Mikami, T. Korenaga, M. Terada, R. Noyori, *J. Am. Chem. Soc.* **1998**, *120*, 1086 – 1087.
[8] a) H. Brunner, *Organometallics* **1984**, *3*, 1354 – 1359; b) H. Nishiyama, M. Kondo, T. Nakamura, K. Itoh, *ibid.* **1991**, *11*, 500 – 508.
[9] E. J. Corey, R. K. Bakshi, S. Shibata, C.-P. Chen, V. K. Singh, *J. Am. Chem. Soc.* **1987**, *109*, 7925 – 7926.
[10] a) T. Ohkuma, H Ooka, S. Hashiguchi, T. Ikariya, R. Noyori, *J. Am. Chem. Soc.* **1995**, *117*, 2675 – 2676: b) H. Doucet, T. Ohkuma, K. Murata, T. Yokozawa, M. Kozawa, E. Katayama, A. F. England, T. Ikariya, R. Noyori, *Angew. Chem.* **1998**, *110*, 1792 – 1796, *Angew. Chem. Int. Ed.* **1998**, *37*, 1703 – 1707.
[11] X. Zhang, T. Taketomi, T. Yoshizumi, H. Kumobayashi, S. Akutagawa, K. Mashima, H. Takaya, *J. Am. Chem. Soc.* **1993**, *115*, 3318 – 3319.
[12] T. Nagata, K. Yorozu, T. Yamada, T. Mukaiyama, *Angew. Chem.* **1995**, *107*, 2309 – 2311; *Angew. Chem. Int. Ed. Engl.* **1995**, *34*, 2145 – 2147. For a recent review on the asymmetric reduction of carbonyl groups with hydrides, see J. Seyden-Penne, *Reductions by the Alumino- and Borohydrides in Organic Synthesis*, 2nd ed., Wiley, New York, **1997**, 55 – 84.
[13] Review: a) R. Noyori, S. Hashiguchi, *Acc. Chem. Res.* **1997**, *30*, 97 – 112; b) K. Matsumura, S. Hashiguchi, T. Ikariya, R. Noyori, *J. Am. Chem. Soc.* **1997**, *119*, 8738 – 8739.
[14] K. Nakamura, T. Matsuda, A. Ohno, *Tetrahedron: Asymmetry* **1996**, *7*, 3021 – 3024.
[15] a) J. Takehara, S. Hashiguchi, A. Fujii, S. Inoue, T. Ikariya, R. Noyori, *Chem. Commun.* **1996**, 233 – 234; b) T. Langer, G. Helmchen, Tetrahedron Lett. **1996**, *37*, 1381 – 1384; c) K. Püntener, L. Schwink, P. Knochel, *ibid.* **1996**, *37*, 8165 – 8168; d) T. Sammakia, E. L. Stangeland, *J. Org. Chem.* **1997**, *62*, 6104 – 6105; e) M. Palmer, T. Walsgrove, M. Wills, *ibid.* **1997**, *62*, 5226 – 5228; f) F. Touchard, P. Gamez, F. Fache, M. Lemaire, *Tetrahedron Lett.* **1997**, *38*, 2275 – 2278; g) S. Inoue, K. Nomura, S. Hashiguchi, R. Noyori, Y. Izawa, *Chem. Lett.* **1997**, 957 – 958.
[16] Y. Jiang, Q. Jiang, G. Zhu, X. Zhang, *Tetrahedron Lett.* **1997**, *38*, 215 – 218.
[17] R. Berengeuer, J. Garcia, J. Villarasa, *Tetrahedron: Asymmetry* **1994**, *5*, 165 – 168.
[18] a) T. Osawa, T. Harada, A. Tai, *J. Mol. Catal.* **1994**, *87*, 333 – 342; b) T. Osawa, T. Harada, A. Tai, *Catal. Today* **1997**, *37*, 465 – 480.
[19] a) M. J. Burk, M. F. Gross, T. G. P. Harper, C. S. Kalberg, J. R. Lee, J. P. Martinez, *Pure Appl. Chem.* **1996**, *68*, 37 – 44; b) J. Albrecht, U. Nagel, *Angew. Chem.* **1996**, *108*, 444 – 446; *Angew. Chem. Int. Ed. Engl.* **1996**, *35*, 404 – 407.
[20] T. Imai, T. Tamura, A. Yamamuro, *J. Am. Chem. Soc.* **1986**, *108*, 7402 – 7404.
[21] F. Touchard, F. Fache, M. Lemaire, *Tetrahedron: Asymmetry* **1997**, *8*, 3319 – 3326.
[22] R. Stürmer (BASF AG), personal communication.
[23] Review: K. Mori, *Chem. Commun.* **1997**, *13*, 1153 – 1158.
[24] T. Imamoto, J. Watanabe, Y. Wada, H. Masuda, H. Yamada, H. Tsuruta, S. Matsukawa, K. Yamaguchi, *J. Am. Chem. Soc.* **1998**, *120*, 1635 – 1636.

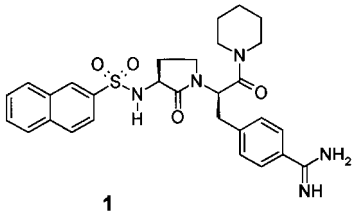
# Metal Ions as Co-Inhibitors of Serine Proteases: A New Approach in the Search for Specific High-Affinity Ligands

**Tanja Schirmeister***

Serine proteases are key enzymes for a variety of biological processes and are therefore of great importance in the design of new drugs.[1] The trypsin-like proteases of the blood-clotting cascade, thrombin and factor Xa, are of particular interest in this context.[2] A common characteristic is their specificity for basic amino acids in P1.[3] Apart from trypsin, the proteases of fibrinolysis (plasmin, t-PA, urokinase),[4] those of the complement system (factors C1r, C1s),[5] and the mast-cell tryptase[6] belong to this family. Leukocyte elastase is also of therapeutic interest, which, although it uses the same hydrolysis mechanism, possesses a small hydrophobic S1 pocket and thus exhibits only P1 specificity for the short-chain amino acids alanine and valine.[7] Pathological processes for which serine protease inhibitors could be of therapeutic value include blood-vessel diseases (thrombin), tumors (plasmin, urokinase), infectious processes and autoimmune diseases (tryptase, complement proteases), lung emphysema (elastase), and pancreatitis (trypsin).[1]

[*] Dr. T. Schirmeister
Pharmazeutisches Institut der Universität
Hermann-Herder-Strasse 9, D-79104 Freiburg (Germany)
Fax: (+49) 761-203-6351
E-mail: schirmei@sun2.ruf.uni-freiburg.de

Up to now, there were three approaches to developing inhibitors for trypsin-like proteases which differed in the type of reaction between the inhibitor and the active site. Compound **1** is one of numerous benzamidines which employ their



**1**

positively charged amidinium group to occupy reversibly and noncovalently the S1 pocket of the enzymes; the S1 pocket carries a negative charge due to the amino acid Asp 189.[8] The petidyl aldehyde GYKI 14766 (*N*-Me-D-Phe-Pro-Arg-H),[9] a potent thrombin inhibitor, additionally reacts reversibly with the serine residue at the active site to yield a hemiacetal. Irreversible inhibitors can be found, for example, in the group of peptidyl chloromethyl ketones (e.g. D-Phe-Pro-Arg-$CH_2Cl$).[9]

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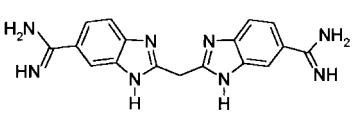
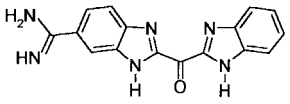
The recently published discovery by Katz et al.—that the effect of weak inhibitors such as amidinobenzimidazole BABIM (**2**)[10] can be greatly enhanced by the addition of $Zn^{2+}$ or $Co^{2+}$ ions—represents a new approach for the development of selective, high-affinity serine protease inhibitors.[11] The activity of the inhibitor **3**, for example, increases



**2**



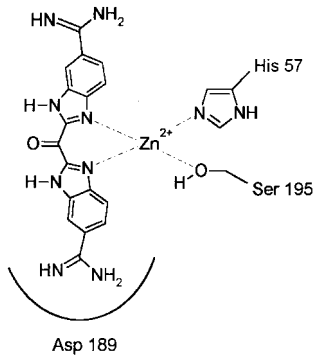
**3**

upon the addition of $Zn^{2+}$ from a $K_I$ value in the micromolar range (87.5 μM) by a factor of 17000 to $K_I = 5.3$ nM.[11] The inhibiting effect of metal cations on proteases has so far only been known for cysteine and aspartate proteases. In these cases, complexation blocks the active site, for aspartate proteases the two catalytically relevant Asp residues and for cysteine proteases the thiolate in the active site. Exceptions in the case of serine proteases are kallikreins[12] and herpes virus proteases such as the cytomegalovirus protease hCMV.[13] These are, however, not typical for serine proteases, as they do not utilize Asp/Glu-Ser-His as the catalytic triad, but rather His-Ser-His; this then actually allows the complex formation. In the case of the trypsin-like kallikreins, complexation is made possible by additional histidine-containing loops.

There are also known serine and cysteine proteases which contain an additional $Zn^{2+}$ binding site. However, these do not require $Zn^{2+}$ ions for hydrolytic activity, but rather for the stabilization of the active conformation. Examples are the NS3 protein of the hepatitis C virus (HCV), which is a chymotrypsin-like serine protease, and the 2A proteinases of picornaviruses, a group of chymotrypsin-like cysteine proteases.[14]

In addition, a number of trypsin mutants have been synthesized. Replacement of different amino acids by histidine allows inhibition, activation, or a change in substrate specificity by $Zn^{2+}$ and other metal cations.[15]

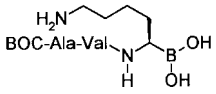
The enormous increase in the inhibitor activity of the benzamidines of type **2** upon addition of $Zn^{2+}$ ions can be traced to chelation of the metal ion by the amino acids of the active site of the protease (His 157, Ser 195) and the nitrogen atoms of the two imidazole rings.[11] The complexation is achieved by the chelating elements of the inhibitor, and not just by histidine residues in the enzyme, as is the case in the above-mentioned metal-dependent trypsin variants or virus proteases. X-ray structure analyses[19] on keto-BABIM/trypsin (Scheme 1) show that the distance between the S1 recognition




Scheme 1. Binding mode of keto-BABIM to trypsin.

element, the amidinium group, and the chelating element of the inhibitor is of crucial importance for the exhibited high affinity.[11] Thus, the S1 pocket as well as both catalytically relevant amino acid residues in the enzyme can be blocked.

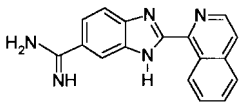
A similar phenomenon was already observed by Katz et al. for the inhibition of trypsin by peptidylboronates (e.g. **4**; BOC = *tert*-butoxycarbonyl) in combination with alcohols.[16] The behavior of the enzyme to "select" components from the reaction medium for binding was termed episelection.[16]



**4**

The main problem for the development of new inhibitors, apart from pharmacokinetics (e.g., oral availability), is the lack of selectivity. This can be improved by choosing substituents that take into account not only the P1 specificity of the individual proteases, but also larger substrate sequences. For example, the selectivity between thrombin and trypsin could be significantly increased by exploiting the fact that thrombin, in contrast to trypsin, contains a distinct, lipophilic P3 pocket. This has been realized, for example, in GYKI 14766.

Katz et al. were able to show that an improvement in the selectivity can also be achieved by using $Zn^{2+}$ ions as co-inhibitors.[11] For example, the inhibition of thrombin by inhibitor **5** is increased by a factor of 760 upon the addition of $Zn^{2+}$ ions ($K_I = 41$ nM with $Zn^{2+}$, 31 μM without $Zn^{2+}$), whereas the inhibition of trypsin is barely affected ($K_I = 22.5$ μM with $Zn^{2+}$, 31.2 μM without $Zn^{2+}$). The selectivity is even a factor of 4750 higher between thrombin and tryptase (for tryptase, $K_I =$ 54.5 μM with $Zn^{2+}$, 8.8 μM without $Zn^{2+}$). Apparently , the chelate formation increases the differences between the individual enzymes at substrate binding sites outside S1.



**5**

As the increased selectivity and affinity can be observed at $Zn^{2+}$ concentrations of 100 nM (i.e., far below physiological levels), there are promising possibilities for the development of active drugs. On the other hand, it is necessary to critically review the results obtained with inhibitors that contain functional groups that can participate in chelate formation, and which were obtained without the absolute exclusion of complex-forming metal cations.

When applied to other serine proteases, these results imply that the combination of a suitable S1 recognition element—hydrophobic residues for chymotrypsin or short alkyl chains for elastase—and a chelating group can transform weak, unselective ligands into specific, high-affinity inibitors of low molecular weight by complexation with metal ions. In the case of cysteine proteases, of which the lysosomal cathepsins are especially interesting targets for the therapeutic application of inhibitors,[17] it must be taken into account that the thiolate at the active site represents a functional group with a stronger tendency for complexation, that the geometry of the active site is of opposite handedness to the structure found for serine proteases,[18] and that there is a very distinct P2 specificity for hydrophobic amino acids.

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**Keywords:** cations · chelates · enzyme inhibitors · serine proteases · zinc

[1] Y. Nakayama, K. Senokuchi, H. Nakai, T. Obata, M. Kawamura, *Drugs Future* **1997**, 22, 285 – 293.
[2] E. Davie, K. Fujikawa, W. Kisiel, *Biochemistry* **1991**, 30, 10363 – 10370.
[3] J. J. Perona, C. S. Craik, *Prot. Science* **1995**, *4*, 337 – 360.
[4] D. Collen, H. R. Lijinen in *Hematology Basic Principles & Practice* (Eds.: R. Hoffman, E. J. Benz, S. J. Shattil, Jr., B. Furie, H. J. Cohen) , Churchill Livingstone, New York, **1991**, pp. 1232 – 1242.
[5] M. M. Frank, L. F. Fries in *Fundamental Immunology* (Ed.: W. E. Paul), Raven, New York, **1989**, pp. 679 – 701.
[6] G. H. Caughey, W. W. Raymond, E. Bacci, R. Lombardy, R. R. Tidwell, *J. Pharmacol. Exp. Ther.* **1993**, *264*, 676 – 682.
[7] A. Krantz, *Ann. Rep. Med. Chem.* **1993**, *29*, 195 – 204.
[8] a) D. Banner, P. Hadváry, *J. Biol. Chem.* **1991**, *266*, 20085 – 20093; b) D. Banner, J. Ackermann, A. Gast, K. Gubernator, P. Hadváry, K. Hilpert, L. Labler, K. Müller, G. Schmid, T. Tschopp, H. van de Waterbeemd, B. Wirz in *Perspectives in Medicinal Chemistry* (Eds.: B. Testa, E. Kyburz, W. Fuhrer, R. Giger), VCH, Weinheim, **1993**, pp. 27 – 43.
[9] H.-J. Böhm, G. Klebe, H. Kubinyi, *Wirkstoffdesign*, Spektrum, Heidelberg, **1996**, pp. 469 – 485.
[10] a) R. Tidwell, J. Geratz, O. Dann, G. Volz, D. Zeh, H. Loewe, *J. Med. Chem.* **1978**, *21*, 613 – 623; b) R. Tidwell, J. Geratz, E. Dubovi, *ibid.* **1983**, *26*, 294 – 298.
[11] B. A. Katz, J. M. Clark, J. S. Finer-Moore, T. E. Jenkins, C. R. Johnson, M. J. Ross, C. Luong, W. R. Moore, R. M. Stroud, *Nature* **1998**, *391*, 608 – 612.
[12] a) K. Watt, P.-J. Lee, T. M'Timkulu, W.-P. Chan, R. Loor, *Proc. Natl. Acad. Sci. USA* **1986**, *83*, 3166 – 3170; b) M. Fujinaga, M. James, *J. Mol. Biol.* **1987**, *195*, 373 – 396; c) F. Fiedler, E. Werle, *Eur. J. Biochem.* **1968**, *7*, 27 – 33.
[13] L. Tong, C. Qian, M.-J. Massariol, P. Bonneau, M. Cordingley, L. Lagacé, *Nature* **1996**, *383*, 272 – 275.
[14] R. De Francesco, A. Urbani, M. Nardi, L. Tomei, C. Steinkühler, A. Tramontano, *Biochemistry* **1996**, *35*, 13282 – 13287.
[15] a) W. Willett, L. Brinen, R. Fletterick, C. S. Craik, *Biochemistry* **1996**, *35*, 5992 – 5998; b) L. Brinen, W. Willett, C. S. Craik, R. Fletterick, *ibid.* **1996**, *35*, 5999 – 6009; c) J. Higaki, B. Haymore, S. Chen, R. Fletterick, C. S. Craik, *ibid.* **1990**, *29*, 8582 – 8586; d) S. Halfon, C. S. Craik, *J. Am. Chem. Soc.* **1996**, *118*, 1227 – 1228.
[16] B. A. Katz, J. Finer-Moore, R. Mortezaei, D. Rich, R. Stroud, *Biochemistry* **1995**, *34*, 8264 – 8280.
[17] T. Schirmeister, H.-H. Otto, *Chem. Rev.* **1997**, *97*, 133 – 171.
[18] a) R. M. Garavito, M. G. Rossmann, P. Argos, W. Eventoff, *Biochemistry* **1977**, *16*, 5065 – 5071; b) W. Bode, R. Engh, D. Musil, B. Laber, M. Stubbs, R. Huber, V. Turk, *Biol. Chem. Hoppe-Seyler* **1990**, *371*, *Suppl.*, 111 – 118.
[19] Katz et al.[11] describe three binding modes, which have been crystallographically verified, of BABIM and keto-BABIM to trypsin: 1) with $Zn^{2+}$ or $Co^{2+}$, 2) with $SO_4^{2-}$, 3) without ions.

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