collagenase (cdMMP-8) as the screening enzyme. From this analysis, a small number of compounds (**1**−**3**) were determined

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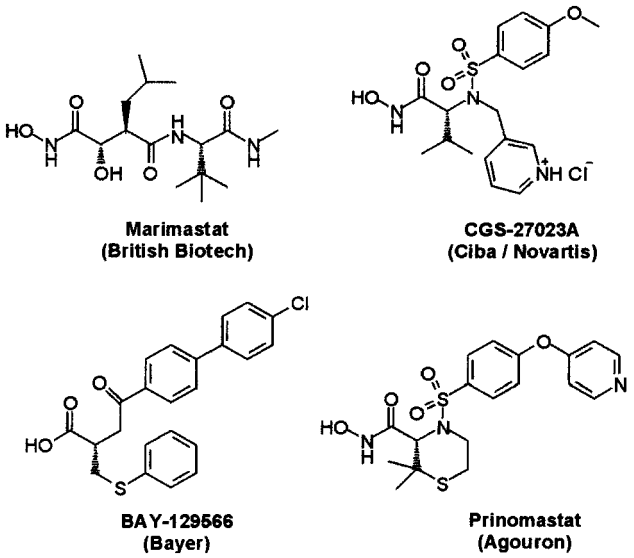


Figure 1. Selected MMP inhibitors.

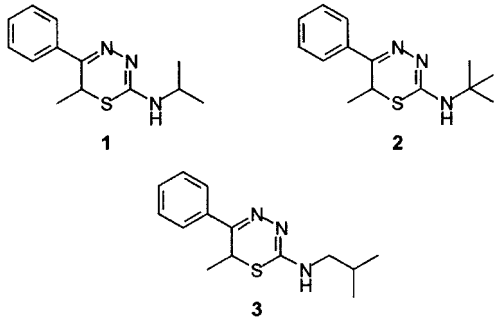


Figure 2. Thiadiazine screening leads.

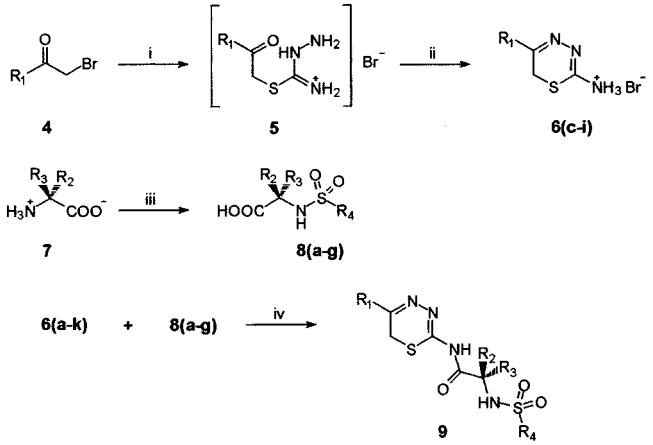
to be competitive inhibitors with weak (K_i > 40 μM) inhibitory activity (Figure 2).

Further chemical modifications including the lack of the 6-methyl group resulted in novel 6*H*-1,3,4-thiadiazine derivatives that are potent inhibitors of matrix metalloproteinases with an exceptional binding mode to the active site. The account below will focus on the synthesis, structure−activity relationship (SAR) studies, and in vitro activity of this series of compounds.

Synthesis

Synthesis of the 6*H*-1,3,4-thiadiazine-based inhibitors was accomplished using two methods. In the first method, a substituted α-bromo-keto compound (**4**) was allowed to react with thiosemicarbazide in chilled ethanol (Scheme 1). The resulting linear intermediate (**5**) was ring-closed by heating this compound in an ethanol/H_2O/HBr mixture to afford the 5-substituted 6*H*-1,3,4-thiadiazine-2-amines (**6c**−**i**) as their hydrobromide salts.[16] A D- or L-configurated amino acid (**7**) was heated with the appropriate sulfonyl chloride in an aqueous potassium carbonate solution to produce the desired sulfonamides (**8a**−**g**). Acylation of the 5-substituted 6*H*-1,3,4-thiadiazine-2-amine hydrohalides (**6a**−**k**) (Table 1) with the carboxylic group of the sulfonamides (**8a**−**g**) (Table 2) was mediated by a mixture of *N*-ethyl-*N*′-(3-dimethylaminopropyl)-carbodiimide hydrochloride, 1-hydroxybenzotriazole, and 4-methylmorpholine in DMF at 5 °C. The compounds with the general structure **9** were obtained as white or yellow solids that could be

Scheme 1. Method A[a]



[a] Reagents and conditions: (i) thiosemicarbazide, EtOH, 0−20 °C; (ii) EtOH, 48% aq HBr, reflux; (iii) (a) K_2CO_3/H_2O, R_4SO_2Cl, reflux, (b) concd HCl; (iv) EDC, HOBt, NMM, DMF, 5 °C.

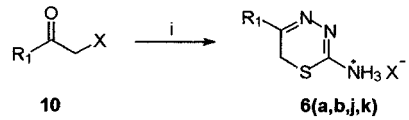
Table 1. Synthesized 5-Substituted 6*H*-1,3,4-Thiadiazin-2-amine Hydrohalides (**6a**−**k**)

compd	R1	formula	mp (°C)
6a	Ph	$C_9H_{10}ClN_3S$	206
6b	4-FPh	$C_9H_9ClFN_3S$	231
6c	4-ClPh	$C_9H_9BrClN_3S$	227
6d	4-BrPh	$C_9H_9Br_2N_3S$	218
6e	4-O_2NPh	$C_9H_9BrN_4O_2S$	228
6f	4-NCPh	$C_{10}H_9BrN_4S$	248
6g	4-F_3CPh	$C_{10}H_9BrF_3N_3S$	224
6h	4-H_3COPh	$C_{10}H_{12}BrN_3OS$	185
6i	4-H_3CPh	$C_{10}H_{12}BrN_3S$	220
6j	1-adamantyl	$C_{13}H_{20}BrN_3S$	251
6k	5-Cl-2-thienyl	$C_7H_7BrClN_3S_2$	249

Table 2. Synthesized *N*-Sulfonylated Amino Acids (**8a**−**g**)

compd	R_2	R_3	R_4	formula	mp (°C)
8a(*S*)	H	CH_3	Ph	$C_9H_{11}NO_4S$	123−125
8b(*R*)	CH_3	H	Ph	$C_9H_{11}NO_4S$	124−126
8c(*S*)	H	CH_3	2-thienyl	$C_7H_9NO_4S_2$	85−87
8d(*S*)	H	CH_3	CH_2Ph	$C_{10}H_{13}NO_4S$	125−127
8e(*S*)	H	$CH(CH_3)_2$	Ph	$C_{11}H_{15}NO_4S$	149
8f(*R*)	$CH(CH_3)_2$	H	Ph	$C_{11}H_{15}NO_4S$	148−149
8g	CH_3	CH_3	Ph	$C_{10}H_{13}NO_4S$	146−147

Scheme 2. Method B[a]



[a] Reagents and conditions: (i) thiosemicarbazide hydrohalide; MeOH; reflux.

crystallized from methanol/acetonitrile mixtures as described in the Experimental Section.

In the second method, the thiadiazine ring was prepared in one step from the substituted α-halogeno-keto compound and thiosemicarbazide hydrochloride or thiosemicarbazide hydrobromide[16] (Scheme 2). Compounds (**10**) were slowly heated with the corresponding thiosemicarbazide hydrohalides in methanol to produce the 5-substituted 6*H*-1,3,4-thiadiazine-2-amine hydrohalides (**6a,b,j,k**) as crystalline solids that could be purified by recrystallization from an appropriate solvent as described in the Experimental Section. With this method the novel compound 5-(5-chloro-2-thienyl)-6*H*-1,3,4-thiadiazin-2-amine hydrobromide could be ob-

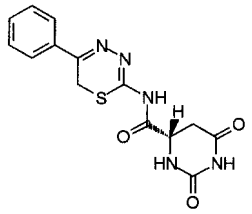


Figure 3. Structure of compound **11**.

tained in an acceptable yield. The remaining transformation leading to compounds with the general structure **9** was performed as illustrated in Scheme 1.

Results and Discussion

Structure−Activity-Relationship (SAR) Analysis. All compounds were tested in vitro for the inhibition of PMNL-gelatinase (MMP-9) and the recombinant catalytic domains of human neutrophil collagenase (cdMMP-8), human gelatinase A (cdMMP-2), macrophage elastase (cdMMP-12), collagenase-3 (cdMMP-13), and membrane-type-1 MMP (cdMMP-14). Selected compounds have also been tested for the inhibition of collagenase-1 (MMP-1) and the ectodomain of membrane-type-1 MMP (MMP-14). The first molecule prepared to test the concept of 5-substituted-6*H*-1,3,4-thiadiazine-2-amide linked MMP inhibitors was the dihydroorotic acid derivative (**11**) (Figure 3). This compound showed a promising potency of $K_i = 1.2\ \mu$M against MMP-9. It was recently discovered that sulfonylated amino acid hydroxamates and sulfonylated amino acids (carboxylates) act as efficient MMP inhibitors.[17] The most active compounds from this class of nonpeptide MMP inhibitors possess an arylsulfonyl group, occupying the specificity S_1' pocket of the enzyme. It was also shown that the $-SO_2-$ moiety of the inhibitors is involved in several strong hydrogen bonds with amino acid residues from the active site cleft, which considerably stabilize the enzyme−inhibitor adduct.[18] Assuming that the phenyl ring of **11** occupies the unprimed side of MMP-9, we focused on preparing 5-substituted-6*H*-1,3,4-thiadiazine-2-amides with a sulfonamide moiety to improve the primed side affinity. The promising concept of these novel sulfonamide inhibitors prompted us to establish SAR considerations that apply to most of the 6*H*-1,3,4-thiadiazines described in this paper (Figure 4).

The class in general inhibits cdMMP-2, cdMMP-8, MMP-9, and cdMMP-14 selectively in the nanomolar range. Depending on functional group manipulations

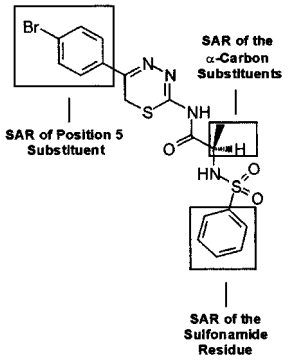


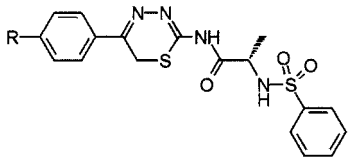
Figure 4. Proposed 6*H*-1,3,4-thiadiazine SAR studies.

within the compound series, the inhibition of these MMPs showed great variability. On the other hand, the inhibition of MMP-1 and cdMMP-12 was less potent relative to the other enzymes and demonstrated very few variations in potency as functional groups were altered. Surprisingly, the inhibition of cdMMP-13, in general, occurs in the micromolar to submicromolar range within the tested 6*H*-1,3,4-thiadiazine series (Tables 3−7).

SAR of the Position 5 Substituent. As a first modification, we prepared analogues substituting the phenyl ring in position 5 of the 6*H*-1,3,4-thiadiazine moiety with various halogens (**12a**−**c**), electron-withdrawing groups (**12d**−**f**), and moderately electron-donating groups (**12g,h**) (Tables 3 and 4) and assessed the effects that these changes have on MMP inhibitory activity.

As can be seen in Table 3, halogens attached to the 4-position of the phenyl ring increased the potency against cdMMP-8, MMP-9, and cdMMP-14. This increase had the maximum level with an appended chloro substituent in the case of MMP-9 and cdMMP-14 and with an appended bromo substituent in the case of cdMMP-8. However, electron-withdrawing groups and moderately electron-donating groups at the 4-position of the phenyl ring were well-tolerated by the tested MMPs (Table 4). Surprisingly, the electron-withdrawing CN group of compound (**12e**), which can be described as a pseudohalogen,[19] also improved inhibitory activity against MMP-9 in a manner comparable to the halogens. The moderately electron-donating CH_3 group of compound (**12h**) tended to have selective potency against cdMMP-12. Substitution by electron-withdrawing substituents produces an electron-deficient phenyl ring,

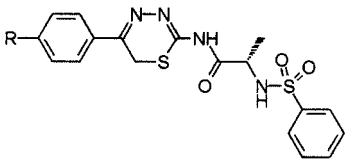
Table 3. In Vitro Activity of Halogenated 6*H*-1,3,4-Thiadiazine Derivatives



					MMPs K_i (μM)[b]							
compd	R	formula[a]	mp (°C)	method	1[c]	2	8	9[c]	12	13	14	14E[d]
12a	F	$C_{18}H_{17}FN_4O_3S_2$	166−167	B	0.44	0.45	0.19	0.16	0.28	0.65	0.44	nt[e]
12b	Cl	$C_{18}H_{17}ClN_4O_3S_2$	184−185	A	0.65	0.14	0.73	0.06	0.52	0.18	0.10	0.21
12c	Br	$C_{18}H_{17}BrN_4O_3S_2$	180−181	A	nt	0.27	0.11	0.16	0.44	0.37	0.34	nt

[a] Analytical results are within ±0.4% of the theoretical values. [b] MMP inhibition in vitro. Assays were run at pH 7 against the catalytic domains of the enzymes. See the Experimental Section for complete protocols. Standard deviations were typically ±15% of the mean or less. [c] Full-length version of the enzyme was used. [d] The ectodomain of the enzyme was used. [e] nt denotes not tested.

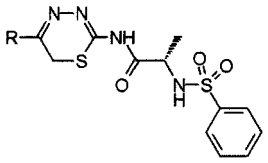
Table 4. In Vitro Activity of 6*H*-1,3,4-Thiadiazine Derivatives Substituted with Electron-Withdrawing or Electron-Donating Functionalities



					MMPs K_i (μM)[b]							
compd	R	formula[a]	mp (°C)	method	1[c]	2	8	9[c]	12	13	14	14E[d]
12d	NO_2	$C_{18}H_{17}N_5O_5S_2$	201−202	A	0.26	0.30	0.17	0.13	0.28	0.26	0.29	nt[e]
12e	CN	$C_{19}H_{17}N_5O_3S_2$	186−187	A	0.39	0.24	0.22	0.08	0.37	0.25	0.24	0.40
12f	CF_3	$C_{19}H_{17}F_3N_4O_3S_2$	187−188	A	0.43	nt	0.26	0.24	0.30	0.57	0.58	0.39
12g	OCH_3	$C_{19}H_{20}N_4O_4S_2$	192−193	A	0.58	0.52	0.18	0.32	0.34	1.33	0.59	nt
12h	CH_3	$C_{19}H_{20}N_4O_3S_2$	184−185	A	nt	0.41	0.30	0.16	0.11	0.62	0.38	nt

[a−e] See footnotes in Table 3.

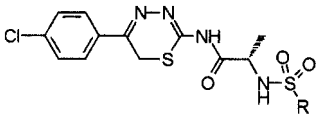
Table 5. In Vitro Activity of 6*H*-1,3,4-Thiadiazine Derivatives with Different Position 5 Residues



					MMPs K_i (μM)[b]							
compd	R	formula[a]	mp (°C)	method	1[c]	2	8	9[c]	12	13	14	14E[d]
13a[f]	1-adamantyl	$C_{22}H_{28}N_4O_3S_2$	183−184	B	nt[e]	>15	>15	>15	0.41	>15	3.07	nt
13b	5-Cl-thienyl	$C_{16}H_{15}ClN_4O_3S_3$	198−199	B	0.45	1.87	0.18	0.17	0.24	0.47	0.32	0.20

[a−e] See footnotes in Table 3. [f] At high assay concentrations (>5 μM) the compound showed fluorescence quenching effects.

Table 6. Modifications of the Sulfonamide Residue



					MMPs K_i (μM)[b]							
compd	R	formula[a]	mp (°C)	method	1[c]	2	8	9[c]	12	13	14	14E[d]
12b	Ph	$C_{18}H_{17}ClN_4O_3S_2$	184−185	A	0.65	0.14	0.73	0.06	0.52	0.18	0.10	0.21
14a	CH_2Ph	$C_{19}H_{19}ClN_4O_3S_2$	169−170	A	0.36	0.09	0.20	0.04	0.48	0.12	0.19	0.18
14b	2-thienyl	$C_{16}H_{15}ClN_4O_3S_3$	175−176	A	1.04	0.55	0.06	0.80	0.36	3.81	0.25	nt[e]

[a−e] See footnotes in Table 3.

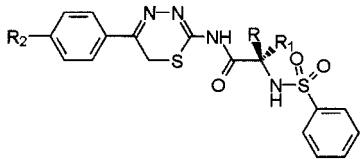
which in turn can improve aryl−aryl stacking interactions with aromatic side chains in the active site. The reduction in affinity, resulting from a reduced aryl−aryl stack by phenyl rings para-substituted by moderate electron-releasing groups (e.g. CH_3), appears to be more than offset by the increase in steric bulk/lipophilic contacts that these groups provide in the case of cdMMP-12. Consequently, the replacement of the 4-methylphenyl ring in compound (**12h**) with the bulky adamantyl residue verified in compound (**13a**) resulted in a 10−1000-fold decrease of inhibitory activity against the tested MMPs, with the exception of cdMMP-12 (Table 5). This enzyme was inhibited by **13a** very selectively in the submicromolar range. The bioisosteric[20] replacement of the 4-chlorophenyl residue with a 5-chlorothienyl moiety represented by compound **13b** demonstrated enzyme selectivity between cdMMP-2 and MMP-9 by a factor of 10.

SAR of the Sulfonamide Residue. The sulfonamide portion of the molecules and the influence of this group on enzyme inhibition was also proven (Table 6).

The phenylsulfonamide **12b**, one of the first compounds we examined, was determined to be a potent broad-spectrum inhibitor. The more flexible benzylsulfonamide analogue **14a** was also prepared and tested for in vitro activity. This compound possessed a slight increase of inhibitory activity with cdMMP-2, cdMMP-13, and MMP-9, all of which are characterized by a deep S_1' pocket.[21] The results obtained with the thienylsulfonamide **14b** were remarkable. Replacement of the phenyl group with a thienyl substituent led to a significant shift in enzyme selectivity. The compound was found to be even more selective for cdMMP-8 (K_i = 60 nM) compared with the other tested MMPs.

SAR of Variations of the α-Carbon Substituents. The substituents at the α-carbon of the *N*-sulfonylated amino acid residue of the molecules were also modified. To determine if either the *R*-isomer or the *S*-isomer binds tighter to the active site, we synthesized enantiomeric pairs of most of the compounds. The results of the in vitro tests revealed that the corresponding *R*-isomers were, in general, more potent than the parent *S*-isomers (Table 7). Replacement of the methyl group with the bulky isopropyl substituent (**15f,g**) led to a decrease of the inhibitory activity against the tested MMPs. Attachment of a second methyl group at the

Table 7. Modifications of the α-Carbon Substituents



					MMPs K_i (μM)[b]							
compd	R, R_1, R_2	formula[a]	mp (°C)	method	1[c]	2	8	9[c]	12	13	14	14E[d]
12a(*S*)	H, CH_3, F	$C_{18}H_{17}FN_4O_3S_2$	166−167	B	0.44	0.45	0.19	0.16	0.28	0.65	0.44	nt[e]
15a(*R*)	CH_3, H, F	$C_{18}H_{17}FN_4O_3S_2$	167−168	B	0.63	0.34	0.35	0.05	0.35	0.30	0.39	0.33
12b(*S*)	H, CH_3, Cl	$C_{18}H_{17}ClN_4O_3S_2$	184−185	A	0.65	0.14	0.73	0.06	0.52	0.18	0.10	0.21
15b(*R*)	CH_3, H, Cl	$C_{18}H_{17}ClN_4O_3S_2$	183−184	A	0.21	0.50	0.15	0.08	0.32	0.14	0.05	0.21
12c(*S*)	H, CH_3, Br	$C_{18}H_{17}BrN_4O_3S_2$	180−181	A	nt	0.27	0.11	0.16	0.44	0.37	0.34	nt
15c(*R*)	CH_3, H, Br	$C_{18}H_{17}BrN_4O_3S_2$	179−180	A	0.42	0.15	0.21	0.04	0.34	0.13	0.20	0.18
12e(*S*)	H, CH_3, CN	$C_{19}H_{17}N_5O_3S_2$	186−187	A	0.39	0.24	0.22	0.08	0.37	0.25	0.24	0.40
15d(*R*)	CH_3, H, CN	$C_{19}H_{17}N_5O_3S_2$	185−186	A	0.40	0.21	0.23	0.08	0.36	0.21	0.24	0.40
12h(*S*)	H, CH_3, CH_3	$C_{19}H_{20}N_4O_3S_2$	184−185	A	nt	0.41	0.30	0.16	0.11	0.62	0.38	nt
15e(*R*)	CH_3, H, CH_3	$C_{19}H_{20}N_4O_3S_2$	183−184	A	0.46	0.44	0.22	0.21	0.09	0.32	0.29	0.15
15f(*S*)[f]	H, $(CH_3)_2CH$, Cl	$C_{20}H_{21}ClN_4O_3S_2$	201−202	A	nt	0.88	0.78	0.20	0.38	>15	0.74	nt
15g(*R*)[f]	$(CH_3)_2CH$, H, Cl	$C_{20}H_{21}ClN_4O_3S_2$	189−190	A	0.55	0.85	4.37	>15	0.35	>15	0.59	0.33
15h	CH_3, CH_3, Cl	$C_{19}H_{19}ClN_4O_3S_2$	204−205	A	0.30	0.08	0.48	0.09	0.20	0.18	0.15	0.11

[a−e] See footnotes in Table 3. [f]At high assay concentrations (>5 μM) the compound showed fluorescence quenching effects.

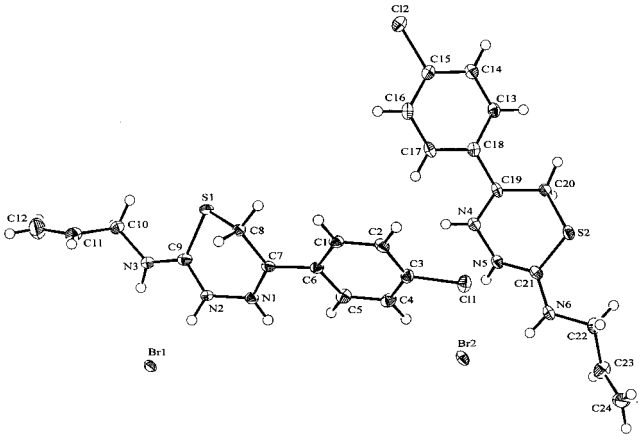


Figure 5. The ORTEP-III view of *N*-allyl-5-(4-chlorophenyl)-6*H*-1,3,4-thiadiazin-2-amine hydrobromide showing the two independent molecules and the atom-numbering scheme. Displacement ellipsoids are drawn at the 50% probability level and H atoms are shown as small spheres of arbitrary radii. The hydrogen positions on all endocyclic nitrogens are calculated as half-occupied.

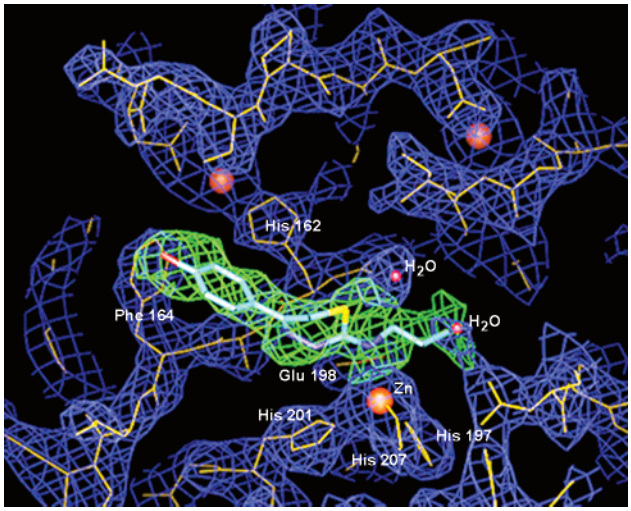


Figure 6. The electron density at the active site of the catalytic domain of human neutrophil collagenase (cdMMP-8) complexed with *N*-allyl-5-(4-chlorophenyl)-6*H*-1,3,4-thiadiazin-2-amine.

α-carbon was verified in compound **15h**. This compound is the first example of a potent nonchiral 6*H*-1,3,4-thiadiazine-based MMP inhibitor with nanomolar affinity for cdMMP-2 and MMP-9.

X-ray Crystallography. A crystal structure of the catalytic domain of human neutrophil collagenase (cdMMP-8) complexed with *N*-allyl-5-(4-chlorophenyl)-6*H*-1,3,4-thiadiazin-2-amine hydrobromide (**16**) (K_i = 41 μM) was determined at 2.7 Å resolution and is shown in Figure 6. This structure provides insights into the enzyme−inhibitor interactions that play a role in the exceptional binding of 6*H*-1,3,4-thiadiazine-based MMP inhibitors. These interactions are represented schematically in Figure 7.

Notably, the inhibitor is coordinated to the catalytic zinc cation via the exocyclic nitrogen of the thiadiazine moiety. The ring nitrogens are involved in specific hydrogen bonds with the backbone of cdMMP-8. The crystal structure of free inhibitor (**16**) reveals that the thiadiazine ring deviates from planarity (Figure 5).

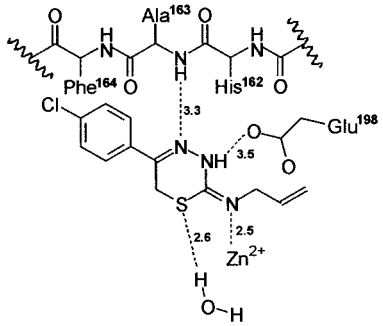


Figure 7. Schematic diagram of binding interactions between *N*-allyl-5-(4-chlorophenyl)-6*H*-1,3,4-thiadiazin-2-amine and cdMMP-8. Interatomic distances given in ångstroms are those observed between protein and inhibitor heteroatoms. Hydrogen positions are inferred from heavier atom positions. Zn^{2+} is the catalytic zinc of cdMMP-8.

Consistent with previous reports,[22] the puckering of the thiadiazine ring may be described as out-of-plane displacements of the vertexes of the symmetric flat polygon

using group theory. The method applicable to any real cyclic compound was introduced by Cremer and Pople and defines a unique mean plane for the ring atoms. The displacements of atoms from this plane are treated as an adequate measure of ring puckering. The calculated puckering parameters are $Q = 0.651$ Å for both molecules in the asymmetric unit, $\theta = 109.9°$ (molecule 1) and 109.7° (molecule 2) and $\varphi = 218.8°$ (molecule 1) and 218.3° (molecule 2), respectively. The large φ value for both molecules indicates that the direction of the ring distortion is toward an inverted screw-boat conformation.[23] The space group $P2_1/c$ of the uncomplexed **16** crystals implies that there is an equal subset of molecules with the non-inverted screw-boat conformation in the crystal structure due to an inversion-center. By contrast, when complexed with cdMMP-8 the puckering parameters of the thiadiazine ring are $Q = 0.619$ Å, $\theta = 71.3°$, and $\varphi = 36.9°$. This corresponds to the non-inverted screw-boat conformation of the heterocycle and fits the 4-chloro-substituted phenyl residue into the S_3 subsite formed by Phe-164, His-162, and Ser-151 on the unprimed side. Consequently, stabilization of the non-inverted screw-boat conformation of the thiadiazine core structure is expected to result in an inhibitor with higher affinity. The allyl substituent is directed toward the S_1' pocket of cdMMP-8. The hydrogen on the endocyclic nitrogen closest to the exocyclic nitrogen is prototropically shifted from the exocyclic nitrogen and stabilized by a hydrogen bond to the carboxylate oxygen of Glu-198. This glutamate is conserved in all zinc proteinases, serving as the general base crucial for catalysis.[24] The other double-bound nitrogen accepts a hydrogen bond from the backbone amide of Ala-163. The endocyclic sulfur does not appear to interact with any protein group but makes a specific hydrogen bond to a structural water molecule. It is further required for electronic delocalization of charge within the thiadiazine ring, which is necessary for Zn^{2+} coordination. The electron density of the exocyclic nitrogen is directed to the catalytic zinc and does not interact with the backbone. The unique hydrogen bonding of the thiadiazine core explains why other related thiadiazines were not identified through the screening effort. Furthermore, the complex structure rationalizes the selectivity profile of the compounds described herein toward certain MMPs. Significant enzyme differences are found for the MMPs in the unprimed side of the enzyme cleft (S_1-S_3), as compared to the primed side ($S_1'-S_3'$), where most of the known inhibitors bind. In fact, the potency of *N*-allyl-5-(4-chlorophenyl)-6*H*-1,3,4-thiadiazin-2-amine hydrobromide is remarkable, given that the inhibitor lacks a substituent capable of filling the S_1' pocket. Consequently, it makes sense to attach a sulfonamide moiety to the thiadiazine core structure to improve primed-side affinity.

Docking Experiments. We examined the interaction of compound **14b** with the active site of cdMMP-8 to understand the observed SARs and to rationalize the specificity profile. Since suitable crystals of a cdMMP-8/compound (**14b**) complex could not be obtained for X-ray studies, we modeled **14b** with the help of the program FlexX[25] into the protein by using coordinates from the above-described cdMMP-8/inhibitor complex structure as a reference. The free binding energy of a protein−ligand complex is estimated in FlexX as the sum of free energy contributions from hydrogen bonds, ion-pair interactions, hydrophobic and π-stacking interactions of aromatic groups, and lipophilic interactions. The underlaying assumption for the docking experiments is that the orientation of the 5-(4-chlorophenyl)-6*H*-1,3,4-thiadiazin-2-amine moiety in the binding site of cdMMP-8 is highly conserved. This assumption appears to be reasonable, since the interactions of this residue (Zn^{2+} coordination, a.o.) are highly favorable and specific. The flexible ligand docking results (highest scoring binding modes) for **14b** in cdMMP-8 were calculated in the form of rmsd values between the crystallographic binding mode and the docked binding mode of the core fragment. The rmsd value calculated for the docked structure of Figure 8 was 3.6 Å with a corresponding free binding energy of $\Delta G = -24.2$ kJ/mol.

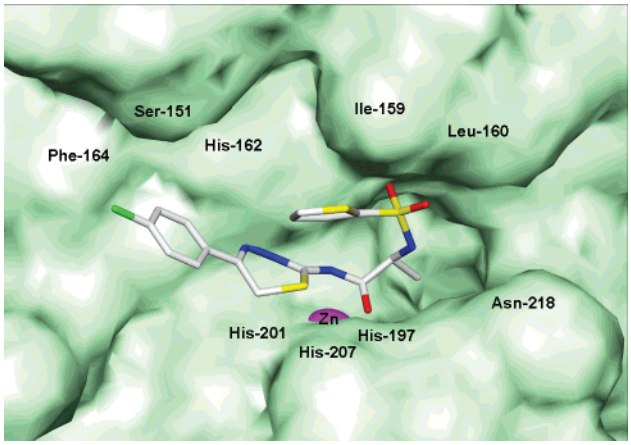


Figure 8. View of a model of compound (**14b**) in the active site of cdMMP-8. Amino acid residues that form the binding cleft are labeled, and a solid surface has been added.

Surprisingly, in this binding prediction the thiophene ring does not occupy the S_1' pocket but is positioned above the catalytic zinc. Such electrostatic interactions between the positive charge of a cation and the negative partial charge of aromatic π clouds have been proposed in molecular recognition processes and recently explored by experimental and theoretical works.[26] A physical model of this interaction has been reported in a review.[27] The catalytic zinc ion is positioned on the normal vector to the aromatic thiophene ring at 5.1 Å distance. A similar interaction involving the catalytic zinc ion has already been described for the complex between cdMMP-8 and batimastat,[28] where the thiophene aromatic ring of the inhibitor faces the cation at a distance of 4.5 Å. The contribution of these attractive electrostatic interactions, at a relatively large distance, should not be neglected in the evaluation of the stabilization of the complex model and should be taken into account in the selectivity of compound **14b** as well as other compounds of this series.

Conclusion

A series of potent matrix metalloproteinase inhibitors based on a 6*H*-1,3,4-thiadiazine scaffold was discovered and optimized. In contrast to the established MMP-inhibitors, which exclusively bind to the primed side, the compounds described interact with both the primed

and the unprimed side of the MMP. Attachment of a phenylsulfonamide moiety to the thiadiazine core structure led to an increase of inhibitory potency against the tested MMPs. Furthermore, the conformational rigidity of the series may be advantageous as a means to obtain the observed selectivity. Using X-ray crystallography, a novel binding mode of *N*-allyl-5-(4-chlorophenyl)-6*H*-1,3,4-thiadiazin-2-amine in the active site of the catalytic domain of human neutrophil collagenase was elucidated. This inhibitor binds differently than peptide-based inhibitors. An extensive network of hydrogen bonds and chelation of the catalytic zinc stabilize the latter in the active site. Hydrophobic interactions in the specificity pockets play a relatively minor role for the binding energy. In the presence of nonpeptide thiadiazine-based inhibitors, loss of binding affinity due to a reduced number of hydrogen bonds is compensated by significant hydrophobic interactions and, as suggested by docking experiments, cation−aromatic interactions. Thus, the unique combination of the observed SARs produced the best selective inhibitor, (2*R*)-*N*-[5-(4-bromophenyl)-6*H*-1,3,4-thiadiazin-2-yl]-2-[(phenylsulfonyl)-amino]propanamide (**15c**), with high affinity for MMP-9 (K_i = 40 nM). This compound may be regarded as a novel, nonpeptidic, and low molecular weight lead for continued development of MMP inhibitors as drugs.

Experimental Section

General. All moisture-free reactions were performed in oven-dried glassware under a positive pressure of argon and were stirred magnetically. Sensitive liquids and solutions were transferred via syringe or cannula and were introduced into reaction vessels through rubber septa. P.a. grade reagents and solvents were used without further purification, except that methylene chloride was distilled under argon from calcium hydride and DMF was distilled under argon on molecular sieve 3 Å. Many of the special organic starting materials and reagents were obtained from Sigma-Aldrich (Deisenhofen, Germany) and Lancaster-Synthesis (Mühlheim, Germany). Solvents were generally obtained from Merck (Darmstadt, Germany) and Baker (Gross-Gerau, Germany). Abbreviations that have been used in the descriptions of the schemes and the descriptions that follow are APMA for *p*-aminophenylmercuric acetate, DCI for direct chemical ionization, dec for decomposition, DEI for direct electron impact, DHB for 2,5-dihydroxybenzoic acid, DMF for dimethylformamide, DMSO for dimethyl sulfoxide, EDCI for *N*-ethyl-*N*′-(3-dimethylaminopropyl)-carbodiimide hydrochloride, HEPES for *N*-[2-hydroxyethyl]piperazine-*N*′-[2-ethanesulfonic acid], HOBt for 1-hydroxy-benzotriazole, MALDI for matrix assisted laser desorption/ionization, MCA for (7-methoxycoumarin-4-yl)-acetyl-, MeOH for methanol, MRB for microfluorometric reaction buffer, NMM for 4-methylmorpholine, PEG 8000 for poly(ethylene glycol) av mol wt 8000, PMNL for polymorphonuclear neutrophil leucocytes, RP-HPLC for reverse-phase high-performance liquid chromatography, TFA for trifluoroacetic acid, TKI for trypsin-kallikrein-inhibitor, TLC for thin-layer chromatography, ΔTM for without transmembrane moiety, TMS for tetramethylsilane, and TOF for time of flight. Analytical TLC was performed on Alugram Sil G/UV_{254} precoated aluminum-backed silica gel plates (Macherey-Nagel, Düren). Visualization of spots was effected by one of the following techniques: (a) ultraviolet illumination and (b) immersion of the plate in a 3% solution of ninhydrin in ethanol followed by heating. Analytical purity was assessed by RP-HPLC using an Applied Biosystems 130A Separation System. Compounds were detected at 230 nm. The stationary phase was an Aquapore OD-300 C_{18} column (2.1 mm × 220 mm). The mobile phase employed aqueous trifluoroacetic acid with acetonitrile as the organic modifier and a flow rate of 250 μL/min. Solvent A = 0.1% TFA in water. Solvent B = 0.09% TFA and 80% CH_3CN in water. The following gradient was used: t = 0 min (0% B), t = 5 min (40% B), t = 25 min (60% B). Analytical data is reported as retention time, t_R, in minutes. Melting points (mp) were determined with a Büchi 510 melting point apparatus and are uncorrected. Proton (^{1}H) nuclear magnetic resonance (NMR) spectra were measured with a Bruker DRX-500 (500.1 MHz) spectrometer, and ^{13}C NMR spectra were measured with a Bruker DRX-500 (125.8 MHz) spectrometer, both with TMS as an external standard. Chemical shifts are reported in parts per million (ppm, δ units). Coupling constants are reported in units of hertz (Hz). All of the compounds synthesized in the experiments below were analyzed by NMR, and the spectra were consistent with the proposed structures in each case. Mass spectral (MS) data were obtained on a Fisons Autospec VG spectrometer by the DCI/methane and DEI/isobutane method. MALDI-TOF mass spectral data were obtained on a PerSeptive Biosystems Voyager-DE spectrometer with DHB as the matrix and PEG 200 as the calibration standard. Most of the compounds synthesized in the experiments below were analyzed by mass spectrometry, and the spectra were consistent with the proposed structures in each case. Elemental analysis were performed on a Leco CHNS-932 elemental analyzer. All inhibitors synthesized in the experiments below were analyzed by elemental analysis, and the compounds had values within the acceptable range of ±0.4% for C, H, N. 2-Bromo-1-(5-chloro-2-thienyl)ethanone[29] was prepared following literature procedures.

2-Bromo-1-[4-(trifluoromethyl)phenyl]ethanone. A solution of 4-trifluoromethylacetophenone (1.88 g, 10 mmol) in 40 mL of methylene chloride was heated to reflux. Within 1 h a solution of bromine (1.6 g, 10 mmol) in 20 mL of methylene chloride was added dropwise to the boiling solution under rapid stirring. During reaction the resulting HBr gas was removed by a positive argon pressure. The orange reaction mixture was stirred overnight at room temperature within which time it becomes clear. The mixture was then evaporated under reduced pressure to give an off-white solid. Recrystallization from petrolether/*n*-hexane afforded 2.36 g (88%) of the desired product as colorless plates. Mp: 53−54 °C. ^{1}H NMR ($CDCl_3$): δ 4.43 (s, 2H), 7.74 (d, ^3J = 8.3 Hz, 2H), 8.08 (d, ^3J = 8.2 Hz, 2H). ^{13}C NMR ($CDCl_3$): δ 30.31 (CH_2), 123.37 (q, $^1J_{CF}$ = 272.9 Hz, CF_3), 125.90, 129.31 (CH_{arom}), 135.05 (q, $^2J_{CF}$ = 33.09 Hz, *C*-CF_3), 136.56 (*C*-CO), 190.40 (C=O).

General Procedure for Preparing 2-Amino-6*H*-1,3,4-thiadiazine Hydrohalides (Method A). Synthesis of 2-Amino-5-(4-chlorophenyl)-6*H*-1,3,4-thiadiazine Hydrobromide (6c). 4-Chlorophenacylbromide (2.34 g, 10 mmol) and thiosemicarbazide (0.91 g, 10 mmol) were suspended in 30 mL of ethanol at 0 °C. The mixture was allowed to warm to room temperature overnight under stirring. The resulting slurry was cooled to −20 °C and the precipitate was collected by filtration, washed with cold ethanol, and dried in vacuo. The pale yellow solid was again suspended in 20 mL of ethanol that contained 1 mL of 48% aqueous hydrobromic acid. The mixture was heated to reflux for 30 min and was then cooled to room temperature overnight. The precipitate was filtered, recrystallized from ethanol, and dried in high vacuo at 40 °C over phosphorus pentoxide. Yield: 1.84 g (60%) of colorless needles. Mp: 227 °C. ^{1}H NMR (DMSO-d_6): δ 4.31 (s, 2H), 7.59 (d, ^3J = 8.6 Hz, 2H), 7.90 (d, ^3J = 8.7 Hz, 2H), 9.46 (br s, 1H), 10.11 (br s, 1H), 13.32 (br s, 1H). ^{13}C NMR (DMSO-d_6): δ 22.10 (CH_2), 128.81, 129.11 (CH_{arom}), 131.86, 136.22, 150.24, 164.17 (C). DEI MS: m/z 225 $[M - HBr]^+$.

2-Amino-5-(4-bromophenyl)-6*H*-1,3,4-thiadiazine Hydrobromide (6d). The compound was prepared starting from 4-bromophenacyl bromide as described for compound **6c**. Yield: 2.33 g (66%) of colorless crystals. Mp: 218 °C. ^{1}H NMR (DMSO-d_6): δ 4.30 (s, 2H), 7.73 (d, ^3J = 8.6 Hz, 2H), 7.83 (d, ^3J = 8.6 Hz, 2H), 9.34 (br s, 1H), 10.09 (br s, 1H), 13.31 (br s, 1H). ^{13}C NMR (DMSO-d_6): δ 22.04 (CH_2), 125.16 (C), 128.96, 132.03 (CH_{arom}), 132.21, 150.34, 164.16 (C). DEI MS: m/z 270 $[M - HBr]^+$.

2-Amino-5-(4-nitrophenyl)-6*H*-1,3,4-thiadiazine Hydrobromide (6e). The title compound was prepared starting from 4-nitrophenacyl bromide as described for compound **6c**. Yield: 1.58 g (49%) of orange crystals. Mp: 228 °C. ^{1}H NMR (DMSO-d_6): δ 4.38 (s, 2H), 8.14 (d, ^3J = 8.9 Hz, 2H), 8.34 (d, ^3J = 8.9 Hz, 2H), 9.61 (br s, 1H), 10.21 (br s, 1H), 13.42 (br s, 1H). ^{13}C NMR (DMSO-d_6): δ 22.22 (CH_2), 124.07, 128.36 (CH_{arom}), 139.00, 148.76, 149.34, 164.25 (C). DEI MS: *m*/*z* 236 [M − HBr]$^+$.

2-Amino-5-(4-cyanophenyl)-6*H*-1,3,4-thiadiazine Hydrobromide (6f). The compound was prepared starting from 4-cyanophenacylbromide as described for compound **6c**. Yield: 1.80 g (61%) of yellow needles. Mp: 248 °C. ^{1}H NMR (DMSO-d_6): δ 4.35 (s, 2H), 8.00 (d, ^3J = 8.4 Hz, 2H), 8.05 (d, ^3J = 8.4 Hz, 2H), 10.02 (br s, 1H), 10.19 (br s, 1H), 13.33 (br s, 1H). ^{13}C NMR (DMSO-d_6): δ 22.08 (CH_2), 113.41, 118.30 (C), 127.74, 132.92 (CH_{arom}), 137.30, 149.68, 164.22 (C). DEI MS: *m*/*z* 216 [M − HBr]$^+$.

2-Amino-5-(4-(trifluoromethyl)phenyl)-6*H*-1,3,4-thiadiazine Hydrobromide (6g). The compound was prepared starting from 2-bromo-1-[4-(trifluoromethyl)phenyl]ethanone as described for compound **6c**. Yield: 1.54 g (45%) of colorless crystals. Mp: 224 °C. ^{1}H NMR (DMSO-d_6): δ 4.36 (s, 2H), 7.90 (d, ^3J = 8.3 Hz, 2H), 8.10 (d, ^3J = 8.2 Hz, 2H), 9.92 (br s, 1H), 10.12 (br s, 1H), 13.13 (br s, 1H). ^{13}C NMR (DMSO-d_6): δ 22.17 (CH_2), 123.88 (q, $^1J_{CF}$ = 272.3 Hz, CF_3), 125.92, 127.86 (CH_{arom}), 130.97 (q, $^2J_{CF}$ = 32.2 Hz, *C*-CF_3), 137.01, 149.92, 164.18 (C). DEI MS: *m*/*z* 259 [M − HBr]$^+$.

2-Amino-5-(4-methoxyphenyl)-6*H*-1,3,4-thiadiazine Hydrobromide (6h). The title compound was prepared starting from 4-methoxyphenacylbromide as described for compound **6c**. Yield: 1.65 g (55%) of pale yellow needles. Mp: 185 °C. ^{1}H NMR (DMSO-d_6): δ 3.81 (s, 3H), 4.27 (s, 2H), 7.06 (d, ^3J = 8.9 Hz, 2H), 7.85 (d, ^3J = 8.9 Hz, 2H), 9.26 (br s, 1H), 9.85 (br s, 1H), 13.10 (br s, 1H). ^{13}C NMR (DMSO-d_6): δ 22.07 (CH_2), 55.54 (CH_3), 114.46 (CH_{arom}), 125.06 (C), 128.82 (CH_{arom}), 151.04, 161.85, 163.99 (C). DEI MS: *m*/*z* 221 [M − HBr]$^+$.

2-Amino-5-(4-methylphenyl)-6*H*-1,3,4-thiadiazine Hydrobromide (6i). The compound was prepared starting from 4-methylphenacylbromide (20 mmol) as described for compound **6c**. Yield: 3.13 g (55%) of colorless crystals. Mp: 220 °C. ^{1}H NMR (DMSO-d_6): δ 2.35 (s, 3H), 4.29 (s, 2H), 7.33 (d, ^3J = 8.0 Hz, 2H), 7.78 (d, ^3J = 8.1 Hz, 2H), 9.31 (br s, 1H)), 10.04 (br s, 1H)), 13.23 (br s, 1H). ^{13}C NMR (DMSO-d_6): δ 21.01 (CH_2), 22.11 (CH_3), 126.96, 129.61 (CH_{arom}), 130.13, 141.60, 151.28, 164.19 (C). DEI MS: *m*/*z* 205 [M − HBr]$^+$.

***N*-Allyl-5-(4-chlorophenyl)-6*H*-1,3,4-thiadiazin-2-amine Hydrobromide (16).** 4-Chlorophenacyl bromide (2.34 g, 10 mmol) and 4-allylthiosemicarbazide (1.31 g, 10 mmol) were suspended in 30 mL of ethanol at 0 °C. The mixture was allowed to warm to room temperature overnight with stirring. The yellow solution was added dropwise to 50 mL of ethyl acetate at 0 °C, and the precipitate was collected by filtration, washed with cold ethanol, and dried in vacuo. The yellow solid was again suspended in 20 mL of ethanol that contained 1 mL of 48% aqueous hydrobromic acid. The mixture was heated to reflux for 30 min and was then cooled to room temperature overnight. After addition of ethyl acetate, the precipitate was filtered, recrystallized from ethanol/ethyl acetate (1:3), and dried in high vacuo at 40 °C over phosphorus pentoxide. Yield: 1.49 g (43%) of colorless crystals. Mp: 210 °C. HPLC t_R: 11.97. ^{1}H NMR (DMSO-d_6): δ 4.17 (br s, 2H), 4.31 (s, 2H), 5.24−5.31 (m, 2H), 5.8 g (m, 1H), 7.61 (d, ^3J = 8.6 Hz, 2H), 7.92 (d, ^3J = 8.6 Hz, 2H), 10.71 (br s, 1H), 12.87 (br s, 1H). ^{13}C NMR (DMSO-d_6): δ 22.23 (CH_2), 46.01 (CH_2), 117.95 (=CH_2), 128.82, 129.16 (CH_{arom}), 131.53 (=CH), 131.86, 136.28, 151.29 (C). DEI MS: *m*/*z* 265 [M − HBr]$^+$. MALDI-TOF-MS: *m*/*z* calcd for [M − Br]$^+$ 266.1, found 266.1. Anal. ($C_{12}H_{13}BrClN_3S$) C, H, N.

General Procedure for Preparing 2-Amino-6*H*-1,3,4-thiadiazine Hydrohalides (Method B). Synthesis of 2-Amino-5-phenyl-6*H*-1,3,4-thiadiazine Hydrochloride (6a). Thiosemicarbazide Hydrochloride. This compound was obtained as colorless crystals by evaporation of a hydrochloric acid solution of thiosemicarbazide.

A suspension of phenacyl chloride (1.55 g, 10 mmol) and thiosemicarbazide hydrochloride (1.72 g, 10 mmol) in 25 mL of methanol was heated to reflux for 15 min. After cooling to room temperature the white crystalline solid was filtered, recrystallized from methanol, and dried in high vacuo at 40 °C over phosphorus pentoxide. Yield: 0.99 g (43%) of colorless needles. Mp: 206 °C. ^{1}H NMR (DMSO-d_6): δ 4.29 (s, 2H), 7.50−7.56 (m, 3H), 7.87−7.89 (m, 2H), 9.63 (br s, 1H), 10.51 (br s, 1H), 13.73 (br s, 1H). ^{13}C NMR (DMSO-d_6): δ 21.97 (CH_2), 126.97, 129.03, 131.40 (CH_{arom}), 133.06, 150.85, 164.95 (C). DEI MS: *m*/*z* 225 [M − HCl]$^+$.

2-Amino-5-(4-fluorophenyl)-6*H*-1,3,4-thiadiazine Hydrochloride (6b). The compound was prepared starting from 4-fluorophenacyl bromide as described for compound **6a**. Yield: 1.79 g (73%) of colorless crystals. Mp: 231 °C. ^{1}H NMR (DMSO-d_6): δ 4.28 (s, 2H), 7.34−7.38 (m, 2H), 7.92−7.96 (m, 2H), 9.59 (br s, 1H), 10.54 (br s, 1H), 13.74 (br s, 1H). ^{13}C NMR (DMSO-d_6): δ 22.02 (CH_2), 116.02, 116.19, 129.53 (CH_{arom}), 129.60 (C), 129.64 (CH_{arom}), 149.95, 162.92, 164.94 (C). DEI MS: *m*/*z* 209 [M − HCl]$^+$.

Synthesis of 2-Amino-5-(2-adamantyl)-6*H*-1,3,4-thiadiazine Hydrobromide (6j). Thiosemicarbazide Hydrobromide. This compound was obtained as colorless crystals by evaporation of a 48% hydrobromic acid solution of thiosemicarbazide.

A suspension of 1-(2-bromoacetyl)adamantane (2.57 g, 10 mmol) and thiosemicarbazide hydrobromide (1.72 g, 10 mmol) in 25 mL of methanol was heated to reflux for 15 min. After cooling to room temperature, the white crystalline solid was filtered, recrystallized from methanol, and dried in high vacuo at 40 °C over phosphorus pentoxide. Yield: 1.97 g (60%) of colorless crystals. Mp: 251 °C. ^{1}H NMR (DMSO-d_6): δ 1.61−1.68 (m, 6H), 1.75 (s, 6H), 1.98 (s, 3H), 3.79 (s, 2H), 9.11 (br s, 1H), 9.84 (br s, 1H), 12.91 (br s, 1H). ^{13}C NMR (DMSO-d_6): δ 20.39 (CH_2), 27.21 (CH), 35.82 (CH_2), 38.18 (C), 162.87 (C), 164.84 (C). DEI MS: *m*/*z* 249 [M − HBr]$^+$.

2-Amino-5-(5-chloro-2-thienyl)-6*H*-1,3,4-thiadiazine Hydrobromide (6k). The title compound was prepared starting from 2-bromo-1-(5-chloro-2-thienyl)ethanone as described for compound **6j**. Yield: 0.35 g (11%); off-white solid. Mp: 249 °C. ^{1}H NMR (DMSO-d_6): δ 4.35 (s, 2H), 7.28 (d, ^3J = 4.1 Hz, 1H), 7.68 (d, ^3J = 4.1 Hz, 1H), 9.63 (br s, 1H), 10.03 (br s, 1H), 13.19 (br s, 1H). ^{13}C NMR (DMSO-d_6): δ 21.69 (CH_2), 128.36, 131.47 (CH), 133.86, 135.94, 146.13, 163.71 (C). DEI MS: *m*/*z* 231 [M − HBr]$^+$.

General Procedure for Preparing *N*-Sulfonylated Amino Acids. Synthesis of (2*S*)-2-[(Phenylsulfonyl)amino]propanoic Acid (8a). Benzenesulfonyl chloride (1.80 g, 10 mmol) was added to a solution of L-alanine (1.07 g, 12 mmol) in aqueous potassium carbonate (20 mL, 1.1 M). Under vigorous stirring, the mixture was carefully heated to 70 °C for 30 min. The resulting clear solution was cooled in an ice bath and then acidified to pH 2.5 by the dropwise addition of concentrated hydrochloric acid under stirring. The precipitate was collected by filtration and washed with a minimum amount of ice-cold water. Recrystallization from distilled water yielded 1.42 g (62%) of (2*S*)-2-[(phenylsulfonyl)amino]propanoic acid as a white crystalline solid. Mp: 123−125 °C. ^{1}H NMR (DMSO-d_6): δ 1.13 (d, ^3J = 7.2 Hz, 3H), 3.76 (m, 1H), 7.54−7.61 (m, 3H), 7.78 (d, ^3J = 7.4 Hz, 2H), 8.14 (d, ^3J = 8.3 Hz, 1H), 12.64 (s, 1H). ^{13}C NMR (DMSO-d_6): δ 18.43 (CH_3), 51.15 (CH), 126.40, 129.06, 132.35 (CH_{arom}), 141.34 (C), 173.22 (COOH).

(2*R*)-2-[(Phenylsulfonyl)amino]propanoic Acid (8b). This compound was prepared starting from D-alanine as described for compound **8a**. Yield: 1.65 g (72%) of white solid. Mp: 124−126 °C. ^{1}H NMR (DMSO-d_6): δ 1.13 (d, ^3J = 7.1 Hz, 3H), 3.76 (m, 1H), 7.54−7.62 (m, 3H), 7.78 (d, ^3J = 7.5 Hz, 2H), 8.15 (d, ^3J = 8.3 Hz, 1H), 12.65 (s, 1H).

(2*S*)-2-[(2-Thienylsulfonyl)amino]propanoic Acid (8c). The title compound was prepared starting from thiophene-2-sulfonyl chloride as described for compound **8a**. Yield: 1.57 g

(67%) of white solid. Mp: 85−87 °C. ^{1}H NMR (DMSO-d_6): δ 1.17 (d, ^3J = 7.3 Hz, 3H), 7.14 (m, 1H), 7.56 (d, ^3J = 3.1 Hz, 1H), 7.89 (d, ^3J = 4.8 Hz, 1H), 8.35 (d, ^3J = 8.2 Hz, 1H), 12.71 (s, 1H). ^{13}C NMR (DMSO-d_6): δ 27.81 (CH_3), 60.83 (CH), 137.09, 141.07, 141.99 (CH_{arom}), 151.69 (C), 182.67 (COOH).

(2*S*)-2-[(Benzylsulfonyl)amino]propanoic Acid (8d). This compound was prepared starting from benzylsulfonyl chloride as described for compound **8a**. Yield: 1.97 g (81%) of white solid. Mp: 125−127 °C. ^{1}H NMR (DMSO-d_6): 1.23 (d, ^3J = 7.3 Hz, 3H), 3.79 (m, 1H), 4.33 (AB_q, ^2J = 13.7 Hz, 2H), 7.33−7.39 (m, 5H), 7.55 (d, ^3J = 7.9 Hz, 1H), 12.71 (s, 1H). ^{13}C NMR (DMSO-d_6): δ 18.67 (CH_3), 51.30 (CH), 58.55 (CH_2), 128.01, 128.26 (CH_{arom}), 130.40 (C), 130.91 (CH_{arom}), 174.20 (COOH).

(2*S*)-3-Methyl-2-[(phenylsulfonyl)amino]butyric Acid (8e). The compound was prepared starting from L-valine as described for compound **8a**. Yield: 1.75 g (68%) of colorless needles. Mp: 149 °C. ^{1}H NMR (DMSO-d_6): δ 0.77 (d, ^3J = 6.7 Hz, 3H), 0.80 (d, ^3J = 6.7 Hz, 3H), 1.92 (m, 1H), 3.51 (m, 1H), 7.52−7.60 (m, 3H), 7.77 (d, ^3J = 7.5 Hz, 2H), 8.02 (d, ^3J = 9.2 Hz, 1H), 12.58 (s, 1H). ^{13}C NMR (DMSO-d_6): δ 17.85 (CH_3), 19.03 (CH_3), 30.41 (CH), 61.26 (CH), 126.52, 128.90, 132.27 (CH_{arom}), 141.19 (C), 172.21 (COOH).

(2*R*)-3-Methyl-2-[(phenylsulfonyl)amino]butyric Acid (8f). This compound was prepared starting from D-valine as described for compound **8a**. Yield: 1.73 g (67%) of colorless needles. Mp: 148−149 °C. ^{1}H NMR (DMSO-d_6): δ 0.77 (d, ^3J = 6.7 Hz, 3H), 0.80 (d, ^3J = 6.7 Hz, 3H), 1.92 (m, 1H), 3.51 (m, 1H), 7.52−7.60 (m, 3H), 7.77 (d, ^3J = 7.5 Hz, 2H), 8.02 (d, ^3J = 9.2 Hz, 1H), 12.58 (s, 1H).

2-Methyl-*N*-(phenylsulfonyl)alanine (8g). The title compound was prepared starting from 2-aminoisobutyric acid as described for compound **8a**. Yield: 1.84 g (76%) of colorless crystals. Mp: 146−147 °C. ^{1}H NMR (DMSO-d_6): δ 1.25 (s, 6H), 7.52−7.59 (m, 3H), 7.79−7.83 (m, 2H), 7.99 (s, 1H), 12.55 (s, 1H). ^{13}C NMR (DMSO-d_6): δ 25.76 (CH_3), 57.77 (C), 126.13, 128.92, 131.99 (CH_{arom}), 143.73 (C), 175.32 (COOH).

General Procedure for Preparing Substituted Thiadiazine Amides. Synthesis of (4*S*)-2,6-Dioxo-*N*-(5-phenyl-6*H*-1,3,4-thiadiazin-2-yl)hexahydro-4-pyrimidinecarboxamide (11). To a suspension of 2-amino-5-phenyl-6*H*-1,3,4-thiadiazine hydrochloride (**6a**) (314 mg, 1.38 mmol), (*S*)-2,6-dioxohexahydropyrimidine-4-carboxylic acid (221 mg, 1.40 mmol), and 1-hydroxybenzotriazole (190 mg, 1.40 mmol) in 5 mL of DMF at 0 °C was added 4-methylmorpholine (190 μL, 1.72 mmol) followed by the addition of *N*-ethyl-*N*′-(3-dimethylaminopropyl)-carbodiimide hydrochloride (275 mg, 1.43 mmol). After 48 h at 5 °C, the mixture was diluted with 20 mL of 0.1 M hydrochloric acid and stirred for 30 min at room temperature. The precipitate was filtered and washed successively with water, ice-cold methanol, and diethyl ether. After drying in vacuo the solid was recystallized from methanol/acetonitrile (5:1) to yield 225 mg (49%) of the title compound as colorless crystals. Mp: 198 °C (dec). HPLC t_R: 11.30. ^{1}H NMR (DMSO-d_6): δ 2.61 (m, 1H), 2.94 (m, 1H), 3.80 (AB_q, ^2J = 14.9 Hz, 2H), 4.18 (m, 1H), 7.48−7.50 (m, 3H), 7.63 (s, 1H), 7.88−7.90 (m, 2H), 10.07 (s, 1H), 12.45 (br s, 1H). ^{13}C NMR (DMSO-d_6): δ 21.63, 33.27 (CH_2), 51.64 (CH), 126.95, 128.87, 130.61 (CH_{arom}), 133.97, 148.58, 153.63, 163.32 (C), one C and one C═O were not detected. DCI MS: *m*/*z* 332 [M + H]$^+$. MALDI-TOF-MS: *m*/*z* calcd for [M + H]$^+$ 332.1, found 332.2; calcd for [M + Na]$^+$ 354.1, found 354.2; calcd for [M + K]$^+$ 370.0, found 370.1. Anal. ($C_{14}H_{13}N_5O_3S$) C, H, N.

The following compounds were synthesized following the general procedure as described for compound **11**.

(2*S*)-*N*-[5-(4-Nitrophenyl)-6*H*-1,3,4-thiadiazin-2-yl]-2-[(phenylsulfonyl)amino]propanamide (12d). Yield: 278 mg (45%) of yellow crystals. Mp: 201−202 °C; HPLC t_R: 13.30. ^{1}H NMR (DMSO-d_6): δ 1.15 (d, ^3J = 7.0 Hz, 3H), 3.73 (AB_q, ^2J = 14.7 Hz, 2H), 4.03 (m, 1H), 7.53−7.61 (m, 3H), 7.79 (d, ^3J = 7.4 Hz, 2H), 8.15 (m, 3H), 8.31 (m, 2H), 12.15 (br s, 1H). ^{13}C NMR (DMSO-d_6): δ 18.82 (CH_3), 20.99 (CH_2), 53.04 (CH), 123.93, 126.47, 128.19, 129.04, 132.33 (CH_{arom}), 140.32, 141.09, 146.65, 148.17 (C), one C and one C═O were not detected. DCI MS: *m*/*z* 448 [M + H]$^+$. MALDI-TOF-MS: *m*/*z* calcd for [M + H]$^+$ 448.1, found 448.0; calcd for [M + Na]$^+$ 470.1, found 470.1; calcd for [M + K]$^+$ 486.0, found 486.0. Anal. ($C_{18}H_{17}N_5O_5S_2$) C, H, N.

(2*S*)-*N*-[5-(4-Cyanophenyl)-6*H*-1,3,4-thiadiazin-2-yl]-2-[(phenylsulfonyl)amino]propanamide (12e). Yield: 375 mg (64%) of yellow crystals. Mp: 186−187 °C; HPLC t_R: 12.98. ^{1}H NMR (DMSO-d_6): δ 1.15 (d, ^3J = 7.1 Hz, 3H), 3.69 (AB_q, ^2J = 14.7 Hz, 2H), 4.02 (m, 1H), 7.53−7.61 (m, 3H), 7.78−7.83 (m, 2H), 7.95 (d, ^3J = 8.4 Hz, 2H), 8.07 (d, ^3J = 8.3 Hz, 2H), 8.12 (d, ^3J = 7.6 Hz, 1H), 12.11 (br s, 1H). ^{13}C NMR (DMSO-d_6): δ 18.83 (CH_3), 20.89 (CH_2), 53.03 (CH), 112.48, 118.51 (C), 126.46, 127.69, 129.03, 132.32, 132.74 (CH_{arom}), 138.55, 141.09, 146.96 (C), one C and one C═O were not detected. DCI MS: *m*/*z* 428 [M + H]$^+$. MALDI-TOF-MS: *m*/*z* calcd for [M + H]$^+$ 428.1, found 428.0; calcd for [M + Na]$^+$ 450.1, found 450.0; calcd for [M + K]$^+$ 466.0, found 466.0. Anal. ($C_{19}H_{17}N_5O_3S_2$) C, H, N.

(2*S*)-2-[(Phenylsulfonyl)amino]-*N*-{5-[4-(trifluoromethyl)phenyl]-6*H*-1,3,4-thiadiazin-2-yl}propanamide (12f). Yield: 396 mg (61%) of colorless needles. Mp: 187−188 °C; HPLC t_R: 14.17. ^{1}H NMR (DMSO-d_6): δ 1.17 (d, ^3J = 7.0 Hz, 3H), 3.71 (AB_q, ^2J = 14.6 Hz, 2H), 4.03 (m, 1H), 7.54−7.61 (m, 3H), 7.79 (d, ^3J = 7.4 Hz, 2H), 7.84 (d, ^3J = 8.2 Hz, 2H), 8.10−8.12 (m, 3H), 12.10 (br s, 1H). ^{13}C NMR (DMSO-d_6): δ 18.85 (CH_3), 21.07 (CH_2), 53.07 (CH), 122.14 (C), 124.03 (q, $^1J_{CF}$ = 272.3 Hz, CF_3), 125.11, 125.69, 127.76, 129.03 (CH_{arom}), 130.14 (q, $^2J_{CF}$ = 31.9 Hz, *C*-CF_3), 132.32 (CH_{arom}), 138.55, 141.09, 146.96 (C), one C═O was not detected. DCI MS: *m*/*z* 471 [M + H]$^+$. MALDI-TOF-MS: *m*/*z* calcd for [M + H]$^+$ 471.1, found 471.1; calcd for [M + Na]$^+$ 493.1, found 493.1. Anal. ($C_{19}H_{17}F_3N_4O_3S_2$) C, H, N.

(2*S*)-*N*-[5-(4-Methoxyphenyl)-6*H*-1,3,4-thiadiazin-2-yl]-2-[(phenylsulfonyl)amino]propanamide (12g). Yield: 345 mg (58%) of colorless plates. Mp: 192−193 °C; HPLC t_R: 12.95. ^{1}H NMR (DMSO-d_6): δ 1.15 (d, ^3J = 7.0 Hz, 3H), 3.62 (AB_q, ^2J = 14.5 Hz, 2H), 3.80 (s, 3H), 3.99 (m, 1H), 7.03 (d, ^3J = 8.6 Hz, 2H), 7.53−7.61 (m, 3H), 7.79 (d, ^3J = 7.4 Hz, 2H), 7.86 (d, ^3J = 8.6 Hz, 2H), 8.06 (d, ^3J = 7.3 Hz, 1H), 11.99 (br s, 1H). ^{13}C NMR (DMSO-d_6): δ 18.98 (CH_3), 21.22 (CH_2), 53.22 (CH), 55.38 (CH_3), 114.22, 126.47, 128.69, 129.02 (CH_{arom}), 129.70 (C), 132.30 (CH_{arom}), 141.15, 148.03, 161.11 (C), one C and one C═O were not detected. DCI MS: *m*/*z* 433 [M + H]$^+$. MALDI-TOF-MS: *m*/*z* calcd for [M + H]$^+$ 433.1, found 433.2; calcd for [M + Na]$^+$ 455.1, found 455.2. Anal. ($C_{19}H_{20}N_4O_4S_2$) C, H, N.

(2*S*)-*N*-[5-(1-Adamantyl)-6*H*-1,3,4-thiadiazin-2-yl]-2-[(phenylsulfonyl)amino]propanamide (13a). Yield: 420 mg (66%) of colorless prisms. Mp: 183−184 °C. HPLC t_R: 14.70. ^{1}H NMR (DMSO-d_6): δ 1.11 (d, ^3J = 7.1 Hz, 3H), 1.64−1.70 (m, 6H), 1.77 (s, 6H), 1.99 (s, 3H), 3.17 (AB_q, ^2J = 14.5 Hz, 2H), 3.91 (m, 1H), 7.51−7.59 (m, 3H), 7.77 (d, ^3J = 7.2 Hz, 2H), 7.95 (d, ^3J = 7.5 Hz, 1H), 11.89 (br s, 1H). ^{13}C NMR (DMSO-d_6): δ 18.99 (CH_3), 20.07 (CH_2), 27.32 (CH), 35.95 (CH_2), 38.55 (C), 53.59 (CH), 126.38, 128.91, 132.14 (CH_{arom}), 141.19, 159.80 (C), one C and one C═O were not detected. DCI MS: *m*/*z* 461 [M + H]$^+$. MALDI-TOF-MS: *m*/*z* calcd for [M + H]$^+$ 461.2, found 461.0; calcd for [M + Na]$^+$ 483.2, found 483.1. Anal. ($C_{22}H_{28}N_4O_3S_2$) C, H, N.

(2*S*)-*N*-[5-(5-Chloro-2-thienyl)-6*H*-1,3,4-thiadiazin-2-yl]-2-[(phenylsulfonyl)amino]propanamide (13b). Yield: 216 mg (35%) of colorless needles. Mp: 198−199 °C; HPLC t_R: 13.67. ^{1}H NMR (DMSO-d_6): δ 1.13 (d, ^3J = 6.9 Hz, 3H), 3.65 (AB_q, ^2J = 14.7 Hz, 2H), 4.03 (m, 1H), 7.22 (d, ^3J = 3.7 Hz, 1H), 7.53−7.61 (m, 4H), 7.77−7.79 (m, 2H), 8.13 (d, ^3J = 7.5 Hz, 1H), 11.85 (br s, 1H). ^{13}C NMR (DMSO-d_6): δ 18.80 (CH_3), 20.27 (CH_2), 52.54 (CH), 127.30, 127.97, 129.04, 129.83, 132.35 (CH_{arom}), 132.49, 138.18, 141.03, 143.49 (C), one C and one C═O were not detected. DCI MS: *m*/*z* 443 [M + H]$^+$. MALDI-TOF-MS: *m*/*z* calcd for [M + H]$^+$ 443.0, found 443.0; calcd for [M + Na]$^+$ 465.0, found 465.0. Anal. ($C_{16}H_{15}ClN_4O_3S_3$) C, H, N.

(2*S*)-2-[(Benzylsulfonyl)amino]-*N*-[5-(4-chlorophenyl)-6*H*-1,3,4-thiadiazin-2-yl]propanamide (14a). Yield: 288 mg (46%) of colorless needles. Mp: 169−170 °C. HPLC t_R:

14.03. ^{1}H NMR (DMSO-d_6): δ 1.26 (d, ^3J = 7.1 Hz, 3H), 3.75 (AB_q, ^2J = 13.8 Hz, 2H), 4.00 (m, 1H), 4.33 (AB_q, ^2J = 14.0 Hz, 2H), 7.33−7.39 (m, 5H), 7.50 (d, ^3J = 6.2 Hz, 1H), 7.56 (d, ^3J = 8.5 Hz, 2H), 7.93 (d, ^3J = 8.4 Hz, 2H), 12.12 (br s, 1H). ^{13}C NMR (DMSO-d_6): δ 19.18 (CH_3), 21.19 (CH_2), 53.12 (CH), 58.64 (CH_2), 127.98, 128.24, 128.74, 128.90 (CH_{arom}), 130.32 (C), 130.86 (CH_{arom}), 133.10, 135.20, 147.41 (C), one C and one C=O were not detected. DCI MS: *m/z* 451 $[M + H]^+$. MALDI-TOF-MS: *m/z* calcd for $[M + H]^+$ 451.1, found 451.3; calcd for $[M + Na]^+$ 473.1, found 473.3; calcd for $[M + K]^+$ 489.0, found 489.3. Anal. ($C_{19}H_{19}ClN_4O_3S_2$) C, H, N.

(2*S*)-*N*-[5-(4-Chlorophenyl)-6*H*-1,3,4-thiadiazin-2-yl]-2-[(2-thienylsulfonyl)amino]propanamide (14b). Yield: 301 mg (49%) of colorless plates. Mp: 175−176 °C. HPLC t_R: 13.62. ^{1}H NMR (DMSO-d_6): δ 1.19 (d, ^3J = 7.1 Hz, 3H), 3.67 (AB_q, ^2J = 14.6 Hz, 2H), 4.08 (m, 1H), 7.14 (m, 1H), 7.55−7.56 (m, 3H), 7.89 (d, ^3J = 4.9 Hz, 1H), 7.90 (d, ^3J = 8.5 Hz, 2H), 8.30 (d, ^3J = 6.9 Hz, 1H), 12.07 (br s, 1H). ^{13}C NMR (DMSO-d_6): δ 18.81 (CH_3), 21.11 (CH_2), 53.12 (CH), 127.54, 128.75, 128.88, 131.62, 132.51 (CH_{arom}), 133.11, 135.18, 142.02, 147.37 (C), one C and one C=O were not detected. DCI MS: *m/z* 443 $[M + H]^+$. MALDI-TOF-MS: *m/z* calcd for $[M + H]^+$ 443.0, found 443.0; calcd for $[M + Na]^+$ 465.0, found 465.0. Anal. ($C_{16}H_{15}ClN_4O_3S_3$) C, H, N.

(2*R*)-*N*-[5-(4-Fluorophenyl)-6*H*-1,3,4-thiadiazin-2-yl]-2-[(phenylsulfonyl)amino]propanamide (15a). Yield: 225 mg (39%) of colorless needles. Mp: 167−168 °C. HPLC t_R: 13.17. ^{1}H NMR (DMSO-d_6): δ 1.15 (d, ^3J = 7.1 Hz, 3H), 3.65 (AB_q, ^2J = 14.6 Hz, 2H), 4.02 (m, 1H), 7.30−7.34 (m, 2H), 7.53−7.61 (m, 3H), 7.78−7.79 (m, 2H), 7.94−7.97 (m, 2H), 8.09 (d, ^3J = 8.3 Hz, 1H), 12.02 (br s, 1H). ^{13}C NMR (DMSO-d_6): δ 18.91 (CH_3), 21.22 (CH_2), 53.10 (CH), 115.74, 115.91, 126.46, 129.03, 129.39, 129.46 (CH_{arom}), 130.80 (C), 132.31 (CH_{arom}), 141.15, 147.44, 162.40, 164.38 (C), one C=O was not detected. DCI MS: *m/z* 421 $[M + H]^+$. MALDI-TOF-MS: *m/z* calcd for $[M + H]^+$ 421.1, found 421.0; calcd for $[M + Na]^+$ 443.1, found 443.0. Anal. ($C_{18}H_{17}FN_4O_3S_2$) C, H, N.

(2*R*)-*N*-[5-(4-Chlorophenyl)-6*H*-1,3,4-thiadiazin-2-yl]-2-[(phenylsulfonyl)amino]propanamide (15b). Yield: 308 mg (51%) of colorless needles. Mp: 183−184 °C. HPLC t_R: 13.18. ^{1}H NMR (DMSO-d_6): δ 1.15 (d, ^3J = 7.1 Hz, 3H), 3.65 (AB_q, ^2J = 14.6 Hz, 2H), 4.01 (m, 1H), 7.53−7.61 (m, 5H), 7.78−7.80 (m, 2H), 7.92 (d, ^3J = 8.6 Hz, 2H), 8.09 (d, ^3J = 8.2 Hz, 1H), 12.04 (br s, 1H). ^{13}C NMR (DMSO-d_6): δ 18.88 (CH_3), 21.04 (CH_2), 53.07 (CH), 126.46, 128.76, 128.87, 129.02, 132.30 (CH_{arom}), 133.12, 135.18, 141.12, 147.34 (C), one C and one C=O were not detected. DCI MS: *m/z* 437 $[M + H]^+$. MALDI-TOF-MS: *m/z* calcd for $[M + H]^+$ 437.1, found 437.1; calcd for $[M + Na]^+$ 459.0, found 459.1. Anal. ($C_{18}H_{17}ClN_4O_3S_2$) C, H, N.

(2*R*)-*N*-[5-(4-Bromophenyl)-6*H*-1,3,4-thiadiazin-2-yl]-2-[(phenylsulfonyl)amino]propanamide (15c). Yield: 365 mg (55%) of colorless crystals from methanol/acetonitrile (10:1). Mp: 179−180 °C. HPLC t_R: 13.90. ^{1}H NMR (DMSO-d_6): δ 1.15 (d, ^3J = 7.1 Hz, 3H), 3.65 (AB_q, ^2J = 14.6 Hz, 2H), 4.00 (m, 1H), 7.53−7.61 (m, 3H), 7.69 (d, ^3J = 8.4 Hz, 2H), 7.78 (d, ^3J = 7.3 Hz, 2H), 7.84 (d, ^3J = 8.4 Hz, 2H), 8.10 (d, ^3J = 8.0 Hz, 1H), 12.05 (br s, 1H). ^{13}C NMR (DMSO-d_6): δ 18.89 (CH_3), 21.01 (CH_2), 53.07 (CH), 124.05 (C), 126.46, 128.98, 129.03, 131.80, 132.31 (CH_{arom}), 133.48, 141.11, 147.45 (C), one C and one C=O were not detected. DCI MS: *m/z* 481 $[M + H]^+$. MALDI-TOF-MS: *m/z* calcd for $[M + H]^+$ 481.0, found 481.0; calcd for $[M + Na]^+$ 503.0, found 503.0; calcd for $[M + K]^+$ 519.0, found 519.0. Anal. ($C_{18}H_{17}BrN_4O_3S_2$) C, H, N.

(2*R*)-*N*-[5-(4-Methylphenyl)-6*H*-1,3,4-thiadiazin-2-yl]-2-[(phenylsulfonyl)amino]propanamide (15e). Yield: 308 mg (54%) of colorless needles. Mp: 183−184 °C. HPLC t_R: 13.42. ^{1}H NMR (DMSO-d_6): δ 1.15 (d, ^3J = 7.0 Hz, 3H), 2.34 (s, 3H), 3.63 (AB_q, ^2J = 14.5 Hz, 2H), 4.00 (m, 1H), 7.29 (d, ^3J = 7.9 Hz, 2H), 7.53−7.61 (m, 3H), 7.78−7.80 (m, 4H), 8.06 (d, ^3J = 7.9 Hz, 1H), 12.02 (br s, 1H). ^{13}C NMR (DMSO-d_6): δ 18.94 (CH_3), 20.94 (CH_3), 21.23 (CH_2), 53.22 (CH), 126.46, 126.94, 129.01, 129.40 (CH_{arom}), 131.41 (C), 132.29 (CH_{arom}), 140.37, 141.15, 148.30 (C), one C and one C=O were not detected. DCI MS: *m/z* 417 $[M + H]^+$. MALDI-TOF-MS: *m/z* calcd for $[M + H]^+$ 417.1, found 417.1; calcd for $[M + Na]^+$ 439.1, found 439.1; calcd for $[M + K]^+$ 455.1, found 455.1. Anal. ($C_{19}H_{20}N_4O_3S_2$) C, H, N.

(2*S*)-*N*-[5-(4-Chlorophenyl)-6*H*-1,3,4-thiadiazin-2-yl]-3-methyl-2-[(phenylsulfonyl)amino]butanamide (15f). Yield: 324 mg (51%) of colorless needles. Mp: 201−202 °C. HPLC t_R: 14.42. ^{1}H NMR (DMSO-d_6): 0.77 (d, ^3J = 6.8 Hz, 3H), 0.80 (d, ^3J = 6.7 Hz, 3H), 1.93 (m, 1H), 3.59 (AB_q, ^2J = 14.6 Hz, 2H), 3.76 (m, 1H), 7.49−7.57 (m, 5H), 7.76 (d, ^3J = 7.2 Hz, 2H), 7.91−7.93 (m, 3H), 11.95 (br s, 1H). ^{13}C NMR (DMSO-d_6): 17.95 (CH_3), 19.11 (CH_3), 21.00 (CH_2), 30.90 (CH), 50.66 (CH), 126.55, 128.82, 132.18 (CH_{arom}), 133.18, 135.15, 140.99, 147.31 (C), one C and one C=O were not detected. DCI MS: *m/z* 465 $[M + H]^+$. MALDI-TOF-MS: *m/z* calcd for $[M + H]^+$ 465.1, found 465.0; calcd for $[M + Na]^+$ 487.1, found 487.0. Anal. ($C_{20}H_{21}ClN_4O_3S_2$) C, H, N.

***N*-[5-(4-Chlorophenyl)-6*H*-1,3,4-thiadiazin-2-yl]-2-methyl-2-[(phenylsulfonyl)amino]propanamide (15h).** Yield: 239 mg (38%) of colorless needles. Mp: 204−205 °C. HPLC t_R: 14.17. ^{1}H NMR (DMSO-d_6): 1.31 (s, 3H), 1.78 (s, 3H), 3.57 (m, 1H), 3.75 (m, 1H), 7.42 (m, 1H), 7.54−7.57 (m, 3H), 7.67 (m, 1H), 7.74 (m, 1H), 7.82 (m, 1H), 7.90 (m, 1H), 8.05−8.10 (m, 2H), 12.11 (br s, 1H). ^{13}C NMR (DMSO-d_6): 21.63 (CH_2), 24.13 (CH_3), 66.85 (C), 126.30, 127.77, 128.15, 128.88, 129.04, 129.36 (CH_{arom}), 133.82 (C), 134.01 (CH_{arom}), 134.52, 135.16, 139.09, 149.85, 160.63, 173.79 (C). DCI MS: *m/z* 451 $[M + H]^+$. MALDI-TOF-MS: *m/z* calcd for $[M + H]^+$ 451.1, found 451.7; calcd for $[M + Na]^+$ 473.1, found 473.7. Anal. ($C_{19}H_{19}ClN_4O_3S_2$) C, H, N.

The stereoisomers **12a**−**c**, **12h**, **15d**, and **15g** were synthesized in a similar manner. Analytical data were consistent with the proposed structures in each case.

Enzyme Preparations. MMP-1 from human rheumatoid synovial fibroblasts was purchased from Calbiochem-Novabiochem Corp. (La Jolla, CA). Preparation of the recombinant catalytic domain of human gelatinase A (cdMMP-2): The catalytic domain of pro-gelatinase A was prepared using an *Escherichia coli* expression system.[30] The proenzyme was activated with 0.5 mM APMA at 37 °C prior to use in the assay. Preparation of the recombinant catalytic domain of human neutrophil collagenase (cdMMP-8): The enzyme was expressed in *E. coli* as an active variant.[31] Preparation of PMNL-gelatinase (MMP-9): Latent PMNL-pro-gelatinase was prepared from human plasma buffy coat as described by Tschesche et al.[32] PMNL-pro-gelatinase was activated prior to use by incubation with trypsin at 37 °C for 10 min. Inactivation of trypsin was accomplished with TKI. Preparation of the recombinant catalytic domain of human macrophage elastase (cdMMP-12): The catalytic domain of MMP-12 was expressed in *E. coli*. The overexpressed protein was isolated as inclusion bodies, and the renatured protein was purified by affinity chromatography with a hydroxamate inhibitor coupled column.[33] Preparation of the recombinant catalytic domain of human collagenase-3 (cdMMP-13): Human pro-cdMMP-13 was prepared using an *E. coli* expression system.[34] The isolated pro-cdMMP-13 was activated prior to use by incubation with 5 mM $HgCl_2$ for 2 h at 37 °C. Preparation of the recombinant catalytic domain of membrane-type-1 MMP (cdMMP-14): The catalytic domain of MMP-14 was expressed in *E. coli* and was activated by autocatalysis.[35] Preparation of the recombinant human ectodomain of membrane-type-1 MMP (ΔTM-MMP-14): The ectodomain of MMP-14 was expressed in *Pichia pastoris* by the method of Roderfeld et al. and activated by yeast proteinases (furin-like-proteinases) during maturation.[36]

Crystallography. Crystals of *N*-allyl-5-(4-chlorophenyl)-6*H*-1,3,4-thiadiazin-2-amine hydrobromide suitable for diffraction analysis were obtained by slow crystallization from an ethanol/ethyl acetate (1:3) solution. The crystals grew in space group $P2_1/c$ [a = 21.3350(6) Å, b = 9.3150(2) Å, c = 14.4910(4) Å, β = 102.9950(19)°] and contain two molecules per asymmetric unit. Diffraction data were collected under cryogenic conditions (100 K) on the Nonius Kappa CCD station

to 0.71 Å resolution. All calculations were performed using maXus.[37] Data reduction was done with Denzo and Scalepak.[38] SHELXS-97[39] was used to solve the structure, SHELXL-97[40] was used to refine the structure, and ORTEP-III[41] was used to generate the molecular graphics. The catalytic domain of human neutrophil collagenase (cdMMP-8) was concentrated to 8 mg/mL and then mixed with a 3-fold molar excess of an aqueous solution of *N*-allyl-5-(4-chlorophenyl)-6*H*-1,3,4-thiadiazin-2-amine hydrobromide for a final cdMMP-8 concentration of 6 mg/mL. Three microliters of protein/inhibitor complex was mixed with 2 μL of precipitant solution containing 100 mM cacodylate (pH 5.5−6.5), 10 mM $CaCl_2$, 100 mM NaCl, and 10% PEG 6000. The hanging drop was equilibrated by vapor diffusion at room temperature against a reservoir containing 1.0−1.5 M phosphate buffer. Data were collected on an imaging plate detector (MAR Research) to 2.7 Å resolution and processed using CCP4 data reduction software. The space group of the crystal was determined as $P2_12_12_1$ with unit cell dimensions $a = 33.67$ Å, $b = 69.64$ Å, and $c = 73.40$ Å. The orientation and translation of the molecule within the crystallographic unit cell was determined with Patterson search techniques[42] using the program AMoRe.[43] Electron density calculation and model building proceeded using the program MAIN.[44] The structure was refined using the program X-PLOR.[45] Ring puckering parameters were calculated using the program RICON.[46] Atomic coordinates of the complex have been deposited in the Protein Data Bank under accession code 1JH1.

Computational Docking. The docking package FlexX (GMD-SCAI) was used under standard conditions (autodock mode) in combination with the molecule visualization program InsightII version 98 (MSI, Germany).

MMP Inhibition Assay. Enzymatic activity was measured using a modified version of a resonance energy transfer fluorogenic assay.[47] Progress curves were monitored by following the increase in fluorescence at 393 nm ($\lambda_{ex} = 328$ nm), induced by the cleavage of the (7-methoxycoumarin-4-yl)acetyl-Pro-Leu-Gly-Leu-(3-[2,4-dinitro-phenyl]-L-2,3-diaminopropionyl)-Ala-Arg-NH_2 (Mca-Pro-Leu-Gly-Leu-Dpa-Ala-Arg-NH_2) fluorogenic substrate by MMPs. Enzyme inhibition assays were carried out in MRB that consisted of 50 mM HEPES/NaOH (pH 7.0), 10 mM $CaCl_2$ and 0.02% (w/v) PEG 8000 at 25 °C. The MMP microfluorometric profiling assay was done in white U-bottomed 96-well plates (Microfluor 1 White, Dynex) with a final substrate concentration of 6 μM Mca-peptide, approximately 0.3−5 nM MMP with variable inhibitor concentrations, and 2% DMSO vehicle. From a 1.5 mM stock (100% DMSO) the inhibitors were serially diluted with MRB to 15, 10, 5, 3, 1, 0.6, 0.3, 0.1, 0.05, 0.01, and 0.005 μM final assay concentration. Column 1, rows A−H contained only DMSO for the "enzyme-only" wells in the assay. After preincubation for 30 min at 25 °C, the reaction was started by addition of substrate, and the plate was read on a SpectraFluor Plus (Tecan) plate reader with excitation at 330 nm and emission at 405 nm with the gain set to 150. Each measurement was done in triplicate to ensure statistically significant results. The experiment was further controlled for background fluorescence of the substrate, for fluorescence of fully cleaved substrate, and for fluorescence quenching or augmentation from solutions containing the test compounds.

Determination of the K_i Values. Datapoints from eight different MMPs generated on the SpectraFluor Plus were directly visualized on a master Excel spreadsheet. The response of inhibition was determined for each inhibitor concentration by comparing the amount of hydrolysis (fluorescence units generated over 30 min of hydrolysis) of wells containing compound with the "enzyme only" wells in column 1. With the program GraFit (Erithacus Software Limited) a four-parameter logistic fit to the dose−response data was used to calculate IC_{50} values for each compound.

For each MMP, initial rate measurements in the absence of inhibitor were made for eight different substrate concentrations. From these data, K_m values were determined by nonlinear fit using the program GraFit. The K_m values determined for MMP-1, cdMMP-2, cdMMP-8, MMP-9, cdMMP-12, cdMMP-13, cdMMP-14, and ΔTM-MMP-14 were 40.3, 9.1, 5.9, 1.8, 27.3, 7.5, 6.8, and 7.5 μM, respectively. K_i values were determined using the method proposed by Horovitz and Levitski.[48] This approach explicitly takes into account the effect of the substrate, enzyme, and inhibitor concentrations and applies to the situation of both standard and tight-binding inhibition.

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Supporting Information Available: X-ray crystallographic data for **16** and analysis data for **11**, **12a**−**h**, **13a**, **13b**, **14a**, **14b**, **15a**−**h**, and **16**. This material is available free of charge via the Internet at http://pubs.acs.org.

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