[3] H. Zhu, M. Bilgin, R. Bangham, D. Hall, A. Casamayor, P. Bertone, N. Lan, R. Jansen, S. Bidlingmaier, T. Houfek, T. Mitchell, P. Miller, R. A. Dean, M. Gerstein, M. Snyder, *Science* **2001**, *293*, 2101.
[4] A.-C. Gavin, M. Bösche, R. Krause, P. Grandi, M. Marzioch, A. Bauer, J. Schultz, J. M. Rick, A.-M. Michon, C.-M. Cruciat, M. Remor, C. Höfert, M. Schelder, M. Brajenovic, H. Ruffner, A. Merino, K. Klein, M. Hudak, D. Dickson, T. Rudi, V. Gnau, A. Bauch, S. Bastuck, B. Huhse, C. Leutwein, M.-A. Heurtier, R. R. Copley, A. Edelmann, E. Querfurth, V. Rybin, G. Drewes, M. Raida, T. Bouwmeester, P. Bork, B. Seraphin, B. Kuster, G. Neubauer, G. Superti-Furga, *Nature* **2002**, *415*, 141.
[5] M. Mann, R. C. Hendrickson, A. Pandey, *Annu. Rev. Biochem.* **2001**, *70*.
[6] A. Dongre, J. K. Eng, J. R. Yates, *Trends Biotechnol.* **1997**, *15*, 418.
[7] M. R. Wilkins, K. L. Williams, R. D. Appel, D. F. Hiochstrasser, *Proteome Research: New Frontiers in Functional Genomics*, Springer, Berlin, **1997**.
[8] Y. Ho, A. Gruhler, A. Heilbut, G. D. Bader, L. Moore, S.-L. Adams, A. Millar, P. Taylor, K. Bennett, K. Boutilier, L. Yang, C. Wolting, I. Donaldson, S. Schandorff, J. Shewnarane, M. Vo, J. Taggart, M. Goudreault, B. Muskat, C. Alfarano, D. Dewar, Z. Lin, K. Michalickova, A. R. Willems, H. Sassi, P. A. Nielsen, K. J. Rasmussen, J. R. Andersen, L. E. Johansen, L. H. Hansen, H. Jespersen, A. Podtelejnikov, E. Nielsen, J. Crawford, V. Poulsen, B. D. Sørensen, J. Matthiesen, R. C. Hendrickson, F. Gleeson, T. Pawson, M. F. Moran, D. Durocher, M. Mann, C. W. V. Hogue, D. Figeys, M. Tyers, *Nature* **2002**, *415*, 180.
[9] L. Wang, A. Brock, B. Herberich, P. G. Schultz, *Science* **2001**, *292*, 498.
[10] L. Wang, A. Brock, P. G. Schultz, *J. Am. Chem. Soc.* **2002**, *124*, 1836.
[11] J. W. Chin, S. W. Santoro, A. B. Martin, D. S. King, L. Wang, P. G. Schultz, *J. Am. Chem. Soc.* **2002**, *124*, 9026.
[12] J. C. Kauer, S. Erickson-Viitanen, H. R. Wolfe, W. F. DeGrado, *J. Biol. Chem.* **1986**, *261*, 10695.
[13] J. W. Chin, A. B. Martin, D. S. King, L. Wang, P. G. Schultz, *Proc. Natl. Acad. Sci. USA* **2002**, *99*, 11020.
[14] G. Dorman, G. D. Prestwich, *Biochemistry* **1994**, *33*, 5661.
[15] D. A. Fancy, K. Melcher, S. A. Johnston, T. Kodadek, *Chem. Biol.* **1996**, *3*, 551.
[16] Y. Maru, D. E. Afar, O. N. Witte, M. Shibuya, *J. Biol. Chem.* **1996**, *271*, 15353.
[17] M. A. McTigue, D. R. Williams, J. A. Tainer, *J. Mol. Biol.* **1995**, *246*, 21.
[18] W. A. Houry, D. Frishman, C. Eckerskorn, F. Lottspeich, F. U. Hartl, *Nature* **1999**, *402*, 147.
[19] The near-UV irradiation per unit area (fluence) at 365 nm used in these studies for a five-minute exposure was approximately $10-30\ kJ\ m^{-2}$. Classic studies on the effects of bacterial exposure at this wavelength have shown that lethal effects of such radiation on bacterial cells require fluences one to two orders of magnitude greater than this value. This observation raises the possibility that the phenotypes of live cells may be altered by photocrosslinking between protein surfaces in vivo.
[20] B. Alberts, *Cell* **1998**, *92*, 291.

Received: May 17, 2002
Revised version: September 3, 2002 [Z421]

De Novo Design, Synthesis, and In Vitro Evaluation of a New Class of Nonpeptidic Inhibitors of the Malarial Enzyme Plasmepsin II

David A. Carcache,[a] Simone R. Hörtner,[a] Andreas Bertogg,[a] Christoph Binkert,[b] Daniel Bur,*[b] Hans Peter Märki,[c] Arnulf Dorn,[c] and François Diederich*[a]

KEYWORDS:

de novo design · enzymes · inhibitors · malaria · structure–activity relationships

Malaria, a life-threatening disease caused by parasites of the genus *Plasmodium*, affects 500 million people annually, of which more than one million die.[1] The emergence of multi-drug-resistant strains of *Plasmodium falciparum*, the parasite that causes the deadliest form of malaria, exacerbates the situation and necessitates new medicines with novel modes of action.[2] Plasmepsin II (PII; EC 3.4.23.39),[3] a parasitic aspartic protease involved in the hemoglobin degradation process that takes place in an acidic vacuole, has been identified as a potential target for antimalarial therapy. Several groups reported PII inhibitors that mimic the natural substrate and display up to single-digit nanomolar activity.[4] Inhibition of PII is expected to block the life cycle of the parasite.[5] Here we report the synthesis and in vitro evaluation of a new class of nonpeptidic PII inhibitors developed with the help of structure-based de novo design that show up to single-digit micromolar inhibitory activities.[6–8]

A major conformational change around the active site of the human aspartic protease renin (EC 3.4.23.15) upon complexation of 3,4-disubstituted piperidines has been observed, which unveils unexpected flexibility of the enzyme.[9] The flap that lies over the catalytic dyad, and a tryptophan side chain of the core domain, move and thereby unlock a new hydrophobic pocket (flap pocket). The high sequence homology between renin and PII prompted us to hypothesize that an induced-fit adaptation such as that of the active site of renin might also be operative

[a] *Prof. F. Diederich, Dipl.-Chem. D. A. Carcache, Dipl.-Natw. S. R. Hörtner, Dipl.-Chem. A. Bertogg*
Laboratorium für Organische Chemie
ETH-Hönggerberg, HCI
8093 Zürich (Switzerland)
Fax: (+41) 1-632-1109
E-mail: diederich@org.chem.ethz.ch

[b] *Dr. D. Bur, Dr. C. Binkert*
Actelion Pharmaceuticals Ltd.
Gewerbestrasse 16, 4123 Allschwil (Switzerland)
Fax: (+41) 61-487-4500
E-mail: daniel.bur@actelion.com

[c] *Dr. H. P. Märki, Dr. A. Dorn*
Pharma Research
F. Hoffmann-La Roche Ltd.
4070 Basel (Switzerland)

upon complexation of adequate inhibitors to the enzyme found in *Plasmodium*.[10] Further evidence for the structural flexibility of PII arose from the crystal structure of proplasmepsin II.[11] The conformational changes observed in renin complexes have, therefore, been modeled into the X-ray structure of PII[3] and exploited in our design approach (Figure 1). A major hallmark of the active-site modification found in renin complexes is the disruption of the H bond between Tyr75 and Trp39 (pepsin numbering) caused by lifting of the flap and movement of these two side chains into new positions. This conformational change unlocks a large and very hydrophobic pocket, which we call the flap pocket (Figure 2).

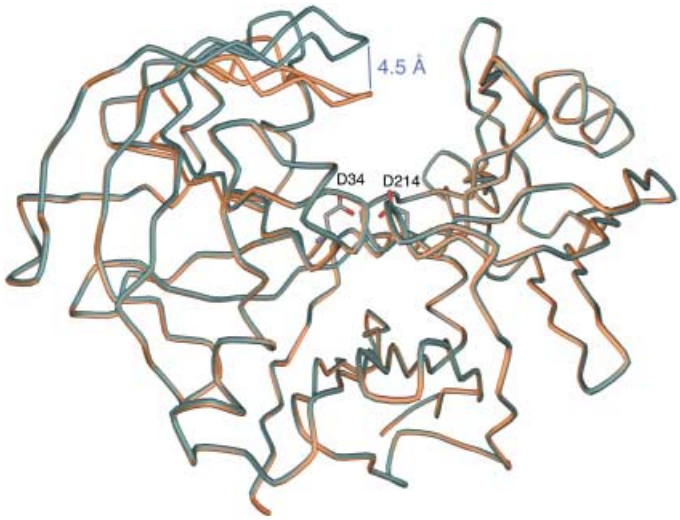


Figure 1. *Superimposition of the X-ray crystal structure of PII (orange) with the modeled structure (green), with the flap moved away from the catalytic dyad.*

We used the molecular modeling package MOLOC[12] and the molecular recognition principles of protein–ligand systems to design small molecules that are well accommodated by the active site of our modeled structure of PII. The 11-azatricyclo[6.2.1.$0^{2,7}$]undeca-2(7),3,5-triene tricycle was identified as a suitable central scaffold for PII inhibitors. The positioning of this tricycle in the active site of PII is dominated by the effects of two charge-reinforced H bonds between its nitrogen atom and each of the two Asp residues of the catalytic dyad.

Substitution at positions 5 and 6 of the central scaffold was expected to provide two exit vectors pointing into the large hydrophobic flap pocket and the combined S1/S3 pocket, respectively (Figure 2). Our analysis revealed that a naphthyl moiety could be accommodated by the S1/S3 pocket. Modeling also suggested that the rear of the flap pocket, which is rather spacious, should be well filled by hydrophobic heterocyclic residues linked to the central scaffold by aliphatic linkers.

Based on this analysis, we prepared a series of inhibitors (±)-**1a**–**k** (Scheme 1) by variation of the heterocyclic residue that points into the flap pocket and the linker that attaches it to the central scaffold, as well as variation of the linker to the differently substituted naphthyl moieties used to occupy the S1/S3 pocket.

Diels–Alder reaction of protected pyrrole **3**[13] with a benzyne species generated in situ from **2**,[14] gave the tricyclic scaffold **4** (Scheme 2). Hydrogenation, followed by a sequence of nucleophilic substitutions afforded first (±)-**5a**–**c**, then the *N*-protected inhibitors (±)-**6a**–**f**.[15] Deprotection by intermediate formation of the *tert*-butyldimethylsilyl carbamate followed by cleavage under basic conditions provided the inhibitors (±)-**1a**–**f**. Compounds **1g** and (±)-**1h**,**i** were obtained by a similar route.[15]

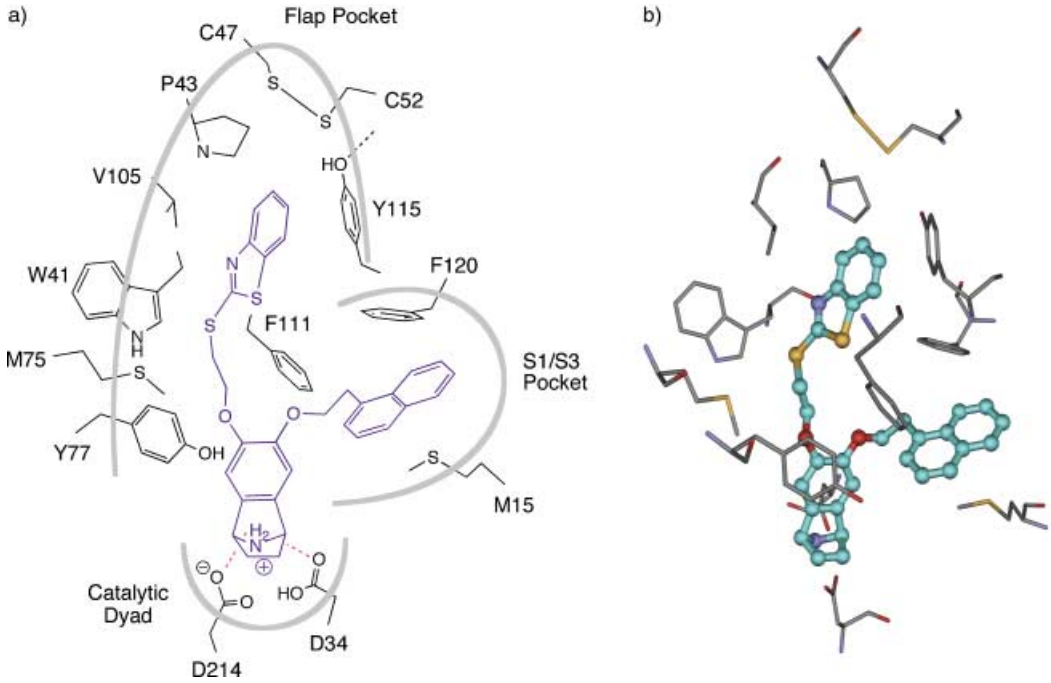
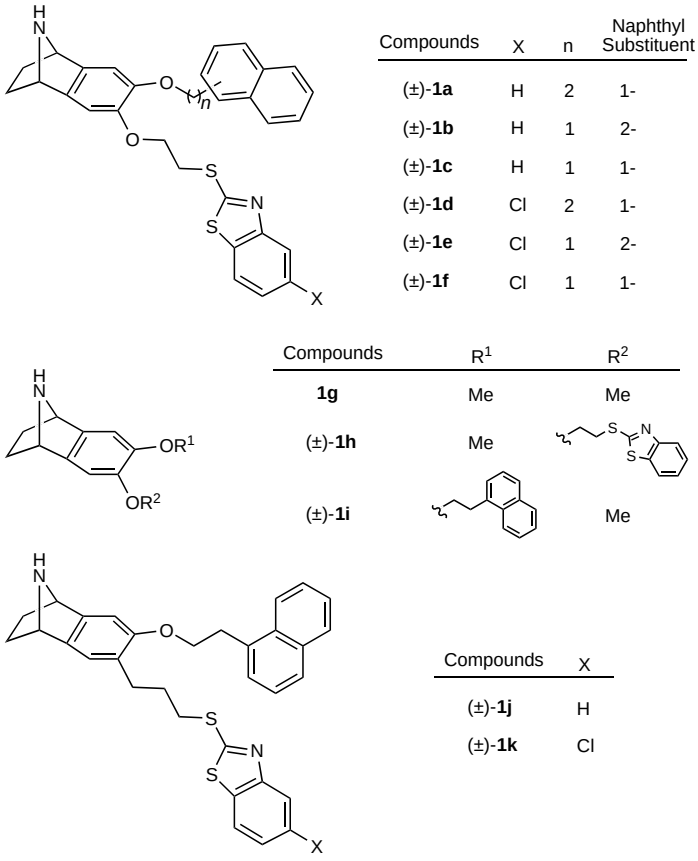


Figure 2. *Schematic (2a) and ball-and-stick (2b) representations of (±)-**1a** in the active site of the modeled structure of PII. The complexation of the (1S,8R)-**1a** enantiomer is shown. See Scheme 1 for the structure of **1a**.*

  1439-4227/02/03/11 $ 20.00+.50/0 ChemBioChem **2002**, No. 11



Compounds	X	n	Naphthyl Substituent
(±)-**1a**	H	2	1-
(±)-**1b**	H	1	2-
(±)-**1c**	H	1	1-
(±)-**1d**	Cl	2	1-
(±)-**1e**	Cl	1	2-
(±)-**1f**	Cl	1	1-

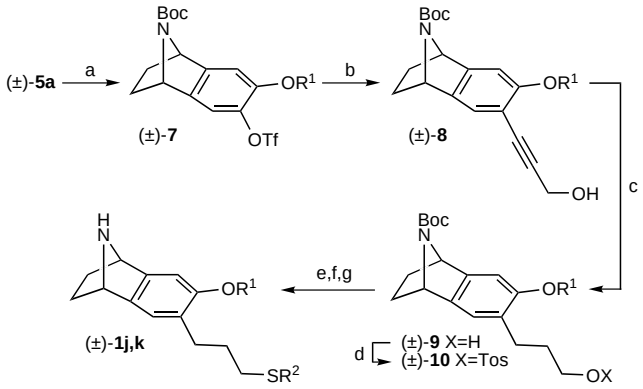
Compounds	R^1	R^2
1g	Me	Me
(±)-**1h**	Me	
(±)-**1i**		Me

Compounds	X
(±)-**1j**	H
(±)-**1k**	Cl

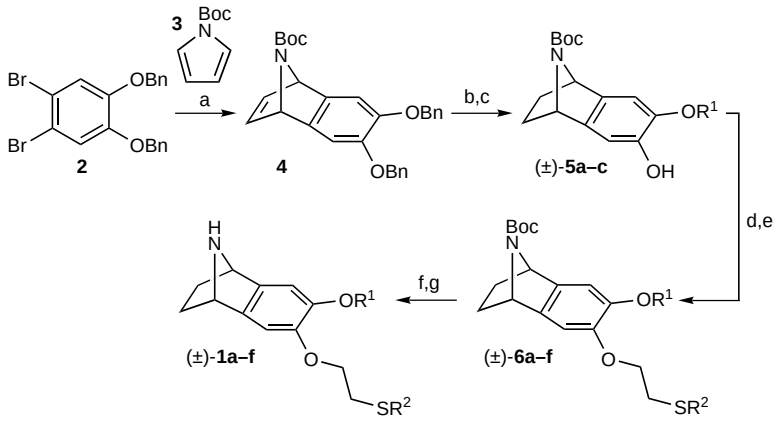
Scheme 1. *The series of inhibitors prepared.*

that replacement of one of the aryl ether oxygen atoms by a CH_2 group should permit the new inhibitors (±)-**1 j,k** to adopt a more favorable conformation.

The synthesis of (±)-**1 j,k** started from (±)-**5 a**, which was transformed into aryl triflate (±)-**7** (Scheme 3). Sonogashira cross-coupling[16] with propargyl alcohol furnished (±)-**8**. Reduction of the triple bond gave (±)-**9**, which was converted into tosylate (±)-**10**. Final nucleophilic substitution and *N*-deprotection yielded the inhibitors (±)-**1 j,k.**[15]



Scheme 3. *Synthesis of inhibitors (±)-**1 j,k**. Reagents and conditions: a) $(CF_3SO_2)_2O$, pyridine, CH_2Cl_2, $-40\,^\circ C$, 2 h, 86%; b) propargyl alcohol, piperidine, $[Pd(PPh_3)_4]$, $80\,^\circ C$, 36 h, sealed tube, 57%; c) H_2, Pd/C (10%), MeOH, RT, 12 h, 100%; d) TosCl, pyridine, CH_2Cl_2, $0\,^\circ C \rightarrow$RT, 90 min, 51%; e) R^2SH, NaH, DMF, RT, 12–23 h, 72–100%; f) $Me_2(tBu)SiOSO_2CF_3$, 2,6-lutidine, CH_2Cl_2, RT, 45–80 min; g) aq K_2CO_3, MeOH/THF, RT, 60–90 min, 41% (over two steps). See Scheme 1 for the nature of the R^1 and R^2 groups.*



Scheme 2. *Synthesis of inhibitors (±)-**1 a–f**. Reagents and conditions: a) tBuLi, toluene/hexane, $-40\,^\circ C \rightarrow 0\,^\circ C$, 3 h, 65%; b) H_2, Pd/C (10%), CH_3CN, RT, 3 h, 86%; c) R^1X, K_2CO_3, dimethylformamide (DMF), 60–80 °C, 12–18 h, 53–64% (X = Br, OTos ($OSO_2C_6H_4$-p-Me)); d) 1,2-dibromoethane, KOH, nBu_4NOH, H_2O, 50 °C, 12 h, 92–100%; e) R^2SH, NaH, DMF, 80 °C, 1–2 h, 63–77%; f) $Me_2(tBu)SiOSO_2CF_3$, 2,6-lutidine, CH_2Cl_2, RT, 30 min; g) aq K_2CO_3, tetrahydrofuran (THF)/MeOH, RT, 45 min, 71–87% (over two steps). See Scheme 1 for the nature of the R^1 and R^2 groups.*

During the syntheses, we noticed that the conformations adopted by the two ether substituents of (±)-**1 a–f** were not optimal in the bound state, with torsional angles $C(sp^2)$–$C(sp^2)$–O–$C(sp^3)$ that deviate substantially from planarity in the modeled PII complexes. Computational studies suggested

IC_{50} values of (±)-**1 a–k** against PII and several other aspartic proteases (plasmepsin IV, cathepsins D and E, and renin) were determined in automated assays.[17] The inhibitors bearing two substituents displayed promising inhibitory activity and good selectivity towards renin (Table 1). Activity against plasmepsin II is higher in each case than the affinities for the other aspartic proteases as determined in the assay. The modest selectivity of the inhibitors (factors of 2–3) with respect to cathepsin D and E could suggest high homology in the active sites of the enzymes.

The data for the first series of inhibitors (±)-**1 a–f** (Scheme 1) tend to support our hypothesis that conformational changes observed in renin[9] in the flap atop the active site are also operative in PII. Chloro-substituted benzothiazole inhibitors ((±)-**1 d–f**) were predicted by the molecular modeling to have a better shape complementarity with respect to the flap pocket than the benzothiazole derivatives that lack the halogen atom ((±)-**1 a–c**). This prediction was confirmed by experimental results; the chlorinated inhibitors showed a three- to fourfold increase in affinity with respect to their nonchlorinated analogues. These results indicate that our inhibitors could adopt the expected conformation in the active site of PII.

Table 1. *Biological activities and selectivities of inhibitors (±)-**1 a** – **k**.*

Inhibitor	PII[a] [μM]	PIV[b] [μM]	IC_{50} Cat.D[c] [μM]	Cat.E[d] [μM]	Renin [μM]
(±)-**1 a**	13	60	18	34	83
(±)-**1 b**	10	85	23	42	81
(±)-**1 c**	14	70	25	46	> 100
(±)-**1 d**	3	15	12	6	> 100
(±)-**1 e**	4	50	11	14	> 100
(±)-**1 f**	5	17	12	7	> 100
1 g	> 100	> 100	> 100	> 100	> 100
(±)-**1 h**	34	54	71	47	> 100
(±)-**1 i**	24	81	82	70	> 100
(±)-**1 j**	4	33	12	9	93
(±)-**1 k**	2	10	7	4	> 100

[a] Plasmepsin II. [b] Plasmepsin IV. [c] Cathepsin D. [d] Cathepsin E.

The negligible effects of naphthyl substitution pattern and linker length on affinities displayed by compounds (±)-**1 a** – **f** suggest that the combined S1/S3 pocket is capable of accommodating residues up to the size of naphthyl groups in different orientations with similar affinity. These observations furnish further evidence for substantial flexibility of PII and an induced-fit adaptation of the active site upon complexation with our inhibitors.

Comparison of **1 g** and (±)-**1 h**,**i** with the other inhibitor series indicates that at least one of the two hydrophobic pockets (S1/S3 or flap) needs to be occupied in order to obtain measurable inhibitory activity. Compound **1 g**, which lacks suitably sized substituents, showed no activity at all. Compounds (±)-**1 h**,**i** showed measurable binding affinities that were, however, weaker than those displayed by the corresponding derivatives (±)-**1 a** – **c**, which feature two suitably sized substituents.

Replacement of the oxygen atom (in (±)-**1 a** and (±)-**1 d**) in the linker of the substituent that points into the flap pocket by a CH_2 group (in (±)-**1 j** and (±)-**1 k**) gave a two- to threefold enhancement in activity, as expected from the conformational analysis. This result emphasizes the importance of preorganization in low-energy states of the inhibitors in the protein – ligand complexation process. Introduction of a chloro substituent into the benzothiazole moiety of (±)-**1 k** again enhanced the affinity by a factor of two, as previously observed by comparison of inhibitors (±)-**1 d** – **f** with (±)-**1 a** – **c**. It is further anticipated that introduction of more rigid linkers between the central scaffold and the bicycles located in the S1/S3 and flap pockets might improve the binding potency of our compounds by decreasing the entropy loss associated with binding of inhibitors with floppy substituents.

In conclusion, de novo design based on a modeled structure of PII followed by convenient synthesis afforded a new class of PII inhibitors that display promising IC_{50} values and good selectivity towards renin, and little selectivity with respect to other aspartic proteases used. The investigations support the speculation that the active site of PII undergoes major conformational changes upon binding of adequate inhibitors. The flap pocket found to be unlocked and filled by inhibitors in renin complexes could turn out to play a similar role in PII. Efforts to ultimately validate the proposed binding modes by X-ray crystal structure analysis are ongoing.

We thank the Eidgenössische Technische Hochschule (ETH) research council and F. Hoffmann – La Roche for support of the work done at the ETH.

[1] U. Weiss, *Nature* **2002**, *415*, 669.
[2] R. G. Ridley, *Nature* **2002**, *415*, 686 – 693.
[3] A. M. Silva, A. Y. Lee, S. V. Gulnik, P. Majer, J. Collins, T. N. Bhat, P. J. Collins, R. E. Cachau, K. E. Luker, I. Y. Gluzman, S. E. Francis, A. Oksman, D. E. Goldberg, J. W. Erickson, *Proc. Natl. Acad. Sci. USA* **1996**, *93*, 10034 – 10039.
[4] For peptidic and peptidomimetic inhibitors of plasmepsins, see: a) C. D. Carroll, H. Patel, T. O. Johnson, T. Guo, M. Orlowski, Z.-M. He, C. L. Cavallaro, J. Guo, A. Oksman, I. Y. Gluzman, J. Connelly, D. Chelsky, D. E. Goldberg, R. E. Dolle, *Bioorg. Med. Chem. Lett.* **1998**, *8*, 2315 – 2320; b) T. S. Haque, A. G. Skillman, C. E. Lee, H. Habashita, I. Y. Gluzman, T. J. A. Ewing, D. E. Goldberg, I. D. Kuntz, J. A. Ellman, *J. Med. Chem.* **1999**, *42*, 1428 – 1440; c) S. Jiang, S. T. Prigge, L. Wei, Y.-E. Gao, T. H. Hudson, L. Gerena, J. B. Dame, D. E. Kyle, *Antimicrob. Agents Chemother.* **2001**, *45*, 2577 – 2584.
[5] G. H. Coombs, D. E. Goldberg, M. Klemba, C. Berry, J. Kay, J. C. Mottram, *Trends Parasitol.* **2001**, *17*, 532 – 537.
[6] a) U. Obst, V. Gramlich, F. Diederich, L. Weber, D. W. Banner, *Angew. Chem.* **1995**, *107*, 1874 – 1877; *Angew. Chem. Int. Ed. Engl.* **1995**, *34*, 1739 – 1742; b) U. Obst, P. Betschmann, C. Lerner, P. Seiler, F. Diederich, V. Gramlich, L. Weber, D. W. Banner, P. Schönholzer, *Helv. Chim. Acta* **2000**, *83*, 855 – 909; c) P. Betschmann, S. Sahli, F. Diederich, *Helv. Chim. Acta* **2002**, *85*, 1210 – 1245.
[7] a) B. Masjost, P. Ballmer, E. Borroni, G. Zürcher, F. K. Winkler, R. Jakob-Roetne, F. Diederich, *Chem. Eur. J.* **2000**, *6*, 971 – 982; b) C. Lerner, A. Ruf, V. Gramlich, B. Masjost, G. Zürcher, R. Jakob-Roetne, E. Borroni, F. Diederich, *Angew. Chem.* **2001**, *113*, 4164 – 4166; *Angew. Chem. Int. Ed.* **2001**, *40*, 4040 – 4042.
[8] E. A. Meyer, R. Brenk, R. K. Castellano, M. Furler, G. Klebe, F. Diederich, *ChemBioChem* **2002**, *3*, 250 – 253.
[9] C. Oefner, A. Bingeli, V. Breu, D. Bur, J.-P. Clozel, A. D'Arcy, A. Dorn, W. Fischli, F. Grüninger, R. Güller, G. Hirth, H.-P. Maerki, S. Matthews, M. Müller, R. G. Ridley, H. Stadler, E. Vieira, M. Wilhelm, F. K. Winkler, W. Wostl, *Chem. Biol.* **1999**, *6*, 127 – 131.
[10] For a broader application of 3,4-disubstituted piperidine inhibitors of the aspartic proteases renin and pepsin, which undergo in both cases a conformational change of the flap atop the catalytic site, see: a) M. G. Bursavich, C. W. West, D. H. Rich, *Org. Lett.* **2001**, *3*, 2317 – 2320; b) M. Bursavich, D. H. Rich, *Org. Lett.* **2001**, *3*, 2625 – 2628.
[11] N. K. Bernstein, M. M. Cherney, H. Loetscher, R. G. Ridley, M. N. G. James, *Nat. Struct. Biol.* **1999**, *6*, 32 – 37.
[12] P. R. Gerber, K. Müller, *J. Comput.-Aided. Mol. Des.* **1995**, *9*, 251 – 268.
[13] L. Grehn, U. Ragnarsson, *Angew. Chem.* **1984**, *96*, 291 – 292; *Angew. Chem. Int. Ed. Engl.* **1984**, *23*, 300 – 301.
[14] M. Hu, N. Brasseur, S. Z. Yildiz, J. E. v. Lier, C. C. Leznoff, *J. Med. Chem.* **1998**, *41*, 1789 – 1802.
[15] All new compounds were fully characterized by ^1H and ^{13}C NMR and IR spectroscopies, MS, and elemental analysis or high-resolution MS.
[16] K. Sonogashira in *Metal-catalyzed Cross-coupling Reactions* (Eds.: F. Diederich, P. J. Stang), Wiley-VCH, Weinheim, **1998**, pp. 203 – 230.
[17] The proteolytic activities of plasmepsins II and IV and cathepsins D and E were determined in a fluorescence resonance energy transfer based assay with a substrate purchased from Bachem (#M-2120). The enzyme (approximately 1 nM) was incubated with substrate (1 nM) at 37 °C in NaOAc (50 mM, pH 5), glycerol (12.5% w/v), bovine serum albumin (BSA; 0.1% w/v), and Me_2SO (10%). Enzyme activity was derived from the turnover rate of the substrate and monitored by observation of the increase of the fluorescent signal over time. Fluorescence was determined with a FLUOstar Galaxy spectrometer from BMG with excitation and emission filters at 355 and 520 nm. The proteolytic activity of renin was determined in two steps. Renin (approximately 2 pM) was incubated at 37 °C with a peptide substrate (Bachem, #M-11200; 5 mM) whose sequence is derived from the renin cleavage site in angiotensinogen. The assay buffer contained phosphate-buffered saline (pH 7.4), ethylenediamine tetraacetic acid (1 mM), 8-hydroxyquinoline sulfate (1 mM), BSA

  1439-4227/02/03/11 $ 20.00+.50/0 ChemBioChem **2002**, No. 11

(0.1%), and Me_2SO (5%). The angiotensin 1 produced by the renin was subsequently quantified with an enzyme immunoassay by comparison with an angiotensin-1 standard curve. Test compounds were dissolved and diluted in 100% Me_2SO. Inhibitory activities are expressed as IC_{50} values, which represent the concentration of compound that inhibits 50% of the maximal (uninhibited) enzyme activity. IC_{50} values for some of the inhibitors were also determined by a second method, not reported here; good agreement between the data was found.

Received: July 19, 2002 [Z458]

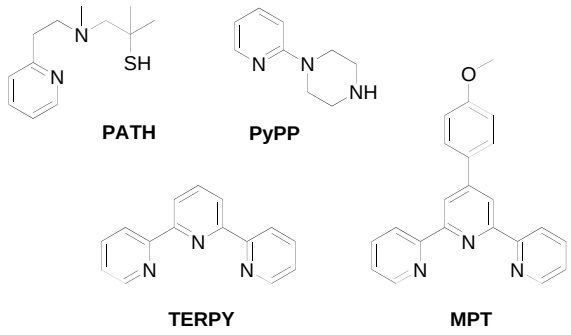
Protease Inhibitors Formed In Situ from Copper and Tridentate Chelates: A Generalized Approach towards Metal-Based Pharmaceuticals

Christopher J. Brinkerhoff,[+[a]] Paul Podsiadlo,[+[a]] Tomoko Komiyama,[b] Robert S. Fuller,[b] and Ofer Blum*[a]

KEYWORDS:

bioinorganic chemistry · chelation · drug research · furin · N ligands

Metal-based pharmaceuticals are expected to bind to their targets with high affinity, which may translate to reduced drug intake and safer treatment. Their distinct mode of action suggests they have excellent prospects in therapy against drug-resistant pathogens.[1] By their sheer number, they vastly increase the pool from which new drugs can be chosen. Metal compounds find wide use in medical diagnostics.[2] However, these compounds are coordinatively saturated and unable to bind biological molecules and bring about therapeutic effects. Of the few metallodrugs currently in use, most target DNA.[2] We therefore set out to develop a generalized methodology to obtain protein-targeted, metal-based drugs. To eliminate any fear of toxicity associated with ingesting metals, our strategy involves a prodrug intended to extract the metal from within the patient's body. Our in vitro model for this new approach involves the in situ formation of a ternary complex containing a chelate (the prodrug), a metal, and the target enzyme. We now report that the readily available tridentate chelates 2,2′:6′,2″-terpyridine (TERPY), 2-methyl-1-[methyl(2-pyridine-2-yl-ethyl)amino]-propane-2-thiol (PATH),[3] and 1-[2-pyridyl]-piperazine (PyPP) coordinate Cu^{2+} ions in situ to enhance the potency of Kex2



inhibition beyond that of the solvated metal ion. Furthermore, TERPY makes the inhibition irreversible. The variability among these chelates and the fact that none inhibits the target by itself suggest that our model applies to a range of chelates. As the single criterion we used to choose our enzyme was its inhibition by solvated metal ions (its structure is unknown), we suggest that our model has the potential to apply to additional targets. An approach *complementary* to ours, in which enzyme inactivation by a potent bidentate chelating inhibitor is enhanced by the coordination of Zn^{2+} ions, was demonstrated with trypsin-like serine proteases.[4, 5]

Kex2 is a yeast endopeptidase that converts protein precursors into biologically active proteins to be secreted from the cell.[6] Most Kex2 inhibitors also affect the mammalian homologue furin. Furin activates the envelope glycoproteins of many viruses, including Ebola, avian influenza, and rabies prior to exocytosis, thereby enabling their uptake by new host cells.[7] As furin is expressed in most body cells, its inhibitors may stop proliferation of these aggressive viruses after exposure and possibly even after the related disease symptoms appear. Furin blockers may also affect toxins of bacteria such as *Bacillus anthracis* (which causes anthrax) and Pseudomonas by hindering maturation of their protoxins during endocytosis.[7] Such antidote-like treatment is needed when toxin levels render antibiotics ineffective.

Both Zn^{2+} and Cu^{2+} ions inhibit Kex2 in vitro,[8] with Cu^{2+} ions being more potent. Likewise, chelate enhancement of inhibition was much better with Cu^{2+} ions. We report only results with copper.

In the present study we used tridentate chelates as our prodrug models. Their binding constants to metals are much higher than those of bidentates.[9] Thus, tridentates are predicted to compete more effectively for the endogenous metals against natural cellular factors. Tetradentates will bind more strongly, but are less likely to generate sufficient free sites on the metal for target coordination. We tested chelates that seemed likely to bind to Cu^{2+} ions, but target inhibition was not among the criteria in choosing them. None of our chelates inhibited Kex2 on

[a] *Prof. Dr. O. Blum, C. J. Brinkerhoff,[+] P. Podsiadlo[+]*
Department of Chemical Engineering
The University of Michigan
Ann Arbor, MI 48109 (USA)
Fax: (+1) 734-763-0459
E-mail: oferblum@umich.edu

[b] *Dr. T. Komiyama, Prof. Dr. R. S. Fuller*
Department of Biological Chemistry
The University of Michigan, Ann Arbor (USA)

Supporting information for this article is available on the WWW under http://www.chembiochem.com or from the author.

[+] *These authors contributed equally to this work.*