Bioorganic & Medicinal Chemistry Letters 20 (2010) 4038–4044

Contents lists available at ScienceDirect

Bioorganic & Medicinal Chemistry Letters

journal homepage: www.elsevier.com/locate/bmcl

Subtype-selectivity of metal-dependent methionine aminopeptidase inhibitors

Markus A. Altmeyer, Aline Marschner, Rolf Schiffmann, Christian D. Klein *

Department of Medicinal Chemistry, Universität Heidelberg, Im Neuenheimer Feld 364, D-69120 Heidelberg, Germany

ARTICLE INFO

Article history:
Received 30 April 2010
Revised 21 May 2010
Accepted 22 May 2010
Available online 27 May 2010

Keywords:
Methionine aminopeptidase
Benzimidazole
Hydroxyquinoline
Picolinic acid
Selectivity

ABSTRACT

Inhibitors of methionine aminopeptidases (MetAPs) are treatment options for various pathological conditions. Several inhibitor classes have been described previously, but only few data on the subtype selectivity, which is of crucial importance for these enzymes, is available. We present a systematic study on the subtype- and species-selectivity of MetAP inhibitors that require the binding of an auxiliary metal ion. This includes, in particular, compounds based on the benzimidazole pharmacophore, but also hydroxyquinoline and picolinic acid derivatives. Our data indicates that a significant degree of selectivity can be attained with metal-dependent MetAP inhibitors.

Methionine aminopeptidases (MetAPs) are ubiquitous enzymes that are pursued as drug targets in the context of various pharmacological interventions, including the treatment of parasitic and bacterial infections and the therapy of cancer.[1,2] The biological function of MetAPs is to remove the N-terminal initiator methionine from nascent polypeptide chains, in order to recycle methionine and prepare further post-translational modifications. MetAPs play a central role in the processing and quality control of proteins and are therefore essential for cell survival.[3]

The active site of MetAPs contains two transition metal ions. While the nature of the biologically relevant element is debated, cobalt ions (Co^{II}) are usually employed as the metal for in vitro enzymological studies.[4] There exist two major classes of MetAPs, one associated with eubacteria (MetAP-1) and one associated with archaebacteria (MetAP-2). Eukaryotic organisms possess both classes. The structural scaffold of the two classes of MetAPs is highly conserved.[5,6]

The most prominent inhibitory compound in the context of MetAPs is fumagillin, which selectively targets the human subtype 2 of the enzyme.[2,7] Fumagillin and its derivative TNP-470 have been studied as inhibitors of tumor angiogenesis.[8,9] Various other classes of compounds have been described as inhibitors of bacterial or human MetAPs, including compounds that bind via an auxiliary metal ion.[10,11] Under in vitro-conditions, the auxiliary metal ion may be 'recruited' from excess metal present in the buffer. For example, the benzimidazole derivatives first described in our group are potent inhibitors of *Escherichia coli* MetAP that require an auxiliary metal ion. The binding mode of this class of compounds has been elucidated by X-ray crystallography.[10] The application of a metal-mediated binding mode for inhibitor development offers novel pharmacological opportunities, since it couples the inhibition of an enzyme to the presence of metal ions. Furthermore, recent work from the group of Qi-Zhuang Ye indicates that metal-dependent MetAP inhibitors show activity under in vivo-conditions.[12]

Relatively few data—and, in particular, practically no systematic SAR studies on groups of analogs—exist on the subtype- and species-selectivity of MetAP inhibitors. We have established a panel of different MetAPs which allows us to perform selectivity studies under uniform conditions (concentrations of enzymes and buffer constituents, assay-readout).[13] We would like to report here on the activity and selectivity of metal-dependent inhibitors, in particular from the benzimidazole class,[14] in those MetAPs with the largest therapeutical relevance.

The results are summarized in Table 1, sorted according to the structural scaffold and the inhibitory potency against *E. coli* MetAP. The structure–activity relationships can be summarized as follows:

A. Benzimidazoles[15]

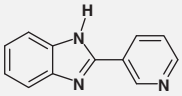
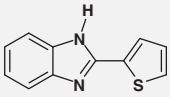
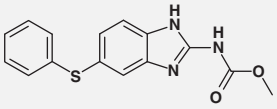
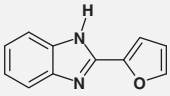
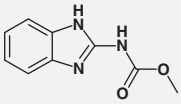
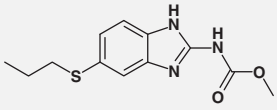
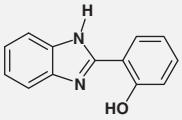
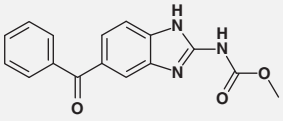
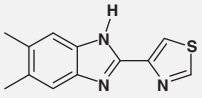
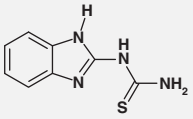
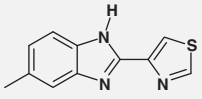
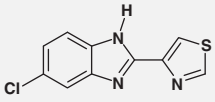
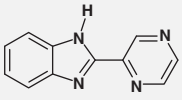
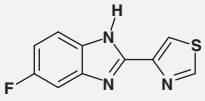
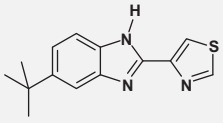
- Benzimidazoles with a chelating substructure are potent inhibitors of *E. coli* MetAP. Compounds that lack chelating properties are inactive against all tested MetAPs (compounds **1**, **2**, **3**, **4** and the clinical drugs except thiabendazole). This clearly indicates that binding to an auxiliary transition metal ion is important for the activity of benzimidazoles, irrespective of the enzyme subtype.

Abbreviations: MetAP, methionine aminopeptidase; AAO, amino acid oxidase; HRP, horseradish peroxidase.

* Corresponding author. Tel.: +49 6221 544875; fax: +49 6221 546430.
E-mail address: c.klein@uni-heidelberg.de (C.D. Klein).

0960-894X/$ - see front matter © 2010 Elsevier Ltd. All rights reserved.
doi:10.1016/j.bmcl.2010.05.093

Table 1
Structure of the compounds and their activities against various MetAPs, sorted with respect to structural class

Compound ID/ generic name	Structure	*E. coli* MetAP		*S. aureus* MetAP		*H. sapiens* MetAP-1		*H. sapiens* MetAP-2		Ref.
		% Inh. (25 μM)	IC_{50} [μM]	% Inh. (25 μM)	IC_{50} [μM]	% Inh. (25 μM)	IC_{50} [μM]	% Inh. (25 μM)	IC_{50} [μM]	
1		n.i.		20		n.i.		n.i.		14
2		n.i.		n.i.		n.i.		n.i.		14
Fenbendazole		n.i.		12.5		n.i.		n.i.		[a]
3		26		10		n.i.		n.i.		14
Carbendazim		34		n.i.		n.i.		n.i.		[a]
Albendazole		35		10.5		n.i.		10		[a]
4		66		12		n.i.		18		14
Mebendazole		47		n.i.		n.i.		n.i.		[a]
5			10.0 ± 3.5	17		34	38.8 ± 0.7	n.i.		14
6			9.0 ± 1.3	31	35.9 ± 0.6	25		10		14
7			7.2 ± 0.1	n.i.		34	35.5 ± 2.5	39	40.6 ± 2.4	14
8			5.2 ± 1.0	15		28	47.0 ± 3.8	46	28.0 ± 1.3	14
9			4.6 ± 0.4	n.i.		15		43	32.9 ± 2.7	14
10			4.3 ± 0.6	21		26	45.8 ± 3.1	46	38.4 ± 5.5	14
11			3.9 ± 0.5	30.2	41.7 ± 2.3	n.i.		14		14

(*continued on next page*)

Table 1 (*continued*)

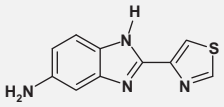
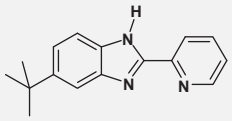
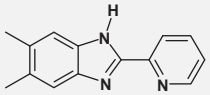
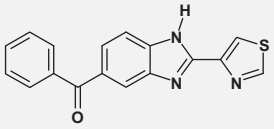
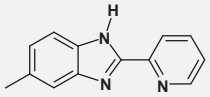
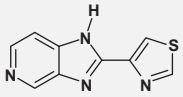
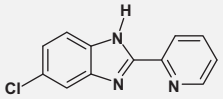
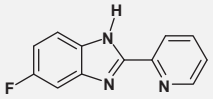
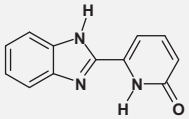
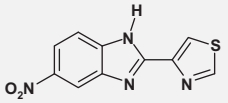
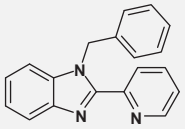
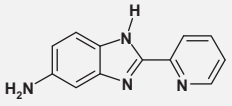
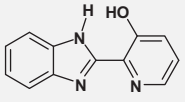
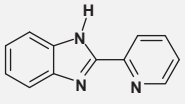
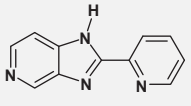
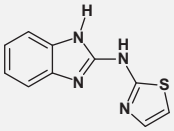
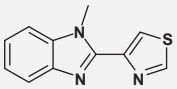
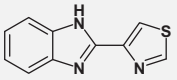
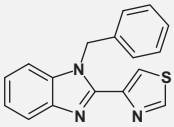
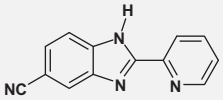
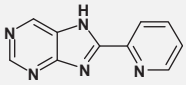
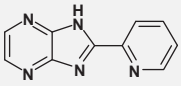
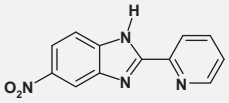
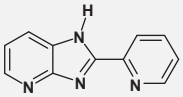
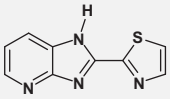
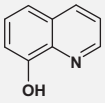
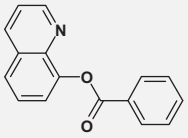
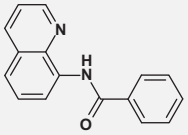
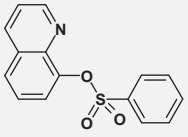
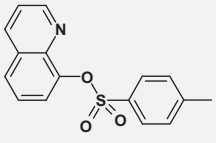
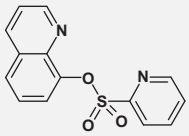
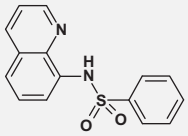
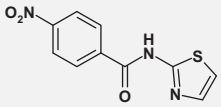
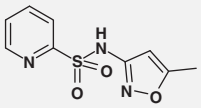
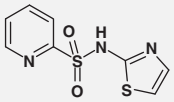
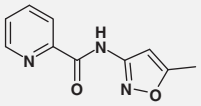
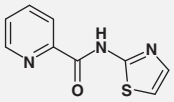
Compound ID/ generic name	Structure	*E. coli* MetAP		*S. aureus* MetAP		*H. sapiens* MetAP-1		*H. sapiens* MetAP-2		Ref.
		% Inh. (25 μM)	IC_{50} [μM]	% Inh. (25 μM)	IC_{50} [μM]	% Inh. (25 μM)	IC_{50} [μM]	% Inh. (25 μM)	IC_{50} [μM]	
12			3.4 ± 0.2	92	2.3 ± 0.7	14		78	10.1 ± 1.3	14
13			2.6 ± 0.4	40.9	38.9 ± 3.7	n.i.		36	44.1 ± 1.6	14
14			2.4 ± 0.3	87	17.9 ± 1.9	37	32.6 ± 2.4	39	28.4 ± 2.9	14
15			2.4 ± 0.5	n.i.		36	41.0 ± 2.8	14		14
16			2.1 ± 0.3	68	19.4 ± 3.9	49	32.6 ± 4.9	77	7.7 ± 1.1	14
17			1.7 ± 0.2	32	45.5 ± 3.4	53	19.0 ± 0.9	18		14
18			1.7 ± 0.1	16		25	48.8 ± 2.3	63	18.1 ± 2.0	14
19			1.5 ± 0.2	31	40.1 ± 1.5	43	34.7 ± 3.3	64	14.9 ± 1.9	14
20			1.3 ± 0.3	47	29.2 ± 1.3	15		11		14
21			1.2 ± 0.1	n.i.		26	48.5 ± 0.5	15		14
22			0.99 ± 0.13	n.i.		n.i.		n.i.		14
23			0.97 ± 0.02	92	4.9 ± 0.1	n.i.		92	9.3 ± 0.4	14
24			0.78 ± 0.03	97	4.4 ± 1.7	15		22		14
25			0.57 ± 0.08	56	19.9 ± 2.2	40	28.4 ± 0.7	62	13.3 ± 2.7	14
26			0.55 ± 0.08	55	22.8 ± 1.9	74	10.3 ± 0.8	74	8.0 ± 1.4	14

Table 1 (*continued*)

Compound ID/ generic name	Structure	*E. coli* MetAP		*S. aureus* MetAP		*H. sapiens* MetAP-1		*H. sapiens* MetAP-2		Ref.
		% Inh. (25 μM)	IC_{50} [μM]	% Inh. (25 μM)	IC_{50} [μM]	% Inh. (25 μM)	IC_{50} [μM]	% Inh. (25 μM)	IC_{50} [μM]	
27			0.54 ± 0.03	52	22.4 ± 2.4	25		12		14
28			0.50 ± 0.01	n.i.		38	32.4 ± 1.1	49	24.3 ± 2.8	14
Thiabendazole		86.3	0.47 ± 0.06	28		28	47.8 ± 1.4	32	44.7 ± 3.1	[a]
29			0.46 ± 0.01	n.i.		n.i.		n.i.		14
30			0.43 ± 0.02	29	33.0 ± 4.0	33	37.3 ± 3.6	42	32.9 ± 1.7	14
31			0.38 ± 0.02	18		28	33.9 ± 3.9	36	36.4 ± 3.7	14
32			0.24 ± 0.04	51	22.0 ± 2.7	29	40.1 ± 1.2	24	48.4 ± 2.4	14
33			0.16 ± 0.02	n.i.		45	28.8 ± 1.6	n.i.		14
34			0.11 ± 0.00	76	18.8 ± 0.7	39	27.6 ± 1.9	49	26.7 ± 1.5	14
35			0.08 ± 0.01	21		26	39.8 ± 1.9	17		14
8-Hydroxy quinoline		95	0.77	65	18.3 ± 1.3	54	21.5 ± 2.3	85	5.6 ± 0.8	[a]
36		32		10		29	42.8 ± 1.9	39	34.8 ± 2.8	19
37		n.i.		n.i.		n.i.		17		19
38		n.i.		62	16.0 ± 1.9	29	41.2 ± 1.2	25	41.7 ± 4.1	19
39		n.i.		n.i.		15		18		19

(*continued on next page*)

Table 1 (*continued*)

Compound ID/ generic name	Structure	*E. coli* MetAP		*S. aureus* MetAP		*H. sapiens* MetAP-1		*H. sapiens* MetAP-2		Ref.
		% Inh. (25 μM)	IC_{50} [μM]	% Inh. (25 μM)	IC_{50} [μM]	% Inh. (25 μM)	IC_{50} [μM]	% Inh. (25 μM)	IC_{50} [μM]	
40		38		n.i.		15		24		19
41		93	11.0	47	22.9 ± 1.9	28	44.9 ± 1.9	32	39.8 ± 2.4	19
42		n.i.		n.i.		n.i.		n.i.		16
43		21		n.i.		23		15		20
44		23				24		12		21
45		80	2.8	20		n.i.		14		16
46		100	0.06 ± 0.01	58	21.2 ± 1.3	89	8.1 ± 3.0	48	25.8 ± 1.3	16

Most compounds were screened at a single concentration (25 μM) and IC_{50} values were determined only if the initial screen showed relevant activity. To ease the interpretation of the data, IC_{50} values below 10 μM are highlighted with borders and IC_{50} values between 10 and 20 μM are highlighted with dashed borders. The enzyme concentration was 22 nM. n.i., no inhibition.

[a] These compounds are part of the in-house compound collection and were originally purchased or donated by commercial suppliers.

- Only a few compounds show activity against the MetAPs from organisms other than *E. coli*. These are, in particular, **24**, **23**, **16**, and **12**. These compounds tend to have hydrogen-bonding functional groups in a position that allows specific interactions at the entrance of the MetAP active site, as is illustrated in Figure 1 for compound **24**. A detailed discussion of the binding mode and its implications is given in the figure legend.
- Most notably, it is possible to achieve subtype selectivity with metal-dependent inhibitors. This is illustrated by compound **24**, which has a submicromolar IC_{50} at the bacterial MetAPs and practically no affinity towards eukaryotic MetAPs. This is particularly relevant for the suggested application of MetAP inhibitors as antibacterial drugs. In this context, compound **24** is one of the very few compounds with documented selectivity for bacterial MetAPs and may serve as a starting point for further optimization.
- It is very remarkable that the *N*-benzyl-substituted analogs (**22** and **29**) are highly selective at the *E. coli* enzyme and possess no activity against the other MetAPs. The selectivity is not attributable to differences in the hydrogen-bonding or metal-binding properties but rather to the steric bulk of the benzyl group, as the methyl analog **28** retains some activity against the human MetAPs. Because of their significant selectivity, the *N*-benzyl-congeners may serve as leads for the development of MetAP inhibitors with selective, antibiotic activity against *E. coli* or other Gram-negative pathogens.
- Compound **20** is tautomeric and may, in theory, be present as the pyridone or hydroxypyridine species. The pyridone tautomer is not expected to bind to MetAPs via the chelating mechanism and the auxiliary metal ion. We therefore studied the interaction of compound **20** with cobalt ions in solution by UV–vis spectrometry and compared this to the non-hydroxylated analog **25**. The latter forms a cobalt complex, which is evident from a bathochromic shift of the main UV–vis absorption band from 320 to 350 nm. No changes in the UV–vis spectrum upon addition of cobalt ions could be observed for compound **20**. We can therefore conclude that this compound exists exclusively or predominantly as the pyridone tautomer and most likely has an alternative binding mode. This would also explain the SAR of this compound, which is, in comparison to compound **25**, practically inactive against the human MetAP-1 and MetAP-2. It may therefore be considered as a novel starting point for the development of MetAP inhibitors with selectivity for the bacterial isoforms. The logical next step in that direction would be to combine this structural feature with the *N*-benzyl substitution discussed above.

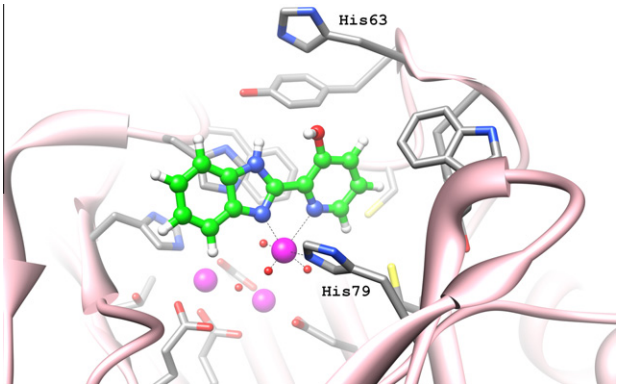


Figure 1. Compound **24** docked at the auxiliary cobalt ion at the entrance of the *E. coli* MetAP active site. The docking pose is identical to the X-ray structure of thiabendazole complexed with *E. coli* MetAP (PDB code 1YVM).[10,17] His79 is highly conserved in MetAPs and it appears likely that all MetAPs can, in principle, bind a third metal ion at this residue.[18] The binding of inhibitors to this metal ion, however, depends on additional, spatial requirements at the active site entry region of MetAPs. This is clearly demonstrated by the selectivity profiles of the benzimidazole derivatives and the other compound classes presented in this article. In the case of *E. coli* MetAP, there is an additional histidine residue (His63) which is not conserved among the MetAPs studied here and may (upon rotation of the side-chain) form a hydrogen bond to the phenolic hydroxy group of **24**, thus explaining the high affinity of the compound to this enzyme.

B. Quinolines

- Our lead compound for this small set of compounds was 8-hydroxyquinoline. We initially tested this compound because it is a well-known, bi-dentate complexing agent used in analytical chemistry. The significant activity against *E. coli* MetAP prompted us to perform a very limited exploration of the SAR (compounds **36**–**41**), which—not unexpectedly—indicated that the activity depends on the chelator properties of the molecule. Because of the ubiquitous binding of the unsubstituted 8-hydroxyquinoline to various metal ions, we did not further pursue it as a lead structure. Independent of our work, Huang et al. described quinolinyl sulfonamides such as **41** as inhibitors of Mn-loaded *E. coli* MetAP[11] that bind to an auxiliary, third manganese ion.
- The selectivity data shown here indicates that 8-hydroxyquinoline has a remarkable selectivity towards HsMetAP-2 (as compared to HsMetAP-1) and is the most potent inhibitor of this enzyme in the present dataset. Thus, chelating quinoline derivatives, where the chelator pharmacophore is conserved, may be promising lead compounds for the development of metal-dependent HsMetAP-2 inhibitors. Since a wealth of structural data is already available on this compound class, the structure-guided development of inhibitors with certain selectivity profiles may be a promising approach.

C. Picolinic acid derivatives

- This class of compounds, first described by Luo et al.,[16] also has metal-chelating properties. **46** is the most potent inhibitor of *E. coli* MetAP in the present dataset. It is also a moderately active inhibitor of the *Homo sapiens* MetAP-1, but has a 100-fold selectivity for the bacterial enzyme. We prepared and tested a few compounds from this class, including sulfonamide analogs, but were unable to increase their activity or modify the selectivity profile.

The data demonstrates that a subtype- and species-selective inhibition of MetAPs is possible with inhibitors that bind to an auxiliary metal ion. This can be realized with various structural scaffolds, including the highly drug-like benzimidazole pharmacophore and the hydroxyquinoline moiety. Furthermore, the data shows that even very moderate structural variations, like the *N*-benzyl group in compounds **29** and **22**, lead to significant changes in the selectivity profiles of the compounds. When applied to the hydroxyquinoline moiety, analogous modifications in the positions opposite to the chelator function may lead to highly potent and subtype-selective MetAP inhibitors.

Compound **20** has significant selectivity for the bacterial MetAPs and is not a metal chelator. A combination of the pyridone group with a *N*-benzyl substituted benzimidazole core with may yield potent and selective inhibitors of bacterial MetAPs that are no longer prone to form undesired metal complexes.

The binding of inhibitors to auxiliary metal ions in MetAPs may be advantageous with respect to subtype- and species-selectivity: Whereas the core of the active site with its two 'native' metal ions and the methionine binding pocket is highly conserved among different MetAPs, this is not the case for the entrance region of the active site. First, the binding affinity of the auxiliary metal ion is already highly variable among different MetAPs; Second, inhibitors that rely on the auxiliary metal ion will experience a highly variable binding environment, as has been discussed in this article for several selective compounds. Therefore, it is the entrance region of MetAPs and not the catalytic core that needs to be addressed to generate subtype-selective compounds, and metal-dependent inhibitors may be a viable approach to attain this goal.

Acknowledgments

This work was supported by the Deutsche Forschungsgemeinschaft (KL 1356). The authors thank Dr. S. Schütt of the Medical Microbiology Department at Heidelberg University Clinic for the *S. aureus* DNA and Dr. D. Kuck of the German Cancer Research Center for advice on the expression of *H. sapiens* MetAP-2 in Sf9 cells. *E. coli* MetAP was obtained using the expression plasmid generously provided by Profs. B. Matthews and T. Lowther.

Supplementary data

Supplementary data associated with this article can be found, in the online version, at doi:10.1016/j.bmcl.2010.05.093.

References and notes

1. Chang, S. Y.; McGary, E. C.; Chang, S. *J. Bacteriol.* **1989**, *171*, 4071.
2. Sin, N.; Meng, L.; Wang, M. Q.; Wen, J. J.; Bornmann, W. G.; Crews, C. M. *Proc. Natl. Acad. Sci. U.S.A.* **1997**, *94*, 6099.
3. Lowther, W. T.; Matthews, B. W. *Biochim. Biophys. Acta* **2000**, *1477*, 157.
4. Lowther, W. T.; Orville, A. M.; Madden, D. T.; Lim, S.; Rich, D. H.; Matthews, B. W. *Biochemistry* **1999**, *38*, 7678.
5. Arfin, S. M.; Kendall, R. L.; Hall, L.; Weaver, L. H.; Stewart, A. E.; Matthews, B. W.; Bradshaw, R. A. *Proc. Natl. Acad. Sci. U.S.A.* **1995**, *92*, 7714.
6. Bazan, J. F.; Weaver, L. H.; Roderick, S. L.; Huber, R.; Matthews, B. W. *Proc. Natl. Acad. Sci. U.S.A.* **1994**, *91*, 2473.
7. Griffith, E. C.; Su, Z.; Turk, B. E.; Chen, S.; Chang, Y. H.; Wu, Z.; Biemann, K.; Liu, J. O. *Chem. Biol.* **1997**, *4*, 461.
8. Ingber, D.; Fujita, T.; Kishimoto, S.; Sudo, K.; Kanamaru, T.; Brem, H.; Folkman, J. *Nature* **1990**, *348*, 555.
9. Kruger, E. A.; Figg, W. D. *Expert Opin. Investig. Drugs* **2000**, *9*, 1383.
10. Schiffmann, R.; Heine, A.; Klebe, G.; Klein, C. D. *Angew. Chem., Int. Ed.* **2005**, *44*, 3620.
11. Huang, M.; Xie, S. X.; Ma, Z. Q.; Hanzlik, R. P.; Ye, Q. Z. *Biochem. Biophys. Res. Commun.* **2006**, *339*, 506.
12. Chai, S. C.; Ye, Q. Z. *Bioorg. Med. Chem. Lett.* **2009**, *19*, 6862.
13. The enzymes were obtained by heterologous expression in *E. coli* (*Staphylococcus aureus* MetAP, *E. coli* MetAP, *H. sapiens* MetAP-1) or in Sf9 cells (*H. sapiens* MetAP-2) and subsequent chromatographic purification. For the cloning of the *S. aureus* MetAP gene, genomic DNA from a multi-resistant COL strain was used. *E. coli* MetAP was obtained using the expression plasmid

described by Profs. B. Matthews and T. Lowther (Lowther, W. T.; McMillen, D. A.; Orville, A. M.; Matthews, B. W. *Proc. Natl. Acad. Sci. U.S.A.* **1998**, *95*, 12153) The genes of the *H. sapiens* MetAPs were synthesized by a commercial supplier. A detailed description of the cloning and expression of *H. sapiens* MetAP-2 in Sf9 cells is given in the supporting information. Enzymatic assays were performed as described previously (Schiffmann, R.; Neugebauer, A.; Klein, C. D. *J. Med. Chem.* **2006**, *49*, 511). The concentration of all MetAPs in the enzymatic assays was 22 nM. Interactions with the detection enzymes AAO and HRP were excluded for all tested compounds.

14. Schiffmann, R.; Neugebauer, A.; Klein, C. D. *J. Med. Chem.* **2006**, *49*, 511.
15. The syntheses of some of the compounds presented here (cf. Table 1) were described previously. Synthetic and analytical data for the other compounds are given in the supporting information. Commercial drug substances (thiabendazole, fenbendazole, etc.) were taken from the in-house compound collection.
16. Luo, Q. L.; Li, J. Y.; Liu, Z. Y.; Chen, L. L.; Li, J.; Qian, Z.; Shen, Q.; Li, Y.; Lushington, G. H.; Ye, Q. Z.; Nan, F. J. *J. Med. Chem.* **2003**, *46*, 2631.
17. Docking was based on the 1YVM structure of *E. coli* MetAP (without the thiabendazole ligand) as receptor and the GOLD 4.1 software (Jones, G.; Willett, P.; Glen, R. C. *J. Mol. Biol.* **1995**, *245*, 43) with default settings. Visualization was performed using the Chimera software from the Resource for Biocomputing, Visualization, and Informatics at the University of California, San Francisco (Pettersen, E. F.; Goddard, T. D.; Huang, C. C.; Couch, G. S.; Greenblatt, D. M.; Meng, E. C.; Ferrin, T. E. *J. Comput. Chem.* **2004**, *25*, 1605).
18. Multiple sequence alignments were generated by ClustalW (Thompson, J. D.; Higgins, D. G.; Gibson, T. J., *Nucleic Acids Res.* **1994**, *22*, 4673) and analyzed with BioEdit (Hall, T. A. *Nucleic Acids Symp. Ser.* **1999**, *41*, 95).
19. Clark-Lewis, J. W.; Thompson, M. J. *J. Chem. Soc.* **1957**, 442.
20. Caldwell, W. T.; Kornfeld, E. C. *J. Am. Chem. Soc.* **1942**, *64*, 1695.
21. Goulaouic-Dubois, C. A.; Guggisberg, A.; Hesse, M. *J. Org. Chem.* **1995**, *60*, 5969.