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Protease Inhibitors: Synthesis of Matrix Metalloproteinase and Bacterial Collagenase Inhibitors Incorporating 5-Amino-2-mercapto-1,3,4-thiadiazole Zinc Binding Functions

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Abstract—Matrix metalloproteinase (MMP)/bacterial collagenase inhibitors incorporating 5-amino-2-mercapto-1,3,4-thiadiazole zinc binding functions are reported. A series of compounds was prepared by reaction of arylsulfonyl isocyanates or arylsulfonyl halides with phenylalanyl-alanine, followed by coupling with 5-amino-2-mercapto-1,3,4-thiadiazole in the presence of carbodiimides. These new compounds were assayed as inhibitors of human MMP-1, MMP-2, MMP-8 and MMP-9, and of the collagenase isolated from the anaerobe *Clostridium histolyticum* (ChC). The new derivatives proved to be powerful inhibitors of these metalloproteases, with activities in the low micromolar range for some of the target enzymes, depending on the substitution pattern at the arylsulfonyl(ureido) moieties.

Introduction

Proteases, such as the matrix metalloproteinases (MMPs)[1,2] or the bacterial proteases (BPs),[3,4] have recently become interesting targets for the drug design, in the search of novel types of anticancer, anti-arthritis, antibacterial or other pharmacological agents useful in the management of inflammatory processes.[1–5] All these conditions are generally associated with enhanced activity of several zinc endopeptidases, of which the different MMPs actually known (more than 25 such enzymes were reported for the moment)[1–7] and the large number of BPs[3] isolated in many pathogenic bacterial species, are responsible for the efficient degradation of all components of the extracellular matrix (ECM). ECM turnover is involved in crucial physiological and physiopathological events, such as embryonic development, blastocyst implantation, nerve growth, ovulation, morphogenesis, angiogenesis, tissue resorption and remodeling (such as in the case of wound healing), bone remodeling, apoptosis, cancer invasion and metastasis, arthritis, atherosclerosis, aneurysm, breakdown of blood–brain barrier, periodontal disease, skin and corneal ulceration, gastric ulcer, or liver fibrosis in the case of the vertebrate enzymes mentioned above.[1–7] In bacteria, proteases are involved in critical processes such as colonization and evasion of host immune defenses, acquisition of nutrients for growth and proliferation, facilitation of dissemination, or tissue damage during infection.[3]

The most powerful MMP inhibitors reported up to now incorporate a hydroxamate zinc binding function, which generally induces toxicity to these derivatives.[1–7] After the report that CGS-27023A **1** acts as a broad spectrum, potent inhibitor of some MMPs,[8] a large number of other types of sulfonylated amino acids and their hydroxamate derivatives have been investigated in the search of more potent and selective inhibitors.[9–15] Such derivatives incorporating a zinc-binding function of the hydroxamate (derivatives **1**–**3**) or carboxylate (derivatives **4**–**6**) type, bind within the primed side of the protease active site, generally interacting with the $S_{1'}$–$S_{3'}$ sites.[9–15] The main problem (except toxicity) with such protease inhibitors is their lack of selectivity towards the different MMPs, which are generally classified into two main types, depending on their $S_{1'}$ pocket: (i) the deep pocket enzymes (such as MMP-2, MMP-3, MMP-8, MMP-9 and MMP-13), possessing a relatively big $S_{1'}$ pocket, and (ii) the shallower pocket enzymes (MMP-1,

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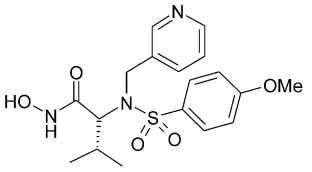
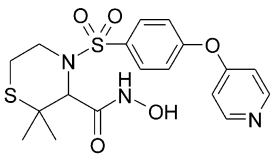
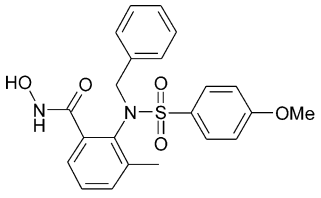


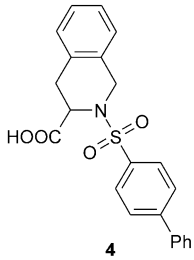
1: CGS-27023A K_I (MMP-3): 43 nM



2: AG-3340 K_I (MMP-2): 0.08 nM



3: K_I (MMP-9): 23 nM



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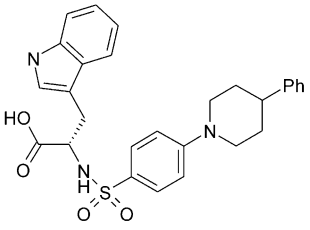
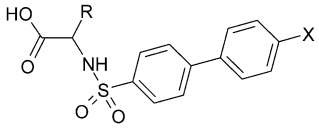
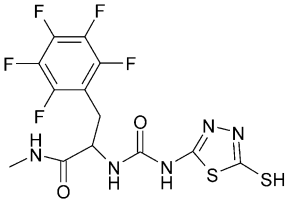


5: K_I (MMP-3): 19 nM



6: R = i-Pr; Bn; X H, Cl, Br
K_I (MMP-2): 2-30 nM



7: PNU-142372
K_I (MMP-3): 18 nM

MMP-7 and MMP-11 among others) which possess a somehow smaller specificity $S_{1'}$ pocket due to its partial occlusion by bulkier amino acid residues, such as those in position 193 (MMP-8 numbering) which from Leu in MMP-8, becomes Arg in MMP-1, Tyr in MMP-7 and Gln in MMP-11.[2b,4] The $S_{2'}$ and $S_{3'}$ subsites are important for the binding of inhibitors as well as for their specificity towards the different proteases. The $S_{2'}$ subsite is generally a solvent-exposed cleft with a preference for hydrophobic $P_{2'}$ residues, in both substrates and MMP inhibitors.[1–4] The $S_{3'}$ subsite on the other hand is a relatively ill-defined, solvent exposed region.[1–4]

Very few MMP/BP inhibitors binding on the S side of the cleavage point were reported up to now, that is inhibitors that interact with the unprimed sites of these metalloproteases.[1–5] Recently, scientists from Pharmacia & Upjohn reported a series of stromelysin (MMP-3) inhibitors of type **7**, incorporating a 5-amino-2-mercapto-1,3,4-thiadiazole zinc binding function, which were shown to bind to the left side of the enzyme cleft.[16] By means of NMR spectroscopy it was shown that the exocyclic sulfur belonging to the mercapto-thiadiazole ring of **7** coordinates to the zinc ion of the metalloprotease active site, whereas the urea-thiadiazole moiety is accommodated within the S_1 site, the methylamide carbonyl moiety within the S_2 site and the pentafluorophenyl moiety within the S_3 site of the protease.[16] Using this relatively unexplored zinc binding function, we report in this paper the preparation of a series of MMP and *Clostridium histolyticum* collagenase (ChC, EC 3.4.24.3) inhibitors incorporating arylsulfonyl(ureido)-phenylalanyl-alanyl-5-amino-2-mercapto-1,3,4-tyhiadiazole in their molecule, with low micromolar affinity for these metalloproteases.

Chemistry

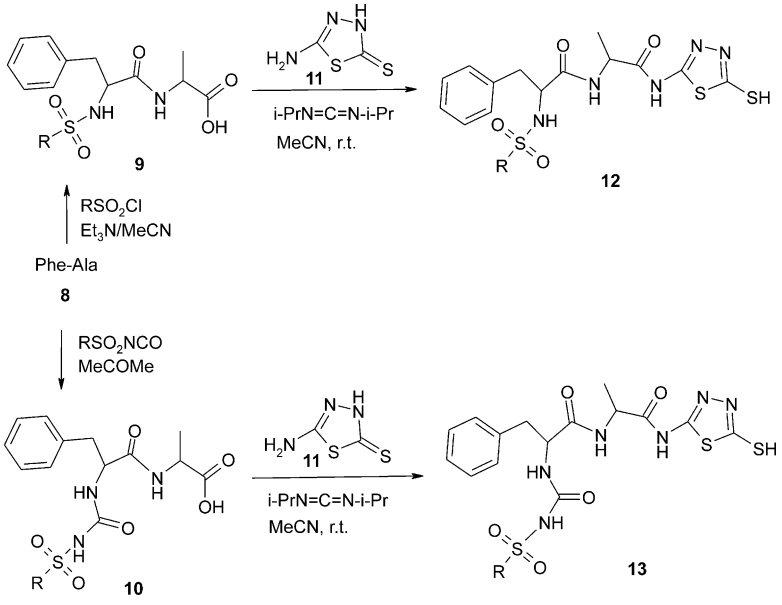
Except for derivatives of type **7** mentioned above, few other MMP inhibitors bind to the unprimed site of these proteases. One of them is Pro-Leu-Gly-hydroxamate,[17] which binds very similarly with the substrate of the protease, that is collagen, with the Gly moiety occupying the S_1 site, the Leu one the S_2, and Pro accommodating within the S_3 site. Considering such left hand MMP inhibitors, **7** and Pro-Leu-Gly-hydroxamate as leads,[16,17] for which the structure of adducts with

MMP-3 has been determined by means of NMR or X-ray crystallography, it is obvious that the S_1-S_3 sites are more shallow with respect to the corresponding $S_{1'}$–$S_{3'}$ sites, in most of the MMPs of interest from the pharmacological point of view, such as MMP-1,-2,-3,-8 and-9. This finding may be important for the potential selectivity of such metalloprotease inhibitors and thus, we decided to use derivatives of type **7** as leads for the design of tight binding MMP/BP inhibitors, taking into consideration the following structural requirements of such a compound: (i) a zinc binding function of the amino-mercapto-1,3,4-thiadiazole, which should probably occupy the S_1 pocket of the protease; (ii) a rather compact, and preferentially hydrophilic P_2 residue. In **7**, this moiety is represented by the methylamide carbonyl group, whereas in the substrate generally by the more hydrophobic Leu residue. On the other hand, the S_2 environment is more hydrophilic in MMP-1 and MMP-8 as compared to MMP-3,[1–4,16,17] and these are the reasons why we opted for an Ala residue as P_2 group (we considered the Gly as a too compact moiety, but such a possibility was also taken into account); (iii) since the S_3 pocket is the best defined and deeper left-hand pocket in MMPs,[1–4,16,17] we decided to employ a bulky Phe scaffold in this position, and thus, a Phe-Ala moiety was the most logical synthon to be used for the coupling with 5-amino-2-mercapto-1,3,4-thiadiazole (which is stable in the thione tautomeric form);[18] (iv) the amino-terminal moiety of the Phe-Ala part of the molecule should be derivatized in order to assure better interactions with the S_4 site of the protease and also to diminish the peptidomimetic character of these derivatives. A sulfonamido and sulfonylureido derivatization appeared of interest, since in other protease inhibitors previously reported by us, these groups induced good activity.[14,15]

The syntheses of the new MMP/BP inhibitors **12** and **13** reported here were achieved starting from Phe-Ala which was derivatized with either sulfonyl halides leading to sulfonamides **9**, or with arylsulfonyl isocyanates, leading to arylsulfonylureido derivatives **10** (Scheme 1).[14,15] These intermediates were then coupled with 5-amino-2-mercapto-1,3,4-thiadiazole **11** in the presence of carbodiimides, leading with excellent yields to **12** and **13**.[19] A large series of sulfonamido derivatives **12** were obtained, incorporating different aromatic/heterocyclic R moieties, whereas the number of arylsulfonylureido derivatives **13** was more restricted (Table 1).

MMP/ChC inhibition

The new 5-mercapto-1,3,4-thiadiazoles **12** and **13** reported in the present paper showed broad metalloprotease inhibitory activity, as illustrated by data of Table 1, where inhibition against four MMPs and a bacterial protease (ChC) are presented.[23] The following SAR may be observed: (i) most of the new thiadiazoles **12** and **13** are micromolar inhibitors of MMP-1, -2, -8, -9 and ChC; (ii) substitution pattern at the sulfonamido/sulfonylureido moiety of **12** and **13** is the primary factor influencing MMP/BP inhibitory properties, with the sulfonylureido derivatives **13** generally more active than the sulfonamides **12**. Thus, for the first subgroup of compounds (**13**), low micromolar inhibitory activity was observed against MMP-2,-8,-9 and ChC (with inhibition constants in the range of 0.1–8 μM), whereas these derivatives were slightly less active against MMP-1 (inhibition constants in the range of 10–19 μM). All the substitution patterns of the aryl moieties in the sulfonylureido function of these derivatives were inducing good metalloprotease inhibitory activities, with the 4-fluorophenyl/4-chlorophenyl ones being the most effective; (iii) among the sulfonamides **12**, only few derivatives were as active as the sulfonylureido compounds **13**. Thus, the poly-halogenosubstituted compounds **12m**–**12o** as well as the 4-acetamidophenylsulfonyl derivative **12k** were among the most effective inhibitors in this subseries, with K_Is in the range of 1–17 μM against MMP-2, -8, -9 and ChC, and 9–19 μM against MMP-1,



Scheme 1.

Table 1. Inhibition of MMPs and ChC with the thiadiazoles **12a–x** and **13a–e**

No.	R	K_I[a] (μM)				
		MMP-1[b]	MMP-2[b]	MMP-8[b]	MMP-9[b]	ChC[c]
12a	C_6H_5–	22	18	24	15	15
12b	$PhCH_2$–	24	16	25	16	19
12c	4-F–C_6H_4–	19	17	18	14	21
12d	4-Cl–C_6H_4–	20	16	18	15	20
12e	4-Br–C_6H_4–	24	15	20	21	16
12f	4-I–C_6H_4–	21	17	19	18	14
12g	4-CH_3–C_6H_4–	30	20	23	20	15
12h	4-O_2N–C_6H_4–	15	12	10	18	13
12i	3-O_2N–C_6H_4–	18	12	13	18	12
12j	2-O_2N–C_6H_4–	29	15	17	14	15
12k	4-AcNH–C_6H_4–	14	8	9	10	16
12m	C_6F_5–	9	6	1	2	5
12n	3-CF_3–C_6H_4	13	11	3	5	9
12o	2,5-$Cl_2C_6H_3$	19	10	9	17	15
12p	4-MeO–C_6H_4–	28	18	20	21	14
12q	2,4,6–$Me_3C_6H_2$–	33	24	25	29	17
12r	1-Naphthyl	80	36	40	44	35
12s	2-Naphthyl	72	33	45	53	40
12t	5-Me_2N–1-naphthyl–	87	40	48	50	43
12u	2-Thienyl	14	13	12	10	9
12v	Quinoline-8-yl	74	41	39	45	36
12x	Camphor-10-yl	69	35	34	40	25
13a	C_6H_5–	19	5	5	4	3
13b	4-F–C_6H_4–	12	2	0.1	0.3	0.2
13c	4-Cl–C_6H_4–	10	4	0.2	0.4	0.3
13d	4-Me–C_6H_4–	15	7	5	5	4
13e	2-Me–C_6H_4–	19	8	6	7	7

[a]K_Is values were obtained from Easson–Stedman[20] plots using a linear regression program, from at least three different assays. Standard errors were of 5–10%.
[b]With the thioester substrate Ac-ProLeuGly-*S*-LeuLeuGlyOEt, spectrophotometrically.[21]
[c]With FALGPA as substrate, spectrophotometrically.[22]

respectively. Other substitution patterns wich led to effective MMP/BP inhibitors were those incorporating monohalogeno-phenylsulfonyl, nitrophenylsulfonyl-, 2-thienylsulfonyl-, benzylsulfonyl of phenysulfonyl among others. These compounds were slightly less effective than the previously mentioned ones, with K_Is in the range of 10–33 μM against MMP-2,-8,-9 and ChC, and 14–30 μM against MMP-1, respectively. The most ineffective inhibitors in this subgroup were those incorporating bicyclic aromatic moieties, of the naphthyl or quinoline type (**12r–12t** and **12v**) as well as the 10-camphor-sulfonyl derivative **12x**. These compounds showed K_Is in the range of 25–53 μM against MMP-2, -8, -9 and ChC, and 69–80 μM against MMP-1; (iv) the affinity of the different metalloproteases investigated here for this class of inhibitors generally varied in the following order: MMP-8 > ChC > MMP-9 > MMP-2 > MMP-1 for the sulfonyureido derivatives **13**, and in the order: MMP-9 > MMP-2 ≅ ChC > MMP-8 > MMP-1 for most of the derivatives **12** (although for some derivatives, such as **12m–12o**, the best inhibition was seen against MMP-8 as for compounds **13** discussed above). It is rather hard at the present time to explain this type of susceptibility to inhibition of these metalloproteases with the new clas of inhibitors reported here.

In conclusion, the present paper reports the first broadly active metalloprotease inhibitors incorporating 5-mercapto-1,3,4-thiadiazole zinc-binding functions. Starting from some stromelysin inhibitors recently reported by Pharmacia,[16] we designed effective MMP-1, -2, -8, -9 and ChC inhibitors incorporating this zinc-binding function and a Phe-Ala peptidomimetic derivatized at the aminoterminal moiety by means of sulfonamido or sulfonylureido functionalities. SAR for this class of metalloprotease inhibitors showed that arylsulfonylureido- or perfluorophenylsulfonyl-substituted such compounds act as low micromolar MMP/BP inhibitors. Although these compounds are not as effective as the hydroxamates, presumably the lower toxicity of 1,3,4-thiadiazoles compared to that of the hydroxamates, makes the present class of compounds interesting for more detailed pharmacological evaluation.

References and Notes

1. Supuran, C. T.; Scozzafava, A. Matrix Metalloproteinases (MMPs). In *Proteinase and Peptidase Inhibition: Recent Potential Targets for Drug Development*; Smith, H. J., Simons, C., Eds.; Taylor & Francis: London and New York, 2002, p 35.
2. (a) Woessner, J. F., Jr.; Nagase, H. *Matrix Metalloproteinases and TIMPS*; Oxford University Press: Oxford, 2000, p 1. (b) *Metalloproteinases as Targets for Anti-Inflammatory Drugs*; Bottomley, K. M. K., Bradshaw, D., Nixon, J. S., Eds; Birkhäuser: Basel, Boston-Berlin, 1999, p 1.
3. (a) Leung, D.; Abbenante, G.; Fairlie, D. P. *J. Med. Chem.* **2000**, *43*, 305. (b) Whittaker, M.; Floyd, C. D.; Brown, P.; Gearing, A. J. H. *Chem. Rev*. **1999**, *99*, 2735. (c) Skotnicki, J. S.; Levin, J. I.; Zask, A., Killar, L. M. Matrix Metalloproteinase Inhibitors. In *Metalloproteinases as Targets for Anti-Inflammatory Drugs*, Bottomley, K. M. K., Bradshaw, D., Nixon, J. S., Eds.; Birkhäuser: Basel, Boston, Berlin, 1999, p 17. (d) Bottomley, K. M. K.; Johnson, W. H.; Walter, D. S. *J. Enzyme Inhib*. **1998**, *13*, 79.
4. (a) Supuran, C. T.; Scozzafava, A.; Mastrolorenzo, A. *Exp. Opin. Ther. Pat*. **2001**, *11*, 221. (b) Supuran, C. T.; Scozzafava, A.; Clare, B. W. *Med. Res. Rev*. **2002**, *22*, 329.
5. (a) Nagase, H.; Woessner, J. F., Jr. *J. Biol. Chem*. **1999**, *274*, 21491. (b) Johnson, L. L.; Dyer, R.; Hupe, D. J. *Curr. Opin. Chem. Biol*. **1998**, *2*, 466.
6. (a) Giavazzi, R.; Taraboletti, G. *Crit. Rev. Oncol. Hematol*. **2001**, *37*, 53. (b) Hidalgo, M.; Eckhardt, S. G. *J. Natl. Cancer Inst*. **2001**, *93*, 178.
7. (a) Gearing, A. J. H.; Adams, S. E.; Clements, J. C.; Miller, K. M. Matrix Metalloproteinases in Neuroinflammatory Disease. In *Metalloproteinases as Targets for Anti-Inflammatory Drugs*; Bottomley, K. M. K., Bradshaw, D., Nixon, J. S., Eds.; Birkhäuser: Basel, Boston, Berlin, 1999, p 85. (b) Hooper, N. M. Overview of the Biological Roles of Metalloproteinases in Health and Disease. In *Metalloproteinases as Targets for Anti-Inflammatory Drugs*; Bottomley, K. M. K., Bradshaw, D., Nixon, J. S., Eds.; Birkhäuser: Basel, Boston, Berlin, 1999; p 145.
8. MacPherson, L. J.; Bayburt, E. K.; Capparelli, M. P.; Carroll, B. J.; Goldstein, R.; Justice, M. R.; Zhu, L.; Hu, S.; Melton, R. A.; Fryer, L.; Goldberg, R. L.; Doughty, J. R.; Spirito, S.; Blancuzzi, V.; Wilson, D.; O'Byrne, E. M.; Ganu, V.; Parker, D. T. *J. Med. Chem*. **1997**, *40*, 2525.
9. (a) Matter, H.; Schwab, W.; Barbier, D.; Billen, G.; Haase, B.; Neises, B.; Schudok, M.; Thorwart, W.; Schreuder, H.;

Brachvogel, V.; Lonze, P.; Weithmann, K. U. *J. Med. Chem.* **1999**, *42*, 1908. (b) Matter, H.; Schwab, W. *J. Med. Chem.* **1999**, *42*, 4506.
10. (a) Natchus, M. G.; Bookland, R. G.; Laufersweiler, M. J.; Pikul, S.; Almstead, N. G.; De, B.; Janusz, M. J.; Hsieh, L. C.; Gu, F.; Pokross, M. E.; Patel, V. S.; Garver, S. M.; Peng, S. X.; Branch, T. M.; King, S. L.; Baker, T. R.; Foltz, D. J.; Mieling, G. E. *J. Med. Chem.* **2001**, *44*, 1060. (b) Almstead, N. G.; Bradley, R. S.; Pikul, S.; De, B.; Natchus, M. G.; Taiwo, Y. O.; Gu, F.; Williams, L. E.; Hynd, B. A.; Janusz, M. J.; Dunaway, C. M.; Mieling, G. E. *J. Med. Chem.* **1999**, *42*, 4547. (c) Cheng, M.; De, B.; Almstead, N. G.; Pikul, S.; Dowty, M. E.; Dietsch, C. R.; Dunaway, C. M.; Gu, F.; Hsieh, L. C.; Janusz, M. J.; Taiwo, Y. O.; Natchus, M. G. *J. Med. Chem.* **1999**, *42*, 5426. (d) Hanessian, S.; Bouzbouz, S.; Boudon, A.; Tucker, G. C.; Peyroulan, D. *Bioorg. Med. Chem. Lett.* **1999**, *9*, 1691.
11. (a) Kiyama, R.; Tamura, Y.; Watanabe, F.; Tsuzuki, H.; Ohtani, M.; Yodo, M. *J. Med. Chem.* **1999**, *42*, 1723. (b) O'Brien, P. M.; Ortwine, D. F.; Pavlovsky, A. G.; Picard, J. A.; Sliskovic, D. R.; Roth, B. D.; Dyer, R. D.; Johnson, L. L.; Man, C. F.; Hallak, H. *J. Med. Chem.* **2000**, *43*, 156. (c) Jeng, A. Y.; Chou, M.; Parker, D. T. *Bioorg. Med. Chem. Lett.* **1998**, *8*, 897.
12. (a) Levin, J. I.; Gu, Y.; Nelson, F. C.; Zask, A.; DiJoseph, J. F.; Sharr, M. A.; Sung, A.; Jin, G.; Cowling, R.; Chanda, P.; Cosmi, S.; Hsiao, C. L.; Edris, W.; Wilhelm, J.; Killar, L. M.; Skotnicki, J. S. *Bioorg. Med. Chem. Lett.* **2001**, *11*, 239. (b) Levin, J. I.; Du, M. T.; DiJoseph, J. F.; Killar, L. M.; Sung, A.; Walter, T.; Sharr, M. A.; Roth, C. E.; Moy, F. J.; Powers, R.; Jin, G.; Cowling, R.; Skotnicki, J. S. *Bioorg. Med. Chem. Lett.* **2001**, *11*, 235. (c) Barta, T. E.; Becker, D. P.; De Crescenzo, G. A.; McDonald, J. J.; Munie, G. E.; Rao, S.; Shieh, H. S.; Stegeman, R.; Stevens, A. M.; Villamil, C. I. *Bioorg. Med. Chem. Lett.* **2000**, *10*, 2815.
13. (a) Gavuzzo, E.; Pochetti, G.; Mazza, F.; Gallina, C.; Gorini, B.; D'Alessio, S.; Pieper, M.; Tschesche, H.; Tucker, P. A. *J. Med. Chem.* **2000**, *43*, 3377. (b) Pavlovsky, A. G.; Williams, M. G.; Ye, Q.; Ortwine, D. F.; Purchase, C. F., II; White, A. D.; Dhanaraj, V.; Roth, B. D.; Johnson, L. L.; Hupe, D.; Humblet, C.; Blundell, T. L. *Protein Sci.* **1999**, *8*, 1455. (c) Tamura, Y.; Watanabe, F.; Nakatani, T.; Yasui, K.; Fuji, M.; Komurasaki, T.; Tsuzuki, H.; Maekawa, R.; Yoshioka, T.; Kawada, K.; Sugita, K.; Ohtani, M. *J. Med. Chem.* **1998**, *41*, 640.
14. (a) Clare, B. W.; Scozzafava, A.; Supuran, C. T. *J. Med. Chem.* **2001**, *44*, 2253. (b) Scozzafava, A.; Supuran, C. T. *J. Med. Chem.* **2000**, *43*, 1858. (c) Scozzafava, A.; Supuran, C. T. *J. Med. Chem.* **2000**, *43*, 3677.
15. (a) Scozzafava, A.; Supuran, C. T. *Eur. J. Med. Chem.* **2000**, *35*, 299. (b) Supuran, C. T.; Scozzafava, A. *Eur. J. Pharm. Sci.* **2000**, *10*, 67. (c) Supuran, C. T.; Briganti, F.; Mincione, G.; Scozzafava, A. *J. Enzyme Inhib.* **2000**, *15*, 111. (d) Scozzafava, A.; Supuran, C. T. *Bioorg. Med. Chem.* **2000**, *8*, 637. (e) Scozzafava, A.; Supuran, C. T. *Bioorg. Med. Chem. Lett.* **2000**, *10*, 499. (f) Scozzafava, A.; Supuran, C. T. *Eur. J. Pharm. Sci.* **2000**, *11*, 69.
16. (a) Jacobsen, E. J.; Mitchell, M. A.; Hendges, S. K.; Belonga, K. L.; Skaletzky, L. L.; Stelzer, L. S.; Lindberg, T. J.; Fritzen, E. L.; Schostarez, H. J.; O'Sullivan, T. J.; Maggiora, L. L.; Stuchly, C. W.; Laborde, A. L.; Kubicek, M. F.; Poorman, R. A.; Beck, J. M.; Miller, H. R.; Petzold, G. L.; Scott, P. S.; Truesdell, S. E.; Fallace, T. L.; Wilks, J. W.; Fisher, C.; Goodman, L. V.; Staples, D. J.; Baldwin, E. T.; Finzel, B. C. *J. Med. Chem.* **1999**, *42*, 1525. (b) Stockman, B. J.; Waldon, D. J.; Gates, J. A.; Scahill, T. A.; Kloosterman, D. A.; Mizsak, S. A.; Jacobsen, E. J.; Belonga, K. L.; Mitchell, M. A.; Mao, B.; Petke, J. D.; Goodman, L. V.; Powers, E. A.; Ledbetter, S. R.; Kaytes, P. S.; Vogali, G.; Marshall, V. P.; Petzold, G. L.; Poorman, R. A. *Protein Sci.* **1998**, *7*, 2281.
17. Reinemer, P.; Grams, F.; Huber, R.; Kleine, T.; Schnierer, S.; Piper, M.; Tschesche, H.; Bode, W. *FEBS Lett.* **1994**, *338*, 227.
18. (a) Kornis, G. In *Comprehensive Heterocyclic Chemistry*; Katritzky, A. R., Ed.; Pergamon: Oxford, UK, 1984; Vol. VI, p 545. (b) Supuran, C. T.; Banciu, M. D.; Botez, G.; Balaban, A. T. *Rev. Roum. Chim.* **1992**, *37*, 1375.
19. Derivatives **9** were prepared as described in: Scozzafava, A.; Supuran, C. T. *Eur. J. Med. Chem.* **2000**, *35*, 299, whereas derivatives **10** as described in: Scozzafava, A.; Supuran, C. T. *J. Med. Chem.* **2002**, *45*, 284. A typical procedure for the synthesis of one such compound is described below: an amount of 252 mg (1 mmol) of Phe-Ala (Sigma-Aldrich, Milan, Italy) was suspended in 30 mL of anhydrous acetone and the equivalent amount (185 mg, 1 mmol) of 4-tosyl-isocyanate (Sigma-Aldrich, Milan, Italy) dissolved in a small volume of the same solvent was added dropwise. The mixture was stirred for 1 h at room temperature, the solvent was evaporated in vacuo and the obtained sulfonylurea **10** crystallized from 120 mL of ethanol–water (1:1, v/v). The yield was quantitative. The obtained compound **10** was then suspended in 50 mL of anhydrous acetonitrile together with the equivalent amount of 5-amino-2-mercapto-1,3,4-thiadiazole (133 mg), and 1 mmol (126 mg = 98 μL) of diisopropylcarbodiimide was added dropwise. The mixture was magnetically stirred at room temperature for 12 h (TLC control), the solvent was evaporated in vacuo, the raw product washed with 2×10 mL of water for removing the diisopropylurea formed in the reaction and the desired product **13d** was recrystallized from 70 mL of methanol-water (2:1, v/v). The yield was of 83%. Pale yellow crystals, mp 212–213 °C (dec.); IR (KBr), cm^{-1}: 1150 (SO_2^{sym}), 1283 (amide III), 1375 (SO_2^{as}), 1584 (amide II), 1609 (C=N of thiadiazole); 1710 (amide I), 3060 (NH); ^1H NMR (300 MHz, DMSO-d_6), δ, ppm: 2.37 (d, 3H, CH_3 of Ala); 2.50 (s, 3H, $CH_3C_6H_4$); 3.33 (m, 2H, CH_2CH of Phe), 3.62 (q, 1H, CH of Ala), 4.10 (dd, $^3J_{HH} = 5.0$, $^3J_{HH} = 7.8$, 1H, CH_2CH of Phe), 7.11–7.35 (m, 5H, H_{arom} of Phe); 7.38 (2H, H_{ortho} of $CH_3C_6H_4$); 7.72 (d, $^3J_{HH} = 8.2$, 2H, H_{meta} of $CH_3C_6H_4$); ^{13}C NMR (75 MHz, DMSO-d_6), δ, ppm: 22.1 (s, CH_3 of Ala); 26.0 (s, $CH_3C_6H_4$), 34.5 (s, $CHCH_3$ of Ala); 41.7 (s, CH_2CH of Phe), 59.3 (s, CH_2CH of Phe), 132.3 (s, C_{meta} of $CH_3C_6H_4$), 132.7 (s, NHCONH), 133.8 (s, C_{meta} of Phe), 134.4 (s, C_{ortho} of Phe), 135.1 (s, C_{ortho} of $CH_3C_6H_4$), 141.5 (s, C_{ipso} of Phe), 145.0 (s, C_{ipso} of $CH_3C_6H_4$), 148.6 (s, C_{para} of $CH_3C_6H_4$), 154.5 (C-2 of thiadiazole), 166.9 and 172.3 (CONH), 183.1 (C=S). Anal., found: C, 48.37; H, 4.66; N, 15.14; S, 17.82%; $C_{22}H_{24}N_6O_5S_3$ requires C, 48.16; H, 4.41; N, 15.32; S, 17.53%..
20. Bieth, J. G. *Meth. Enzymol.* **1995**, *248*, 59.
21. (a) Powers, J. C.; Kam, C. M. *Meth. Enzymol.* **1995**, *248*, 3. (b) Johnson, L. L.; Bornemeier, D. A.; Janowicz, J. A.; Chen, J.; Pavlovsky, A. G.; Ortwine, D. F. *J. Biol. Chem.* **1999**, *274*, 24881.
22. Van Wart, H. E.; Steinbrink, D. R. *Anal. Biochem.* **1981**, *113*, 156.
23. Human purified MMPs (MMP-1, MMP-2, MMP-8 and MMP-9) were from Calbiochem (Inalco, Milan, Italy). They were activated in the assay buffer by adding bovine trypsin (50 μL, 0.6 mg/mL) to the proenzyme, followed by incubation at 37 °C for 10 min. The trypsin was then inactivated with aprotinin (50 μL, 1.2 mg/mL). Initial rates for the hydrolysis of the thioester substrate AcProLeuGly-*S*-LeuLeuGlyOEt, coupled to the reaction with 5,5′-dithiobis-(2-nitrobenzoic acid) were used for assessing the catalytic activity and inhibition of the four MMPs mentioned above, by the spectrophotometric method of Powers and Kam,[21a] modified by Johnson et al.[21b] The change of absorbance ($\varepsilon = 19{,}800\ M^{-1}\ cm^{-1}$)[21] at 405 nm was monitored continuously at room temperature, using a Cary 3 spectrophotometer interfaced with a PC. A typical 100 μL reaction contained 50 mM MES, pH 6.0, 10 mM $CaCl_2$,

100 μM substrate, 1 mM 5,5′-dithiobis-(2-nitrobenzoic acid) and 5 nM MMP. For the K_I determinations, DMSO solutions of the inhibitor were included in the assay, resulting in a final concentration of 2% DMSO in the reaction mixture. In these conditions, K_I values varied from 5 to 10% in replicate experiments. K_Is were then determined by using Easson–Stedman[20] plots and a linear regression program. *C. histolyticum* highly purified collagenase and its substrate, FALGPA (furanacryloyl-leucyl-glycyl-prolyl-alanine) were from Sigma-Aldrich (Milan, Italy), and their concentrations were determined from the absorbance at 280 nm and the extinction coefficients furnished by the supplier. The activity of such preparations was in the range of 10 NIH (National Institute of Health) units/mg solid. The potency of standard and newly obtained inhibitors was determined from the inhibition of the enzymatic (amidolytic) activity of the collagenase, at 25 °C, using FALGPA as substrate, by the method of van Wart and Steinbrink.[22] The substrate was reconstituted as 5 mM stock in 50 mM Tricine buffer, 0.4 M NaCl, 10 mM $CaCl_2$, pH 7.50. The rate of hydrolysis was determined from the change in absorbance at 324 nm using an extinction coefficient for FALGPA $\varepsilon_{305} = 24{,}700\ M^{-1}\ cm^{-1}$ in the above-mentioned reaction buffer.[22] Measurements were made using a Cary 3 spectrophotometer interfaced with a PC. Initial velocities were thus estimated using the direct linear plot-based procedure reported by van Wart and Steinbrink.[22] K_Is were then determined according to Easson–Stedman[20] plots and a linear regression program.